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Co-stimulation with anti-CD28 (Kolt-2) enhances DNA synthesis by defective T cells in common variable immunodeficiency

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SUMMARY

In normal T cells, an anti-CD28 MoAb (Kolt-2) will synergize with the mitogenic stimuli phytohaemagglutinin (PHA), anti-CD3 (OKT3) or a combination of anti-CD2 antibodies (OKT11 and GT2) in the induction of DNA synthesis. A subgroup of patients with common variable immunodeficiency (CVID) show a defect in DNA synthesis by T cells stimulated *in vitro* with the above mitogens. We have now investigated whether anti-CD28 will correct the defect. This strategy partially restored DNA synthesis, providing evidence that the CD28 co-stimulatory pathway in CVID T cells is normal. Ligation of CD28 acts through co-stimulating IL-2 secretion. The natural ligand (B7) for CD28 on antigen-presenting cells from CVID patients is expressed normally. We conclude that the defect in CVID T cells lies in pathways that lead to transcription of the IL-2 gene other than that induced by ligation of CD28 with Kolt-2.

Keywords CD28 common variable immunodeficiency IL-2 T cells

INTRODUCTION

Common variable immunodeficiency (CVID) is a disorder characterized by hypogammaglobulinaemia, a severe defect in antibody production [1,2] and a failure of T cells to proliferate in response to recall antigens [3]. A subgroup of patients have more profound T cell abnormalities, with low DNA synthesis and depressed production of IL-2 to various non-antigenic stimuli such as phytohaemagglutinin (PHA), anti-CD3 and anti-CD2 [4–9]. In normal T cells, stimulation by antigen through the T cell receptor (TCR) leads to anergy [10]. For T cell proliferation, a co-stimulus is also required. It has been shown that the major co-stimulatory pathway in human T cells is through the CD28 antigen [11–13], resulting in IL-2 secretion and proliferation [11,12].

The CD28 antigen is a 44-kD homodimer member of the immunoglobulin supergene family, and is expressed on thymocytes, peripheral T cells and activated B cells [14]. It is recognized by a number of different MoAbs, although not all of them will provide an appropriate co-signal [15]. MoAbs to CD28 do not induce DNA synthesis alone, but they can act as a co-stimulus for non-antigenic stimuli; for example, anti-CD28 synergizes with two MoAbs directed against distinct epitopes of the CD2 molecule [16] or with suboptimal concentrations of PHA or OKT3 [17–23]. The co-stimulus of anti-CD28 induces transcription of the IL-2 gene through a CD28 response element in the enhancer region [24,25]. Anti-CD28 can also stabilize the mRNA of cytokine genes including IL-2 [26]. The natural ligand for CD28 is B7, and it is expressed on activated antigen-presenting cells (APC; e.g. monocytes, dendritic cells and B cells) [12,14].

The defects in antigen- and mitogen-driven responses by T cells from most patients with CVID are associated with depressed IL-2 production [5,6,27]. This suggests that CVID T cells resemble normal cells which have not received the necessary co-stimulus. In some patients, the DNA synthesis can be enhanced by the addition of IL-2 [2,4,28]. Against this background we have tested whether CVID T cells will respond to co-stimulus through CD28 in the presence of different mitogenic stimuli.

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MATERIALS AND METHODS

Selection of donors

CVID patients whose B cells are unable to produce either IgM or IgG *in vitro* in response to solid phase anti-μ and IL-2 are classified as Group A [29]. The CVID patients used in this study were a subgroup of Group A with T cells that consistently failed to proliferate within the normal range in response to PHA [8,9]. Donors of normal T cells were healthy laboratory personnel.

Medium

Unless otherwise stated, the culture medium used was Iscove's serum-free medium adapted for human lymphocyte culture [30]. The additives, bovine serum albumin (BSA), soybean lipid and transferrin, were obtained from Boehringer Mannheim and the final concentrations in the medium were as follows: BSA 1 mg/ml; soybean lipid 20 μg/ml; transferrin 45 μg/ml; L-glutamine 2 mM; penicillin 100 U/ml; and streptomycin 100 U/ml.

