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IgE production *in vitro* by peripheral blood mononuclear cells of patients with parasitic helminth infections

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SUMMARY

Helminth parasites induce production of high levels of IgE antibodies but the immunoregulatory mechanisms determining this IgE biosynthesis are poorly understood. To investigate these mechanisms, peripheral blood mononuclear cells were obtained from six normal controls, six atopic patients and eight patients with parasitic helminth infections (three with schistosomiasis, two with loiasis, three with onchocerciasis). Cells were cultured at 1×10^6 cells/ml for 8 days in the presence of media alone or media supplemented with pokeweed mitogen (PWM) or cycloheximide; the supernatant fluids from these cultures were then assayed quantitatively for total and parasite specific IgE and IgG using an avidin–biotin amplified (for IgE) or standard (for IgG) microelisa assay. The geometric mean spontaneous IgE production was markedly elevated in peripheral blood mononuclear cells from parasitized individuals (2,487 pg/ml) when compared to those from atopics (358 pg/ml) or normals (152 pg/ml). Spontaneous IgG synthesis was equivalent in all three groups (range 140–420 ng/ml). PWM did not induce IgE production in any group and in the parasitized group even caused significant suppression of total IgE synthesis. Antigen specific antibody production (both IgE and IgG) paralleled total immunoglobulin synthesis. These findings demonstrate for the first time spontaneously enhanced IgE production *in vitro* in patients with helminth infections and provide a model system for studying the suppressive and regulatory mechanisms controlling IgE secretion.

Keywords IgE *in vitro* biosynthesis helminthiasis pokeweed mitogen

INTRODUCTION

Though IgE antibody responses in most individuals are tightly regulated both quantitatively and qualitatively (Marsh, Bico & Ishisaka, 1974), the presence of helminth infection appears to overcome these regulatory mechanisms so that high levels of IgE are consistently produced *in vivo* (Kojima, Yokagawa & Tada, 1972). Furthermore, this IgE in experimental models (Jarrett *et al.*, 1974) and perhaps even in man (Turner, Holt & Hull, 1981) has been shown to result not only from specific antibody responses to the parasites, but also from non-specific polyclonal activation of differentiated B cells already possessing the capacity for IgE secretion.

In the present study we have examined the requirements for production of IgE by lymphocytes obtained from patients with parasitic infections. Because parasitized individuals produce IgE at levels far exceeding those of normal and atopic individuals (Kojima *et al.*, 1972) and because the parasite-induced polyclonal activation of B_ϵ cells presumably occurs in these patients, cultures of

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peripheral blood mononuclear cells (PBMC) from such patients should provide an ideal *in vitro* system for the study of spontaneous IgE production in a naturally activated state. At the same time, we examined the ability of a known B cell polyclonal activator, pokeweed mitogen (PWM), to affect this *in vitro* IgE biosynthesis in order to gain further understanding of the mechanisms involved in the regulation of this particular immune response.

MATERIALS AND METHODS

Patient population. Three groups of subjects served as donors of the PBMC studied. (a) Six normal volunteers aged 21–41 years with no history of allergic or parasitic disease. (b) Six atopic individuals (aged 24–41 years) with symptomatic allergic rhinitis. (c) Eight patients with single helminth infections (three schistosomiasis, two loiasis and three onchocerciasis) between the ages of 18 and 34.

Isolation of PBMC. Mononuclear cells were obtained from heparinized venous blood by Ficoll-Diatrizoate (Litton Bionetics, Inc., Kensington, Maryland, USA) density gradient centrifugation after the method of Böyum (1968). Cells were washed three times and resuspended in RPMI 1640 supplemented with 2 mM glutamine, gentamicin (20 μg/ml), 25 mM HEPES and 10% heat-inactivated fetal calf serum (FCS) (complete medium). FCS from two lots were used throughout the present study with equivalent results.

