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Defects in antigen-driven lymphocyte responses in common variable immunodeficiency (CVID) are due to a reduction in the number of antigen-specific $CD4^+$ T cells

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SUMMARY

T cells from patients with CVID have defects that may relate to the failure *in vivo* of B cell production of antibodies. Antigen-driven responses of T cells from CVID patients and normal subjects have been assessed by measuring DNA synthesis *in vitro*. Low density cells enriched for antigen-presenting dendritic cells were pulsed with purified protein derivative (PPD) and cultured with autologous T cells. Overall, T cells from CVID patients showed a significantly low mean response to PPD, although non-specific DNA synthesis induced in CVID T cells by IL-2 was within the normal range. However, mean PPD-specific T cell responses in CVID were not restored by IL-2 irrespective of the presence of monocytes. Depletion of $CD8^+$ cells also failed to restore the mean PPD response of CVID $CD4^+$ T cells. Limiting dilution analysis showed that in CVID there was a reduced frequency of antigen-specific cells within the T cell preparations. The mean frequency of the PPD-specific T cells in cultures from patients vaccinated with bacille Calmette–Guérin (BCG) was reduced to 1 in 109 000 T cells compared with 1 in 18 600 T cells in BCG-vaccinated normal donors. These data show that the reduced PPD-specific response in CVID is due to a partial peripheral loss of antigen-specific cells.

Keywords common variable immunodeficiency $CD4^+$ T cells $CD4^+$ T cell depletion purified protein derivative

INTRODUCTION

CVID is an acquired disease of unknown cause, clinically characterized by recurrent respiratory and gastrointestinal infections [1]. Patients present with low serum levels of immunoglobulins of all isotypes, with variable defects in cellular immunity, particularly with absent DTH skin responses to both primary and recall antigens [2]. A subgroup of CVID patients show very poor T cell proliferation on stimulation with mitogens or phorbol with ionomycin [3,4]. This is associated with depression of IL-2 production, and some workers claim that proliferation can be restored to near normal levels by the addition of recombinant IL-2 (rIL-2) [5]. The finding that mRNA for IL-2 is grossly reduced in phytohaemagglutinin (PHA)-stimulated T cells from CVID patients [6], whether or not these cells proliferate normally *in vitro*, provides further evidence that a failure of IL-2 production might be of central importance in the mechanism of CVID. Also, it has been reported that extrinsic IL-2 could restore normal proliferative responses to tetanus toxoid antigen *in vitro* in some patients [7], and also reconstitute antibody production to the same antigen in some patients [8]. These findings have provided impetus for a clinical trial in CVID of polyethylene glycol conjugated with rIL-2 [9].

Recently, we have shown that T cells from most CVID patients have very low proliferative responses to the antigen purified protein derivative (PPD) when presented on autologous circulating low density cells (LDC) which are enriched for dendritic cells, regardless of whether these same T cells respond normally to PHA [10]. One explanation is that potentially responding T cells are present but have been rendered anergic by inappropriate stimuli, and remain in that state because of the lack of IL-2. Such a mechanism may mimic the mouse clone rendered anergic by antigen in the absence of co-stimuli, this anergy being reversed by adding IL-2 [11]. We have investigated this by testing whether IL-2 corrects the

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antigen unresponsiveness of T cells from CVID patients. An alternative mechanism is that antigen-specific memory cells are lacking. We have therefore measured the frequency of PPD-responding cells by limiting dilution.

PATIENTS AND METHODS

Patients

Defibrinated peripheral blood (40 ml) was obtained from CVID patients attending the clinic for routine immunoglobulin replacement therapy (i.v. immunoglobulin injection every 2–4 weeks). Adult patients selected for the study were already classified into one of the three established patient subgroups (A, B and C) [12]. This classification depends on the isotypes of immunoglobulin secreted *in vitro* by T-depleted CVID B cells driven by IL-2 with or without anti-μ: in group A, no IgM or IgG is produced *in vitro*; in group B, IgM alone; and in group C, both IgM and IgG. Healthy adult volunteer laboratory workers served as normal donors. All the normal control donors had been immunized with bacille Calmette–Guérin (BCG) in adolescence. Because of their diagnosed immunodeficiency only about half of the patients had been vaccinated with BCG. One half of the patients without a history of BCG vaccination were dignosed as CVID in early life.

