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HEAVY CHAIN DISEASES IN MAN *

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INTRODUCTION

Studies of patients with disorders of cells producing immunoglobulins (Ig's) have provided much insight into the molecular structure of antibodies, their genetic control and their biosynthetic origin. Heavy chain disease (HCD), one of the most unusual anomalies of immunoglobulin synthesis, was originally described by FRANKLIN et al. [45, 48, 53]. The hallmark of the syndrome was the synthesis of a fragment of the γ heavy chain with absent light chain production. The spectrum of HCD has expanded considerably. Today we recognize heavy chain diseases of the 3 main immunoglobulin classes. In addition, monoclonal immunoglobulins have been described in which both the heavy and light chains of the same molecule were structurally abnormal, although the heavy and light were still linked to each other.

If one views the HCD cell as a mature secretory B cell in which the structural gene for the Ig heavy chain has undergone either a point mutation or some other deletional events (*vide infra*), one can study the structure of the aberrant protein for the insights it offers concerning the nature and synthesis of normal heavy chains. If the HCD cell is viewed as an earlier non-secreting B cell, which in the course of oncogenic transformation has had a portion of its previously unexpressed genome (i.e. that coding for a portion of the heavy chain) derepressed, albeit imperfectly, yielding a structurally abnormal product, the cell and the nature of its normal counterpart become the focus of our attention. Alternatively, the oncogenic event may have altered the genes which normally code for heavy and light chains and caused imperfect expression of the heavy chain and prevented light chain synthesis completely. Additional views have suggested that these diseases may be genetic defects in regulation in normal Ig producing cells which are reacting either to external antigens or to malignant lymphoid cells proliferating in their immediate vicinity. All of these discard intra- or extracellular degradation of an intact primary gene product as a possibility, however they can accept some post-synthetic modification of the incomplete chain which results in a structure of the serum and urine proteins which may vary slightly from that of the primary gene product.

What kind of information is available supporting any or all of these explanations? By examining the available clinical data one can compare the clinical pictures of the

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various HCD's with the findings seen in patients bearing malignancies of lymphoid or plasma cells in which intact Ig's are synthesized. The tissue distribution of the cells, their surface markers, and their responsiveness to various chemotherapeutic agents can be utilized as measures of the biologic behaviour of the cells. The structure of the protein products can be analyzed in exquisite detail and these findings correlated with the structures of intact Ig's. Synthesis and secretion can also be compared in cells synthesizing proteins with normal and abnormal structure to determine what, if any, differences in process are responsible for the differences in end product.

CLINICAL ASPECTS

When the clinical features of γHCD were compared with those of IgG producing multiple myeloma the disparity between the 2 clinical syndromes was evident [10, 17, 18, 19, 65, 69, 73, 75, 89, 100, 109, 123, 125, 131]. Of the main clinical features the 2 entities shared 3 phenomena. Anemia occurred almost uniformly in both. Infections occurred in approximately 50 % of patients with each, usually late and were frequently the cause of death. They also shared a variability of clinical course and response to therapy. Some patients died rapidly within weeks of diagnosis, while others followed indolent protracted courses. Do these shared characteristics indicate that the cell populations are similar? Probably not. Anemia and variability of course are features shared by many neoplastic diseases regardless of the cell of origin. Susceptibility to infection is also a characteristic of many malignancies, however it is possible that these 2 conditions share the relatively specific deficit in B cell function which results in multiple gram-positive infections [13, 31, 81, 133].

Two features associated with myeloma are absent in most patients with γHCD. 30-72 % of myeloma patients have lytic bone lesions, a rarity in γHCD. The skeletal lesions may reflect the distribution of the malignant cells, with myeloma producing pressure necrosis of bony tables or the production of the recently described osteoclast activating factor (OAF) by myeloma cells and not by γHCD cells [84].

The second feature found more frequently in myeloma was severe, frequently fatal renal disease. Only 10 % of patients with γHCD had renal disease usually due to other causes. In myeloma, renal disease was detected in 20-55 % of patients and was somewhat pleiomorphic [1, 132]. Chronic renal failure was frequently seen and usually associated with excessive urinary light chain excretion [78]. Acute renal failure has also been seen, usually related to hypercalcemia, dehydration and excess L chain excretion [13, 24, 88]. Rarely a true nephrotic syndrome has been the chief renal manifestation [106].