Cell cultures. Human PBMC were cultured in complete medium at a concentration of 1×10^6 cells/ml in a volume of 1 ml in 12 × 75 mm round bottomed tubes (Falcon Plastics, Oxnard, California, USA). Cell cultures were set up in triplicate and were incubated in a humidified atmosphere at 37°C in 5% CO_2 for 8 days. As controls, one set of cultures either had cycloheximide (100 μg/ml) added or were frozen and thawed five times prior to initial culture; each gave equivalent results. Data expressed as the synthetic ratio are determined by dividing the amount of immunoglobulin produced in unstimulated cultures by that produced in control cultures.

Stimulation with PWM. PWM was obtained from GIBCO (Grand Island, New York, USA) and was added at a final dilution of 1/200; this concentration was shown to be optimal for the generation of IgG and IgM antibodies *in vitro* in our laboratory. Two different lots were used throughout the study with equivalent results.

Preparation of anti-IgE antibodies. Both the goat and rabbit anti-human IgE used in the enzyme linked immunosorbant assays were derived from serum of animals immunized with PS myeloma IgE (a kind gift of Dr T. Ishizaka). This serum was exhaustively absorbed on a human gamma globulin (HGG)-Sepharose column and purified on a PS myeloma immunosorbent. The biotinylated goat anti-human IgE was prepared as described by Subbarao, McCartney-Francis & Metcalfe (1983). The specificity of the anti-IgE used in the avidin–biotin microelisa (described below) was established by demonstrating that no reactivity was seen when pooled IgG subclasses (WHO reference standards kindly supplied by Dr F. Skvaril) were added to the ELISA system until a ratio of 1,000,000:1 IgG/IgE was attained (Fig. 1). This ratio is well beyond those occurring in the *in vitro* system. There was also no reactivity demonstrable between this anti-IgE preparation and IgM and IgA.

Avidin–biotin microelisa assay for total and parasite specific IgE. The microelisa assay for total and specific IgE is a modification of the assay described by Subbaro *et al.* (1983). Briefly, flat bottomed microtitre plates (Dynatech laboratories, Inc., Alexandria, Virginia, USA) were coated a 0·5 μg/ml solution of rabbit anti-human IgE or 5 μg/ml of one of the soluble parasite antigen extracts prepared as previously described: schistosome egg antigen (SEA) (Boros & Warreb, 1970); *Brugia malayi* adult antigen (BMA) (Hussain *et al.*, 1981) and *Onchocerca volvulus* adult antigen (OVA) (Weiss, Spieser & Hussain, 1981). The plates were washed in phosphate-buffered saline (PBS)-0·05% Tween-20 (Sigma Chemical Co., St Louis, Missouri, USA) and blocked with 200 μl of PBS-5% BSA for 1 h. They were next incubated with 100 μl of culture supernatant fluid or diluted IgE standard at 4°C for 72 h. The wells were washed as before, blocked with PBS-5% BSA, washed again and further incubated with goat anti-human IgE-biotin (final dilution of 1/500). Four hours later, the wells were washed and avidin-HRP (Vector Laboratories, Inc., Burlingame, California,

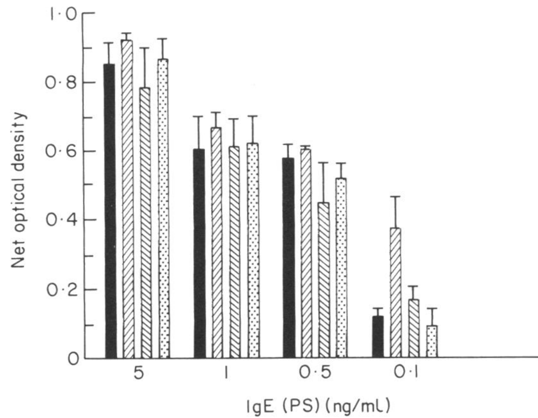

Fig. 1. Effect of IgG antibodies (pooled IgG subclasses) on the detection of IgE in the avidin–biotin microelisa. Dark bars indicate the optical density achieved with PS myeloma alone. Hatched bars indicate the optical density achieved when known concentrations of PS myeloma were first mixed with different concentrations of IgG: 100,000 ng/ml (▨), 10,000 ng/ml (▧) or 1,000 ng/ml (▢). Data are expressed as mean net optical density ± s.d.

