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Immunoglobulin E-dependent stimulation of human alveolar macrophages: significance in type 1 hypersensitivity

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SUMMARY

Human alveolar macrophages were obtained during diagnostic bronchoalveolar lavage. Cells were cultured, and morphological examination (including electron microscopy) revealed that not more than 5% of the cultured cells were identifiable as cells other than alveolar macrophages. The cells were sensitized with human myeloma immunoglobulin E, and then challenged with anti-immunoglobulin E anti-sera. The experiments employed a highly specific monoclonal antibody and three affinity purified reagents. The formation of immunoglobulin E/anti-immunoglobulin E complexes facilitated release from alveolar macrophages of leukotriene B_4, prostaglandin $F_{2\alpha}$, thromboxane B_2 and the lysosomal hydrolase *N*-acetyl-β-D-glucosaminidase. There was no release of active oxygen species, with this stimulus, as measured by lucigenin chemiluminescence. Immunoglobulin E receptors were identified histochemically on the surface of human alveolar macrophages, and were visualized as conjugates with colloidal gold by electron microscopy. These results support the view that human alveolar macrophages may contribute to type 1 hypersensitivity reactions in the lung.

Keywords macrophages immunoglobulin E allergy leukotriene B_4 thromboxane B_2

INTRODUCTION

Type 1 hypersensitivity reactions have been implicated in the cause of extrinsic (allergic) asthma. These responses are mediated by the E subclass of immunoglobulins (Ig) (Ishizaka, Ishizaka & Hornbrook, 1966a,b; Ishizaka, Tomioka & Ishizaka, 1970), and release of stored histamine is facilitated by cross-linking IgE on tissue mast cells (Ishizaka & Ishizaka, 1968; Ishizaka *et al.*, 1975). The effects of histamine are augmented by the simultaneous release of oxidative metabolites of arachidonic acid, specifically prostaglandin D_2 (Holgate *et al.*, 1984) and leukotrienes C_4 and D_4 (Bach *et al.*, 1980; Lewis & Austen, 1981).

Human asthma is not however explained fully by this simple model. Many inhaled antigens have a particle size of >5 μm (Tovey *et al.*, 1981), which would not allow transit beyond the smaller airways, nor through the epithelium. Cells within the lumen that are retrieved by bronchoalveolar lavage comprise $<0{\cdot}25\%$ mast cells, even in asthma (Tomioka *et al.*, 1984). The interaction

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therefore between inhaled antigen and intraluminal mast cells is limited significantly. Furthermore, inhibition of histamine (H_1 or H_2) receptors produces little significant reduction in airway resistance in asthma (Partridge & Saunders, 1979). The most abundant cell (> 80%) retrieved by airway lavage is the alveolar macrophage (Tomioka *et al.*, 1984). These cells have a central role in numerous immune responses, and during phagocytosis release high concentrations of thromboxane A_2 (MacDermot *et al.*, 1984), leukotriene B_4 (Fels *et al.*, 1982; MacDermot *et al.*, 1984), numerous lysosomal hydrolases (Joseph *et al.*, 1980; MacDermot *et al.*, 1984; Tonnel *et al.*, 1983) and active oxygen species (Joseph *et al.*, 1980; Williams & Cole, 1981). A role for macrophages in allergic responses was suggested by the demonstration in rodent macrophages of IgE Fc receptors (Boltz-Nitulescu & Spiegelberg, 1981), and the capacity to synthesise leukotriene C_4 and leukotriene D_4 (Scott, Rouzer & Cohn, 1983). Furthermore, antigen bridging of surface IgE facilitates release of leukotriene E_4 (Rankin *et al.*, 1982), a product of leukotriene C_4 and D_4 metabolism. A possible role for alveolar macrophages in human type 1 hypersensitivity reactions in the lung was suggested by the release of β-glucuronidase from these cells following challenge with antigen or anti-IgE (Joseph *et al.*, 1983). The present report describes experiments in which the role of alveolar macrophages in human type 1 hypersensitivity is explored further. The confidence with which IgE is implicated in macrophage responses is dependent on the specificity of the anti-IgE employed. Thus, experiments have been performed with a highly specific monoclonal anti-IgE, and three affinity purified polyclonal anti-IgE reagents.