Cell separation

Mononuclear cells were isolated from defibrinated venous blood by centrifugation over Ficoll–Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) at 600 *g* for 30 min. After washing the cells twice in RPMI 1640 containing 2% fetal calf serum (FCS), the mononuclear cells were incubated overnight at 5×10^6/ml in 21-cm^2 Petri dishes (Nunc, Roskilde, Denmark) in complete medium (RPMI 1640 containing 10% FCS, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin). Non-adherent cells were centrifuged over hypertonic Metrizamide (made as 14·5 g plus 100 ml complete medium) at 600 *g* for 10 min. LDC from the Metrizamide interface were used as a source of antigen-presenting cells (APC) rich in dendritic cells [13,14]. Approximately one third of LDC have the appearance of dendritic cells by light and electron microscopy and can be labelled with the MoAb RFD1 which labels dendritic cells but not monocytes [15]. In the LDC preparation, contaminating cells are CD14$^+$ monocytes with negligible lymphocytes. When required, adherent cells were removed from the Petri dish with a cell scraper (Falcon 3086; Becton Dickinson, Franklin Lakes, NJ) and used as the source of monocytes. The pellet cells from the Metrizamide gradient containing the lymphocytes were further enriched for T cells by rosetting with sheep erythrocytes treated with neuraminidase for 2 h at 4°C. The rosette-forming cells were isolated by centrifugation over Ficoll–Paque at 600 *g* for 30 min and collecting the pellet. The sheep erythrocytes bound to T cells were lysed by mixing the pellet with distilled water for 3–5 s. The same volume of 2 × PBS pH 7·4 with a large excess of RPMI 1640 supplemented with 2% FCS was then added to restore normal molarity. The T cells were washed twice and used for culture.

Purification of T cell subpopulations

T cells were further purified by depleting CD4$^+$ or CD8$^+$ cells. T cells were incubated with anti-CD4 or anti-CD8 mouse MoAb (RFT4 or RFT8, respectively, being kind gifts from Dr A. Akbar (Royal Free Hospital School of Medicine, London, UK)) for 30 min at 4°C. The concentration of these antibodies was determined by preliminary experiments. After washing the cells with RPMI 1640 containing 2% FCS, anti-mouse immunoglobulin-coupled dynabeads (Dynal AS, Oslo, Norway) were added to the T cells with a bead/T cell ratio of 3:1. The beads and T cells were spun down at 300 *g* for 5 min and resuspended; this procedure was repeated four more times. T cells were then exposed to a magnet for 5 min before the cells in suspension were transferred to another tube. One more round of depletion was performed, and the resulting cells were used for antigen-specific responses. CD4$^+$- or CD8$^+$-depleted T cells contained <0·1% CD4$^+$ or CD8$^+$ cells, respectively, by flow cytometry.

Cell cultures

LDC were prepulsed with PPD (50 μg/ml; Statens Serum Institut, Copenhagen, Denmark) by incubation for 2 h at 37°C. After washing, 2×10^3 LDC were mixed with 8×10^4 T cells per well, and cultured in a total volume of 20 μl in 60-well Terasaki plates. Control cultures contained T cells and LDC not exposed to PPD. The Terasaki plates were inverted over sterile saline in a plastic box in a humidified incubator gassed with 5% CO_2 for 5 days [16,17]. For further experiments, human rIL-2 (R&D Systems, Abingdon, UK), at a concentration of 10 or 50 U/ml, was added to the above cultures at the beginning of incubation. To investigate the effect of IL-2 on the T cell response to PPD in the presence of monocytes, the same number of T cells and LDC as above were cultured in 200 μl in 96-well round-bottomed microplates (Nunc). Monocytes (8×10^3 or 2×10^4 in 10 μl) were added to these cultures. For each experimental condition, the culture was performed in triplicate. The response of T cells without the need for antigen processing was tested using two synthetic peptides (38A, 38G) derived from the 38-kD antigen of *Mycobacterium tuberculosis* [18,19]. LDC were pulsed with the peptides (50 μg/ml for each) in the same way as for the PPD response and the pulsed LDC were then mixed with T cells.

