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The capacity of interleukin-4 to induce *in vitro* IgE synthesis by B cells of patients with common variable immunodeficiency

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SUMMARY

Interleukin-4 (IL-4) has been shown to induce IgE synthesis by peripheral blood mononuclear cells (PBMC) of normal donors *in vitro*. However, induction of PBMC of patients with common variable immunodeficiency (CVI) with IL-4 resulted in IgE production in only two out of eight cases tested. PBMC of the first patient that produced IgE in response to IL-4 also secreted normal levels of IL-4 upon activation. PBMC of the second patient secreted very low levels of IL-4 *in vitro* which may account for the very low serum IgE levels in this patient. Of the other six patients who had very low serum IgE levels and whose PBMC failed to produce IgE in response to IL-4 *in vitro*, five did not secrete IL-4 upon *in vitro* activation. The capacity of the T cells to produce IL-4 was intact in the sixth patient. Collectively our data indicate the PBMC of the majority of patients with CVI are defective since they failed to respond appropriately to IL-4 and they failed to produce IL-4, contributing to the view that CVI is a heterogeneous disorder in which a variety of T and B cell defects occur.

Keywords interleukin-4 IgE synthesis immunodeficiency patients

INTRODUCTION

Common variable immunodeficiency (CVI) is a heterogeneous disorder characterized by the failure of B cells to differentiate into antibody-secreting plasma cells (Cooper, Lawton & Bockman, 1971; Preud'homme, Griscelli & Seligman, 1973; Geha *et al.*, 1974; Wu, Lawton & Cooper, 1974; de la Concha *et al.*, 1977; Siegal & Good, 1977; Schwaber, Lazaurus & Rosen, 1978; Saiki *et al.*, 1982). Patients with CVI consequently have reduced levels of serum immunoglobulins and are unable to generate antibody responses against common antigens. The block in B cell differentiation has been attributed to intrinsic B cell defects (Schwaber *et al.*, 1978; Saiki *et al.*, 1982; Jones, 1984; Perri & Weisdorf, 1985), defects in immunoregulatory T cells (Reinherz *et al.*, 1981a, 1981b; Lopez-Botet *et al.*, 1982; Saiki *et al.*, 1984; Matheson & Green, 1987), defects in monocyte functions (Siegal, Siegal & Good, 1976) or to increased T suppressor cell activity (Waldman *et al.*, 1974; Siegal *et al.*, 1976; Mitsuya *et al.*, 1981; Jones, 1984).

Normal B cell differentiation proceeds through a series of events which require B cell growth and differentiation factors produced by T cells or monocytes (Kishimoto, 1987). However, attempts to induce immunoglobulin production by B cells of

patients with CVI by Epstein-Barr virus (EBV), pokeweed mitogen (PWM) or supernatants of activated T cells containing the necessary B cell growth and differentiation factors, generally results in IgM secretion with no or very low levels of IgG and IgA production (de Gast *et al.*, 1980; Pereira, Webster & Platts-Mills, 1982; Haber, Kubagawa & Cooper, 1983; Mayer *et al.*, 1984; Perri & Weisdorf, 1985). In addition to the reduced levels of the three major immunoglobulin isotypes, patients with CVI generally have reduced serum IgE levels. Recently we demonstrated that both recombinant and natural interleukin-4 (IL-4) can induce IgE synthesis by mononuclear cells derived from healthy donors (Pène *et al.*, 1988a). This IgE-inducing effect of IL-4 is completely dependent of autologous CD4⁺ T cells (Pène *et al.*, 1989). In addition, it was shown that the IgE inducing effect of IL-4 was inhibited by interferon-gamma (IFN- γ) and interferon-alpha (IFN- α) (Pène *et al.*, 1988a) and enhanced by interleukin-5 (IL-5) (Pène *et al.*, 1988c) and interleukin-6 (IL-6) (Pène *et al.*, unpublished results). Furthermore, recent murine studies have demonstrated that IL-4 induces B cells pre-activated by lipopolysaccharide (LPS) to switch to IgE-producing cells (Rothman *et al.*, 1988). These observations prompted us to investigate whether B cells of patients with CVI could be induced to switch to IgE-producing cells in response to IL-4.

Here we demonstrate that peripheral blood mononuclear cells (PBMC) of two out of eight patients could be induced to produce IgE. In addition, PBMC of these two patients secreted IL-4 upon activation, whereas PBMC of five out of the six other patients failed to do so.

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These results confirm the heterogeneity of the syndrome and indicate that either B or T cell defects, or a combination of both may contribute to the pathogenesis of CVI.

