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Peripheral blood lymphocytes of patients with common variable immunodeficiency (CVI) produce reduced levels of interleukin-4, interleukin-2 and interferon-gamma, but proliferate normally upon activation by mitogens

G. PASTORELLI, M. G. RONCAROLO, J. L. TOURAINE*, G. PERONNE, P. A. TOVO† & J. E. DE VRIES

*UNICET, Laboratory for Immunological Research, Dardilly; and *INSERM U-80, Hôpital Ed. Herriot, Lyon, France; and †Department of Paediatrics, University of Turin, Turin, Italy*

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SUMMARY

Peripheral blood lymphocytes (PBL) of 11 patients with CVI produced reduced levels of interleukin-4 (IL-4) upon activation by mitogens as compared with those secreted by PBL of healthy donors. The interleukin-2 (IL-2) and interferon-gamma (IFN-γ) production by PBL of a series of 15 patients with CVI was also reduced. Decreased levels of IL-4 or IL-2 and IFN-γ production were not only observed after activation by phytohaemagglutinin (PHA) at concentrations of 10 and 1 μg/ml, but also after activation by concanavalin A (Con A, 10 μg/ml). Longitudinal studies indicated that this defective lymphokine production was consistent upon testing periods up to 5 months. No correlation between reduced IL-4, IL-2 or IFN-γ production was observed. PBL of patients that produced reduced levels of one lymphokine generally secreted normal levels of the other two lymphokines. Despite the reduced synthesis of the T cell growth factors IL-2 and IL-4, the proliferative responses of the PBL of the patients were in the normal range, which is compatible with the finding that IL-2 and IL-4 have synergistic effects on lymphocyte proliferation, particularly when one of these lymphokines is present at suboptimal concentrations. Since IL-2, IL-4 and IFN-γ can act as B cell growth and differentiation factors, our data suggest that the reduced synthesis of these lymphokines may contribute to the deficient immunoglobulin production in patients with CVI.

Keywords interleukin-2 interleukin-4 interferon-gamma production T cell proliferation common variable immunodeficiency

INTRODUCTION

CVI represents a heterogeneous group of diseases characterized by B cell dysfunction resulting in reduced levels of serum immunoglobulins (Rosen, Cooper & Wedgwood, 1984). In addition, approximately 50% of the patients have defective T cell function, including reduced responsiveness to mitogenic lectins (Cunningham-Rundles *et al.*, 1981; Lopez-Botet *et al.*, 1982; Kruger *et al.*, 1984), reduced production of interleukin-2 (IL-2) (Lopez-Botet *et al.*, 1982; Kruger *et al.*, 1984; Saiki *et al.*, 1984) and interferon-gamma (IFN-γ) (Virelizier *et al.*, 1979), defective synthesis of B cell growth and/or differentiation factors (Reinherz *et al.*, 1981; Mayer *et al.*, 1984; Perri & Weisdorf, 1985; Callard *et al.*, 1986; Matheson & Green, 1987). Antibody production normally requires the interaction of B cells, T cells, antigen-presenting cells and growth/differentiation factors synthesized by these cells (Kishimoto, 1987). Therefore, defective antibody production in CVI patients may be related to defective T cell help and soluble factors produced by these cells. However, defective immunoglobulin synthesis may be due to intrinsic B cell abnormalities, like the failure to respond appropriately to B cell growth and differentiation factors.

IL-2, interleukin-4 (IL-4) and IFN-γ produced by human T cells have been shown to act as B cell growth and differentiation factors (Sidman *et al.*, 1984; Kehrl *et al.*, 1985; Nakagawa *et al.*, 1986; Miyawaki *et al.*, 1987; Bich-Thuy & Fauci, 1986; Defrance *et al.*, 1986, 1987). In order to investigate whether deficient immunoglobulin production in CVI patients was related to defective T cell functions, proliferative responses of peripheral blood lymphocytes (PBL) to mitogenic lectins, IL-2, IFN-γ and IL-4 production by these cells were measured in a series of 15 CVI patients.

