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Histochemical studies for 5′-nucleotidase and alpha-naphthyl (non-specific) esterase in lymphocytes from patients with primary immunoglobulin deficiencies

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SUMMARY

Lymphocytes from patients with primary immunodeficiency were tested histochemically for ecto 5′-nucleotidase (5′N) and alpha-naphthyl (non-specific) esterase. More than half the patients with 'common variable' hypogammaglobulinaemia, all those with X-linked hypogammaglobulinaemia and some of those with selective IgA deficiency had a very low percentage of lymphocytes staining for 5′N as compared to controls. A lack of B cells probably explains the finding in X-linked hypogammaglobulinaemia, but does not fully explain the results in the other groups.

Most patients with 'common variable' hypogammaglobulinaemia had a very low percentage of lymphocytes with granular staining for α-naphthyl (non-specific) esterase in contrast to normal numbers in those with X-linked hypogammaglobulinaemia and most of those with selective IgA deficiency.

Granules containing non-specific esterase are characteristically found in 'mature' T lymphocytes. The enzyme abnormalities in the T and B cells of 'common variable' hypogammaglobulinaemic patients could be explained by 'immature' cell types.

INTRODUCTION

Previous reports from this laboratory have shown that many patients with primary 'common variable' hypogammaglobulinaemia have low or absent activity of lymphocyte 5′-nucleotidase (5′N). These investigations employed a quantitative radiochemical assay using intact lymphocytes (Johnson *et al.*, 1977; Webster *et al.*, 1978). The low levels in some patients may have been due to a deficiency in a sub-population having 5′N activity or a generalized lowering of activity in all types of lymphocytes. In order to clarify the situation we performed histochemical studies on patients with 'common variable' hypogammaglobulinaemia, selective IgA deficiency and X-linked hypogammaglobulinaemia.

Alpha-naphthyl (non-specific) esterase is an intracellular enzyme present throughout the cytoplasm of monocytes and macrophages. More recently, activity has been found in discrete granules within most T lymphocytes (Kulenkampff, Janossy & Greaves, 1977; Horwitz *et al.*, 1977a). Grossi *et al.* (1978) have shown that this enzyme is confined to the sub-population of T lymphocytes with Fc receptors for IgM. Functional T lymphocyte defects, as shown by poor *in vitro* responses to PHA, occur in about 25% of patients with 'common variable' hypogammaglobulinaemia. (Webster & Asherson, 1974). This has prompted us to investigate whether these patients also have low numbers of esterase positive T lymphocytes.

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MATERIALS AND METHODS

Subjects. The patients studied comprised: twenty-two patients with 'common variable' hypogammaglobulinaemia (mean age 43, range 19–65 years). All but two developed their disease as adults and all were receiving regular weekly immunoglobulin replacement therapy. Their mean serum immunoglobulin levels as mg/100 ml were as follows: IgG 273, range 95–560; IgA < 5, range < 1–8; IgM < 10, range < 5–60. Five patients with X-linked hypogammaglobulinaemia (mean age 14 years, range 8–16). All had affected male relatives and were receiving weekly gammaglobulin injections. Mean serum immunoglobulins (mg/100 ml) were: IgG 416, range 350–500; IgA < 5, range < 5–8; IgM 14, range < 5–33. Five patients (mean age 33 years, range 18–70) with selective IgA deficiency (serum IgA 0·5 μg–0·03 mg/ml), three of whom were suffering from recurrent upper and lower respiratory infections, one with recurrent herpes simplex infection and one patient with systemic lupus erythematosus. Twenty-one healthy laboratory personnel acted as controls (aged 20–50 years).

Lymphocyte 5′-nucleotidase. Lymphocytes from fifteen patients with 'common variable' hypogammaglobulinaemia, five patients with X-linked hypogammaglobulinaemia and five with selective IgA deficiency were studied using the method of Silber *et al.* (1975). Lymphocytes were purified on a Ficoll–Triosil gradient, washed twice in 0·25 M tris-maleate buffer, pH 7·8, and re-suspended in the same buffer to give a final cell concentration of 10^6 per ml. Cytocentrifuge preparations were found to give the best results. 0·2 ml of the cell suspension was pelleted onto a slide in a Shandon–Elliot cytocentrifuge and then briefly dried in air. Better preparations were obtained when the cells were used within 30 min of washing.

