Antigenic Determinants Common to Human Immunoglobulins G and M: Importance of Conformational Antigens

Abstract. *Antiserums produced against certain isolated human myeloma IgG-globulins and absorbed in order to show only individual antigenic specificity cross-react with certain Waldenström IgM-globulins. Some of the common antigenic determinants revealed by these cross-reactions depend on the tertiary and quaternary structure of the IgG and IgM molecules and are independent of their K and L light-chain antigenic types.*

It is generally assumed that the antigenic determinants common to the various classes of human immunoglobulins are located on their common light polypeptide chains which belong mainly to either K or L antigenic types. During studies on the individual antigenic specificity of IgG myeloma globulins (*1*), we have found that some of the antiserums made specific to the myeloma globulin used for immunization cross-react with some Waldenström macroglobulins. The common antigenic determinants involved in some of these cross-reactions require the combination of heavy and light polypeptide chains of the myeloma globulin and are independent of the K and L light-chain antigenic types of the immunoglobulins.

Rabbits were hyperimmunized with isolated preparations of individual myeloma IgG-globulins in which no IgM was detectable by immunoelectrophoresis or agar double-diffusion studies. The antiserums were absorbed by the minimum amounts of fraction II and either agammaglobulinemic serum or normal human serum necessary to neutralize the occasional antibodies against impurities and to obtain negative precipitin reactions (in agar or liquid medium) with normal IgG-globulins (at concentrations ranging from 0.1 to 100 mg/ml) and with 40 myeloma IgG-globulins of different antigenic subgroups.

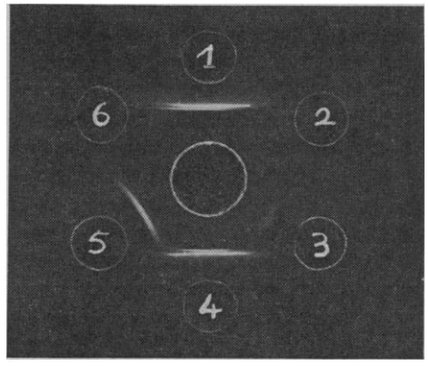

Fig. 1. Demonstration of the cross-reaction between the myeloma IgG-globulin Sa and a Waldenström-type IgM-globulin. Central well: absorbed antiserum to Sa myeloma globulin. Outer wells: Nos. 1 and 4, myeloma globulin Sa; No. 5, Waldenström macroglobulin; No. 2, the same Waldenström macroglobulin after reduction and alkylation (subunits); No. 3, recombined subunits; No. 6, saline.

These absorbed antiserums (absorption A), each of which gave a strong precipitin reaction with the homologous myeloma globulin, were tested in gel diffusion with 50 macro-globulins of the Waldenström type. Twelve of these absorbed antiserums contained antibodies which cross-reacted with one to eight of the Waldenström IgM-globulins. We now report cross-reactions observed with two absorbed antiserums specific for the myeloma IgG-globulin Sa of the K light-chain antigenic type and for the myeloma IgG-globulin Du of the L type, respectively. These antiserums gave strong precipitin reactions with six and five, respectively, of the 50 Waldenström IgM-globulins, at a concentration of 0.5 to 1 mg/ml. None of these macroglobulins had rheumatoid factor activity. Only one of them cross-reacted with both antiserums.

Both absorption-A antiserums gave a faint precipitin line with purified preparations of normal IgM-globulin and of IgM-globulin isolated from serums of patients with African trypanosomiasis when these preparations were tested at a concentration of 3 mg/ml or more. We therefore postulate that the antigenic determinants involved in these cross-reactions occur on normal or "polyclonal" IgM-globulins, but presumably on only a small proportion of these IgM molecules.

Similar antigenic determinants are probably also present on some molecular species of normal IgG-globulins since, in both absorption-A antiserums, the antibodies giving the cross-reaction with IgM-globulins were inhibited by great amounts of pure normal IgG-globulins. Previous studies (*1, 2*) have demonstrated that the antibodies to most "individual-specific" antigens of myeloma IgG-globulins are neutralized by great amounts of pooled normal IgG-globulins; in addition, we have shown that this neutralization is due to an inhibition process.

