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The mutual clonal origin of the lymphoplasmocytic and lymphoma cell in alpha-heavy chain disease

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SUMMARY

Biosynthetic studies in α-heavy chain disease were performed on the gut tumour which was composed mainly of lymphoplasmocytic cells and on the mesenteric lymph node tumour composed mainly of immunoblasts. The gut tumour cells synthesised alpha-heavy chains and secreted them during 2·5 hr culture, whereas the lymph node tumour cells synthesized alpha-heavy chains which were shed into the culture medium only after 20 hr. These chains were shown to be present on the surface of the immunoblastic tumour cells by enzymatic radioiodination. Both the surface and the secreted alpha-heavy chains of the lymph node and gut tumour were found to be smaller than the alpha-heavy chain of myeloma proteins.

These results suggest that the lymphoblasmocytic and the immunoblastic tumour cells originate from the same defective clone.

INTRODUCTION

α-heavy chain disease (α-HCD) is a well-described clinical entity (Seligmann *et al.*, 1968; Seligmann *et al.*, 1971; Ramot, 1971; Doe *et al.*, 1972). Although it is characterized by a diffuse lymphoplasmocytic infiltration of the gut, its clinicopathological evolution and its relation to intestinal lymphoma in general is not clear (Ramot, Shahin & Bubis, 1965; Ramot & Hulu, 1975). Few patients with α-HCD have remitted completely after antibiotic therapy (Rogé, Druet & Marche, 1970; Ramot & Hulu, 1975). In most cases however, the intestinal, mesenteric and finally abdominal lymph nodes become involved by a lymphoma (Rappaport *et al.*, 1972) most probably of the immunoblastic type.

This type of lymphoma could possibly result from either the dedifferentiation of the same clone of 'reactive' lymphoplasmocytic cells which infiltrate the gut and synthesize the α-heavy chain (α-HC), or it may originate from an additional clone of poorly differentiated lymphocytes of the immunoblastic type.

In this study evidence is provided for active synthesis of α-HCs' by both plasmocytes and tumour cells and the presence of α-HCs on the surface of the tumour cells. These results support the hypothesis suggesting that the lymphoma arose from dedifferentiated cells originating from the same defective clone.

CASE REPORT

A 37-year-old Yemenite male living in Israel for the last 20 years, and healthy up to 1974, at which time he developed chills and fever accompanied by mid-abdominal pain with severe constipation and abdominal distention. The diagnosis of α-HCD was not made until early 1976, when the patient was rehospitalized because of severe abdominal pain, marked weight loss and fever. An upper GI tract study showed diffuse involvement of the gut from the duodenum and extending to the terminal ileum. Biopsy of the gut revealed a subtotal villous atrophy with a diffuse lymphoplasmocytic infiltration. Among the lympho-

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plasmocytes were large stem cells that appeared pathological. Relevant laboratory findings were: Hb 12·7 g/dl; total protein g/dl; albumin 3·3 g/dl; globulin 2·7 g/dl; IgG 280 mg/dl; IgA 600 mg/dl; IgM 43 mg/dl; stool fat 7 g/24 hr. Serum immunoelectrophoresis revealed α-heavy chain protein (Fig. 1). At exploratory laparotomy, a diffusely thickened gut as well as a small tumour mass in the mid ileum and a large conglomerate of mesenteric lymph nodes were observed. The tumour of the gut was resected and six lymph nodes were removed for histological and biosynthetic studies. The histological diagnosis of the lymph nodes was malignant lymphoma of the immunoblastic type most probably, since all the cells of the tumour reacted only with fluorescent anti-IgA serum while the intestinal tumour was composed of plasma cells as well as large poorly differentiated malignant cells.

The patient was given weekly cyclophosphamide, oncovin, prednisone (COP) with a marked clinical improvement.

