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Distribution of Allotypic Specificities on the Peptide Chains of Human Gamma-Globulin

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Summary. The peptide chains (A and B) isolated from the 7S γ-globulin of a single donor known to be Gm(a+ b+) and Inv(a+), were tested for the presence of allotypic specificities. Gm factors were present only on the A chain and the Inv factor was found only on the B chain. Ten pathological B chains, of which five were antigenic type I and five type II, were studied; all were negative for Inv(a), but Inv(b) specificity appeared to be associated with B chains of both antigenic types.

INTRODUCTION

During recent years individual variants of immunoglobulins having distinct serological properties have been observed in several species. Oudin (1956) called these variants allotypes. Allotypic forms of human immunoglobulins were originally recognized by differences in the ability of individual γ-globulins to inhibit agglutination of sensitized cells by sera containing substances serologically related to rheumatoid factors (Grubb, 1956); similar substances are found in some normal human sera and in certain patients after multiple transfusions. Since the introduction of this technique several genetically determined types of human immunoglobulins have been described. Of these the Gm factors are determined by genes at one locus, while Inv factors are determined by genes at an independent locus (Steinberg, 1962; Fudenberg, 1963; Ropartz, Lenoir and Rivat, 1961).

The structural basis of allotypic specificity is unknown, but the association of distinct specificities with different types of immunoglobulins and with fragments obtained by papain digestion has been investigated (Fahey and Lawler, 1961; Mårtensson, 1961; Franklin, Fudenberg, Meltzer and Stanworth, 1962; Harboe, Osterland and Kunkel, 1962). The development of a method for isolating the constituent peptide chains of immunoglobulins in biologically active form (Fleischman, Pain and Porter, 1962) provided an opportunity for studying the distribution of Gm and Inv specificities on the chains of normal human 7S γ-globulin; the results of this study were referred to in a preliminary paper (Cohen, 1963a) and are reported in detail below. In addition, data concerning the Inv activity of myeloma B chains belonging to different antigenic types and having distinct electrophoretic mobilities are recorded.

MATERIALS AND METHODS

Gamma-Globulin Preparations

Normal and pathological human 7S γ-globulins were isolated from serum samples by chromatography on diethylaminoethyl cellulose. Pathological 7S and γ_{1A}-globulins were

prepared by zone electrophoresis or by gradient elution from diethylaminoethyl cellulose columns using conditions described by Cohen and Freeman (1960). Pathological macroglobulins were prepared according to the method previously described (Cohen, 1963b). Solutions of γ-globulin were concentrated by pressure dialysis, Seitz filtered and stored at 4°. Cryoglobulins were prepared by centrifuging serum at 4° followed by chromatography of the washed precipitates on Sephadex G-200 at room temperature.

Reduction and Isolation of B Chains

This was carried out according to the method of Fleischman *et al.* (1962) using 0·95 M mercaptoethanol as described by Cohen (1963b). The partially reduced normal γ-globulin was fractionated on a Sephadex G-200 column equilibrated with N acetic acid. Pathological proteins were fractionated on Sephadex G-75 columns in N acetic acid; the second peak (B) was usually concentrated and re-run on Sephadex G-75 to obtain chromatographically homogeneous material. Fractions were concentrated by pressure dialysis against several changes of water and then dialysed against 0·9 per cent NaCl. In all instances the yield of B was 24–30 per cent of the reduced protein.

Electrophoresis

Starch gel electrophoresis was carried out in vertical trays using (i) 8 M urea and 0·05 M formic acid, pH 3·5 (Edelman and Poulik, 1961) or (ii) 8 M urea and 0·035 M glycine buffer, pH 8·8 (Cohen and Porter, 1964a).

Gel Diffusion

Double diffusion in agar was carried out as described by Ouchterlony (1953). Myeloma proteins were classified as 7S or γ_{1A}-globulins by using antisera absorbed so that they reacted specifically with each type of protein; myeloma B chains were classified into antigenic types I and II using antisera specific for each type.

Assay of Gm and Inv Activity

The presence of Gm and Inv factors was determined by an inhibition test using: (a) human group O Rhesus positive red cells; (b) human Rhesus incomplete antibody usually with anti-D specificity; (c) human sera capable of agglutinating (a) coated with (b).