We demonstrated here that IL-2, IFN-γ and IL-4 production by PBL of CVI patients activated by phytohaemagglutinin (PHA) or concanavalin A (Con A) was significantly lower than

Correspondence: Dr J. E. de Vries, DNAX Research Institute, 901 Caliornia Avenue, Palo Alto, CA 94304-1104, USA.

Table 1. Characteristics of 15 patients with CVI

Patient	Sex	Age (years)	Lymphocyte count (mm^3)	Serum immunoglobulin levels (g/l)*			Positive cells (%) with MoAbs				
				IgM	IgG	IgA	CD3	CD4	CD8	CD20	slg
A	F	58	565	0·02	7·0	0·08	53	20	24	6	9
B	F	42	1575	0·03	7·2	0·06	74	24	26	5	7
C	M	7	3450	0·14	4·0	0·02	63	40	12	NT	7
D	F	36	2530	0·23	6·2	0·24	85	70	19	3	NT
E	M	40	1890	0·36	4·2	0·23	76	22	41	3	NT
F	M	21	2035	0·31	4·3	0·21	80	43	35	2	2
G	M	56	2550	0·10	6·1	0·29	79	30	34	10	10
H	M	26	2300	0·18	5·3	0·12	67	25	31	4	NT
I	M	6	2500	0·37	5·0	0·40	70	56	27	7	6
J	F	24	2700	0·13	3·6	0·08	80	58	24	12	9
K	M	17	1035	0·10	3·7	0·06	70	48	25	3	NT
L	M	6	2300	0·25	4·0	0·30	73	48	22	1	NT
M	F	14	2050	0·26	0·3	0·17	87	65	23	3	NT
N	F	54	1600	0·19	3·2	0·70	58	46	12	NT	9
O	M	19	2235	0·03	1·6	0·02	70	55	13	2	4
Normal range			1200–3000	0·6–1·5	7–15	1·2–2·5	50–75	35–45	15–25	5–12	6–14

* Determined after the periodic i.v. immunoglobulin injection, as described in Materials and Methods.
NT, not tested.

that obtained by PBL of healthy donors tested in parallel. However, no correlation was found between reduced production of IL-2, IFN-γ or IL-4 by PBL of the patients with CVI. Despite the overall reduced IL-2 and IL-4 production, proliferation of patients PBL in response to PHA, Con A and pokeweed mitogen (PWM) was in the normal range.

MATERIALS AND METHODS

Patients

Fifteen patients with primary immunodeficiency (six women and nine men, age range 6–58 years) were studied. The cases included 14 patients with CVI and one child with hypogammaglobulinaemia associated with an isolated ACTH defect unresponsive to ovine synthetic corticotropin-releasing factor (patient M). At the time of our investigation, the patients were free of acute infections. All but one (patient M), of the patients were being treated with immunoglobulins. Generally, the blood samples were taken 2–3 weeks after the patients received immunoglobulins, with the exception of patients A, B, D and G whose PBL were tested 1 week after immunoglobulin therapy. All patients except patient A had normal lymphocyte counts and normal percentages of $CD3^+$ T cells (Table 1). $CD4^+$:$CD8^+$ ratios were normal, with the exception of patients A, B, G and H, who had 1:1 ratios and patient E who had an inverse $CD4^+$:$CD8^+$ ratio (1:2). Normal percentages of B cells were observed in seven patients, whereas B cell numbers, as defined by the anti-CD20 monoclonal antibody (MoAb) and by anti-serum against surface immunoglobulin, were reduced in eight other patients. Fifteen sex- and age-matched healthy donors were tested simultaneously as controls.

Lymphokine production

PBL were isolated from heparinized venous blood by centrifugation over Ficoll/Hypaque (Pharmacia, Uppsala, Sweden) density-gradient centrifugation and subsequently washed three times in phosphate-buffered saline (PBS). PBL were resuspended in culture medium consisting of RPMI 1640 (GIBCO, Paisley, Scotland) supplemented with 10% heat-inactivated fetal calf serum (FCS; GIBCO), L-glutamine (2 mM), penicillin (100 U/ml) and streptomycin (100 μg/ml) at a concentration of 10^6 cells/ml. The cells were incubated at 37°C in humidified atmosphere with 5% CO_2 in 5-ml tubes (Falcon, Oxnard, CA) in the presence of either PHA (Wellcome Laboratories, Beckenham, UK) at a final concentration of 10 μg/ml or 1 μg/ml respectively, or Con A (Pharmacia), at a final concentration of 10 μg/ml. 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, IFN-γ and IL-4

