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In Vitro IgE-secretion in Atopic Eczema: Influence of Allergens and Mitogens and Role of CD8 T Cell Subpopulation

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In cultures of peripheral blood mononuclear cells (PBMC) from 23 atopic patients and 14 controls the influence of mitogens, allergens and CD8 suppressor T lymphocytes on the in vitro IgE response was studied. The in vitro IgE levels in lymphocyte culture supernatants reached a plateau after 6 days of culture, whereby low levels of IgE could be reproducibly measured down to 0.5 ng/ml. The spontaneous in vitro IgE secretion from PBMC of atopic eczema patients was elevated in comparison to the control group and showed a direct correlation ($r=0.72$) to the serum IgE levels. Pokeweed mitogen rather suppressed the in vitro IgE production. After removal of the CD8 subpopulation of T lymphocytes by using an indirect erythrocytes rosetting technique we found increased in vitro levels pointing to a role of IgE regulating T suppressor subpopulations.

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Although much has been learned about the regulation of IgE synthesis in rodent species (1, 5), little is known about the mechanisms involved in the control of human IgE production, since analogous in vivo studies are not possible.

Lymphocyte culture studies in atopic patients (especially patients with atopic eczema) showed elevated spontaneous in vitro IgE levels (2, 4, 11) with controversial results on the influence of antigenic or mitogenic stimulation (9, 11, 14, 18). Disturbed T cell mechanism has been described to be of importance for the high IgE synthesis in atopic people. Patients with atopic eczema have been reported to possess low T cell levels (8) as well as depressed T cell functions (16) or reduced T suppressor cell numbers (7).

Here we studied the influence of allergens and mitogens upon the in vitro IgE secretion and the effect of depletion of a T cell subpopulation.

MATERIAL AND METHODS

Cell suspensions

Heparinized venous blood was obtained from 23 atopic patients (among them 15 patients with atopic eczema only) and 14 non-allergic volunteers. Peripheral blood mononuclear cells (PBMC) were subsequently isolated by Ficoll-Paque density gradient centrifugation (Pharmacia, Uppsala, Sweden). CD8 T cells were removed by first incubating the PBMC with the mouse monoclonal antibody MT-811 (anti CD8, IgGl isotype), subsequent rosetting with goat anti mouse Ig bearing ox erythrocytes (10) and final Percoll density gradient centrifugation (Pharmacia, Uppsala, Sweden).

Culture conditions

Cells (1×10^6/ml) were suspended in RPMI 1640 growth medium supplemented with 10% fetal calf serum, 1% penicillin/streptomycin and 1% L-glutamine (Seromed, Munich and Gibco Europe, Karlsruhe, FRG) and cultured up to 6 days at 0.2 ml per well in round-bottomed microtiter plates (Bioplast, Berlin, FRG). Stimulation was performed by adding the following lectins and antigens in a 10 µl volume of culture medium: Purified phytohemagglutinin (PHA), 1 µg/ml (Gibco Europe, Karlsruhe, FRG), concanavalin A (ConA), 3 µg/ml (Roth, Karlsruhe, FRG), pokeweed mitogen (PWM), 1 µg/ml (Seromed, Munich, FRG), cat epithelium 0.5% w/v 1 : 10 (Beecham-Wülfing, Neuss, RFT). Control cultures contained medium only. At the end of the culture period IgE concentration in respectively 0.1 ml of cell-free supernatant was determined by a modified PRIST technique (12) (Pharmacia, Uppsala, Sweden).

RESULTS

Correlation between in vitro and serum IgE

Freshly isolated PBMC from 15 patients with atopic eczema were kept in culture without stimulation. The IgE secreted after 6 days was measured in the supernatants and compared with the serum IgE levels. The results are shown in Fig. 1. All supernatant fluids showed measurable IgE levels. Spontaneous IgE se-

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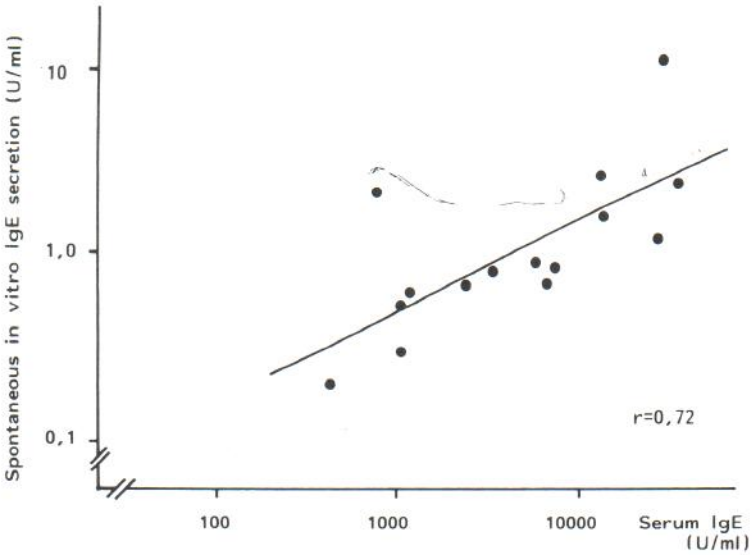

Fig. 1. Correlation of serum and in vitro IgE concentration in patients with atopic eczema ($n=15$).

cretion and corresponding serum IgE level were significantly correlated ($r=0.72$, $p<0.01$).