Cells and cultures

Mononuclear cells were obtained from the peripheral blood of normal donors or CVID patients. The blood was defibrinated, diluted with a fifth volume of medium, layered onto Ficoll–Paque (Pharmacia, Uppsala, Sweden) and centrifuged at 650 ***g*** for 35 min. The cells harvested from the interface were washed twice and resuspended in Iscove's supplemented medium at a concentration of 2×10^6/ml. In some experiments, T cells were separated from the mononuclear cells as follows. Mononuclear cells were adjusted to approximately 3×10^6/ml in RPMI 1640 containing penicillin/streptomycin (100 U/ml), glutamine (2 mM), 10% fetal calf serum (FCS) and 1% neuraminadase-treated sheep erythrocytes. The cell suspension was left to rosette overnight at room temperature. The sedimented cells were gently resuspended and layered on to Ficoll–Paque and centrifuged at 650 ***g*** for 35 min. The T cells were recovered by lysis of the sheep erythrocytes with ammonium chloride lysis fluid. The cells were washed twice in RPMI 1640 and resuspended in Iscove's supplemented medium at 2×10^6/ml. The cells (50 μl) were cultured in plates with round-bottomed wells (Nunc, Roskilde, Denmark) in a 5% CO_2 humidified incubator. Stimulants were added to triplicate wells in a 5 μl volume.

Stimuli

MoAbs used to stimulate cells were as follows: anti-CD3 (OKT3, Ortho) at a final dilution in culture ranging from 1 : 1000 to 1 : 10 000; anti-CD28 (Kolt-2, Dr K. Sagawa, Kurume University, Japan) at a final concentration of a 1 : 20 000 dilution of ascites and anti-CD2 antibodies (GT2-1D7, Life Technologies Ltd, Paisley, UK, and OKT11, ATCC, Rockville, MD) at a final dilution in culture of 1 : 20. PHA (Wellcome) was used at a final concentration of 1 μg/ml unless otherwise stated.

Proliferation assay

Mononuclear or T cell cultures were pulsed at 72 h with methyl-^{3}H-thymidine (ICN Biomedicals, High Wycombe, UK; specific activity 2 Ci/mmol, 1 μCi/well). After a further 2 h, the cultures were harvested onto glass fibre sheets by washing with water and methanol using an LKB/Skatron automatic cell harvester. The sheets were air dried, sealed into sample bags with 9 ml of scintillation fluid and counted in an LKB/Wallac 1205 flat-bed Betaplate liquid scintillation counter.

Flow cytometric studies

Three-part differential cell counts for lymphocytes, monocytes and granulocytes (expressed as a percentage of leucocytes) were performed on the separated cell populations used for culture (Becton Dickinson Simulset program). The expression of CD28 on $CD4^+$ and $CD8^+$ lymphocyte subsets was determined as follows. Cell suspensions were incubated with 1 μg/ml of purified anti-CD28 (clone Kolt-2, IgG1) together with culture supernatants of anti-CD4 (clone T4-407, IgG2a, Oxoid Ltd, Basingstoke, UK) or anti-CD8 (clone RFT8, IgM). After washing the cells, isotype-specific second layers conjugated to either PE or FITC (Southern Biotechnology Associates, Birmingham, AL) were added. Negative controls consisted of cells incubated with purified normal mouse immunoglobulin (Sigma Ltd, Poole, UK) at a final concentration of 1 μg/ml followed by the conjugated second layers. B7 expression was monitored on monocytes of patients and normal donors after 24 h culture of a mononuclear cell preparation with PHA (1 μg/ml) or interferon-gamma (IFN-γ; 1000 U/ml) using a PE-conjugated anti-B7 MoAb kindly provided by Dr E. Clark (Seattle, WA). All samples were read on the Facscan (Becton Dickinson).

RESULTS

Synergy between Kolt-2 and anti-CD2 antibodies on T cells from CVID patients and normal donors

The combination of the two anti-CD2 MoAbs (OKT11 and GT2) did not stimulate DNA synthesis unless Kolt-2 was also present (Fig. 1) in T cells from normal donors or CVID patients. The effects of each of the antibodies alone was negligible.