The IL-2 activity of the supernatants was determined by its ability to support the proliferation of the IL-2-dependent murine T cell line (CTLL). A 100-μl sample containing 4×10^5 CTLL cells was mixed with 100 μl serial dilution of the test sample in 96-well microplates (Nunc, Roskilde, Denmark). Cultures were performed in duplicate. After 48 h, 1 μCi ^{3}H-thymidine (=37 kBq/well, New England Nuclear, Dreieich, FRG) was added to each well. After further incubation for 4 h, the cells were harvested on glass fibre strips and the [^{3}H]TdR incorporation was measured by a liquid scintillation counter. The IL-2 activity in the experimental samples, expressed as U/ml, was compared with that of a biological response modifier program standard (BRMP $13{\cdot}1 \times 10^6$ reference units/mg protein) and calculated according to the method of Gillis *et al.* (1978).

IFN-γ present in the supernatant of the cultures was determined with an immunoenzymatic assay, as described

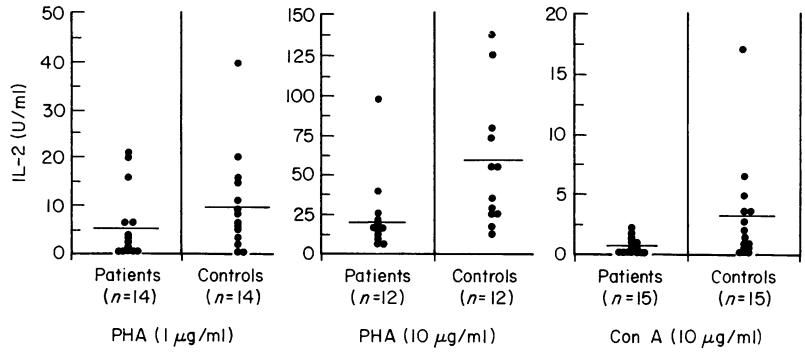

Fig. 1. Activation of the peripheral blood lymphocytes (PBL) of the patients with CVI by phytohaemagglutinin (PHA) (10 μg/ml) and concanavalin A (Con A) (10 μg/ml) resulted in levels of IL-2 production that were significantly reduced compared with those produced by PBL of healthy control donors. PHA, $P<0.02$; Con A, $P<0.01$, as determined by Student's t-test on unpaired samples. No significant differences were observed after activation by PHA 1 μg/ml ($P>0.05$).

previously (Favre *et al.*, 1989). Briefly, flat bottom wells of Nunc microtitre plates were coated with 10 μg/ml of the anti-IFN-γ MoAb A35 in a volume of 200 μl for 16 h at 4°C. Next, the wells were incubated with 300 μl PBS supplemented with 5% bovine serum albumin (BSA; Sigma) and 0·05% Tween 20 (Merck, Darmstadt, FRG) for 1 h at room temperature. After washing, 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 duplicate. Serial dilutions of purified IFN-γ (Schering-Plough Research, Bloomfield, NJ) were included 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, Burlingame, CA) diluted 1:5000 in PBS, BSA, Tween 20, followed by washing, positive samples were revealed by adding *p*-nitrophenyl phosphate (Sigma). Sensitivity of the assay was 300 pg IFN-γ/ml.

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 *E. coli*-derived recombinant IL-4. The plates were then washed by an automatic washer (Titertek, Flow laboratories, Irvine, Scotland) 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). Enzyme activity was determined with the substrate 2,2-aminobis-3-ethylbenzthiazolin sulphonic acid (ABTS, Sigma) at a concentration of 1 mg/ml in a citric acid buffer and 0·1 μl/ml H_2O_2. 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 50 pg IL-4/ml.