Measurement of DNA synthesis

Tritiated ^{3}H-thymidine (TdR; ICN Biomedicals, High Wycombe, UK; specific activity 2 Ci/mmol) 2 μg/ml was added 2 h before harvesting the cells on a filter paper (Whatman GF/C, Maidstone, Kent, UK) using a hanging drop technique [16,17], or using an automatic cell harvester (LKB/Skatron, Milton Keynes, UK) for the 96-well microplate. Radioactivity was measured by liquid scintillation counters (Canberra, Packard, Amstelveen, Holland) for the Terasaki wells or by Betaplate (LKB/Wallac, Milton Keynes, UK) for the 96-well microplate.

Statistical analysis

Statistical analysis was performed by Student's *t*-test in the usual way. For all experiments, $P < 0.05$ was considered significant.

Limiting dilution analysis

Various doubling dilutions of T cells (from 2500 to 16 000 per well) were cultured each with 2×10^3 LDC, which were pulsed with PPD, with 16–24 replicates at each T cell concentration in the plates with 96 round-bottomed wells. Individual wells were considered to be positive for a PPD-specific response if the TdR uptake was greater than the mean + 3 s.d. of that observed in

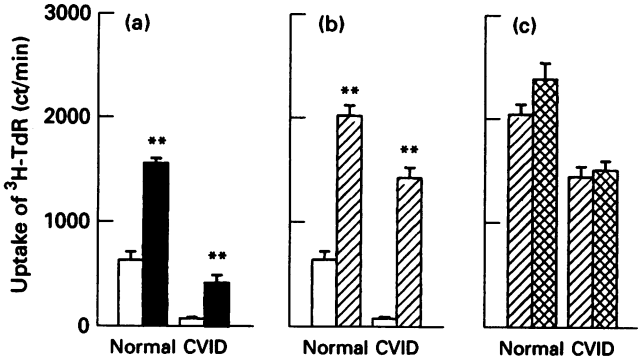

Fig. 1. Uptake of ^{3}H-thymidine (TdR) into DNA (ct/min) on day 5 of culture by T cells from healthy normal donors (n = 3) and from 12 CVID patients (four in group A, five in group B and three in group C). (a) Comparison of mean responses between T cells cultured with unpulsed (□) and purified protein derivative (PPD)-pulsed (■) low density cells (LDC). (b) Comparison of mean responses between T cells cultured with (▨) or without (□) IL-2 (50 U/ml). LDC (not pulsed with antigen) were also present whether IL-2 was added or not. (c) Comparison of mean DNA synthesis of T cells cultured with unpulsed (▨) or PPD-pulsed (▩) LDC both with IL-2 (50 U/ml) present. The data show mean ct/min ± s.e.m. $^{**}P < 0.001$.

