Clin. exp. Immunol. (1989) **78**, 1–6

Accessory and T cell defects in acquired and inherited hypogammaglobulinaemia

K. E. ROZYNSKA, G. P. SPICKETT,* M. MILLRAIN, A. EDWARDS, A. BRYANT, A. D. B. WEBSTER & J. FARRANT *Division of Immunological Medicine, Clinical Research Centre, Harrow, England*

(Accepted for publication 19 June 1989)

SUMMARY

Cellular defects in patients with common variable hypogammaglobulinaemia (CVH) and X-linked agammaglobulinaemia (XLA) have been studied *in vitro*, using a mitogen-driven system of immunoglobulin production. We have confirmed our previous finding of impaired low-density (dendritic) accessory cell function in CVH and now show that accessory cell function is normal in XLA. We demonstrate that macrophage accessory function is normal in CVH. T cell help for IgM production is also deficient in CVH, and T cell help in XLA is also abnormal for both IgG and IgM. Some XLA patients have excessive T suppressor activity. The contribution of these defects to the clinical states is discussed.

Keywords common variable hypogammaglobulinaemia T cell defects
X-linked agammaglobulinaemia accessory cell defects

INTRODUCTION

Acquired (common variable) hypogammaglobulinaemia (CVH) is a rare disease in which patients present with a range of bacterial infections, due to a failure of specific antibody production (WHO Report, 1986). CVH occurs in patients of both sexes and at any age, although there are peaks of incidence in childhood and in the third decade (Asherson & Webster, 1980). The cause is unknown, although a retroviral aetiology has been postulated (Webster *et al.*, 1986); however, most patients do not have evidence for persistent infection (Spickett *et al.* 1988).

Many studies *in vitro* have shown that the B cells in CVH are defective (De La Concha *et al.*, 1977; De Gast *et al.*, 1980; Filipovich & Kersey, 1983). A sub-group of patients has excessive T cell suppression *in vitro*, often associated with a thymoma (Waldmann *et al.*, 1974; Platts-Mills *et al.*, 1981). Early reports indicated that T cell helper function was normal (De La Concha *et al.*, 1977), but others have demonstrated variable defects (De Gast *et al.*, 1980; Ashman, Saxon & Stevens, 1980; Smith *et al.* 1985; Fiedler *et al.*, 1987). We have previously shown a defect of T cell help for IgM production (Farrant *et al.*, 1985).

X-linked agammaglobulinaemia (XLA) is a clinically similar, but genetic disease that presents in the first 2 years of life. There is a block in the differentiation of B cell precursors at the pre-B cell stage (Pearl *et al.* 1978; Filipovich & Kersey, 1983). However, other aspects of immune function have been thought to be normal, although excessive T cell suppression has been reported in occasional patients (Siegal, Siegal & Good, 1976; Dosch & Gelfand, 1978).

Eibl and co-workers have provided evidence that the accessory function of macrophages from CVH patients is poor (Eibl *et al.* 1982a; 1982b; Mannhalter, Ahmad & Eibl, 1986). We have previously demonstrated that low-density cells, enriched for dendritic cells, from patients with CVH have poor accessory cell function for pokeweed mitogen (PWM) with allogeneic B cells (Farrant *et al.*, 1985). We have now extended these observations to ask the following questions in relation to cellular function in CVH: (i) is the defect in dendritic cells accessory function independent of the defect in T cells? (ii) is the same defect of accessory function present in macrophages as well as in dendritic cells? and (iii) what are the roles of impaired T cell help and excess T cell suppression? CVH cells were compared against both cells from normal subjects and patients with XLA.

MATERIALS AND METHODS

Patients

A total of 12 patients with CVH and six patients with XLA were studied. For the comparison of XLA with CVH, only male CVH patients were studied, but for the comparison of dendritic cell and macrophage function, both male and female CVH patients were used. Normal controls comprised healthy laboratory staff. Informed consent was obtained from all participants.

* Present address: Department of Immunology, John Radcliffe Hospital, Headington, Oxford, England.

Correspondence: J. Farrant, Division of Immunological Medicine, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, England.

