γ - and μ -Heavy chain diseases and related disorders¹

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During the last ten years studies of myeloma proteins have shown them to be similar to or identical with normal immunoglobulins. In addition, the not infrequent occurrence of structural variants of immunoglobulins in association with neoplasms of lymphocytes or plasma cells has become widely recognized. Since the discovery of the group of heavy chain diseases in man (Franklin, Meltzer, Guggenheim, and Lowenstein, 1963; Franklin, Lowenstein, Bigelow, and Meltzer, 1964) and shortly afterwards of half-molecule IgA proteins in the mouse (Potter and Kuff, 1964), many structural variants of both heavy and light chains have been recognized (see review by Franklin and Frangione, 1975). It seems likely that studies of these proteins will provide insights into the genetic control of immunoglobulins (Igs) which cannot be obtained from analyses of intact molecules.

This report summarizes the clinical features of γ - and μ -heavy chain disease (HCD) and discusses some of the biochemical and biosynthetic studies which have clearly established that these abnormal proteins are products of disordered synthesis and not the result of degradation. In addition, several other structural abnormalities of heavy chains will be mentioned, excluding α -chain disease which is dealt with elsewhere.

A detailed classification of the known alterations of heavy and light chains is given in a review by Franklin and Frangione (1975). Only alterations in man will be considered, although similar changes in heavy chains have recently been discovered in the mouse (Scharff, 1974; Milstein, Adetiegbo, Cowan, and Secher, 1974).

In this article discussion will be limited to four major types: (1) heavy chain disease proteins; (2) myeloma proteins with altered heavy chains; (3) half-molecules; and (4) myelomas with degraded heavy chains.

Heavy Chain Diseases

CLINICAL FEATURES

γ -Heavy chain disease (γ -HCD)

Studies of more than 30 patients with γ -HCD (Frangione and Franklin, 1973; Seligmann, 1972) have shown that the clinical features of this disorder are characteristic despite some variability in its manifestations. The disease resembles a malignant lymphoma more than myeloma in most cases. Diagnosis, however, must be made on careful immunochemical characterization of the serum and urine proteins; other pathological features are generally of little help in making the diagnosis as they are variable and not specific.

The disease occurs in males more than in females and most frequently in the elderly; only four reported patients have been under 40 years of age. The onset is usually gradual, often with prolonged prodromata, but is occasionally sudden. Commonly the initial manifestations of lymphadenopathy, anaemia and fever, often accompanied by malaise, weakness, and hepato- or splenomegaly, suggest a granulomatous disease such as toxoplasmosis, histoplasmosis, or Hodgkin's disease. As the disease progresses, generalized lymphadenopathy is the rule, but occasionally there may be only local lymph node enlargement involving tracheobronchial, abdominal, cervical, axillary, or mediastinal nodes. Not infrequently lymphadenopathy waxes and wanes and in almost one half of the patients, probably because of involvement of the nodes in Waldeyer's ring, there is palatal erythema and oedema often giving rise to respiratory difficulties. Recurrent infections are common. In no case has bone involvement been demonstrated by radiology, although bony lesions were demonstrable at necropsy in one case whose bones had not been x-rayed during life. The course of the disease can be rapidly progressive, death occurring usually from infection or progressive malignancy in a few months. Some patients, however, have survived for five years or longer. No spontaneous or induced remissions similar to those seen in α -HCD have been encountered to date.

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Laboratory findings include mild to moderate anaemia, often associated with variable leukopenia and thrombocytopenia, eosinophilia, and atypical lymphocytes or plasma cells in the blood. Plasma cell leukaemia has been encountered terminally. The erythrocyte sedimentation rate tends to be elevated, and about one half of the patients have raised serum uric acid levels.

Bone marrow examination, while usually abnormal, is of little help diagnostically. Most often there is a significant increase in plasma cells, lymphocytes or both, often accompanied by eosinophilia which, on occasion, has been noted as the sole abnormality. In several instances bone marrow examination was normal. The diagnosis is generally based on the discovery by immunoelectrophoresis of a protein in serum and/or urine, which migrates in the β - γ region and reacts with antisera to γ chains but not to Fab fragments or L chains. In about half of the patients the protein concentration in serum exceeded 20 g/l. In one subject, no abnormal serum protein was noted on paper or cellulose acetate electrophoresis. In most instances the abnormal band was broad and heterogeneous and accompanied by hypogammaglobulinaemia and a depressed immune response. The same protein can generally be detected in the urine and was excreted in amounts varying from 0.5 to 20 g/day in half the patients; in the remainder the amount was too small to detect in unconcentrated urine samples. None of the patients had Bence-Jones proteinuria.