MATERIALS AND METHODS

Macrophage culture

Bronchoalveolar lavage with 200 ml of warm normal saline was performed during diagnostic bronchoscopy on 32 patients (17 with carcinoma, nine with infection or sarcoidosis, six normal). The lavage was performed with informed patient consent as part of a routine diagnostic service. Cells harvested from the lavage were filtered through a fine nylon mesh and centrifuged at 150 ***g*** for 10 min. The cells were resuspended in Dulbecco's modified minimal essential medium (DMEM), and cultured for 1 h at 37°C in 2 ml of DMEM in 35 mm diameter plastic dishes (Falcon Plastics) to allow adhesion of macrophages to the surface. The cells were then washed with DMEM at 4°C to remove non-adherent cells before incubation in 1 ml of DMEM in the absence or presence of 10,000 iu/ml of human myeloma IgE at 4°C for 1 h. The cells were washed again with DMEM at 4°C before incubation at 4°C for 30 min with 1 ml of DMEM, containing selected concentrations of anti-human IgE immunoglobulin. The dishes were then warmed to 37°C, and cultured for 1 h, except during time-course experiments when cells were cultured for various times up to 160 min. The frequent washing procedures resulted in very great and variable release of mediators if cells were not cooled as described above. DMEM supernatant fractions (0·9 ml) were mixed with 0·1 ml 0·5 M Tris-HCl buffer, pH 7·4, before storage at −20°C. Cell protein content of the dishes was then analysed using a modification of the method of Lowry *et al.* (1951).

Immunoglobulins

Human myeloma IgE was a kind gift from Dr P. Richies, Protein Reference Laboratory, Westminster Hospital Medical School. The serum contained $6{\cdot}5 \times 10^5$ iu/ml IgE.

Anti-human IgE

1. Mouse monoclonal anti-human IgE was a kind gift from Dr A. Saxon, Los Angeles (Hassner & Saxon, 1983).

2. Rabbit anti-human IgE (antibodies a and b). The first injection into hind leg muscle of New Zealand white rabbits used 0·5 mg IgE (a kind gift from Professor Platts-Mills) and complete Freund's adjuvant. Rabbits were boosted five times at monthly intervals with 0·25 mg IgE and incomplete Freund's adjuvant (subcutaneously). The antiserum was adsorbed on Sepharose 4B coated with 30 mg IgE, and eluted with 0·2 M glycine-HCl buffer, pH 2·8. The yield of anti-IgE was 80 mg. Gel diffusion of this antibody (antibody a) revealed a strong precipitation line against human

IgE, and weak precipitation against human IgG. Further adsorption with IgG coated Sepharose 4B produced an affinity purified antibody (antibody b) that did not bind to human IgG in a radioimmunoassay.

3. Goat anti-human IgE (antibody c). Goat anti-human IgE was purified from crude antibody by passing it through a polyclonal IgG affinity column to adsorb anti-light chain activity. Immunoassay of the column effluent showed that immunoglobulin directed against kappa and lambda chains constituted $<0{\cdot}01\%$ of the purified immunoglobulin (Holgate *et al.*, 1984).

Thromboxane B_2 analysis

Thromboxane B_2 was measured by radioimmunoassay (Fuller *et al.*, 1984). The thromboxane antibody and thromboxane B_2 were generous gifts from Dr P.V. Halushka (Medical University of South Carolina) and the Upjohn Company respectively. [^{3}H]Thromboxane B_2 (140 Ci/mmol) was obtained from Amersham International.

Prostaglandin $F_{2\alpha}$ analysis

An internal standard of 10 ng of [$^2H_4$]prostaglandin $F_{2\alpha}$ (a generous gift from the Upjohn Co, USA) was added to each sample. Analysis involved capillary column gas chromatography coupled to negative ion chemical ionization mass spectrometery (Waddell, Blair & Wellby, 1983).

