

In Vitro Production of IgE and IgG Protein by Blood Mononuclear Cells from Non-Atopic and Atopic Donors

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The production of IgE and IgG protein by human peripheral blood mononuclear cells in culture has been examined. Cells obtained from 18 grass-sensitive donors during the grass pollen season and from 11 atopic dermatitis patients (total serum IgE levels >960 ng/ml), spontaneously produced significant amounts of IgE but not IgG with time. Similar results were obtained using B-cell enriched preparations from both groups. Cells from 16 non-atopic donors had mean levels of pre-formed IgE similar to those of grass pollen-sensitive donors, but there was no increase in culture IgE with time. Treatment of mononuclear cells with pokeweed mitogen did not influence the production of IgE but markedly increased the amount of IgG synthesized by non-atopic and atopic donor cells. Slight, but significant increases in culture IgE, but not IgG, were seen following a 7-day mixed lymphocyte reaction involving both unrelated non-atopic donor cells and the lymphoblastoid B-cell line, Raji. Treatment of cells with 50 HA units of influenza A/Hong Kong/1/68 (H3N2) virus or with 0.02% v/v (240 IU/ml) of a purified β -interferon preparation did not alter IgE or IgG produced.

Key words: IgE; IgG; lymphocytes.

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Studies from a number of laboratories have demonstrated IgE biosynthesis *in vitro* using human peripheral blood mononuclear cells (3, 10, 13, 17, 23). Synthetic activity may be spontaneous or induced following stimulation with mitogens (17, 27) or specific antigens (10, 25) and appears to involve different subpopulations of B-cells under varying degrees of T-cell control (17, 27). Despite disagreement as to the effect of the conventional polyclonal B-cell mitogen pokeweed (PWM) to stimulate IgE synthesis *in vitro* (3, 5, 13, 17, 21, 27), it is known that a number of clinical disorders including virus infection (18) and acute graft versus host reaction (4) can lead to excess IgE production in ostensibly normal individuals. This suggests that under appropriate conditions it should be possible to stimulate IgE syn-

thesis *in vitro* using lymphoid tissue from non-atopic donors. In this report we describe the production of IgE and IgG protein by peripheral blood cells from non-atopic, grass pollen-sensitive and atopic dermatitis patients and describe attempts at inducing IgE synthesis in non-atopic donor cells.

MATERIAL AND METHODS

Cell donors

Blood was obtained from 58 individuals. 20 grass pollen-sensitive adult donors with seasonal rhinitis had detectable serum IgE antibody to rye allergens and were skin test-positive to rye grass pollen extract. One individual was undergoing pollen hyposensitisation therapy. 14 do-

nors had elevated serum IgE levels and clinical findings compatible with a diagnosis of atopic dermatitis; all were receiving topical corticosteroid therapy. 24 non-atopic donors with normal serum IgE concentrations were used as controls.

Isolation of mononuclear cells

Mononuclear cells were separated from heparinized peripheral venous blood by Ficoll-Paque (Pharmacia, Uppsala, Sweden) density gradient centrifugation. They were washed three times and resuspended in HEPES-buffered RPMI 1640 tissue culture medium (Gibco Europe Ltd.), supplemented with 1% L-glutamine, 2% penicillin-streptomycin and 15% heat-inactivated foetal bovine serum (FBS).

B-cell rich and T-cell rich lymphocyte populations were prepared by rosetting mononuclear cells with sheep red cells (E) pretreated with 2-aminoethylisothiuronium bromide (Sigma) as described by Madsen et al. (9), followed by centrifugation over Ficoll-Paque. Cells were aspirated from the gradient interface, washed twice and used as B-cell rich populations. They contained < 5% E-rosette-forming cells. Red cells in the cell pellet were lysed with tris-buffered ammonium chloride, the remaining cells washed twice and used as T-cell enriched populations.