Immunofluorescence assay

One-hundred thousand cells were incubated in V-bottomed microtitre plates with the apropriate dilutions of MoAbs for 30 min at 4°C. After two washes in PBS supplemented with 0·02 mM sodium azide and 1% BSA, the cells were incubated for 30 min at 4°C with 40 μl of a 1:40 diluted fluorescein isothiocyanate (FITC) labelled F(ab')2 fragments of a goat anti-mouse antiserum (Bioart, Meudon, France). After three washes, the cells were analysed with a FACS IV (Becton Dickinson, Mountain View, CA).

Lymphocyte proliferation

In order to measure the proliferative responses, 2×10^5 PBL were incubated in the presence of mitogens in round-bottomed microtitre plates (Nunc) in a final volme of 100 μl. As mitogens, we used purified PHA, Con A, both at a final concentration of 10 μg/ml, and PWM (Gibco) at a final dilution of 1:100. After incubation for 72 h at 37°C, in a humidified atmosphere with 5% CO_2, the cultures were pulsed with 1 μCi ^{3}H-thymidine and then harvested 4 h later onto glass filter strips, using a semi-automatic cell harvester. ^{3}H-thymidine incorporation was determined by liquid scintillation counting. The results are presented as mean ct/min in triplicate cultures ± s.d.

RESULTS

IL-2 production by PBL of CVI patients and healthy donors

In order to determine IL-2 production by PBL of patients with CVI and healthy donors, the PBL were activated by PHA at concentrations of 1 and 10 μg/ml and by Con A (10 μg/ml). The overall results shown in Fig. 1 indicate that activation by PHA (10 μg/ml) and Con A (10 μg/ml) resulted in a significantly reduced IL-2 production by PBL of the patients with CVI compared with healthy donors. Activation with PHA (10 μg/ml) resulted in average levels of IL-2 production of 22·3 U/ml ± 7·0 (mean ± s.e.m.) and 56·3 ± 11·4 U/ml by PBL of the patients and healthy donors respectively ($P<0.02$). Activation by Con A resulted in much lower IL-2 levels. Average IL-2 production by PBL of patients with CVI and healthy donors was 0·5 ± 0·2 and 2·7 ± 1·0 U/ml, respectively ($P<0.01$), indicating that Con A under these conditions was a less efficient inducer of IL-2 production than was PHA. Activation of the PBL with suboptimal doses of PHA (1 μg/ml) resulted in considerable levels of IL-2 production by both PBL of patients with CVI and healthy donors, 5·6 ± 2·0 and 10·2 ± 2·7 U/ml, that were not significantly different ($P>0.5$).

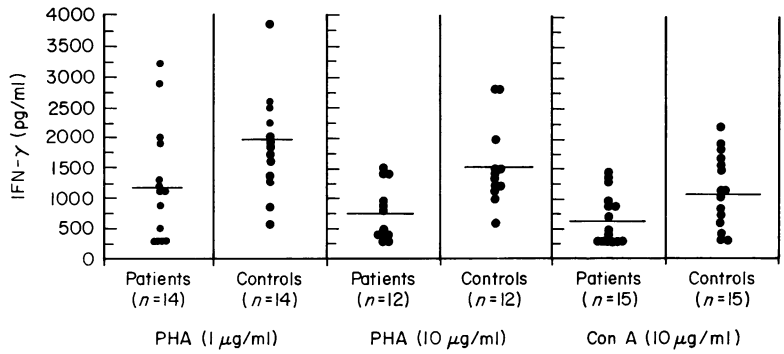

Fig. 2. Activation of the peripheral blood lymphocytes (PBL) of the patients with CVI by phytohaemagglutinin (PHA) 1 μg/ml and 10 μg/ml resulted in levels of interferon-gamma (IFN-γ) production that were significantly reduced compared with those produced by PBL of healthy control donors. PHA 10 μg/ml, $P < 0.002$; PHA 1 g/ml, $P < 0.03$, as determined by Student's *t*-test on unpaired samples. No significant differences were observed after activation of concanavalin A (Con A) ($P > 0.05$).