Kinetics of in vitro IgE secretion

PBMC from 4 atopic eczema patients were stimulated with PHA, PWM and grass pollen extract. After 2, 4 and 6 days the IgE levels in the supernatants were measured. Most of the in vitro IgE levels came to a plateau after 4 days. Stimulation with PWM resulted in inhibition of in vitro IgE production, allergen led to an increase of the in vitro IgE secretion after prolongated culture in single patients (Fig. 2). Patients with marked in vitro IgE response after antigenic stimulation generally showed a specific sensitization

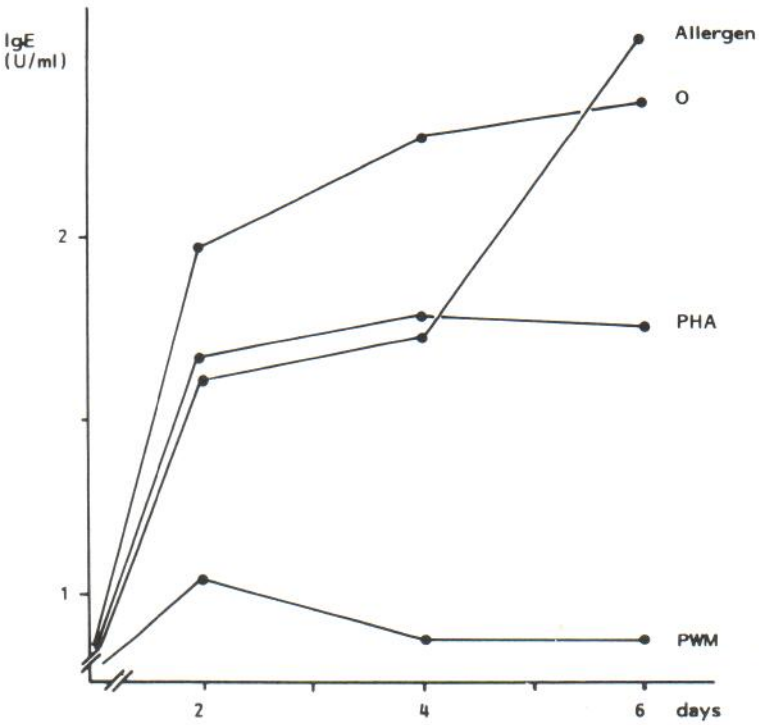

Fig. 2. Kinetics of in vitro IgE secretion ($n=4$).

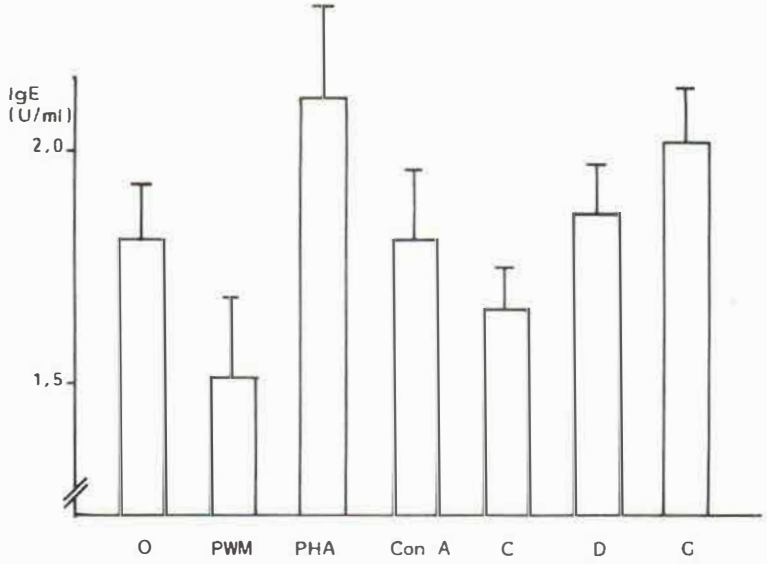

Fig. 3. Influence of mitogens/allergens upon in vitro IgE secretion of patients with atopic eczema ($n=15$). Because of minimal IgE levels controls are not shown. C = cat epithelium, D = house dust mite (D. pteronyssinus), G = grass pollen.

towards the same allergen in the radio-allergo-sorbent-test (RAST) (Table I).

Effect of mitogen or allergen stimulation on in vitro IgE secretion

To determine the effect of different lectins and allergens, we conducted experiments in which IgE secretion from unfractionated mononuclear cells after stimulation was compared with the spontaneous in vitro IgE secretion. The results of these studies are presented in Fig. 3. Cells from normal controls showed very low in vitro IgE levels with a modest augmentation after addition of PWM, while other lectins and antigenic stimulation had no remarkable effect. The spontaneous in vitro IgE secretion of atopic dermatitis patients was inhibited by PWM and ConA allergen eczema was of no statistically significant effect.

