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Defective low-density cells of dendritic morphology from the blood of patients with common variable hypogammaglobulinaemia: low immunoglobulin production on stimulation of normal B cells

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SUMMARY

Low-density cells (LDC) of dendritic morphology from the blood of patients with common variable (late-onset) hypogammaglobulinaemia (CVH) did not induce allogeneic immunoglobulin production by normal B cells unlike LDC from normal blood. When LDC from patients were treated with pokeweed mitogen (PWM), a lower allogeneic secretion of IgM and IgG was induced in normal B cells than that induced by allogeneic normal LDC treated with PWM. B cells from hypogammaglobulinaemic patients were non-responsive to both normal and patient LDC treated with PWM under all conditions tested.

Keywords hypogammaglobulinaemia dendritic cells immunoglobulin production accessory cell defect

INTRODUCTION

B cells from patients with common variable (late-onset) hypogammaglobulinaemia (CVH) secrete low amounts of IgM and very little IgG in pokeweed mitogen (PWM)-driven cultures (De Gast *et al.*, 1980) and do not produce antibody to tetanus toxoid (Brenner *et al.*, 1984). De la Concha *et al.* (1977) and Brenner *et al.* (1984) showed normal T-cell helper functions for PWM-driven immunoglobulin production by normal B cells. Certain B-cell growth and differentiation factors made by CVH cells (probably T cells) are also normal (Brenner *et al.*, 1984).

Eibl *et al.*, 1982 showed a defect in antigen presentation by adherent monocytes from late-onset hypogammaglobulinaemic patients. Van Voohis *et al.* (1982) reported antigen-presenting dendritic cells in the blood of normal individuals and we have found up to 5% of blood mononuclear cells in normal individuals to be non-adherent, of low density, of monocytic phenotype and with dendritic morphology (Knight *et al.*, in preparation). We now report that low-density cells (LDC) from the blood of patients with CVH are defective as judged by their ability to induce immunoglobulin production by normal B cells.

MATERIALS AND METHODS

To prepare low-density cells (LDC). Peripheral blood (50 ml) was taken from four CVH patients and from eight normal individuals (laboratory workers). Low-density cells were obtained following

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Knight *et al.* (in preparation). Briefly, the blood was defibrinated, diluted with medium (RPMI 1640, bicarbonate-buffered, with penicillin/streptomycin 100 IU/ml and L-glutamine 2 mM; 3 volumes blood with 1 volume medium) and separated on Ficoll-Paque. The cells from the interface were incubated in medium with fetal calf serum (FCS) (10%) on Petri dishes (Nunc) at 37°C in a CO_2 incubator for 90 min. The non-adherent cells were removed and layered on a hypertonic gradient of Metrizamide (14·5% w/v, analytical grade, Nygaard; Knight *et al.*, 1982). After centrifugation, the cells from the interface were washed in medium with FCS (10%) (complete medium).

T and non-T cells were separated from the cells in the pellet from the Metrizamide gradient by rosetting overnight at room temperature with sheep red blood cells treated with neuraminidase (Sigma). The T-cell rosettes were separated on a Ficoll-Paque gradient, the red cells were lysed (with ammonium chloride solution) and the T cells X-irradiated (2,000 rads). The cells from the interface were used as the responder non-T (B-cell) preparation.

LDC were incubated overnight in complete medium in 1 ml ($2–5 \times 10^6$ cells/ml). Aliquots were then incubated for 1 h at 37°C in PWM (Sigma). After three washes the treated LDC were X-irradiated (2,000 rads) using a Cobalt 60 source.

Immunoglobulin production was measured after 9 days of culture in 20 μl hanging drops in Terasaki plates using a modification of Iscove's serum-free medium (Farrant *et al.*, 1984). Each well contained 80,000 irradiated T cells, 40,000 responder non-T cells and usually 2,000 LDC. Cells were cultured together in various allogeneic and autologous combinations. Each experiment used cells from only two individuals, either a normal individual and a patient, or two normals.