MATERIALS AND METHODS

Patients

Eight patients (four women and four men, age range 7–21 years) with CVI were studied. At the time of our investigation, two patients, B and C, were not having gammaglobulin replacement therapy, despite the reduced levels of serum IgM, IgG and IgA, because they did not have severe recurrent infections. The other patients received regular i.v. gammaglobulin every 2–3 weeks. Eight age- and sex-matched healthy donors were tested simultaneously as controls.

Cytokines and reagents

Purified rIL-4 (specific activity 10^7 U/ml) was kindly provided by Dr P. Trotta (Schering-Plough Corporation, Bloomfield, NJ). One unit of IL-4 was defined as the concentration of IL-4 inducing half-maximal proliferation of 5×10^5 T cells that were preactivated for 3 days with phytohemagglutinin (PHA). Recombinant IFN- γ (rIFN- γ) (specific activity 10^7 IU/mg) was a gift of Drs P. Trotta and S. Nagabhushan (Schering-Plough Corporation). The monoclonal antibodies (MoAbs) OKT3 (anti-CD3), OKT4 (anti-CD4) and OKT8 (anti-CD8) were purchased from Ortho Pharmaceutical Corporation (Raritan, NJ). The B cell-specific MoAb Leu16 (anti-CD20) was obtained from Becton Dickinson (Mountain View, CA). The polyclonal rabbit anti-IFN- γ antiserum (BEA) was raised and characterized as described previously (Favre *et al.*, 1988). The immunoglobulin fraction was purified by affinity chromatography on protein A columns according to the instructions of the manufacturer (BioRad, Richmond, CA). This antiserum blocks the induction of class I and class II MHC antigen on human melanoma cells by rIFN- γ and neutralizes the inhibitory effects of IFN- γ on the cytopathic effects of encephalomyocarditis virus. The BEA antibody is specific for IFN- γ and does not react with rIL-1 α and β , IL-2, IL-3, IL-4, IL-5, granulocyte-macrophage colony-stimulating factor (CSF), and IFN- α .

The rabbit anti-IL-4 antibody was raised in our laboratory (Chrétien *et al.*, 1989). The immunoglobulin fraction was purified on protein A columns (BioRad). This anti-serum is specific for IL-4. It binds in solution and blocks IL-4 induced T and B cell proliferation and IL-4-induced expression of CD23 on the Burkitt lymphoma cell line Jijoye. In addition, the anti-IL-4 antiserum does not react with rIL-1 α and rIL-1 β , IL-5, low mol. wt B cell growth factor, IFN- γ , or granulocyte-macrophage CSF.

IL-4-induced IgE synthesis

Recently we demonstrated that a two-step culture procedure is optimal of maximal IL-4-induced IgE production (Chrétien *et al.*, 1990). Briefly, PBMC were isolated from heparinized blood by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density-gradient centrifugation. After three washings in phosphate-buffered saline (PBS) (Eurobio, Paris, France), the cells were resuspended in Yssel's medium (Yssel *et al.*, 1984), and cultured at a concentration of 10^6 cells/ml in 24 flat-bottomed wells (Becton Dickinson) in the presence or absence of saturating concentrations of rIL-4 (200 U/ml) for 48 h at 37 °C in

humidified atmosphere with 5% CO $_2$. After this pre-incubation step, the non-adherent cells were collected and washed twice in PBS and cultured in the presence of various concentrations of rIL-4 (100, 500 or 2000 U/ml) with or without anti-IL-4 antiserum (final dilution 1:500), anti-IFN- γ antiserum (final dilution 1:250) or rIFN- γ (final concentration 100 U/ml). The appropriate pre-immune rabbit sera were used as controls. The cultures were carried out in 1 ml culture medium at concentrations of 10^6 PBMC/ml in 2 ml tissue culture wells (Becton Dickinson) for 9 days at 37 °C and 5% CO $_2$. The supernatants of these cultures were then harvested and tested for their IgE, IgM, IgG and IgA content.

ELISAs for IgE, IgM, IgG and IgA

IgE production was measured in a sandwich ELISA as described previously (Pène *et al.*, 1988b). Briefly, 96-well flat-bottomed microtitre plates (Nunc, Roskilde, Denmark) were coated with rabbit anti-IgE immunoglobulin (Dako, Copenhagen, Denmark) diluted 1/2000 in a hydrogen carbonate buffer, pH 9.6. After incubation for 18 h at 4 °C the plates were washed four times with PBS/0.05% Tween (Merck, Darmstadt, FRG) and incubated with RPMI 1640/10% FCS to saturate protein-binding sites for 1 h at 20 °C. The plates were emptied and a murine anti-IgE MoAb (Hybritech, San Diego, CA) was added at a final dilution of 1/5000, incubated for 18 h at 20 °C and finally incubated for 4 h at 20 °C with goat anti-mouse immunoglobulin coupled to alkaline phosphatase, dilution 1/100 (Sigma, St Louis, MO), pre-adsorbed on normal rabbit serum. After washing, IgE was visualized by incubation with *p*-nitrophenyl phosphate (Sigma) in 1 M diethanolamine HCl buffer, pH 9.8, and the plates were read in a micro-ELISA autoreader, MR 580 (Dynatech, Alexandria, VA), at 405 nm. A standard IgE serum (Behringwerke, Marburg, FRG) was used as a reference. The limit of the sensitivity of the assay was 150 pg of IgE/ml.