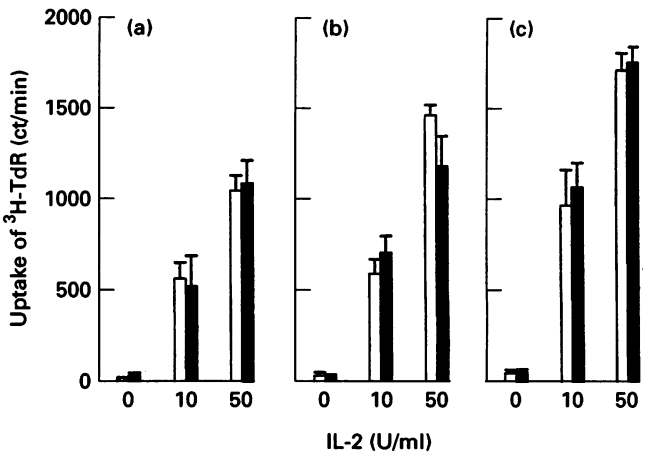

Fig. 2. Effect of monocytes on the possible interaction of IL-2 with purified protein derivative (PPD)-driven T cell responses. IL-2 at the different concentrations shown was added to the culture of 8×10^4 T cells with 2×10^3 unpulsed (□) or PPD-pulsed (■) low density cells (LDC) from a CVID patient (group A) without adding autologous monocytes (a), with 10% monocytes (b), or with 25% added monocytes (c) in relation to the number of T cells. The data show mean ct/min ± s.e.m. with the number of replicate wells = 4.

the wells containing T cells at each concentration with unpulsed LDC. To obtain this background level the number of replicate wells with LDC without antigen was never less than eight. The percentage of non-responder wells was plotted against the number of T cells added to each well and a linear regression performed through the point of origin. The responder cell frequency was interpolated or extrapolated to the 37% non-responder well level, as described by Henry *et al.* [20].

RESULTS

Mean T cell responses to PPD are defective in CVID

Initially, we looked at the ability of T cells (8×10^4/well) to synthesize DNA when cultured in the presence of LDC pulsed with PPD. T cells from the normal controls responded to PPD significantly (Fig. 1a); the mean PPD-induced responses of T cells from the patients of all CVID groups (A, B and C) were significantly reduced ($P < 0.001$) from these normal levels, yet their response was still significant (Fig. 1a). In this initial block of experiments the BCG vaccination status of the patients was not taken into account (see below). It is worth noting that the background levels for the CVID cells were consistently and significantly ($P < 0.001$) lower than normal background levels.

Response to rIL-2 is normal

In the same experimental series, T cells from the same normal control donors and from the same CVID patients responded well to rIL-2 (50 U/ml) (Fig. 1b). T cells with IL-2 were cultured with LDC but without PPD.

Response to PPD not improved by IL-2

In order to investigate whether IL-2 could improve the response of the antigen-driven CVID T cells, the mean response of T cells to unpulsed and PPD-pulsed APC in the presence of the same dose of rIL-2 was also compared within the same experimental series (Fig. 1c). When IL-2 was present, there was no extra significant T cell response specifically attributable to PPD either in the normal control donor group or in any of the patient groups. Additional experiments using lower concentrations of rIL-2 (1–10 U/ml) also gave no synergy with the PPD-specific response in any donor group (data not shown).

Response to PPD not improved by IL-2 and monocytes

To see whether the effect of IL-2 on PPD-driven T cell responses depends on the presence of monocytes, various concentrations of rIL-2 were added to the T cell and LDC culture with or without monocytes (at a number equal to 10% or 25% of T cells) with cells from three CVID patients (one patient from each CVID group A, B or C). Figure 2 shows one example of the result of these experiments illustrated by the group A patient's cells. Despite the presence of monocytes, IL-2 did not show any significant synergy with the T cell response to PPD-pulsed LDC. T cells from the other patients gave similar results.

T cell responses to PPD peptides not requiring processing are defective in CVID

Figure 3 shows that T cells from two CVID patients not only failed to respond to PPD but also to two synthetic peptides (38A and 38G) derived from the 38-kD antigen of *Myco. tuberculosis*. One normal donor (N2) had cells that responded significantly to both peptides. The second normal donor we tested (N1) failed to respond to 38A and only weakly responded to 38G (Fig. 3). However, cells from the two patients, both of whom had been vaccinated with BCG in the past, did not respond to PPD or to either peptide.

