

IgE induces secretion of prostaglandin E₂ by human monocytes

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SUMMARY

IgE was isolated from a patient with the hyper IgE, recurrent infection syndrome by immunoabsorption on sepharose bound goat anti-human IgE. Addition of this IgE to a monolayer culture of human monocytes resulted in a dose-dependent increase in PGE₂ secretion. The addition of F(ab')₂ fraction of goat anti-human IgE in the presence of sub-stimulating doses of IgE markedly increased PGE₂ secretion; whereas addition of F(ab')₂ fragment of irrelevant goat IgG had no effect. Similar activation of monocytes which could be enhanced by anti IgE was observed in the presence of the patient's serum. No such effect was seen in the presence of normal human serum. These results indicate that IgE may activate human monocytes and induce PGE secretion.

Keywords IgE human monocytes prostaglandin E₂

INTRODUCTION

The syndrome of recurrent infections, severe atopic dermatitis and extremely elevated serum IgE levels is now being increasingly recognised. Chronic mucocutaneous candidiasis and varying degrees of decreased chemotaxis of polymorphonuclear leucocytes are additional manifestations of this syndrome (Buckley, Wray & Belmaker, 1972; Hill & Quie, 1974). A large proportion of the IgE in the serum of these patients have been shown to be specific for *Staphylococcus aureus* or *Candida albicans* (Schopfer *et al.*, 1979; Berger *et al.*, 1980). Thus, it has been proposed that ineffective opsonization of these organisms by IgE rather than IgG antibodies occurs and recurrent infections result. Since B cell IgE synthesis is controlled by specific helper and suppressor cells, these findings suggested that IgE specific suppressor T cell dysfunction is the primary underlying defect in this syndrome (Fiser & Buckley, 1979).

It is well known that antigen-antibody complexes of the IgE class bind to specific Fc receptors on mast cells and trigger the release of histamine and other mediators (Ishizaka & Ishizaka, 1978). Recently distinct IgE receptors also have been detected on the plasma membrane of animal and human macrophages (Melewicz, Plummer & Spiegelberg 1982; Melewicz *et al.*, 1982). In this study we show that human monocytes may be activated to produce increasing amounts of prostaglandin E₂ (PGE₂) by IgE isolated from a patient with the hyper IgE syndrome.

CASE REPORT

DY, a 16 year old male offspring of a non-consanguineous marriage, has been under our continuous care since birth. During infancy and early childhood he had two episodes of staphylococcal pneumonia with abscess formation, a cold abscess at the site of BCG immunization, from which BCG organisms were cultured and numerous recurrent episodes of furunculosis, infected cysts of the eyelids, dermatitis and deep tissue abscesses from which *S. aureus* was invariably cultured. Since late childhood he has developed severe atopic eczema, chronic otitis media, mild intermittent mucocutaneous candidiasis and chronic bronchiectasis. A right lower lobectomy was done when he was 12 years old. He is being treated at present with intermittent anti-staphylococcal antibiotic therapy for overt infections and continuous aspirin (see below). At present pulmonary function tests show lung functions to be 70% of expected normal for his age.

Laboratory investigations in representative assays during the past 2 years have shown that DY has had persistent eosinophilia 5–10%; slightly elevated gamma-globulins; IgG 1,600 mg/dl; IgA 120 mg/dl; IgM 330 mg/dl and markedly elevated levels of serum IgE 27,800 iu/ml. All laboratory tests on the patient's cellular immune functions were done when he was in good clinical condition and off all medications for 48 h. Eight per cent of his peripheral lymphocytes were identified as B cells by immunofluorescent labelling with F(ab')₂ antibodies (Meloy Laboratories, Springfield, Virginia, USA). Specific antibodies to *S. aureus* and *Clostridium tetanus*, viral antigens and isohaemagglutinins were present in normal titres. Circulating immune complexes (640 µg/ml) were present when measured by the Raji radioimmunoassay (normal values < 16 µg/ml). The patient had normal numbers of T cells determined by E rosette formation (66%) and normal distribution of T cell subsets detected with monoclonal antibodies (Ortho diagnostics, New Jersey, USA) using a fluorescence activated cell sorter. The antibodies used were OKT3 (total T cells—78%); OKT4 (helper cells—44%) and OKT8 (suppressor cells—24%) of total T lymphocytes (Reinherz & Schlossman, 1980). Lymphocyte proliferation responses to phytohaemagglutinin (PHA), pokeweed mitogen (PWM), concanavalin A (Con A) as well as purified protein derivative, Staphylococcal antigen, *C. albicans* and viral antigens were within the normal range. Con A-induced suppressor cell function showed decreased suppressor activity when either Con A or PHA were used as mitogens (Shou, Schwartz & Good, 1976) (Table 1). Quantitative estimations of total haemolytic complement activity and specific components C1q, C3, C4, and C5 were normal. Phagocytosis of