The sera used for agglutination (Table 1) in the Gm(a) (J.M.), Inv(a) (Vir) and Inv(b) (L.) tests were from normal donors; the serum used for Gm(b) (Bomb.) was from a patient with rheumatoid arthritis. Proteins which carry Gm or Inv specificity will inhibit the corresponding agglutinator. Since the reaction is reversible, agglutinates formed by addition of the agglutinator can be dispersed by protein carrying the appropriate factor. This method has previously been referred to as disagglutination and was used for Gm(a), Gm(b), and Inv(a) typing (Lawler, 1960); in this test the presence of allotypic factors on proteins tested is revealed by absence of agglutination (Table 1). The method used for Inv(b) typing was that suggested by Wilson (1962, personal communication).

RESULTS

NORMAL 7S γ-GLOBULIN

The isolation of chains from reduced γ-globulin prepared from a donor known to be Gm(a+ b+) Inv(a+) was carried out on Sephadex G-200 to ensure that the A chain

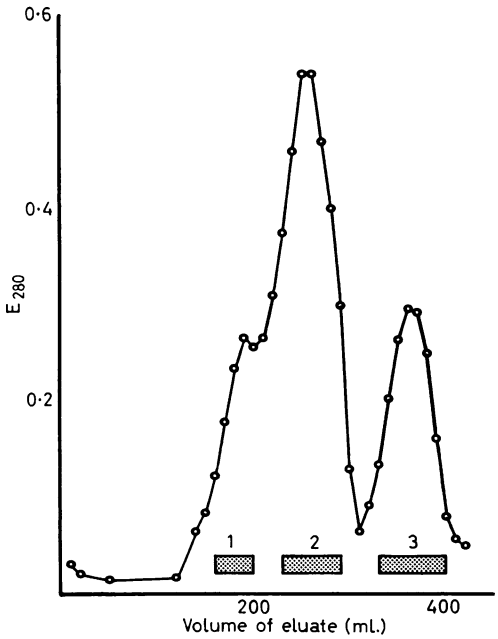

FIG. 1. Fractionation of reduced human 7S γ-globulin on a column (3·5 × 100 cm.) of Sephadex G-200 in N acetic acid at 2°. Starch gel electrophoresis of fractions 1, 2 and 3 is shown in Fig. 2.

was obtained free of unreduced γ-globulin. In fact, the two leading peaks eluted from the column (Fig. 1, fractions 1 and 2), which presumably differed in their degree of association in N acetic acid, appeared similar when examined by electrophoresis on urea formic starch gels; both fractions consisted of A chain, appeared to be free of whole γ-globulin

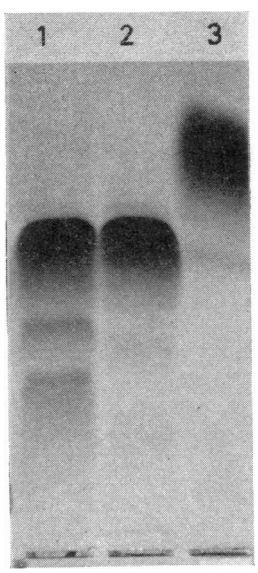

FIG. 2. Electrophoresis in 8 M urea-formic acid starch gel of fractions of reduced human 7S γ-globulin separated on Sephadex G-200 in N acetic acid. Fractions 1, 2 and 3 correspond to the three peaks shown in Fig. 1.

and contained a trace of diffusely staining material having the mobility of B. Minor electrophoretic components of slower mobility which are always present in preparations of A were relatively more prominent in fraction 1 (Fig. 2). Fractions 1 and 2 were tested for allotypic activity at initial concentrations of 3·5 and 7·5 mg./ml. respectively; the initial serum sample contained about 10 mg./ml. of A chain. When tested in a series of doubling dilutions, both preparations of A chains showed greater Gm(a) activity than the corresponding serum; the Gm(b) activity of both fractions was approximately equal to that of the serum and both were negative for Inv(a) activity (Table 1).