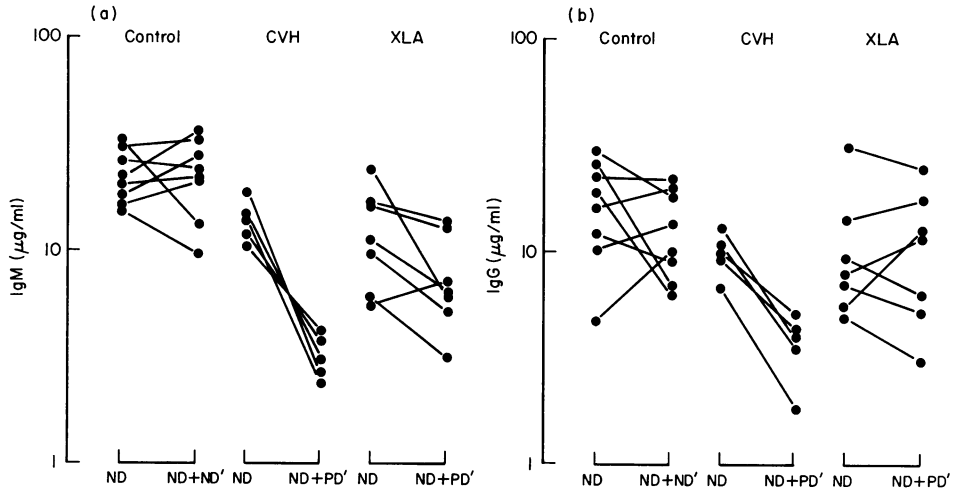

Fig. 1. Effect on accessory function of pokeweed mitogen (PWM) treated autologous normal cells (ND) by PWM-treated allogeneic normal cells (ND'), or patient cells (PD') from CVH or XLA patients. IgM (a) or IgG (b) was measured on day 10. Cells from common-variable hypogammaglobulinaemia (CVH) patients inhibited normal accessory function but this was less marked with X-linked agammaglobulinaemia (XLA) cells.

Table 1. Increase in surface expression of HLA-DR and decrease of CD14 in low-density cells treated with interferon-gamma (IFN-γ)

Donor	IFN-γ dose (U/ml)	Fluorescence Intensity (Mode channel number) HLA-DR IFN-γ −	HLA-DR IFN-γ +	CD14 IFN-γ −	CD14 IFN-γ +
Control (healthy individuals)					
1	200	288	736	656	464
2	200	400	704	672	432
3	200	640	752	720	512
4	100	400	768	752	624
5	100	608	640	768	656
6	100	688	864	784	640
7	50	320	512	640	624
CVH Patients					
1	100	432	688	800	720
2	100	448	832	736	688
3	100	432	752	784	672
4	100	464	768	752	624
5	50	272	656	688	608
6	50	560	752	720	544

Cells were incubated in 1 ml of medium with or without IFN-γ; 200 U/ml were found to increase cell death, and doses of 100 or 50 U/ml were therefore used for patients. Changes in intensity of antigen expression were determined using the mode channel for positive cells for each sample.

Lymphocyte separation

Samples of peripheral blood (40 ml) were taken from patients and controls. The procedures for cell separation and for culture were as previously reported (Farrant *et al.*, 1985). Briefly, the blood was defibrinated, and the serum replaced with medium (RPMI 1640 with penicillin/streptomycin 100 U/ml and glutamine 2 mM) before layering onto Ficoll-Paque (10 ml diluted blood to 10 ml Ficoll). The cells were centrifuged at 680 ***g*** for 30 min.

The cells from the interface of the gradient were washed twice and resuspended at 5×10^6/ml in medium containing 10% fetal calf serum (FCS). They were then incubated in 5-ml aliquots on Petri dishes (Nunc, Roskilde, Denmark) at 37°C for 90 min, after which the non-adherent cells were removed by gentle pipetting. These were layered onto metrizamide (14·5% w/v, analytical grade, Nygaard) and centrifuged for 10 min (Knight *et al.*, 1982). Low-density cells from the interface of the metrizamide gradient were washed twice in medium with FCS and kept overnight at 37°C. This preparation is enriched for dendritic cells, but contains some non-adherent monocytic cells (Knight *et al.*, 1986). Half the cells were treated with pokeweed mitogen (PWM) (1 μg/ml) at 37°C for 1 h, and excess PWM was removed by washing twice with medium containing FCS. Adherent cells were removed from the Petri dishes using gentle pressure from the rubber piston of a 1-ml syringe, and were collected into polypropylene tubes, to minimize further losses. The cells were washed and kept at 37°C overnight, and treated with PWM as for the low-density cells.