Leukotriene B_4 analysis

Samples for analysis were spiked with about 7,500 d/min of [^{3}H]leukotriene B_4. The samples were diluted 1:9 in $0{\cdot}04\%$ (v/v) acetic acid, pH 3·8. These samples (10 ml) were then run onto the loading column of an HPLC system by a 5 ml loop, and switched into an aqueous solvent stream ($0{\cdot}04\%$ acetic acid v/v, pH 3·8) flowing at 2 ml/min through a Brownlee holder/Varian MCH-10 C_{18} cartridge. Samples were eluted from these cartridges onto an analytical column (25 cm × 0·5 cm with 5 μm Hypersil ODS) with a methanol gradient. The methanol gradient was programmed (methanol:water:acetic acid 67:43:0·017 to 96:4:0·0016 v/v/v) at a flow rate of 1 ml/min over 90 min. The time zero eluant was adjusted to pH 5·3 with NH_4OH. The column was conditioned with 30 ml of $0{\cdot}04\%$ (v/v) acetic acid pH 3·8, 90 ml of $0{\cdot}3\%$ (w/v) EDTA (tetra-Na salt) containing $0{\cdot}1\%$ (w/v) Desferal and finally 40 ml of $0{\cdot}04\%$ (v/v) acetic acid, pH 3·8. Fractions (2 ml) around each [^{3}H]leukotriene B_4 peak (retention time 36–38 min) were pooled, dried in a vacuum, and resuspended in distilled water. The leukotriene B_4 content was measured by radioimmunoassay (Fuller *et al.* 1984; Salmon, Simmons & Palmer, 1982); the leukotriene antibody was a generous gift from Wellcome Diagnostics, UK. Some of the redissolved leukotriene B_4 (50–100 μl) was set aside for liquid scintillation counting to estimate recovery. Recovery varied between 30 and 71% (mean = $50{\cdot}4\%$).

N-acetyl-β-D-glucosaminidase (EC 3.2.1.30) activity

Enzyme activity was measured as the hydrolysis of *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide to release *p*-nitrophenol (MacDermot *et al.*, 1984). One unit of activity = 1 μmol product per min.

Chemiluminescence

The cells from bronchoalveolar lavage were washed and resuspended in colourless medium 199 (Flow Laboratories). The cell concentration was adjusted to 4×10^5 cells/ml. In all experiments 500 μl of cell suspension was added to polythene tubes at 4°C, in the absence or presence of human myeloma IgE (final concentration = 10,000 units/ml IgE). The cells were kept at 4°C for 60 min. Anti-human IgE antibodies (monoclonal 50 or 500 ng/ml; anti-IgE (a) 10 or 100 μg/ml; anti-IgE (b) 10 or 50 μg/ml) were added to the tubes which were maintained at 4°C for a further 30 min. To each tube was added 900 μl of 100 μM lucigenin with 1% (w/v) gelatin, and the tubes then incubated at 37°C for the times shown. To one of the control tubes was added 100 μl of 20 mg/ml zymosan A (Sigma), which had been opsonized previously by incubation for 30 min with 10% pooled human serum at 30°C. Following the addition of lucigenin, the chemiluminescence was recorded in an LKB Wallac luminometer 1250 every 5 min for 30 min (Williams & Cole, 1981).

Electron microscopy

The monoclonal anti-IgE immunoglobulin was labelled with dinitrophenyl residues (DNP) as follows. The antibody (1 mg) in 100 μl of 1 M $NaHCO_3$ was mixed with 30 μl 1-fluoro-2, 4-dinitrobenzene (Eastman Kodak). The tube was incubated at 37°C for 7 min with vigorous mixing. The labelled protein was then separated from the free reagents by gel filtration through Sephadex G25 (fine) equilibrated with Dulbecco's phosphate-buffered saline (no Ca^{2+} or Mg^{2+} ions). Recordings of the absorbancies at 280 nm and 358 nm revealed that the molar ratio of DNP to immunoglobulin was 20·8:1.

