Alpha-Chain Disease: A New Immunoglobulin Abnormality

Abstract. *A new type of pathological immunoglobulin was found in the serum, urine, and saliva of a young Arab patient with abdominal lymphoma and diffuse lymphoplasmacytic infiltration of the small intestine. This protein is devoid of light chains and is closely related to the alpha polypeptide chains of the γ_{A1} (Le) subclass of immunoglobulin A. It is characterized by electrophoretic heterogeneity, tendency toward polymerization, and a high carbohydrate content. No intracellular synthesis of light chain was detected.*

An uncommon form of malignant lymphoma (called heavy γ-chain disease) is characterized by the presence in serum and urine of a naturally occurring immunoglobulin representing a portion of the γ heavy chains mainly related to the Fc fragment (*1*). When this syndrome was described, it was anticipated that similar conditions involving heavy chains of immunoglobulin A and M (IgA and IgM) would ultimately be identified.

We have found a corresponding abnormality for IgA globulin in a young Syrian woman (TL) with an abdominal lymphoma, presenting as a severe malabsorption syndrome with a diffuse lymphoplasmacytic infiltration of the entire length of the small intestine. The clinical picture of this patient, analogous to previous observations in young Arabs and non-European Jews (*2*), has been described (*3*). The pathologic IgA globulin (TL protein), devoid of light chains, was found in serum, urine, saliva, and in the proliferating cells.

The electrophoretic pattern of the serum showed a broad abnormal band in the β region, contrasting with a decrease of all the other electrophoretic fractions. This β band, mainly due to the TL protein, accounted for 40 percent of the total serum proteins and represented approximately 4 g/100 ml in the initial samples and 2 g/100 ml in subsequent samples. Concentrations of IgG and IgM were 6 mg and 0.8 mg/ml, respectively. Immunoelectrophoretic analysis of the serum showed an abnormal precipitin line extending from the $\alpha 2$ to the $\beta 2$ region (Fig. 1A). This line corresponding to the TL protein was revealed by all antiserums specific for IgA. Some normal IgA globulins were detected inside the TL protein line with some of the antiserums to IgA. The electrophoretic heterogeneity of the TL protein was also demonstrated by polyacrylamide-gel electrophoresis which showed multiple bands.

The TL protein did not precipitate with any antiserum specific for κ or λ light chains, whether prepared to Bence Jones proteins or to myeloma globulins.

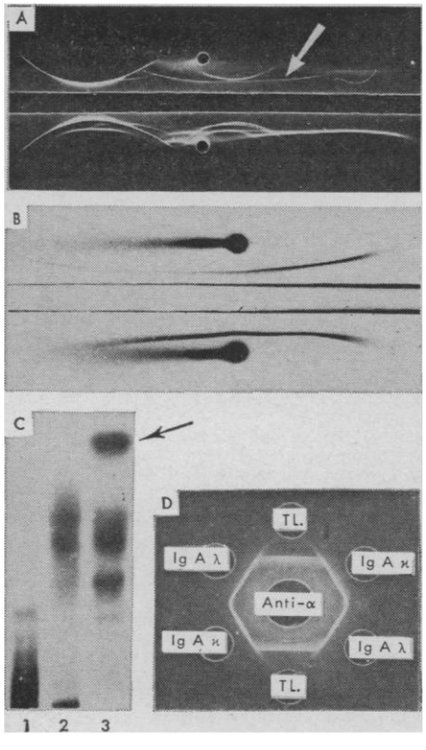

Fig. 1. (A) Immunoelectrophoresis of 0.5 μl of serum TL (top), compared to 1 μl of normal serum (bottom), and revealed by an antiserum to whole normal serum. Anode is at the left. The TL protein line is shown by an arrow. (B) Autoradiographic pattern of immunoelectrophoretic analysis of a cell extract from an intestine biopsy culture in presence of ^{14}C-labeled amino acids. Normal serum (below) and serum TL (top) were used as carriers, and the antiserum was directed to whole normal serum. (C) Starch-gel electrophoresis in 8*M* urea-formate buffer *p*H 3.5 of (strip 1) the polymerized TL protein recovered in the void volume of a G 200 Sephadex column; (strip 2) the same material after reduction with 0.3*M* 2-mercaptoethanol and alkylation; (strip 3) a reduced and alkylated γ_{A1} myeloma protein. The light chain band is shown by an arrow. (D) Comparison of the TL protein and different γ_{A1} myeloma proteins of K or L types with an antiserum to a heterologous IgA myeloma protein. This antiserum had been absorbed with the serum of a patient selectively lacking IgA.