Role of BCG vaccination on mean PPD responses in CVID

In the initial series of experiments showing a defect in CVID in response to PPD and its lack of restoration with IL-2 (Fig. 1), we tested patients without being aware whether or not they had a history of receiving BCG. We therefore began a new series designed to address this point. All the normal donors had a

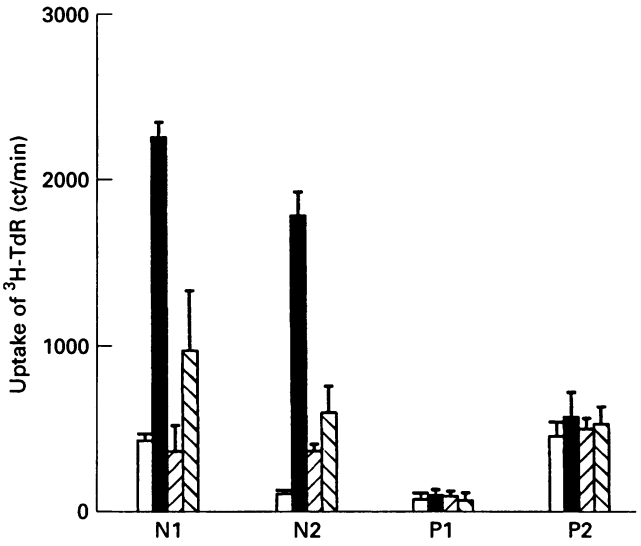

Fig. 3. Uptake of ^{3}H-thymidine (TdR) into DNA (mean ct/min ± s.e.m., number of replicate wells = 3 for normals and 4 for patients) by T cells from two normal donors (N1, N2) and two CVID group B patients (P1, P2) in response to low density cells (LDC) pulsed with purified protein derivative (PPD; ■), peptide 38A (▨), peptide 38G (▧) or unpulsed (□). T cells from N1 responded significantly to PPD ($P < 0{\cdot}001$) but not to peptide 38A; their response to peptide 38G more than doubled the background response, but did not quite reach significance ($P = 0{\cdot}06$). T cells from N2 responded significantly to PPD ($P < 0{\cdot}001$), to 38A ($P < 0{\cdot}001$) and to 38G ($P < 0{\cdot}01$). No response by T cells from the two patients to PPD or the peptides reached significance.

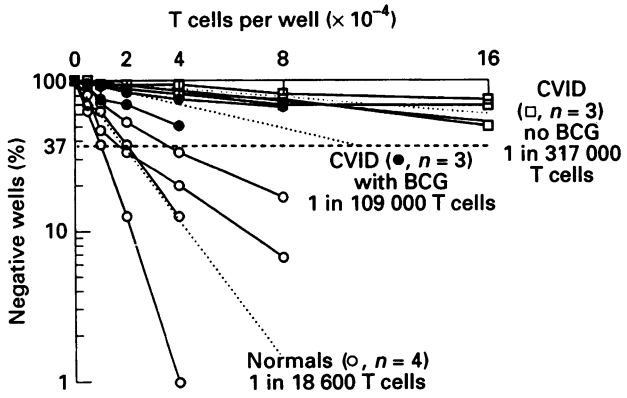

Fig. 5. Limiting dilution analysis of the frequency of purified protein derivative (PPD)-responsive T cells from four normal donors and six CVID patients in two groups; those patients who had received bacille Calmette–Guérin (BCG) vaccination (two group C, one group B) and those who had not (one each from groups A, B and C). Limiting dilution cultures were done with 16–24 replicates of various dilutions of T cells (two-fold serial dilutions beginning with 4–16 × 10^4 T cells per well) and always with 2 × 10^3 PPD-pulsed low density cells (LDC) per well. Positive wells were those with uptake of ^{3}H-thymidine (TdR) greater than mean +3 × s.d. of the cultures containing the same concentration of T cells and 2 × 10^3 unpulsed LDC. The minimum number of replicate wells for the negative controls without PPD was never less than eight. The percentage of negative wells in the PPD cultures was plotted against the number of T cells per well. For each of the three donor groups, the mean responder cell frequency was interpolated or extrapolated at the 37% non-responder well level by a calculated linear regression fixed to the point of origin using a best fit from the data from all the donors in each normal or CVID donor group.

