Structural Differences between Two Types of "Heavy Chain" Disease Proteins and Myeloma Globulins of Corresponding Types

Abstract. *Structural differences between two antigenic types (designated C and Z) of γ-globulins, both of low molecular weight, produced in "Heavy chain" disease are revealed by their relative susceptibilities to enzymatic degradation by papain and the nature of the cleavage products. Corresponding structural differences exist between the respective type C and type Z myeloma γ_2-globulins. These structural differences are tentatively assigned to the H-chains, and more specifically to the portion of the H-chain contained in the fast-moving (F) fragment of papain-treated γ_2-globulins.*

In the past year, five cases of a previously unrecognized form of plasma cell dyscrasia, tentatively designated as "Heavy ($H_{\gamma 2}$) chain" disease (*1*) have been described (*2–5*). The predominant clinical features of this disease are generalized lymphadenopathy, splenomegaly, hepatomegaly, anemia, and a marked susceptibility to bacterial infections. Histologic studies have shown a proliferation of reticulum cells, plasmacytic, and lymphocytic forms. The distinguishing biochemical abnormality in this syndrome is the excessive production of proteins of low molecular weight (of the order of 53,000), which are related to the heavy (H) polypeptide chains of 7*S* γ_2-globulin and more specifically to the fast-migrating (F) fragment of papain-treated 7*S* γ_2-globulin. These proteins are unrelated to the light (L) polypeptide chains and the slow (S) fragment resulting from papain digestion of 7*S* γ_2-globulin, and to the Bence Jones proteins.

The first of these cases, case I(C) (*6*), was reported by Franklin and associates (*2*), and the subsequent four cases, II to V, by us (*3, 5*). In the initial comparative immunochemical studies of these five proteins, we used antiserums prepared against the protein from case I(C) and against 7*S* γ_2-globulin. These studies indicated that the proteins from cases I, II, III, and IV were antigenically identical, whereas the protein from case V (Z), although closely related to these four proteins, was, in comparison, antigenically deficient when examined with the antiserum to the case I (C) protein (*5*).

Subsequent immunochemical studies by Ballieux *et al.* (*7*), which have been confirmed in this laboratory, demonstrated that the protein from case V (Z) contained antigenic determinant(s) which were lacking in the case I (C) protein. After the Z-specific antigenic determinant(s) and the antigenic specificity of C had been demonstrated, it was found (*7*) that myeloma γ_2-globulins could be antigenically typed as either C (the more common) or Z. The Ouchterlony analyses shown in Fig. 1 illustrate this point. The pattern on the left, developed with an antiserum to the case II (type C) protein demonstrates a reaction of complete identity between the case II protein and the type C myeloma γ_2-globulin in well 4. A reaction of partial identity (spur formation) is seen between the case II protein and type Z myeloma γ_2-globulin in well 1. Conversely, the pattern on the right, developed with an antiserum to the case V (Z) protein, shows a reaction of complete identity between the case V (Z) protein and the type Z myeloma γ_2-globulin in well 9, and a reaction of partial identity (spur formation) with the type C myeloma γ_2-globulin in well 6. Analyses of 20 myeloma γ_2-globulins showed that 18 (90 percent) were of type C, and only 2 (10 percent) were type Z (*8*). Figure 1 also demonstrates that pooled normal human 7*S* γ_2-globulin (Cohn fraction II) contains both types C and Z molecules, with a predominance of the C. These findings are in confirmation of the observations of Ballieux *et al.* (*7*).

As reported previously (*5*), further evidence of a structural difference between the case I to IV (type C) proteins and the case V (Z) protein was obtained by the demonstration of differences in their behavior after enzymatic digestion with papain (*9*). These differences are illustrated in the immunoelectrophoretic patterns in Fig. 2 which were developed with the antiserum to the case II (type C) protein. The case II protein (type C), after treatment with papain for 4 hours, shows a major precipitin arc (F^c) with a bimodal distribution and a minor arc (F') which gives a reaction of partial identity with the major arc. After digestion for 18 hours, a marked attenuation of the major arc (F^c) is evident, where-

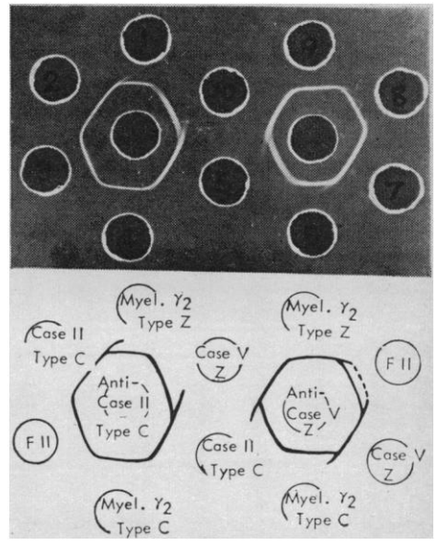

Fig. 1. Immunodiffusion analyses of case II (type C) and case V (Z) proteins, normal 7*S* γ_2-globulin (F II), and type C and type Z myeloma γ_2-globulins. Antiserums to the case II type C and case V (Z) proteins were used.

