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Immunological findings in immunoblastic lymphadenopathy

A DETAILED CASE STUDY

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SUMMARY

Several immunological parameters were investigated in a patient with immunoblastic lymphadenopathy (IBL). An increased concentration of polyclonal immunoglobulins, the presence of autoantibodies in the serum and an increased level of B lymphocytes with an abnormal DNA synthesis response to LPS in peripheral blood were the most salient features.

The findings suggest that in IBL there is a numerical as well as a functional alteration of peripheral blood lymphocytes.

We propose that the alteration was due to either an escape of lymphocytes from central lymphatic organs or a defect in maturation of peripheral blood lymphocytes.

INTRODUCTION

Immunoblastic lymphadenopathy (IBL) is a new syndrome differentiated from Hodgkin's disease by Luckes & Tindel (1973). This syndrome is morphologically characterized by a proliferation of arborizing small vessels, prominent immunoblastic proliferations and amorphous acidophilic interstitial material (Luckes & Tindel, 1975). The clinical evolution is irregular and the prognosis uncertain. The aetiology and the pathogenesis are unknown. A consistent finding in the disease is dysproteinemia (Frizzera, Moran & Rappaport, 1974) and often, haemolytic anaemia (Luckes & Tindel, 1975). Frizzera *et al.* (1974) have emphasized the similarity between this syndrome and the graft-*vs*-host reaction. Luckes & Tindel (1975) have described the basic process in IBL as a non-neoplastic hyperimmune proliferation of the B-cell system with a 'switch on' of B lymphocytes, whereas in Hodgkin's disease a defect in T cells appears to be the fundamental abnormality.

We have studied the presence of autoantibodies, the levels of B and T lymphocytes and the lymphocyte response to specific mitogens for B and T cells in a patient with IBL. Our results indicate numerical and functional abnormalities of B lymphocytes.

CASE REPORT

A 49-year-old man was admitted to the hospital in April 1975 with a history of weakness, anorexia, weight loss, intermittent fever and multiple adenopathies. Since September 1973 he had had several recurrent episodes of fever, diffuse maculopapular skin lesions and adenopathies. He recovered from such episodes without treatment after 10–15 days, and improved sooner when he was treated with ACTH.

On physical examination he appeared mildly ill. He had generalized lymphadenopathies with firm, movable and non-tender nodes ranging from 1–4 cm in diameter. The spleen was felt 6 cm below the left costal margin and the liver 6 cm below the right costal margin and both were firm but non-tender. Haemoglobin, haematocrit and platelet count were normal. The white cell count was 10,500 with 52% segmented neutrophils, 1% basophils, 2% eosinophils, 22% lymphocytes, 14% monocytes, 8% band

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forms and 1% metamyelocytes. A peripheral blood film showed morphologically immature lymphocytes. Serological tests for brucellae, salmonellae and infectious mononucleosis were negative. Singer–Plotz test for rheumatoid factor was negative. Antistreptolysin O test gave a positivity of 833 Todd U. Several haemocultures were negative. Direct and indirect Coombs tests were negative. The PPD skin test was negative. A radiological film of the chest and lymphography revealed hilar, paravertebral and iliac adenopathies. Other routine studies on serum and urine were without significance. A liver biopsy and a liver scan did not reveal substantial lesions. Lymph-node biopsy showed diffused alteration of lymph-node architecture by infiltration of large pyroninophilic cells with characteristics of immunoblasts, proliferation of arborizing small vessels and a deposition of acidophilic staining interstitial material.

The patient was treated with prednisone (40 mg/day) for 3 months. In July 1975 while in the hospital the patient became febrile and the white cell count fell to 1300 with 76% of lymphocytes in the peripheral blood. He did not respond to antibiotic treatment and died from septic shock two days after the onset of the fever. It was not possible to perform an autopsy.