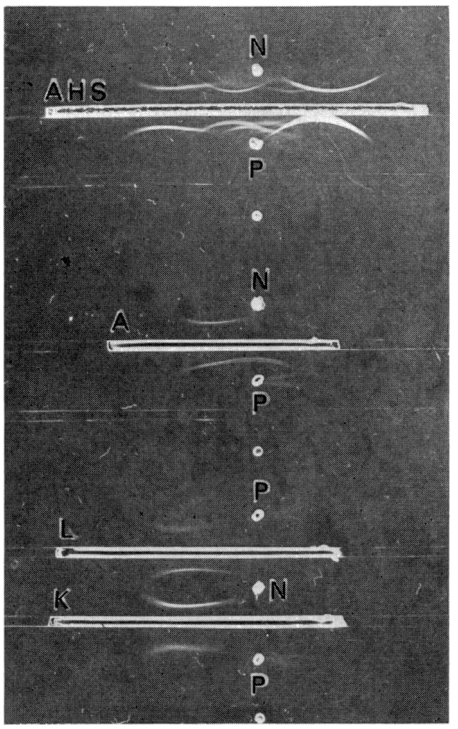

FIG. 1. Immunoelectrophoretic characterization of IgA from a patient with α-heavy chain disease. P=patient serum; N=normal serum; AHS=anti-human serum; A=anti-IgA serum; L=anti-lambda chains serum; K=anti-kappa chains serum.

MATERIALS AND METHODS

Biosynthetic studies were performed both on the tumour of the gut (TG) and mesenteric lymph node tumour (TLN). Since the amount of tissue obtained from the gut tumour was relatively small, only short term, 2·5 hr *in vitro* biosynthesis was performed on cells from this source. The lymph node cells were subjected to 2·5 hr as well as to 20 hr *in vitro* biosynthesis and to radioiodination of their surface proteins.

Treatment of cells. Cells from TG and TLN were teased filtered through a stainless steel screen and washed three times with 0·15 M NaCl in 0·01 M phosphate buffer (PBS) pH 7·2. Mononuclear cells were separated on a Ficoll–Metrizoate gradient and washed three times with Spinner medium containing 1/100 the standard amount of amino acids.

In vitro biosynthesis. Cell suspensions (5×10^7 cells/ml) of TG and TLN were preincubated for 15 min at 37°C followed by the addition of 5/μC of ^{14}C amino acid mixture (uniformly labelled, 45 mCi/milliatom of carbon, Amersham). The cells were incubated at 37°C for 2·5 hr in a 5% CO_2–95% air atmosphere, then chilled to 4°C and spun at 500 *g* for 6 min. The supernatant 'extracellular' fraction was collected. The pellet was washed three times with cold Spinner medium and the cells lysed at 4°C for 15 min with 0·5% Nonidet P_{40} (NP_{40}) in PBS containing Trasylol, 0·2 M iodoacetamide. Debris were removed by centrifugation at 18,000 *g* for 15 min at 4°C and the supernatant collected. This lysate will be designated 'intracellular' fraction.

TLN cells (5×10^6/ml) suspended in Spinner medium without leucine with 10% foetal calf serum, were incubated with

20 μCi [^{3}H]leucine (leucine 3,4,5-3H, New England Nuclear) for 20 hr at 37°C in a 5% CO_2 95% air atmosphere. Both the 'extracellular' and 'intracellular' fractions were obtained as described above.

The radioactivity incorporated into the proteins of the two fractions was determined following precipitation with 5% trichloroacetic acid (TCA), as previously described Moroz & Lahat (1974).

Radioiodination of surface proteins. TLN cells were radioiodinated as previously described (Moroz & Lahat, 1974). Prior to iodination the cells were treated with 0·05 M iodoacetamide in PBS for 60 min at 4°C. For iodination 4×10^7 cells suspended in 0·15 ml PBS were mixed with 1 mCi ^{125}I (10 μl carrier free Na ^{125}I, Amersham), 10 μl 0·3 mg/ml lactoperoxidase (Sigma) and three additions of 10 μl H_2O_2 0·03% during an incubation of 20 min at 4°C. Iodination was terminated by addition of 10 ml cold PBS, and the enzyme was removed by three washes with cold PBS. The washed cells were lysed with 0·5% NP_{40}-trasylol-0·2 M iodoacetamide and the 'intracellular' fraction which included the radioiodinated surface proteins was obtained as described above.

Electrophoresis. The labelled proteins in the 'extracellular' and 'intracellular' fractions were characterized by electrophoresis on 7% polyacrylamide gels containing 0·1% sodium dodecyl sulphate (SDS) Shapiro *et al.* (1966), Moroz & Hahn (1973). Electrophoresis was run at 15 mA/gel for 2·5 hr and the gels were sectioned into fractions. The radioactivity in each fraction was determined with a Packard Model 3380 Tricarb spectrophotometer. The reduction of proteins when performed prior to electrophoresis was done by treatment of samples with 0·2 M mercaptoethanol (2ME) for 1 hr followed by alkylation with 0·22 M iodoacetamide at pH 8 for 1 hr at room temperature. Standards for comparison of electrophoretic mobility of immunoglobulin (Ig) heavy and light chains, were obtained from reduced and alkylated myeloma IgG and IgA proteins.