Cell culture

Cells were cultured at a concentration of 1.5×10^6 /ml in a final volume of 2 ml in 14 mm-diameter round-bottomed tissue culture tubes (Nunc). Cultures were prepared in duplicate or triplicate and incubated in 5% CO₂ at 37°C for an optimum time of 7 days. At the end of each experiment cells were lysed with 1% v/v triton X-100 in 1.0 M HEPES buffer in a final volume of 100 µl and stored at -20°C. Before assay, supernatants were harvested by centrifugation at $800 \times g$ for 10 min. Preliminary experiments indicated that this procedure yielded higher extraction values for immunoglobulin compared with either repeated cycles of freeze-thaw or acid extraction (24). Levels of preformed im-

munoglobulin were determined by a) lysing cells at the start of each experiment and storing at -20°C, b) maintaining lysed cells in culture for 7 days and c) setting up parallel cultures containing 10 µg/ml cycloheximide and lysing as usual at termination. The values obtained using these methods were essentially similar for both immunoglobulin isotypes measured.

Treatment of cells

Pokeweed mitogen (PWM) was purchased from Sigma Chemical Company Ltd., Bethesda Research Laboratories Inc. and Pierce and Warriner (UK) Ltd. Cells were either cultured with 1 and 10 µg/ml mitogen or stimulated with 1 µg/ml PWM for 2 days, washed and recultured in fresh medium for a further 7 days, after the method of Zuraw et al. (27).

Influenza A/Hong Kong/1/68 (H3N2) virus was obtained from J.S. Oxford, National Institute of Medical Research, London, and had a stock titre of 5120 haemagglutination units (HAU)/ml. Mononuclear cells (6×10^6 /ml) were exposed to 50 HAU/ml of virus in serum-free medium for 2 h at 37°C. They were washed three times, resuspended to 1.5×10^6 /ml in culture medium and incubated with an equal number of untreated autologous cells.

Interferon-β (IFN-β) purified to homogeneity was obtained by courtesy of Dr. R. A. Vere Hodge (Beecham Pharmaceuticals Research Division). The freeze-dried preparation, which was not re-assayed before use, was reconstituted in 10 mM glycine/human serum albumin buffer (pH 3.5) to give a nominal specific activity of 1.2×10^6 IU/ml. Mononuclear cells were incubated with a final concentration of 0.02% v/v (= 240 IU/ml) IFN-β and control cultures received medium containing the appropriate dilution of buffer.

Mixed lymphocyte reaction

Mononuclear cells were suspended at 1.5×10^6 /ml in culture medium. 1 ml of mitomycin-C-treated cells from one non-atopic donor was mixed with an equal number of untreated cells from an unrelated non-atopic donor and in-

cubated at 37°C for 7 days. Control cultures included mitomycin-C-treated cells alone and untreated cells at 1.5×10^6 and 3×10^6 /culture. In two experiments, non-atopic donor cells were incubated with 0.375×10^6 mitomycin-C-treated human lymphoblastoid B-cells (Raji, Flow Labs., Ltd.), and immunoglobulin levels measured 7 days later.

Preparation of immunological reagents

A γ -globulin preparation of antihuman IgG (GAHG) was obtained by immunising goats with human IgG, followed by ammonium sulphate precipitation and ion exchange chromatography on DEAE cellulose. Anti-human IgE (RAHE) was prepared by immunising rabbits with purified IgE myeloma Y.U. (kindly supplied by Professor M. Sela, Weizmann Institute of Science, Rehovot, Israel) and isolating the γ -globulin fraction as above. This fraction was adsorbed with normal human serum and human IgG. An amount was further purified by affinity chromatography using Y.U. IgE-sepharose as the immunosorbent. This was radiolabelled with ^{125}I (Amersham Ltd.) and chloramine-T to a specific activity of 2.9 mCi/mg. Staphylococcal protein A (Pharmacia) was similarly iodinated to 29 mCi/mg.

Radioimmunoassay for IgG

IgG concentrations were assayed using GAHG coated microtitre plates, after the method of Zuraw et al. (27). Bound human IgG was detected using ^{125}I -Protein A (3 ng/well). IgG concentrations were interpolated from a plot of bound radioactivity versus log concentration of purified IgG (10 ng/ml to 100 $\mu\text{g/ml}$), calibrated with the WHO International Reference Preparation for Human Serum IgG (batch 67/86, supplied by the National Institute for Biological Standards and Control, U.K.).