USA) was added at 1/250 final dilution in PBS with 1% BSA, 0·5% gelatin and 0·05% Tween-20 added. The wells were incubated at 37°C for 10 min, washed, and allowed to react with *o*-phenylenediamine (Sigma) in a potassium phosphate buffer, pH 7·0 containing 0·0006% H_2O_2. The reaction was terminated with 2N HCl. Subsequent development of color was measured using a MR 600 microplate reader (Dynatech).

For total IgE, standard curves were constructed using dilutions of an IgE standard serum referenced against Pharmacia standards and the US/UK IgE reference serum (a kind gift of Drs F. Adkinson and R. Hamilton). The concentration of IgE in the supernatant fluid was read from the standard curves in their linear portion. The working range of the assay was between 50–5,000 pg/ml. The interassay % coefficient of variation (% CV) was < 13% and the interdilutional % CV was < 15%.

For parasite specific IgE, arbitrary units were defined based upon a reference serum to which all other samples were compared.

Microelisa for total and specific IgG. IgG in culture supernatants was measured in an enzyme linked immunoabsorbent assay. Flat bottomed microtitre plates (Dynatech) were sensitized with either 10 μg/ml Fab fragment goat anti-human IgG (N.L. Cappel Laboratories, Inc., West Chester, Pennsylvania, USA) or 5 μg/ml of parasite specific antigen (as described above) in 0·1ml of carbonate buffer, pH 9·6. The assay was then performed as described previously (Nutman *et al.*, 1984).

Determination of serum IgE levels. IgE levels in the patients' sera were measured by paper radioimmunosorbent test (PRIST; Pharmacia Diagnostics AB, Uppsala, Sweden).

Statistical analysis. The correlation coefficient was determined using both linear regression and Spearman rank analysis. The remainder of the statistical analyses were performed comparing the mean logs using Student's *t*-test.

RESULTS

Spontaneous IgE synthesis by PBMC in vitro

De novo synthesis of IgE *in vitro* was measured in PBMC from six normal, six atopic and eight parasitized subjects. As shown in Table 1, only minimal amounts of IgE were produced spontaneously in unstimulated cultures from normal subjects (geometric mean 152 pg/ml), whereas

Table 1. Spontaneous IgE synthesis in cultures of PBMC

Patient No.	Diagnosis	Serum IgE (ng/ml)	Supernatant IgE (pg/ml) in PBMC cultures: FT/Cyclo*	Supernatant IgE (pg/ml) in PBMC cultures: Media†	Synthetic ratio
1	Normal	12	120	208	1·73
2	Normal	20	103	268	2·60
3	Normal	10	100	100	1
4	Normal	20	272	170	0·675
5	Normal	25	100	160	1·6
6	Normal	30	87	100	1·15
	Normals (geometric mean)		119·6	152·3	1·32 $\overset{\times}{\div}$ 1·22§
	(95% confidence interval)				(0·78–2·21)
7	Allergic rhinitis	66	362	294	0·81
8	Allergic rhinitis	94	81	202	2·49
9	Allergic rhinitis	166	295	909	3·08
10	Allergic rhinitis	48	397	323	0·81
11	Allergic rhinitis	500	100	262	2·6
12	Allergic rhinitis	260	50	460	9·0
	Atopics (geometric mean)		160·6	357·8	2·23 $\overset{\times}{\div}$ 1·45
	(95% confidence interval)				(0·85–5·82)
13‡	Loiasis	1,050	100	1,322	13·22
14	Loiasis	1,220	95	5,167	54
15	Schistosomiasis	1,720	590	10,000	16·9
16	Schistosomiasis	140	165	1,000	6·06
17	Schistosomiasis	640	240	990	4·25
18	Onchocerciasis	125	586	1,931	3·3
19	Onchocerciasis	4,000	277	4,413	15.93
20	Onchocerciasis	1,810	670	2,533	3·78
	Patients (geometric mean)		264·8	2,487	9·39 $\overset{\times}{\div}$ 1·41
	(95% confidence interval)				(4·16–21·19)

