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Role of interleukin-2 and interleukin-6 in the mitogen responsiveness of T cells from patients with 'common-variable' hypogammaglobulinaemia

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SUMMARY

We have assessed the ability of interleukin-2 (IL-2) and interleukin-6 (IL-6) to augment the proliferative response of T lymphocytes from 'common-variable' hypogammaglobulinaemia (CVH) patients and from normal controls, to the mitogens phytohaemagglutinin (PHA) and OKT3. We show that with cells from the control group and from those patients whose T cells respond to PHA within the control range, both IL-2 and IL-6 will significantly augment the response to OKT3. However, in those patients with a T cell defect in which the PHA response is below the control range, neither IL-2 nor IL-6 could restore the PHA or OKT3 response to normal. Responses to IL-2 or IL-6 alone were always in or above the control range.

Keywords hypogammaglobulinaemia T cells mitogen responses interleukin-2 interleukin-6

INTRODUCTION

Peripheral blood mononuclear cells (PBMC) from some patients with 'common variable' hypogammaglobulinaemia (CVH) show depressed DNA synthesis in response to mitogens, including phytohaemagglutinin (PHA) and the monoclonal antibody OKT3 (Webster & Asherson, 1974; Kruger *et al.*, 1984; Cunningham-Rundles, 1989). Responses to interleukin-2 (IL-2) are unaffected; depressed mitogen responses of some patients are reported to be restored by addition of IL-2 (Kruger *et al.*, 1984), and we have previously shown normal responses to IL-2 alone in patients whose T cells showed depressed responses to mitogens (North *et al.*, 1989).

The defect in DNA synthesis may relate to abnormal accessory function of CVH macrophages (Eibl *et al.*, 1982; Mannhalter, Zlabinger & Eibl, 1984; Fiedler *et al.*, 1987). T cell mitogenesis is influenced by signals from accessory cells including IL-1 (Davis & Lipsky, 1986; Chatila *et al.*, 1987) and IL-6 (Habetswallner *et al.*, 1988; Houssiau *et al.*, 1988). For example, responses of human T cells to suboptimal doses of PHA are augmented by IL-6 (Houssiau *et al.*, 1988). Ceuppens *et al.* (1988) have demonstrated that this is independent of IL-2. However, Le *et al.* (1988) have shown that IL-6 stimulates thymocytes via IL-2-dependent as well as independent pathways. In view of these findings, we have used recombinant IL-2 (rIL-2) and rIL-6 to try to modify responses to PHA and OKT3 of purified T lymphocytes from a random group of CVH patients and from healthy individuals.

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MATERIALS AND METHODS

Cells and cultures

Mononuclear cells and T cells were obtained from healthy volunteers and from nine randomly selected CVH patients, as previously described (North *et al.*, 1989). A range of directly conjugated monoclonal antibodies (Becton Dickinson) were used to stain the T cell preparations and establish the phenotypes by flow cytometry (FACStar). Positive cells within the total live-gate were calculated.

Aliquots (20 μl) of T cells at 4×10^6/ml were dispensed into Terasaki plates and stimulants were added in a 1-μl volume to give the required final concentration, with six replicates for each sample. The cells were cultured in hanging drops as described by Farrant *et al.* (1980) in Iscove's medium adapted for human cell culture (Farrant *et al.*, 1984).

Stimulants

PHA (Wellcome) was obtained in freeze-dried form reconstituted in sterile water, stored at -70°C and used at 1 μg/ml. Recombinant IL-2 (Biogen) was reconstituted according to the manufacturer's instructions and stored at -70°C. Dilutions in

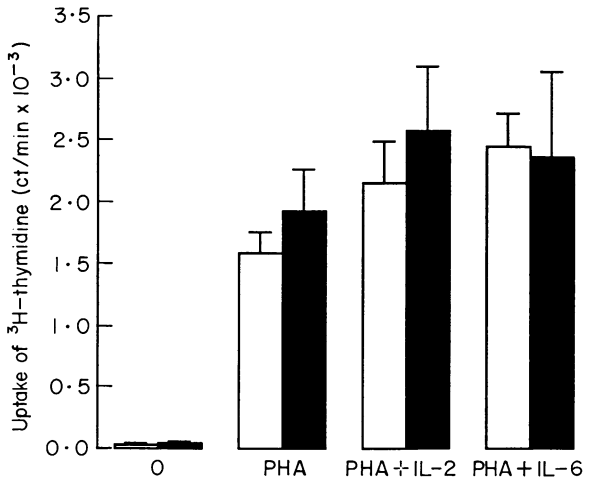

Fig. 1. Effect of IL-2 or IL-6 on the PHA-induced uptake of ^{3}H-Tdr (mean ct/min + 1 s.e.m.) by T cells from nine randomly selected CVH patients (■) and eight healthy donors (□). There were no significant differences between the two groups.

