Clin Exp Immunol 1997; **108**:9–13

Lack of specific antibody response in common variable immunodeficiency (CVID) associated with failure in production of antigen-specific memory T cells

I. KONDRATENKO, P. L. AMLOT*, A. D. B. WEBSTER & J. FARRANT *MRC Immunodeficiency Group and *Department of Clinical Immunology, Royal Free Hospital School of Medicine, London, UK*

(Accepted for publication 6 January 1997)

SUMMARY

Several T cell defects have been described in the antibody deficiency disease, CVID, but there have been few data on the generation of responses of specific T cell populations to primary neoantigens. We have now used immunization with the neoantigens, keyhole limpet haemocyanin (KLH) and DNP-Ficoll, to evaluate immune responses in CVID patients and normal donors. B and T cell responses were examined 2 and 4 weeks post-immunization. Sera were examined for IgM and IgG anti-KLH responses by ELISA and for anti-DNP-Ficoll activity by haemagglutination. The frequency of KLH-responsive T cells was measured by DNA synthesis in a limiting dilution culture system. Low density cells enriched for dendritic cells were pulsed with KLH and cultured with different numbers of autologous T cells. T cells from normal donors and from patients showed a low frequency of antigen-specific precursor T cells ($\leq$1:200 000). After KLH immunization the frequency increased in normal donors (1:60 000 and 1:30 000 at 2 and 4 weeks, respectively), while in CVID patients it did not change from the pre-immunization level. The defect may extend to a dysfunction of antigen-specific cells, rather than being solely due to the reduced numbers of cells, since mean responses of 'positive' wells were also reduced. The serum-specific antibody response paralleled the T cell data, in that all normal donors but none of the CVID patients generated IgG KLH-specific antibodies. CVID patients did produce IgM antibodies against the T-independent DNP-Ficoll, but at a lower level than normal controls. These data show that both T and B cells from CVID patients have defective responses to specific antigen, implicating both lineages in the antibody deficiency.

Keywords neoantigen common variable immunodeficiency primary antibody immunodeficiency limiting dilution antigen-specific T cell defect

INTRODUCTION

CVID is a disease of unknown cause characterized by recurrent bacterial respiratory and intestinal infection and an increased frequency of autoimmune and neoplastic disorders [1,2]. Patients with CVID have low levels of serum immunoglobulin of all isotypes and variable defects in cellular immunity [1–3]. Most CVID patients have circulating B cells, but these fail to secrete sufficient immunoglobulin *in vivo*.

B cells from some CVID patients can secrete IgM and occasionally IgG after stimulation *in vitro* with IL-2 [3]. This has provided the basis for dividing CVID patients into three groups (A, B and C) according to whether their B cells can be induced to secrete IgG + IgM (group C), only IgM (group B) or neither (group A). In general, the severity of the *in vitro* defect of immunoglobulin production corresponds with the severity of clinical features and T cell abnormalities.

Correspondence: Dr J. Farrant, MRC Immunodeficiency Group, Department of Clinical Immunology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, UK.

A number of T cell defects have been described in CVID patients. These include lymphopenia, low numbers of $CD4^+$ cells, a decreased *in vitro* response to phytohaemagglutinin (PHA), decreased IL-2 production and a lack of delayed hypersensitivity on skin testing with primary and recall antigens [4–13]. The proliferative response of T cells to recall antigen (e.g. purified protein derivative (PPD)) is low in most CVID patients, regardless of their ability to respond to PHA [14]. Previous work with limiting dilution analysis showed that this defect is related to a decrease in the number of antigen-specific T cells [5,14]. Using the same limiting dilution system, we now investigate the frequency of antigen-specific T cells to a neoantigen, keyhole limpet haemocyanin (KLH). KLH is the copper-containing protein which can be obtained in pure state from haemolymph of the keyhole limpet, *Megathura crenulata*. Since it is an inedible marine mollusc, prior human contact and sensitization is unlikely [15–17]. Normal donors and patients were immunized with KLH and the precursor frequency measured *in vitro*. DNP-Ficoll immunization was also done and specific antibody in serum was assessed for both the T-dependent and T-independent antigens.