After absorption with the purified homologous myeloma globulin at the equivalence point, the antiserums no longer reacted with the Waldenström IgM-globulins. The cross-reacting IgM-globulins precipitated with only some of the multiple antibodies (*1*) involved in the "individual-specific" reaction of antiserum to Sa, since Sa myeloma globulin spurred over all IgM-globulins and reacted with the antiserum absorbed, in antigen excess, by the Waldenström IgM-globulins.

After reduction with 0.1*M* mercaptoethanol at neutral *p*H and subsequent alkylation, the IgM-globulins failed to precipitate with both absorption-A antiserums. However, these subunits in-

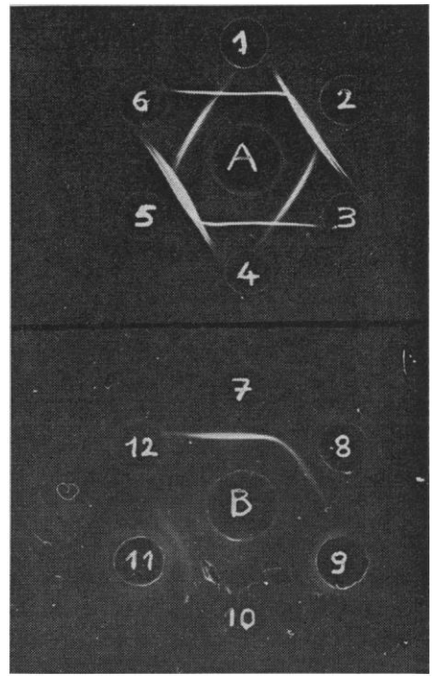

Fig. 2. Agar double-diffusion analysis showing that the antiserum to Sa myeloma globulin, absorbed in order to reveal the individual antigenic specificity, reacts only with the recombined chains of the Sa myeloma globulin. (Top) Reaction with the unabsorbed antiserum in central well A. (Bottom) Reaction with the absorbed antiserum in central well B. Outer wells: Nos. 1, 4, 9, and 12, isolated heavy chains of myeloma globulin Sa; Nos. 2, 5, and 7, myeloma globulin Sa; Nos. 3, 6, and 10, isolated light chains of myeloma globulin Sa; Nos. 8 and 11, recombined chains of myeloma globulin Sa.

hibited the precipitating antibodies involved in the cross-reaction. The reassociation of the reduced subunits resulted in the recovery of some precipitation with the antiserum (Fig. 1). These observations suggest that the involved antigenic determinants could be uni- or bivalent in the IgM subunits. In this respect, one might recall the recent findings of Onoue *et al.* (*3*) which suggest that the subunits of IgM from rabbit antibody are univalent.

For both antiserums, the precipitin reaction with the homologous myeloma globulin is confined to the Fab-fragment and requires the combination of heavy and light chains. The heavy and light chains were prepared by reduction-alkylation and gel filtration (*4*); the Fab- and Fc-fragments were prepared by papain digestion by the Porter method (*5*). The Fc-fragment did not precipitate with the absorption-A antiserums, nor did the isolated light and heavy chains, although they reacted with the unabsorbed antiserums (Fig. 2). The homologous chains, recombined in the weight ratio of two heavy to one light, gave a reaction of complete identity with the native myeloma globulin (Fig. 2) and removed, by absorption, all reactivity with this myeloma globulin. Similar results concerning the localization of individual specific antigens of some myeloma globulins were obtained by Grey *et al.* (*2*).

The cross-reactions with Waldenström IgM-globulins were not inhibited by the homologous myeloma light chain (5 mg per milliter of antiserum). Among the five Waldenström macroglobulins which cross-reacted with the absorbed antiserum to Du myeloma globulin (type L), two were of antigenic type K.

These observations demonstrate the existence of similar antigenic determinants, shared by some IgG and IgM molecules, which depend upon the conformational structure of the molecule. The importance of conformational structure for the antigenic specificity of several proteins (*6*) and for the Gm(bw) and Gm(f) factors of IgG-globulins (*7*) has been pointed out. That interaction between heavy and light chains of IgG is necessary in order to observe the present reaction revealing the common antigenic determinants suggests that both chains could be involved in the antigenic sites; each of the chains could contain amino acid residues contributing to these antigenic sites. However, another possibility is that these antigenic determinants common to IgG- and IgM-globulins are located on a single chain but that they become exposed or able to react with the antibody only when the double (heavy-light) chain configuration exists.

