Alpha-chain disease

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Alpha-chain disease (α -HCD) is the most frequent of the heavy chain diseases. Since it was first described (Seligmann, Danon, Hurez, Mihaesco, and Preud'homme, 1968; Rambaud, Bognel, Prost, Bernier, Le Quintrec, Lambling, Danon, Hurez, and Seligmann, 1968), α -HCD has been recognized in about 80 patients to our knowledge. The purpose of this paper is to summarize the structural and cellular studies carried out in a few cases, to evaluate the methods of immunochemical diagnosis, to review the main clinical and pathological features and to discuss briefly some views on the pathogenesis of the disease.

The Nature of the Protein Abnormality

Alpha-chain disease is defined as the production of a homogeneous population of molecules consisting of incomplete heavy α -chains, devoid of light chains. About 50 such proteins have been typed and all belong to the α_1 subclass. The absence of a single case of α_2 -HCD in this series is probably not accidental but its significance is unknown.

The molecular weight of the monomeric polypeptide subunit was found to vary between 29 000 and 34 000 (Dorrington, Mihaesco, and Seligmann, 1970; Seligmann, Mihaesco, and Frangione, 1971). The length of these chains was thus greater than half but smaller than three-quarters of normal α_1 heavy chains. Antigenic analysis (Seligmann, Mihaesco, Hurez, Mihaesco, Preud'homme, and Rambaud, 1969) and chemical studies (Seligmann *et al*, 1971) indicated that the entire Fc fragment was present in α -HCD proteins, that their C-terminus was identical with that of normal α_1 chains and that the heavy-light peptide was missing. The hinge region was shown by chemical methods to be present in all eight proteins so far studied. All attempts to raise individually specific antibodies to α -HCD proteins have failed, indicating the paucity of antigenic determinants in the variable portion of the chain. In view of these results and of the molecular weight data, the missing portion of the chain is located in the Fd segment and involves both the V and C1 regions

The N-terminal sequences of several α -HCD proteins were shown to be heterogeneous (Seligmann *et al*, 1971). Even for those proteins with the same N-terminal amino acid marked heterogeneity became apparent after two steps in degradation. Attempts to obtain the N-terminal sequence on an automated sequencer were unsuccessful. The N-terminal residues were different from those found in any of the subgroups of the variable regions of normal heavy chains. The most likely explanation of this heterogeneity is that it is the consequence of intracellular proteolysis occurring after synthesis. The fact that the N-terminal residues found in the seven proteins studied were valine and/or isoleucine suggests that the degradation stops at this level for some reason which could possibly be enzyme specificity, steric hindrance or the presence of a carbohydrate moiety.

The demonstration of a large internal deletion in a γ -heavy chain disease protein (Frangione and Milstein, 1969) led us to postulate that in α -chain disease we were dealing with a similar primary deletion followed and obscured by a secondary limited intracellular proteolysis (Seligmann *et al*, 1971). This hypothesis has been confirmed by biosynthetic and structural studies in a case of α -HCD. Cellular biosynthetic studies excluded the possibility of the synthesis of a normal α -chain with subsequent degradation to a smaller fragment after its release from the ribosomes (Buxbaum and Preud'homme, 1972). Comparison of the amino acid sequence of the hinge region of this protein (Def) with that of a normal IgA₁ showed that, after a short segment corresponding with the variable region, protein Def displays a gap which comprises almost the whole Fd segment including the CH1 domain (Wolfenstein-Todel, Mihaesco, and Frangione, 1974). Normal synthesis resumes at the valine residue in the hinge region just preceding the segment which contains a partially duplicated fragment and the interheavy chain disulphide bonds. From there on the molecule is apparently normal with the exception of a substitution of threonine for serine in position 12. Protein Def is therefore synthesized as an internally deleted α_1 heavy chain. It is of interest that valine at position 9 of the hinge

peptide, where the identity with a normal α_1 chain starts, could be the equivalent of glutamine at position 216 of γ chains, the site where normal synthesis resumes in several γ -HCD proteins with internal deletions (Frangione and Franklin, 1973).

Thus the primary defect in α -HCD proteins appears to be a deletion affecting in both instances the variable and first constant regions of the heavy chains, which are under independent genetic control. Any genetic hypothesis about α -HCD proteins should also take into account the fact that immunofluorescent and radioimmuno-electrophoretic studies of proteins synthesized *in vitro* have failed to detect any light chain production in the cells which secrete α -HCD proteins (Seligmann *et al*, 1969). This failure of light chain synthesis has been confirmed by biosynthetic studies of nascent Ig subunits in such patients (Buxbaum and Preud'homme, 1972). Since light and heavy chains are under the control of unlinked genes, this peculiar situation raises a puzzling problem for the cellular geneticist.