T and non-T lymphocytes were separated from the pellet obtained from the metrizamide gradient. After washing twice in medium with FCS, the cells were resuspended to 3×10^6 cells/ml. Cells were mixed 3:1 with 1% neuraminidase-treated sheep erythrocytes (n-SRBC) in phosphate-buffered saline (PBS) and rosetted overnight. The rosettes were then gently resuspended and spun on Ficoll-Paque. The cells from the interface were washed twice and used as the responder non-T population. To

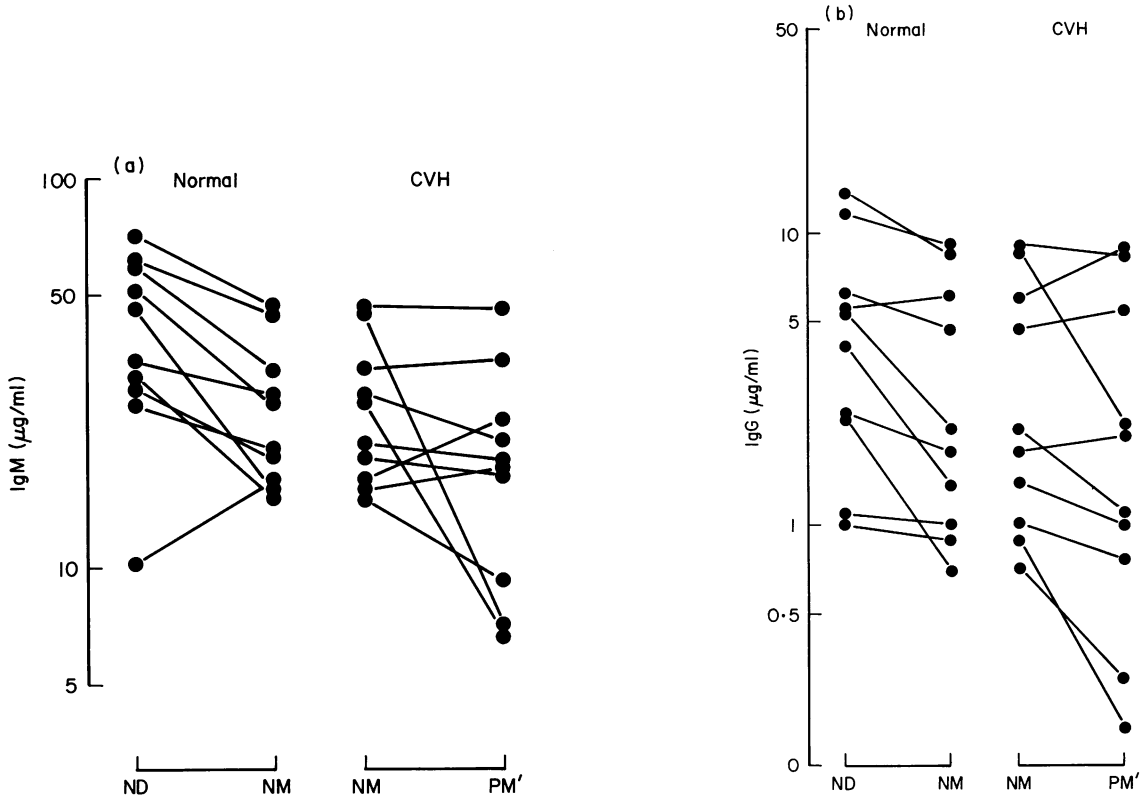

Fig. 2. Comparison of accessory function between normal dendritic cells (ND), normal macrophages (NM) and patient macrophages (PM') for IgM production (a) and IgG production (b). In general, normal macrophages were less effective than dendritic cells but there were no differences between normal and patient macrophages in most patients. CVH, common-variable hypogammaglobulinaemia.

obtain the T cells, the n-SRBC were lysed with buffered ammonium chloride solution, and the remaining cells were washed twice.

Both the T cells and the accessory cells were irradiated (2000 rad) before culture.

Lymphocyte culture

Hanging-drop cultures (20 μl/well) were set up in Terasaki plates, using a modification of Iscove's serum-free medium (Farrant *et al.*, 1984). Each well contained 2000 accessory cells, 80000 T cells and 40000 non-T cells in various autologous and allogeneic mixes. For each set of experimental conditions there were six replicate 20-μl wells.