ELISAs for IgM, IgG, IgA were carried out as described (Siegal & Good, 1977). The sensitivity of these ELISAs was 2 ng/ml (IgM), 1 ng/ml (IgG) and 4 ng/ml (IgA).

Lymphokine production

PBMC were resuspended in culture medium at a concentration of 10^6 cells/ml. The cells were incubated at 37 °C in humidified atmosphere with 5% CO $_2$ in 24-well plates (Becton Dickinson) in the presence of (PHA) (Difco, Detroit, MI) at a final concentration of 10 or 100 μ g/ml in combination with the phorbol ester TPA (1 ng/ml) (Sigma).

The supernatants of the cultures were harvested after 48 h of incubation and stored at -20 °C until testing for lymphokine activity. Incubation times of 48 h were found to result in optimal lymphokine production (not shown).

Determination of IL-2, IL-4 and IFN- γ

The IL-2 activity of the supernatant was assessed by its ability to support the proliferation of an IL-2-dependent murine cytotoxic T cell line (CTLL). A 100- μ l sample containing 10^4 CTLL cells was mixed with 100 μ l serial dilution of the test samples in 96-well microplates. Cultures were performed in triplicate. After 24 h, 1 μ Ci of ^{3} H-thymidine (^{3} H-TdR) was added to each well and cells were further incubated for 16 h. Cultures were harvested and the incorporated radioactivity was determined by a liquid scintillation counter. Every assay was compared with a

Table 1. Characteristics of patients with common variable immunodeficiency (CVI)

Patient	Sex	Age (years)	Lymphocyte count (mm ³)	Serum immunoglobulin levels*				Positive cells with MoAbs (%)			
				IgM (mg/dl)	IgG (mg/dl)	IgA (mg/dl)	IgE (gU/l)	CD3	CD4	CD8	CD20
A	M	15	4500	94	223	96	0.8	80	52	24	6
B	M	7	3200	38	276	18	41.7	68	42	19	7
C	F	17	4200	28	286	17	0.2	83	45	13	3
D	F	7	3700	26	20	5	0.5	52	43	8	12
E	M	10	3250	27	182	2.6	4.3	73	52	33	3.5
F	M	8	3500	177	24	7.4	0.8	63	40	12	6
G	F	21	1200	11	206	8	0.2	60	52	12	3.5
H	F	15	1500	36	5.2	<1	0.02	77	12	49	2
Normal values†			1200–3500	40–350	300–1350	30–350	14–120	50–75	35–45	15–25	5–13

* Diagnostic values at onset of the disease

† The lower limit of the normal values represent normal values for the age at which diagnosis was made.

laboratory standard IL-2 supernatant (100 IU/ml) which was calibrated against the Biological Response Modifiers Program (BRMP) reference reagent human IL-2 (Jurkat) (National Cancer Institute, Frederick, MD). To determine IL-2 units, the logarithm of ct/min of ³H-TdR uptake was plotted against the logarithm of the dilution of the sample. The IL-2 titre was calculated by the reciprocal of the test samples that gave 50% of the maximal ct/min of our laboratory standard.

IL-4 was quantified by an immunoenzymatic assay performed as follows: polyvinylchloride microtitre plates (Dynatech, Alexandria, VA) were coated with 10 µg/ml of purified immunoglobulin fraction of rabbit anti-IL-4 that was raised against *Escherichia coli*-derived recombinant IL-4. The plates were then washed by an automatic washer (Titertek, Flow Laboratories, Irvine, UK) with PBS supplemented with 0.05% Tween 20. Next, various dilutions of the culture supernatants in RPMI + 10% FCS were added to the coated wells and incubated for 90 min at room temperature. The samples were tested in duplicate. After washings, 0.1 µg/ml purified rat anti-IL-4 MoAb 11B4 (Chrétien *et al.* 1989) was added in 50 µl RPMI + 10% FCS and incubated for 90 min. Subsequently, the wells were washed and incubated with 1/2000 dilution of goat anti-rat antibody coupled to peroxidase (Tago, Burlingame, CA). Enzyme activity was determined with the substrate 2,2-aminobis-3-ethyl-benzthiazolin sulphonic acid (ABTS; Sigma) at a concentration of 1 mg/ml in a citric acid buffer and 0.1 µl/ml H₂O₂. The plates were read with an ELISA reader. Calibration curves were made with serial dilutions of highly purified *E. coli*-derived IL-4. Sensitivity of the assay was 25–50 pg/ml (Chrétien *et al.*, 1989).