IFN-γ production by PBL of CVI patients and healthy donors

IFN-γ production by PBL of patients with CVI and healthy donors was measured simultaneously with IL-2 production. Interestingly, activation of PBL by 1 μg/ml of PHA that was suboptimal for IL-2 production (see above) and for induction of proliferation (not shown), induced slightly higher synthesis of IFN-γ than did PHA tested at a concentration of 10 μg/ml (Fig. 2). However, in both situations IFN-γ production by PBL of patients with CVI was significantly lower than IFN-γ levels produced by PBL from healthy donors tested in parallel. The mean IFN-γ production by PBL of the patients was 1259 ± 250 (s.e.m.) pg/ml and 760 ± 127 pg/ml after activation with 1 or 10 μg/ml of PHA, respectively; the amounts of IFN-γ produced by PBL of healthy donors were 2000 ± 245 pg/ml and 1540 ± 192 pg/ml ($P < 0.03$, and $P < 0.002$, respectively).

These differences in IFN-γ production between PBL of patients with CVI and healthy donors were also observed after activation by Con A. The mean IFN-γ levels produced by patients, PBL were 665 + 106 pg/ml compared with 1140 ± 158 pg/ml by PBL of healthy donors.

IL-4 production by PBL of patients with CVI and healthy donors

Recently, we demonstrated that IL-4 acts as a growth factor for activated T cells, natural killer cells and B cells (Spits *et al.*, 1987; Defrance *et al.*, 1987). Therefore it was important to investigate whether the levels of IL-4 production by PBL of CVI patients differed from those produced by PBL of healthy donors. In Fig. 3 we show that the patients tested so far produced varying levels of IL-4 after activation by PHA (10 μg/ml) or Con A (10 μg/ml). Although we were able to test only a limited number of patients and controls, statistical analysis revealed that the average IL-4 production upon activation by PHA (10 μg/ml) of patients' PBL (160 ± 50 pg/ml, mean ± s.e.m.) differed significantly from IL-4 produced by PBL of the healthy donors tested in parallel (370 ± 80 pg/ml) ($P < 0.04$). Also, activation by Con A resulted in reduced IL-4 production by PBL of the patients (181 ± 66 pg/ml), as compared with that observed with PBL of healthy donors (350 ± 110 pg/ml); this difference was not significant ($P > 0.05$).

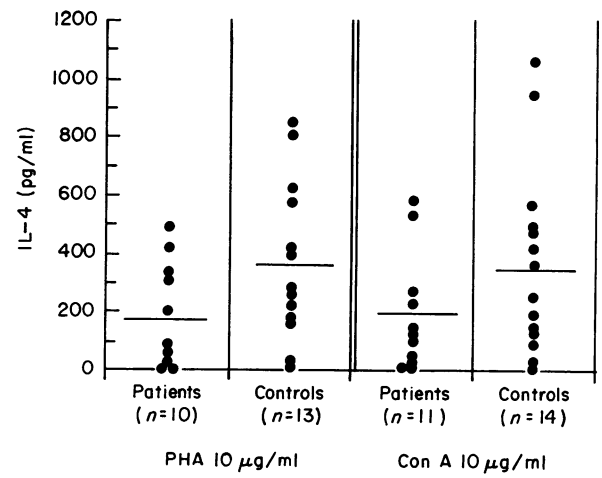

Fig. 3. Activation of the peripheral blood lymphocytes (PBL) of the patients with CVI by phytohaemagglutinin (PHA) 10 μg/ml resulted in levels of IL-4 production that were significantly reduced ($P < 0.04$) compared with those produced by PBL of healthy control donors, as determined by Student's *t*-test on unpaired samples. No significant differences were observed after activation by concanavalin A (Con A) ($P > 0.05$).

Defective lymphokine production is consistent in longitudinal testing

Defective production of lymphokines by PBL of patients with CVI is consistent. Table 2 shows that PBL of patients that could not be induced to produce detectable levels of IL-2 and IL-4 (patient A) or IFN-γ (patients K and M) the first time tested also failed to produce detectable levels of these lymphokines when tested 1–3 months later. In addition, the relatively high levels of IL-2 and IFN-γ production by PBL of patients M and E, respectively, and the marginal and low levels of IL-4 production by PBL of patients M and H were consistently observed in longitudinal testing. Although some degree of variation was observed, the low levels of IL-2 production and the moderate levels of IFN-γ released by PBL of patients E, K and H, and A and H, respectively, remained in a comparable range when tested at intervals of up to 5 months.

