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B cells in patients with X-linked and 'common variable' hypogammaglobulinaemia

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SUMMARY

E^- cells from three patients with X-linked and three patients with non-familial 'common variable' hypogammaglobulinaemia were stained by indirect immunofluorescence with a panel of B cell specific monoclonal antibodies (anti-Bp95, Bp35 and Bp135). The number of B cells detected with the pan-B cell antibodies was variable between patients. One patient in each group was found to have near normal numbers of circulating B cells although those of the X-linked patient were clearly immature.

Keywords hypogammaglobulinaemia B lymphocytes

INTRODUCTION

Patients with X-linked agammaglobulinaemia were traditionally thought to have a selective defect in B cell differentiation because of the absence of surface immunoglobulin positive cells in the circulation (Frøland & Natvig, 1972; Preud'homme, Griscelli & Seligmann, 1973). However, the defect is not complete since there is some IgG immunoglobulin in their serum, and the occasional surface immunoglobulin positive (SIg^+) B cell has been found in the circulation of some patients (Chiao *et al.*, 1976). There was some criticism that the surface immunoglobulin may have originated from the serum (Schwaber, Lazarus & Rosen, 1978; Horwitz, Webster & Newton, 1977).

More recently at least two groups of workers have shown that immunoglobulin producing lines can be obtained from these patients by stimulating peripheral blood mononuclear cells with Epstein-Barr virus (EBV), suggesting that 'immature' B cells are present (Levitt, Ochs & Wedgwood, 1984; Tsuchiya *et al.*, 1980). Pre-B cells are present in the bone marrow (Fu *et al.*, 1980), so the block is thought to involve the differentiation of pre-B cells to immature circulating B cells. The recent availability of a variety of monoclonal antibodies (MAb) to B cells at various stages in their development should enable us to locate the block in differentiation more precisely. Using a panel of B cell specific antibodies, we have investigated the phenotype of circulating mononuclear cells in three patients with X-linked agammaglobulinaemia and three patients with non-familial 'common variable' hypogammaglobulinaemia.

MATERIALS AND METHODS

Subjects. Three unrelated patients with X-linked agammaglobulinaemia, known to have

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Table 1. Staining pattern of E^- cells from hypogammaglobulinaemic patients and normal controls

			Percentage positive cells*						E^-
Patients	Age	Treatment	2D1	UCHT1	UCHM1	Bp95	Bp35	Bp135	recovered§ (%)
X-linked									
1	23	IVG	92	12	51	32	37	1	15
2	24	IVG	60	15	26	3	9	1	37
3	16	IVG	86	4	58	3	5	1	6
NF‡									
4	41	IVG	69	2	9	7	12	1	40
5	45	IVG	43	2	19	2	3	2	33
6	62	IVG	74	6	17	25	36	15	12
Controls									
7 expt 1	26	—	91	3	16	7	38	6	33
7 expt 2	26	—	83	4	15	25	26	16	33
8	27	—	78	1	16	23	44	24	13

* Percentage background staining subtracted (background was between 1 and 5%, except for patient 6 where it was 12%).

† Values for B cell specific antibodies are the means of percentage positive cells for at least two antibodies of the same specificity.

‡ NF: non-familial.

§ Percentage of total PBMC.

affected maternal male cousins, were studied. Three (two male, one female) patients with 'common variable' hypogammaglobulinaemia, selected at random from our Immunodeficiency Clinic, were also studied. Table 1 shows their ages and treatment received at the time of the study. All patients had < 100 mg/100 ml of IgG before treatment with unrecordable IgA and IgM (Table 1). Two adult normal healthy subjects provided the control data.

Cell separation. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood (about 30 ml) by centrifugation over a Ficoll-Hypaque (FH) gradient. Non T-cells were separated by rosetting with aminoethylisothiouronium-treated sheep red blood cells (AET-SRBC) and centrifugation over FH.

