Abnormal chromosomal marker (D_{14} q+) in a patient with alpha heavy chain disease

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SUMMARY A patient with alpha heavy chain disease (αHCD), who showed an abnormal chromosomal marker (D_{14} q+) in 10% of the bone marrow cells, is described. The mesenteric lymph nodes, which showed reactive hyperplasia in the first biopsy, transformed later to a malignant lymphoma and finally to a plasma cell tumour. The small intestine revealed villous atrophy, diminished crypts, and intact surface epithelium. The ultrastructure of the goblet and epithelial cells appeared to be normal, and the microvilli were preserved except for circumscribed areas of destruction. The lamina propria was heavily infiltrated with mononuclear cells, mainly mature plasma cells. Alpha heavy chains (αHC) were found in the patient's saliva.

Alpha heavy chain disease (αHCD) is a well-established entity (Rambaud *et al.*, 1968; Seligmann *et al.*, 1968; Doe *et al.*, 1972). It seems to include most patients with Mediterranean lymphoma (Rambaud and Matuchansky, 1973). The pathogenesis and pathological evolution of the disease are still unclear. However, most of the patients were found finally to develop a lymphoma in their abdominal lymph nodes (Rappaport *et al.*, 1972; Rambaud and Matuchansky, 1973; Lewin *et al.*, 1976), probably of the immunoblastic type (Ramot *et al.*, 1977). We had the opportunity to treat a patient with αHCD in whom the evolution of the disease from a benign pathological picture to a malignant one was shown on sequential intestinal and lymph node biopsies, as well as by simultaneous determination of the serum immunoglobulin level. Chromosomal analysis of the bone marrow cells showed an abnormal chromosomal marker (D_{14} q+), which has not been reported in other cases of αHCD.

Material and methods

Immunoelectrophoresis of the serum and saliva was performed by the method of Osserman and Lawler (1961). Quantitative examination of the serum

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immunoglobulins was carried out by the radial immunodiffusion method of Mancini *et al.* (1965). For the determination of immunoglobulins in the saliva the low-level Diffu-Gen immunodiffusion plates (Oxford Laboratories, Foster City, California, USA) were used.

For electron microscopic examination the jejunal mucosa and lymph nodes were cut in small segments and immediately fixed in cold 1% glutaraldehyde in phosphate buffer, pH 7·4, postfixed in 1% osmium tetroxide, dehydrated in graded alcohols, and embedded in Epon 812. Thin sections were cut with an LKB ultratome III and examined with a Philips 300 transmission electron microscope.

Chromosomal analysis was done on bone marrow cells by a direct technique (Tjio and Whang, 1962). G-banding was carried out by the method of Seabright (1971), whereas C-banding was by the method of Sumner (1972)

Case report

A 14-year-old Israeli-Arab boy was admitted in October 1974 because of intermittent diarrhoea of two years' duration, abdominal pain, and severe weight loss. The patient weighed 34 kg. The abdomen was tender with hyperactive peristalsis. The liver was palpable 2 cm below the costal margin, and spleen and lymph nodes were not palpable.

Laboratory examinations were within normal limits, except for alkaline phosphatase of 4·6 Bessey

Lowry units and IgA of 780 mg/dl (normal range 90-450 mg/dl). Tests for malabsorption, as well as *x*-ray of the small intestine, were compatible with a malabsorption syndrome. Peroral biopsy of the duodenal mucosa showed villous atrophy and intensive infiltration of the lamina propria with mature plasma cells. Exploratory laparotomy revealed a thickened intestinal wall and mesenteric lymphadenopathy. A few mesenteric lymph nodes and the spleen were removed. The nodes revealed hyperplastic germinal centres of different diameters (Fig. 1) and widely

Fig. 2

Fig. 1

Fig. 1 *Lymph node with hyperplastic germinal centres* (× *150*).