Table 1. Con A inducible suppressor cell activity in patient

Stimulating mitogen	% suppression	
	Patient	Control
PHA (1 µg/ml)	23 ± 3	48 ± 20
Con A (10 µg/ml)	0*	50 ± 13

Induction of suppressor cells was done by incubating 1×10^5 mononuclear cells with Con A (50 µg) for 48 h. One hundred thousand mononuclear cells were added as responder cells. Results are the mean ± s.e. of quadruplicate cultures from two different experiments.

* The uptake of ³H-thymidine by the lymphocytes in the cultures following prior treatment with Con A were higher than the control cultures. Control results were obtained from eight normal individuals.

yeast particles, the bactericidal capacity of polymorphonuclear leucocytes and monocytes for both *S. aureus* and *Escherechia coli* as well as chemotactic activity of both polymorphonuclear leucocytes and monocytes were normal (Quie *et al.*, 1967; Baum, Mowat & Kirk, 1971).

MATERIALS AND METHODS

Purification of IgE. Goat antiserum to human IgE was obtained from Dr J. Haimovich. The IgG fraction of this antiserum was isolated by ammonium sulphate precipitation followed by DEAE cellulose chromatography. A reverse immunoabsorbant column for IgE purification was prepared by binding the goat anti-human IgE antibodies to glutaraldehyde activated polyacrylyhydrazide sepharose CL-4B (Pharmacia, Uppsala, Sweden). The sepharose beads were prepared by Miron & Wilchek from the Department of Biophysics, The Weizmann Institute as described by Miron & Wilchek, (1981). The patient's serum was passed through this anti-IgE column (5 ml serum per 11 ml bed volume) which was washed with phosphate-buffered saline (PBS) until the absorbance at 280 nm was lower than 0.02. The bound IgE was subsequently eluted by 0.2 M HCl-glycine buffer pH 2.7. Aliquots of 0.5 ml were collected. Purity of the subsequent fractions was measured by using commercially available immunodiffusion plates for albumin, IgG and the third component of complement (Behringwerke, Frankfurt, FRG) and IgE determinations were obtained by using Phadebas PRIST reagent kits (Pharmacia). These results are expressed as iu/ml according to commercially supplied standards. One iu is equivalent to 2.2 ng of IgE. In order to remove aggregates the isolated IgE preparation was centrifuged at 100,000g for 1 h in a Beckman ultracentrifuge. Gel filtration through an analytical Sephacryl 300 column (Pharmacia) indicated that the eluted IgE was monomeric.

Monocyte monolayers were prepared as described previously (Passwell, Dayer & Merler, 1979). In brief, blood was drawn in preservative free heparin and the mononuclear cells were separated by Ficoll-hypaque density centrifugation at 400g for 20 min. The isolated mononuclear cells were resuspended in medium RPMI 1640 (Microbiological Associates, Bethesda, Maryland, USA) supplemented with 10% pooled heat-inactivated millipore filtered AB positive serum, penicillin (100 units/ml) and streptomycin (100 µg/ml) (complete medium). Two hundred thousand cells were added to wells of plastic trays and adherence of the monocyte was achieved by gentle rocking of the trays for 1 h at 37°C (8 mm diameter, Falcon, Oxnard, California, USA). The non-adherent cells were removed by vigorous washing with Hanks' balanced salt solution (HBSS) and the adherent cell population (which contained more than 90% monocytes and less than 10% lymphocytes) was cultured in complete medium at 37°C in an atmosphere of 5% CO₂. Extracellular medium was removed for assay of PGE₂ after 48 h of incubation.