Myeloma patients whose cells synthesized and secreted large amounts of light chains unassociated with heavy chains were particularly prone to renal dysfunction. Since patients with α- and γHCD usually did not synthesize L chains it was unlikely that L chains would play a role in their renal disease. It is possible that excessive exposure of renal tubular cells to HCD proteins also produces renal disease but this has yet to be conclusively demonstrated. Both myeloma and γHCD have been accompanied by renal amyloid deposition. In myeloma the amyloid protein is structurally related to the light chain of the monoclonal protein [57]. Recent evidence suggests that at least one γHCD protein, when digested under proper conditions, can form fibrils with amyloid properties [99].

γHCD was more of a disease of lymphoid tissue than multiple myeloma (tab. 1). Some 90 % of the HCD patients had lymphadenopathy (*versus* 1-5 % of patients with myeloma). Almost all had splenomegaly (seen in 5-25 % of patients with myeloma). A large majority had clinically detectable hepatomegaly. The last was least distinctive since a recent autopsy survey indicated that 58 % of myeloma patients

cases	myeloma (%)	γHCD (%)
anemia	36-91	90
renal disease	16-55	12
lytic lesions	30-72	0
hepatomegaly	19-58	100
splenomegaly	5-25	100
lymphadenopathy	1-5	90
infections	5-56	40-50
marrow plasmacytosis	100	75

Table 1 - Comparison of multiple myeloma and γ heavy chain disease.

had some myelomatous infiltration of the liver, however, clinical hepatomegaly was much more common in patients with HCD [120].

Although occasional myeloma patients manifested only soft tissue plasmacytomata, myeloma cells were almost always found in the bone marrow. The diagnosis of myeloma was predicated on finding either large numbers and/or abnormal plasma cells in the bone marrow. In the individual patient the cells tended to be homogeneous in appearance although there were some exceptions noted in some series. In γHCD 25 % of patients have either normal marrow examinations (frequently on multiple aspirations or biopsies) or mild eosinophilia. The remaining 75 % showed plasmacytosis, eosinophilia and increased reticulum cells in variable proportions.

These differences in the distribution of the malignant cells may reflect differences between myeloma cells and γHCD cells in their nutritional requirements for optimal growth. More likely is a difference in some surface characteristics of the different cell populations. Studies of surface immunoglobulin (SIg), as detected by immunofluorescence, have indicated that some 62 % of myeloma patients have readily detectable SIg on their plasma cells [98]. Many of these patients also had lymphocytes in their marrows and peripheral blood with the same SIg. SIg may be present on plasma cells and lymphocytes both in the marrow and in the peripheral blood. In other lymphomas the cells isolated from pathologic nodes have been demonstrated to bear monoclonal SIg's. Hence there can be no absolute relationship between tissue localization and the presence of SIg. In some cases of γHCD, in which these studies have been reported, the cells which showed cytoplasmic fluorescence for the HCD protein antigenic determinants did not show these determinants on the cell surface [16]. More recent studies have demonstrated the antigenic determinants of the HCD proteins on the cell surface [96]. No light chain determinants have been noted. Not enough information is available at this time to specifically account for apparent differences in tissue localization of γHCD cells and myeloma cells.

From these considerations it appears that there are far too many differences between γHCD and myeloma to call the HCD cell a typical plasma cell which has merely undergone structural mutation in the genes coding for the heavy and light chains of immunoglobulin. Are there proliferations of other clearly defined lymphoid cell populations which more closely resemble that seen in HCD?

A number of patients with lymphomas have been described whose sera contained monoclonal immunoglobulins [6, 71]. Most of these have been of the IgM type. But at least one series indicates that the incidence of monoclonal IgG proteins in patients

with lymphomas is higher than that found in age matched controls [4]. Immunofluorescent studies in other patients have demonstrated monoclonal IgG production by malignant lymphocytes, creating a *prima facie* case for a relationship between the lymphocyte and the Ig protein [55, 86].

The mixed nature of some immunocytic proliferations cannot be denied [80]. Lymphocyte-reticulum cell-plasma cell transition in the course of normal differentiation has frequently been hypothesized. It may be that this type of transitional morphologic picture reflects the proliferation of a cell population which is erratic in the expression of the proteins coded by its genome.