Fig. 2 *Serum immunoelectrophoresis. Right column: N—normal serum; P—patient's serum. Left column: K—antikappa antiserum; P—polyvalent antiserum; L—antilambda antiserum; G—anti IgG antiserum; A—anti IgA antiserum. The arc is induced by the alpha heavy chain with anti IgA antiserum; M—anti IgM antiserum.*
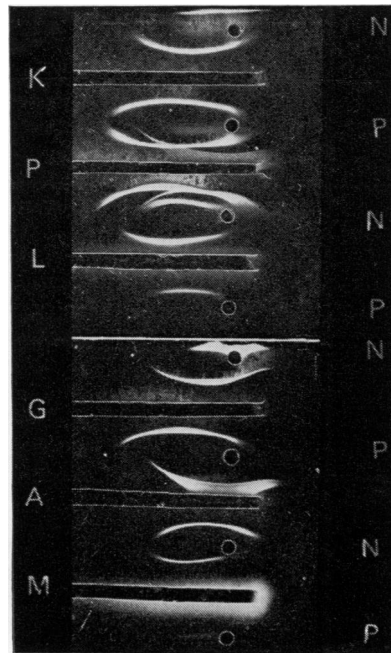
Fig. 3 *Saliva immunoelectrophoresis. Right column; N—normal saliva; P—patient's saliva. Left column: K—antikappa antiserum; A—anti IgA antiserum. The prominent arc with anti IgA is due to alpha heavy chain. L—anti lambda antiserum; G—anti IgG antiserum; M—anti IgM antiserum.*
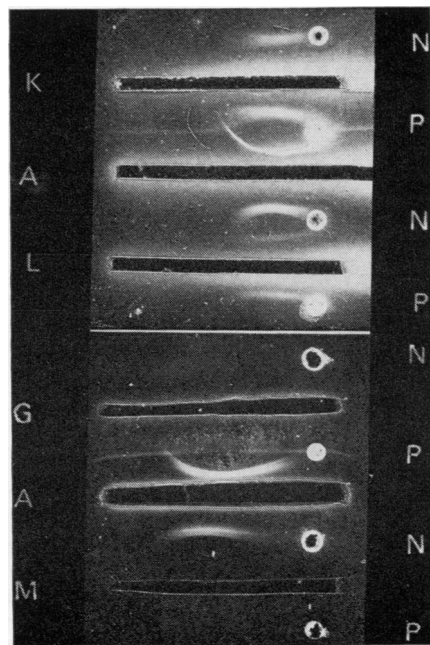

Fig. 3

Table 1   *Sequential determinations of the patient's globulin, albumin, and immunoglobulins*

Date	Globulin (g/dl)	Albumin (g/dl)	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)
29 Oct '74	2·5	3·3	1200	780	110
11 Mar '76	4·5	3·3	800	10800	45
12 July '76	4·3	2·6	330	11300	32
Control values	2·0-3·2	3·9-4·9	800-1800	90-450	60-250

dilated sinusoids. The sinusoids were infiltrated with mature plasma cells. Occasionally, large, primitive plasma cells were seen. The spleen was normal. The diagnosis of Mediterranean lymphoma was suspected, and combined treatment with cyclophosphamide, vincristine, procarbazine, and prednisone was started. The diarrhoea subsided and the patient gained weight up to 44 kg. Severe diarrhoea and weight loss reappeared in March 1976. Serum protein electrophoresis revealed a broad β band. Serum immunoelectrophoresis showed the presence of αHC (Fig. 2), confirming the diagnosis of αHCD. Quantitative examination showed an extremely high level of IgA with a low level of IgM and IgG (Table 1). Subsequently, the level of IgA increased, whereas that of IgM and IgG decreased. The albumin level was also decreased (Table 1).

Immunoelectrophoresis of the patient's saliva revealed a precipitation line with anti-IgA without accompanying precipitation lines with anti-light chain antisera (Fig. 3). Quantitative examination of the saliva revealed a high IgA level in comparison with the saliva of control subjects. IgG was normal, IgM was not found, and the kappa to lambda ratio was similar in both patient's and controls' saliva (Table 2), which confirmed the existence of αHC in the patient's saliva.