Histological studies of tissues obtained at biopsy or necropsy have shown a variety of changes, none of which is diagnostic. Usually there is infiltration with plasma cells and lymphocytes, often in association with eosinophils and/or reticulum cells. In three of the patients the histological appearance and the clinical course were those of a benign condition, in two others overt plasma cell leukaemia was noted, and in the remainder a neoplastic disorder of plasma cells, lymphocytes, and/or reticulum cells was considered likely. Early in the disease a biopsy often suggests Hodgkin's disease or a granulomatous condition. In two of the patients amyloid deposits were found at postmortem examination. Heavy chain disease is often associated with other chronic disorders such as tuberculosis, rheumatoid arthritis, Sjögren's syndrome, myasthenia gravis, lupus, hypereosinophilic syndrome, thyroiditis, and thyroid tumours, but the relationship is obscure. Virus-like particles were seen in one subject. Chromosome studies were normal in the three patients examined.

μ -Heavy chain disease (μ -HCD)

This is probably the least common of the recognized types of heavy chain disease. Of the eight subjects

reported with this rare biochemical abnormality, six had chronic lymphocytic leukaemia, often of long duration. An unusual feature in most was the presence of vacuolated plasma cells in the marrow. More than half of the patients produced large amounts of Bence-Jones protein, two had pathological fractures, and one had amyloidosis. Most had mainly involvement of the spleen, liver and abdominal nodes, with little if any peripheral lymphadenopathy. The only serum abnormality on routine electrophoresis was marked hypogammaglobulinaemia, and in the majority of these patients the diagnosis was made by immunoelectrophoresis which showed a rapidly migrating component reacting with antisera to μ chains but not to light chains. This protein was not found in the urine of any of the patients. Only two had a readily detectable abnormal band on routine electrophoresis.

STRUCTURAL AND BIOSYNTHETIC STUDIES

γ -HCD proteins

The available structural information is greatest for γ -HCD proteins, since these generally occur in the serum and urine in relatively large amounts. It is now recognized that they represent a heterogeneous group of molecules ranging from an intact heavy chain (Adlersberg, Franklin, and Frangione, 1975) to proteins having various types of internal deletions. In addition some are probably the result of proteolytic digestion of a larger precursor molecule. In general the sedimentation coefficient lies between 3.5 and 4S; the molecular weight ranges from 45 000 to 80 000 for the dimer, the electrophoretic mobility is in the fast- β region, and the molecules tend to be rich in carbohydrate. Among the proteins studied to date there is an unexpectedly high incidence of γ_3 proteins. None of the γ -HCD proteins has been shown to possess antibody activity.

The proteins studied most carefully to date have been those with internal deletions. As shown in fig. 1A and B, three proteins—Zuc (γ_3), Cra (γ_1), and Gif (γ_2)—resemble each other in having an internal deletion with resumption of the normal sequence at residue 216, with glutamic acid. One of these, Zuc (Frangione and Milstein, 1969), is a γ_3 protein containing a gap of about 200 residues starting at 17-18 residues from the N-terminus, and including the two intrachain loops of the Fd¹ fragment, the inter-heavy chain-light chain disulphide bridge and a major part of the extended hinge normally present in γ_3 heavy chains (Adlersberg *et al*, 1975). Normal sequence is resumed with the invariant sequence of

¹The Fd fragment is the heavy chain portion of Fab. Ed.

Fig 1 Models of major structural variants of heavy chains.
A Normal heavy chain
B HCD proteins with deletions of part of V_H and all of C_{H1} domains with resumption of normal sequence at residue 216 (Glu) at the hinge ($\uparrow$).
C HCD proteins lacking part of V_{H1} and all of C_{H1} domains as well as the hinge. Normal sequence in one of these resumes at residue 252 (Met) ($\uparrow$).
D Proteins resulting from proteolytic cleavage in the hinge region.
E A crystalline myeloma heavy chain with a deletion of the hinge only (216-232).