Cells from a single patient were cultured on Thermanox R plastic cover slips (Miles Laboratories Inc) and then maintained for 1 h at 4°C in the absence or presence of 10,000 iu/ml of human myeloma IgE. The cells were then washed with DMEM, and incubated for a further 2 h at 4°C in the presence of 0·5 μg DNP_{21}-anti-IgE. The cover slips were then washed three times with DMEM containing 5 mg/ml bovine serum albumin, and then once with DMEM alone. The cells were fixed for 45 min in 2·5% (v/v) ultrapure glutaraldehyde in 0·1 M phosphate buffer, pH 7·4. Control experiments in the presence of 50 μg of unlabelled monoclonal anti-IgE revealed the non-specific binding of the DNP-labelled monoclonal anti-IgE reagent.

The cells were then subjected to a mouse monoclonal IgM anti-DNP reagent (diluted 1:10) for 60–90 min at 30°C and immunoreactive sites visualized using a colloidal gold-DNP complex (Newman & Jasani, 1984). The cells were then treated with 1% (w/v) OsO_4 in 0·1 M phosphate buffer, pH 7·2, dehydrated and processed in Araldite resin using a conventional procedure. Sections (70–

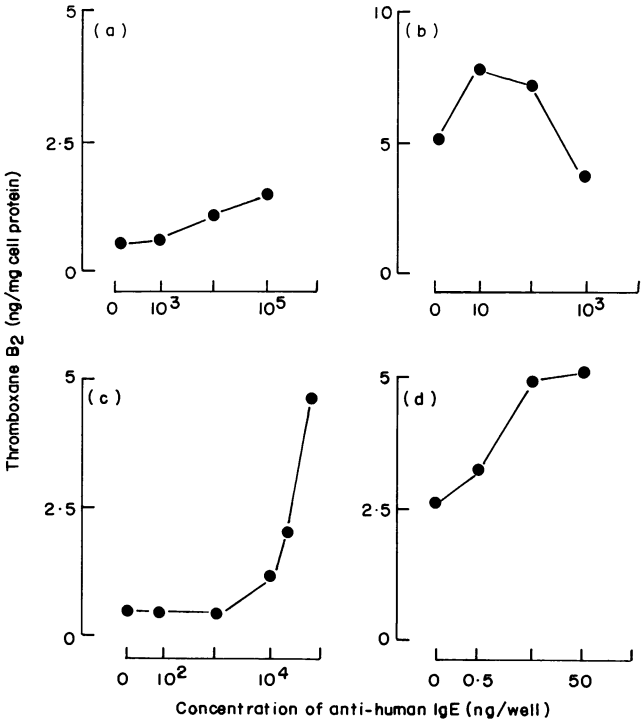

Fig. 1. IgE-dependent release of thromboxane B_2 from human alveolar macrophages. Cells were cultured as described in Materials and Methods, and sensitized *in vitro* with 10,000 iu/ml of human myeloma IgE. The culture plates were then washed and the cells challenged for 1 h in the presence or absence of selected concentrations of one of four anti-IgE antisera. The results show the concentration-dependent changes in thromboxane B_2 release following the addition of: (a) rabbit anti-IgE(a); (b) goat anti-IgE(c); (c) rabbit anti-IgE(b); and (d) monoclonal anti-IgE. Results show the mean values for duplicate plates harvested at the times shown. Each concentration curve is the result from a single patient.

100 nm) were mounted on to 100–200 mesh nickel grids, counterstained with heavy metal salts and viewed in a transmission electron microscope.

Electron micrographs were prepared, and gold particle counts performed direct from the negative. The number of gold particles on the surface of each cell section was determined from counts of 154–167 cells in each experimental group.

Statistical analysis

Results are expressed as the mean ± 1 s.d. Statistical analysis was performed using 2-way analysis of variance or Student's *t*-test for paired samples where appropriate.