Table 2   *Immunoglobulins in the patient's and controls' saliva*

Subject	Globulins				
	IgA (mg/dl)	IgG (mg/dl)	IgM (mg/dl)	K %	l %
Controls					
1	8	4	0	56	44
2	7	4			
3	8	4			
4	8	4			
Patient	50	4	0	54	46

At that stage, a second peroral biopsy of the jejunal mucosa was performed. It showed villous atrophy, intact surface epithelium, and diminished crypts. The lamina propria was heavily infiltrated with mature plasma cells (Fig. 4). The ultrastructure of the microvilli was relatively normal, except for destruction of small superficial epithelium and its microvilli (Fig. 5). The epithelial and goblet cells were

well preserved except for slight vacuolisation. There was intensive infiltration of the lamina propria with plasma cells (Fig. 6) and lymphocytes. The nuclei of the plasma cells were eccentric, round, or oval and contained fair amounts of heterochromatin. The endoplasmic reticulum was well developed and the cisternae were dilated. The lymphocytes revealed an advanced degree of nuclear maturation, but the cytoplasmic membrane appeared discontinuous and even completely destroyed. In addition, cells probably epithelial in type with highly irregular nuclear outline were seen (Fig. 7). The nucleus was almost completely deprived of heterochromatin. Numerous small, round, or oval mitochondria were present in the cytoplasm.

The previous treatment was resumed with the addition of tetracycline. The diarrhoea stopped, but the remission was short. Three months later the patient developed watery diarrhoea, up to 5 litres a day, high fever, and severe electrolyte disturbances. He was given intravenous hyperalimentation, antibiotics, melphalan, and corticosteroids. His condition improved, but he developed intestinal obstruction, which was operated on successfully. During the operation, which revealed adhesions, a few mesenteric lymph nodes were removed. Their architecture was indistinct. The picture was monomorphous with the predominance of immature lymphoplasmacytoid cells (Fig. 8). The diagnosis was diffuse malignant lymphoma.

Electron microscopic examination of the lymph nodes showed an almost completely blurred structure. Most of the cells possessed irregularly shaped nuclei, scanty heterochromatin, and poorly defined cytoplasmic membrane (Fig. 9). In other areas, the predominant cells were plasma cells at various stages of maturation. Immature cells with nucleo-cytoplasmic asynchronism (Fig. 10) and mature plasma cells were almost equally found (Fig.11). Lymphoblasts were also seen.