The cultures were continued for 10 days in a 5% CO_2 humidified incubator at 37°C; then the Terasaki plates were carefully wrapped, frozen and stored at −70°C until assayed for immunoglobulin production.

ELISA

Flat-bottomed ELISA plates (96-well, Dynatech) were coated with aliquots (50 μl/well) of goat anti-human IgG (Sigma Chemical Co., St Louis, MO; diluted × 500 in coating buffer) or with rabbit anti-human IgM (Dako, High Wycombe, UK; diluted ×800 in coating buffer) and left overnight at room temperature. Non-specific binding to the plates was then blocked with aliquots (150 μl/well) of PBS containing 0·2% gelatin, 0·2% bovine serum albumin (BSA) and 0·1% sodium azide for 1 h at 37°C.

After thawing, each set of three replicate culture wells on the Terasaki plates was pooled, producing two samples to assay for every 6-well replicate set of culture conditions. An aliquot (10 μl) from each sample was diluted with 90 μl of blocking buffer in the wells of the top row of the ELISA plate. Eight doubling dilutions of the samples and of the common immunoglobulin standard (LAS-R, Hyland) both in blocking buffer (final volume in each well 50 μl) were then carried out and the plates were incubated for 1 h at 37°C. After adding 50 μl of goat anti-human IgG or goat anti-human IgM conjugated to alkaline phosphatase (Sigma, diluted ×1000 in blocking buffer) and incubating for 60 min at 37°C, substrate (1 mg/ml disodium *p*-nitrophenyl phosphate hexahydrate, 100 μl, Sigma) was added to each well and the colour read at 405 nm on a Multiscan ELISA reader. Between each step of the assay the plates were washed three times with PBS containing 0·02% Tween. Absorbance curves for diluted standards and sample were plotted and immunoglobulin concentrations calculated by microcomputer. Zero sample controls gave negligible absorbance.

Flow cytometry

For flow cytometric analysis, low-density cells prepared as above from healthy volunteers and CVH patients were incubated overnight at 37°C with either medium alone or varying doses of recombinant human interferon-gamma (IFN-γ) (kindly provided by Dr W. Fiers, Ghent, Belgium). Cells were then washed in PBS with 2% FCS. Aliquots of cells (1×10^6) were incubated for 30 min with 5 μl each of anti-HLA-DR-FITC and

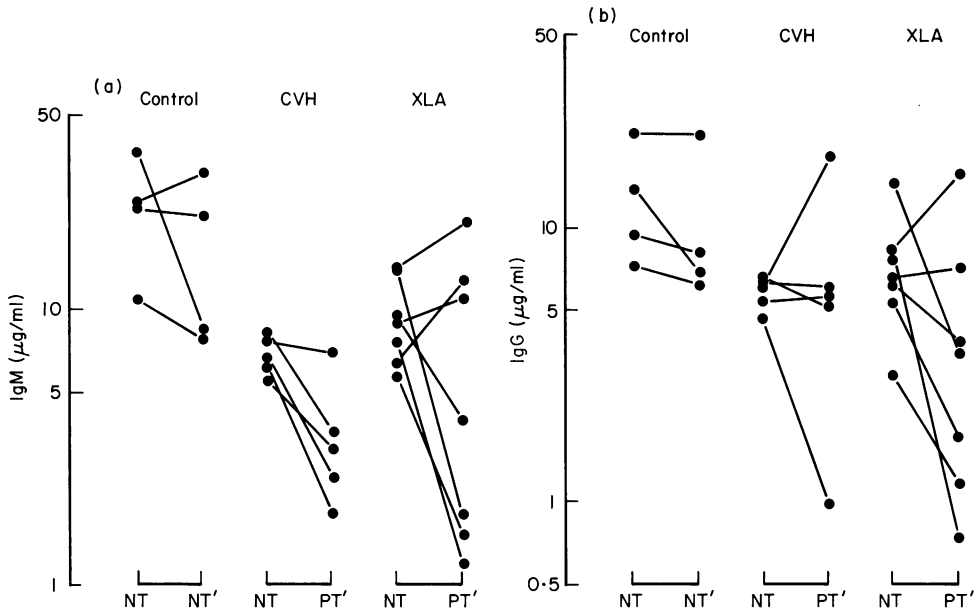

Fig. 3. Effect of allogeneic T lymphocytes from normal individuals (NT') or patients (PT') with either common-variable hypogammaglobulinaemia (CVH) or X-linked agammaglobulinaemia (XLA) on production of IgM (a) or IgG (b) in cultures with autologous normal B lymphocytes and pokeweed mitogen (PWM) treated dendritic cells. T cells from both CVH and XLA are less effective as helper cells than normal T cells.