RESULTS

The number of cells adherent to the culture dishes varied from patient to patient (0·12–2·7 mg cell protein/well, 0·43–9·7 × 10^6 cells). There was < 10% variation in protein content between dishes with cells from any one patient. Electron microscopy revealed that > 95% of adherent cells were macrophages. In an extensive morphological study by electron microscopy, 128 mononuclear cells were examined and their identity confirmed by their characteristic organelles. Of this total, one cell was identified as a mast cell (0·78%) and the rest were macrophages. Measurement of the histamine concentration in the medium above three dishes of 10^6 unstimulated cells, revealed a level of 742 ± 81 pg. Assuming a level of about 5 pg of histamine in each mast cell, and no more than 10% degranulation under these conditions, these results would suggest that about 0·15% of the total cell population were mast cells.

Table 1. Thromboxane B_2 (ng/mg cell protein) released by selected concentrations of the three polyclonal anti-IgE sera, and of monoclonal anti-IgE.

		Immunoglobulin (μg/ml)			
	n	0	0·1	1·0	10
Anti-IgE					
Rabbit (a)	8	1·72 ± 1·02	1·92 ± 0·99	2·04 ± 1·56	2·77 ± 1·38
Rabbit (b)	6	1·14 ± 0·87	1·07 ± 0·55	1·27 ± 0·73	3·94 ± 3·45
Goat (c)	5	4·0 ± 2·45	3·78 ± 2·88	3·11 ± 1·70	4·79 ± 2·93
		Immunoglobulin (ng/ml)			
	n	0	5	50	
Monoclonal	12	1·26 ± 1·43	1·86 ± 2·01	2·33 ± 2·39	

Results show means ± s.d. of *n* patients. There was a significant concentration-dependent increase ($P < 0·05$, 2-way analysis of variance) of thromboxane B_2 following challenge with rabbit anti-IgE (a and b) and monoclonal anti-IgE. The values of thromboxane B_2 at 10 μg of rabbit anti-sera and 50 ng mouse monoclonal antibody differed significantly from controls ($P < 0·01$, paired Student's *t*-test). The peak thromboxane B_2 concentration following addition of goat anti-IgE(c) was observed at varying antibody concentrations. The mean of the maximum thromboxane B_2 values was significantly greater than controls ($P < 0·05$, paired Student's *t*-test).

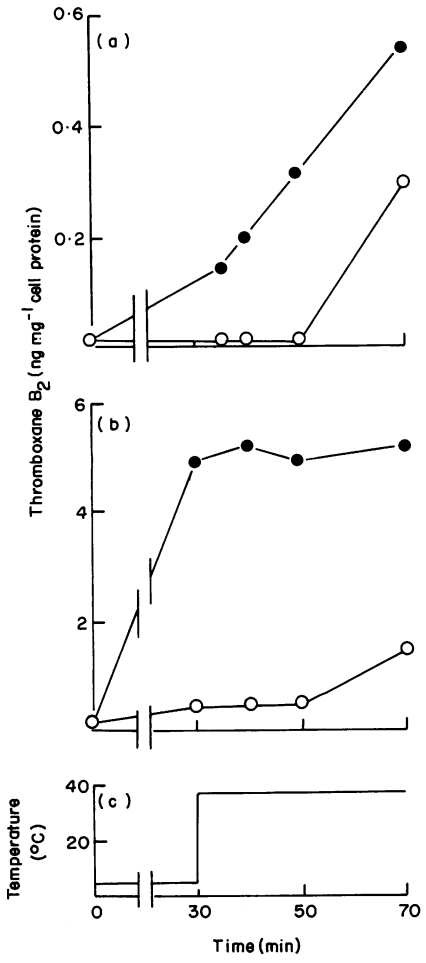

Fig. 2. Time-course for the IgE-dependent release of thromboxane B_2 from alveolar macrophages. Cells were cultured as described in Materials and Methods, and sensitized at 4°C with human myeloma IgE (10,000 iu/ml). The culture plates were then washed, and the cells challenged in the presence (●) or absence (○) of (a) 50 ng/ml monoclonal anti-IgE, or (b) 10 μg/ml rabbit anti-IgE(b). The IgE/anti-IgE complex was allowed to form on the surface of the cells for 30 min at 4°C and the plates then warmed to 37°C. The results show the mean values for duplicate plates harvested at the times shown.