* Freeze thawed five times (FT)/or cycloheximide 100 μg/ml (Cyclo).
† Spontaneous production of IgE.
‡ Treated approximately 6 months prior to *in vitro* PBMC culture.
§ Geometric mean $\overset{\times}{\div}$ relative s.e.

those PBMC cultured from the six atopics were able to produce measurable but low quantities (geometric mean 358 pg/ml). In marked contrast, significant amounts of IgE were detected in PBMC culture supernates obtained from the parasitized subjects (geometric mean 2,487 pg/ml). When the synthetic ratios were determined, there were significant differences between the amount of IgE synthesized by PBMC of the parasitized individuals and the IgE synthesized by the PBMC of either normals ($P<0·001$) or atopics ($P<0·02$).

When all study individuals were grouped, there was a highly significant correlation between total serum IgE levels and IgE production *in vitro* ($r=0·68$, $P<0·001$ by linear regression analysis; $r=0·89$, $P<0·001$ by Spearman rank correlation) (Fig. 2).

Effect of PWM on in vitro *antibody production*

Fig. 3 shows that PWM had minimal effect on the *in vitro* IgE production by cultures of PBMC from

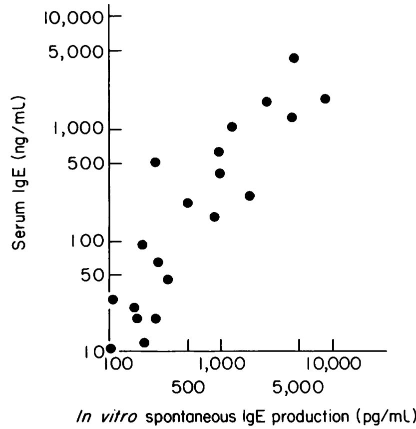

Fig. 2. The correlation between serum IgE levels and spontaneous *in vitro* IgE production in PBMC culture supernatants. Each dot (●) represents an individual patient.

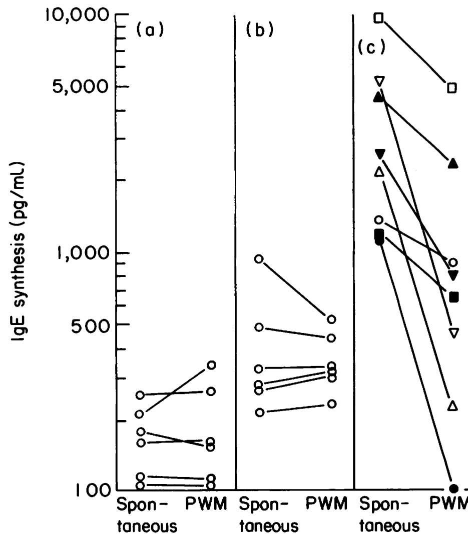

Fig. 3. The effect of PWM on *in vitro* IgE production in (a) normals, (b) atopics and (c) patients with helminth infections (c). Value at the left end of each line indicates level of spontaneous IgE production by PBMC in the presence of media alone while that at the right indicates the effect of PWM on this production. The various symbols in (C) each represent different patients (○—○, ▲—▲—loiasis; ▽—▽, □—□, ●—●—schistosomiasis; ■—■, ▽—▽, △—△—onchocercaisis), and these symbols are utilized consistantly in (c) of Fig. 4 and Fig. 5.

normal (a) and atopic subjects (b). However, when cultures from parasitized individuals were studied, PWM was seen to cause a marked decrease in IgE biosynthesis in all cases (c). Regardless of how cell culture conditions were changed (use of flat bottomed microtitre wells, stimulation of PWM over a wide range of concentrations or use of a two stage culture procedure where PWM was washed out after 4 days), there was no alteration of this PWM-induced inhibition of IgE biosynthesis (data not shown).