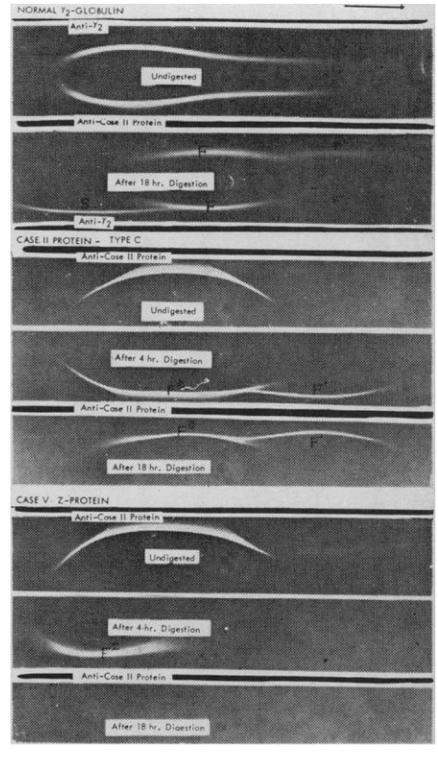

Fig. 2. Immunoelectrophoretic analyses demonstrating the differences in behavior between the case II (type C) protein and the case V (Z) protein after papain-digestion for 4 and 18 hours. The patterns of normal 7*S* γ_2-globulin (fraction II) prior to and following 18-hour papain-digestion are shown at the top. The slow (S) fragment of papain-treated normal γ_2-globulin is demonstrated by the antiserum to normal 7*S* γ_2-globulin (anti-γ_2) but not with the antiserum to the case II protein.

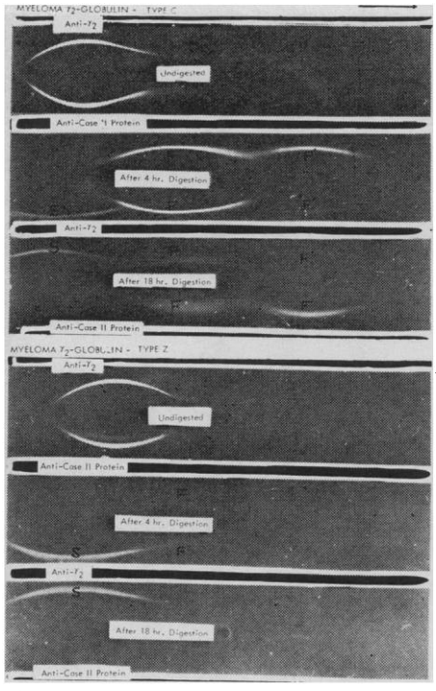

Fig. 3. Immunoelectrophoretic analyses of type C and type Z myeloma γ_2-globulins before and after digestion with papain for 4 and 18 hours.

as the F′ arc is unchanged in intensity as compared with the pattern of the 4-hour digest. In contrast to this behavior, the case V (Z) protein, after 4 hours of papain digestion, shows a marked diminution in intensity and a cathodal displacement of the major precipitin arc (F*) without the development of an F′ arc. Treatment of the case V (Z) protein for 18 hours destroyed all material precipitable by the antiserum to the case II protein.

We then studied the comparative effects of papain treatment on six type C and the two type Z myeloma γ_2-globulins. Representative results of these studies are shown in Fig. 3. These patterns were made with the antiserum to the case II protein, which develops only the F and F′ arcs, and an antiserum to normal 7*S* γ_2-globulin (anti-γ_2) which also demonstrates the S (slow) fragment. The type C myeloma γ_2-globulin, after 4 hours of papain digestion, shows strong F and F′ arcs in addition to an S arc. After 18 hours of digestion, these three arcs are still visible, although attenuated, and it is evident that the F arc has been diminished to a greater degree than the F′ and S arcs. In contrast to this behavior, the type Z myeloma γ_2-globulin, after 4 hours of papain treatment, shows a prominent S arc, only a very faint trace of an F arc, and no F′ arc. After digestion for 18 hours, only the S arc remains. The faint inner arc visible in the pattern of the undigested type Z myeloma γ_2-globulin developed with the antiserum to the case II protein probably represents a small amount of normal 7*S* γ_2-globulin, and the very faint F arc seen after 4 hours of digestion may have been derived from that source.