Such a failure to precipitate has been encountered in this and other (*4*) laboratories with a few myeloma proteins, mainly IgA globulins with λ light chains. However, in contrast to these myeloma proteins, the TL protein was unable to combine with antibodies to κ or λ light chains and inhibit their precipitin reaction with Bence Jones proteins. The actual absence of light chains in the TL protein was demonstrated after reduction of the isolated protein by 0.3*M* 2-mercaptoethanol, *p*H 8.0, and alkylation with iodoacetamide. No light chain band was found in urea-acid starch-gel electrophoresis (Fig. 1C). Ultracentrifugal analysis of the reduced and alkylated protein showed a single symmetrical peak with a sedimentation coefficient of 3.2*S* at a concentration of 7 mg/ml.

Total proteinuria varied between 0.05 and 1 mg/ml. Electrophoretic, immunoelectrophoretic, and gel-diffusion studies of the urinary proteins concentrated by vacuum dialysis (23/32 Visking tubing) showed that TL protein was the main component and that its electrophoretic mobility and antigenic characteristics were similar to those of the serum protein. TL protein was poorly precipitable in 10 percent trichloroacetic or sulfosalicylic acid. Only trace amounts of free light chains of both antigenic types were detected in the urine.

A characteristic feature of TL protein is its high tendency to polymerize. Ultracentrifugal analysis showed that the TL protein was polydisperse, manifesting peaks with sedimentation coefficients up to 11*S*. In gel-filtration experiments on Sephadex G-200 columns (in 0.1*M* tris HCl buffer, *p*H 8.0, 1*M* in NaCl), a significant proportion of the TL protein was eluted in the void volume. No evidence of association between the TL protein and serum albumin or haptoglobin was found by immunoelectrophoretic analysis of this large-sized material or of native serum. When submitted to urea-acid starch-gel electrophoresis, this polymerized material poorly penetrated into the gel, in contrast to the monomers obtained by reduction and alkylation (Fig. 1C). When run on a Sephadex G-100 column in 6*M* guanidine, more than 50 percent of this large-sized material was recovered in the void volume. Thus the TL polymers are partly held by disulfide bonds, presumably due to disulfide interchanges. Since blood and urine were not collected in the presence of alkylating agents, this high tendency

Table 1. Carbohydrate content (milligrams per 100 mg of protein) of protein TL and of the alpha heavy chain of a γ_{A1} myeloma globulin.

Total hexoses	Fucose	Sialic acid	Total hexosamines
Protein TL			
6.67	0.84	3.72	5.36
Alpha chain of γ_{A1} myeloma			
3.19	0.53	0.79	2.80

toward polymerization precluded purification of the "native" monomer.

Papain digestion of the purified TL polymers in the presence of 0.01*M* cysteine hydrochloride was ineffective. When carried out in the presence of 0.1*M* 2-mercaptoethanol, papain degradation resulted in the production of small peptides without any immunologically identifiable component.