MATERIALS AND METHODS

Donors

Peripheral blood (50 ml) was obtained from CVID patients attending the clinic for routine intravenous immunoglobulin replacement therapy and was collected in 25 U/ml of preservative-free heparin (CP Pharmaceuticals Ltd, Wrexham, UK). Healthy adult volunteer laboratory workers served as normal donors. In both donor groups the sex and age distributions were similar; there were twice as many men as women in both groups and the ages were between 26 and 71 years.

KLH immunization

KLH (Calbiochem) had been purified from an $(NH_4)_2SO_4$ slurry (Pacific Biomarine Lab. Inc., Venice, CA) as previously described [18], filter sterilized and stored at 30 mg/ml with 0·01% merthiolate at 4°C. Normal donors and CVID patients were immunized intradermally with 0·2 mg KLH in 0·1 ml saline in the upper arm [17]. The KLH was endotoxin-free, which was a criterion for its use [17]. In this series two patients were chosen to be from each of the three CVID groups A, B or C [3]. The response to KLH was examined before, 2 and 4 weeks after immunization [17]. Ethical committee approval was obtained.

DNP-Ficoll immunization

DNP-Ficoll was prepared as previously described [17] and 0·2 mg in 0·1 ml was injected subcutaneously in the opposite arm to KLH.

Cell separation

Mononuclear cells were isolated from blood by centrifugation over Ficoll–Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) at 600 ***g*** for 30 min. After washing the cells twice in Dulbecco's PBS without Ca and Mg (ICN Pharmaceuticals, Thame, UK), the mononuclear cells were incubated overnight at 5×10^6/ml in 21-cm^2 Petri dishes (Nunc Intermed, Roskilde, Denmark) in complete culture medium (CCM): RPMI 1640, containing L-glutamine 2 mM, penicillin 100 U/ml, and streptomycin 100 U/ml. Human AB serum (10%) was used. Non-adherent cells were centrifuged at 600 ***g*** for 10 min over hypertonic Metrizamide: 14·5 g of Metrizamide (Nicomed Pharma AS, Oslo, Norway) with 90 ml of RPMI 1640 (Dutch Modification: HEPES buffer 20 mM, $NaHCO_3$ 1·0 g/*l*, and NaCl 6·4 g/*l*, all from ICN, but without glutamine), together with serum (10 ml). Low-density cells (LDC) from the Metrizamide interface were used as the source of antigen-presenting cells (APC) rich with dendritic cells [14]. T cells were obtained from the Metrizamide gradients by rosetting the lymphocyte-containing pellet cells with sheep erythrocytes treated with neuraminidase (Calbiochem Novabiochem Ltd, Beeston, UK) 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 T cells obtained by lysing the neuraminidase-treated sheep erythrocytes were washed twice and used for culture.

Cell cultures

LDC were incubated with KLH (50 μg/ml) for 2 h at 37°C and then washed twice. For each well, 2000 LDC were mixed with different numbers of T cells, resuspended in CCM in the 96-well round-bottomed plates (Nunc Intermed) at a final volume of 50 μl. Control cultures contained T cells mixed with LDC not exposed to KLH. The plates were cultured in a humidified incubator (5% CO_2 at 37°C) for 5 days.

Measurement of DNA synthesis

Tritiated thymidine (ICN Pharmaceuticals), specific activity 2 Ci/mM, at 1 μCi/well was added 2 h before harvesting the cells on filter paper (Printed Filtermat A; Wallac Oy, Turku, Finland), using an automatic cell harvester (Tomtec; Wallac) for the 96-well microplate. Radioactivity was measured in a liquid scintillation counter (Betaplate 1205; Wallac).

Limiting dilution analysis

Various doubling dilutions of T cells (from 10 000 to 160 000 per well) were cultured each with 2000 LDC, previously pulsed with KLH, with 24 or 36 replicate wells at each T cell concentration. For controls without KLH, 6–12 replicate wells were used. Individual wells in the cultures with KLH-treated LDC were considered to be positive for a KLH-specific response if their thymidine uptake in ct/min was greater than the mean ct/min + 3 s.d. observed in wells containing the same number of T cells and control untreated LDC. The log 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 cell level, as described by Henry *et al.* [19], to calculate the number of T cells containing one antigen-specific cell. In addition, for the different conditions, mean levels of the uptake of ^{3}H-thymidine were calculated for the positive wells alone.