Table 2. T cell suppression in patients with X-linked agammaglobulinaemia (XLA)

		Index of suppression*	
	Healthy control	IgM	IgG
Patient 1	1	0·35 s	0·53 s
	1	1·07	0·56 s
	2	1·06	1·31
	2	1·54	1·67
Patient 2	3	0·45 s	0·56 s
	3	0·29 s	0·25 s
	4	1·20	0·88
	4	0·98	0·90
Patient 3	5	0·34 s	0·17 s
	6	1·63	3·90
Patient 4	7	1·22	2·24
Patient 5	8	0·11 s	0·19 s
Patient 6	2	0·43 s	0·71 s

Excessive suppressor activity occurs when T cells from XLA patients are mixed with cells from some normal individuals but not others.

* Mean ratios of secreted immunoglobulin concentrations between a 1:1 mixture of patient T cells and normal cells and the appropriate control of twice the usual concentration of normal T cells. All cultures were done with control low-density and B cells. ⩾1·00, No suppression; s, suppression.

CD14-PE (Leu-M3) (Becton Dickinson). After washing, the cells were resuspended in PBS containing propidium iodide, and analysed on a FACStar-Plus flow cytometer. Dead cells and debris were excluded using propidium iodide, and analysis was confined to the 'monocytic' population, as determined by forward-*versus*-side scatter; this gated population comprised 50–70% of total cells.

RESULTS

Dendritic cells

The ability of dendritic cells from patients with CVH to act as accessory cells for PWM and allow production of IgM and IgG was compared with normal dendritic cells, always using normal T and B cells. As this combination is allogeneic, combinations of allogeneic normal cells served as controls. We also used mixtures of dendritic cells from XLA patients with normal dendritic cells as an allogeneic patient control group.

Figure 1 shows that, using PWM, the addition of CVH dendritic cells to normal dendritic cells inhibits the ability of the normal cells to act as accessory cells. An allogeneic mixture of dendritic cells from two different normals did not show this effect. The effect was also absent when dendritic cells from XLA patients were combined with normal dendritic cells (Fig. 1 a and b).

Table 1 shows that exposure of patients' low-density cells to IFN-γ leads to a reduction in CD14 expression, and an increase in DR-antigen expression, similar to that seen in normal control low-density cells.

Macrophages

When normal adherent cells, containing mainly macrophages but also a few cells of dendritic morphology, were treated with PWM and added to normal T and B cells, they were, on a cell-for-cell basis, less efficient than the dendritic cell preparation. However, substitution of CVH macrophages for normal macrophages failed to demonstrate any significant abnormality of accessory cell function for PWM in most of the patients (Fig. 2).

T cell help

Using the same system, but this time with cultures of autologous normal B cells and normal dendritic cells together, mixed with either autologous normal, allogeneic normal or patient T cells, it is possible to demonstrate abnormal T helper function for immunoglobulin production in some CVH patients and more dramatically in some patients with XLA. Figure 3 shows the results. In CVH, the lack of help (when present) is more marked for IgM than for IgG, whereas both isotypes are equally affected in XLA.

T cell suppression

When patient T lymphocytes are combined with normal T lymphocytes in the presence of normal dendritic and B cells, some, but not all XLA patients exhibit excessive suppressor activity towards normal T cells (Table 2). A feature of this effect was its dependence on the donor of the normal cells. Apart from one patient (not known to have a thymoma), there was no evidence of an increase in suppressor activity in CVH (data not shown).

DISCUSSION

The significant immunological defect in CVH is a failure to make specific antibody in response to antigenic challenge. It is not known whether this failure results entirely from a B cell defect, or whether defects in other cell lineages contribute. In a sub-group of CVH patients, mature B lymphocytes—as determined by a panel of monoclonal antibodies—are absent (Farrant *et al.*, 1989) but most patients have normal or near normal numbers of B cells. In the larger group with apparently mature B cells, combination with normal T cells and accessory cells does not provide a sufficient set of signals to induce significant immunoglobulin production (Farrant *et al.*, 1985; 1989).