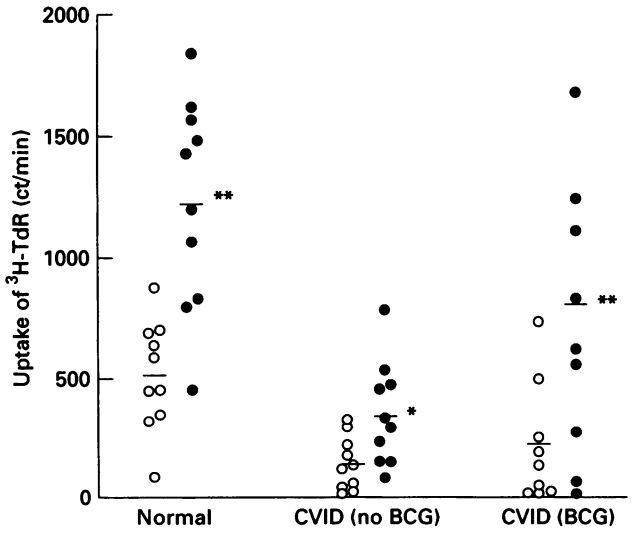

Fig. 4. Individual uptake of ^{3}H-thymidine (TdR) into DNA (ct/min) on day 5 by T cells cultured with unpulsed (○) and purified protein derivative (PPD)-pulsed (●) low density cells (LDC) from healthy normal donors (n = 10) and from CVID patients with ($n = 9$) and without ($n = 10$) a history of bacille Calmette–Guérin (BCG) vaccination. The numbers of patients in the different subgroups were five group A, one B, four C without BCG and two group A, six B and one C with a history of BCG vaccination. Mean values are also shown. *$P < 0{\cdot}05$; **$P < 0{\cdot}001$.

history of BCG vaccination, but some of the CVID patients had not received vaccination often because they were diagnosed as immunodeficient before their adolescence. Figure 4 shows the individual uptake of ^{3}H-TdR into DNA by T cells from normal donors (n = 10) and from CVID patients with ($n = 9$) and without (n = 10) a history of BCG vaccination. When the mean values were calculated, normal T cells had a significant mean response to PPD ($P < 0{\cdot}001$), as did the patients who had a history of BCG vaccination ($P < 0{\cdot}001$) (Fig. 4). The mean response of the patients who had received BCG was significantly less than the response of normal cells ($P < 0{\cdot}05$). Many of the CVID patients with BCG had individual responses within the normal range. Patients without BCG had a very small but still significant response (Fig. 4).

Frequency of PPD-specific T cells is reduced in CVID

Limiting dilution analysis was performed with T cells from four normal donors and six CVID patients. Figure 5 shows that within the T cells put into the cultures, the estimated mean frequency of the PPD-primed T cells for the control preparations from normal donors was 1/18 600 (range 1/10 000–1/35 000), for cells from those patients (n = 3) who had been vaccinated with BCG the frequency was 1/109 000 (range 1/52 800–1/166 000), and for those patients (also n = 3) who had no history of BCG vaccination the frequency was 1/317 000 (range 1/230 000–1/495 000). Using the data from Fig. 5, the mean regression coefficient for the patient group with BCG was significantly different from the normal regression coefficient

Table 1. Effects of CD8 T cell depletion in purified protein derivative (PPD)-specific responses (ct/min) in normal controls and CVID patients