Table 2. Lymphokine production by peripheral blood lymphocytes of patients with CVI tested at different occasions

Patient	Day of testing	IL-2 (U/ml)	IFN-γ (pg/ml)	IL-4 (pg/ml)
A	0	0	850	0
	32	0	1100	0
E	0	12	1500	NT
	42	4	2860	NT
	66	7	3500	NT
M	0	98	<300	42
	85	82	<300	60
K	0	13	<300	NT
	54	15	<300	NT
	94	10	<300	NT
H	0	21	850	205
	148	9	1100	120

Production was measured after activation of the lymphocytes with 10 μg/ml phytohaemagglutinin (PHA) as indicated in Materials and Methods. No spontaneous lymphokine production was observed in unstimulated cultures.

IL-2, interleukin-2; IFN-γ, interferon-gamma; IL-4, interleukin-4.

Comparison of simultaneous IL-2, IFN-γ, and IL-4 production by PBL of patients with CVI

In order to investigate whether PBL of patients with CVI that produced reduced levels of IL-2 were also low producers of IFN-γ, IL-2 production was plotted against IFN-γ production. Figure 4 shows that no correlation was found between defective IL-2 and defective IFN-γ production after activation by PHA (10 μg/ml or 1 μg/ml). Also, after stimulation with Con A no correlation between defective IL-2 and IFN-γ production was observed (results not shown). Similar data were obtained when the levels of IL-2 and IL-4 or IL-4 and IFN-γ production were compared (Table 3). After stimulation with PHA (10 μg/ml), no correlation between defective IL-2, IL-4 or IFN-γ-synthesis was observed. These results indicate that low production of each of these lymphokines is probably due to different independent defects.

Comparison of proliferative responses to mitogenic lectins by PBL of patients with CVI and healthy donors

Having demonstrated that PBL of patients with CVI produce lower levels of IL-2 and IL-4 than PBL of healthy donors, we tested whether this reduced IL-2 and IL-4 production affected mitogen induced proliferation of PBL of patients with CVI. Table 4 shows that T cell proliferation in response to PHA, Con A, or PWM, as measured by ^{3}H-thymidine incorporation, was in the normal range and not significantly different from proliferation observed with T cells of healthy donors tested in parallel. These results indicate that reduced endogenous IL-2 or IL-4 production does not affect significantly the overall T cell proliferation in patients with CVI.

DISCUSSION

We have demonstrated here that IL-2, IL-4 and IFN-γ production by PBL of a relatively large series of patients with CVI varies considerably. However, the overall production of these lymphokines after activation of the PBL by both PHA or Con A is significantly reduced compared with that by PBL of healthy donors tested in parallel. Other investigators have demonstrated that the levels of IL-2 production by PBL of patients with CVI can vary; normal IL-2 production by PBL of a CVI patient was reported by Matheson & Green (1987), and others have demonstrated that PBL of patients with CVI had low levels of IL-2 production and reduced proliferative responses to PHA (Cunningham-Rundles *et al.*, 1981; Lopez-Botet *et al.*, 1982; Kruger *et al.*, 1984). Furthermore, Saiki *et al.* (1984) described one case of CVI, whose PBL failed to produce IL-2 after activation by Con A. In our hands, Con A was far less effective than PHA in inducing IL-2 production, despite the finding that no significant differences were observed between the proliferative responses to these mitogens. The variation in IFN-γ production by PBL of our patients with CVI and the overall reduced IFN-γ production were consistent with those observed by Virelizier *et al.* (1979). In contrast, Matricardi *et al.* (1984) showed that in a series of eight patients with CVI, IFN-γ production was generally in the normal range. These differences may be related to the larger series ($n = 15$) of patients with CVI tested here.