Synergy between Kolt-2 and anti-CD2 antibodies on mononuclear cells from CVID patients and normal donors

With normal mononuclear cells, Kolt-2 increased the DNA synthesis to the combination of the two anti-CD2 antibodies almost to the level of the normal PHA response (Fig. 2). By contrast, with CVID mononuclear cells, the response to the combined antibodies was subnormal ($P<0{\cdot}005$), although it was greater than the CVID PHA response ($P<0{\cdot}025$). The small response of the CVID mononuclear cells to the combination of the two anti-CD2 antibodies without Kolt-2 was less than that of normal cells ($P<0{\cdot}05$).

When comparing these mononuclear cell responses (Fig. 2) to those of purified T cells (Fig. 1), the synergy between Kolt-2 and anti-CD2 was greater with the mononuclear cells. Only with

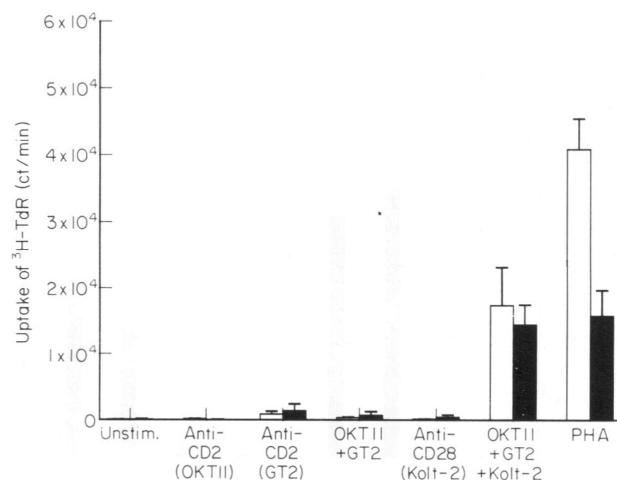

Fig. 1. Comparison of the synergy between Kolt-2 and anti-CD2 MoAbs on the DNA synthesis of purified T cells from five normal donors (□) and seven common variable immunodeficiency (CVID) patients (■). The mean uptake of ^{3}H-TdR (±1 s.e.m.) was measured on day 3 of cell culture with the antibodies as indicated on the abscissa. The response to phytohaemagglutinin (PHA; 1 μg/ml) is also shown. In this and subsequent figures the patients were selected to have subnormal responses to PHA.

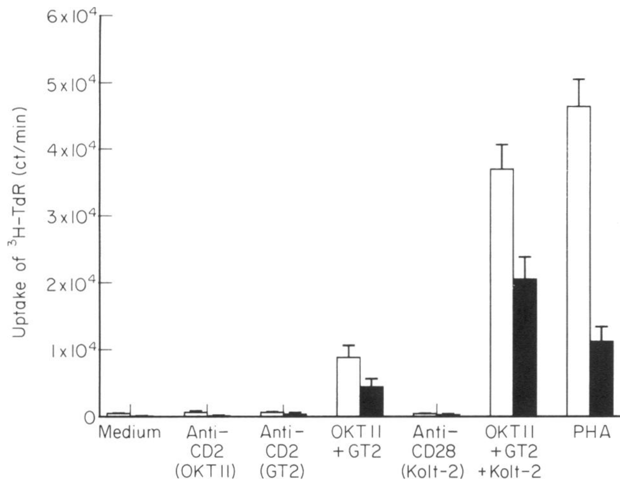

Fig. 2. Comparison of the synergy between Kolt-2 and anti-CD2 MoAbs on the DNA synthesis of mononuclear cells from nine normal donors (□) and 10 common variable immunodeficiency (CVID) patients (■). Other details as in Fig. 1. PHA, Phytohaemagglutinin.

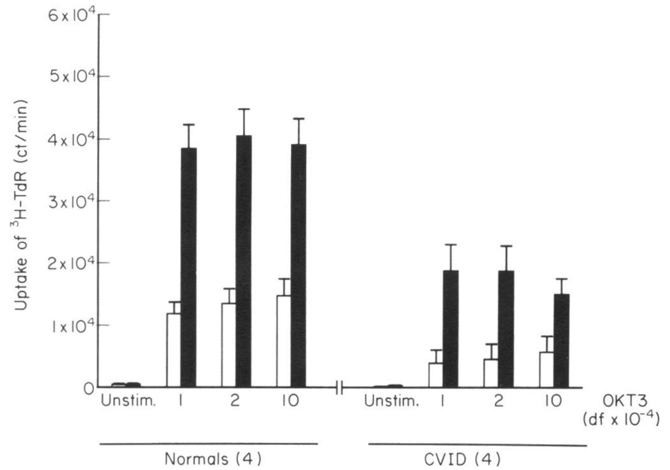

Fig. 4. Comparison of the effect of the presence (■) or absence (□) of Kolt-2 with different dilutions (df) of anti-CD3 (OKT3) (as shown on the abscissa) on the DNA synthesis of mononuclear cells from four normal donors and four common variable immunodeficiency (CVID) patients. Other details as in Fig. 3.