MATERIALS AND METHODS

Serum immunoglobulins and C_3 levels were determined by radial immunodiffusion on commercial plates (ICL Scientific, Fountain Valley, California), antinuclear factor (ANF), antimitochondria (A.mit.), anti-smooth muscle (A.sm.), anti-skeletal muscle (A.sk.) and anti-thyroid (A.th.) antibodies were determined in serum with indirect immunofluorescence technique according to Holborow (Johnson & Holborow, 1973). FITC rabbit anti-human Ig sera, used at the appropriate dilutions were purchased from Hyland Labs (Division Travenol Labs Incorporated, Costa Mesa, California).

Surface immunoglobulin-bearing cells (S-Igs-C) and complement rosette cells (EAC) and spontaneous rosette cells (E) from blood lymphocytes were detected according to the technique described by Jondal, Holm & Wigzell (1972). Lymphocytes were obtained from heparinized peripheral blood after iron treatment and isolation by flotation on a Ficoll-Isopaque density gradient (Böyum, 1968). A small contamination of other cells (less than 5% of granulocytes and monocytes) was demonstrated by May–Grunwald–Giemsa stained smears and acridine orange staining of the rosette-forming cells.

Lymphocyte culture procedure was as follows: lymphocytes from peripheral blood were separated by Ficoll–Isopaque flotation technique. Cells were washed in balanced salt solution (BSS) and then, subsequently resuspended in MEM Eagle's medium at a final concentration of 1×10^6 cells/ml in plastic tubes (Falcon Plastics, Los Angeles, California). Cells were cultured in the presence of 10% heat-inactivated pooled human AB serum and incubated in an atmosphere of 5% CO_2 at 37°C., for 3, 4 or 5 days. After 2, 3 or 4 days of incubation ^{3}H-labelled thymidine (2 μCi per culture) (Radiochemical Centre, Amersham, Buckinghamshire) was added to test DNA synthesis. Twenty-four hours after labelling, the cultures were harvested and filtered on glass fibre filters. All filters were dried overnight and thereafter transferred to scintillation vials. Radioactivity was measured in a scintillation spectrophotometer (Packard 3320).

In order to stimulate DNA synthesis response in lymphocytes in culture the following substances were used: purified derivative antigen (PPD) (RT 32, Statens Serum Institute, Copenhagen, Denmark), phytohaemagglutinin mitogen (PHA) (PHA Reagent Grade, Wellcome Research Laboratory, Beckenham) and lipopolysaccharide of *E. coli* 055:B5 (LPS) (kindly donated by Professor G. Moller, Karolinska Institute, Stockholm, Sweden).

RESULTS

At the time of the patient's admission to the hospital the following findings were obtained.

Serology

The level of C_3 was within normal limits. There were no cryoglobulins or cryoagglutinins in the serum. The protein electrophoretic pattern showed a pronounced increase of gammaglobulin fraction. IgG, IgA and IgM levels are shown in Table 1. Serum immunoelectrophoresis studies revealed a polyclonal increase of the three immunoglobulins studied. Neither Bence–Jones nor other proteins were found in the urine.

Table 2 shows the serum autoantibodies found by immunofluorescence. It is remarkable that A.mit, A.Sm., A.Sk., and A.th. antibodies were positive whereas ANF was negative.

Cellular findings

In Table 3 the levels of E, EAC and S-Igs-C lymphocytes are shown. A marked increase of the B-lymphocyte subpopulation was found. The T-lymphocyte level was slightly decreased. DNA synthesis

TABLE 1. Serum immunoglobulin levels

	mg/100 ml	
Igs	IBL patient	Controls
IgG	4300	700–1700
IgA	1200	100–350
IgM	480	50–150

TABLE 2. Serological findings in IBL

Test	Results
Cryoagglutinins	Neagative (1/1)
Cryoglobulins	Negative
Rheumatoid factor	Negative (1/20)
Streptolysin O	833 Todd U
Heterophile abs.	Negative (1/2)
Antinuclear factor	Negative (1/20)
Anti-smooth muscle abs.	Positive (1/10)
Anti-skeletal muscle abs.	Positive (1/10)
Anti-mitochondria abs.	Positive (1/10)
Anti-thyroid abs.	Positive (1/10)