IFN-γ present in the supernatant of the cultures was determined with an immunoenzymatic assay carried out as described previously (Favre *et al.*, 1989). Briefly, flat-bottomed wells of Nunc microtitre were coated with 10 µg/ml of the anti-IFN-γ MoAb A35 in a volume of 200 µl for 16 h at 16 °C. Next, the wells were incubated with 300 µl PBS supplemented with 5% BSA (Sigma) and 0.05% Tween 20 (Merck) for 1 h at room temperature. After washings, dilutions of culture supernatants were added in PBS supplemented with 1% BSA and 0.05% Tween 20 and incubated for 90 min. The samples were tested in

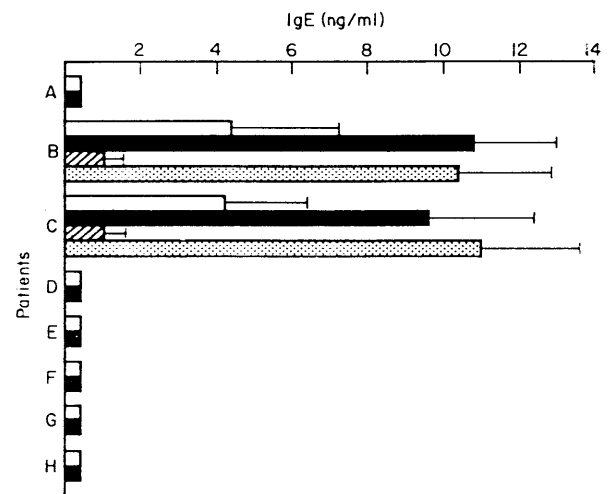


Fig. 1. Induction of IgE synthesis by PBMC of patients with common variable immunodeficiency by IL-4. IgE production is expressed as mean $\pm$ s.d. of duplicate cultures. Spontaneous IgE synthesis in the absence of IL-4 was negative (< 150 pg IgE/ml). □, IL-4 (100 U/ml); ■, IL-4 (500 U/ml); ▨, IL-4 (500 U/ml) + anti-IL-4 (1/500); ▤, IL-4 (500 U/ml) + control rabbit serum (1/500).

duplicate. Serial dilutions of purified IFN-γ (Schering-Plough Research) were incubated to establish a calibration curve. After washings, the samples were incubated for 90 min with the anti-IFN-γ MoAb B27 that was labelled with biotin. After an additional incubation for 1 h with alkaline phosphatase coupled to avidin (Tago) diluted 1/500 in PBS, BSA, Tween 20, followed by washing, positive samples were revealed by the adding of the substrate *p*-nitrophenyl phosphate (Sigma). Sensitivity of the assay was 300 pg IFN-γ/ml (Favre *et al.*, 1989).

Cell surface marker analysis

PBMC (10^6) were resuspended in PBS, in a volume of 100 µl, and incubated for 30 min at 4 °C with 5 µl of anti-CD3, anti-CD4, anti-CD8 or anti-CD20 MoAbs. After washing the cells twice in PBS, the cell pellet was resuspended in 50 µl of goat antibody to