Table 1. ^{3}H-thymidine uptake (ct/min) of CVH patients A and B in relation to control range

Stimulus	Control range (n=8)	Patient A	Patient B
0	26–57	51	31
PHA	979–2351	756	52
OKT3	40–299	157	33
IL-2	99–542	114	247
IL-6	36–288	308	70

medium were made immediately before use at a final concentration of 500 U/ml. One unit (Biogen) is defined as the amount of IL-2 reported to cause half maximal incorporation of ^{3}H-thymidine in 4×10^3 CTLL-2 cells in culture. Recombinant IL-6 from Dr T. Kishimoto (University of Osaka) was used at 100 U/ml. One unit of IL-6 is defined as the activity able to induce 50% of the maximum response of IgM production in 1×10^4 SKW6-CL4 cells (Hirano et al., 1987). OKT3 (Ortho) was reconstituted according to the manufacturer's instructions and stored at −70°C. A final concentration of OKT3 of 1/1000, found to give maximal proliferation, was used.

Incorporation of ^{3}H-TdR into DNA

Proliferation was measured on day 3 by pulsing the hanging drops with methyl-^{3}H-TdR thymidine (specific activity 2 Ci/mmol, 0·4 Ci/well). After a further 2-h culture, cells were harvested as described by Farrant *et al.* (1980). The glass fibre discs were dried and counted in 1-ml liquiscint (National Diagnostics) in a liquid scintillation spectrometer. Counts from these 20-μl microcultures are much lower than those from 200-μl cultures, but the ratio of response to background is superior (Farrant *et al.*, 1980). All comparisons between patients and controls were done in pairs on the same occasion.

Table 2. Phenotypes of purified T cell preparations (% positive cells)

Group		CD3	CD4	CD8	CD14	CD16	HLA-DR
Controls	mean	84·3	28·7	37·5	1·7	7·8	4·8
	s.d.	7·6	16·5	16·7	2·3	4·6	3·6
	n	8	8	8	8	8	6
CVH	mean	82·8	37·4	36·0	1·5	10·1	9·1
	s.d.	7·3	10·6	11·8	1·1	7·1	5·1
	n	8	8	7	8	8	6

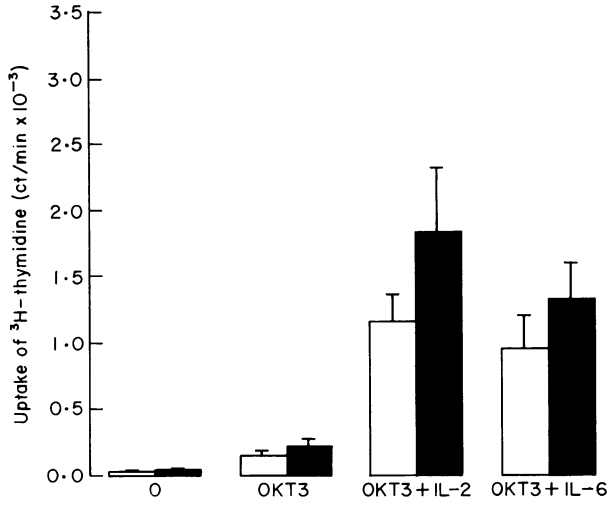

Fig. 2. Effect of IL-2 or IL-6 on the OKT3-induced uptake of ^{3}H-Tdr (mean ct/min + 1 s.e.m.) by T cells from the group of nine randomly selected CVH patients (■) and eight healthy donors (□). There was no significant difference between the two groups. Both showed a significant increase in the OKT3 response on the addition of IL-2 ($P < 0{\cdot}004$) or IL-6 ($P < 0{\cdot}003$).