The slides were incubated in freshly made and filtered 0·25 M tris-maleate buffer pH 7·8 containing: 0·02 M β-glycerophosphate, 0·01 M Na-K tartrate, 0·01 M Mg $(NO_3)_2$, 0·002 M Ph $(NO_3)_2$ and 0·003 M 5′ AMP (Sigma Chemical Company Ltd, USA) for 20 hr at room temperature. For the negative control, the 5′ AMP was omitted and substituted with 2′3′ AMP (0·003 M). After two washes in distilled water, the preparations were fixed in 0·6% gluteraldehyde for 10 min and washed twice in distilled water. They were placed in freshly made 2% ammonium sulphide solution for 30 sec, rinsed and allowed to dry. 200 cells were counted. Positive cells stained dark brown or black. The number of positive cells were expressed as a percentage of the total cells counted.

Lymphocyte alpha-naphthyl (non-specific) esterase. Lymphocytes from nineteen patients with 'common variable' hypogammaglobulinaemia, four with X-linked hypogammaglobulinaemia and three with selective IgA deficiency were studied using the method of Horwitz *et al.* (1977a). In brief, cytocentrifuge preparations were prepared as for the 5′-nucleotidase procedure above except that the cells were washed in medium after the Ficoll–Triosil gradient.

The slides were briefly dried in air, fixed immediately in buffered formalin–acetone solution, pH 6·6, for 30 sec and washed three times in distilled water. After air-drying, the slides were kept at 4°C until stained. The best results were obtained when the slides were stained the same day or within 72 hr.

The cytocentrifuge preparations were incubated for 45 min in freshly prepared medium pH 6·1, containing 4·0 mg α-naphthyl acetate (Sigma Chemical Co.) dissolved just before use in 0·5 ml ethylene glycol monomethyl ether and mixed with 8·9 ml M/15 phosphate buffer pH 7·6, and 0·6 ml hexazotised pararosanilin. Hexazotised paraosanilin was prepared by taking 0·3 ml stock solution (2·0 g pararosanilin hydrochloride and 50 ml 2N hydrochloric acid) and 0·3 ml of freshly made 4% sodium nitrite. Cells were counterstained in a 2% solution of methyl green (chloroform extracted) for 5 min, rinsed in three changes of distilled water and air-dried. Finally, they were rinsed in xylene and mounted in Ralmount. Cells with discrete granules were counted and expressed as a percentage of total lymphocytes (i.e. mononuclear cells not showing diffuse cytoplasmic esterase staining characteristic of monocytes).

E-rosettes. The method recommended by Brown & Greaves (1974) was used. The numbers of E-rosettes in peripheral blood had previously been assessed in all the patients, in many on more than one occasion. Seven patients in the 'common variable' hypogammaglobulinaemic' group were re-tested at the time of the enzyme studies.

RESULTS

Fig. 1 shows the percentage of cells staining for 5′-nucleotidase in healthy controls, patients with primary hypogammaglobulinaemia and selective IgA deficiency. Compared with the controls, 87% of the patients with 'common variable' hypogammaglobulinaemia had reduced numbers of positive cells and the majority of these showed no positive cells. Some patients with X-linked hypogammaglobulinaemia and selective IgA deficiency also had no positive cells and all but one of these patients had values that were outside the normal range.

Fig. 2 shows the percentage of lymphocytes staining for non-specific esterase. About half the patients with 'common variable' hypogammaglobulinaemia showed a low percentage of positive cells in contrast to normal numbers in all four patients with X-linked hypogammaglobulinaemia.

E-rosetting cells and monocytes

All the patients had normal percentages of E-rosetting lymphocytes (mean 64%, range 44–81). The 95% range of results for normal subjects in our laboratory is 50–74%.

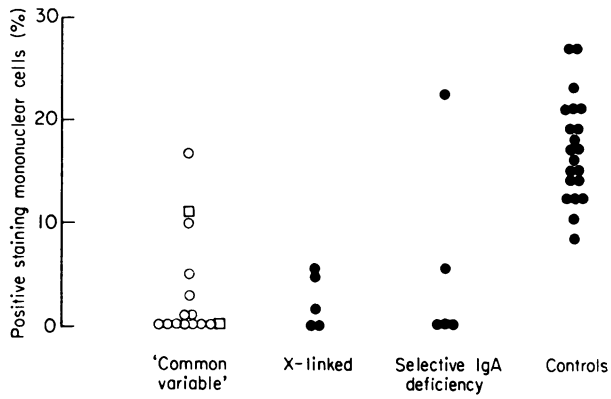

FIG. 1. 5′-nucleotidase positive cells in immunoglobulin deficient patients. (□) Childhood onset 'common variable' hypogammaglobulinaemia, (○) adult onset 'common variable' hypogammaglobulinaemia.