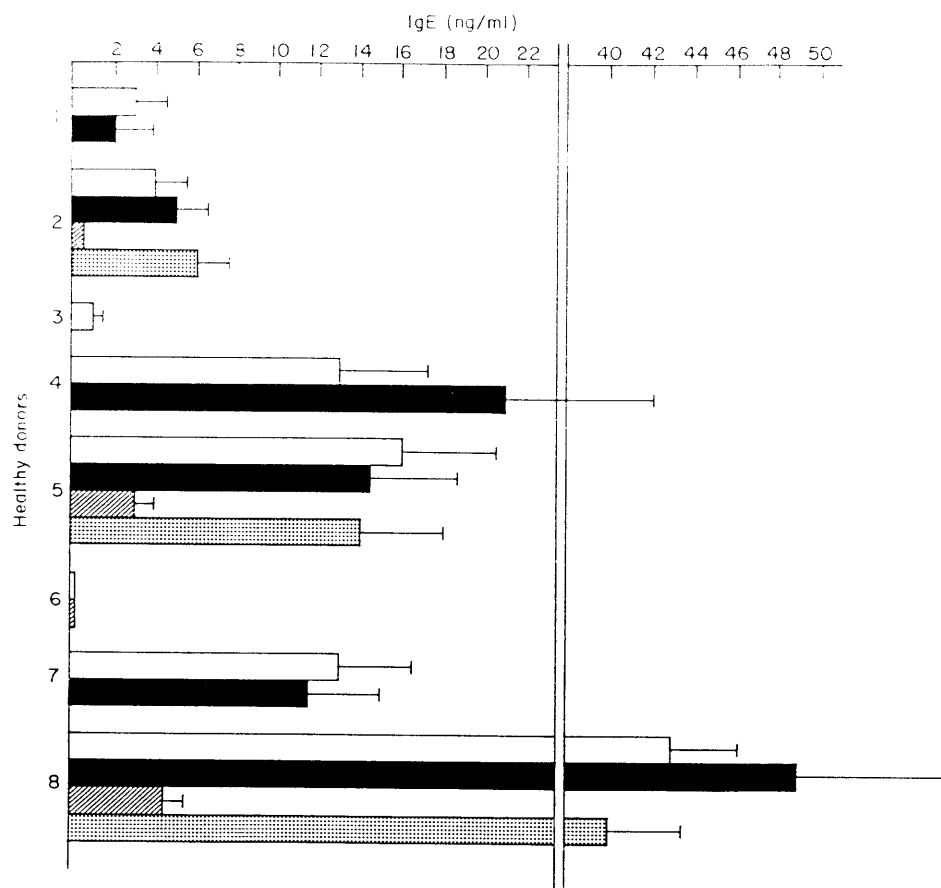


Fig. 2. Induction of IgE synthesis by PBMC of healthy donors by IL-4. PBMC of donor 3 were not tested in the presence of IL-4 (500 U/ml). Inhibition studies with anti-IL-4 antiserum were only carried out with PBMC of donors, 2, 5 and 8. IgE production is expressed as mean $\pm$ s.d. of duplicate cultures. $\square$, IL-4 (100 U/ml); $\blacksquare$, IL-4 (500 U/ml); $\square$ (hatched), IL-4 (500 U/ml) + anti-IL-4 (1/500); $\square$ (dotted), IL-4 (500 U/ml) + rabbit serum (1/500).

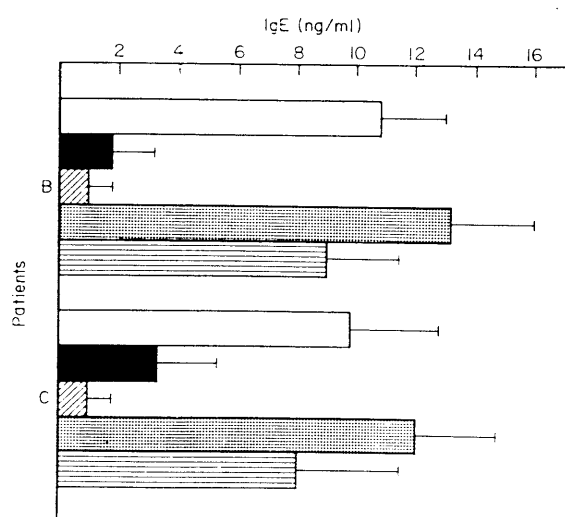


Fig. 3. The effect of IFN- γ and anti-IFN- γ antibody on IgE synthesis by PBMC of CVI patients. IgE production is expressed as mean $\pm$ s.d. of duplicate cultures. $\square$, IL-4 (500 U/ml); $\blacksquare$, IL-4 (500 U/ml) + IFN- γ (100 U/ml); $\square$ (hatched), IL-4 (500 U/ml) + anti-IL-4 (1/500); $\square$ (dotted), IL-4 (500 U/ml) + anti-IFN- γ (1/250); $\square$, IL-4 (500 U/ml) + IFN- γ (100 U/ml) + anti-IFN- γ (1/250).

mouse IgG and IgM labelled with fluorescein (Bionetics, Labtek, Milan, Italy) and 100 μ l of PBS. After incubation for 30 min at 4 $^{\circ}$ C, the cells were washed twice, resuspended in PBS and analysed with the FACScan (Becton Dickinson).

RESULTS

Cell counts, serum immunoglobulin values, and phenotyping of PBMC of CVI patients

Immunoglobulin serum value, lymphocyte counts and phenotyping of PBMC of the patients are shown in Table 1. Lymphocyte counts of the eight patients were in the normal range. All patients presented low or non-detectable serum levels of the IgM, IgG and IgA isotypes with the exception of patient A, who had normal serum IgM and IgA levels, and patient F whose serum IgM concentrations were in the normal range. Serum IgE levels were normal in patient B, patient E had very reduced serum IgE levels, whereas the other patients had serum IgE levels that were below the detection level. Normal percentages of CD3 $^{+}$, CD4 $^{+}$ and CD8 $^{+}$ T cells were found, with the exception of patient D who had decreased numbers of CD8 $^{+}$ T cells, and patient H who had reduced numbers of CD4 $^{+}$ T cells.