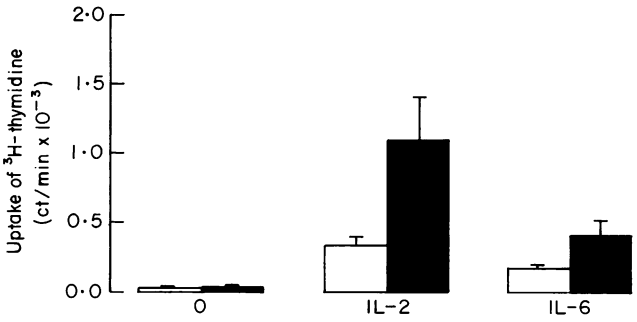

Fig. 3. Effect of IL-2 or IL-6 alone on the uptake of ^{3}H-Tdr (mean ct/min + 1 s.e.m.) by T cells from the group of nine randomly selected CVH patients (■) and eight healthy control donors (□). There was a significant increase both with IL-2 for control ($P < 0{\cdot}002$) and CVH ($P < 0{\cdot}05$) cells, and for IL-6 for control ($P < 0{\cdot}004$) and patient ($P < 0{\cdot}004$) cells. However, the mean responses of CVH cells were significantly higher than normal cells both for IL-2 ($P = 0{\cdot}04$) and for IL-6 ($P = 0{\cdot}05$).

RESULTS

Figure 1 shows that there was no significant difference in the mean T cell response to PHA between the groups of nine randomly selected CVH patients and eight healthy donors. The addition of IL-2 or IL-6 did not affect significantly the mitogenic response to PHA in both groups.

When the mitogenic stimulus was provided by OKT3 (Fig. 2), again there was no difference in the responses of the randomly selected patient and normal groups. However, the addition of either IL-2 or IL-6 with OKT3 did increase the uptake of ^{3}H-TdR significantly above that seen with OKT3 alone, but still did not induce any significant difference between patients and controls.

Figure 3 illustrates the DNA synthesis of T cells from the group of nine randomly selected CVH patients and from the group of eight normal individuals in response to IL-2 or IL-6 alone. In both donor groups, DNA synthesis was increased

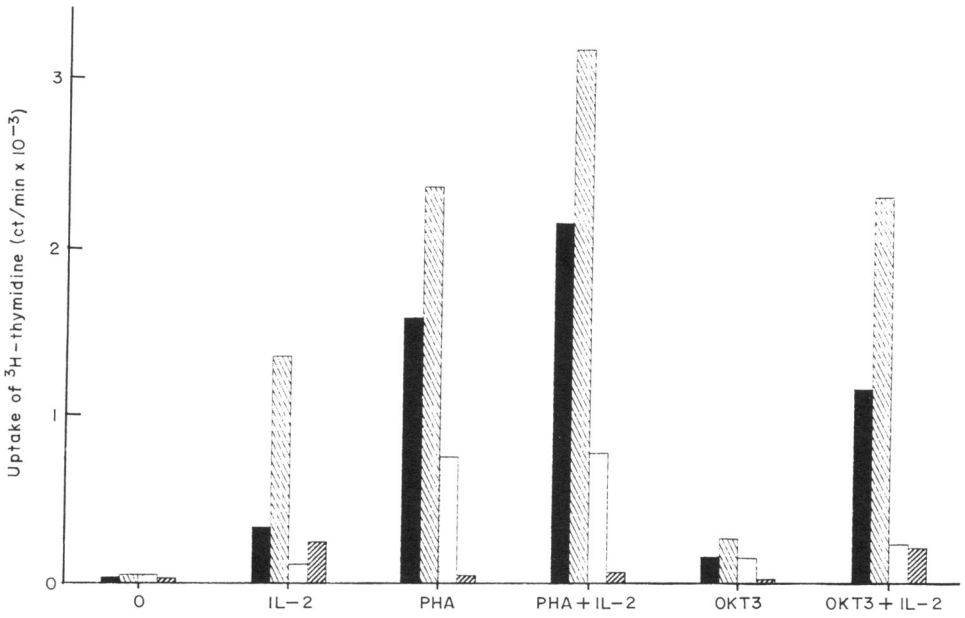

Fig. 4. Effect of IL-2 on the PHA and OKT3 induced uptake of ^{3}H-Tdr (ct/min) by T cells from two patients with impaired responses to PHA (see Table 1). IL-2 did not restore to normal levels the responses to PHA or OKT3 of T cells from patients A (□) or B (■). However, IL-2 significantly augmented ($P < 0{\cdot}001$) the mean responses to OKT3 of both normal T cells (■) and T cells from the group of seven patients whose responses to mitogens were in the control range (▧).