We have previously shown that accessory function of low-density dendritic cell preparations from CVH patients is less efficient than that of normal cells in an allogeneic system (Farrant *et al.*, 1985). We have now shown that this defect occurs independently of any T cell defect, and, moreover, that the abnormal patient low-density dendritic cells are capable of interfering with normal accessory cell function. The significance of this finding is enhanced by the fact that in the absence of PWM treatment, the allo-induced, background immunoglobulin production is reduced when CVH dendritic cells are mixed with normal dendritic cells (Farrant *et al.*, 1985; and data not shown). In contrast, dendritic cells from patients with XLA showed no such abnormalities.

The low-density cell population is not pure, and contains cells of dendritic morphology with and without monocyte markers. One group of investigators has previously suggested that monocyte-negative dendritic cells are the most potent antigen presenters (Nussensweig *et al.*, 1980; Van Voorhis, Witmer & Steinman, 1983). An abnormality of major histocompatibility complex (MHC) class II antigen expression on CVH dendritic cells may explain poor accessory function, but would not explain the allogeneic suppression, and studies on CVH dendritic cells in our laboratory so far have not revealed any significant abnormalities of surface antigen expression. For example, the results obtained by flow cytometric analysis of class II MHC and CD14 antigen expression on the surface of low-density cells from CVH patients after exposure to IFN-γ strongly suggest that the observed defects in the functional capacity of the low-density cells are not due to a lack of class II on the cells, nor to a failure of up-regulation of class II by lymphokines.

It is known that monocytes may suppress immune responses (Fernandez & MacSween, 1981). Our adherent cell population, containing predominantly macrophages and monocytes, did not show evidence of suppression.

Previous work, using an antigen-driven system (Eibl *et al.*, 1982 a; b; Mannhalter *et al.*, 1986) has suggested that macrophage function is also abnormal in CVH. In contrast, we have shown that for a PWM-driven system, most CVH patients' macrophages function as well as normal macrophages, although both are less efficient than dendritic cells. This would be expected on the basis of previous work suggesting that dendritic cells are more potent than macrophages (Nussensweig *et al.*, 1980). Two patients showed a reduction in accessory function by their macrophages, and it may be that the defect is expressed only in some patients.

T cell function has been reported both as normal (De la Concha *et al.* 1977; Brenner *et al.*, 1984) and abnormal (Ashman *et al.*, 1980; Fiedler *et al.*, 1987). We have recently confirmed that T cells from some patients have abnormally low DNA synthesis in response to mitogens but not to interleukin-2 (IL-2) (North *et al.* 1989). The results presented here show that T cell helper function is abnormally poor in both CVH and XLA, and the effect seems to be more marked for IgM than for IgG, despite the clinically greater depression of IgG, especially in CVH. Smith *et al.* (1985) have also reported a T helper cell defect using PWM in one of two patients studied, and earlier De Gast *et al.* (1980) found defective T help in two of six CVH patients.

We have confirmed the finding that some patients with XLA may have excessive suppressor activity (Siegal *et al.*, 1976; Dosch & Gelfand, 1978). This activity seemed to be most marked in patients with the most severe disease, and occurred with B cells from some normal donors but not with others—indicating possibly that MHC antigens may be involved in its generation, although random mixes of cells from normal individuals did not exhibit this effect (data not shown). The T cell populations were irradiated prior to use, and a possible explanation is that there is an increase in radioresistant suppressor cells in XLA. Flow cytometry data have not shown any marked change in the numbers of $CD5^+$ or $CD8^+$ cells in the XLA patients included in this study (Spickett & Edwards, unpublished data). The presence of T cell defects in XLA, a primary B cell disorder, was unexpected although there is evidence of immaturity of the T cell phenotype (Matamoros, Abad & Webster, 1982; Tedder *et al.*, 1985).

We have described defects in both T cells and dendritic cells in CVH, but show that macrophage accessory function is normal. It is also clear that CVH is not a homogeneous disease

with much variability between patients. In XLA, dendritic cell function is normal whereas T cell help is poor, and T-cell mediated suppression may occur. In CVH, the low-density dendritic cell defect is not related to lack of antibody or to chronic infection, as the function of the dendritic cells from XLA patients under identical conditions is normal. It is still not clear, however, which of the cellular defects is primarily responsible for the fundamental defect, the failure of antibody production *in vivo*.

