Immunochemical evidence for a common variable region in three immunoglobulin classes in the same individual

(idiotyp/M-component)

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ABSTRACT One IgCl(x), one IgM(x), and one IgAl(x) monoclonal (M)-component were purified from one human serum. Rabbit antisera were raised against the IgG and IgM M-components and were absorbed until specific for idiotypic determinants on these molecules. All three M-components gave reactions of immunological identity when tested by double radial immunodiffusion with either of the two idiotyp-specific antisera. Both heavy and light chains were isolated from each of the three M-components and all preparations inhibited formation of idiotypic precipitates. None of these preparations formed precipitates with idiotyp-specific antisera alone. When heavy or light chains of one M-component were hybridized with light or heavy chains from the other M-components the resultant molecules precipitated with anti-idiotypic serum. Hybrids with chains from polyclonal IgG were not precipitable with such antisera. These results indicate that the variable region of the heavy chains of these M-components of three different immunoglobulin classes are closely similar, if not identical.

Much of the knowledge about immunoglobulin structure has developed from the study of patients with monoclonal dysproteinemia. Monoclonal immunoglobulins usually occur alone, but a few patients have been studied who had two monoclonal proteins (1–8). Such studies have, by use of antisera against idiotypic determinants, permitted examination of the relationship between variable and constant regions of different immunoglobulin peptide chains.

We had the rare opportunity to study a patient who had three monoclonal immunoglobulins of different classes in his serum. We isolated each of the M-components and their heavy and light chains, raised idiotyp-specific antisera, and found immunochimcal evidence for the similarity, if not identity, between the variable regions of the heavy chains from all three M-components.

MATERIALS AND METHODS

Case History. An 86-year old man (G.Å.) was admitted to Malmö General Hospital in October 1970 after the detection of a lung tumor. As plasma protein analysis showed an IgA, IgG, and an IgM M-component, a diagnosis of lymphoma was made and treatment with cyclophosphamide was instituted. During the next year plasma IgG increased from 20 to 35 g/liter and IgM from 43 to 60 g/liter, whereas plasma IgA remained constant at 4 g/liter. At post-mortem in December 1971 the tumor was found to be composed of a mixture of lymphocytes, reticulum cells, and plasma cells.

Immunochemical procedures

Antisera. Antisera against γ-, α-, μ-, κ-, and λ-chains were raised in rabbits by subcutaneous injections of suitable antigens emulsified in Freund’s complete adjuvant. The antisera were rendered class- and type-specific by appropriate absorptions.

Classification of the M-Components. Classical microimmunoelectrophoresis according to Scheidegger (9) and crossed immunoelectrophoresis according to Ganrot (10) were used to demonstrate the presence of one IgG(x), one IgM(x), and one IgA(x) M-component in the serum of the patient. The subclass of the IgG M-component was determined by papain digestion as earlier described (11). The IgA subclass was determined by use of commercial subclass-specific antisera (Nordic Pharmaceuticals, Tilbury, Holland).

Immunochemical Quantiation. Single radial immunodiffusion was used (12). In the quantitation of IgA and IgM the samples (and the standards) were reduced for 1 hr at room temperature by mixing one part of the sample with one part of 0.2 M mercaptoethanol in 0.075 M barbitral buffer, pH 8.6, before the samples were diluted and applied to the gel.

Insolubilization of Antigens. Normal human serum and several M-component-containing sera were insolubilized by the glutaraldehyde procedure of Avrameas and Ternynck (13).

Immunosorents. The globulin fractions of rabbit antisera against either human γ- or μ-chains were covalently attached to agarose beads by a modification (14) of the cyanogen bromide procedure of Porath et al. (15).

Purification of M-components

IgM M-Component. Euglobulin precipitate from G.Å. serum was dissolved in 0.1 M, pH 5.2 sodium acetate buffer containing 0.8 M NaCl. The protein was re-precipitated by dialysis in distilled water, again dissolved in barbitral buffer, and purified by preparative agarose gel electrophoresis (16).

IgG M-Component. The supernatant from the first euglobulin precipitation was dialyzed in barbitral buffer and the IgG M-component was purified by preparative electrophoresis.

IgA M-Component. IgA-containing fractions from the last-mentioned preparative electrophoresis were selected, pooled, and passed through anti-IgG and anti-IgM immunosorbent columns.