Immunoprecipitation. Radioactive Igs were isolated from 'intracellular fractions by immunoprecipitation using the following monospecific antisera: goat antiserum to human γ chains, μ chains, α chains, κ chains, λ chains (Meloy, Springfield, Virginia). The antisera were rendered monospecific by further purification on immunoadsorbents of non-related myeloma proteins. Intracellular or extracellular aliquots were incubated for 2 hr at 4°C with either one of the antisera in antibody excess to form soluble complexes, which were then precipitated by the addition of rabbit antiserum to goat γ globulin (Meloy) for 16 hr at 4°C. Non-specific coprecipitable radioactivity was determined using normal goat serum in the same system. Each precipitate was collected by centrifugation at 12,000 *g* for 15 min at 4°C, washed five times with cold PBS, suspended in PBS containing 1% SDS and 0·5 M urea, placed in a boiling water bath for 1 min and the radioactivity in the dissolved precipitate determined. The precipitated Igs were reduced and alkylated and further characterized by electrophoresis on acrylamide SDS-gel as described above.

RESULTS

Synthesis and secretion of nascent proteins of TG and TLN cells in culture

Results presented in Table 1 demonstrate that radioactive amino acids were incorporated into intracellular proteins of TG cells during 2·5 hr incubation. Nascent radiolabelled proteins were also secreted into the culture medium during the same period of time. TLN cells cultured for 2·5 hr incorporated only about 20% of the radioactive amino acids into 'intracellular' proteins and no nascent proteins were secreted. However, when the TLN cells were cultured for 20 hr, radioactive protein was demonstrated in the culture medium.

α-HCD protein synthesis in TG and TLN cells in culture

IgA was the only nascent immunoglobulin precipitated from the cytoplasm of TG cells (Table 2). 65% of the radioactivity incorporated into intracellular proteins during 2·5 hr incubation was precipitated with anti-α chain serum. In contrast, when the cytoplasm of TLN cells was reacted with anti-α chain serum, the amount of precipitable radioactivity was similar to that of non-specific coprecipitation

TABLE 1. Incorporation of radioactive amino acids into intracellular and extracellular proteins synthesized by TG and TLN cells in culture

Source of cells	Time of culture (hr)	Isotope	Radioactivity ct/min/10^6 cells	
			Intracellular	Extracellular
TG	2·5	^{14}C	6650	1100
TLN	2·5	^{14}C	1400	140
TLN	20·0	^{3}H	17000	16000

TABLE 2. Incorporation of radioactive amino acids into intracellular Ig synthesized by TG and TLN cells in culture

Source of cells	Time of culture (hr)	Antiserum	Radioactivity in immunoprecipitate/10^6 cells	
			ct/min	(%)
TG	2·5	Anti-α-chain	4200	65·0
		Anti-μ-chain	240	4·0
		Anti-γ-chain	240	4·0
		Normal goat serum	280	4·4
TLN	2·5	Anti-α-chain	250	3·8
		Normal goat serum	210	3·2
TLN	20	Anti-α-chain	380	2·3
		Normal goat serum	340	2·0

with normal goat serum, suggesting that either this protein is not synthesized by these cells or that the intracellular pool of nascent Ig is very small. The latter possibility was more likely, since ^{3}H-labelled protein which appeared in the extracellular culture medium of TLN cells following incubation for 20 hr with [^{3}H]leucine (Table 1) was precipitated with anti-α-chain serum, indicating active synthesis of α-chains by these cells (Fig. 2C).