ACKNOWLEDGMENTS

We would like to thank Mrs J. Gilbert for secretarial help, Dr G. L. Asherson for helpful discussions and Dr W. Fiers for his kindness in providing the IFN-γ and for his patience.

REFERENCES

ASHERSON, G.L. & WEBSTER, A.D.B. (1980) *Diagnosis and Treatment of Immunodeficiency Diseases.* Blackwell Scientific Publications, Oxford.

ASHMAN, R.F., SAXON, A. & STEVENS, R.M. (1980) Profile of multiple lymphocyte functional defects in acquired hypogammaglobulinaemia, derived from *in vitro* cell recombination analysis. *J. Allergy clin. Immunol.* **65,** 242.

BRENNER, M.K., NORTH, M.E., CHADDA, H.R., NEWTON, C.A., MALKOVSKY, M., WEBSTER, A.D.B. & FARRANT, J. (1984) The role of B cell differentiation factors and specific T cell help in the pathogenesis of primary hypogammaglobulinaemia. *Eur. J. Immunol.* **14,** 1021.

DE GAST, G.C., WILKINS, S.R., WEBSTER, A.D.B., RICKINSON, A. & PLATTS-MILLS, T.A.E. (1980) Functional immaturity of isolated B cells from patients with hypogammaglobulinaemia. *Clin. exp. Immunol.* **42,** 535.

DE LA CONCHA, E.G., OLDHAM, G., WEBSTER, A.D.B., ASHERSON, G.L. & PLATTS-MILLS, T.A.E. (1977) Quantative measurements of T- and B-cell function in 'variable' primary hypogammaglobulinaemia: evidence for a consistent B-cell defect. *Clin. exp. Immunol.* **27,** 208.

DOSCH, H.M. & GELFAND, E.W. (1978) Functional differentiation of B lymphocytes in agammaglobulinaemia. III. Characterisation of spontaneous suppressor activity. *J. Immunol.* **121,** 2097.

EIBL, M.M., MANNHALTER, J.W., ZIELINSKI, C.C. & AHMAD, R. (1982a) Defective macrophage–T cell interaction in common varied immunodeficiency. *Clin. Immunol. Immunopathol.* **22,** 316.

EIBL, M.M., MANNHALTER, J.W., ZLABINGER, G., MAYR, W.R., TILZ, G.F., AHMAD, R. & ZIELINSKI, C.C. (1982b) Defective macrophage function in a patient with common variable immunodeficiency. *N. Engl. J. Med.* **307,** 803.

FARRANT, J., BRYANT, A., ALMANDOZ, F., SPICKETT, G., EVANS, S.W. & WEBSTER, A.D.B. (1989) B cell function in acquired 'common-variable' hypogammaglobulinaemia: proliferative response to lymphokines. *Clin. Immunol. Immunopathol.* **51,** 196.

FARRANT, J., BRYANT, A.E., LEVER, A.M.L., EDWARDS, A.J., KNIGHT, S.C. & WEBSTER A.D.B. (1985) Defective low-density cells of dendritic morphology from the blood of patients with common variable hypogammaglobulinaema: low immunoglobulin production on stimulation of normal B cells. *Clin. exp. Immunol.* **61,** 189.

FARRANT, J., NEWTON, C.A., NORTH, M.E., WEYMAN, C. & BRENNER, M.K. (1984) Production of antibody by human B cells under serum-free conditions. *J. Immunol. Methods,* **68,** 25.

FERNANDEZ, L.A. & MACSWEEN, J.M. (1981) The suppressive effects of monocytes in the autologous mixed lymphocyte reaction. *Immunology,* **44,** 653.

FIEDLER, W., SYKORA, K.W., WELLE, K., KOLITZ, J.E., CUNNINGHAM-RUNDLES, C., HOLLOWAY, K., MILLER, G.A., SOUZA, L. & MERTELSMANN, R. (1987) T cell activation defect in common variable immunodeficiency: restoration by phorbol myristate acetate (PMA) or allogeneic macrophages. *Clin. Immunol. Immunopathol.* **44,** 206.

FILIPOVICH, A.H. & KERSEY, J.H. (1983) B-lymphocyte function in immunodeficiency disease. *Immunol. Today,* **4,** 50.