Normal percentages of CD20 $^{+}$ B cells were observed in four patients (A, B, D, F) whereas the B cell numbers were reduced in the other four patients (C, E, G, H).

Table 2. Production of IL-4, IL-2 and IFN- γ by peripheral blood mononuclear cells of patients with common variable immunodeficiency and healthy controls

Patient	Activation	IL-4 (pg/ml)	IL-2 (IU/ml)	IFN- γ (ng/ml)	Healthy donor	IL-4 (pg/ml)	IL-2 (IU/ml)	IFN- γ (ng/ml)
A	Medium	≤ 25	< 0.1	< 0.3	1	≤ 25	< 0.1	< 0.3
	PHA + TPA*	39 ± 3	56	1.2 ± 0		426 ± 2	56	3.6 ± 0.1
	PHA + TPA†	30 ± 2	20	3.6 ± 0		584 ± 8	95	3.3 ± 0.1
B	Medium	≤ 25	< 0.1	< 0.3	2	≤ 25	< 0.1	< 0.3
	PHA + TPA*	NT	20	2.3 ± 0.1		285 ± 4	80	6.5 ± 1
	PHA + TPA†	300 ± 8	8.7	3.1 ± 0.1		370 ± 8	72	8.1 ± 0.2
C	Medium	≤ 25	< 0.1	< 0.3	3	≤ 25	< 0.1	< 0.3
	PHA + TPA*	45 ± 0	120	3.5 ± 0.1		97 ± 3	58	5.4 ± 0.2
	PHA + TPA†	54 ± 0	85	2.1 ± 0.1		493 ± 0	72	4.7 ± 0.2
D	Medium	≤ 25	< 0.1	< 0.3	4	≤ 25	< 0.1	< 0.3
	PHA + TPA*	≤ 25	120	1.6 ± 0		120 ± 4	56	1.5 ± 0.1
	PHA + TPA†	≤ 25	90	4.3 ± 0		290 ± 8	87	7.4 ± 0.3
E	Medium	≤ 25	< 0.1	< 0.3	5	≤ 25	< 0.1	< 0.3
	PHA + TPA*	NT	NT	NT		NT	NT	NT
	PHA + TPA†	≤ 25	26	3.0 ± 0.1		560 ± 4	72	4.1 ± 0.1
F	Medium	≤ 25	< 0.1	< 0.3	6	≤ 25	< 0.1	< 0.3
	PHA + TPA*	NT	NT	NT		NT	NT	NT
	PHA + TPA†	≤ 25	4	2.0 ± 0.1		140 ± 4	85	11.1 ± 0.4
G	Medium	≤ 25	< 0.1	< 0.3	7	≤ 25	< 0.1	< 0.3
	PHA + TPA*	≤ 25	20	6.0 ± 0.4		508 ± 9	70	8.3 ± 0.5
	PHA + TPA†	≤ 25	26	11.3 ± 0.2		NT	91	14.3 ± 0.2
H	Medium	≤ 25	< 0.1	< 0.3	8	≤ 25	< 0.1	< 0.3
	PHA + TPA*	292 ± 11	10.7	9.7 ± 0		360 ± 8	> 300	6.6 ± 0.3
	PHA + TPA†	218 ± 4	5	10.4 ± 0.1		136 ± 7	24.6	5.7 ± 0.2

* PHA 10 μ g/ml + TPA 1 ng/ml† PHA 100 μ g/ml + TPA 1 ng/ml

NT, not tested.

Induction of IgE synthesis by PBMC of CVI patients by IL-4

We demonstrated previously that IL-4 can induce IgE synthesis by mononuclear cells isolated from peripheral blood, tonsils or spleen of healthy non-allergic, non-atopic individuals (Pène *et al.*, 1988a, 1988b, 1988c, 1989). IL-4 concentrations of 200 U/ml were found to be optimal to induce maximal IgE synthesis by PBMC of healthy donors. In order to investigate whether the PBMC of CVI patients could also be induced to synthesize IgE *in vitro* in the presence of IL-4, the mononuclear cells of eight CVI patients were incubated with varied concentrations of IL-4. In Fig. 1 it is shown that IL-4 induced IgE synthesis in two patients (B and C), whereas PBMC of 6 other patients (A, D, E, F, G, H) failed to synthesize IgE on multiple occasions tested. Maximal IgE synthesis (approximately 10 ng/ml) was obtained at IL-4 concentrations on 500 U/ml, whereas IL-4 at 100 U/ml was suboptimal in these two patients (Fig. 1). IgE levels induced by IL-4 at 2000 U/ml were similar to those induced by IL-4 at 500 U/ml (not shown). The IgE synthesis is specifically induced by IL-4, since the anti-IL-4 antiserum completely blocked IgE synthesis (Fig. 1). Spontaneous IgM, IgG or IgA synthesis by PBMC of the patients was extremely low or absent and could not be up-regulated by IL-4 (not shown).