T cell preparation	In the presence of: Unpulsed LDC	In the presence of: PPD-pulsed LDC	*P*
Normal controls			
Exp. 1			
Whole T cells	267[a] ± 48	1514 ± 161	<0·01
CD8-depleted	344 ± 39	2251 ± 170	<0·001
Exp. 2			
Whole T cells	520 ± 41	1615 ± 263	<0·01
CD8-depleted	838 ± 196	2293 ± 380	<0·01
CVID patients			
Exp. 1 (BCG)			
Whole T cells	264 ± 27	299 ± 37	NS
CD8-depleted	371 ± 143	406 ± 156	NS
Exp. 2 (no BCG)			
Whole T cells	304 ± 155	538 ± 158	NS
CD8-depleted	242 ± 69	473 ± 164	NS

[a]T cells from two normal donors and two CVID-B patients (8×10^4/well) with or without depletion of $CD8^+$ T cells were cultured in the presence of autologous unpulsed or PPD-pulsed low density cells (LDC) (2000 per well) for 5 days. ^{3}H-thymidine incorporation by the T cells during the last 2 h was counted. The numbers represent mean ct/min ± s.d. of three replicates. The difference in the responses due to PPD were significant in normal controls, but not in patients. When the T cells were depleted of $CD8^+$ cells, the mean response was significantly increased in one normal control (Exp. 1) and not in the other normal (Exp.2) or in either of the CVID patients. One patient had had bacille Calmette–Guérin (BCG) vaccination and the other had not.

($P = 0{\cdot}05$). The number of PPD-specific T cells from those CVID patients with a history of BCG was six times less than normal (Fig. 5).

CVID PPD-specific responses are still depressed when $CD8^+$ cells are depleted

Having confirmed by depletion experiments that normal PPD responses require $CD4^+$ cells (data not shown), we determined the effect of depletion of $CD8^+$ cells on the responses to PPD in T cell preparations from two normal donors and from two CVID patients (Table 1) (both belonged to CVID B group). All responses by whole T cell preparations or by $CD8^+$ cell-depleted T cells from normal controls showed significant PPD-specific T cell responses. The increase in PPD-specific responses when the $CD8^+$ cells were depleted reached significance in one normal control but not in the other. On the other hand, T cells from both CVID patients still failed to show significant PPD-specific responses after $CD8^+$ cell depletion. One patient had a history of BCG vaccination and the other had not.

DISCUSSION

In the present study, antigen responsiveness was measured by culturing T cells with PPD-pulsed LDC. In all subgroups of CVID (A, B and C), which correlate with clinical status and B cell defect in this disease, mean PPD-specific responses were significantly reduced from normal values, similar to our previous report [10]. That this occurs in all subgroups of CVID patients contrasts with the defect in responses to mitogens which we and others have previously reported in only about 30% of patients [5,21,22], suggesting that the problem with antigen-driven T cell responses is more fundamental to the pathogenesis of CVID.

When the vaccination status of the CVID patients was taken into account it was clear that even those who had received BCG during adolescence had a significantly sub-normal mean PPD-driven DNA synthesis.

We have found a clear explanation for these differences in antigen-driven responses, since by limiting dilution analysis the frequency of PPD-specific T cells in CVID was reduced compared with that in normal controls. In this limiting dilution study it is interesting to note that the mean response of the smaller number of wells that were 'positive' for the PPD response in the patients was not significantly different from the mean response from positive wells for normal subjects. This indicates no impairment in the proliferative capacity of these cells. It is also clear that those patients who had not been vaccinated with BCG, as expected, had an extremely low frequency of specific cells, but surprisingly, the cells were not absent. This could imply an effect due to environmental exposure to mycobacteria. Since CVID patients are not generally prone to mycobacterial disease, our experiments may establish that the low level of antigen-specific cells in this group is sufficient for immune protection.