These observations establish the existence of major structural differences between type C and type Z myeloma γ_2-globulins which are comparable to the demonstrated differences in the type C and Z proteins in "Heavy ($H\gamma_2$) chain" disease. These structural differences are reflected in greater susceptibility to papain digestion of type Z than of type C proteins, and the development of an F′ component after papain treatment of type C but not of type Z proteins. Because of the greater susceptibility of type Z molecules to papain degradation, it would be anticipated that the isolated F fragment of papain-treated pooled normal 7*S* γ_2-globulin would contain exclusively type C molecules, and this has been demonstrated.

Within the obvious and generally recognized limitations of the nomenclature systems now used, the structural differences demonstrated in these studies are tentatively assigned to the H-chains, and more specifically to the portion of the H-chain contained in the F fragment of papain-treated 7*S* γ_2-globulin.

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References and Notes

1. Since the precise structure and polypeptide composition of the γ-globulins are unknown, the designations of the enzymatically and chemically produced subunits as well as the subunits which are found in the various plasma cell dyscrasias, including this disease, must be recognized as tentative.
2. E. C. Franklin, M. Meltzer, F. Guggenheim, J. Lowenstein, *Federation Proc.* **22**, 264 (1963).
3. E. F. Osserman and K. Takatsuki, *Medicine* **42**, 357 (1963).
4. E. C. Franklin, J. Lowenstein, B. Bigelow, M. Meltzer, *Am. J. Med.*, in press.
5. E. F. Osserman and K. Takatsuki, *ibid.*, in press.
6. It is a useful coincidence that the initial, C, of Franklin's first case corresponds to the "common" antigenic type, and that the initial of our case which is representative of the rare antigenic type is Z.
7. R. E. Ballieux, G. Bernier, K. Tominaga, F. W. Putnam, unpublished observations.
8. The group of 18 myeloma γ_2-globulins which were demonstrated to be of type C included proteins of both antigenic group I and group II with respect to their constituent L-chains. The two type Z myeloma γ_2-globulins were of antigenic group I. At present, therefore, there appears to be no correlation between the antigenic types of the constituent H-chains (that is, C or Z) and the constituent L-chains (I or II) of these proteins.
9. Papain digestion was carried out according to the procedure of R. R. Porter, *Biochem. J.* **73**, 119 (1956). We used twice-crystallized papain (Worthington Biochemical Corp.) (1 mg papain per 100 mg protein) in the presence of 0.01*M* cysteine and 0.002*M* ethylenediamine-tetraacetic acid (EDTA) at *p*H 5.6 and at 37°C.
10. These studies were supported by NIH grant CA–02332.
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Resistance to the Chemical Sterilant, Apholate, in Aedes aegypti

Abstract. *Increased resistance to the sterilizing effects of apholate was observed in two colonies of* Aedes aegypti *(L.) exposed in the larval stage of each generation to concentrations of apholate that induced about 90 to 40 percent sterility in the eggs laid by the ensuing adults.*

Since the inception of research on the potential of chemosterilants as insect control agents, many persons have asked whether insects could develop resistance to the sterilizing effects of these compounds. On the other hand, because the action of certain chemosterilants influences the induction of dominant lethal mutations, it has been theorized that continued exposure to substerilizing dosages or to dosages causing only partial sterility might result in accumulation of genetic defects and ultimate death of a colony.

In 1962 efforts were initiated to determine whether the yellow fever mosquito, *Aedes aegypti* (L.), could develop resistance to apholate (*1*). Weidhaas *et al.* (*2*) had reported that sterility could be induced in *A. aegypti* by feeding adult mosquitoes on honey solution containing 0.1 percent of apholate. Weidhaas (*3*) had also demonstrated that exposure of larvae of *A. aegypti*, from the third instar to pupation, in water containing 10 parts of apholate per million produced about 90 percent sterility in the ensuing males and about 50 percent sterility in females. When both sexes were treated, sterility was about 98 percent. In later experiments at this laboratory sterility induced by this larval treatment sometimes reached 100 percent. For our experiments in development of resist-