TABLE 3. Percentages of EAC, E and S-Igs-C lymphocytes

	EAC	E	S-Igs-C	S-IgG	S-IgA	S-IgM
Control	13	55	16	7	1·5	6
(±s.d.)	(3)	(10)	(4)	(2)	(1)	(2)
Patient	40	39	38·6	35	8	7

response of peripheral blood lymphocytes with optimal dose of PHA was normal in intensity as well as in the kinetics of the response when compared to the response of control lymphocytes from healthy donors. The stimulation with PPD at two different doses also gave a normal response. When peripheral blood lymphocytes were stimulated with LPS (100 μg/ml) an increase of DNA synthesis was obtained, whereas control peripheral blood lymphocytes from healthy donors did not show any response. The maximal response to LPS was on day 4 of the culture (Fig. 1).

When the patient was readmitted 3 months later with a pronounced deterioration, a part of the study was repeated. Levels of serum Igs were decreased, being 1500 mg% for IgG, 290 mg% for IgA and 89 mg% for IgM. B-lymphocytes were dramatically lower, EAC lymphocytes were 1% and S-Igs-C lymphocytes were 0%. DNA synthesis response to LPS was negative at the time.

DISCUSSION

The case reported is histologically identical to the cases described in the literature (Frizzera *et al.*, 1974, Luckes & Tindel, 1975). Clinically it has some similarities with other cases, in particular the presence of dysproteinemia (Frizzera *et al.*, 1974) and lacks other characteristics such as the presence of haemolytic anaemia (Frizzera *et al.*, 1974, Luckes & Tindel, 1975) and cryoglobulinaemia (Schultz & Yunis, 1975).