Anti-KLH responses in sera

The anti-KLH response was estimated by ELISA using a peroxidase system similar to the radioimmunoassay and ELISA used previously [17,20]. Serum and standard samples diluted with PBS–Tween were incubated in KLH-coated plates before detection with peroxidase-conjugated goat anti-human IgG or IgM (SBA, Birmingham, AL). The standard serum was obtained from an individual (PLA) multiply immunized with KLH. Absorbance was read using a Titertek Multiscan plate reader. It is possible that some intravenous gammaglobulin preparations contain antibodies to KLH. However, this does not invalidate the differences observed between the patient and normal donor groups.

Anti-DNP-Ficoll responses in sera

These were assayed before and after immunization by haemagglutination of trinitrophenol (TNP)-horse erythrocytes with doubling dilutions of serum starting at 1:20 as previously described [17].

RESULTS

T cell responses to KLH

Frequency of KLH-specific T cells varied between different individuals. Limiting dilution analysis is shown for a single normal donor (Fig. 1a) and a CVID patient (Fig. 1b) in response to the neoantigen KLH before, 2 and 4 weeks after immunization.

Detecting KLH-specific T cells in non-immunized individuals

Initially the possibility of detecting KLH-specific T cells in non-immunized individuals to induce a primary response *in vitro* was evaluated. Before immunization, T cells from both normal donors and patients were able to respond to KLH in high cell concentrations (80 000–160 000 cells per well). This primary *in vitro* response was high in four (< 30% of negative wells), low in three (> 30% of negative wells) and undetectable in one of the normal donors. In comparison, the primary response was high in one, low in four and undetectable in three CVID patients (not

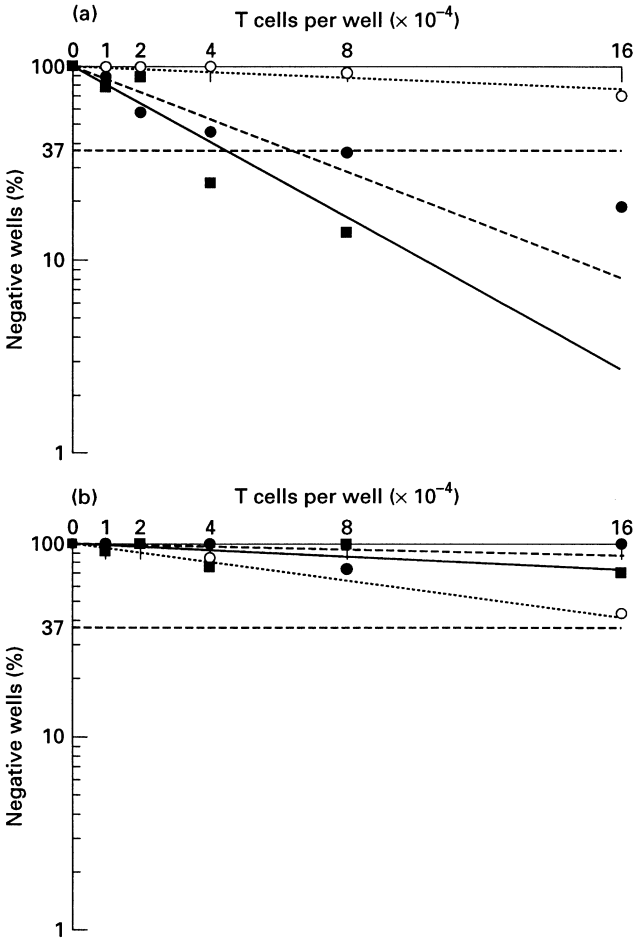

Fig. 1. Limiting dilution plot of keyhole limpet haemocyanin (KLH)-specific precursors from (a) a normal donor and (b) a CVID patient. The numbers of T cells (abscissa) in replicate wells is plotted against the log percentage of negative wells (ordinate). Positive wells responding to KLH are defined in Materials and Methods. A best fit linear regression for the data forced through the point of origin is used. The individual linear regressions correspond to T cell responses before (○), 2 (●) and 4 (■) weeks after KLH immunization. The intersection of a regression line with the 37% negative wells denotes one specific cell in that number of T cells (see Materials and Methods).