Table 1. Mean three part differential cell counts (% of leucocytes, $n = 4$)

	Lymphocytes (%)	Monocytes (%)	Granulocytes (%)
Normal PBMC	82	11	7
CVID PBMC	76	15	10
Normal T cells	92	2	7
CVID T cells	88	2	10

PBMC, Peripheral blood mononuclear cells; CVID, common variable immunodeficiency.

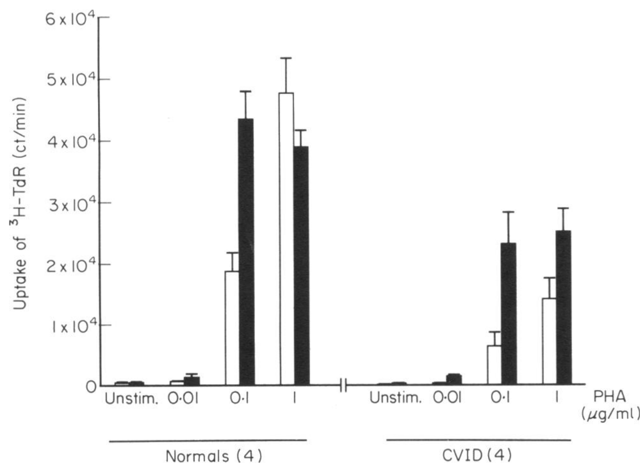

Fig. 3. Comparison of the effect of the presence (■) or absence (□) of Kolt-2 with different concentrations of phytohaemagglutinin (PHA) (as shown on the abscissa) on the DNA synthesis of mononuclear cells from four normal donors and four common variable immunodeficiency (CVID) patients. The mean uptake of ^{3}H-TdR (± 1 s.e.m.) was measured on day 3 of cell culture.

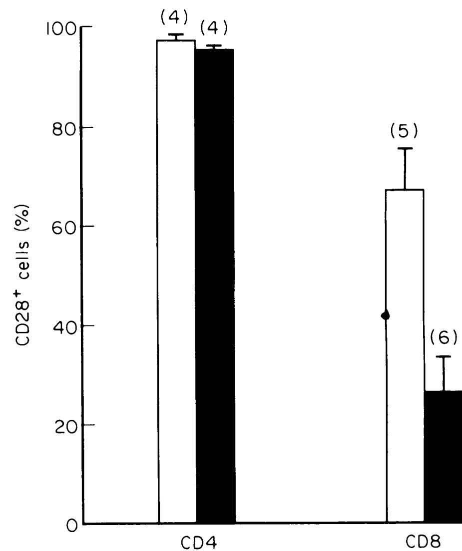

Fig. 5. Expression of CD28 on CD4 and CD8 lymphocyte subsets. The mean proportion of circulating CD4 and CD8 cells that were positive for CD28 for normal donors (□) or common variable immunodeficiency (CVID) patients (■).

the mononuclear cell preparation was there a significant difference between normal and CVID responses. Furthermore, the presence of monocytes allowed a small response to the two anti-CD2 antibodies which was not seen with purified T cells.

Kolt-2 co-stimulus and the numbers of monocytes

There were different responses by normal and CVID mononuclear cells (Fig. 2) to Kolt-2 and the two anti-CD2 antibodies despite equivalent numbers of monocytes in the cell preparations (Table 1).