There are two possible mechanisms to explain the lower frequency of PPD-specific cells in CVID. The first is the lack of development of normal numbers of PPD-specific primed T cells *in vivo* in CVID. This would imply a problem with primary responses to antigen *in vivo*. A second explanation is that normal priming occurs, but that there is a defect that we can detect *in vitro*, in the secondary response to antigen during the process of APC presenting antigen to primed T cells. This defect could be in the APC, the T cell or both. If there was a defect in the APC then it is likely to involve the processing and presentation of peptides in the context of HLA class II. We approached this question using synthetic peptides from the 38-kD antigen fom *Myco. tuberculosis*. The finding that both patients' cells failed to respond to both peptides implies that the defect is unlikely to be related to the processing of antigen. It is also unlikely that there was a more general defect in the APC, since they had not lost their capacity to stimulate a normal allogeneic response [10].

We could not detect any improvement in the PPD-specific T cell response in CVID by adding monocytes *in vitro*, although it has been reported that T cell proliferation to antigen can be restored in some CVID patients by the addition of normal monocytes [23].

Previously, we have reported that the defect in mitogen responses in a more restricted group of CVID patients may relate to abnormalities in the activation within the T cells at the level of protein kinase C (PKC) or downstream of PKC [24]. IL-2 is known to play a central role in the amplification of immune responses, and there is evidence that the defect in some CVID patients in proliferation in response to mitogens is accompanied by a reduction in secretion of IL-2 [5,8,24]. We have detected no impairment of non-specific responses to extrinsic IL-2, which are known to be PKC-independent [26],

suggesting that there are no defects in the IL-2-induced signaling pathway that involve tyrosine kinase [27], in IL-2 receptor function, or in the apparatus of DNA synthesis. More importantly, we have shown that rIL-2 will not reverse the antigen-specific defect. This is consistent with the finding of a reduced frequency of otherwise normal antigen-specific T cells rather than an IL-2-reversible anergy of antigen-specific cells.

We have studied CVID patients in all of the different patient subgroups [12]. These subgroups have different laboratory and clinical features, ranging from patients with low numbers of circulating CD4$^+$ T cells and defective T cell proliferation with mitogens, to those with no T cell phenotypic abnormalities or gross functional defects. The CVID patient described by Fischer *et al.* whose T cell response to tetanus toxoid recovered after the addition of rIL-2 [7] might be an exceptional patient, or the difference may relate to the use of a different antigen.

Many CVID patients have a relative expansion of circulating CD8$^+$CD28$^-$ T cells which could suppress CD4$^+$ T cell proliferation [28]. However, in two patients the depletion of CD8$^+$ cells from cultures did not restore the PPD response to normal levels. This adds weight to the hypothesis that the defect is due to a reduction in the numbers of CD4$^+$ antigen-specific cells rather than aberrant suppression.

Our report that many CVID patients (within the CVID A and B groups) have low numbers of circulating CD4$^+$ T cells [29] supports the hypothesis of partial deletion of CD4$^+$ antigen-responsive cells. In addition, our present data show that even in CVID group C patients, whose circulating numbers of CD4$^+$ cells are normal [29], depletion of the antigen-specific cells still occurs. The patients in this study have not been HLA-typed, so it is not possible to comment on the relationship between the HLA haplotype skewing reported in CVID [30] and the loss of antigen-specific T cells reported here.

One obvious question is why patients with severe CD4$^+$ cell depletion in advanced HIV-related disease do not develop hypogammaglobulinaemia. The answer appears to lie in the ability of HIV or factors stimulated by the virus to override the B cells' requirements for CD4$^+$ cells. This view is strongly supported by the observation that antibody production in some CVID patients is switched on when they become infected with HIV [31].

In conclusion, we have shown that the ability of CVID T cells to respond to antigen *in vitro* may be affected by reduced numbers of antigen-specific responding T cells within the CD4$^+$ population. This reduction of responding T cells would help explain the variable and inconclusive results obtained by others when rIL-2 has been added *in vitro* to CVID T cells or given *in vivo* to patients.

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