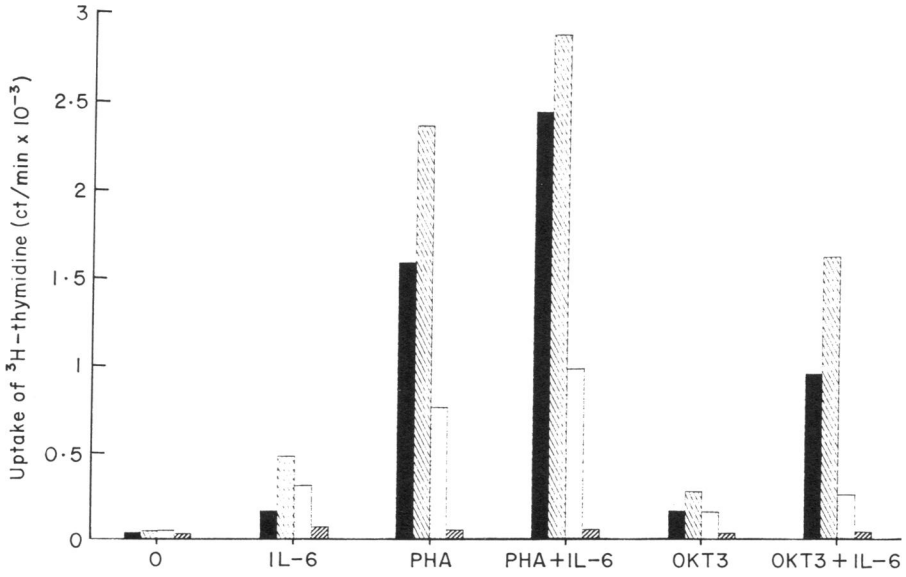

Fig. 5. Effect of IL-6 on the PHA and OKT3 induced uptake of ^{3}H-Tdr (ct/min) by T cells from two patients with impaired responses to PHA (see Table 1). IL-6 did not restore to normal levels the responses to PHA or OKT3 of T cells from patients A (□) or B (■). However, IL-6 significantly augmented ($P < 0{\cdot}001$) the mean responses to OKT3 of both normal T cells (■) and T cells from the group of seven patients whose responses to mitogens were in the control range (▧).

significantly by both cytokines but the mean response of the patients' T cells was significantly greater than that of cells from normal individuals (for IL-2, $P=0{\cdot}04$; for IL-6, $P=0{\cdot}05$).

All the above data considered the mean responses of the randomly chosen group of nine CVH patients. However, within the group of nine CVH patients there were two whose T cell responses to PHA were below the control range (Table 1). In Fig. 4 the effects of IL-2 are compared on the mean responses of the control group, the mean responses of the seven CVH patients who have normal PHA responses, and the individual responses of patients A and B. In these two patients, IL-2 did not enhance the depressed T cell responses to PHA or OKT3 (Fig. 4). As expected from the data of Figs 1 and 2, in the group of seven CVH patients, IL-2 had little effect on the mean PHA response but did increase the mean OKT3 responses.

The comparable data for IL-6 is given in Fig. 5. Again, IL-6 did not alter the response to PHA or OKT3 of the two patients A and B (Fig. 5).

Despite the failure of IL-2 or IL-6 to restore the mitogen responses of patients A and B, their T cell responses to IL-2 or IL-6 alone (always lower than responses to mitogens) were in the control range (Table 1).

Cell preparations were screened for CD3, CD4, CD8, CD14, CD16 and HLA-DR (Table 2). There was no statistically significant difference in the level of these markers or the number of cells expressing them in the group of CVH patients when compared with controls.