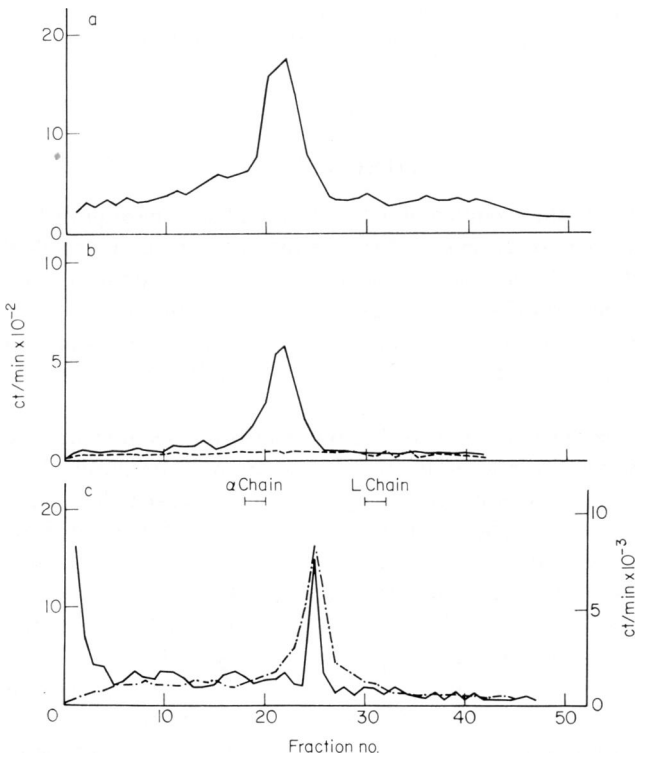

FIG. 2. Electrophoresis on acrylamide-gel-SDS radiolabelled α-HC isolated from TG and TLN cells by immunoprecipitation. All immunoprecipitates were reduced and alkylated prior to electrophoresis. (a) ^{14}C-labelled 'extracellular' fraction of TG cells reacted with anti-α chain serum. (b) ^{14}C-labelled 'intracellular' fraction of TG cells reacted with anti-α chain serum (——) and anti-light chain serum (– – – –). (c) ^{3}H-labelled 'extracellular' fraction of TLN cells reacted with anti-α chain serum (– · – · –) and ^{125}I-labelled 'intracellular' fraction reacted with anti-α chain serum (——).

The nascent IgA precipitated from intracellular and extracellular fractions obtained from TG and TLN cells was further characterized by electrophoresis on acrylamide gel. As seen in Fig. 2, ^{14}C-labelled, IgA precipitated from the extracellular and intracellular fractions of TG cells, when reduced and alkylated, exhibited a single radioactive peak corresponding to α-heavy chains with no peak corresponding to light chains (Fig. 2a, b). Moreover, no radioactive peak corresponding to light chains could be demonstrated in the cytoplasm of TG cells after reaction with anti-κ or anti-λ chain sera (Fig. 2b). The electrophoretic mobility of the α-HC from TG cells was faster than normal α-chains obtained from reduced and alkylated IgA myeloma protein. Nascent IgA precipitated from the extracellular fraction of TLN cells cultured for 20 hr equally exhibited a single peak corresponding to α-HC and lacking a peak corresponding to light chains. However, TLN α-HC was of faster electrophoretic mobility than the α-HC obtained from TG cells, indicating a difference in mol. wt. (Fig. 2c).

α-HCD protein on the surface of TLN cells

Enzyme radioiodination of TLN cells followed by reaction with anti-α-chain serum indicated that 6·6% of the iodinated proteins on the surface of the cells were immunoprecipitated as IgA. Further characterization of the reduced alkylated radioiodinated immunoprecipitate by electrophoresis on acrylamide gel indicated that the IgA on the surface of TLN cells consists of a radioactive peak corresponding to α-HC with no peak corresponding to light chains (Fig. 2c). The radioactivity at the beginning of the gel may represent polymerized iodinated α-HC which were not dissociated after reduction and alkylation. The electrophoretic mobility of the iodinated surface α-HC was found to be identical to that of ^{3}H nascent α-HC isolated from the culture medium of these cells, indicating identity of their molecular weight (Fig. 2c). On the other hand, both the surface and the secreted α-HC isolated from the TLN had a faster mobility than those secreted by the TG cells indicating an α-chain fragment of smaller size.

DISCUSSION

It is well known that during the evolution of α-HCD a lymphoma, most probably of the immunoblastic type evolves. The question however remains open whether this tumour is a result of the dedifferentiation of the lymphoplasmocytic cells, or is due to the appearance of an additional clone of pathological cells.

Biosynthetic studies performed on lymphoplasmocytic cells from the gut of patients with α-HCD showed synthesis of α-HC protein only (Seligmann *et al.*, 1969). No answer however has been given as to the biosynthetic capacity of the α-HC by the tumour cells (WHO meeting on α-heavy chain to be published).