Radioimmunoassay for IgE

RAHE γ -globulin fraction was coupled to Matrex Pel 102 (Amicon Ltd.) using 50 mg protein/g beads. The suspension (50 μl , 0.2 mg

beads) was incubated with 0.9 ml volumes of culture supernatant or 50 μl of serum for 24 h at 4°C, with mixing. After centrifugation, the supernatant was aspirated and the immunosorbent incubated with 50 μl of ^{125}I -RAHE (30 ng) solution for 24 h at room temperature with constant agitation. The beads were washed three times with 2 ml 0.05% Tween 20, phosphate buffered saline, and the pellets counted in a Packard 5260 γ counter. IgE concentrations were determined in a similar fashion to those for IgG, using pooled sera from atopic donors calibrated against the WHO Second International Reference Preparation for IgE. The lower limit of assay sensitivity was 100 pg/ml.

Statistical analysis

Immunoglobulin assays were performed in duplicate. Replicate IgE and IgG assays using the same samples were consistent with a standard error of the mean (SEM) of <6%. Variations between individual donors tested on either the same or different occasions were greater, with an SEM for IgE and IgG of 9.8% and 6.6% respectively. All results are expressed as geometric means. Significance on log-transformed data ± 1 SD was determined by Student's *t* test.

RESULTS

Mononuclear cells from 18 grass-sensitive donors obtained during the pollen season (June-July, 1983) were cultured for 7 days and total IgE and IgG protein determined. Basal levels of IgE varied considerably between individuals, ranging from below the level of detection (~ 100 pg/ml) to a maximum of 2000 pg/ml, with a geometric mean of 397 pg/ml. Following culture, cells from 15 of the volunteers showed a net increase in IgE values with a mean of 1177 pg/ml (Table 1). There were similar variations in the amount of performed IgG between individuals but no increase in values after culture (Table 1). Levels of performed IgE in cells of atopic dermatitis patients also varied, ranging from a low of 480 pg/ml

up to 4000 pg/ml, with a mean of 1471 pg/ml. As with the grass-sensitive donors there was a significant increase in the amounts of spontaneous IgE detected after 7 days, with a mean of 3429 pg/ml. Eight of the 11 donors examined showed a $> 50\%$ increase in IgE and three patients gave ≥ 2 -fold increases. Levels of culture IgG did not increase with time (Table 1).

Cells from non-atopic donors had a mean basal IgE level of 377 pg/ml which was similar to the value seen with the grass-sensitive donor

cells. Six of the 16 non-atopic volunteers, however, had preformed IgE values below the limit of assay detection, whereas only two grass-sensitive donors had values this low. In contrast to the atopic donor cells, those from the non-atopics did not show any increase in spontaneous IgE in culture. Both preformed and culture IgG values were similar to those seen using atopic donor cells (Table 1).

B-cell enriched preparations from non-atopic, grass-sensitive and atopic dermatitis patients were also considered. Non-atopic donor cells

Table 1

Detection of IgE and IgG protein in 7-day cultures of mononuclear cells from non-atopic, grass-sensitive and atopic dermatitis patients

Subjects	n	Serum IgE levels (ng/ml)	IgE protein (pg/ml)		IgG protein (ng/ml)	
			Preformed	7-day culture	Preformed	7-day culture
Non-atopic	16	206 (5.33 $\pm$ 0.93)	377 (5.93 $\pm$ 0.54)	384 (5.95 $\pm$ 0.49)	157 (5.06 $\pm$ 0.36)	148 (5.00 $\pm$ 0.30)
Grass-sensitive	18	1462 (7.29 $\pm$ 0.59)	397 (5.98 $\pm$ 0.82)	1177** (7.07 $\pm$ 0.48)	74 (4.30 $\pm$ 0.99)	105 (4.65 $\pm$ 0.88)
Atopic dermatitis	11	5271 (8.57 $\pm$ 1.10)	1471 ² (7.29 $\pm$ 0.63)	3429 ¹ * (8.14 $\pm$ 0.69)	165 (5.11 $\pm$ 0.44)	200 (5.30 $\pm$ 0.70)

Values presented as geometric means. Ln means $\pm$ SD in parenthesis.