DISCUSSION

We and others have demonstrated that some CVH patients (usually less than 50% of those tested) have depressed DNA synthesis in response to mitogens such as PHA, Con A and anti-CD3 monoclonal antibody (Webster & Asherson, 1974; Lopez-Botet *et al.*, 1982; Kruger *et al.*, 1984; Fiedler *et al.*, 1987; Cunningham-Rundles, 1989; North *et al.*, 1989). In the present study, two out of nine patients were in this category.

It is known that total depletion of monocytes from a T cell preparation will abolish T cell mitogenesis to PHA or anti-CD3 (de Vries *et al.*, 1979; Umetsu *et al.*, 1987; Ceuppens *et al.*, 1988; Halvorsen *et al.*, 1988). This requirement for monocytes consists of the provision of soluble factors and direct cell–cell contact. The aim of our study is to further our understanding of the depressed DNA synthesis seen in T cells from some CVH patients, and in particular, to see whether the defect lies in the accessory monocyte or in the T cell itself. To do this we have assessed the ability of a T cell-derived cytokine (IL-2) and a monocyte-derived cytokine (IL-6) to augment the depressed responses of purified T cell preparations to mitogens. IL-6 may be made by several cell types, but in our system it is most likely to be derived from monocytes.

It is now clear that IL-6 derived from monocytes (Andersson *et al.*, 1988) provides an accessory signal for normal T cell mitogenesis (Houssiau *et al.*, 1988; Ceuppens *et al.*, 1988). The IL-6 can act both independently of IL-1 and IL-2 but it can also synergize with IL-1 and partially act in an IL-2-dependent manner. Our T cell preparations contained up to 1·6% monocytes, a level of depletion that did not abolish T cell mitogenesis to PHA but was sufficient to allow the synergistic effects of added IL-6 to be observed for the OKT3-induced response.

However, we were not able to restore the DNA synthesis to normal levels by adding IL-6 to either PHA- or OKT3-stimulated cultures of cells from the two affected patients, although cells from all patients responded to IL-6 or IL-2 alone. This argues that the defect in these two patients does not lie solely in the provision of the monocyte accessory signal provided by IL-6 or the T cell signal provided by IL-2. The phenotypic data suggest that the defects seen in the subgroup of CVH patients were not due to a difference in the number of monocytes or natural killer (NK) cells present.

If the defect does not lie with the IL-6-related accessory signal from monocytes, it could be within the T cell itself, perhaps at the level of a specific activation pathway. The key finding in the literature appears to be the depression of IL-2 production on stimulation with mitogens in T cells from affected CVH patients (Kruger *et al.*, 1984; Fiedler *et al.*, 1987). The present data showing normal or supranormal responses to IL-2 alone indicate that the activation pathways used when extrinsic IL-2 is added are functional in CVH patients with depressed mitogenic responses. This pathway involves the inducibility of IL-2 receptor (IL-2R) by added IL-2 (Cantrell & Smith 1984). Responsiveness to IL-2 alone are a recognized property of partially activated cells; our data thus suggest that patient cells may be more 'activated' than control cells. That we see such responses from control cells may reflect the sensitivity of our culture system.

Neither our data nor those of Lopez-Botet *et al.* (1982) support the idea of IL-2-induced 'restoration' of mitogenic responses in CVH patients as proposed by Kruger *et al.* (1984). However, on examining the data of Kruger *et al.* (1984) in detail, it appears that the patients with the most severe depression in mitogenesis (their Group C) did not respond to IL-2. If both IL-2 and IL-6 do not 'restore' the responses but merely increase DNA synthesis by an unaffected pathway, then the defective molecular component in CVH T cells could lie elsewhere. Responsiveness to IL-2 requires induction of IL-2R, but IL-2 production and T cell proliferation to mitogens (e.g. to anti-CD3) requires not only the induction of IL-2R but also the elevation of intracellular calcium levels (Cantrell, Collins & Crumpton, 1988).

Fiedler *et al.* (1987) showed that the depressed IL-2 production in some patients in response to OKT3 could be 'corrected' using the protein kinase C (PKC) activating phorbol ester (PMA). The inference from their work that the defect involves PKC is also supported by our data in which IL-2 known to be acting through the alternative calcium channel (Cantrell *et al.*, 1988) fails to 'restore' T cell responses in affected patients.

We conclude that the depressed responses to mitogens in a minority of CVH patients may involve a defect in the activation pathway containing PKC.

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