PGE₂ was measured by radioimmunoassay using specific antisera for PGE (Serogen Inc., Boston, Massachusetts, USA). ³H-PGE₂ was obtained from New England Nuclear, Boston (Levine, Gitierrez-Gittierrez & van Vunakis, 1971). Fifty microlitres of extracellular medium without prior extraction was used in the assay. The assay was done in 0.01 M potassium phosphate buffer, pH 7.4 containing 0.15 M sodium chloride, 0.1% sodium azide and 0.1% bovine serum albumin. Formed complexes were separated from free radioactive PGE₂ by dextran coated charcoal. This assay detects 10 pg of PGE₂/tube and the antiserum shows no cross-reactivity with F prostaglandins. Standards for the assay were prepared after dissolving PGE₂ (Upjohn Co., Kalamazoo, Ohio, USA) in absolute ethanol and the appropriate dilutions in buffer were made.

Micro-organisms. *S. aureus* cultured from the patient's skin infection and *S. aureus* Wood strain were cultured in trypticase-soy-broth. *C. albicans* was cultured in Sabouraud liquid medium. Cultures were harvested, centrifuged at 3,000g for 10 minutes and washed and resuspended in PBS pH 7.4 and stored in suspension. Agglutination of these organisms was recorded after addition of dilutions of the patient's serum and the isolated IgE preparation.

Proteins. F(ab')₂ fragments of the goat anti-IgE were prepared by pepsin digest as previously described (Nissonof *et al.*, 1960). F(ab')₂ fragments to acetylcholine receptor prepared in identical fashion were kindly provided by Dr R. Tarrab-Hasdai and were used as a control IgG preparation.

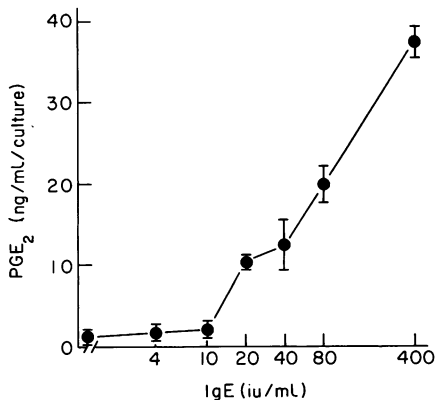


Fig. 1. Dose-dependent effect of isolated IgE (from DY) on PGE₂ secretion by human monocytes. Each point represents the mean \pm s.e. of duplicate or triplicate cultures from four different experiments.

RESULTS

IgE was isolated from the serum of DY by affinity chromatography on sepharose-anti-IgE. The serum specimen used contained 66.7 μ g/ml of IgE and its concentration in the eluted bound fraction was 29.9 μ g/ml; while IgE could not be detected in the unbound fraction (sensitivity of PRIST assay \sim 1 ng/ml). Normal concentrations of albumin, IgG and the third component of complement were found in the serum and in the unbound fraction; while none of these serum proteins were detected in the eluted bound fraction (sensitivity of immunodiffusion assays \sim 5 mg/ml). The anti-human IgE antibody used in this column reacted specifically with the patient DY serum and did not show significant reactivity with normal human serum as revealed by double immunodiffusion in agar. A similar line of immunoprecipitation was observed with the purified IgE, but not with IgG (results not shown).

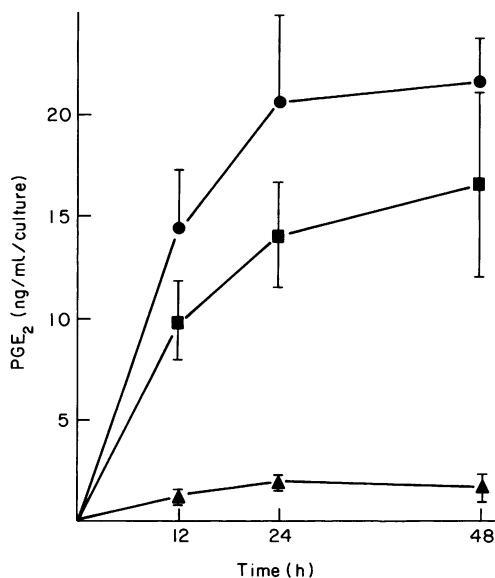


Fig. 2. Kinetics of production of PGE₂ from human monocytes when stimulated with IgE (●, 100 iu/ml) or Con A (■, 50 μ g/ml), control = ▲. Each point is the mean \pm s.e. and represents data from two separate experiments.