Our results indicate that the gut as well as the immunoblastic tumour synthesizes α-HC. The tumour cells however did not actively secrete α-HC into the culture medium during 2·5 hr incubation. On the other hand, 20 hr culture of the same cells was followed by marked shedding of the α-HC into the culture medium. The size of the shed protein was smaller than that secreted by the gut tumour containing plasma cells in large numbers. The surface α-HC on the tumour cells was found to be identical in size to that of the shed α-HC from the same tumour cells.

In both the lymph node tumour (TLN) and the gut tumour (TG) however, the molecular size of the α-HC was smaller than α-HC obtained from myeloma protein. The difference in mol. wt. of α-HC obtained from the two tissues studied, as evidenced by a difference in electrophoretic mobility on acrylamide-gel, could be the result of proteolytic degradation occurring intracellularly or extracellularly, in spite of the measures taken to inhibit proteolysis during the isolation of the protein. The size similarity of the intracellular and extracellular α-HC within the same cell type (Fig. 2), does not favour proteolysis as a plausible explanation. The second possibility suggested previously in the literature is a deletion in α-HC protein, which would imply a defective messenger. The observation that the α-HC fragment on the surface and shed by the tumour cells is smaller than the protein isolated from the gut tumour culture, would imply an additional mutation in the same clone of cells, possibly during their

dedifferentiation. Either of these explanations could be proven by the isolation of the messenger for α-HC from gut plasma cells infiltrating the reactive gut as compared with the tumour cells.

Our data confirm those of Seligmann *et al.* (1969) concerning the absence of light chain synthesis by the lymphoplasmocytic cells. Furthermore, we were unable to show light chain synthesis by the tumour cells.

We conclude that the proliferating plasmocytes and the immunoblastic tumour cells originate from the same defective clone.

Since antigen can regulate the pathway, the rate of differentiation and replication of immune cells (Uhr & Horibata, 1967), the surface α-HC fragment on the immunoblastic tumour cells may represent a defective receptor which cannot be regulated by a specific antigen.

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REFERENCES

Doe, W.F., Henry, K., Hobbs, J.R., Avery, Jones, F., Dent, C.E. & Booth, C.C. (1972) Five cases of alpha chain disease. *Gut*, **13**, 947.

Moroz, C. & Lahat, N. (1974) Surface immunoglobulin of mouse thymus cells and its *in vitro* biosynthesis. *Cell. Immunology*, **13**, 397.

Moroz, C. & Hahn, Y. (1973) Cell-surface immunoglobulin of human thymus cells and its biosynthesis *in vitro*. *Proc. nat. Acad. Sci.* (*Wash.*), **70**, supplement II.

Ramot, B. (1971) Malabsorption due to lymphomatous disease. *Ann. Rev. Med.* **22**, 19.

Ramot, B., Shahin, N. & Bubis, J.J. (1965) Malabsorption syndrome in lymphoma of small intestine: a study of 13 cases. *Israel J. med. Sci.* **1**, 221.

Ramot, B. & Hulu, N. (1975) Primary intestinal lymphoma and its relation to alpha heavy chain disease. *Brit. J. Cancer*, **31**, supplement II.

Rappaport, H., Ramot, B., Hulu, N. & Park, J.K. (1972) The pathology of so-called Mediterranean abdominal lymphoma with malabsorption. *Cancer*, **29**, 1502.

Rogé, J., Druet, P.H. & Marche, C. (1970) Lymphoma Mediterranean avec maladie des chaines alpha. Triple remission clinique, anatomique et immunologique. *Path. Biol.* **18**, 851.

Seligmann, M., Danon, F., Hurez, D., Mihaesco, E. & Preud'Homme, J.L. (1968) Alpha chain disease: a new immunoglobulin abnormality, *Science*, **162**, 1396.

Seligmann, M., Mihaesco, E. & Fragnione, B. (1971) Studies on alpha chain disease, *Ann. N.Y. Acad. Sci.* **190**, 487.

Seligmann, N., Mihaesco, E., Hurez, D., Mihaesco, C., Preud'Homme, J.L. & Rambaud, J.C. (1969) Immunochemical studies in four cases of alpha chain disease. *J. clin. Invest.* **48**, 2374.

Shapiro, A.L., Scharff, M.D., Maizel, J.V. Jr & Uhr, J.W. (1966) Polyribosomal synthesis and assembly of the heavy and light chains of gamma globulin. *Proc. nat. Acad. Sci.* (*Wash.*) **56**, 216.

Uhr, J.W. & Horibata, K. (1967) The role of antigen in the regulation of antibody formation. *Immunity Cancer and Chemotherapy*, p. 187. Acad. Press, New York.