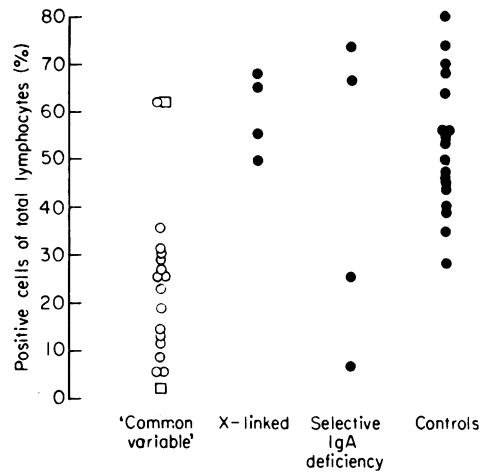

FIG. 2. Alpha-naphthyl esterase positive lymphocytes in immunoglobulin deficient patients. (□) Childhood onset 'common variable' hypogammaglobulinaemia, (○) adult onset 'common variable' hypogammaglobulinaemia.

The percentage of cells showing the characteristic esterase staining of monocytes was no greater in patients as compared to the controls (mean 22, range 7–49%).

DISCUSSION

The histochemical studies on lymphocyte 5′N confirm our previous finding of low 5′N activity using a radiochemical method (Webster *et al.*, 1978). Presumably, the histochemical method is less sensitive as some of the patients studied here had shown low but measurable 5′N activity when measured previously by the radiochemical method. This suggests that the low levels of activity found in patients by the radiochemical method is not due to the presence of a few cells with normal 5′N activity but rather results from a generalized lowering of activity below that detectable by the histochemical method.

The finding that some patients with selective IgA deficiency also lack 5′N positive cells is of particular interest and complements other findings that indicate an aetiological link between this disorder and 'common variable' panhypogammaglobulinaemia (Nell *et al.*, 1972; Platts-Mills *et al.*, 1979). Work in this laboratory has shown that B lymphocytes have about four times the 5′N activity of T lymphocytes and that in most normal subjects the histochemical method mainly detects B lymphocytes (Rowe *et al.*,

1979). However, some normal subjects (Fig. 1) have between 20 and 30% of cells staining for 5′N, indicating that cells other than B lymphocytes may stain for the enzyme. The lack of B lymphocytes in X-linked hypogammaglobulinaemia presumably explains the very low numbers of 5′N positive cells found in these patients. Some patients with 'common variable' hypogammaglobulinaemia also lack circulating B lymphocytes which might explain the absence of 5′N positive cells in some of these patients (Horwitz, Webster & Newton, 1977b). Correlation of B cell numbers with 5′N positive cells will answer this question. However, it is unlikely to be the explanation in all patients since one of the patients with 5′N positive cells has previously been shown to have normal proportions of circulating B lymphocytes.

A role for lymphocyte ecto 5′-nucleotidase has not yet been found, but cord blood lymphocytes (Kramers *et al.*, 1977) and B cells from most patients with chronic lymphatic leukaemia (Silber *et al.*, 1975) have very low activity. There is evidence that the B cells in both these situations are immature (Hayward & Lawton, 1977; Gordon, Howlett & Smith, 1978). The question arises whether the low activity seen in the lymphocytes from patients with 'common variable' hypogammaglobulinaemia reflects immaturity rather than the enzyme defect being the primary cause of the disease.

The finding that more than half of the patients with 'common variable' hypogammaglobulinaemia have low numbers of circulating lymphocytes staining for α-naphthyl (non-specific) esterase supports previous observations of functional T lymphocyte defects (Webster & Asherson, 1975). Recent work has shown that lymphocytes staining for non-specific esterase have receptors for the Fc of IgM (T_M) (Grossi *et al.*, 1978). On the other hand, E-rosetting T lymphocytes with receptors for the Fc of IgG, (T_G) do not stain for non-specific esterase but have been shown, under suitable culture conditions, to differentiate into T_M cells (Pichler, Lum & Broder, 1978). This, together with the absence of non-specific esterase activity in thymocytes (Mueller *et al.*, 1976), suggests that esterase negative T lymphocytes are immature cells. Analysis of T cell subsets in these patients should answer this question and may show that those patients with low numbers of esterase positive cells, and by inference a high percentage of T_G cells, may be those patients whose T cells show evidence of suppressor activity *in vitro* for immunoglobulin production (Waldman *et al.*, 1974). Although there is good evidence of a fundamental B cell defect in most, if not all, patients with 'common variable' hypogammaglobulinaemia (de la Concha *et al.*, 1977), an unbalanced proportion of T_G cells may exacerbate the immunoglobulin deficiency.

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