The E^- cells recovered were stained with a panel of monoclonal antibodies by indirect immunofluorescence. Briefly 2×10^5 cells/well were stained in flexiplates for 30 min at 4°C with 50 μl of ascites diluted 1/250 in Hepes-buffered minimal essential medium (MEM) with 5% fetal calf serum and 0·002% azide. This concentration of antibody was at least twice that required for optimal staining by indirect immunofluorescence. After washing the cells were incubated with 50 μl affinity-purified sheep anti-mouse Ig-FITC conjugate for 30 min at 4°C. Analysis was performed by flow cytometry on a fluorescence activated cell sorter (FACS IV, Beckton Dickinson).

Monoclonal antibodies. The monoclonal antibodies used were part of a panel of anti-B cell monoclonals obtained from the 2nd International Workshop on Human Leucocyte Antigens. There were four antibodies against Bp95 (i.e. B4, HD37, 4G7 and SJ25-C1), three antibodies specific for Bp35 (i.e. B1, 1F5 and 2H7), and five antibodies against Bp135 (i.e. 29–110, HD6, HD39, SJ10-1H11, SHCL-1). All three antigens are B cell specific. Bp95 and Bp35 are present at the surface of all or most B cells (pan-B) (Stashenko *et al.*, 1981; Nadler *et al.*, 1983) whereas Bp 135 appears later in ontogeny and may not be present on all B cells thereafter (Leucocyte Typing II, Report from the second International Workshop on Human Leucocyte Antigens, in press). For each patient, two or more antibodies of each specificity were used and found to give percentage staining consistent with each other.

UCHT1 is an anti-CD3 (pan-T) antibody (Beverley & Callard 1981) UCHM1 is a monocyte-specific antibody (Hogg *et al.*, 1984) 2D1 stains all human leucocytes (Beverley, 1980).

RESULTS

The number of PBMC recovered from all patients were similar to those of controls ($1\text{–}2 \times 10^6$ cells/ml). The yield of E^- cells obtained from most patients was within the normal range except for patient 3 from whom 6% or less of his PBMC were recovered as E^- on two occasions. 2D1 stains all human leucocytes (Beverley, 1980) and was used as a positive control. The percentage of UCHT1 positive cells (Beverley & Callard, 1981) showed that most T cells had been effectively removed by E-rosetting. E^- cells from the X-linked patients tended to give a higher percentage staining with the monocyte-specific antibody UCHM1 (Hogg *et al.*, 1984). More interestingly, there were differences in the number of cells staining with monoclonal antibodies specific for B cells in the three patients with X-linked agammaglobulinaemia. Two of these patients had very few cells detectable with these antibodies, and in the third patient, up to 40% of the non-T cells stained with the two pan-B monoclonal antibodies, Bp95 and Bp35 (Table 1). The E^- from patient 1 were, however, clearly different from those of the controls. They contained a higher proportion of large cells and the majority of the cells positive with the pan-B cell antibodies were large. The control B cells, on the other hand, were mainly small and stained more brightly with the anti-Bp35 monoclonals. Patient 1 was studied on two separate occasions with an interval of several months and the results were