PBMC of seven out of eight healthy controls tested in parallel with CVI patients produced variable levels (1–49 ng/ml) of IgE in response to IL-4 (Fig. 2). With the exception of donor 4, no significant differences were observed between the levels of IgE induced by 100 or 500 U/ml of IL-4. In the cases tested

(donors 2, 5 and 8), IL-4-induced IgE synthesis was strongly blocked by anti-IL-4 antiserum. These results are similar to those described previously with PBMC of other series of healthy individuals (Pène *et al.*, 1988a; Chrétien *et al.*, 1989, 1990). In addition, spontaneous IgM, IgG and IgA production by PBMC of the healthy donors was low and was not significantly affected by IL-4 (not shown).

IL-4-induced IgE synthesis by PBMC of CVI patients is blocked by IFN- γ

We demonstrated that IL-4 induced IgE synthesis by PBMC of healthy donors was strongly inhibited by IFN- γ or IFN- α , indicating that these IFNs act as antagonists of IL-4 (Pène *et al.*, 1988a, 1988b). In order to establish whether IFN- γ had similar inhibitory effects on IL-4-induced IgE synthesis by PBMC of patients with CVI, blocking studies with IFN- γ were carried out with PBMC of patients B and C. Figure 3 shows that IFN- γ at 100 U/ml significantly reduced IgE synthesis induced by IL-4 (500 U/ml). This inhibitory effect is neutralized by an anti-IFN- γ antiserum, illustrating the specificity of the blocking effect. In the presence of this anti-IFN- γ antiserum, IL-4-induced IgE synthesis by PBMC of patients B and C was only slightly enhanced, indicating that the levels of endogenous IFN- γ production by T cells present in the culture are insufficient to diminish IL-4-induced IgE synthesis significantly.

Furthermore, we excluded the possibility that the failure of the PBMC of the six other CVI patients to produce IgE in

response to IL-4 alone was due to high levels of endogenous IFN- γ production by T cells in the culture, since cultures carried out in the presence of the anti-IFN- γ antiserum did not result in IL-4 induced IgE synthesis (not shown).

IL-2, IL-4 and IFN- γ production by PBMC of patients with CVI

The results obtained so far suggest that two out of the six patients have B cells, or B cell precursors that can produce IgE in response to IL-4 *in vitro*. However, since IL-4 has been shown to be the sole inducer of IgE synthesis (Pène *et al.*, 1988b), defective production of IL-4 by T cells may account for the low levels of IgE observed in some patients *in vivo*. Therefore, we investigated the capacity of T cells of our patients to produce IL-2, IL-4 and IFN- γ . Table 2 shows that the levels of IL-4 produced by PBMC of patients B and H after activation by combinations of PHA (10 μ g/ml) + TPA (1 ng/ml), or PHA (100 μ g/ml) + TPA (1 ng/ml) were in the normal range. PBMC of patient C produced low but significant levels of IL-4, which were, however, reduced compared with IL-4 production by PBMC of healthy donors tested in parallel. PBMC of patient A produced IL-4 (30–40 pg/ml), but these levels have to be considered as borderline because of the sensitivity of our ELISA (25–50 pg/ml). PBMC of all eight healthy donors tested in parallel produced significant levels of IL-4 which varied between 120 and 584 pg/ml (Table 2).

Although the series of patients with CVI tested here is relatively small, it seems that in addition to defective IL-4 production by PBMC, there is also impaired production of IL-2 and IFN- γ .

DISCUSSION

The reduced levels of immunoglobulin produced in CVI patients have been attributed to defects in regulatory T cells, and/or B cells or defective monocyte functions (Waldman *et al.*, 1974; Siegal *et al.*, 1976; Schwaber *et al.*, 1978; Mitsuya *et al.*, 1981; Reinherz *et al.*, 1981a, 1981b; Lopez-Botet *et al.*, 1982; Saiki *et al.*, 1982, 1984; Jones, 1984; Perri & Weisdorf, 1985; Matheson & Green, 1987). Here we demonstrate that PBMC of two out of eight patients with CVI (patients B and C) could be induced to produce IgE *in vitro* by IL-4, showing that their B cells, like B cells of the majority of healthy donors, can be induced to switch to IgE producing cells upon interaction with IL-4.

Although IL-4 is the sole inducer of IgE synthesis (Pène *et al.*, 1988a, 1988b) the capacity of IL-4 to induce IgE synthesis by B cells of healthy individuals is completely dependent on CD4⁺ T cells (Pène *et al.*, 1989), enhanced by monocytes (Pène *et al.*, 1988a, 1989) and suppressed by autologous CD8⁺ T cells (Pène *et al.*, 1989, manuscript in preparation) indicating the multiple cells or factors are involved in a cascade of events finally resulting in IgE synthesis.