Table 2. Effect of IgE and F(ab')₂ anti-IgE on monocyte PGE₂ production

Addition*	PGE ₂ † (ng/ml/culture)
None	1.8
IgE (40 iu/ml)	12.6
IgE (10 iu/ml)	2.2
IgE (10 iu/ml) + F(ab') ₂ anti-IgE	140.0
IgE (10 iu/ml) + F(ab') ₂ IgG‡	2.7
F(ab') ₂ anti-IgE	1.3
F(ab') ₂ IgG‡	1.2

*Monocyte cultures were incubated for 48 h in the continued presence of the various additions.

† Results are the average of duplicate cultures. Similar results were obtained in two other experiments.

‡ F(ab')₂ fragments of goat IgG to the acetylcholine receptor were used as the negative control.

Only small amounts of PGE₂ were found in the extracellular medium of monocyte monolayers in culture. Addition of Con A to these cultures resulted in increased secretion of PGE₂. The addition of purified monomeric IgE to normal human monocyte monolayers resulted in an increased production of PGE₂. The effect of IgE was dose-dependent (Fig. 1). There was a similar progressive increase in PGE₂ production when human monocyte monolayers were stimulated by both IgE and Con A (Fig. 2). The addition of F(ab')₂ fragments of anti-IgE markedly enhanced the production of PGE₂ in the presence of suboptimal amounts of IgE, while F(ab')₂ fragments which were not IgE specific had no enhancing effect. Neither the IgG nor the anti-IgE specific F(ab')₂ antibodies when added alone induced an increase in monocyte PGE₂ secretion (Table 2).

Similar results were observed when serum from the patient DY was used. The patient's monocyte monolayers produced relatively larger amounts of PGE₂ (3.1 ± 0.3 ng/ml/culture) compared to several controls (1.1 ± 0.4 ng/ml/culture). Addition of the patient's serum to either control or autologous monocyte cultures resulted in an increase of PGE₂ production. Moreover, the

Table 3. Comparison of patient's and control monocyte PGE₂ production

Addition	Control monocytes (ng/ml/culture)	Patient monocytes (ng/ml/culture)
AB serum 2% (normal)	0.9	3.6
DY serum 2%	5.4	21.2
AB serum (2%) + F(ab') ₂ anti-IgE	1.8	11.0
DY serum (2%) + F(ab') ₂ anti-IgE	24.0	40.0
AB serum (2%) + Con A (50 µg/ml)	ND	8.0
DY serum (2%) + Con A (50 µg/ml)	ND	15.2

Results are the average of duplicate cultures. The values of duplicate cultures were within 5% of each other. Similar results were obtained in two other experiments.

ND = not done.

addition of F(ab')₂ anti-IgE in the presence of the patient's serum greatly increased the PGE₂ production both from his monocytes and from normal monocyte monolayers (Table 3).

The levels of PGE₂ produced by the monocyte monolayers could be decreased after washing the IgE 1 h after it had been added to the monolayers (IgE [40 iu/ml] 13.6 ± 0.6 and after washing 2.9 ± 0.2 ng/ml/culture, $n=6$); however, once the specific F(ab')₂ anti-IgE antibody was added, washing the monocyte monolayer did not abrogate the monocyte response of PGE₂ production (IgE [10 iu/ml] + F(ab')₂ anti IgE 135.0 ± 25.1 and after washing 135.0 ± 21.0 ng/ml/culture, $n=6$).