Synergy between Kolt-2 and PHA or OKT3 on both normal and CVID mononuclear cells

Kolt-2 augments the DNA responses to suboptimal mitogenic concentrations of PHA (Fig. 3) and anti-CD3 (OKT3) (Fig. 4) in both normal and patient groups. With normal cells, Kolt-2 increased the suboptimal response to PHA (0·1 μg/ml) up to the maximal response observed with 1 μg/ml of PHA alone (Fig. 3). With CVID cells, Kolt-2 significantly augmented the responses to PHA, but to levels less than that of normal cells (Fig. 3). A similar increase with OKT3 and Kolt-2 was seen in both patient and normal groups (Fig. 4).

Abnormal kinetics were not responsible for the differences between normal and CVID responses, since in a normal individual and a CVID patient the kinetics of the response to PHA or the combination of the anti-CD2 MoAbs with Kolt-2 were similar (data not shown).

Phenotypic expression of CD28 on cells from CVID patients and normal donors

We observed that there was no difference in the proportion of $CD4^+$ cells that were $CD28^+$ between normal donors and CVID patients (Fig. 5). By contrast, there was a significant increase in the proportion of $CD8^+$ cells that were $CD28^-$ in the CVID patients. The $CD8^+$ cells were $CD3^+$ and $CD16^-$.

DISCUSSION

We chose CVID patients with the most severe defect in proliferation and attempted to correct this by providing a co-signal using anti-CD28 (Kolt-2). The results show that DNA synthesis could be substantially increased with Kolt-2 when combined with the two anti-CD2 MoAbs, with OKT3 or with PHA. These data suggest strongly that the pathway activated via CD28 is not defective in CVID.

The B7/CD28 interaction is essential for T cell function [14]. The defective mitogen responses in CVID do not seem to be due to a lack of the natural ligand (B7), since in two patients we have shown that B7 is expressed on monocytes after activation (data not shown). Since B7 is expressed and Kolt-2 clearly acts as an effective co-stimulus, the defect must lie elsewhere.

It is clear that IL-2 production is depressed in CVID [4,5], and that the normal co-stimulation with anti-CD28 acts through IL-2 [30]. Our finding that the anti-CD28 pathway is normal in CVID implies that the ability of anti-CD28 to increase IL-2 production in CVID is not impaired. This is supported by a preliminary experiment with PHA-stimulated mononuclear cells from a CVID patient showing that co-stimulation with Kolt-2 resulted in an increase in the production of IL-2 (data not shown). The defect must thus lie in another pathway that triggers transcription of the IL-2 gene. Unlike the pathway activated by anti-CD28 [20,23,24], IL-2 production induced through the TCR or by other stimuli like PHA is protein kinase C (PKC)-dependent. Previously we have reported that direct activation of PKC by phorbol esters and ionomycin is defective [9]. The data are compatible with a defect involving PKC-dependent response elements of the IL-2 gene (e.g. AP-1) [31].

There are several explanations as to why the defect can not be completely reversed by the anti-CD28 co-stimulus. The first is that insufficient IL-2 is produced. Second, monocytes and monocyte-derived factors are crucial for some proliferative responses by T cells. For both normal and patient cells, the monocyte dependence of the synergy between Kolt-2 and the stimuli we have used (anti-CD2, OKT3 or PHA) is identical to the monocyte dependence of these stimuli in the absence of Kolt-2. This shows that signals through the CD28 antigen can not substitute for monocytes. Nevertheless, it is possible that the extent of the response could be affected by a defect in the accessory function of CVID monocytes. Finally, the responses could be influenced by the abnormal numbers of cells in different lymphocyte subsets. There is a severe circulating $CD4^+$ cell depletion in the group of CVID patients we have studied (Group A) [32,33], but no difference from normal in the numbers of circulating $CD8^+$ cells [32]. This imbalance is also seen in the cell preparations used (data not shown). The proportion of CVID $CD4^+$ cells that are $CD28^+$ is at the normal level. By contrast, compared with normal cells, there is a marked decrease in the proportion of CVID $CD8^+$ cells that express CD28. These imbalances do not prevent the responses to Kolt-2 and PHA by CVID T cells, but they may contribute to their inability to reach completely normal levels under all conditions [34].

We conclude that the CD28 pathway in CVID T cells is normal, and that the defect lies in other pathways that normally induce transcription of the IL-2 gene. Further understanding of this mechanism may lead to strategies to reverse the proliferative defect of CVID T cells, not only to mitogens but more importantly to antigens.

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