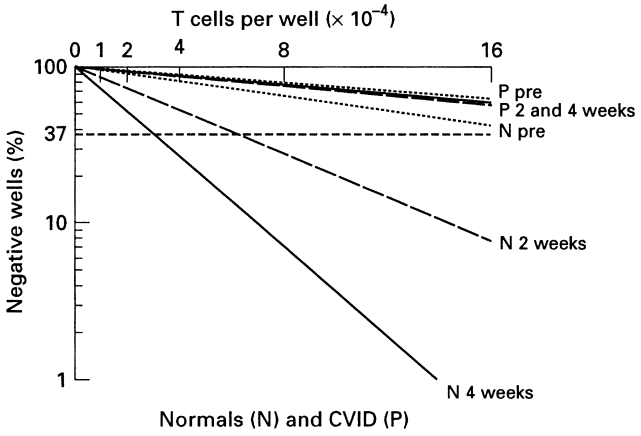

Fig. 2. Limiting dilution plot of keyhole limpet haemocyanin (KLH)-specific precursors from six normal donors and six CVID patients. There are two patients from each of the three groups in CVID (A, B and C) [3]. The mean regressions combine all of the data for each donor group for each cell preparation (at pre-immunization, and at 2 and 4 weeks after immunization). All other details are as in Fig. 1.

significant by $r \times c$ contingency table analysis). Before immunization, the extrapolated mean frequency of antigen-responding T cells in normal donors was 1:183 000 and in CVID patients 1:344 000. Despite the frequency of KLH-responding cells in patients being nearly half that in normal, this difference was not statistically significant.

T cell responses to KLH in immunized individuals

All normal donors and half of the patients were tested 2 weeks after immunization and all donors and patients were tested at 4 weeks. In one normal donor with a low primary *in vitro* response the frequency of KLH-responding cells did not increase significantly, in one normal individual with a high primary response the frequency increased 2 weeks after immunization but did not change at 4 weeks. In all other normal donors the number of antigen-responding T cells rose by 2 weeks and still further by 4 weeks after immunization. Figure 2 shows the best-fit mean regression lines for the limiting dilution experiments from all donors calculated for the normal and CVID groups at each of the three time points. The data are from six normal donors and six CVID patients. Deliberately, for this functional test, the CVID donors were chosen from all patient subgroups (two each from groups A, B and C) [3], thus including patients with and without $CD4^+$ cell depletion [21]. The overall frequency of KLH-responding cells in normal subjects was significantly increased and after 2 weeks was 1:62 000 and after 4 weeks became 1:30 000. In the patient with the high primary response before immunization, and in two with the low response, the frequency of specific T cells decreased at 2 and 4 weeks. For the average responses by CVID patients, there was no significant increase in the mean number of antigen-responding cells after immunization either at 2 or at 4 weeks (1:287 000 and 1:297 000 antigen-responding cells).

Table 1 shows the statistical analysis of these regressions. In the normal group there was a significant increase in the mean frequency of KLH-responsive cells after immunization by three-fold at 2 weeks and six-fold at 4 weeks. In the CVID group the frequency did not change significantly following immunization. There were no significant differences between the patient subgroups (data not shown).

Figure 3 shows the mean value, in the positive wells only, of the uptake of ^{3}H-thymidine in response to KLH in the same experimental series used for limiting dilution. It can be seen that the increase in mean KLH-driven DNA synthesis which occurred with normal T cells following KLH immunization, was absent in the CVID group.

Anti-KLH antibody synthesis

All normal donors (9/9) but none of the CVID patients (0/8) made IgG anti-KLH responses at 2 and 4 weeks after immunization (χ^2 P value < 0·0001). For IgM anti-KLH, the corresponding figures were 8/9 for normal donors and for the CVID patients 0/8 (χ^2 P value < 0·0004).

Anti-DNP-Ficoll antibody synthesis

Table 2 shows that there was no significant difference in the

Table 1. Increase in the frequency of keyhole limpet haemocyanin (KLH)-specific T cells in normal but not CVID donors after immunization with KLH: statistics for the limiting dilution study of Fig. 2

Donor group (*n*)	Immunization status	Regression slope (mean)	Regression slope (s.d.)	Frequency of KLH-positive T cells	Increase of KLH-positive T cells	*P**
	Pre	0·012 55	0·006 05	1:344 000	—	—
CVID	2 weeks post	0·015 02	0·005 94	1:287 000	×1·20	0·52 (NS)
(6)	4 weeks post	0·014 53	0·006 59	1:297 000	×1·16	0·62 (NS)
	Pre	0·023 60	0·018 00	1:183 000	—	—
Normal	2 weeks post	0·069 62	0·030 48	1:62 000	×2·95	0·001 33
(6)	4 weeks post	0·143 60	0·060 98	1:30 000	×6·08	0·000 05

*Compared with pre-immunization values (*t*-test).

number of donors in both the normal and CVID groups that responded to the T cell-independent antigen DNP-Ficoll. However, Table 2 also shows that in normal donors the titre of anti-DNP-Ficoll antibody was significantly greater than in CVID patients. Almost all of the antibody detected in both groups of donors was IgM (dithiothreitol-sensitive).