A chromosomal analysis of the bone marrow cells was also performed. Forty cells were examined, and 15 were karyotyped. The modal count was 46 (XY). Two cells had 45 chromosomes, and two cells had 44 chromosomes with random loss. By C-banding a pericentric inversion of the heterochromatic part in one of the No. 9 chromosomes could be noted. The breakage rate was increased (as compared with the
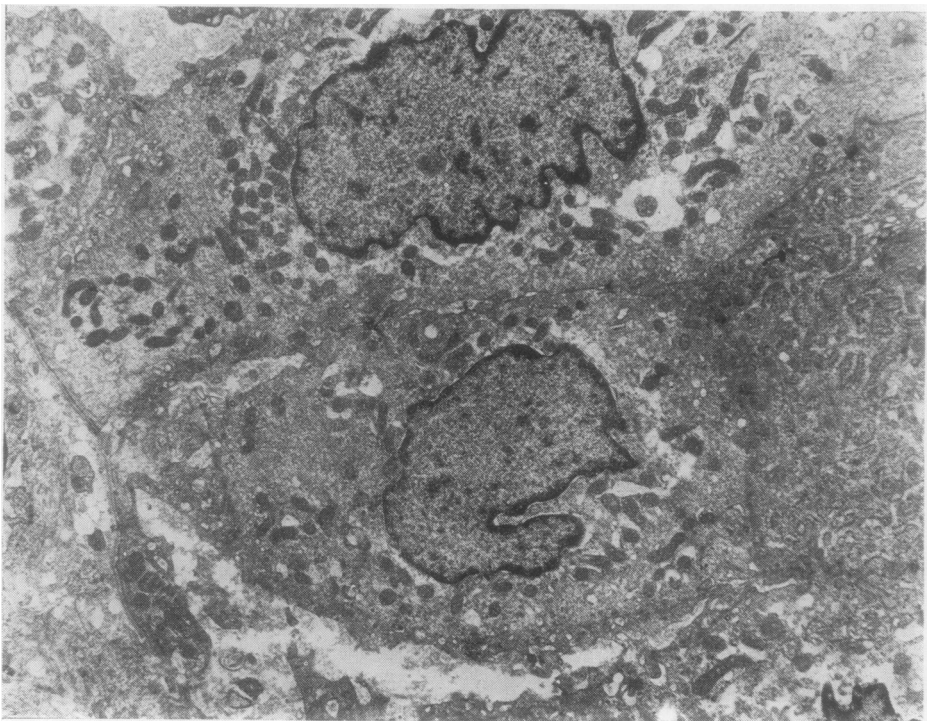
Fig. 4 *Jejunum with villous atrophy, intact surface epithelium, and heavy infiltration of the lamina propria with plasma cells* (× 360).
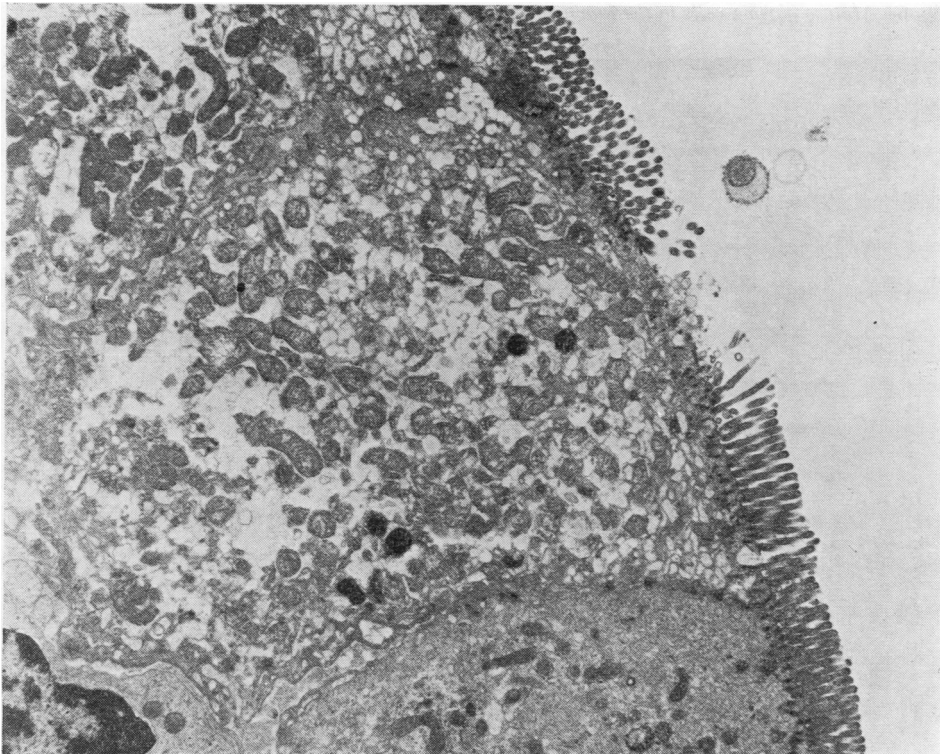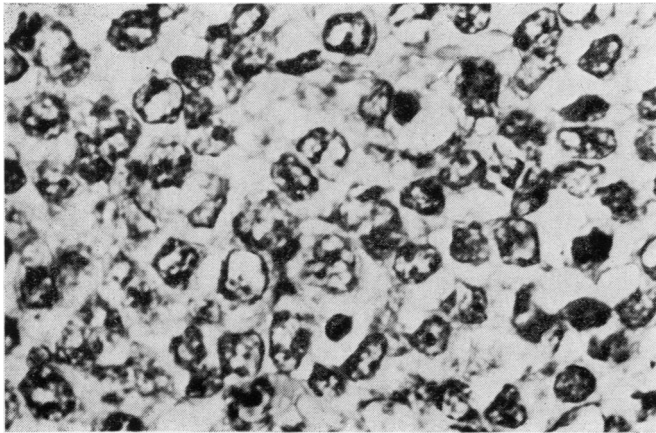