KNIGHT, S.C., BALFOUR, S.M., O'BRIEN, J., BUTTIFANT, L., SUMERSKA, T. & CLARKE, J. (1982) Role of veiled cells in lymphocyte activation. *Eur. J. Immunol.* **12,** 1057.

KNIGHT, S.C., FARRANT, J., BRYANT, A.E., EDWARDS, A.J., BURMAN, S., LEVER, A., CLARKE, J. & WEBSTER, A.D.B. (1986) Non-adherent low-density cells from human peripheral blood contain dendritic cells and monocytes both with veiled morphology. *Immunology,* **57,** 595.

MANNHALTER, J.W., AHMAD, R. & EIBL, M.M. (1986) Deficient antigen presentation in a patient with common variable immunodeficiency. *Immunol. Pol.* **11,** 173.

MATAMOROS, N., ABAD, E. & WEBSTER, A.D.B. (1982) LDH isoenzymes in the T lymphocytes of patients with primary hypogammaglobulinaemia. *Clin. exp. Immunol.* **50,** 617.

NORTH, M.E., SPICKETT, G.P., ALLSOP, J., WEBSTER, A.D.B. & FARRANT, J. (1989) Defective DNA synthesis by T cells in acquired 'common-variable' hypogammaglobulinaemia on stimulation with mitogens. *Clin. exp. Immunol.* **76,** 19.

NUSSENSWEIG, M.C., STEINMAN, R.M., GUTCHINOV, B. & COHN, Z.A. (1980) Dendritic cells are accessory cells for the generation of anti-TNP cytotoxic T cells. *J. exp. Med.* **152,** 1070.

PEARL, E.R., VOGLER, L.B., OKOS, A.J., CRIST, W.M., LAWTON, A.R. & COOPER, M.D. (1978) B lymphocyte precursors in human bone marrow: an analysis of normal individuals and patients with antibody deficiency states. *J. Immunol.* **120,** 1169.

PLATTS-MILLS, T.A.E., DE GAST, G.C., WEBSTER, A.D.B., ASHERSON, G.L. & WILKINS, S.R. (1981) Two immunologically distinct forms of late-onset hypogammaglobulinaemia. *Clin. exp. Immunol.* **44,** 383.

SIEGAL, F.R., SIEGAL, M. & GOOD, R.A. (1976) Suppression of B cell differentiation by leukocytes from hypogammaglobulinaemic patients. *J. clin. Invest.* **58,** 109.

SMITH, C.I.E., HAMMARSTROM, L., LINDHAL, M. & LOCKNER, D. (1985) Kinetics of spontaneously occurring common variable hypogammaglobulinaemia; an analysis of two individuals with previously normal immunoglobulin levels. *Clin. Immunol. Immunopathol.* **37,** 22.

SPICKETT, G.P., MILLRAIN, M., BEATTIE, R., NORTH, M., GRIFFITHS, J., PATTERSON, S. & WEBSTER, A.D.B. (1988) Role of retroviruses in acquired hypogammaglobulinaemia. *Clin. exp. Immunol.* **74,** 177.

TEDDER, T.F., CRAIN, M.J., KUBAGAWA, H., CLEMENT, L.T. & COOPER, M.D. (1985) Evaluation of lymphocyte differentiation in primary and secondary immunodeficiency diseases. *J. Immunol.* **135,** 1786.

VAN VOORHIS, W.C., WITMER, M.D. & STEINMAN, R.M. (1983) The phenotype of dendritic cells and macrophages. *Fed. Proc.* **42,** 3114.

WALDMAN, T.A., BRODER, S., BLAESE, R.M., DURM, M., BLACKMAN, M. & STROBER, W. (1974) Role of suppressor T cells in the pathogenesis of 'common variable' hypogammablobulinaemia. *Lancet,* **ii,** 609.

WEBSTER, A.D.B., MALKOVSKY, M., PATTERSON, S., NORTH, M., DALGLEISH, A.G., BEATTIE, R.E., ASHERSON, G.L. & WEISS, R.A. (1986) Isolation of retroviruses from two patients with 'common variable' hypogammaglobulinaemia. *Lancet,* **i,** 581.

WHO REPORT (1986) Primary immunodeficiency diseases, a report of a WHO scientific group. *Clin. Immunol. Immunopathol.* **40,** 166.