DISCUSSION

An important contribution to the hypogammaglobulinaemia in CVID is made by T cell defects preventing normal B cell function. Data suggest that whereas defective mitogen responses by T cells can occur in some groups of CVID patients, it is the defective antigen-specific responses in all patients which may be the qualifying and determining feature leading to the clinical manifestation of CVID. For example, we have reported that CVID T cells have a significantly reduced mean response to the recall antigens PPD or tetanus toxoid, whereas responses to stimulation with alloantigen were normal [14]. We subsequently measured the precursor frequency by limiting dilution and showed that for PPD there was a reduced frequency of antigen-specific precursors within the T cell preparations from the patients [5]. Other reports confirm that the defect in T cell antigen responses in CVID can occur even when responses to anti-CD3 or protein kinase C (PKC) stimulation, using phorbol ester, are normal [22,23]. Eibl *et al.* have also shown that the defect occurs on stimulation with superantigen as well as with antigen, and that there was a reduced expression of IL-2 mRNA and of inositol 1,4,5 triphosphate [24,25]. These data suggest a defect in the helper capacity of $CD4^+$ T cells for B cell differentiation, although in some patients there may be additional suppressor mechanisms.

The present data extend the previous findings to demonstrate that defective T cell responses are also seen with a neoantigen such as KLH. Unlike bacille Calmette–Guérin (BCG), for the previous PPD data, KLH was a new antigen for both groups of donors. This neoantigen status for KLH is not invalidated by the small 'primary' T cell response which occurs *in vitro* from some normal donors and even a few patients. The response seen to KLH seems to be a primary response, despite some cross-reactive antibodies thought to be natural antibodies [17]. The data also demonstrate for the first time within the same experiment that it is possible for the T cell defect to precede the failure of antigen-specific IgG antibody production. This defect occurred in patients from all three subgroups (A, B, C), previously defined by B cell responses to IL-2 [3]. Since gross $CD4^+$ T cell depletion is only a feature of group A and B patients with CVID [21], it does not seem to be closely

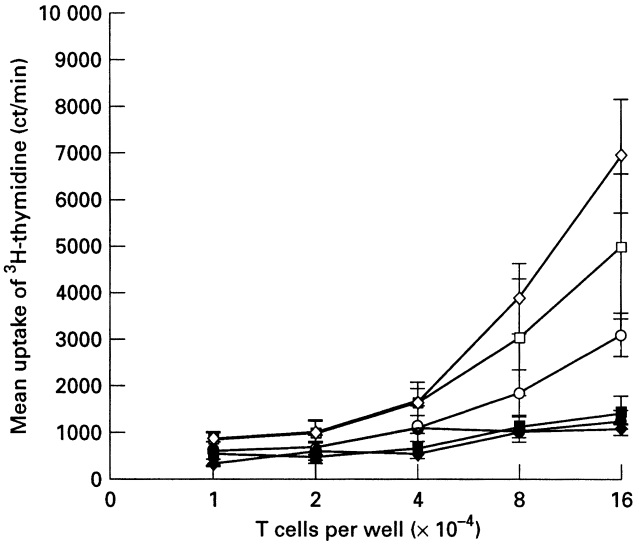

Fig. 3. The mean responses to keyhole limpet haemocyanin (KLH) (ct/min ± s.e.m.) of the uptake of ^{3}H-thymidine of the positive wells alone from the same limiting dilution series of experiments shown in Fig. 2. Positive wells are as defined in Materials and Methods and in Fig. 1. Pre-immunization: ○, normal; ●, CVID. Post 2 weeks: □, normal; ■, CVID. Post 4 weeks: ◇, normal; ◆, CVID.