Of the two patients (B and C) whose PBMC could be induced to synthesize IgE *in vitro*, patient B had very low levels of serum IgM, IgG and IgA, but his serum IgE levels were in the normal range. Our *in vitro* data reflected exactly the *in vivo* situation, since the PBMC of this patient were 'normal' in their capacity to produce IgE in response to IL-4. Furthermore, we demonstrated that the T cells of patient B produced quantities of IL-4 *in vitro* that were in the normal range. These results indicate that the regulatory circuits finally resulting in IgE synthesis *in vivo* and *in vitro* are intact in this patient, whereas regulation of

IgG, IgA and IgM production, as measured *in vivo*, is defective. PBMC of patient C, which also produced IgE in response to recombinant IL-4 *in vitro* synthesized minimal amounts of IL-4 *in vitro* and failed to produce significant IgE levels *in vivo*. This shows that in this patient the cells required for the cascade of events finally resulting in IL-4-induced IgE synthesis are functioning normally *in vitro*, and suggests that the very low levels of IgE *in vivo* could be due to the reduced capacity to produce IL-4 upon T cell activation. However, it may be possible that other, yet to be defined mechanisms are responsible for the very reduced serum IgE levels in this patient.

PBMC of the six other patients failed to produce IgE *in vivo* and *in vitro* despite normal, or slightly reduced numbers of B cells present in their peripheral blood. We excluded that the lack of *in vitro* IgE synthesis was due to excessive endogenous IFN- γ production by T cells present in the cultures, since in the presence of anti-IFN- γ antibody, IL-4 was unable to induce IgE synthesis (not shown). This indicates that the B cells of these six patients may be intrinsically defective.

Apart from possible defects in B cells, it is clear that patients with CVI also have defects in regulatory T cells. PBMC of five out of six patients failed to produce IL-4 upon activation. PBMC of these five patients also failed to secrete IgE in response to exogenous IL-4 *in vitro*. The only exception was patient H, whose PBMC failed to produce IgE despite normal levels of IL-4 secretion.

Collectively, our data indicate that failure to produce normal IgE levels *in vivo* may be attributed to both defective IL-4 production by T cells of these five patients and to failure of the B cells to respond to IL-4 appropriately, whereas the absence of IgE synthesis *in vitro* in the sixth patient seems to be related to a B cell defect. On the other hand, it is possible that the lack of IgE synthesis is due to other defects in helper functions of the CD4⁺ T cells, increased suppressive effects of the CD8⁺ T cells or defective monocyte functions. The lack of production of detectable levels of IL-4 confirms our previous observations which demonstrated that the capacity to secrete IL-4 by PBMC of a group of 11 CVI patients was significantly reduced as compared with that of PBMC of healthy donors (Pastorelli *et al.*, 1989). In addition, in that study overall IL-2 and IFN- γ production were also found to be reduced, compared with those obtained by PBMC of an age- and sex-matched group of control donors (Pastorelli *et al.*, 1989); this is confirmed in the present study.

Furthermore, it is clear that although the precise cascade of events leading to IgE production induced by IL-4 *in vivo* still remains to be determined, the frequency of successful IgE production *in vitro* by PBMC of patients with CVI is much lower than that by PBMC of healthy donors (2/8 *versus* 7/8). It remains to be clarified why PBMC of healthy donor 6 failed to produce IgE, but recent studies in our laboratory have indicated the PBMC of a small proportion of healthy donors do not produce IgE under the present culture conditions (Chrétien *et al.*, 1990).

It has been shown that B cells of patients with CVI behave functionally like immature cord blood B cells (de Gast *et al.*, 1980; Tosato *et al.*, 1980; Platts-Mills *et al.*, 1981; Perreira *et al.*, 1982; Haber *et al.*, 1983; Brenner *et al.*, 1984; Mayer *et al.*, 1984). Stimulation of B cells of patients with CVI by EBV, PWM or supernatants of activated T cells results, as with cord blood B cells, predominantly in secretion of IgM and no or very low

levels of IgA and IgG. However, we have found that cord blood cells from 8 donors produced IgE in the presence of IL-4, indicating that cord blood B cells and B cells of CVI patients differ in their capacity to produce IgE in response to IL-4 (Pastorelli *et al.*, unpublished). Therefore, B cells from patients with CVI cannot be compared functionally with cord blood B cells.

The study of the mechanisms underlying defective IgE synthesis by PBMC of certain patients with immunodeficiencies may contribute to our understanding of the various steps in the cascade of events leading to IgE production in both normal and allergic individuals.

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