Horizontal hatching = V_H C_{H1} – C_{H1} domain
Vertical hatching = Hinge C_{H2} – C_{H2} domain
C_{H3} – C_{H3} domain

Arrow = site of resumption of normal synthesis
PCA = pyrrolidone carboxylic acid

γ_3 beginning with a glutamic acid residue corresponding to 216 at the middle of the normal heavy chain. Cra (Franklin and Frangione, 1971) is a γ_1 protein which lacks almost the entire Fd fragment. The amino terminal sequence is heterogeneous, and after 10-11 residues that do not resemble any of the known heavy chain variable region subclasses, normal synthesis resumes at the glutamic acid residue corresponding to position 216 (γ_1 numbering) (Edelman, Cunningham, Gall, Gottlieb, Rutishauser, and Waxdal, 1969). There are three inter-heavy chain disulphide bridges instead of the usual two found in γ_1 heavy chains since, in the absence of light chains, the cysteine residue ordinarily

involved in forming the H-L disulphide bonds appears to have joined the homologous residue of the other heavy chain to yield an additional inter-H-H disulphide bond. Gif (Cooper, Franklin, and Frangione, 1972) is a γ_2 protein which has a blocked N-terminus (like Zuc) and contains much of the Fd variable region. It has a gap of about 100 residues corresponding with the Fd constant region and involving one intrachain loop and the H-L disulphide bridge. Here too the normal sequence is resumed at a glutamic acid residue which corresponds with residue 216 in the normal heavy chain.
Four other proteins seem to be closely related to this group in having an internal deletion of part of

the V region and the C_H1 domain. However, since they also lack the whole hinge (fig 1C), these molecules dissociate into monomers in the absence of reducing agents, a property highly suggestive of this type of structural defect. Careful studies of one protein in this group (Hal), a γ_4 -HCD protein, have led to a complete identification of the nature of the internal deletion (Frangione, Lee, Haber, and Bloch, 1973) and have clearly demonstrated that the normal sequence resumes at a methionine residue at position 252. The other three proteins with internal deletions probably resemble Hal in having a deletion which includes the hinge but have not been as carefully studied in sufficient detail to identify where the normal sequence recommences; Hi (Terry and Ohms, 1970; Woods, Blumenschein, and Terry, 1970) probably lacks the C_H1 domain and the hinge, but for Par (Calvanico, N., and Tomasi, T., personal communication) and Baz (Smith, Barton, Garver, Lutchner, and Faguet, 1973; Garver, F. A., and Smith, L. L., 1974, personal communication) the deletions have not been mapped precisely. Perhaps the methionine at position 252 may turn out to be another favoured reinitiation site.

Apparently HCD proteins which begin at the hinge are almost as common, this site being very susceptible to proteolysis by a variety of enzymes. While they appear to represent examples of proteolytic digestion of a larger precursor molecule, the possibility that they too are synthetic products has not been excluded. Two examples of this type have been published (Terry and Ein, 1971) and we have personally observed an additional one (Franklin *et al*, unpublished) (fig 1D). In these three proteins the larger precursor was never found, nor was it possible to discover an increase in proteolytic activity in the serum. However the possibility that some of these patients synthesize an intact heavy chain which is then degraded is supported by studies of the serum of a patient (OMM) with HCD disease of the γ_3 type in whom two abnormal proteins were present (Adlersberg, J., Grann, V., and Franklin, E. C., unpublished, 1974). One was a polypeptide which, after reduction, had a molecular weight of 59 000 daltons and appeared to consist of virtually the entire heavy chain. The second had a molecular weight of 39 000 daltons after reduction, started with glycine and consisted of the entire hinge region and the remainder of the Fc region. While it is tempting to postulate that the smaller molecule was derived from the larger one by proteolysis, and that this is the first instance where the precursor has been found, it is possible also that this represents a two-step mutation, one of which originally gave rise to the intact H chain protein while the second one resulted in the smaller fragment.

The significance of the γ -HCD proteins in terms of a normal counterpart remains uncertain. While it seems likely that these apparently functionless molecules, no two of which so far appear to be identical, are abnormal, Lamm and Stevenson (1973) have reported the isolation from pooled normal serum of a small amount of a heavy chain with a molecular weight of 35 000, a normal amino terminal sequence, PCA¹-Val-Gln and presumably an internal deletion. This raises the distinct possibility that these molecules are normally present in trace amounts. If this finding can be confirmed, it would greatly complicate any explanation of the regulation of H and L chain synthesis.