The B chain was obtained in a yield of 27 per cent and contained a trace of material with the mobility of A on urea formic starch gel electrophoresis (Fig. 2). B was tested at

TABLE 1

THE Gm AND Inv ACTIVITY OF THE A AND B CHAINS OF HUMAN 7S γ-GLOBULIN

Gm(a)

	Dilution										
	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
Whole serum	−	−	−	−	−	+	+++	++++	++++	++++	++++
1 = A	−	−	−	−	−	−	−	−	−	−	++++
2 = A	−	−	−	−	−	−	−	−	−	−	++++
3 = B	+	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++

Cell sensitization: anti-D (1386) 0·1 ml.; 0 (R_1r) cells 0·2 ml.; saline 1·0 ml.
Agglutinator: anti-Gm(a) (J.M.) diluted 1 in 5.

Gm(b)

	Dilution									
	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
Whole serum	−	−	−	−	−	−	+++	+++	++++	++++
1 = A	−	−	−	−	−	+++	++++	++++	++++	++++
2 = A	−	−	−	−	+++	++++	++++	++++	++++	++++
3 = B	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++

Cell sensitization: anti-D (2147) 0·1 ml.; 0 (R_1r) cells 0·1 ml.; saline 1·0 ml.
Agglutinator: anti-Gm(b) (Bomb.) diluted 1 in 10.

Inv(a)

	Dilution							
	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Whole serum	−	−	−	−	+++	+++	+++	+++
1 = A	+++	+++	+++	+++	+++	+++	+++	+++
2 = A	+++	+++	+++	+++	+++	+++	+++	+++
3 = B	−	−	+	+++	+++	+++	+++	+++

Cell sensitization: anti-D (3) 5 parts; 0 (R_1r) cells 1 part; saline 5 parts.
Agglutinator: anti-Inv(a) (Vir) undiluted.
+ Indicates degree of agglutination.
In all tests the protein concentrations of undiluted fractions 1, 2 and 3 were 3·5, 7·5 and 5·0 mg./ml. respectively.
1 = A, 2 = A, and 3 = B refers to fractions 1, 2 and 3 respectively shown in Figs. 1 and 2.

an initial concentration of 5 mg./ml. (approximately equimolar with serum). Neither Gm(a) nor Gm(b) specificity was detectable; Inv(a) activity was present at somewhat lower titre than that of the original serum (Table 1).

PATHOLOGICAL B CHAINS

B chains were isolated from seven 7S myeloma proteins, from two macroglobulins and from one γ_{1A}-myeloma protein; five were antigenic type I and five were type II. The normal B chain can be resolved into ten subfractions by electrophoresis in urea glycine starch gels, pH 7–8; these subfractions have been numbered B_1 to B_{10} from the cathodal end of the gel (Cohen and Porter, 1964a). B chains from pathological γ-globulins, on the other hand, consist of one or two components which usually have mobilities corresponding to one or other of the ten subfractions of normal human B chains. The pathological B chains were tested for Inv(a) and (b) activity. None carried Inv(a) specificity. Three were Inv(b) positive and of these one was antigenic type II with mobilities 4, 5, while two were antigenic type I with mobilities 4, 5 and 5, 6, respectively. Owing to shortage of reagents it was not possible to test the original myeloma proteins for Inv(b) activity; eight out of ten sera available for testing were Inv(a) negative.

DISCUSSION

Studies on the papain fragments of human γ-globulin have shown that Gm specificity is confined to the F fragment while the S fragment carries Inv determinants (Franklin *et al.*, 1962; Harboe, Osterland and Kunkel, 1962). Fleischman *et al.* (1962) showed that papain piece III of rabbit γ-globulin, which is equivalent to human F, consists only of A chain. These findings suggested that Gm determinants must be carried by A and Inv specificity by B chains. This distribution has been confirmed in the present study. Gm activity is found only on 7S γ-globulin (Fahey and Lawler, 1961; Franklin *et al.*, 1962; Harboe, Osterland, Mannik and Kunkel, 1962), is present on the F fragment and, according to the model proposed by Porter (1962), must therefore be located on the C-terminal portion of the human 7S A chain (Fig. 3).