Table 2. Prostaglandin (PG) $F_{2\alpha}$ (ng/mg cell protein), leukotriene (LT) B_4 (ng/mg cell protein) and the lysosymal hydrolase N-acetyl-β-D-glucosaminidase activity (units released/g cell protein) following stimulation with the rabbit anti-IgE sera (a and b)

			Immunoglobulin (μg/ml)			
Mediator	*n*	Anti-sera	0	0·1	1·0	10
$PGF_{2\alpha}$	2	(a)	2·10	2·55	2·40	4·43
LTB_4	3	(b)	5·43 ± 6·53	NA	NA	9·07 ± 11·0
Enzyme	3	(b)	13·3 ± 13·8	14·4 ± 12·6	21·1 ± 14·9	27·6 ± 23·1

Results show means ± s.d. of *n* patients.
NA = not analysed.

Thromboxane B_2 release

The basal release of thromboxane B_2 varied between 0·14 and 8·01 ng/mg cell protein. Figure 1 shows the release of thromboxane B_2 mediated by the four anti-human IgE antibodies tested. The levels of thromboxane B_2 released were similar to those seen when cells were challenged under the same conditions with zymosan A opsonised with human serum. Table 1 summarizes all the results for the release of thromboxane B_2 following stimulation with the four anti-IgE antibodies. Figure 2 shows the time course of release of thromboxane B_2. In preliminary experiments, macrophages from two subjects were challenged with anti-IgE (100 μg/ml of a, and 50 μg/ml of b) without previous passive sensitization with IgE. There was an increase in release of thromboxane B_2 (150% and 409% above control respectively) and *N*-acetyl-β-D-glucosaminidase (100% above basal with antibody b). However, 50 ng/ml of the monoclonal reagent (one subject) did not facilitate thromboxane B_2 release.

Prostaglandin $F_{2\alpha}$ leukotriene B_4 and N-acetyl-β-D-glucosaminidase

The release of these mediators from alveolar macrophages challenged with IgE and two of the affinity purified anti-IgEs are summarized in Table 2.

Chemiluminescence

Figure 3a shows the measurement of lucigenin chemiluminescence in cells from one patient. Results are presented for unstimulated cells, and cells stimulated with opsonised zymosan, anti-IgE (a) or monoclonal anti-IgE. Figure 3b shows the mean results of chemiluminescence at 10 min ($n=5$ patients). Opsonized zymosan increased chemiluminescence, and a small increase was also caused by anti-IgE (a), which has minimal cross-reactivity with IgG. However neither the monoclonal antibody, nor anti-IgE (b) caused any release of oxygen radicals.