Whether α -HCD proteins should be considered as 'abnormal' is still an open question. Polypeptides analogous to the proteins of γ -chain disease were recently reported to be present in very small amount in normal plasma (Lam and Stevenson, 1973). However, if heavy chain disease arises from proliferation of a clone of cells producing such polypeptides, it is necessary to postulate a wide variety of cells carrying such deletions in normal individuals since the site and length of the deletion appears to vary from one α -HCD protein to another.

Deletions are not confined to heavy chain disease proteins but are also encountered in myeloma proteins. Various types of heavy and/or light chain deletions have now been described in human monoclonal immunoglobulins¹. It is of interest that such deletions are also found in immunoglobulin molecules synthesized by clonal variants of murine myeloma cells (Birshtein, Preud'homme, and Scharff, 1974; Milstein, Adetugbo, Cowan, and Secher, 1975).

Immunochemical Diagnosis

The diagnosis of α -HCD relies entirely upon laboratory studies including immunochemical analysis of the serum proteins and, as previously emphasized (Seligmann *et al*, 1969; Seligmann *et al*, 1971), may be difficult in a routine laboratory. It can easily be missed on the serum protein electrophoretogram, and the pathological protein was not noticeable in half of the 64 cases studied in our laboratory. When detectable by electrophoresis, the pathological α -HCD protein shows as an abnormal broad band

usually in the α_2 or β region. The characteristic narrow band, which is suggestive of a monoclonal Ig abnormality, is always lacking. In most of the cases where the pathological protein was not noticeable, serum electrophoresis showed only a decrease in serum albumin and a moderate to severe hypogammaglobulinaemia.

The diagnosis is usually suspected or established by the immunoelectrophoretic analysis of the serum of these patients. The various patterns have been described in detail elsewhere (Seligmann *et al*, 1969; Seligmann *et al*, 1971). In many cases the protein abnormality escaped detection by routine immunoelectrophoresis using polyvalent antiserum to human normal serum, and analysis with monospecific antisera to IgA is essential. The abnormal component usually gives an abnormal precipitin line either extending from the α_1 globulins to the slow β_2 region or showing a faster electrophoretic mobility than normal IgA. However, in a few patients the α -HCD protein had a slow electrophoretic mobility. The anomalous component does not of course precipitate with antisera to light chains. It should, however, be emphasized that this lack of precipitation with anti-k and anti- λ antisera is not a sufficient criterion for the diagnosis of α -HCD since many IgA myeloma proteins, even though they contained light chains (mainly λ chains), failed to precipitate with such antisera.

Selected antisera to IgA which contain antibodies related to the conformational specificity of the Fab region, which precipitate only with α - and light chains combined, were found to be very useful for the diagnosis of α -HCD by immunoelectrophoresis or the Ouchterlony technique (Seligmann *et al*, 1969), as was the immunoselection plate method of Radl (Doe, Henry, Hobbs, Jones, Dent, and Booth, 1972). In all doubtful cases the pathological protein should be purified, reduced and alkylated, and the lack of light chains should be demonstrated directly by starch or polyacrylamide gel electrophoresis or by gel filtration after dissociating the molecule.

The striking and unexpected electrophoretic heterogeneity of these presumably monoclonal α -HCD proteins is certainly due in part to the heterogeneity of their N-terminal sequences as discussed above. It may also be related to two other features, the high carbohydrate content of most α -HCD proteins (Seligmann *et al*, 1971) and their high tendency to polymerize. Indeed on ultracentrifugation α -HCD proteins appear to consist of dimers with a 3-4 S sedimentation constant and, in most instances, of larger polymers of various sizes (Seligmann *et al*, 1969).

The serum levels of normal IgA, IgG, and IgM molecules are usually decreased. In two patients

¹See contribution by Dr Franklin, page 65.

with α -HCD we have found, in addition to the pathological protein, a small homogeneous IgG component.

The diagnosis of α -HCD is made more difficult by the very low concentration of pathological protein in the urine. In most patients, however, it could be detected in concentrated urine and had the same electrophoretic and immunochemical characteristics as that in the serum. Bence-Jones proteinuria was never found. The pathological protein was also found in significant amounts in jejunal fluid, as expected from the involvement of the intestine, whereas the IgA in parotid saliva of these patients was normal (Seligmann *et al*, 1969).