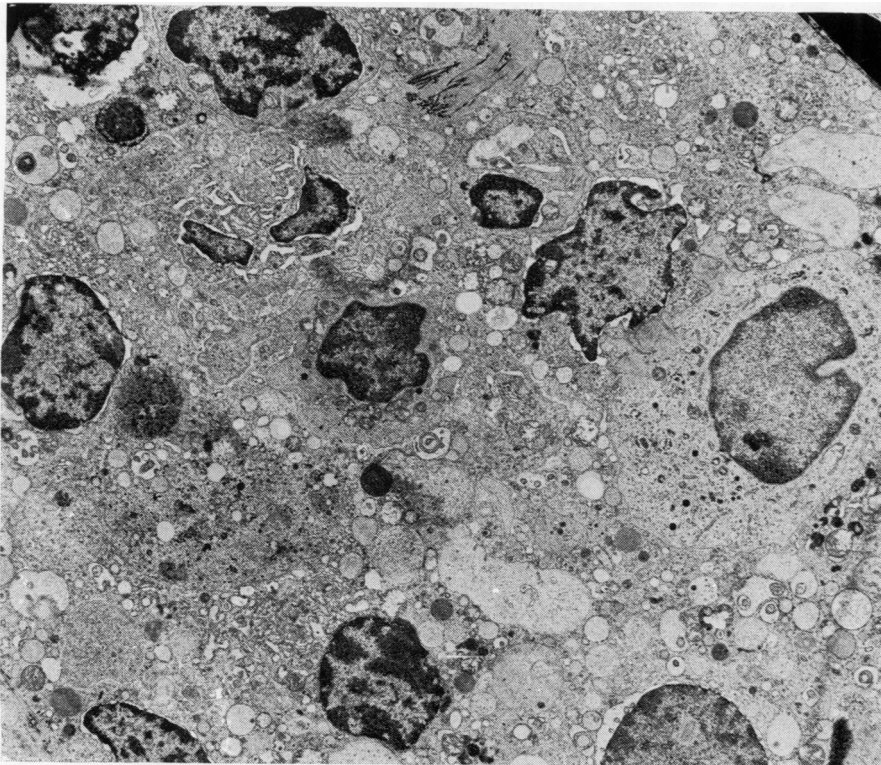Fig. 5 *Jejunum showing normal microvilli, some with destroyed areas* (× 6300).
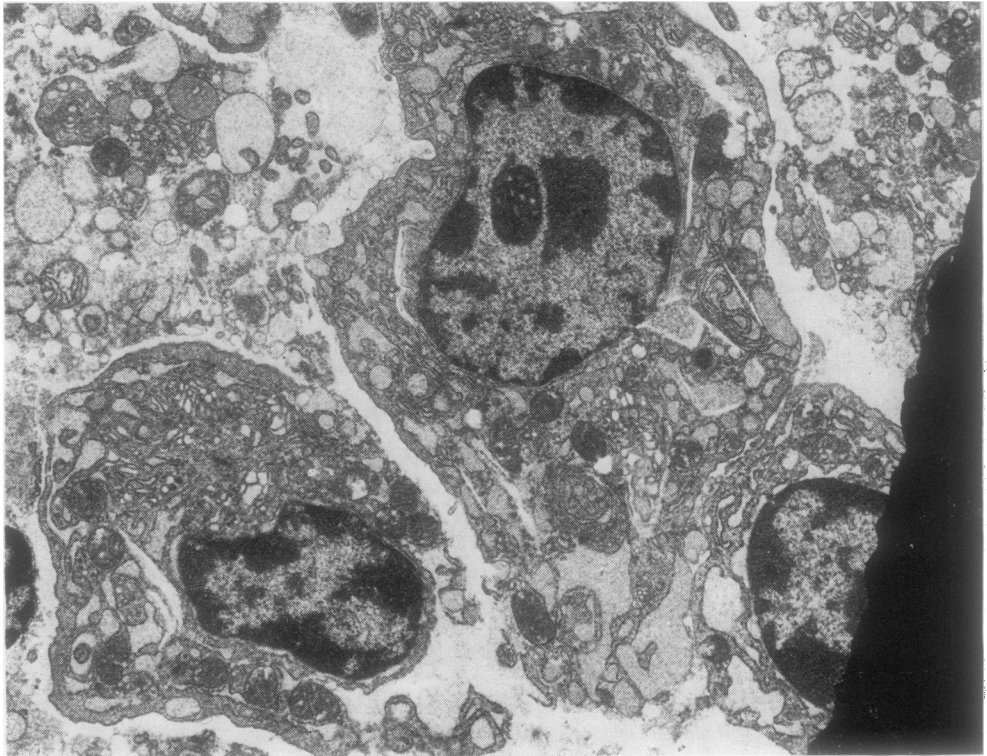
**Fig. 6** *Mature plasma cells in the lamina propria (× 6300).*