The three M-component preparations appeared to be free of contaminating proteins when solutions of approximately 1% (w/v) were analyzed by electrophoresis in agarose gel (16). Similarly, single radial immunodiffusion with class-specific antisera revealed no contamination. The κ/λ ratio of the IgA preparation was used to assess contamination of the monoclonal component with polyclonal immunoglobulin. The ratio was estimated at 50/1 by single radial immunodiffusion. Fig. 1 shows an agarose gel electrophoresis of the native serum and of the purified M-components.
dissolved in Three milligrams spontaneous areas Freund’s complete adjuvant approximately injections were and IgA M-components denoted by M, G, and A.

Production of antiserum against idiosyncratic specificities
Three milligrams of the purified G.A. IgG M-component dissolved in 3 ml 0.15 M NaCl was emulsified with 3 ml of Freund’s complete adjuvant and injected into several subcutaneous areas and into the footpads of two rabbits. Booster injections were given several times at intervals of approximately 2 weeks. Sera from several bleedings of the rabbits were pooled and absorbed with excessive amounts of insolubilized human normal serum and with large quantities of insolubilized human serum containing an IgG1(κ) M-component. The specificity of the extensively absorbed antiserum was tested by both single and double radial immunodiffusion. No precipitation was observed with multiple dilutions of pooled normal human plasma, pooled normal human IgG, or 18 different human sera that contained IgG, IgM, or IgA M-components. Precipitation did occur when the purified G.A. IgG M-component was used as antigen.

A rabbit antiserum against idiosyncratic specificities of the G.A. IgM M-component was raised and tested by completely analogous procedures.

Preparation of heavy and light chains
Polypeptide chains from the IgM, IgG, and IgA M-components of G.A., from an unrelated M-component, and from polyclonal IgG were prepared by reduction with 0.5 M mercaptoethanol at pH 8 for 1 hr, alkylation at the same pH with 0.55 M iodoacetamide, and subsequent separation by gel filtration on Sephadex G-200 in 0.1 M formic acid. The chain preparations were dialyzed in distilled water or neutral buffers and their purity was checked by single and double radial immunodiffusion and immunoelectrophoresis. The three light chains had similar electrophoretic properties.

Recombination of chains
Mixtures of light and heavy chains (molar ratio from A280, 1.5:1) in 0.1 M HCOOH were usually dialyzed against Tris-acetate-buffered NaCl as described earlier (17); in some experiments, chains in distilled water were mixed and the mixtures were used for immunodiffusion experiments; in other experiments chains were applied to adjacent wells in agarose and allowed to diffuse into, and recombine in, the gel.

RESULTS

Idiosyncratic specificities of the native G.A. IgM, IgG, and IgA M-components
The extensively absorbed antiserum against the IgM and IgG M-components of G.A. still precipitated all three of the paraproteins from this patient but did not precipitate with any of 18 unrelated IgM, IgG, or IgA M-components or normal polyclonal immunoglobulin. The three G.A. M-components gave a reaction of identity in Ouchterlony experiments with either of the two anti-idiosyncratic sera (Fig. 2).

The pattern of identity was not due to contamination of the three solutions of purified G.A. M-components with small amounts of the other two M-components since the idiotypic precipitin lines did not change on excess addition to
the purified antigen solutions of antisera against the heavy chains of the possible contaminants.

**Idiotype specificities of the polypeptide chains of the three G.A. M-components**

Idiotypic determinants were studied with the anti-G.A. IgG idiotypic serum. Purified heavy and light chains from the three M-components did not give precipitin lines on single and double radial immunodiffusion. When, however, the heavy (or light) chains from one of the M-components were allowed to recombine with the light (or heavy) chains from itself or the two other M-components, the hybrid molecules always gave precipitin lines with the anti-idiotypic serum on immunodiffusion as well as on immunoelectrophoresis. All the hybrid molecules containing one chain (heavy or light) from the IgG M-component were tested for cross reaction with the native pure IgG M-component in Ouchterlony experiments: a reaction of idiotypic identity was always obtained (Fig. 3).