In order to determine whether the PWM-induced polyclonal B cell activation for IgG production was similar in all three groups, IgG response patterns were also studied. As seen in Fig. 4, cultures from all three groups showed similar, enhanced IgG responses in the presence of PWM.

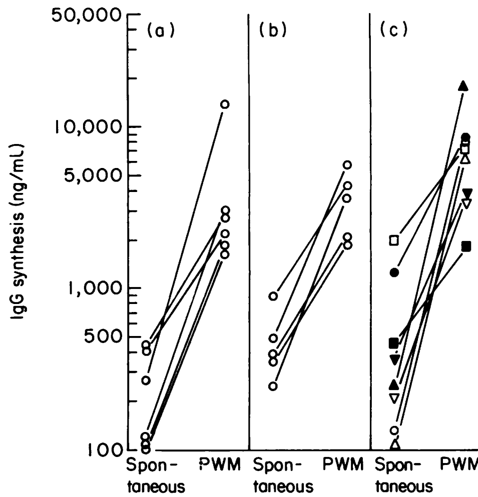

Fig. 4. PWM-induced IgG synthesis *in vitro* by (a) normals, (b) atopics and (c) patients with helminth infections. Value at left end of each line indicates level of spontaneous IgG production in the presence of media alone and that at the right IgG production in the presence of PWM. Symbols are used as described for Fig. 3.

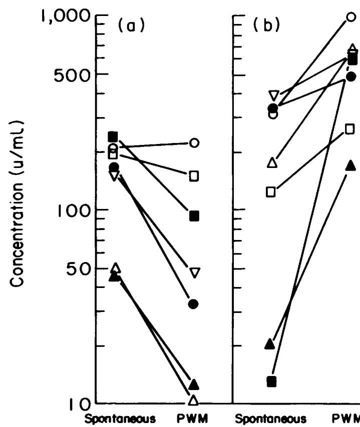

Fig. 5. Antigen specific IgE (a) and IgG (b) responses in spontaneous and PWM driven cultures of PBMC from parasitized individuals. Symbols are used as described for Figs 3 & 4.

Spontaneous and PWM-induced synthesis of parasite antigen specific IgE and IgG antibodies

Culture supernatant fluids from parasitized individuals were examined for antigen specific IgE and IgG. In all cases specific IgE and IgG were detectable in supernates from unstimulated cultures (Fig. 5). When PWM was added, the antigen specific responses generally followed the total immunoglobulin responses in that there was PWM inhibition of IgE specific antibody production but PWM enhancement of IgG specific antibody synthesis. Supernatant fluid from normal and atopic subjects, as expected, produced neither parasite specific IgE nor IgG.

DISCUSSION

The importance of the IgE response in parasitic diseases remains unclear though in some cases IgE production has correlated with resistance to helminth infection (Dessein *et al.*, 1981; Gusmao,

Stanley & Ottesen, 1981), cellular killing of parasites *in vitro* (Joseph *et al.*, 1978) and the pathological processes associated with certain clinical disorders (Ottesen, 1980). Thus, an appreciation of the factors that regulate the production of this particular immunoglobulin isotype should provide insight not only into a general understanding of the mechanisms involved in IgE secreting B cell activation but also into the mechanisms involved in protective immunity and the pathogenesis of the helminth infections of man.