αHCD

The clinical features of αHCD have been reviewed by several authors [101, 110, 114]. It seems to have the most characteristic clinical picture of the 3 types of HCD. Almost all the patients presented with malabsorption secondary to infiltration of the *lamina propria* of the entire bowel wall with lymphocytes and plasma cells [12, 61, 87, 94]. Reticulum cells or histiocytes dominated the infiltrate in some patients [27, 108]. Although mesenteric lymphadenopathy was seen in many patients, hepatosplenomegaly was uncommon and evidence of dissemination in either the peripheral blood or the bone marrow was rare. The amount of α heavy chain fragment in the serum was quite variable usually being low and electrophoretically heterogeneous [29].

Several patients have been described in whom gastrointestinal manifestations were absent, pulmonary pathology was striking and αHCD protein was found in the serum [33, 37]. One of these patients had progressive pulmonary fibrosis and mediastinal lymphadenopathy, but at autopsy displayed no definite evidence of lymphoma [117].

These patients shared very few features with patients with multiple myeloma whose cells were synthesizing intact IgA proteins. IgA myeloma shared many of the features described for IgG myeloma (*vide supra*). In some series hypercalcemia occurred

techniques	substances	γ	α	μ
cellulose acetate electrophoresis	serum	γβ monoclonal diffuse	monoclonal or diffuse β	α monoclonal (often inapparent)
	urine	+ —	±	—
immunoelectrophoresis	serum	$\gamma^+, \varkappa^-, \lambda^-$	$\alpha^+, \varkappa^-, \lambda^-$	$\mu^+, \varkappa^-, \lambda^-$
	urine	$\gamma^\pm, \varkappa^\pm, \lambda^{\pm}$ *	$\varkappa^\pm, \lambda^{\pm}$ *	$\varkappa^\pm, \lambda^\pm$
	isolated serum protein	$\gamma^+, \varkappa^-, \lambda^-$	$\alpha^+, \varkappa^-, \lambda^-$	$\mu^+, \varkappa^-, \lambda^-$
	urine protein	$\gamma^+, \varkappa^-, \lambda^-$	$\alpha^{\pm}$ **	$\varkappa^\pm, \lambda^\pm$
isolated protein size		50-116,000	35-360,000	100-365,000
isolated protein size after reduction and alkylation		25-58,000	35-42,000	35-55,000

* no cases of α or γ heavy chain disease with free ϰ or λ chains in the urine have yet been reported;

** if light chain antigenic determinants are present in urine they must be present on proteins other than the actual HCD protein, i.e. either intact Ig's or free L chains.

Table 2 - Laboratory diagnosis of heavy chain diseases.

question	methodologic approach
1. *Is this a degradative product?* a) Does *in vitro* secretion product have same size as serum and/or urine protein? b) Is the cytoplasmic molecule of the same size as secreted molecule? c) Is there any evidence for normal size heavy and light chains intracellularly?	1. Size analysis serum and urine protein 2. Short-term tissue culture
2. *Is the product clearly monoclonal?* a) After cloning, do all clones synthesize and secrete the same product? b) Is the cultured cell product chemically identical to that seen *in vivo*?	3. Long-term tissue culture 4. Chemical analysis serum and urine protein compared with material synthesized in culture
3. *Is the loss of chain synthesis a structural or regulatory defect?* a) Is the mRNA for normal or heavy or light chains present? b) Is the primary translation product identical to that found in whole cell or in serum of patient? c) If the answer to b) is no, what are the differences? d) What is the size of mRNA?	5. *In vitro* translation of mRNA and analysis of translation products 6. Analysis of mRNA
4. *What is the nature of genetic defect?* a) Is defect due to nonsense mutation or frameshift? b) If no light chain is present, is gene present? c) Is there difference in hybridization kinetics for normal H chain mRNA-cDNA and HCD mRNA-cDNA?	7. Effect of suppressor tRNA's on *in vitro* translation 8. Synthesis of cDNA from mRNA's and DNA-DNA hybridization in comparison with other Ig messages

Table 3 - Approach to delineation of molecular and genetic defects in heavy chain diseases.

more frequently in IgA than in IgG myeloma and a larger percentage of patients with IgA proteins synthesized and secreted excess light chains [65].

Myeloma involved the gut infrequently, when it did the small intestine was involved about 20 % of the time [58, 59, 63, 126]. 25-30 % of these patients had the bowel affected in the course of disseminated disease with diffuse marrow involvement. The remainder had isolated plasmacytomata of the gut. Where data, identifying the nature of the monoclonal protein, were available, 5 of 9 patients had IgG proteins while 1 had an IgA protein. IgA proteins were found in 4 plasmacytomata not involving the gut. None of these patients were reported to show malabsorption as a prominent feature. One patient with the clinical picture of αHCD had an IgG3 myeloma protein in the serum and no αHCD protein [70].