Increased agglutination of *S. aureus* was observed with the patient's serum and with the IgE fraction isolated from it, no agglutination was obtained with *C. albicans*. Dilutions of up to 1:100 of the isolated IgE fraction resulted in the agglutination of *S. aureus*. Addition of *S. aureus* preparations alone to both control and the patient's monocyte monolayers caused a marked increase of PGE₂ production even in the absence of exogenously added antibodies. Therefore the effect of *S. aureus* anti-IgE complexes could not be examined.

DISCUSSION

Circulating human monocytes, which are the precursors of macrophages of the fixed reticuloendothelial system can be stimulated to secrete PGE₂ by interaction with IgG molecules through specific membrane bound Fc receptors. Similarly, Con A binds to specific membrane receptors and stimulates PGE₂ production (Passwell *et al.*, 1979). This phenomenon may have physiological significance since local PGE₂ concentrations correlate with accumulation of inflammatory exudate and since inhibitors of prostaglandin synthesis decrease inflammatory responses (Vane, 1976; Flower, 1976). In addition PGE₂ may decrease lymphocyte proliferative responses either by a direct effect on T cells (Goodwin, Messner & Peake, 1978; Passwell, Rosen & Merler, 1980) or by preferential activation of T suppressor cells. (Stobo, 1977; Stobo, Kennedy & Goldyne, 1979).

Recently, Geha *et al.* (1981) reported that patients with the hyper IgE, recurrent infection syndrome had low suppressor T cell functions. This was manifested by a lower induction of suppressor cells by Con A and an apparently smaller number of total T cells defined by monoclonal antibodies (MoAb) directed to subset specific glycoproteins (Geha *et al.*, 1981). The patient described here exhibited decreased Con A-induced suppressor cell functions but contrary to the findings of Geha *et al.* (1981) had a normal number of suppressor T cells. We consider it unlikely that the T cell population involved in suppression of specific IgE responses will be detected by the T cell MoAb presently available. The induction of Con A suppressor cells is dependent on prostaglandin production, so that prior activation of mononuclear cells of these patients may be responsible for these observed disturbances in suppressor T cell function (Fischer, Durandy & Griscelli, 1981). Since we demonstrated a marked increase in this patient's monocyte PGE₂ secretion, we have prescribed continuous aspirin. However, at present no conclusive clinical evidence of its benefit can be claimed.

We have demonstrated that isolated IgE from a patient with the hyper IgE, recurrent infection syndrome activates normal human monocytes and excess PGE₂ is secreted into the medium. The dose-dependent effect of addition of large amounts of monomeric IgE stimulated PGE₂ production probably suggests that at these concentrations dimers or oligomers of IgE are present in sufficient concentration to cause activation of the cells via their IgE receptors. The fact that small amounts of IgE, which had no effect on the monocyte monolayers induced marked activation when anti-IgE antibodies were added to the culture indicated that activation may be potentiated by an intensive cross-linking of the membrane bound IgE and its receptor. This phenomenon is probably similar to the well described mechanism of activation of mast cells (type I immune reaction). F(ab')₂ anti-IgE molecules were used in these experiments to prove that the reaction was mediated by the Fc portion of the IgE molecules and not via complexes formed *in vitro* and thus via the IgG Fc receptor. The specificity of these reactions was confirmed by the absence of enhancement with F(ab')₂ molecules that were not directed to IgE. The increased PGE₂ secretion by monocytes observed in the presence of the patient's serum may have also been due to the presence of pre-formed IgG immune complexes. However, since F(ab')₂ anti-IgE enhanced PGE₂ production in the presence of this serum, it is likely that stimulation via IgE receptors is a distinct and significant process in the patient described here.

The hyper IgE globulinaemia typical of parasitic infections may also be of similar biological consequence. Capron *et al.* (1982) have shown that both an increase in lysosomal enzyme as well as neutral protease secretion and antibody-dependent cytotoxicity towards *Schistosoma* are mediated by IgE. Joseph *et al.* (1980) have also shown that mixtures of either IgE myeloma and anti-IgE antibody or serum from allergic patients with the corresponding antigen activate human pulmonary macrophages. Our studies provide further direct evidence indicating that IgE can activate mononuclear phagocytes and induce secretion of prostaglandins by these cells.

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