Influence of CD8 cell depletion

To examine the influence of CD8 T lymphocytes on the in vitro IgE secretion, unfractionated and CD8-cell-depleted (less than 1%) PBMC from 7 healthy donors and 19 atopic patients were simultaneously kept under identical culture conditions. T and B cell ratios were kept equal. The IgE production after 6 days of culture from fractionated and unfractionated cells of the same patients was measured and compared. PBMC of atopic patients demonstrated higher spontaneous in vitro IgE secretion after T suppressor cell depletion (Fig. 4). Likewise, CD8 depletion resulted in augmentation of lectin and allergen-stimulated in vitro IgE (Table II). Some of the controls now

Table I. *Six patients with atopic eczema in whom spontaneous in vitro IgE secretion was clearly enhanced by allergen stimulation*

Allergen	Serum RAST (class)	Increase in in vitro IgE (U/ml)
D. pteronyssinus	2	0.4
	3	1.58
Grass pollen	4	4.00
	3	0.50
	0	0.49
		0.40

Table II. *Influence of CD8 depletion upon in vitro IgE secretion from PBMC of atopic patients ($n=19$)*

	Spontaneous	PWM	PHA	Allergen (cat)
Increase	11	10	10	11
Decrease	5	4	3	6
Unchanged	3	5	6	2
	$p<0.05$	$p<0.05$	NS	NS

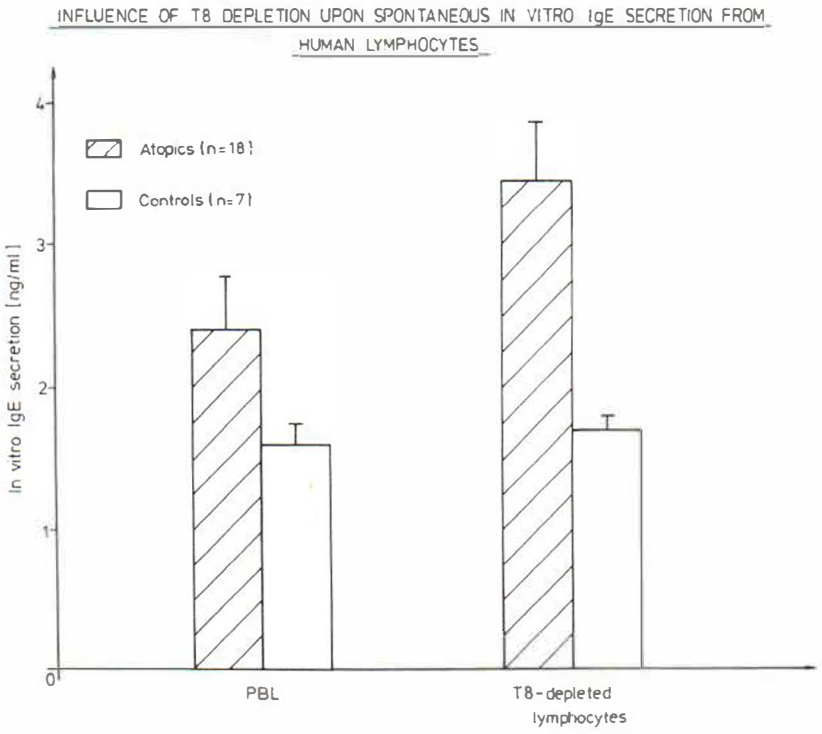

Fig. 4. Spontaneous in vitro IgE secretion in atopics ($n=7$) and normals ($n=10$) after depletion of CD8-T cells by indi-

secreted detectable amounts of spontaneous in vitro IgE (data not shown).

DISCUSSION

Tada et al. (17) first pointed to the role of T cell regulatory mechanisms on IgE biosynthesis when they demonstrated increase of ongoing IgE antibody production in rats after treating the animals with antithymocyte serum, whole body irradiation, adult thymectomy or with various immunosuppressive drugs.

When lymphocyte cultures are used to study human IgE regulation (13), 6 or 7 days of culture are required as our data showed. A recent multicenter study in which our laboratory took part reported a low reproducibility of in vitro IgE measurements below 0.5 ng/ml and stressed the need for appropriate culture conditions and IgE detection methods (3).

Our observation that atopic eczema patients with high serum IgE concentrations also showed a high spontaneous in vitro IgE secretion suggests that the trigger to increased B cell IgE synthesis in vitro may already have occurred in vivo (15). Thus the addition of lectins—especially PWM—could not further increase but rather inhibit spontaneous in vitro IgE production (11, 14).

The marked augmentation of in vitro IgE secretion after removal of CD8 suppressor T cells points to the existence of IgE-isotype-specific regulatory T lymphocytes within this subpopulation. A functionally altered suppressor cell population, extensively investigated by Ishizaka et al. (6) in a rodent model, may be one factor in the impaired T–B-cell cooperation leading to atopic disease. The model of spontaneous in vitro IgE secretion in lymphocyte cultures might be suitable to study possible inhibitory factors of human IgE production.

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