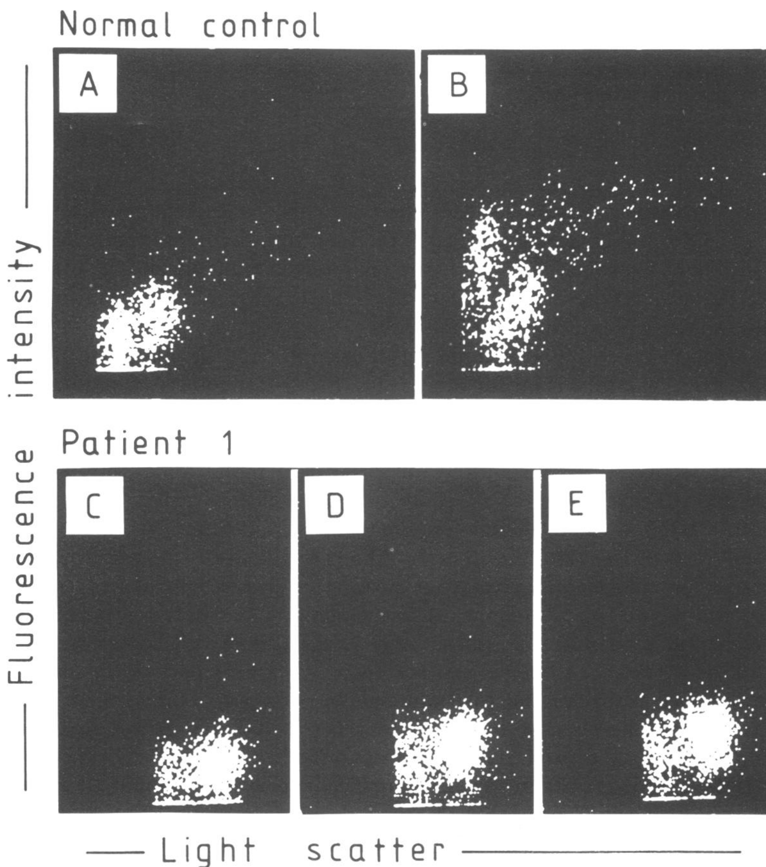

Fig. 1 Staining pattern of patient 1 and control. E^- cells from a normal control (7) were stained with medium only (A) or with 1F5 antibody (Bp35) (B). The E^- cells from patient 1 were stained with medium (C), 1F5 (D) or SJ25-C1 antibody (Bp 95) (E). The pictures were taken from two separate experiments but the results found to be reproducible.

similar. All three patients were negative for Bp135 which recognizes a more mature subset of B cells suggesting an early block in B cell ontogeny. A similar variation in the number of B cells detectable by this method was seen with the three 'common variable' hypogammaglobulinaemia patients. The Bp35 positive cells in patient 6 were mainly small cells unlike those in patient 1 (data not shown). Furthermore, they stained at least weakly with Bp135 antibodies and, therefore, resembled more closely normal B cells than those of patient 1 (X-linked).

DISCUSSION

This data confirms that the block in B cell differentiation in patients with X-linked agammaglobulinaemia is not complete. The induction of EB transformed IgM secreting lines from similar patients (Levitt *et al.*, 1984), and the finding of small numbers of surface IgM and IgD positive B cells in the circulation (Conley, 1985) suggests that a significant number of B cells reach an immature phenotype, but very few progress beyond this stage. However, some probably do develop into plasma cells *in vivo* as shown by the low but measurable levels of serum IgG (usually 10–50 mg/100 ml).

Conley (1985) recently showed that seven patients studied with X-linked agammaglobulinaemia all had between 0·01 and 0·3% of total lymphocytes staining for surface IgM, and all these cells were positive for the B1 monoclonal antibody. However, one of our patients (1) had near normal numbers of circulating B cells which stained with pan-B antisera, although they were clearly different from those of the controls in that they were larger and stained less intensely.

Patients with non-familial 'common variable' hypogammaglobulinaemia have variable numbers of surface Ig positive circulating B cells (Horwitz *et al.*, 1977). Two of the patients studied here had virtually no detectable circulating B cells, suggesting a similar block in differentiation to that seen in X-linked agammaglobulinaemia.

The following points arise from this study. First, it is clear that near normal numbers of circulating immature B cells may be present in some patients with X-linked agammaglobulinaemia. The numbers of these immature B cells vary between patients, and may vary from time to time in the same patient, although this latter possibility has not been studied. Assuming that X-linked agammaglobulinaemia (defined as all serum immunoglobulins $< 10\%$ of lower limit of normal and affected maternal cousins) has a single aetiology, the defect is likely to involve a factor, regulated or encoded by a gene on the X-chromosome, which has a major but not over-riding role in the differentiation of B cells. It is worth emphasizing that although the T lymphocytes of patients with X-linked agammaglobulinaemia are functionally normal, they do show metabolic abnormalities consistent with immaturity (Webster, 1982). It is possible that this putative differentiating factor also has some effect on T cell maturation.

The second point of practical interest is that the presence or absence of B cells, even when measured with new specific monoclonals, is not diagnostically useful in differentiating X-linked agammaglobulinaemia from some patients with non-familial 'common variable' hypogammaglobulinaemia. However, most of the latter patients who lack circulating SIg^+ B cells are also lymphopenic (Horwitz *et al.*, 1977) and their T lymphocytes are usually both functionally and metabolically abnormal (Webster, 1982). It is, therefore, not usually difficult to distinguish between these two groups of patients.

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