Table 2. Anti-DNP-Ficoll responses: number of donors responding to DNP and titre of anti-DNP antibodies

	DNP responders		Median titre of anti-DNP		
	2 weeks	4 weeks	Pre-	2 weeks*	4 weeks*
CVID	2/9	4/8	< 20	40	40
Controls	4/9	7/9	< 20	640	320
P†	0·62	0·33	0·33	0·01	0·014

*Mean rise in titre compared with pre-imunization level.

†For the proportions, a Fisher's exact test was used and for the reciprocal titre $\log_2$, a Mann-Whitney was performed (all pre-immunized donors were analysed but only responders were analysed at 2 and 4 weeks).

linked to the failure of T cells to respond to antigen and the subsequent B cell immunodeficiency. The CVID B cells do retain some ability to make T-independent IgM antibodies to DNP-Ficoll, albeit at a reduced level.

Three further points can be made. First, in CVID the mean response of the low numbers of wells positively responding to the antigen KLH is very low compared with the normal mean response. This suggests that the defect may extend to a dysfunction of antigen-specific cells, rather than being solely due to the reduced numbers of such cells. This is supported by the altered levels of intracellular cytokine protein [26], cytokine mRNA [22] or second messenger (inositol 1,4,5 triphosphate) [24,25] observed on T cell stimulation in CVID. Second, the equivalent (but low) measure of antigen-specific precursor cells before immunization in the two donor groups shows that the 'repertoire' of response to this antigen is normal in CVID. This implies that there is no general loss of antigen-specific T cells, as occurs following thymic atrophy [27], but a post-activation loss of specific T cells following exposure to antigen. The mechanism of the failure to respond is not clear, but disturbed cytokine profiles [26] and increased levels of markers of activation (e.g. HLA-DR) [26] imply that a state of T cell anergy may exist. Third, the defective T cell response cannot automatically be interpreted to mean that this is the primary defect in CVID. The patients studied have had long-term hypogammaglobulinaemia and immunoglobulin replacement therapy, and we cannot exclude the remote possibility that the latter interferes with antigen responses. However, in XLA patients on the same therapy, *in vitro* T cell responses to tetanus toxoid are normal [14]. Despite this apparent gross abnormality of $CD4^+$ T cell function which explains the failure to generate delayed hypersensitivity skin responses in CVID [28], most patients do not suffer from opportunistic viral and fungal infections, unlike AIDS patients.

REFERENCES

1 Cunningham-Rundles C. Clinical and immunologic analyses of 103 patients with common variable immunodeficiency. J Clin Immunol 1989; **9**:22–33.

2 WHO Scientific Group. Primary immunodeficiency diseases. Clin Exp Immunol 1995; **99**:1–24.

3 Bryant A, Calver NC, Toubi E, Webster AD, Farrant J. Classification of patients with common variable immunodeficiency by B cell secretion of IgM and IgG in response to anti-IgM and interleukin-2. Clin Immunol Immunopathol 1990; **56**:239–48.

4 Lopez-Botet M, Fontan G, Garcia Rodriguez MC, de Landazuri MO. Relationship between IL 2 synthesis and the proliferative response to PHA in different primary immunodeficiencies. J Immunol 1982; **128**:679–83.

5 Funauchi M, Farrant J, Moreno C, Webster ADB. 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. Clin Exp Immunol 1995; **101**:82–88.

6 Kruger G, Welte K, Ciobanu N *et al.* Interleukin-2 correction of defective *in vitro* T-cell mitogenesis in patients with common varied immunodeficiency. J Clin Immunol 1984; **4**:295–303.

7 North ME, Akbar AN, Borthwick N *et al.* Co-stimulation with anti-CD28 (Kolt-2) enhances DNA synthesis by defective T cells in common variable immunodeficiency. Clin Exp Immunol 1994; **95**:204–8.

8 North ME, Spickett GP, Allsop J, Webster AD, Farrant J. Defective DNA synthesis by T cells in acquired 'common-variable' hypogammaglobulinaemia on stimulation with mitogens. Clin Exp Immunol 1989; **76**:19–23.

9 North ME, Webster AD, Farrant J. Defects in proliferative responses of T cells from patients with common variable immunodeficiency on direct activation of protein kinase C. Clin Exp Immunol 1991; **85**:198–201.