Idiotype precipitins were never noted when hybrids containing one chain from a G.A. M-component and the other chain from polyclonal IgG or from an unrelated IgG1 M-component were used. This negative result could not be due to an inability of the G.A. polypeptide chains to form heterologous hybrids since hybrid formation was regularly observed when the chain mixtures were tested with heavy and light chain antiserum in immunoelectrophoresis experiments.

Although the preparations of free heavy and light chains from the three M-components did not precipitate with the anti-idiotypic serum, all six preparations inhibited the idiotype precipitation of the purified complete IgG M-component. This was demonstrated by gel immunodiffusion techniques as well as by absorption-in-tube experiments.

**DISCUSSION**

The three M-components (IgA1k, IgG1k, and IgMk) of the G.A.-serum gave a reaction of identity when tested by double radial immunodiffusion with the use of extensively absorbed antisera raised against the purified IgM or IgG M-components. The three M-components therefore shared idiotype determinants. Absorption and inhibition experiments with the isolated heavy and light chains from the three M-components demonstrated that all the polypeptide chains could inhibit the precipitation by anti-idiotypic antiserum of the tested native IgG M-component. All heavy chains, therefore, probably contain at least one common idiotypic determinant. The corresponding is probably true also for the light chains. These conclusions were further corroborated by the recombination experiments which showed that any of the isolated heavy chains could give hybrid molecules with any of the isolated light chains and that these hybrid molecules gave reactions of identity with the tested native IgG M-component when anti-idiotypic serum was used. Since idiotypic determinants are present in the variable part of heavy (and light) immunoglobulin chains, the findings related above imply extensive structural similarities between the variable portions of the \( \gamma_1 \), \( \mu \), and \( \alpha_1 \)-chains of the G.A. M-components. The antigenic identity of the variable portions of the three heavy chains may very well signify identical primary structure of these portions, since such a relation between identical idiotypic antibodies and primary structures has been demonstrated earlier in a serum containing two M-components of different classes (4, 6).

The light chains of the three M-components were all of the \( \kappa \)-type, had common idiotypic determinants, and were found to have similar electrophoretic mobility. They are therefore similar if not identical in both their constant and variable \( (V) \) portion.

Thus, if identical idiotypic determinants mean identical V-regions of immunoglobulin polypeptide chains, the \( \gamma_1 \), \( \alpha_1 \), and \( \mu \)-chains of the three M-components in the G.A.-serum have identical V-regions. Earlier immunochemical and chemical studies of sera containing two M-components have revealed some cases where two heavy chains with different C-regions have had identical \( V \)-regions (2-4, 6-8) but evidence for the presence of one and the same \( V \)-region in three heavy chains of different classes has to our knowledge never been reported before. There are two earlier reports in the literature of the presence of three M-components of different classes in one serum but in one case (18) only two of the M-components shared idiotypic determinants and in the other no studies of the \( V \)-regions of the M-components were performed (19). The present case of a \( V \)-region being shared by an \( \alpha_1 \)-, a \( \gamma_1 \)-, and a \( \mu \)-chain gives further strong support to the two-gene-one-polypeptide chain theory of immunoglobulin synthesis originally proposed by Dreyer and Bennett (20).

Two hypotheses have been proposed about the cellular events which might lead to the appearance of two or more M-components of different classes but with identical \( V \)-regions (5). One states that cells which normally produce immunoglobulin of two, or more, classes with the same \( V \)-regions may commence rapid proliferation. The alternate proposal is that rapidly proliferating cells producing immunoglobulin of one class undergo a "genetic event" resulting in the emergence of a subclone that produces immunoglobulin of another class but with the same \( V \)-region as the original immunoglobulin. Fu et al. (21) recently presented strong evidence for the occurrence of human lymphocytes simultaneously producing two immunoglobulins of different classes.
but with identical V-regions. Gearhart and associates (22) have also recently demonstrated that a single stimulated murine B cell may give rise to a progeny producing monoclonal immunoglobulins of C, M, and A classes which all share idio-
typic determinants. The present findings are not incompatible with any of these hypotheses but it should be pointed out that if only the first hypothesis were valid the presence of cells simultaneously producing three classes of immunoglobulin has to be assumed. Furthermore, additional assumptions have to be made to explain the great difference in the concentrations of the three M-components. The association between the tumor cells of the patient and his three plasma paraproteins is not yet elucidated.

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