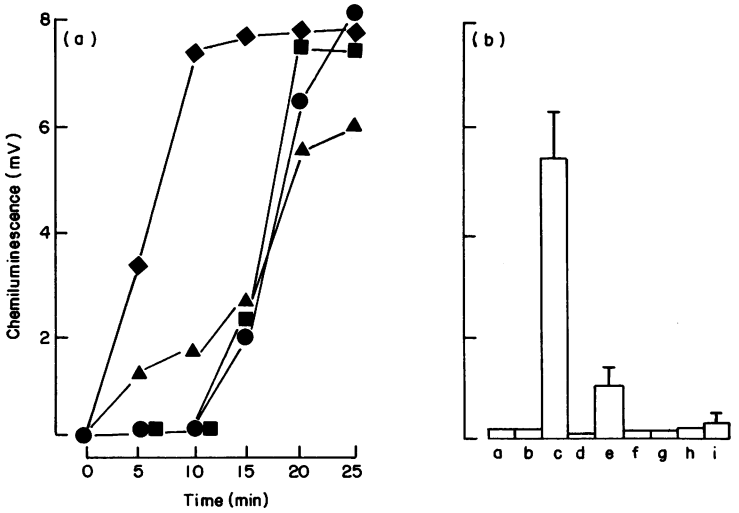

Fig. 3. The release of active oxygen species from human alveolar macrophages as identified by lucigenin chemiluminescence. Cell suspensions were prepared as described in Materials and Methods, and the cells challenged in the cuvette of a luminometer. The cells were sensitized passively at 4°C with 10,000 iu/ml of human myeloma IgE. (a) The sequential increase in lucigenin chemiluminescence of control macrophages (■), and those challenged with 500 ng/ml monoclonal anti-IgE (●), 100 μg/ml rabbit anti-IgE(a) (▲), or 2 mg/ml opsonized zymosan A (♦). (b) Lucigenin chemiluminescence measured 10 min after the addition of selected compounds; the results show mean values ± s.e. of determinations of cells from five patients. Chemiluminescence was measured in: (a) the absence of IgE or anti-IgE; (b) the presence of 10,000 iu/ml human myeloma IgE; (c) the presence of 2 mg/ml opsonized zymosan A; (d and e) 10,000 iu/ml human myeloma IgE and 10 or 100 μg/ml respectively of anti-IgE(a); (f; g) 10,000 iu/ml human IgE + 50 or 500 ng/ml respectively of monoclonal anti-IgE; (h, i) 10,000 iu/ml human myeloma IgE and 10 or 50 μg/ml respectively of anti-IgE(b).

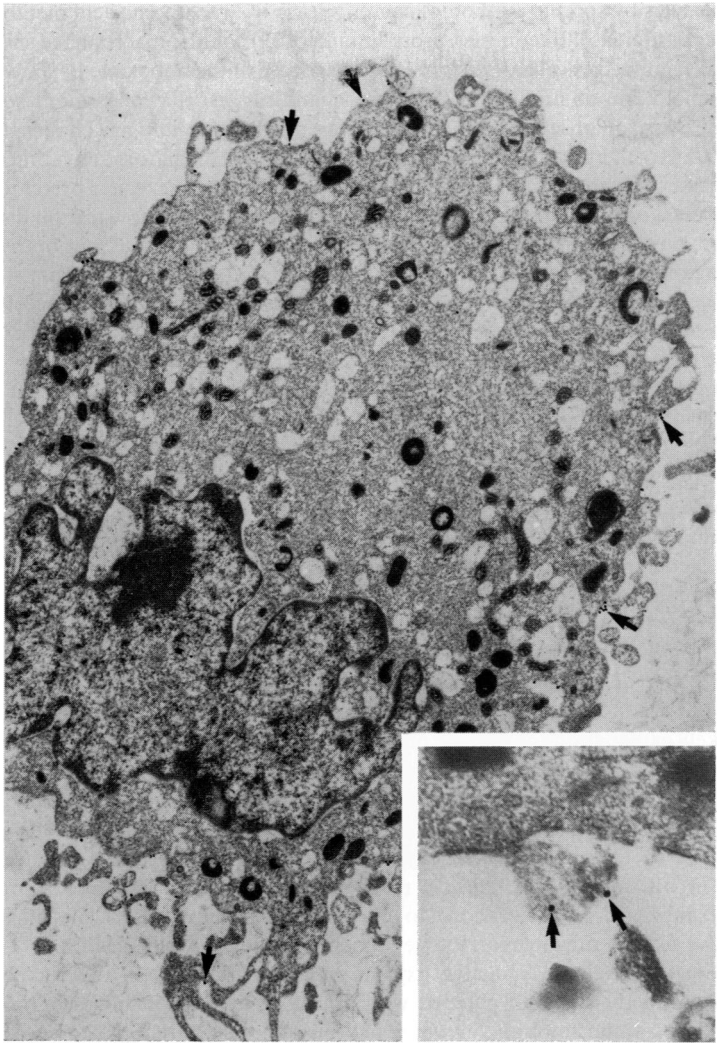

Fig. 4. Electron micrograph of a human alveolar macrophage with surface IgE visualized with colloidal gold as described in Materials and Methods. The inset shows a section of the plasma membrane. Magnification = 3,500 (61,500 in the inset).