Significance: * $P < 0.01$; ** $P < 0.001$.

Positive correlation between individual serum IgE concentrations and levels of IgE in culture.

¹ $P < 0.05$; ² $P < 0.01$.

Table 2

Detection of IgE and IgG protein in 7-day cultures of B-lymphocyte enriched mononuclear cells from non-atopic, grass-sensitive and atopic dermatitis patients

Subjects	n	IgE protein (pg/ml)		IgG protein (ng/ml)	
		Preformed	7-day culture	Preformed	7-day culture
Non-atopic	3	248 (5.51 $\pm$ 0.27)	246 (5.50 $\pm$ 0.36)	58 (4.06 $\pm$ 0.09)	98 (4.59 $\pm$ 0.42)
Grass-sensitive	7	1100 (7.00 $\pm$ 0.59)	2358* (7.77 $\pm$ 0.41)	54 (3.98 $\pm$ 0.41)	61 (4.12 $\pm$ 0.33)
Atopic dermatitis	6	1231 (7.12 $\pm$ 0.55)	2807* (7.94 $\pm$ 0.38)	40 (3.67 $\pm$ 0.30)	71 (4.26 $\pm$ 0.78)

Values presented as geometric means. Ln means $\pm$ SD in parenthesis.

Significance: * $P < 0.02$.

yielded low levels of preformed IgE similar to the results using unseparated cells; there was no increase in values with culture. IgG levels also remained constant (Table 2). Both the grass-sensitive and atopic dermatitis patients' B-cells gave appreciable values of basal IgE increasing 2-fold and 2.3-fold respectively with time. Levels of IgG remained the same (Table 2). As a control, the T-cell enriched preparations were also examined for preformed and culture immunoglobulin. In none of the groups did IgE values exceed 600 pg/ml and the IgG values 14 ng/ml. Only five donors had IgE and IgG levels above the limit of detection.

Although the results are given for a culture period of 7 days, using cells from the grass-sensitive donors we found a progressive increase in spontaneous IgE over the first 4 days in culture; this reached a maximum and remained constant for up to 11 days. Known amounts of exogenous IgE and IgG could be detected throughout culture, and there was no evidence of catabolism either in medium alone or in the presence of disrupted cells for up to 10 days at 37°C.

Because of the controversy surrounding the ability of human blood lymphocytes to synthesize *de novo* IgE in culture, the effects of a number of stimuli were examined. Using a culture system similar to that described by Zuraw et al. (27), mononuclear cells from eight

non-atopic and eight grass-sensitive donors were stimulated with PWM, washed and recultured and immunoglobulin levels compared. Despite giving increases in IgG of 13-fold and 4-fold respectively, neither of the two groups showed any increase in IgE (Table 3). Indeed, in only two of the non-atopic donors examined were IgE values above background. Five of the grass-sensitive donors gave an increase in spontaneous IgE in the same experiments, yet only three showed any increase with PWM. Addition of both 1 and 10 µg/ml PWM to cultures for the duration of the experiments similarly failed to influence IgE values, despite increasing the amount of IgG detected in the non-atopic and grass-sensitive cell cultures 6-fold and 4-fold respectively. The overall increase in PWM-stimulated IgG using atopic dermatitis patient cells was less than that seen with cells from the other donors, with only three individuals giving a >2-fold increase and two of the eight patients failing to respond to either mitogen concentration (Table 4).

Despite the apparent failure of PWM to stimulate IgE synthesis *in vitro*, cultures of non-atopic donor cells mixed with cells from unrelated volunteers or with the Raji cell line, showed a slight but significant ($P < 0.01$) increase in IgE but not IgG with time (Table 5). Four of the seven cultures examined gave >2-fold and two gave >5-fold increases in IgE.