The genetic mechanisms involved in the synthesis of these presumably altered proteins have been the subject of much speculation. It seems possible that a clear-cut understanding of the factors responsible for their synthesis may provide answers to many of the questions related to the genetic control of immunoglobulin heavy chain synthesis.

μ -HCD proteins

The available information on the structure and nature of μ -HCD proteins is much more limited. Most of these molecules appear to exist in the serum as pentamers similar to the (Fc μ)₅² fragments with sedimentation rate of 11.5S and a molecular weight of 180 000 to 300 000, a feature which explains why they are not found in urine. The mechanism of polymer formation is unexplained since J chain was not found in three examples examined though it appeared to be present in a fourth (Dammacco, Bonomo, and Franklin, 1974; Bonhomme, Seligmann, Mihaesco, Clauvel, Danon, Brouet, Bouvry, Martine, and Clere, 1974).

Patients with μ -HCD differ from the other types of HCD in that free light chains were synthesized in more than half (Franklin, 1975); in the one case studied by immunofluorescence the light chains were found in the same cell as the heavy chains (Zucker-Franklin and Franklin, 1971). This finding, in the absence of structural studies, has led to the suggestion that the part of the C_H1 region which contains the H-L bridge is missing, thus accounting for failure of the L-chains to be linked to the H-chains (Forte, Prelli, Yount, Jerry, Kochwa, Franklin, and Kunkel, 1970). Precise structural studies will be needed to prove this, but have been hampered by difficulties in isolating this protein. The limited chemical studies so far carried out on three proteins are consistent with the immunological findings that most if

¹PCA—pyrrolidone carboxylic acid. N-terminal peptides containing this cyclized residue are typical of normal γ chains.

²(Fc μ)₅ consists of five 5 Fc fragments derived from μ chains and linked together.

not all of the Fc region is present (Forte *et al*, 1970; Dammacco *et al*, 1974; Lebreton, J. *et al*, 1974, personal communication). The most carefully analysed μ -chain protein differed from the others in having a lower molecular weight (35 000 daltons) and starting at residue 338 of the normal μ -chain which in this case was alanine instead of valine (Lebreton *et al*, personal communication, 1974; Putman, Florent, Paul, Shinoda, and Shimuzu, 1973).

ORIGIN OF HCD PROTEINS

Since the initial description of γ -HCD it has appeared likely that the HCD proteins represented synthetic products of aberrant plasma cells rather than the results of proteolytic degradation. This view, initially postulated on the basis of a normal turnover of IgG in the first patient with γ -HCD (Franklin *et al*, 1964), has been amply supported by the structural studies and recently by tissue culture studies of patients with γ_1 -, α -, and μ -HCD (Buxbaum, 1973). It is of some interest that even in those instances where structural studies suggested that the protein might have resulted from proteolysis the biosynthetic studies suggested abnormal synthesis of a deleted H-chain fragment.

Myelomas with Altered Heavy Chains

In recent years a number of less striking abnormalities of the heavy chain have been discovered in myeloma proteins, usually through routine screening. Most of these occur as isolated cases associated with a plasma cell neoplasm and are apparently not associated with any particular clinical presentation. The following alterations in heavy chains have been noted.

INTERNAL DELETION SIMILAR TO THE γ -HCD PROTEINS

Such a protein has been encountered only in the mouse (Milstein *et al*, 1974) and will consequently not be discussed here.

DELETION OF THE HINGE ONLY

Two unusual crystalline myeloma proteins, both of the γ_1 type, have been described which are of particular interest since they have a small deletion involving only the hinge (Deutsch and Suzuki, 1971; Fett, Deutsch, and Smithies, 1973; Lopes and Steiner, 1973). These proteins appear to have the normal IgG molecular weight of 145 000 daltons in non-dissociating solvents, but when subjected to acid or urea they yield an L-chain dimer and two free heavy chains with a mean molecular weight of 50 000 daltons. The deletion in the first case, McG, has been mapped and shown to involve residues

216-232 (Fett *et al*, 1973) (fig 1E) but the second has not been defined.

These findings suggest that lack of the hinge may have been partly responsible for their ready crystallizability and that all crystalline molecules may have such a deletion. However, we have studied two other crystalline molecules belonging to the IgG₁ and IgG₂ subclasses respectively both of which were shown to possess an intact hinge (Franklin, 1973, unpublished).