The cultures were terminated by freezing the Terasaki plates and storing then at −70°C until assay. ELISA plates (96-well, Dynatech) were washed three times with phosphate buffered saline with Tween 80 (0·02%) between each step of the assay. Plates were coated with rabbit anti-human IgM (Dako, 1/800) or goat anti-human IgG (Sigma, 1/500) in sodium carbonate-coating buffer (pH 9) overnight at room temperature. Non-specific binding was blocked using an albumin/gelatin/azide buffer at 37°C for 2 h. From each well of the thawed Terasaki culture plates, 15 μl was removed and diluted to 40 μl in the ELISA assay plates with blocking buffer using a Microlab-M diluter (Hamilton). Eight doubling dilutions in blocking buffer of common immunoglobulin standards (LAS-R serum, Hyland) and four of samples were prepared using a multichannel pipette (Titertek). After adding goat anti-human IgM or IgG conjugated with alkaline phosphatase (Sigma) for 2 h at 37°C, substrate (disodium *p*-nitrophenyl phosphate hexahydrate, Sigma) was added and colour read at 405 nm on a Multiscan MC Elisa reader (Titertek). Absorbance curves for diluted standards and samples were plotted and immunoglobulin concentrations calculated by Sirius microcomputer.

Within each experiment, autologous cultures of normal LDC, T cells and non-T cells were used as controls. Results were pooled for all replicates in all experiments. Although the levels of immumoglobulin production varied between experiments, the overall effects were essentially representative of the findings in each experiment. The total number of replicates for each mean are given in the figures.

RESULTS

As few as 250 PWM-treated LDC per well increased IgM production by normal B cells but 2,000 LDC were needed before there was an increase in production of IgG (Fig. 1). In all subsequent experiments 2,000 LDC were used. The T cells were essential for immunoglobulin production (data not shown).

When LDC were not treated with PWM, IgM and IgG produced by B cells from normal individuals was greater with allogeneic LDC and T cells from a single normal donor than with autologous LDC and T cells (Figs 2 and 3). Allogeneic stimulation of IgM production also occurred when only the LDC were from a different normal individual. Allogeneic normal LDC did not stimulate the secretion of IgG significantly but allogeneic normal T cells did.

Compared with allogeneic cells from normal individuals LDC and T cells from hypogammaglobulinaemic patients stimulated less IgM and IgG secretion in normal B cells. LDC from patients

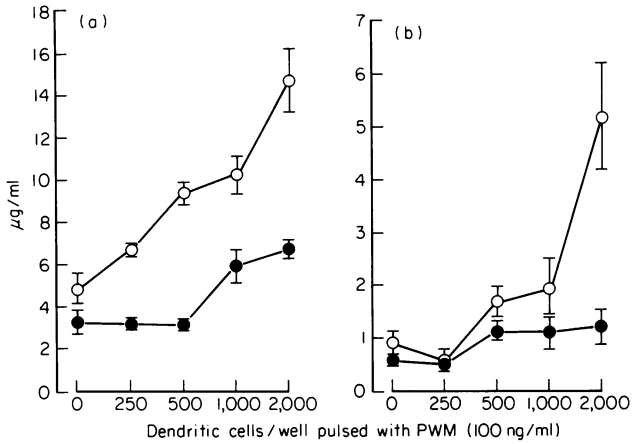

Fig. 1. Effect of number of LDC in each well on production of IgM (a) or IgG (b) by 40,000 normal non-T cells in the presence of 80,000 autologous irradiated T cells. The LDC were either untreated (●) or treated for 1 h at 37°C with PWM (100 ng/ml, ○). More LDC were needed for production of detectable IgG than for IgM. 2,000 LDC/well were used in subsequent experiments.

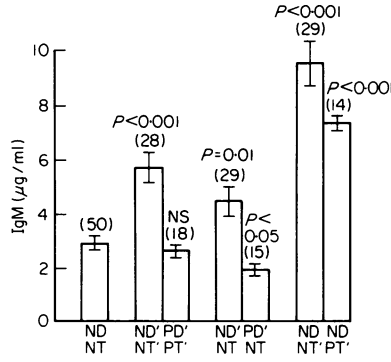

Fig. 2. Effects of CVH and normal LDC on production of IgM by normal B cells in the absence of PWM. N = normal, P = patient (CVH), D = low density cell and T = T cell. Prime (′) indicates the cells are allogeneic to the normal responder B cells (eg. PD′ or NT′). When two cell types are allogeneic to the responder B cells they are autologous to themselves. The probability values are for significance in comparison with the autologous normal controls (i.e. ND NT). The numbers of wells are in brackets. Allogeneic normal LDC or T cells induce IgM production; CVH LDC do not .

induced little or no stimulation of immunoglobulin production with either normal or patient T cells (Figs 2 and 3). Allogeneic patients' T cells with normal LDC increased the production of IgM but not of IgG by normal B cells.