Electron microscopy

Cells were examined by electron microscopy as described in Materials and Methods. Analysis of grain counts revealed that cells sensitized *in vitro* with IgE had $15{\cdot}9 \pm 8{\cdot}1$ gold particles/sectional cell circumference. An example is shown in Fig. 4. Cells not passively sensitized *in vitro* had $13{\cdot}9 \pm 4{\cdot}6$ gold particles/sectional cell circumference, and cells incubated in the presence of the 100-fold excess of unlabelled monoclonal anti-IgE immunoglobulin had $5{\cdot}1 \pm 1{\cdot}7$ gold particles/sectional cell circumference. Thus about 68% of the DNP-labelled anti-IgE was displaced by the unlabelled reagent. Furthermore the results suggest that human alveolar macrophages are sensitized with IgE *in vivo*.

DISCUSSION

The results presented here reveal that human alveolar macrophages bind IgE, and that cross-linking

of these Ig molecules by specific antibodies causes release of arachidonic acid metabolites and *N*-acetyl-β-D-glucosaminidase. In contrast, cross-linking of IgE on the macrophage surface failed to release active oxygen species. The time course for the release of thromboxane B_2 showed significant elevations within 15 min of stimulation of the cells, and suggest that release *in vivo* might occur within the time span of onset of bronchoconstriction following antigen challenge in sensitive subjects (Pepys & Hutchcroft, 1975). The possibility of mast cell mediators activating macrophages in these circumstances is not totally excluded in these experiments. The results presented show a low level ($< 1\%$) of mast cells in the total cell population. However, the demonstration histochemically of IgE receptors on the macrophage surface provide compelling evidence that both cell types are involved in reaginic responses.

The presence of IgE receptors on human alveolar macrophages has been suggested previously by the binding of IgE-labelled sheep erythrocytes to human macrophages (Melewicz *et al.*, 1982), and the release of β-glucuronidase by human alveolar macrophages is increased by IgE/anti-IgE complexes (Joseph *et al.*, 1983). Rabbit alveolar macrophages are capable of synthesizing lipoxygenase metabolites of arachidonic acid, and they are released following cross-linking of IgE on their surface (Rankin *et al.*, 1982). However, the results presented here are the first demonstration of IgE-dependent release from human alveolar macrophages of cyclo-oxygenase and lipoxygenase products, in addition to the lysosomal hydrolase.

There appears to be no IgE-dependent release of active oxygen species. Cross-linking of IgE on the surface of alveolar macrophages demonstrated therefore that the cells are capable of differential production of inflammatory mediators that depends on the nature of the stimulus. The release of lysosomal enzymes but not active oxygen species has been observed following the cross-linking of surface IgE, but both were released when the cells were challenged with zymosan particles coated with IgE (Joseph *et al.*, 1980). It has been suggested that alveolar macrophages cultured in suspension do not produce active oxygen species on particulate challenge (Kunkel & Duque, 1983). This is an unlikely explanation for the lack of an oxygen burst in the present experiments, however, as the cells responded promptly to challenge with opsonised zymosan. In related experiments, human basophils have been shown to release less histamine following challenge with anti-IgE than with C5a complement fragments (Farnan *et al.*, 1985), and human neutrophils show differences in the enhancement of aggregation and oxygen radical production following treatment with cytochalasin B (Whitin & Cohen, 1985). The release of leukotriene B_4, as distinct from its synthesis, by human neutrophils has also been shown to depend upon the nature of the challenge (Williams *et al.*, 1985). Active oxygen may increase tissue damage in many diseases (Halliwell, 1982), and the lack of IgE-dependent release of active oxygen species might explain the lack of permanent structural changes in the lungs of patients with mild allergic asthma. This contrasts with lung disease related to cigarette smoking, in which oxygen radicals are thought to be involved in the pathogenesis of the tissue damage (Greening & Lowrie, 1983).

The identity of cells responsible for bronchoconstriction due to inhaled antigen is controversial, but is widely held to be mast cells. The mast cell is however unlikely to be the only effector cell for the reasons outlined in the Introduction. It is proposed here that alveolar macrophages in susceptible patients are coated with specific IgE, and that antigen bridging of IgE molecules facilitates release of several bronchoconstrictor mediators. The data presented reveal also that macrophages isolated after bronchoalveolar lavage may still be sensitized with IgE. Finally, immunocytochemistry has visualized by electron microscopy surface IgE on alveolar macrophages.

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