OTHER DELETIONS OF THE HEAVY CHAIN

While deletions of the C-terminal domain of the heavy chain appear to be common in the mouse (Scharff, 1974; Milstein *et al*, 1974), they have been only rarely encountered in man. One example is an IgA₁ monomeric myeloma (VO) which seemed to lack more than 100 residues (Despont, Abel, Grey, and Penn, 1974; Despont and Abel, 1974). It was discovered as a result of its lower molecular weight and significant antigenic deficiency compared with other IgA molecules. Molecular weight studies indicated a normal L-chain and an H-chain with a molecular weight of 42 000 instead of the normal 58 000. Chemical analyses revealed the presence of an intact hinge and associated disulphide linked peptides, and suggested strongly that most of the C_H3 domain was lacking. A similar though somewhat larger deletion encompassing the C_H3 and C_H4 domains of the μ chain appears to have occurred in a patient with (Fab')₂ μ fragment disease having a sedimentation coefficient of 6·1S, reported by DeCoteau, Calvanico, and Tomasi (1973). While a synthetic origin of the protein has not been clearly documented, its persistence over a long period of time in the absence of intact IgM or proteolytic activity in the serum strongly suggests a synthetic origin.

Half-molecules without an Apparent Deletion

Two examples of human IgG half-molecules have been reported in recent years and, not unexpectedly, both were present in large amounts in the urine. The first of these, WGS, an IgG k-type myeloma protein with a molecular weight of 75 000 daltons was only superficially characterized and appeared to have normal sized H and L chains (Hobbs and Jacobs, 1969). Another IgG k myeloma protein having a sedimentation coefficient of 4·3S and a molecular weight of about 70 000 daltons was studied in more detail (Spiegelberg *et al*, 1974, personal communication). This molecule did not appear to have a deletion in the heavy chain, and somewhat unexpectedly it was shown to contain peptides similar in composition to those in the γ_1 chain which are normally involved in the formation of the H-H and

H-L disulphide bridges.

Deletions of the L-chain with Concomitant Loss of the V Region of the H-chain Perhaps by Proteolysis

An unusual and rather complicated situation was revealed by studies of two γ_1 myeloma proteins which seemed to have as their primary defect the loss of the variable region of the light chain, one of which (Sac) was shown to represent an internal deletion (Lewis *et al*, 1968; Smithies *et al*, 1971; Parr *et al*, 1972). For reasons that remain to be established, but possibly because of greater susceptibility to proteolysis, the first 103 residues of the heavy chain were also lacking. A similar situation may also pertain to another protein, SM (Isobe and Osserman, 1974), in which degradation of some of the molecules appears to have proceeded even further.

Conclusion

During the past 10 years several practical points have been clearly recognized as important in the recognition of structurally altered immunoglobulins and immunoglobulin polypeptide chains. While a certain amount of clinical intuition is often of help in choosing those sera warranting more than a routine diagnostic study, the major factor leading to the discovery of these proteins has been careful systematic work-up of sera with a battery of antisera having a variety of specificities, at times supplemented by physico-chemical or biochemical analyses. At present immunoelectrophoretic analysis remains the major clinical tool, perhaps coupled with a simple technique for determining the molecular weight under reducing and non-reducing conditions. All the heavy chain variants currently recognized and described here could be detected by these two techniques. Nevertheless the possibility is far from remote that additional more subtle abnormalities are even more frequent and require more precise biochemical techniques for their recognition.

In respect of heavy chain diseases, it seems likely that much new information will be gained from future studies. To mention just a few, ϵ - and δ -chain diseases must exist and await recognition. The delineation of the clinical syndromes associated with all of these disorders will require study of many additional patients. Biochemical and biosynthetic studies, by defining the mechanisms of assembly and the nature of the deletions, may provide important information on such important questions as the number of genes ultimately involved in control of heavy chain synthesis, the true manner of immunoglobulin synthesis and assembly, and the interrelations between heavy and light chain synthesis.

Thus, as has so often been true in medicine, and particularly in immunology, the clinical investigator and the clinician can play a major role in advancing our understanding of basic biology and medicine. In this area in particular such an approach may prove to be the most direct and the most fruitful in answering many of the important questions still facing us today.

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