Autologous normal LDC and T cells significantly increased IgM and IgG production by normal B cells after the LDC had been treated with 10 ng/ml PWM. At high doses of PWM the response declined (Figs 4a and 5a).

Allogeneic PWM-treated normal LDC with T cells stimulated the production of IgM by normal B cells as well as autologous cells but PWM-treated hypogammaglobulinaemic LDC were less effective (Fig. 4a). At high doses of PWM normal allogeneic LDC and T cells gave lower secreted IgG than the autologous control.

When both the normal B cells and T cells were from the same donor allogeneic PWM-treated

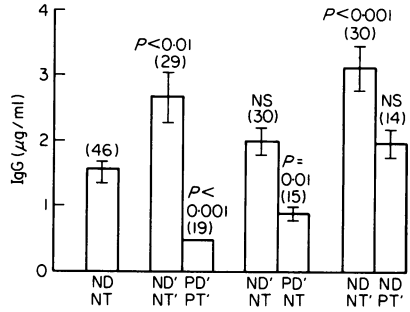

Fig. 3. Effects of CVH and normal LDC on production of IgG by normal B cells in the absence of PWM. The abbreviations are as for Fig. 2. NS = not significant at $P = 0{\cdot}05$. Allogeneic normal LDC induce IgG production when allogeneic normal T cells are present. CVH LDC or T cells do not.

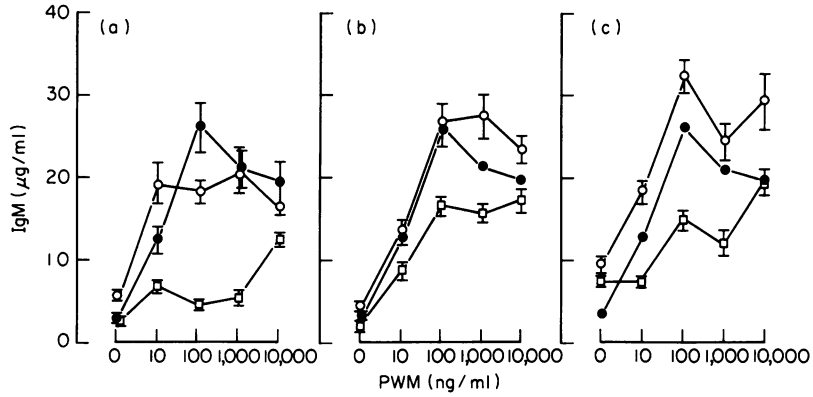

Fig. 4. Production of IgM by normal B cells on stimulation with CVH and normal LDC 2,000/well treated with different doses of PWM. Abbreviations as for Fig. 2. Data shows means with (s.e.m.); number of wells 16 or more. ●——● autologous normal control (ND NT NB). (a) T cells autologous with the LDC; ○——○ allogeneic normal control (ND'NT'NB), □——□ allogeneic CVH patient cells (PD'PT'NB). (b) T cells autologous with the normal B cells; ○ allogeneic normal LDC (ND'NT NB) □ allogeneic CVH patient LDC (PD'NT NB). (c) Dendritic cells autologous with the normal B cells; ○ allogeneic normal T cells (ND NT'NB), □ allogeneic CVH patient T cells (ND PT'NB). Production of IgM is reduced when CVH LDC are used especially in the presence of CVH T cells.

normal LDC stimulated similar levels of IgM production to that obtained with autologous LDC (Fig. 4b); treated hypogammaglobulinaemic LDC were less effective. In contrast, allogeneic PWM-treated LDC stimulated a lower production of IgG than that obtained under autologous conditions (Fig. 5b). With patient LDC the stimulation of IgG production was defective when the lowest concentration of PWM (10 ng/ml) was used. However, the treatment of LDC with higher concentrations of PWM-stimulated IgG secretion to a greater level than that seen under autologous conditions.

When only the T cells were allogeneic (Fig. 4c), patient T cells stimulated a lower production of IgM than that seen with normal T cells. For IgG production, normal or patient allogeneic T cells gave responses similar to that seen with the autologous control (Fig. 5c).

No secretion of IgM or IgG was detected when B cells from patients were cultured with PWM-treated LDC from either patients or normal individuals. This lack of response was the same with either patient or normal T cells (data not shown).

