STUDIES ON HUMAN ANTIBODIES

II. Distribution of Genetic Factors*

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Recent investigations have revealed a variety of types of heterogeneity for human γ-globulin (1). In addition to the three immunoglobulin classes (7S γ, 19S γ, β_{2A}), it may be further divided by the presence of group I and group II molecules (2, 3) and by the presence or absence of various genetic factors controlled by alleles at two genetic loci (the Gm and Inv loci) (4).

Studies of isolated human antibodies against single antigens have revealed that they may be more homogeneous than the whole γ-globulin of their donor in their electrophoretic mobility (5, 6) and in the electrophoretic mobility of their isolated light chains (7). Recent studies have also shown that isolated antibodies may vary in the ratio of group I and group II molecules (8) and may have individual antigenic specificity (6). Thus, isolated human antibodies approach the homogeneity of myeloma proteins in many ways. In view of the selective distribution of genetic (Gm and Inv) factors in myeloma proteins (9), the present study was undertaken to explore the distribution of genetic factors in isolated human antibodies. The findings indicate that many such antibodies are qualitatively or quantitatively limited in their content of genetic factors, compared with the whole γ-globulin of the donor.

Materials and Methods

Antisera.—Human antisera were obtained from fourteen individuals previously immunized against dextran (10, 11) and from two individuals immunized with porcine blood group A substance (To. and Wh.). Antisera were also obtained from three patients with systemic lupus erythematosus, one for antinuclear antibody (Th.) and two (Lu. and An.) for antibody against teichoic acid isolated from *Staphylococcus aureus* (12–14). Antibody against teichoic acid was also obtained from one patient with rheumatoid arthritis (Fr.), from two normal individuals who had been immunized with teichoic acid (Bo. and Da.), and from several of those immunized against dextran. Serum was obtained from one individual (subject 1) who had been immunized with porcine blood group A substance, alum-precipitated tetanus toxoid, levan, and dextran (11); this serum also contained antibody against teichoic acid.

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Isolation and Preparation of Antibody.—Appropriate sera were cleared by ultracentrifugation and absorbed for 1 to 7 days with dextran, levan, teichoic acid, or alum-precipitated tetanus toxoid in ratios at or near the points of maximal precipitation. When more than one antibody was harvested from the same serum, sequential absorptions were used. The resulting precipitates were separated by centrifugation and washed six times in cold phosphate buffer at pH 7.4 and ionic strength 0.15. Antinuclear antibody from patient Th. was absorbed onto calf thymus nuclei (15) and separated from the serum by centrifugation and washing. Teichoic acid antibody in serum from donors 1, 173, 98, 205, and Fo. was precipitated with teichoic acid supplied by Dr. J. L. Strominger, and that in the remaining sera was precipitated with teichoic acid supplied by Dr. S. Morse.

Washed antigen-antibody precipitates of levan, blood group A substance, and teichoic acid were dissolved by addition of excess of the respective antigens. Dextran-immune precipitates were treated with dextranase by the method previously described (16). Teichoic acid precipitates from some patients were digested with papain at pH 7.4 after the method of Porter (17), as was the tetanus toxoid immune precipitate from 1. As a control for the effect of papain treatment of these antibodies on genetic typing, four dextran antibody solutions (176, 49, Ca., 20), anti-A substance Wh., and the teichoic acid immune precipitate from patient An. were digested with papain by this method. In each instance results of Gm genetic typing were identical with those found with the undigested material. Inv(a) typing was not performed on papain-digested antibodies, as it was not clear what effect this treatment had on dissolving the antibody S fragments known to contain both the Inv determinant and the antibody combining site (18, 19). Antiblood group A immune precipitate from subject 1 was dissolved in excess porcine blood group A substance, while antibody from the anti-A immune precipitate of To. and Wh. was eluted with pH 3.8 acetate buffer as previously described (6). Antibody from Th., absorbed on calf thymus nuclei, was eluted by treatment with deoxyribonuclease as described by Holman and Deicher (20).

All antibody solutions were dialysed to a pH of 7 against a phosphate buffer of that pH and 0.1 ionic strength prior to genetic typing. Protein concentrations were determined by the Folin-Ciocalteu method (21) and concentrated where necessary to at least 0.5 mg/ml by membrane filtration at 4°C under pressure. Normal individual γ-globulin of known phenotype was isolated by zone electrophoresis on starch (22) for comparison titrations.

Analysis of Immunoglobulin Composition.—Quantities of 26 of the 33 antibodies studied were adequate to allow semiquantitative estimation of relative proportions of 7S γ-, 19S γ-, and β_{2A}-immunoglobulin classes. This group included 10 of the 13 antibodies found deficient in both Gm factors, and included representatives of all specificities studied. Serial doubling dilutions of the antibody solution were reacted with rabbit antisera specific for 19S or β_{2A}-globulins in a double diffusion in agar system as previously described (8). Comparison titrations with these antisera were made against immunoglobulin solutions of known concentration under the same conditions. The presence of 7S immunoglobulin or its fragments was verified by agar diffusion against a rabbit antiserum specific for the fast fragment of papain-digested human 7S γ-globulin.