αHCD shares all the clinical features of the Mediterranean type of abdominal lymphoma with malabsorption [102, 103]. In fact, when the unusual immunoglobulin was described it was anticipated that the production of the αHCD protein would be found in all cases of this lymphoma, however it has now become apparent that αHCD probably represents a subset of these patients [115].

Is there a situation in which there is a similar gut associated proliferation of cells in which intact IgA is synthesized? Gluten-sensitive enteropathy is characterized by a pleiomorphic small bowel infiltrate with plasma cells and lymphocytes, and substantial amounts of local IgA and IgM synthesis [32]. Evidence has been presented that the Ig production is a response to dietary gluten. Although these patients have been reported to have an increased incidence of lymphoma, monoclonal protein production has not been noted. Several patients with the protein abnormality of αHCD have not followed a course consistent with a neoplasm [110]. Hence some of these may represent a host response to an unknown agent in which there is an abnormality of protein structure without malignancy. In short, αHCD does not appear to be a simple case of myeloma localized to the gut or respiratory tract in which a simple structural mutation can account for the protein abnormality and the clinical features.

μHCD

It is difficult to make general statements about μHCD since the small number of patients described may not be a statistically accurate sample of what will ultimately be the entire population with this disease. It is unusual among the heavy chain diseases in that the cells of all but 2 of the patients described also produced an apparently normal light chain [46].

Most of the μHCD patients exhibited many of the features of chronic lymphocytic leukemia with anemia, increased susceptibility to infection and hepatosplenomegaly [7, 11, 40, 77]. Bone marrow samples showed predominant lymphocytosis with some plasmacytosis. The plasma cells have been reported to show large vacuoles in many of the cases. Peripheral lymphadenopathy was not a regular feature although it has been reported in some patients. Lytic bone lesions were reported in the original patient as was amyloidosis [7].

Two patients have been reported to show μHCD proteins in the presence of intact monoclonal Ig's. One of these was an elderly man with a monoclonal IgA protein and a peculiar ulcerating tumour of the cheek [67]. In the other a protein with μ antigenic determinants and no light chain antigenic determinants was found in the presence of an intact IgM protein [9]. The characterization of the latter 2 proteins was not as extensive as in the other reported cases.

There is little doubt that μHCD is a lymphoid disorder which most closely resembles chronic lymphocytic leukemia. The fact that most of these patients continued to synthesize and secrete light chains makes a simple structural mutation in the heavy chain gene much easier to accept than in γ- or αHCD. The additional feature of long-standing disease, frequently treated with cytotoxic agents which may have been mutagenic at the genetic locus coding for heavy chain, also makes this explanation plausible [97]. The association of substantial intact monoclonal protein production with chronic lymphocytic leukemia is unusual, and the presence of vacuolated plasma cells producing the protein suggest that some lymphocyte-plasma cell transformation may have occurred and that this was associated with unfaithful expression of the structural genes for μ chain.

STRUCTURAL ASPECTS

The diagnosis of HCD is suspected on the basis of the immunoelectrophoresis of serum or urine [45, 54]. The HCD proteins will react with antisera specific for the heavy chain in question but will not precipitate with antisera specific for κ or λ light chains [64]. It is obvious that monospecific antisera are required. Other techniques of diagnosis based on the same principles have been described [54]. Chemical analysis of the isolated protein is necessary to confirm the diagnosis [41, 47].

Molecular weight determinations of the isolated protein by either polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS) or sedimentation equilibrium analysis have revealed molecules smaller than normal Ig's. Most of the proteins studied to date have existed as either covalently or non-covalently bound dimers in the case of γHCD, larger polymers in α- or μHCD. Those molecules linked by non-covalent bonds will dissociate to the monomeric form and halve their molecular weight when exposed to dissociating solvents (e.g. SDS, urea, guanidine or propionic acid). Covalent polymers must be chemically reduced with either dithiothreitol or 2-mercaptoethanol in a dissociating solvent to ascertain the size of the monomer. Normal IgG's retain their molecular size in dissociating reagents, and only when they are exposed to reducing agents they release heavy and light chains [28]. Similar treatment of polymeric IgA and IgM molecules releases a third polypeptide, the J chain [62].