Fig. 7 *Cells, probably epithelial in type, with irregular nuclear outline, scanty heterochromatin, and numerous oval and round mitochondria (× 6600).*
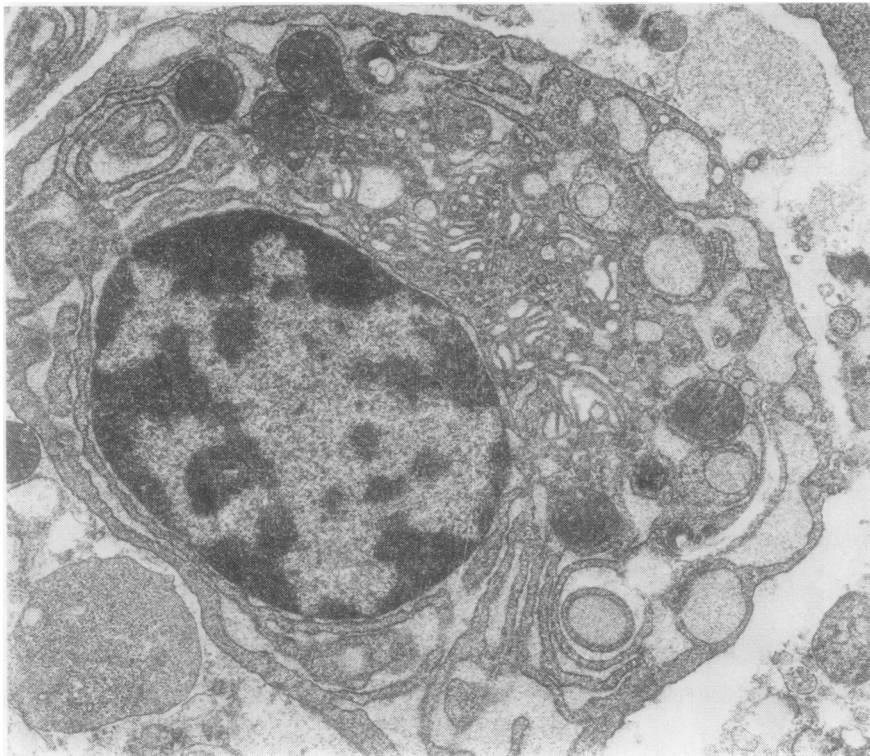
Fig. 8 *Lymph node showing predominance of immature lymphoplas- macytoid cells (× 480).*