Table 3

Detection of IgE and IgG protein in 9-day cultures of mononuclear cells from non-atopic and grass-sensitive donors stimulated with PWM

Subjects	n	IgE protein (pg/ml)		IgG protein (ng/ml)	
		Unstimulated	PWM-stimulated	Unstimulated	PWM-stimulated
Non-atopic	8	121 (4.79 ± 0.53)	133 (4.89 ± 0.53)	33 (3.48 ± 0.45)	433* (6.07 ± 0.34)
Grass-sensitive	8	421 (6.04 ± 0.63)	483 (6.18 ± 0.63)	37 (3.62 ± 0.73)	152* (5.02 ± 0.56)

4×10^6 mononuclear cells were cultured in Nunc tubes for 48 h with or without 1 µg/ml PWM and 10 µg/ml cycloheximide. They were washed twice and the nonadherent cells recultured in the original volume of fresh medium with or without cycloheximide in new tubes for a further 7 days.

Values presented as geometric means. In mean ± SD in parenthesis.

Significance: $P < 0.001$.

Parallel cultures confirmed that neither of the two cell-populations alone showed any increase in culture IgE or IgG.

No such increase in IgE was observed following co-culture of mononuclear cells treated with influenza A virus and untreated autologous cells (Table 5). Additional experiments, in which purified blood monocytes from non-atopic donors were similarly pretreated with virus and then recultured with autologous lymphocytes, also failed to influence IgE or IgG values (results not shown).

Incubation of non-atopic donor cells with 0.02% v/v IFN- β had no effect on culture IgE or IgG values (Table 5), and co-culturing cells with allogeneic mononuclear cells in the presence of IFN- β for 7 days did not increase immunoglobulin levels above that seen using cells alone (Table 5).

DISCUSSION

The results confirm that peripheral blood cells from non-atopic donors do not spontaneously produce *de novo* IgE or IgG protein in culture

Table 4
Detection of IgE and IgG protein in 7-day cultures of mononuclear cells from atopic dermatitis patients stimulated with PWM

	IgE protein (pg/ml)	IgG protein (ng/ml)
Preformed	1299 (7.17 $\pm$ 0.41)	110 (4.70 $\pm$ 0.59)
Unstimulated	2981 (8.00 $\pm$ 0.72)	126 (4.83 $\pm$ 0.38)
1 μ g/ml PWM	3207 (8.07 $\pm$ 0.69)	200* (5.30 $\pm$ 0.43)
10 μ g/ml PWM	2525 (7.83 $\pm$ 0.54)	245** (5.50 $\pm$ 0.27)

PWM added for duration of culture.

Values are presented as geometric means ($n = 8$).

Ln means $\pm$ SD in parenthesis.

Significance: * $P < 0.05$; ** $P < 0.01$ compared to unstimulated cultures.

(5, 17). In contrast, cells obtained from grass pollen-sensitive donors during the height of the pollen season (12, 22) showed an increase in spontaneous IgE but not IgG with time. Atopic dermatitis patients with elevated serum IgE levels showed a similar profile of response to the grass-sensitive donor cells, although the absolute values for both preformed and culture IgE were higher in the former group. In the atopic dermatitis patients there was a positive correlation between individual serum IgE levels and the values for both preformed ($r = 0.76$; $P < 0.01$) and synthesized ($r = 0.61$; $P < 0.05$) IgE. This is in agreement with the findings of Turner et al. (23) who also reported a clear association between *in vitro* synthesis of IgE and serum IgE levels of atopic cell donors. We were not, however, able to detect an individual correlation between the levels of serum and culture IgE in the 18 grass pollen-sensitive donors examined.

Production of spontaneous IgE by atopic donor cells appeared to be independent of T-lymphocytes as depletion of E-rosette cells did not impair activity, and little or no immunoglobulin was associated with the T-lymphocytes themselves. Using cycloheximide-treated cultures as well as day 0 lysates, our results confirm those of other workers and suggest that spontaneous production is a consequence of pre-committed B-cells still capable of restricted synthesis when placed in culture (3, 13, 22).