γHCD

γHCD proteins have been described for all 4 classes of IgG. FRANKLIN and FRANGIONE [49] have recently classified these proteins according to their structural abnormalities.

The first group consists of heavy chains which, when compared with prototype intact myeloma proteins of the same subgroup, have normal amino terminal sequences of varying lengths. The normal sequence at the amino terminus is followed by a deletion and resumes at a glutamic acid which appears to represent residue 216 in the intact IgG sequence. This group of proteins is thus characterized by deletions of different lengths which start in the variable region (V_H) and extend through the C_H region to residue 216, an area just proximal to the hinge region [20, 42, 44, 48, 50, 95]. The resumption of presumably normal sequence at, or near Glu-216 in several proteins has led these authors to suggest that this residue could represent the start of what was once a distinct genetic unit.

The second group of proteins also starts with an apparently normal amino terminus followed by a deletion which extends beyond the hinge and in one instance to normal residue 252, methionine [29, 43, 100, 119]. This resumption of normal sequence at the carboxy terminal end of the hinge region has been interpreted in 2 ways. The initial report of protein HAL suggested that AUG codon which codes for methionine served as an internal initiator [43]. Alternatively the hinge region could be a genetically distinct element and residue 252 represents the start of the genetically defined CH_2 domain [49].

Proteins with hinge region deletions cannot have the cysteines which form the inter-heavy chain disulfides, consequently these proteins occur as non-covalently linked dimers which dissociate when they are exposed to appropriate solvents. This type of behaviour suggests the presence of a hinge region deletion. Four proteins of this type have been described although the precise sequence analysis of only one has been completed.

Three additional proteins have been described which are not true HCD products in that they consist of both heavy and light chains which are covalently linked with the proper stoichiometry, i.e. H_2L_2. In 2 of these, SAC and SM, both chains are shorter than normal. In 2 others (MCG and DOW), only the heavy chain was aberrant with a deletion which encompassed the hinge region (residues 215-230) [25, 34]. The light chain of the SAC protein had an internal deletion while the heavy chain defect was N-terminal. The structural analysis suggested that the light chain defect was primary and that the heavy chain was shortened by post-synthetic proteolysis. In SM a light chain deletion has been established, however the heavy chain defect has not yet been defined. In 2 other patients free light chains and material carrying heavy chain antigenic determinants have been found in the urine [36, 72]. Synthetic studies have not been carried out, nor has the chemical nature of the anti-heavy chain reactive material been established.

Four proteins have been described which appear to start at the hinge region [51, 118, 122]. These proteins may be the result of extensive amino terminal deletions, less extensive deletions followed by post-synthetic degradation or a pure degradative process. Since biosynthetic studies have not been carried out with any of the cell populations producing these proteins (*vide infra*) it is not possible to choose among these possibilities.

αHCD

The αHCD molecules isolated from serum have been very heterogeneous [113]. Those proteins which were isolated, displayed significant amino terminal heterogeneity. Therefore it is difficult to unequivocally accept these as synthetic products of single clones.

These proteins carried the antigenic determinants of the Fc region of the α chain. They exhibited no reactivity with anti-Fd sera [111, 112]. They all were found as dimers or larger polymers suggesting that they retained the cysteines involved in heavy chain disulfide bond formation. Two of these proteins (DEF and AIT) were studied in greater detail [127, 128]. DEF had a molecular weight of 39,000 (without correction for carbohydrate content; 29,000 for the polypeptide portion alone). Amino acid sequence analysis indicated that the amino terminal portion of the deleted protein contained residues homologous with a portion of the normal variable region but probably not the true normal amino terminal residues. These V_H residues were contiguous with a sequence identical to that of the normal hinge region. Hence the deletion included most of the V_H and CH_1 and part of the hinge (as in the γHCD proteins). It was not clear if the absence of the normal amino terminal residues was a post-synthetic modification (*vide infra*). A murine IgA myeloma protein has been described which contains a 100 residue deletion in its Fc region [105]. This differs considerably from the human αHCD proteins which have been studied.