Fig. 9 *Lymph node with blurred structure. The cytoplasmic membranes are scarcely visible (× 4900).*
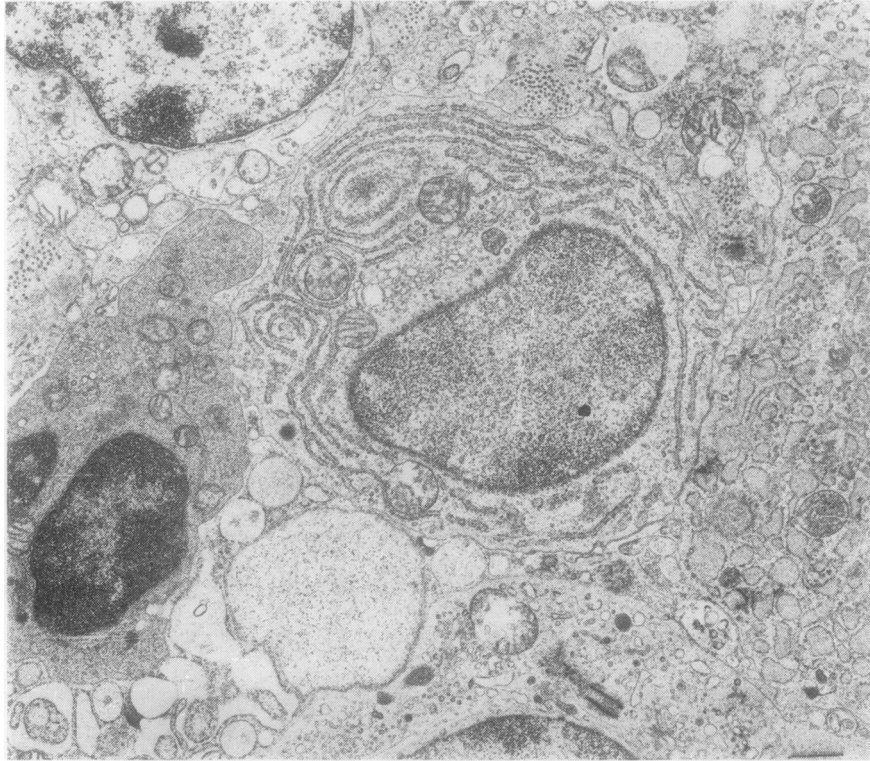
Fig. 10 *An immature plasma cell in the lymph node, with nucleocytoplasmic asynchronism, immature nucleus, and developed cytoplasmic reticulum (× 8000).*

Fig. 11 *A mature plasma cell in the lymph node (× 13 000).*

normal range in our laboratory), with clustering of breakages in groups D and B. Ten per cent of the cells had the same karyotype presenting a marker D chromosome (D_{14} q+) (Fig. 12). No translocation could be found. The No. 8 chromosomes were particularly examined. The karyotype of most of the cells was normal.

About two months after the operation the patient's condition began to deteriorate gradually, and he died about six months later, in March 1977. On postmortem examination huge mesenteric and retroperitoneal lymph nodes were found. Histologically, the predominant cells were immature plasma cells (Fig. 13). The histology of the small intestine was similar to that seen in the previous biopsies. The liver showed plasma cell infiltration in the portal spaces and moderate fatty degeneration.