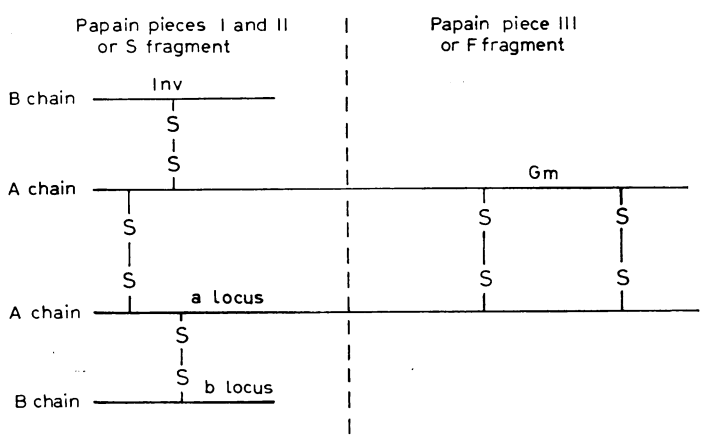

FIG. 3. Diagrammatic configuration of 7S γ-globulin (Porter, 1962) showing probable location of human Gm and Inv specificities (above) and rabbit allotypes determined by a and b loci (below).

Inv specificity is confined to the B chain, but its distribution on the two distinct antigenic types and the ten electrophoretic subfractions of normal B has not been established. Studies on Bence-Jones proteins, which appear to be composed of B chains (Edelman and Gally, 1962; Schwartz and Edelman, 1963) have previously shown that Inv specificity is frequently present on proteins of type I, but is rarely found on type II Bence-Jones proteins (Franklin *et al.*, 1962; Harboe, Osterland, Mannik and Kunkel, 1962). However, Harboe, Osterland, Mannik and Kunkel (1962) found that Inv factors occur in both group I and group II myeloma proteins. The present results also indicate that myeloma B chains of both antigenic types may be associated with Inv activity; however, there is less certainty about the results obtained for Inv(b) than for the other three factors. It is surprising that seven out of the ten myeloma B chains tested were Inv(b) negative since this factor is present in 99 per cent of Caucasians. This finding, together with the diminished reactivity of normal B compared to the original serum (Table 1) suggests that Inv specificity may be reduced during separation and isolation of the polypeptide chains.

B chains are associated with those antigenic determinants which are common to all human immunoglobulins, while A chains carry determinants specific for each type (Cohen, 1963a, b; Fahey, 1963). In addition, the B chains of normal 7S and 19S γ-globulins appear identical in urea–formic acid starch gels, pH 3·5, while A chains of normal 7S, 19S and γ_{1A}-globulins have different mobilities (Cohen, 1963a; Carbonara and Heremans, 1963). The electrophoretic identity of B chains is even more striking in urea glycine gels at neutral pH (Cohen and Porter, 1964a). Amino acid analysis of B chains from normal 7S and 19S γ-globulins are closely similar, while their A chains differ considerably in chemical composition (Chaplin, Cohen and Press, 1964). All these findings indicate that each type of human immunoglobulin has a distinct A chain and that all have B chains of closely similar or identical structure. The fact that Gm specificity, which is confined to 7S γ-globulin, is present on the A chain, while the common allotypic specificity, Inv, is present on B, adds further support to this view.

Since immunoglobulins of all species investigated appear to have a fundamentally similar structure, it is of interest to compare the distribution of the allotypes of human γ-globulin with those of the rabbit. The rabbit specificities are determined by two genetic loci, a and b (Dray, Dubiski, Kelus, Lennox and Oudin, 1962). Unlike the human, both allotypic specificities are present on 19S γ-globulin as well as on 7S γ-globulin (Todd, 1963) and are probably also present on γ_{1A}-globulin (Feinstein, 1963); in addition, after papain digestion of rabbit 7S γ-globulin, the allotypes determined by both genetic loci are found on pieces I and II (Kelus, Marrack and Richards, 1960; Feinstein, Gell and Kelus, 1963). The isolated rabbit B chain carries only those allotypic specificities determined by the b locus (Kelus, 1963; Feinstein *et al.*, 1963; Stemke, 1964). Specificities determined by the a locus occur on the A chain and, since they are also present on papain pieces I and II, they must be localized on the N-terminal portion (A piece) of the A chain (Fig. 3). These findings suggest that both the B chain and the A piece may be common to all γ-globulins and that type specificity may be confined to the C terminal half of the A chain. This raises the question of whether there are, in fact, three different peptide chains in the immunoglobulin molecule (see Cohen and Porter, 1964b). Studies in the rabbit have shown that the A chain which carries specificities determined by the a locus is also consistently associated with factors determined by the b locus; this suggests that rabbit A, unlike that of the human, cannot easily be completely dissociated from the B chain (Kelus, 1963; Feinstein *et al.*, 1963; Stemke, 1964).

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