μHCD

Structural data on μHCD proteins are also scant. The GLI protein, isolated from the first patient with μHCD, had a reduced and alkylated molecular weight of 55,000 [38]. Although the cells of this patient synthesized κ chains, the H and L chains were not disulfide-linked. The absence of the H-L disulfide suggested that one or both of the cysteines involved in the bond were absent. However, recent experiments have indicated that the molecule starts with Ala-131 and that Cys-141, which normally participates in the H-L disulfide, was present. The deletion appeared to be N-terminal with 9 residues present just distal to the H-L cysteine [52]. Since the GLI κ chains were normal, Cys-141 must be stabilized by other residues for the H-L disulfide to be formed. If this stabilization does not occur then formation of an additional inter- or intra-heavy chain -S-S- is favoured. Further studies are in progress to elucidate this point.

μHCD protein BUR was smaller than GLI with a reduced molecular weight of 35,000 [76]. The native serum protein was a non-covalently linked polymer with an approximate molecular weight of 365,000. In dissociating solvents the major species had a molecular weight of 140,000. Two minor proteins with molecular weights of 95,000 and 70,000 were also noted under these conditions. J chain was released by complete reduction but no light chains were found.

Protein BO existed as a disulfide-linked polymer with a molecular weight of 520,000-540,000 daltons. On reduction, a heterodisperse population with an average molecular weight of 52,000 on SDS-acrylamide gel electrophoresis was obtained. J chain was released by reduction but again no light chains were evident [82].

The amino terminal residue of BO was Asp and it appeared to possess the antigenic specificities associated with the constant region of the μ chain and the conformational

determinants of the native IgM molecule. However, the peptide maps of the $(FC\mu)_5$ of BO and that of the $(FC\mu)_5$ of an intact monoclonal IgM protein were quite different. It was not clear if the differences were related to the same process which produced the μHCD protein *in vivo* or represented a genetically determined polymorphic phenomenon [83]. Further detailed chemical data are required before any firm conclusions can be drawn. Less data are available for the protein IA which has a N-terminal Ala and a molecular weight of 54,000 [22].

In summary, γHCD proteins may exhibit clearcut internal deletions or amino terminal truncations which may be synthetic, degradative or both. αHCD proteins exhibit a greater degree of N-terminal heterogeneity which suggests some secondary proteolytic process. The μ chain proteins seem to have homogeneous amino termini with little or no preceding normal sequence. The latter also appear less likely to have lost light chain production.

BIOSYNTHESIS

The genetic interpretation of the structural data is dependent upon the demonstration that the amino acid sequence is a faithful reflection of the DNA base sequences as they are transcribed and translated through the intermediary messenger RNA. Most of the early synthetic studies indicated only that the cells in question synthesized and secreted proteins antigenically identical with those being isolated from the serum and urine [29, 30]. More detailed studies have been performed with cells obtained from patients with each of the 3 classes of heavy chain disease and 5 additional questions have been asked [15, 16].

Was there any evidence for light chain synthesis in the cells of any patient whose serum and urine did not contain light chains? The answer was no. In the one patient with μHCD who was excreting light chains in the urine, the light chains were demonstrated intracellularly by immunofluorescence and by radiolabeling techniques and no heavy-light interaction was observed [134]. Was there any intact heavy chain precursor? None was noted in any experiments, although an extremely short-lived (i.e. less than 5 min) molecule might have been missed. Was the major intracellular molecule defective? Intact heavy chains were not seen in any of the cases studied. Was there any evidence for intracellular degradation? No, however the limit of detection of size differences by the methods employed would have allowed some degradation to be missed. Were the cytoplasmic and secreted proteins identical to those found in the serum and urine? No chemistry was performed to answer this question, however in at least 2 proteins, 1 a γ (VA) and 1 an α (DEF), the protein isolated from the serum may have been smaller than that synthesized in tissue culture by 1,000 daltons [16, 127]. This suggested that some post-synthetic degradation had taken place, probably, but not necessarily, after the protein had left the cell. Finally, it appears that the processes of synthesis, disulfide bond formation and secretion were analogous if not identical to those seen in cells producing Ig's with normal structure. The extent of polymerization was characteristic of the normal Fc region of intact proteins of the same class. γHCD proteins formed no polymers larger than dimers, i.e. $(\gamma F)_2$, while μ and α chain proteins formed tetramers $[(\alpha F)_2]_2$ and $[(\mu F)_2]_5$.

All these studies were carried out with techniques sufficient only to analyze post-ribosomal events. The definitive experiments necessary to answer these questions must be carried out with *in vitro* systems in which the isolated purified mRNA can be translated and the translation products analyzed.