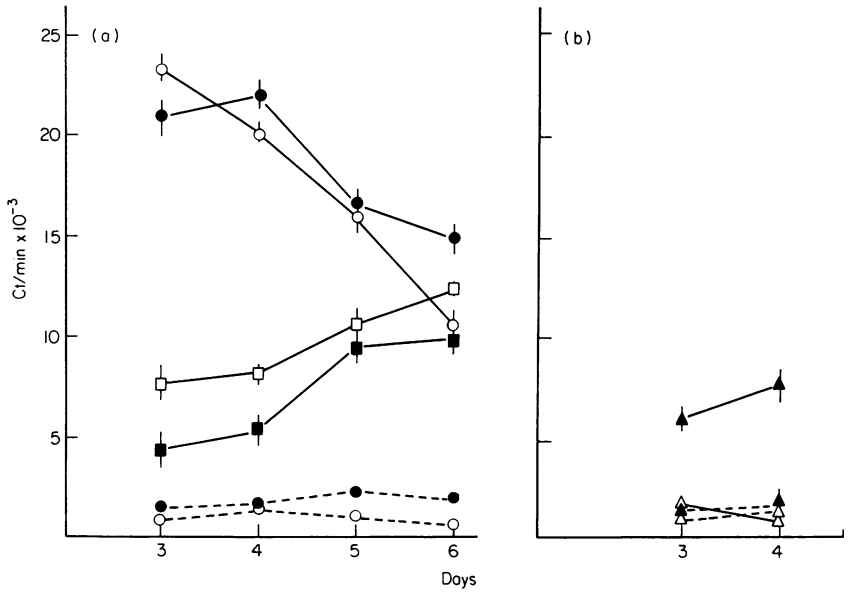

FIG. 1. DNA Synthesis responses of peripheral blood lymphocytes from a healthy donor and from the patient. (a) Stimulation with PHA (final dilution 1/100) and with PPD (5 ug/ml). (○---○) Spontaneous DNA synthesis of control lymphocytes; (●- - - - -●) i.d. of patient lymphocytes. (○———○) DNA synthesis response of control lymphocytes to PHA; (□———□) i.d. of control lymphocytes to PPD; (●———●) i.d. of patient lymphocytes to PHA; (■———■) i.d. of patient lymphocytes to PPD. (b) Stimulation with LPS (100 ug/ml). (△- - - -△) Spontaneous DNA synthesis of control lymphocytes; (▲- - - -▲) i.d. of patient lymphocytes. (△———△) DNA synthesis of control lymphocytes to LPS; (▲———▲) i.d. of patient lymphocytes to LPS.

We have observed three phenomena: (a) the presence of a variety of autoantibodies; (b) a marked increase of the B-lymphocyte subpopulation and (c) an abnormal response of peripheral lymphocytes to LPS.

(a) Smooth muscle autoantibodies, which have been described in I.B.L. before (Tangun *et al.*, 1974), associated with the presence of other autoantibodies as A.mit., A.Sk., and A.th., are usually considered as autoimmune phenomena and represent a clear alteration of the immunological response in which B lymphocytes are implicated to a certain extent.

(b) A marked increase of B-lymphocytes in peripheral blood has been shown and supports the Luckes & Tindel hypothesis (Luckes & Tindel, 1975). On the other hand, a similar increase is often found in autoimmune diseases (Boonpucknavig *et al.*, 1975) and in several types of lymphoproliferative tumours (Moller, 1973).

(c) Finally, peripheral blood lymphocytes showed an increased DNA synthesis response to LPS. LPS at low doses (100 μg/ml) does not stimulate normal peripheral blood lymphocytes but in contrast, it does stimulate lymphocytes from lymphatic organs such as tonsils and spleen (Janossy & Greaves, 1975). In certain immunological disorders, a peripheral lymphocyte response to LPS has been described, but the doses of LPS employed were ten times higher than the dose we have employed (Hammarstrom *et al.*, 1975). Furthermore, it has been claimed that high doses (2000 μg/ml) could stimulate normal peripheral lymphocytes (Hammarstrom *et al.*, 1975). In spite of the fact that we have not studied the response of B lymphocytes independently from T lymphocytes, we can assume that the subpopulation of lymphocytes responding to LPS was the B-cell subpopulation since at the time of the second study we could not detect any response to LPS and the level of B-lymphocytes was dramatically decreased, probably due in part to treatment given to the patient. Furthermore LPS is accepted to be a typical polyclonal activator of B-lymphocytes in all species tested and produces an increase of immunoglobulin secretion as well as an increase of the DNA synthesis (Janossy & Greaves, 1975).

Since, in the case reported, a response to LPS was detected with a low dose, we may speculate on the possibility that in IBL there is not only an alteration in the number of peripheral blood B lymphocytes but in certain functional characteristics of such B lymphocytes as well. The use of mitogens has been described as a tool to characterize different degrees of B-cell maturation in mice (Melchters, Boehmer & Philips, 1975; Gronowitz & Coutinho, 1975). In our case, it can be assumed that the response to LPS can either be indicative of the response in peripheral blood of an abnormal subset of B lymphocytes or can be indicative of the presence of B lymphocytes in peripheral blood from lymph nodes and spleen which, as it is known, are sensitive to 100 μg/ml of LPS (Moller, 1973). The last hypothesis would correlate with the observation that lymph nodes have a marked depletion of B-lymphocytes in IBL (Horne, Fraser & Petrie, 1974).

Since only a slight decrease of E-rosette cells was noted and since the T-cell response to PHA and PPD was normal, we infer that the T-cell population is not substantially affected and that IBL is rather a disease of B cells. The association of the three *in vitro* parameters studied, if they can be confirmed in other cases of IBL, in addition to the findings already described in the literature would support the hypothesis that a quantitative as well as a functional alteration of B-lymphocytes plays a role in the pathogenesis of IBL.

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