10 Rump JA, Jahreis A, Schlesier M, Drager R, Melchers I, Peter HH. Possible role of IL-2 deficiency for hypogammaglobulinaemia in patients with common variable immunodeficiency. Clin Exp Immunol 1992; **89**:204–10.

11 Spickett GP, Webster AD, Farrant J. Cellular abnormalities in common variable immunodeficiency. Immunodef Rev 1990; **2**:199–219.

12 Spickett GP, Matamoros N, Farrant J. Lymphocyte surface phenotype in common variable immunodeficiency. Disease Markers 1992; **10**:67–80.

13 Zielen S, Dengler TJ, Bauscher P, Meuer SC. Defective CD2 T cell pathway activation in common variable immunodeficiency (CVID). Clin Exp Immunol 1994; **96**:253–9.

14 Stagg AJ, Funauchi M, Knight SC, Webster AD, Farrant J. Failure in antigen responses by T cells from patients with common variable immunodeficiency (CVID). Clin Exp Immunol 1994; **96**:48–53.

15 Curtis JE, Hersh EM, Harris JE, McBride C, Freireich EJ. The human primary immune response to keyhole limpet haemocyanin: interrelationships of delayed hypersensitivity, antibody response and *in vitro* blast transformation. Clin Exp Immunol 1970; **6**:473–91.

16 Curtis JE, Hersh EM. The human secondary immune response to keyhole limpet haemocyanin. Clin Exp Immunol 1972; **10**:171–7.

17 Amlot PL, Hayes AE, Gray D, Gordon-Smith EC, Humphrey JH. Human immune responses *in vivo* to protein (KLH) and polysaccharide (DNP-Ficoll) neoantigens: normal subjects compared with bone marrow transplant patients on cyclosporine. Clin Exp Immunol 1986; **64**:125–35.

18 Herscowitz HB, Harold WW, Stavitsky AB. Immunochemical and immunogenic properties of a purified keyhole limpet haemocyanin. Immunology 1972; **22**:51–61.

19 Henry C, Barbrook L, Vann DC, Kodin D, Mishel BB, Shigii SM, eds. Selected methods of cellular immunology. San Francisco: Feeman Press, 1980.

20 Devey ME, Bleasdale-Barr KM, Bird P, Amlot PL. Antibodies of different human IgG subclasses show distinct patterns of affinity maturation after immunization with keyhole limpet haemocyanin. Immunology 1990; **70**:168–74.

21 Farrant J, Spickett G, Matamoros N *et al.* Study of B and T cell phenotypes in blood from patients with common variable immunodeficiency (CVID). Immunodeficiency 1994; **5**:159–69.

22 Fischer MB, Hauber I, Vogel E, Wolf HM, Mannhalter JW, Eibl MM. Defective interleukin-2 and interferon-gamma gene expression in response to antigen in a subgroup of patients with common variable immunodeficiency. J Allergy Clin Immunol 1993; **92**:340–52.

23 Hauber I, Fischer MB, Maris M, Eibl MM. Reduced IL-2 expression upon antigen stimulation is accompanied by deficient IL-9 gene expression in T cells of patients with CVID. Scand J Immunol 1995; **41**:215–9.

24 Fischer MB, Hauber I, Eggenbauer H *et al.* Activation of CVID patients' T cells with conventional antigens and superantigens. Immunodeficiency 1993; **4**:15–16.

25 Fischer MB, Wolf HM, Hauber I *et al.* Activation via the antigen receptor is impaired in T cells, but not in B cells from patients with common variable immunodeficiency. Eur J Immunol 1996; **26**:231–7.

26 North ME, Ivory K, Funauchi M, Webster ADB, Lane AC, Farrant J. Intracellular cytokine production by human $CD4^+$ and $CD8^+$ T cells from normal and immunodeficient donors using directly conjugated anti-cytokine antibodies and three-colour flow cytometry. Clin Exp Immunol 1996; **105**:517–22.

27 Mackall CL, Fleisher TA, Brown MR *et al.* Age, thymopoiesis, and $CD4^+$ T-lymphocyte regeneration after intensive chemotherapy. New Eng J Med 1995; **332**:143–9.

28 Luquetti A, Newton CA, Webster AD. Cellular immunity in primary hypogammaglobulinaemia: evidence for a generalised lymphocyte defect in some patients with 'common' variable hypogammaglobulinaemia. Allergologia et Immunopathologia 1981; **9**:295–306.