Discussion

The natural history of the patient's disease represents a transformation from a relatively benign pathologic state to a malignant tumour. While the histology of the small intestine did not change during the whole follow-up period, the lymph nodes, which showed reactive hyperplasia in the first biopsy, turned to a malignant lymphoma in the second and eventually to a plasma cell tumour in the final stage of the disease. The pathologic development was accompanied by a progressive increase in the serum IgA level, indicating a malignant transformation (Rambaud and Matuchansky, 1973). Bognel *et al.* (1972) described a patient with a similar evolution in whom, unlike in our patient, the malignant transformation involved the small and large intestine and only finally the mesenteric lymph nodes. Two
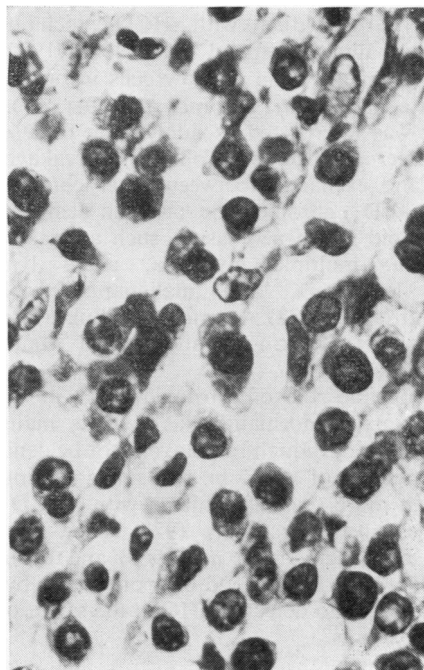

Fig. 13 *A lymph node showing immature plasma cells* ($\times$ *480*).

Fig. 12 *Karyotypes from three cells presenting a marker D chromosome* (D_{14} *q*+).

possible mechanisms may explain the evolution of the disease in our patient: either a dedifferentiation from a mature plasma cell to an immature lymphoid cell, or the appearance of an additional clone (Rambaud and Matuchansky, 1973). The observations of Ramot *et al.* (1977), who have found *in vitro* αHC production by both intestinal and lymph node cells in a patient with αHCD, favour the dedifferentiation mechanism. The progressive rise in αHC serum level in our patient serves as an additional support.

The light microscopic findings of the small intestine, which included villous atrophy, a decreased number of crypts, intact surface epithelium, and heavy infiltration with plasma cells, are typical for αHCD (Guardia *et al.*, 1976; Selzer, G. personal communication). As for the ultrastructure, we found that the goblet and epithelial cells were preserved, and the microvilli appeared to be normal, too, except for circumscribed areas of destruction.

Although the plasma cells in the jejunal biopsy appeared to be mature, when examined with the electron microscope it was difficult to define them as normal, since they showed dilated endoplasmic reticulum observed in other malignant conditions such as multiple myeloma.

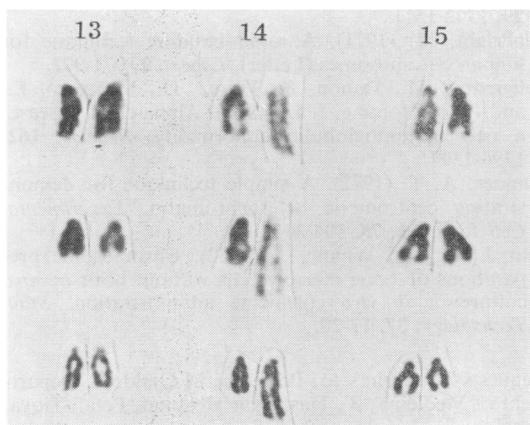In contrast to previous chromosomal studies of

blood cells from patients with αHCD, which revealed no abnormality (Rambaud *et al.*, 1968; 1970), the karyotype of the bone marrow cells in our patient showed a marker D_{14} chromosome (D_{14} q +). This marker was interpreted as duplication of D_{14} long arm material, probably following breakages on D_{14} q and exchanges between sister chromatides. Abnormal D_{14} chromosome has been demonstrated in lymphoid B cell neoplasms, such as plasma cell leukaemia, multiple myeloma, acute lympho-blastic leukaemia, and malignant lymphoma (Oshimura *et al.*, 1977). A common finding in Burkitt's lymphoma is D_{14} q + (Manolov and Manolova, 1972).

The possible role of cytotoxic drugs in causing chromosomal abnormalities as well as malignant transformation, though remote, cannot be denied.

The finding of αHC in the saliva is unusual. Although more than 80 patients with αHCD have been described (Franklin, 1977) since the first reports by Seligmann *et al.* (1968) and Rambaud *et al.* (1968), we have found only one paper by Doe *et al.* (1972), who detected αHC in the saliva of one of their patients.

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