THE ASSOCIATION OF HEREDITARY GAMMA GLOBULIN (a) ACTIVITY WITH A FRAGMENT OF HUMAN GAMMA GLOBULIN PRODUCED BY PAPAIN DIGESTION

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The Gm system of genetically determined differences between normal human gamma globulins was first described by Grubb (10). These differences may be delineated by the capacity of some normal gamma globulins to inhibit a test system in which tannic acid-treated sheep erythrocytes, coated with normal 7S human gamma globulin heated to 56°C., are agglutinated by selected sera from patients with peripheral rheumatoid arthritis (2). Macroglobulin rheumatoid factors in these sera act as anti-Gm agglutinating substances. Seven different Gm determinants have been described to date (8), of which only the Gm (a)-anti-Gm (a) system is discussed in the present study.

Gamma globulin may be digested by the enzyme papain to yield three fragments (11,6). In the present study, papain hydrolysis was applied to 7S human gamma globulin isolated from the sera of three Gm (a+) and two Gm (a-) persons in an effort to find a fraction of the intact molecule that retains the genetically determined specificity.

MATERIALS AND METHODS

1. Gamma globulin. Isolation of human 7S gamma globulin from the sera of donors of previously determined Gm (a) specificity was accomplished by gel filtration, using polymerized dextran followed by anion and cation exchange column chromatography (4). Studies to determine whether the Gm (a+) individuals were homozygous or heterozygous for this trait were not performed.

2. Enzymatic digestion. The method of Porter (11) was followed, using mercuri-papain (Worthington Biochemical, Lot No. 6102). Digestions were carried out for 12 to 17 hours at 37°C.

3. Isolation of digestion fractions. Diethylaminoethyl cellulose and carboxymethyl cellulose were employed. Conditions of chromatographic separation were exactly those of Franklin (6).

4. Serological studies. Tannic acid-treated sheep erythrocytes were coated with human 7S gamma globulin isolated from single donors. When gamma globulin N-4760 is used as the cell coat and rheumatoid serum R-4501 as the agglutinator, the test system has been demonstrated to be specific for Gm (a) (3); that is, sera typed as Gm (a+) cause inhibition to dilutions as great as 1:40,000, whereas those typed as Gm (a-) fail to inhibit this system above dilutions of 1:100.

Inhibition studies were performed by serial dilution of 0.5 ml volumes of equal protein concentrations of intact gamma globulin and the fractions obtained by digestion. A 1:56 dilution of serum R-4501 was added to each tube. After the mixture had been incubated at 37°C. for one hour, 0.5 ml of a 0.25% suspension of cells coated with gamma globulin N-4760 was added to each tube. Tubes were read by the pattern of settled cells after 16 hours at 4°C.

5. Ultracentrifugal analysis. Samples were analyzed at a single protein concentration in a Spinco Model E analytic ultracentrifuge at 56,100 rpm with schleiren optics. Uncorrected sedimentation values were calculated.

6. Immunoelectrophoresis. A microtechnique using 2% agar on 75 mm x 25 mm glass slides was employed. Precipitin bands were developed with antisera from rabbits immunized with commercial Cohn fraction II.

RESULTS

The papain digestion mixture was fractionated into peaks A, B, C and D by a combination of cation and anion column chromatography. Ultracentrifugation of peak A revealed a 3.6S major component and approximately 20% of 6.6

to 6.8S material. Rechromatography of this peak on carboxymethyl cellulose at pH 5.3, 0.01 M phosphate, utilizing constant volume gradient elution with increasing ionic strength to 0.3 M NaCl at constant pH, allowed recovery of an early fraction that contained only the 3.6S component. This fraction will subsequently be referred to as fraction A. Fractions B and C were entirely 3.2 to 3.6S and no heavier material was observed.

Immunoelectrophoretic patterns of fractions A, B and C were essentially the same as those described by Franklin, with the B fragment migrating more rapidly toward the anode than fractions A or C (6). No contamination of the A or C fragments with B, or vice versa, was detected by immunoelectrophoresis.

The results of hemagglutination inhibition studies of the intact 7S gamma globulins and the fragments obtained by papain hydrolysis are shown in Table I. All solutions tested for inhibitory capacity were adjusted to an initial protein concentration of 0.1 mg of protein per ml as measured at 280 mμ in a Beckman DU spectrophotometer. Results similar to those shown for gamma globulin N-4760 were obtained with two other Gm (a+) gamma globulins. One other Gm (a-) globulin yielded similar results to those obtained for N-4757.

As shown in Table I, intact gamma globulin from serum N-4760 (Gm (a+)) had an inhibitory titer greater than $\log_2 = 8$. Papain fraction B of this serum had an inhibitory titer of $\log_2 = 7$, while fractions A and C caused no inhibition, even when tested at an initial concentration of 1.0 mg of protein per ml of solution.

Neither the intact gamma globulin nor any of the papain fractions of serum N-4757 (Gm (a-)) had an inhibitory titer of more than $\log_2 = 1$. Inhibitory activity did not increase when the fractions were tested at a concentration ten times that employed above.

Inhibition was not demonstrated by these gamma globulins or their papain fractions when tested in a system of specificity other than Gm (a)-anti-Gm (a).

Table I
Results of Hemmaglutination Inhibition Studies of
Intact 7S Gamma Globulins and Fragments

Gm Test System		
	(Cell coating:	γN-4760 (Gm (a+))
	(Agglutinating serum:	R-4501 (1:56) (anti-Gm (a))
	(Control:	2+ agglutination

Source of Inhibiting Material	Inhibition Test Pattern*							
	Log_2 Dilution of Inhibiting Material							
	1	2	3	4	5	6	7	8
γN-4760 (Gm (a+))								
Intact γ	0	0	0	0	0	0	0	0
Fraction A	2	2	2	2	2	2	2	2
Fraction B	0	0	0	0	0	0	0	2
Fraction C	2	2	2	2	2	2	2	2
γN-4757 (Gm (a-))								
Intact γ	2	2	2	2	2	2	2	2
Fraction A	1	2	2	2	2	2	2	2
Fraction B	1	2	2	2	2	2	2	2
Fraction C	1	2	2	2	2	2	2	2

*0 = no agglutination; 2 = complete agglutination

DISCUSSION

Fahey and Lawler (5) and Fudenberg (9) have shown that Gm (a) specificity is characteristic only of 7S gamma globulins. The work presented here demonstrates that Gm (a) determinants of human 7S gamma globulin are confined to a fragment of the molecule. Similar observations have been made using the sensitized D cell system (7,8). This fragment is the B fraction of Franklin and probably is identical to Porter's fraction III (11) and Edelman's F component (1). It is characterized by rapid electrophoretic mobility, an S value of about 3.5, and a molecular weight of between 40,000 and 55,000, which is approximately one-third the weight of the intact molecule (6).

The possibility that an even smaller molecular fragment having genetically determined structural specificity may be present in fraction B is under investigation.

SUMMARY

The technique of enzymatic hydrolysis has been utilized to demonstrate that for the five sera investigated the determinant of Gm (a) specificity is confined to a 3.5S fragment of the human 7S gamma globulin molecule.

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