With the use of appropriate antiserums, IgA myeloma proteins can be divided into two major subclasses γ_{A1} and γ_{A2} (Le and He) related to the α-chains (*5*). The TL protein was shown to be related to the γ_{A1} subclass since, in gel-diffusion experiments, it formed a spur over the γ_{A2} (deficient) protein (*5a*). To further characterize TL protein, we attempted to compare its antigenic properties to those of several γ_{A1} myeloma proteins and their subunits. All antiserums were made specific for IgA by absorption with light chains or serum of patients having selective IgA deficiency, or both. Since none of the studied IgA myeloma proteins have yielded Fc fragments after papain hydrolysis, we were unable to compare directly the TL protein to these fragments. No antigenic determinants common to TL protein and Fab fragments of these myeloma globulins could be demonstrated. Moreover several absorbed antiserums (which did not precipitate with light chains) showed a definite spur of heterologous IgA myeloma proteins and of normal IgA over the TL protein. With two of these antiserums, TL protein was found to be antigenically deficient when compared with all tested IgA myeloma proteins of type K as well as of type L (Fig. 1D). However, in view of the poor immunogenicity of the Fd piece and of the importance of the light-heavy chain interaction in establishing the antigenic structure of the immunoglobulin molecules (*6*), these findings do not conclusively indicate that the TL protein lacks Fd piece. Conformational specificity has not been ruled out as the source of these antigenic differences, because antigenic deficiency of TL protein with respect to purified myeloma α-chains could not be convincingly demonstrated. Although the high carbohydrate content of TL protein as compared to α-chains of γ_{A1} subclass (Table 1) suggests that it may represent mainly the Fc portion of the chain, further physicochemical studies are required in order to determine whether TL protein is a complete α-chain or a portion thereof.

Immunofluorescence studies with many different antiserums to κ and λ light chains showed no evidence of light-chain synthesis in the few lymphoid and plasma cells of the bone marrow which produced the TL protein. Samples of small intestine taken at biopsy were submitted to short-term culture in the presence of ^{14}C-labeled amino acids, and the cell extracts were studied by immunoelectrophoresis and subsequent autoradiography (*7*). The only immunoglobulin line labeled was the TL protein line (Fig. 1B). This TL protein synthetized in vitro did not react with antiserums to light chains and no free labeled light chains could be demonstrated in the cell extracts. Therefore it seems reasonable to assume that we are not dealing with an absence of light-heavy chain assembly but with an actual lack of light-chain synthesis in the proliferating cells.

This newly recognized abnormality of IgA is perhaps not uncommon in patients with this peculiar type of abdominal lymphoma, because an analogous protein has been recently detected in our laboratory in two other cases with a very similar clinical pattern. Alpha-chain disease may thus represent a well-defined condition with characteristic clinicopathological and biological features and possibly a genetic predisposition.

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5a. TL protein does not react with an antiserum specific for γ_{A2} subclass provided by Dr. H. G. Kunkel. Since it contains exclusively α chains of the γ_{A1} subclass, this protein may be considered as "monoclonal."
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8. We thank Dr. Y. Le Quintrec for referring the patient, Drs. J. P. Vaerman and J. F. Heremans for supplying the reference proteins Le and He, Dr. R. Van Rapenbusch for ultracentrifugal analysis, Mrs. Y. Signoret, A. Chevalier, and C. Masurel for technical assistance. Supported by grant CR.66.237 from the French National Institute for Health and Medical Research.

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Cucumber Sex Expression Modified by 2-Chloroethanephosphonic Acid

Abstract. *Application of 2-chloroethanephosphonic acid (120 to 240 parts per million) to monoecious cucumber plants when the first true leaf was 2 centimeters in diameter has resulted in as many as 19 continuous pistillate nodes. Control of the flowering habit simplifies the production of hybrid seed and offers the possibility of enhancing cucumber yields.*

Monoecious cucumbers (*Cucumis sativus*) treated with 2-chloroethanephosphonic acid have exhibited gynoecious characteristics for as many as 19 nodes. Other effects on growth regulation such

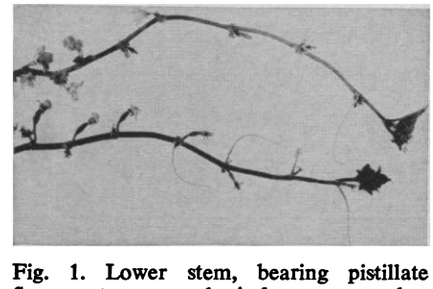

Fig. 1. Lower stem, bearing pistillate flowers at every node, is from a cucumber plant treated with 2-chloroethanephosphonic acid (240 parts per million) at first true-leaf stage. Upper stem, with staminate flowers at every node, is from an untreated plant.