Main Clinicopathological Features

Alpha chain disease appears to be a condition affecting primarily the secretory IgA system and mainly the digestive tract. Its age distribution is in sharp contrast to that of multiple myeloma since it occurs mainly in the second and third decades of life. There is a 3/2 ratio of males to females.

Only three cases of α -HCD without detectable intestinal involvement have been reported (Stoop, Ballieux, Hijmans, and Zegers, 1971; Faux, Crain, Rosen, and Merler, 1973; Florin-Christensen, Doniach, and Newcombe, 1974). They represent the respiratory form of the disease but the pathology of these cases is very poorly documented. It is probable that other non-intestinal forms of α -HCD with involvement of salivary glands and other areas of the secretory IgA system will be described in the future.

All other recognized cases were of the intestinal variety; the clinical features were strikingly uniform and very similar to those of the first reported patient (Rimbaud *et al*, 1968). The disease was usually revealed by chronic diarrhoea, a severe malabsorption syndrome with considerable loss of weight, steatorrhoea and hypocalcaemia, and excessive faecal losses of water and electrolytes. Abdominal pains were often a major presenting symptom and abdominal masses were palpable in several patients. Finger clubbing was common. Radiological studies and multiple intestinal biopsies usually showed the whole length of the small intestine to be involved. Rectal involvement was detected by biopsy in several cases, and at laparotomy diffuse mesenteric lymphadenopathy was often found.

The plasmacytic infiltration of α -HCD has little tendency to spread. In contrast with the diffuse enteromesenteric lesions, there is usually no involvement of the liver, spleen, or peripheral nodes except at a late stage. Enlarged retroperitoneal lymph nodes were found by lymphangiography in a few

patients and enlarged mediastinal nodes in at least one. Radiologically demonstrable bone lesions have not yet been reported. A mild bone marrow invasion of α -chain-producing cells was found in two patients by immunofluorescent studies.

Histological examination of the small intestine showed in all patients a diffuse and massive infiltration of the lamina propria with lymphoid cells. The latter were predominantly plasma cells, as confirmed by electron microscopy, but there were individual variations with sometimes relatively immature plasma cells or intermediate lymphoplasmacytic cells. Occasional lymphocytes and reticulum cells were also found. Secondary villous atrophy and sparsity of crypts were noted in all instances, whereas the epithelium appeared relatively normal (Rimbaud *et al*, 1968; Bonomo, Dammacco, Marano, and Bonomo, 1972; Bognel, Rimbaud, Modigliani, Matuchansky, Bernier, 1972; Doe *et al*, 1972). Sections of the mesenteric nodes sometimes showed similar cellular changes.

In many patients the plasma cell infiltrate in the lamina propria is moderately invasive and consists of apparently normal cells but in a few cases the neoplastic nature of the plasmacytic proliferation has been convincingly demonstrated. For instance, in the patient studied by Bognel *et al* (1972) the plasmacytic proliferation involved the muscularis mucosa of the gut, disorganized the architecture of the mesenteric nodes, and included a great number of atypical and mitotic cells.

In some patients, who presented with symptoms of a tumour and possible intestinal obstruction, pathological features of truly malignant lymphoma were found in addition to the diffuse plasma cell proliferation. These sarcomas involved the gut, often with single or multiple circumscribed intestinal tumours, or the mesenteric lymph nodes or both. These tumours were labelled by the pathologist as 'reticulum cell sarcoma', 'undifferentiated sarcoma' or sometimes as 'Hodgkin's disease'. In fact these tumours usually show the pathological features of what has recently been called immunoblastic lymphoma. These malignant sarcoma cells probably derive from the same B-cell clone as the proliferating plasma cells. Both proliferations can occur together in the lymph nodes and in the gut (Bognel *et al*, 1972). Rough endoplasmic reticulum may be found by electron microscopy in the apparently poorly differentiated malignant cells. Intracytoplasmic α -HCD protein was not detected in the undifferentiated malignant cells found in the lymph nodes and blood of a patient at a terminal stage (Bognel *et al*, 1972) but membrane markers were not studied. However in 'reticulum cell sarcoma' supervening on chronic lymphocytic leukaemia or on Waldenström

macroglobulinaemia, we have been able to show that the membrane-bound monoclonal immunoglobulins of the large malignant cells were identical with those found on the proliferating B lymphoid cells of the preceding leukaemia or macroglobulinaemia. (Brouet, Labaume, and Seligmann, 1975), and the same process probably occurs in α -HCD.