It should be noted that all cultures were lysed before assay, including the cycloheximide-treated controls, so the values recorded represent total immunoglobulin levels not just that proportion released into the culture medium during the incubation period. This may be important as preliminary experiments revealed that although some 90% of total IgG measured after 7 days' culture was extracellular, 40% of IgE was still cell-associated. This may explain some of the anomalies recorded by others using different procedures for assessing levels of IgE (14, 24).

Treatment of non-atopic and grass-sensitive donor cells with PWM under conditions which elevated levels of IgG had no effect on IgE. Varying the length of contact between cells and

the mitogen for 2–11 days, washing and reculturing cells both with and without autologous monocytes, varying the concentration and source of mitogen and only using those batches of PWM containing insufficient amounts of histamine to activate T-suppressor cells (19), were without effect. A number of authors have additionally emphasized the importance of screening batches of FBS, both for their ability to support IgE synthesis and to increase IgG production in the presence but not absence of PWM (17, 27). We have tested

eight batches of FBS from four suppliers and, with the exception of one which suppressed all values (probably by interfering with the assays), no obvious differences were apparent. It should be noted, however, that although we could obtain significant increases in PWM-stimulated IgG, especially in the 2-stage culture system, we never achieved the 20-fold increases reported by others (17, 27). Mononuclear cells from the atopic dermatitis patients stimulated with PWM similarly failed to increase IgE, although in these individuals the increase in IgG

Table 5

Detection of IgE and IgG protein in 7-day cultures of non-atopic donor mononuclear cells stimulated in a mixed lymphocyte reaction, with influenza A/Hong Kong virus and interferon- β

Treatment	n	IgE protein (pg/ml)		IgG protein (ng/ml)	
		Preformed	7-day culture	Preformed	7-day culture
MLR ^a	7	229 (5.43 $\pm$ 0.35)	915** (6.82 $\pm$ 0.85)	201 (5.31 $\pm$ 0.45)	178 (5.18 $\pm$ 0.49)
Unstimulated	10	289 (5.67 $\pm$ 0.64)	487 (6.18 $\pm$ 0.73)	70 (4.25 $\pm$ 0.59)	85 (4.44 $\pm$ 1.02)
Influenza virus ^b			267 (5.59 $\pm$ 0.42)		55 (4.00 $\pm$ 0.42)
Unstimulated	4	262 (5.57 $\pm$ 0.49)	291 (5.67 $\pm$ 0.91)	52 (3.96 $\pm$ 0.19)	89 (4.49 $\pm$ 0.39)
IFN- β ^c			251 (5.52 $\pm$ 0.73)		86 (4.46 $\pm$ 0.55)
MLR ^d	4	361 (5.89 $\pm$ 0.48)	810* (6.70 $\pm$ 0.25)	212 (5.35 $\pm$ 1.08)	133 (4.89 $\pm$ 1.05)
IFN- β			767* (6.64 $\pm$ 0.36)		159 (5.07 $\pm$ 0.97)

^a 1.5×10^6 mononuclear cells incubated for 7 days with mitomycin-C-treated stimulator cells. Five experiments involved cell combinations between eight unrelated donors (3×10^6 cells/culture), the remaining two experiments used Raji cells (0.375×10^6 /ml) as the stimulator population. Non-atopic donor cells (1.5×10^6 /ml) cultured for 7 days gave a mean IgE value of 319 pg/ml; range < 200–700 pg/ml and a mean IgG value of 84 ng/ml; range 20–210 ng/ml.

^b 1.5×10^6 mononuclear cells incubated for 7 days with an equal number of autologous cells pretreated for 2 h at 37°C with 50 HAU influenza A virus or 1 ml serum-free medium.

^c 3×10^6 mononuclear cells incubated for 7 days in the presence of 0.02% v/v interferon- β or 0.02% 10^{-3} M glycine/HSA buffer.