No correlation between reduced IL-2 and reduced IFN-γ production was observed. In general, PBL of individual patients that produced low IL-2 levels, showed normal IFN-γ production or *vice versa*, indicating that defective production of each of these lymphokines by PBL of individual patients with CVI may be due to different independent defects. Defective production of a single lymphokine by PBL of patients with CVI have been also described by others (Saiki *et al.*, 1984; Mayer *et al.*, 1984; Perri & Weisdorf, 1985; Matheson & Green, 1987). Interestingly, the average IL-4 production by PBL of our patients was found to be reduced as compared with IL-4 production by PBL of healthy donors, both after Con A or PHA activation. IL-4 production by PBL of both patients with CVI and healthy donors in general was low and even PBL of some healthy donors did not produce significant amounts of IL-4. This is probably related to the stimulation conditions used, since recently we demonstrated that higher levels of IL-4 were produced when PBL of healthy donors were activated by CA^{2+} ionophore A23187 in combination with the phorbol ester TPA, as compared with Con A or PHA (Paliard *et al.*, 1988). Despite these suboptimal activation conditions, no correlation was observed between defective IL-4 and defective IL-2 or IFN-γ production, supporting the notion that defective production of each of these lymphokines may be the result of different mechanisms. The absence of simultaneous defects in IL-2 and IL-4 production in the limited number of patients with CVI tested here may also explain why IL-2 or IL-4 defects did not finally result in reduced proliferative responses to mitogenic lectins. Both IL-2 and IL-4 have T cell growth promoting effects; we have previously demonstrated that IL-4 and IL-2 can act synergistically maintaining T cell proliferation, particularly when one of those factors is present at suboptimal concentrations (Spits *et al.*, 1987).

It is clear that the failure to produce normal levels of IL-2, IFN-γ or IL-4 represent stable defects, since longitudinal studies in a series of selected patients demonstrated that the failure to produce normal levels of one or more of these lymphokines was a consistent finding on sequential testing over periods of 1–5 months. In addition, defective IFN-γ production by PBL of some of the CVI patients was observed not only after activation

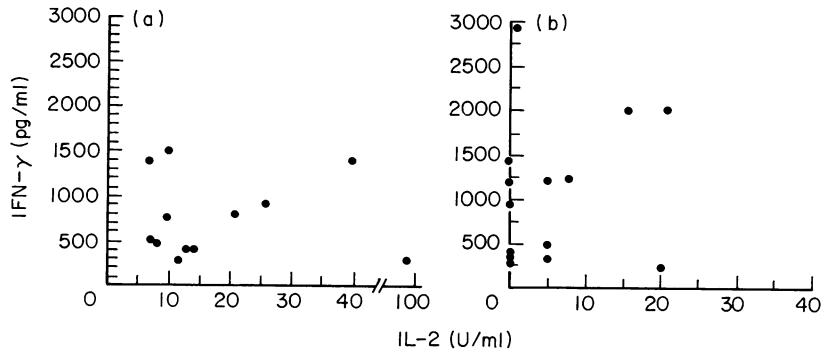

Fig. 4. No correlation between defective IL-2 and defective interferon-gamma (IFN-γ) production after stimulation with (a) 10 μg/ml phytohaemagglutinin (PHA); or (b) 1 μg/ml PHA.

Table 3. Interleukin-2 (IL-2), interferon-gamma (IFN-γ), and interleukin-4 (IL-4) production by peripheral blood lymphocytes of patients with CVI

Patients	IL-2 (U/ml)	IFN-γ (pg/ml)	IL-4 (pg/ml)
A	0	880	0
B	7	1400	377
C	0	<300	183
D	0	1360	NT
E	12	1500	NT
F	8	480	0
G	10	760	87
H	21	850	205
I	40	1400	NT
J	26	880	309
K	13	<300	NT
L	14	410	NT
M	98	<300	42
N	12	400	29
O	7	410	NT
Mean values ± s.d.	22·3 ± 7·0	760 ± 127	161 ± 50

Production was measured after activation of the lymphocytes with 10 μg/ml phytohaemagglutinin (PHA) as indicated in Materials and Methods. No spontaneous lymphokine production was observed in unstimulated cultures.

NT, not tested.