The course of α -chain disease is usually progressive and fatal, and the occurrence of these truly malignant lymphomas probably represents the second stage of the disease. However, complete clinical remission has now been observed in several patients, with disappearance of the α -HCD protein from the serum and intestinal fluid, together with a normal histological appearance and negative immunofluorescent studies. Some of these had received chemotherapy with alkylating agents but it is of considerable interest that an apparently complete remission of the disease was achieved in at least four patients treated only with oral antibiotics. One of these (Rogé, Druet, and Marche, 1970) is still in remission after five years. It seems therefore reasonable to perform a laparotomy in all patients with α -HCD without overt malignancy, so that patients without pathological evidence of true sarcoma can be treated only with antibiotics and general supportive therapy, at least initially.

It was first shown in Israel that abdominal lymphomas in this part of the world frequently present as malabsorption (Ramot, Shanin, and Bubis, 1965; Eidelman, Parkins, and Rubin, 1966). The clinical pattern and the pathological features (Rappaport, Ramot, Hulu, and Park, 1972) of these patients are analogous to those of α -HCD. Immunoglobulin studies were not performed in most of these patients, but we believe that many of these so-called 'Mediterranean abdominal lymphomas' (if the definition of this entity is restricted to cases showing a diffuse plasma cell infiltration) are examples of α -HCD, and this hypothesis has been confirmed by the study of the immunoglobulins in numerous such patients. We have been unable to detect a protein characteristic of α -HCD in the serum of some patients with the same clinicopathological pattern. In order to establish the true incidence of α -HCD within 'Mediterranean lymphomas' it will be necessary to study systematically the immunoglobulins of the intestinal fluid and to perform immunofluorescent and biosynthetic studies of intestinal biopsies from those patients without detectable α -HCD protein in the serum.

Two Turkish patients presenting with the typical clinicopathological features of α -HCD were found to have unexpected immunoglobulin abnormalities. In one patient studied with Dr Inceman we found an IgA myeloma globulin and a Bence-Jones protein.

In the other, a young girl under the care of Dr Bender in Frankfurt, we demonstrated a γ -chain in the serum and, by immunofluorescence, in an intestinal biopsy.

Pathogenesis

The geographical origin of patients affected with α -HCD is of special interest. Whereas the three patients with the respiratory form of the disease originated from the Netherlands, the United States, and Great Britain, the patients with intestinal involvement originated from and had been living in areas with a high degree of infestation by intestinal pathogens: North Africa (Algeria, Tunisia, Morocco), South Africa, the Middle East (Israel, Lebanon, Iran, Syria, Lybia, Iraq), the Far East (Pakistan, Cambodia, India), South America (Columbia, Argentina), and southern Europe (Spain, Turkey, Yugoslavia, Greece, South Italy). The single exception is a patient from Finland. This geographical distribution strongly suggests that intestinal microorganisms which provide a sustained local antigenic stimulus do play a role in the pathogenesis of α -HCD. Limited parasitological and bacteriological studies in patients with α -HCD have been unrewarding and, in view of their chemical structure, there is little chance of demonstrating a specific antibody activity in α -HCD proteins. The specific or non-specific nature of the postulated antigenic stimulus is open to question. These environmental factors could trigger the clonal proliferation directly. Alternatively they may only be predisposing factors causing a non-specific stimulation of immunocytes which potentiates the oncogenic effect of a virus interfering with genes controlling IgA synthesis (Rimbaud and Matuchansky, 1973). In either case the role of these environmental factors does not exclude the possibility of predisposing genetic factors although the few family studies which have been performed have yielded essentially negative results.

It is remarkable that the plasma cell proliferation resulting from the postulated antigenic stimulation appears to lead to heavy chain disease rather than to myelomatosis. Whether this is related to the nature of the stimulated cells or to that of the stimulating agents is at present a matter for speculation.

As discussed above, the benign or malignant nature of the plasma cell infiltrate in α -HCD is open to question. Although the hypothesis of a benign process seems unlikely in those patients with high and rising serum levels of pathological protein, the benign nature of α -chain disease at a relatively early stage is a real possibility. Hence the possibility of reversing the hyperplastic process by the administration

of antibiotics to suppress the stimulating agent is obviously of considerable theoretical and practical interest. Thus α -HCD may well represent a model of a lymphoma characterized by a continuous sequence of events ranging from an apparently benign and reversible hyperplastic process to an overt neoplastic proliferation.

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