^d 1.5×10^6 mononuclear cells incubated with an equal number of mitomycin-C-treated allogeneic cells in the presence of 0.02% v/v interferon- β or glycine/HSA buffer for 7 days. Non-atopic donor cells (4 responder, 4 stimulator) cultured at 1.5×10^6 cells/ml for 7 days gave a mean IgE value of 300 pg/ml; range < 100–700 pg/ml and a mean IgG value of 65 ng/ml; range 20–290 ng/ml.

All values presented as geometric means. Ln means $\pm$ SD in parenthesis.

Significance: * $P < 0.05$; ** $P < 0.01$ compared to preformed immunoglobulin.

was only just significant (Table 5). It may be that lymphocytes from these donors are more susceptible to the effects of PWM on cell viability (7) or that the depression of cell-mediated immunity associated with atopic dermatitis (6) in conjunction with the steroid therapy that all patients were receiving, reduced both T- and B-cell activity.

The failure to induce IgE *in vitro* using PWM has been reported by other workers (3, 13, 23) and is consistent with the hypothesis that there are either insufficient IgE precursor B-cells in peripheral blood or that cells capable of responding to PWM need to have been pre-activated *in vivo* (16). Despite testing atopic donor cells during the height of the grass season our cultures may not have been sufficiently primed (16, 22). Nevertheless, the levels of both culture and serum IgE were similar to those reported by others using comparable subjects (13, 22, 23). The possibility that suppressor cells present in the cultures prepared from the atopic donors were inhibiting IgE production cannot be ruled out. Incubating B-cell-enriched populations with PWM did not increase culture IgE or IgG, presumably because of the absence of helper T-cells required for immunoglobulin production (27). In addition, unpublished observations have indicated that removing the theophylline-sensitive T-cell subpopulation (T γ cells) (20), reconstituting the remaining cells and stimulating with PWM, still failed to affect IgE values, although the mean levels of IgG were increased 2-fold. Similar conclusions were reached by Vela et al. (25) following selective depletion of the T γ cell population from their atopic cell cultures.

Patients undergoing acute GvH reactions have elevated levels of serum IgE (4). The *in vitro* counterpart of GvH is the MLR. An MLR between non-atopic donor cells or between blood mononuclear cells and a B-cell line gave a slight, but significant increase in culture IgE, although the mean values were still < 1 ng/ml (Table 5). It is not known whether the magnitude of this increase could be related to antigenic differences between individuals as HLA-typing was not carried out. In addition, although the number of stimulator cells was kept constant and directionality was ensured by treat-

ing with mitomycin-C, either or both of the populations could have contributed to the increased IgE levels. Separate experiments indicated that mitomycin-C-treated atopic donor cells were still capable of spontaneous IgE production, although, as indicated by Ohta et al. (11), this procedure would be expected to impair *de novo* synthesis.

The mechanism by which an MLR was able to effect an increase in IgE is not known. Certainly soluble factors that selectively regulate IgE synthesis have been described in both man and experimental animals (15, 26), and factors able to suppress IgE production identified in the supernatants of human 1 and 2-way MLR (27). Recently, Lanzavecchia & Parodi (8), described the production of IgE by peripheral blood cells stimulated with alloreactive T-helper cell clones isolated from a secondary MLR. In addition to these cells they identified suppressor/cytotoxic lymphocyte precursors in peripheral blood capable of inhibiting IgE synthesis. Whether the extent of the IgE response depends primarily on the balance between suppressor and helper cell precursors or the various factors derived from them, or on the relative susceptibility of atopic vs. non-atopic cell populations to regulatory molecules, remains to be determined. It did occur to us, however, that the observed increase in IgE may have been a direct consequence of immunologic recognition of foreign antigens, and that procedures aimed at either generating altered self antigens or of increasing surface expression of class I or class II HLA molecules may have similar effects. However, treatment of mononuclear cells with either influenza A virus or IFN- β using schedules that result in the expression of virally-coded surface antigen and HLA-D/DR antigens respectively (1, 2), has so far proved ineffective.

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