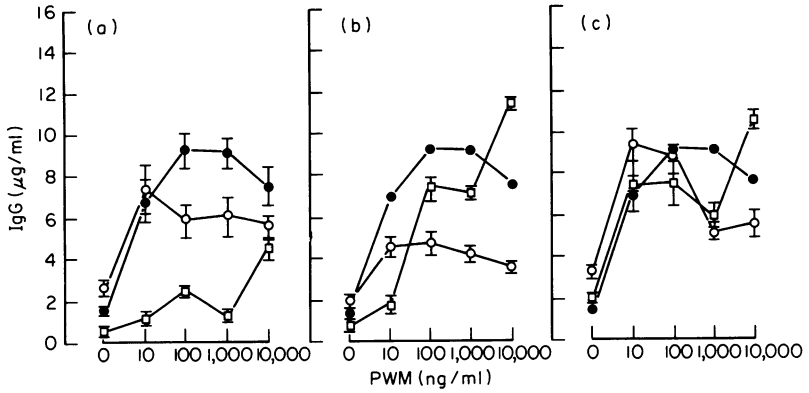

Fig. 5. Production of IgG by normal B cells on stimulation with CVH and normal LDC (2,000/well) treated with different doses of PWM. Data shows means with (s.e.m.); numbers of wells 16 or more. Abbreviations, key to lines and definitions of (a), (b) and (c) are as for Fig. 4. Production of IgG is reduced when CVH LDC are used in the presence of CVH T cells. At the lowest dose of PWM only (10 ng/ml) CVH LDC depress IgG levels even with normal T cells.

DISCUSSION

These data show that PWM-treated LDC from the blood of patients with CVH are defective in stimulating immunoglobulin production by normal B cells. This defect is most profound when patients' T cells are also present but also occurs with normal T cells. There are previous reports (Eibl *et al.*, 1982; Mannhalter, Zlabinger & Eibl, 1984) of a defect in CVH in the interaction between antigen-treated monocytes and patient T cells. The relationship between the low-density cells that we obtain and the dendritic cells reported in blood by Van Voohis *et al.* (1982) is not clear but is discussed in detail elsewhere (Knight *et al.* in preparation). Essentially, the cells used in the present work are non-adherent, of low density and primarily of monocytic phenotype. The lower number of cells obtained by Van Voohis *et al.* (1982) are temporarily adherent, also of low density and do not have some monocytic antigens. It is possible that the LDC we have studied also contain some of the dendritic cells described by Van Voohis *et al.* (1982).

There are several possible explanations for several cell types being defective in late onset hypogammaglobulinaemia.

1. There may be an infectious agent in different cell types in this disease.
2. Alternatively, there could be a defect in some cell which is a precursor of several cell types.
3. Another possibility is that the impaired function of the B cell is not primary but secondary to a defect in antigen presentation or some other signal necessary for the normal differentiation of the B cell.

This last possibility is the easiest to consider from the present data. Several signals to B cells are normal. Patient T cells provide normal helper function for immunoglobulin production by allogeneic normal B cells (De la Concha *et al.*, 1977; Brenner *et al.*, 1984), except in the small subset of patients with CVH with potent T suppressor cells (Waldmann *et al.*, 1974; Platts-Mills *et al.*, 1981). This group has not yet been studied for function of LDC cells. In the present study, hypogammaglobulinaemic T cells provide essentially normal help for B cells except that the level of IgM production are rather low. Mixtures of patients' cells (both T and B) make growth and differentiation factors as effectively as cells from normal individuals (Brenner *et al.*, 1984). The idea that the B cells in hypogammaglobulinaemia may be normal is suggested by one report that cell lines prepared from patient B cells using Epstein Barr Virus make IgG (Brenner *et al.*, 1984). It is clear from the present data, however, that circulating B cells from patients did not produce

immunoglobulin when stimulated by PWM on low-density cells from normal individuals or from CVH patients.

The technique of exposing LDC to mitogen (e.g. PWM) allows more control of experimental variables. For example, it is impossible to obtain 'pure' normal B cells without donor monocytic (including low-density) cells which could acquire PWM from solution. The stimulation of B-cell immunoglobulin production we have obtained with PWM-treated LDC is dependent on T-cell help as is that of having PWM in solution. The defective stimulation of PWM-treated CVH low-density LDC may result from having fewer molecules on the cells since higher doses of PWM appeared to overcome the defect. Another possible reason for the defect is that the LDC of the CVH patients may contain fewer non-monocytic dendritic cells.

It is not clear whether the defect is in the reaction of the LDC with T cells or B cells or both. This is being investigated. Despite the fact that not all of the possible interactions between cellular components have been studied, the present data suggests that abnormalities of function of accessory cells for B-cell differentiation and function may be important in CVH.

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