Although many laboratories have reported *in vitro* IgE production by human peripherial blood cells, the conditions causing its induction or inhibition have been under considerable debate (Katz, 1982). The strong correlation between the serum IgE levels of our subjects and the levels of IgE produced *in vitro* by their PBMC is consistent with the data from some (Turner *et al.*, 1981; Ohta *et al.*, 1980) but not other investigators (Fiser & Buckley, 1979; Saxon & Stevens, 1980). However, in this study it should be noted that within the atopic group, serum IgE levels and the *in vitro* levels of IgE are somewhat lower than those reported previously. Similarly, our finding that PWM does not induce IgE synthesis in PBMC from normal subjects agrees with the results of most investigators (Tjio, Hull & Gleich, 1979; Romagnani *et al.*, 1980; Fiser & Buckley, 1979; Saryan, Ldung & Geha, 1983; but conflicts with those of others (Saxon, Kaplan & Stevens, 1980; Nonaka *et al.*, 1981). The fact that vessel geometry, mode of PWM administration, and varying lots of FCS and PWM had no significant effect on these findings again is in agreement with the results of most (Buckely & Becker, 1978; Tjio *et al.*. 1979; Romagnani *et al.*, 1980; Fiser & Buckley, 1979) but not all (Katz, 1980; Saxon *et al.*, 1980) laboratories.

While helminth parasite infections have been shown to activate ('potentiate') B_e cells polyclonally in animals (Jarrett & Bazin, 1974) and possibly in humans as well (Turner, Feddema & Quinn, 1979), the *in vivo* conditions that result in such activation are unknown. Parasite antigens do not induce either blastogenic responses or *in vitro* synthesis of IgG, IgM, or IgE in cell cultures from normal and atopic individuals (unpublished observations), but it is possible that the persistent antigenic stimulation or subtle T cell imbalances associated with helminth infection may contribute to the activation seen in our patients. Whatever the cause, it is likely that cultured PBMC from patients exposed to these antigens had already been maximally stimulated *in vivo* to produce IgE since the unstimulated PBMC obtained from these patients produced IgE antibodies at levels 10–15 times those of allergic individuals and more than 100 times those of normal subjects (Table 1). Indeed, if the cells had already been maximally stimulated, then the signals triggered by PWM would not be expected to provide additional augmentation of an already potentiated response, and the finding that PWM significantly inhibited *in vitro* production of IgE would become less surprising. Since our studies were performed with the patients' unfractionated PBMC it is not clear whether this suppression was exerted at the T or B cell level. It would appear either that PWM activates IgE specific suppressor cells or that PWM driven production of a T cell helper factor shuts off IgE synthesis. This second possibility is supported by studies in mice that show the IgE response to be extremely sensitive to T cell-mediated help and suppression (Turner *et al.*, 1979; Matsumato *et al.*, 1981). It does appear, however, from the disparity between the IgE and IgG PWM driven responses in our system that IgE production and IgG production in humans, as in experimental models (Chiorrazzi, Fu & Kunkel, 1979; Ishizaka & Adachi, 1976) are under discrete regulatory controls. Work on regulatory factors both in animals (Ishizaka, 1976; Kishimoto *et al.*, 1976) and in man (Zuraw *et al.*, 1981) seem to implicate the participation of T cell-mediated processes. Whether suppressive mechanisms are involved in regulating IgE production in humans infected with helminths is currently under investigation in our laboratory.

When the issue of parasite antigen specific responses *in vitro* was studied, the results tended to parallel those obtained for the total immunoglobulin responses. For parasite antigen specific IgE, enhanced IgE synthesis was suppressed by PWM, and for specific IgG, PWM stimulated production of anti-parasite antibody. In all likelihood this PWM driven suppression of specific IgE antibody reflects a general modulation of IgE responses rather than the existence of any antigen specific regulatory mechanism.

Understanding the mechanisms involved in the modulation of IgE responses may contribute significantly to improved immunological control of these parasitic diseases. Our ability to examine the conditions for the induction and suppression of picogram quantities of antigen specific

immunoglobulins provides a means to explore such immune responses to helminth parasites; also, it should provide a means for investigating the pathogenetic aspects of IgE responses to helminth parasites and in so doing, hopefully gain insight into mechanisms involved in the control of immediate hypersensitivity responses in general.

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