Recently a patient (OMM) has been described whose serum contained 2 proteins bearing γ3 antigenic determinants [2, 3]. One protein was characterized as an apparently intact γ3 myeloma protein. The second had a molecular weight of 40,000 and amino

terminal glycine which appeared to be contiguous with a sequence consistent with the normal hinge region [2]. It was not certain if the smaller chain was a proteolytic digestion product of the larger, if these were products of separate malignant clones, or if the second was the product of a mutant subclone of the first.

A cell line was established from the peripheral blood of the patient (BUXBAUM: unpublished observation). The line synthesized only the smaller protein. When cloned, each clone synthesized and secreted the 40,000-dalton γ3HCD protein. Hence the smaller protein was clearly a clonal product. The cytoplasm and secreted material contained the dimer of the protein, which could be reduced to the 40,000 molecular weight monomer. There was no evidence for the production of light chain or a normal sized γ chain. mRNA was extracted from these cells and translated in a cell-free system derived from wheat germ to yield a protein of 40,000 daltons which could be precipitated with anti-γ3. There was no *in vitro* synthesis of a normal sized heavy chain. There is thus little evidence for the smaller protein being a proteolytic product of the larger. The combined structural and biosynthetic evidence suggests that the cell line is a mutant subclone of a malignant lymphoid population that was originally synthesizing an intact protein.

CONCLUSIONS

The heavy chain diseases comprise a group of conditions in which populations of lymphoid cells synthesize and secrete immunoglobulin heavy chains which are structurally defective. In most of the patients the disease follows a malignant course, however there is some evidence suggesting that there may be a subpopulation of normal B cells which secrete similar molecules into the normal human serum Ig pool. If these experiments are confirmed it may indicate that the production of these defective molecules may not necessarily be associated with malignancy [74].

In all cases the proliferation appears to be more lymphoid than plasmacytic but the cells are usually pleiomorphic. It is possible that the heterogeneity may characterize an Ig-producing population which is particularly susceptible to structural defects in their secreted products. Since the deletions do not appear to be random it is tempting to speculate that the expression of the variable and CH_1 genomes is sensitive to disruption at a time when B lymphocytes are beginning to synthesize entire chains for display on the cell surface as SIg. Recent data (TONEGAWA: unpublished observation) have suggested that the genetic elements coding for variable and constant regions may be spatially separated in the embryo but fused in the adult. HCD could represent malignancy in cells which have undergone faulty gene fusion. The pleiomorphic nature of the HCD cells might also suggest that this event is temporally related to the lymphocyte-plasma cell morphologic transition.

In most cases of μHCD where only a single chain is abnormal, a single genetic event, either mutational or deletional, is easily accepted, but what kind of molecular events can be responsible for the 2 defects seen in α- and γHCD (where there is no light chain production)? It is possible that one clone could have undergone 2 separate mutational events each affecting the structure of an Ig chain. Statistically this should be a very rare occurrence. If this were the case, the defective light chain would have to be too small to detect or rapidly degraded either intra- or extracellularly. This hypothesis is difficult to discard experimentally, however there are data indicating that mutagenized cultured mouse myeloma cells lose H chain production before L chain production [124]. Similarly in humans there are many cases of L chain myeloma and few of HCD. Further, in patients SM and SAC where defects were seen in both chains, degradation did not occur to the extent that the fragments were not detectable. In fact, the L chain deletion was felt to be primary and the N-terminal H chain short-

ening secondary to the presence of the aberrant L chain. No biosynthetic studies were performed in either patient to clarify this point. These data make a double structural mutation highly unlikely.

There is a substantial body of evidence in bacteria which indicates that mutant proteins are readily degraded by enzyme systems specifically present for that purpose [5, 23, 26, 85, 130]. These processes may proceed either from C-terminus or N-terminus, and are quite rapid. The systems have not been as thoroughly explored in eukaryotic cells, but there is evidence that in some mouse myelomas excess light chains can be degraded as well as secreted [8, 93, 109].

The lack of light chain production could also be explained by a regulatory defect. In this case the genetic information necessary to code for the protein is present but for some reason it is either not transcribed or transcribed and not translated. Like the previous hypothesis these possibilities are experimentally testable. Such a biochemical lesion has been proposed in a non-secreting murine myeloma variant in which no Ig was synthesized either in the intact cell or a cell-free system but an RNA species with a fingerprint similar to that of authentic L chain message was detected intracellularly [21].