Table 4. Proliferative responses to mitogenic lectins by peripheral blood lymphocytes of patients with CVI and healthy donors

	CVI patients			Healthy donors		
	PHA	Con A	PWM	PHA	Con A	PWM
A	34 ± 6	14 ± 4	NT	82 ± 10	53 ± 1	NT
B	43 ± 2	31 ± 2	NT	82 ± 10	53 ± 1	NT
C	92 ± 7	55 ± 10	NT	116 ± 2	66 ± 9	NT
D	20 ± 1	27 ± 1	NT	54 ± 5	50 ± 7	NT
E	51 ± 1	30 ± 11	NT	89 ± 25	65 ± 9	NT
F	138 ± 8	65 ± 3	45 ± 0	114 ± 16	58 ± 2	65 ± 2
G	52 ± 4	20 ± 4	18 ± 5	37 ± 2	41 ± 11	30 ± 12
H	19 ± 3	97 ± 1	NT	83 ± 7	110 ± 18	NT
I	177 ± 18	149 ± 3	39 ± 2	192 ± 22	77 ± 4	38 ± 2
J	131 ± 3	NT	43 ± 1	67 ± 3	NT	51 ± 3
K	120 ± 12	65 ± 6	39 ± 2	69 ± 1	54 ± 3	27 ± 2
L	96 ± 13	17 ± 0	80 ± 4	18 ± 12	16 ± 1	40 ± 2
M	85 ± 5	14 ± 0	50 ± 7	147 ± 4	14 ± 0	61 ± 2
N	161 ± 7	75 ± 4	50 ± 7	174 ± 1	85 ± 6	61 ± 2
O	27 ± 2	56 ± 1	63 ± 0	114 ± 0	55 ± 0	79 ± 0
Mean	84 ± 51	51 ± 36	48 ± 16	96 ± 46	57 ± 23	51 ± 16

Data are expressed as ct/min × 10^3 ± s.d. and represent mean ^{3}H-thymidine incorporation of triplicate cultures. Proliferative responses of unstimulated lymphocytes were always < 2 × 10^3 ct/min. Healthy donors were tested simultaneously.

PHA, phytohaemagglutinin; Con A, concanavalin A; PWM, pokeweed mitogen

by PHA or Con A but also after stimulation by anti-CD3 MoAbs and phorbol ester or calcium ionophore plus phorbol ester, indicating that the failure of the production of detectable levels of IFN-γ is not due to the stimulation procedures used here (unpublished results).

IL-2, IL-4 and IFN-γ have also been shown to act as B cell growth and/or differentiation factors (Leibson *et al.*, 1984; Kehrl *et al.*, 1985; Romagnani *et al.*, 1986; Defrance *et al.*, 1986, 1987). Furthermore, it has been shown in murine systems that IL-4 induced IgG1 and IgE production, whereas it suppressed IgM, IgG2a, IgG2b and IgG3 secretion. In addition, IFN-γ was found to induce IgG2a and to suppress IgE synthesis, indicating that the relative quantities of IL-4 and IFN-γ regulate isotype production (Snapper & Paul, 1987). Similar data have been obtained in human systems, where it has been shown that IL-4 induces IgE production, whereas IFN-γ blocks the IgE-inducing activity of IL-4 (Pène *et al.*, 1988). Therefore, reduced synthesis of these lymphokines by T cells from CVI patients may contribute to the failure of B cells to differentiate terminally into antibody-secreting cells, which is the general characteristic of this disease (Rosen *et al.*, 1984).

In addition to the reduced IL-2, IL-4 or IFN-γ production by regulatory T cells, the block in B cell differentiation may be due to defective release of other B cell growth and differentiation factors (or combinations of these factors) including IL-5 and IL-6 (Kishimoto, 1987). IL-6, produced by monocytes, may be of particular interest, since it has been shown to play an essential role for terminal B cell differentiation (Maraguchi *et al.*, 1988). However, the absence of immunoglobulin production in patients with CVI has also been ascribed to intrinsic B cell defects (Rosen *et al.*, 1984). It should be noted that the defective lymphokine production by T cells may be indirectly induced by

aberrant secretion of factors produced by subsets of T cells, monocytes, natural killer cells or B cells, present in the mononuclear cell preparations, that selectively suppress IL-2, IL-4 or IFN-γ production. In addition, production of factors that affect the accessory functions of monocytes cannot be excluded. Studies to define the defects in lymphokine production more precisely are presently in progress. Collectively, our results indicate that overall IL-2, IL-4 and IFN-γ production observed in patients with CVI is reduced; whether this contributes to the pathogenesis of the disease remains to be determined.

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