The demonstration that the antigenic structures are common to an IgG-globulin of the type L and to an IgM-globulin of the type K probably excludes any role of the carboxy-terminal part of the light chain (*8*). These common antigenic determinants could possibly be localized on the Fd-fragment of the heavy chain and depend on its tertiary structure. There is evidence that identical antigenic determinants are present on the Fd-fragment of two distinct classes of immunoglobulins. In the rabbit, the allotypic antigens Aa 1, 2, and 3, which are present on the Fd-fragment of IgG-globulin, have been demonstrated in IgM-globulin (*9*). The Fd-fragments of guinea pig γ_1- and γ_2-immunoglobulins have similar antigenic determinants (*10*). Antiserums prepared against pepsin-digested IgG-globulin from normal serum and absorbed with light chains react with certain IgA myeloma proteins and Waldenström macroglobulins (*11*). However, three antiserums prepared against pepsin-digested Sa myeloma globulin failed to cross-react with any of the 50 Waldenström IgM-globulins.

MAXIME SELIGMANN
CONSTANTIN MIHAESCO*
GILBERT MESHAKA*

Laboratory of Immunochemistry, Hôpital Saint-Louis, Paris 10e, France

References and Notes

1. M. Seligmann, G. Meshaka, D. Hurez, C. Mihaesco, *IVth International Symposium on Immunopathology, Monaco 1965*, P. Grabar and P. Miescher, Eds. (Schwabe, Basel, 1966), p. 229.
2. H. Grey, M. Mannik, H. Kunkel, *J. Exp. Med.* **121**, 561 (1965).
3. K. Onoue, Y. Yagi, A. L. Grossberg, D. Pressman, *Immunochemistry* **2**, 401 (1965).
4. J. B. Fleischman, R. H. Pain, R. R. Porter, *Arch. Biochem. Biophys. Suppl.* **1**, 174 (1962).
5. R. R. Porter, *Biochem. J.* **73**, 119 (1959).
6. E. Haber, J. C. Bennett, J. A. Mills, *Medicine* **43**, 305 (1964).
7. S. Polmar and A. G. Steinberg, *Science* **145**, 928 (1964); E. R. Gold, W. J. Mandy, H. H. Fudenberg, *Nature* **207**, 1099 (1965).
8. N. Hilschmann and L. E. Craig, *Proc. Nat. Acad. Sci. U.S.* **53**, 1403 (1965); K. Titani and F. W. Putnam, *Science* **147**, 1304 (1965).
9. C. W. Todd, *Biochem. Biophys. Res. Comm.* **11**, 170 (1963).
10. V. Nussenzweig and B. Benacerraf, *J. Immunol.*, in press.
11. H. Kunkel, H. M. Grey, A. Solomon, *IVth International Symposium on Immunopathology, Monaco 1965*, P. Grabar and P. Miescher, Eds. (Schwabe, Basel, 1966), p. 220; M. Harboe and J. Deverill, *Acta Med. Scand.* **179**, Suppl. 445, 74 (1966).
12. Supported by grant 61 FR 062 of the Délégation Générale à la Recherche Scientifique. We thank Miss J. Ferber for helping with the English translation.
* Chargé de Recherches of the French National Institute of Health and Medical Research.

1 September 1966

Nucleotide Sequence Repetition: A Rapidly Reassociating Fraction of Mouse DNA

Abstract. *The separated complementary strands of a minor component in mouse DNA reassociate with each other much more rapidly than do the complementary strands of other DNA's including those of the principal part of mouse DNA. This difference in capacity of the strands to reassociate can be used to effect a preparative separation of the minor component from the principal fraction. The rate constant for reassociation of the minor component, compared with those of viral and bacterial DNA's, indicates that the minor component consists of a short nucleotide sequence present in about one million copies.*

High-molecular-weight DNA can be fractionated on the basis of nucleotide composition by centrifugation to equilibrium in concentrated CsCl solutions (*1*). When the DNA of higher organisms is analyzed in this way it is found to be heterogeneous in composition. In many cases (*2, 3*) small discrete (satellite) bands are observed, more-or-less well-resolved from a broad principal band. Mouse DNA exhibits a relatively intense band (*3*) with a density of 0.01 g/cm^3 less than that of the center of the principal band. This component has been found in native DNA from all mouse tissues that have been examined and from mouse cell lines cultured in vitro.

We now report that, when the complementary strands are separated by heating (denatured) and incubated under appropriate conditions, they reassociate (*4*) with each other, quickly finding appropriate partners and re-form-