It is also possible that L chain mRNA is transcribed and is not translated in the intact cell but can be translated in a heterologous cell-free system. Several groups have approached the problem of β° thalassemia using both cell-free translation and DNA/DNA and DNA/RNA hybridization. The difficulties inherent in this approach are indicated by the fact that one laboratory has reported absent mRNA and another nonfunctional mRNA for the β-globin chain in these patients [38, 68]. Other laboratories have demonstrated the presence of the unexpressed gene by molecular hybridization studies in both β° and $\beta^\circ/\delta\beta^\circ$ thalassemia [90, 121].

An absent gene has been demonstrated by cDNA/DNA hybridization studies with cells obtained from patients with hereditary persistence of fetal hemoglobin. In those studies normal α and β chain mRNA were used as templates for reverse transcriptase and cDNA copies of the α and β genes were synthesized. These cDNA's were then hybridized to the DNA from cells with persistent fetal hemoglobin and the extent of hybrid formation determined and compared with the extent of hybrid formation with DNA obtained from cells synthesizing normal α- and β-globin chains. These experiments demonstrated no material hybridizing with α and β genes in the cells of these patients. Hence neither the gene nor the mRNA was present [39].

Several possible events could account for the amino acid sequences seen in the isolated HCD proteins. Single base changes which result in the generation of a nonsense codon cause termination. Normally this would cause a short peptide (a V-region fragment) with a normal amino terminus to be released from the polyribosome. However, in bacterial systems (e.g. the LAC operon), where polycistronic mRNA's code for a number of related sequentially synthesized proteins, nonsense mutations in the first cistron do not always result in lack of synthesis of the succeeding proteins. Those cases in which translation does not take place beyond the mutation are known as polar mutants, referring to the fact that the gene order is critical to the processing of the message [60]. In some cases the mutants exhibit a lesser degree of polarity and the mRNA is read again at the start of the next cistron so that the later proteins in the gene cluster are expressed. The degree of polarity appears to be a function of the distance between the site of mutation and the start of the next cistron or another internal initiation site [35]. This had led to the hypothesis that there is a limit to the length of untranslatable message which will stay on the polyribosome. If this were true in HCD, one would expect the site of reinitiation to be the amino terminal end of the CH_1 region. This is not the case. Residue 216 is the site of resumption of normal sequence in several proteins, suggesting that this may be a preferred site for reinitiation. However, in bacteria, translational reinitiation of this type has resulted in frag-

ments with new N-termini rather than deleted proteins, hence in proteins with structural deletions this mechanism seems unlikely. If there were a nonsense mutation followed by translational reinitiation one would expect that the mRNA would be of normal size with that coding for the normal N-terminus and the restart region held together by a stretch of nonsense RNA.

The final interpretation of the structural data accepts a simple deletional hypothesis created by either a looping out and excision of the DNA after V-C integration or an unequal crossover resulting in a short gene. In this case the mRNA should also be short. The measurement of mRNA size is clearly possible.

The primary defect in heavy chain structure should tell us something about the integration of the separate genetic elements (V and C) which make up the heavy chain. Since the deletions extend well into the constant region and structural and genetic evidence indicate that V and C are coded by 2 separate genes, most investigators have felt that the defect must occur at the DNA level [116, 117, 119]. If the deletional events are random then the chances of having a constant region deletion should be 3 times that of having a variable region abnormality. This is not the case. Further evidence in favour of the non-randomness of these variants is the apparent predisposition for normal sequence to resume around the hinge region.

If V-C gene fusion takes place in embryonic life (*vide supra*) the site of fusion should be at the nucleotide sequence corresponding to the carboxyl end of the variable region and the amino terminus of the constant region. If duplex formation takes place at sticky ends, it is possible that the amino terminal nucleotides and the hinge region nucleotide sequence are particularly sticky and suitable for duplex formation. The overlapping but non-matching DNA sequences could then be cleaved by endonucleases.

It is not clear if any of these mechanisms must be associated with an oncogenic event which results from the integration of a viral genome into a specific or non-specific area of host DNA, which interrupts the sequences coding for the structures of H or C chains; however, it is an intriguing possibility.

SUMMARY

Human heavy chain diseases offer an opportunity to examine the molecular events taking place in cells which produce naturally occurring structural variants of a well-studied complex protein. Extensive structural analyses have been carried out on many of these proteins and have revealed the existence of certain characteristic features. Current studies are beginning to elucidate the molecular biology underlying these structural features.

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