Gm and Inv Typing.—Gm(a) and Gm(b) typing was performed by a slide technique, and Inv(a) by a tube technique, in the inhibition of agglutination procedure as previously reported from this laboratory (9). All preparations were tested at a protein concentration of at least 0.5 mg/ml. Appropriate controls for non-specific agglutination of the coated cells by the antibody solutions were run with each determination. Similarly, control tests showed that none of the antigens used in this study inhibited any of the test systems at appropriate concentrations. Quantitation of inhibiting capacity was performed in simultaneous tests on serial doubling dilutions of those antibodies which were found to inhibit the test system in the undiluted state. The degree of agglutination was visually graded unmagnified in the Inv(a) tube test, and at $\times 45$ with a dissecting microscope for the Gm slide tests. Genetic typing of the whole

γ-globulin of each donor was performed on whole serum diluted 1:10 and 1:20 with normal saline.

Fresh group O, Rh_0 red blood cells from one of two donors were used throughout these experiments. The agglutinators were all from non-rheumatoid donors and were specific only for the factor tested. Specificity of each agglutinator anti-D system was established by typing 20 normal sera of known phenotype. Coating of red cells with the incomplete anti-D sera was performed in a standard manner and gave reproducible Coombs titers.

The specific reagent systems and the dilutions at which they were used in this study are listed below.

Factor	Agglutinator	Anti-D
Gm(a)	Smejsa 1/30	2368 1/5
Gm(b)	A. Berg 1/20	Black 1/5
Inv(a)	LeClerc 1/20	Roehm 1/5

RESULTS

Results of inhibition titrations on the isolated antibodies are presented in Tables I, II, and III for the Gm(a), Gm(b), and Inv(a) factors respectively. Wh. anti-A inhibited the Gm(a) system through a protein concentration of 0.03 mg/ml and both Wh. anti-A and Th. antinuclei inhibited the Gm(b) system through 0.06 mg/ml. These antibodies had inhibiting capacities in these systems one tube higher than that of isolated homozygous whole γ-globulin. Antidextrans from 54, 220, and 1 and antilevan from 1, isolated from Gm(a+) donors, failed to inhibit the Gm(a) system at the highest concentration. Similarly, a number of antibodies isolated from Gm(b+) donors failed to inhibit the Gm(b) system at any concentration tested. Inhibition by other isolated antibodies varied between these extremes. Bo., 1, and Fr. antiteichoic acid antibodies did not totally suppress the Gm(a) and/or Gm(b) system at the highest protein concentration at which they were studied, but they gave patterns of partial inhibition which were different from any pattern seen in titration of antibodies isolated from Gm(a−) or Gm(b−) individuals. In no instance did an antibody isolated from a donor whose γ-globulin lacked the genetic factor being titered show even partial inhibition of the test system.

Fo. antidextran inhibited the Inv(a) system at a protein concentration of 0.12 mg/ml which is one tube lower than that of the Inv(a+) whole γ-globulin. In this system, as in the Gm(a) and Gm(b) systems, a gradation in inhibiting capacity was seen, with antibodies isolated from Inv(a+) donors Fr., 54, and 220 failing to inhibit the Inv(a) system at any concentration tested. It is worth noting that the antiteichoic acid antibodies from Fr. and Fa. were isolated at the same time and under identical conditions. Fa. antiteichoic acid inhibited the Inv(a) test system to 0.5 mg/ml while Fr. antiteichoic acid gave no inhibition at 1 mg/ml.

TABLE I

Inhibition of Gm(a) Typing System by Isolated Antibodies

Donor	Donor γ-globulin phenotype	Antibody	Agglutination of test system at various concentrations of inhibitor (antibody), mg/ml							
			1.0	0.5	0.25	0.12	0.06	0.03	0.015	0
Wi.	Gm(a+)	Whole γ-globulin	0	0	0	0	0	1	2	3
Wh.	"	A substance	—*	0	0	0	0	0	1	
An.	"	Teichoic acid	0	0	0	0	0	1	2	
Ma.	"	Dextran	0	0	0	0	1	2	3	
Th.	"	Nuclei	0	0	0	0	1	2	3	
49	"	Dextran	0	0	0	1	2	3	3	
186	"	"	—	0	0	1	2	3	3	
Fa.	"	Teichoic acid	—	0	0	1	2	3	3	
1	"	Tetanus toxoid	0	0	0	1	2	3	3	
Ca.	"	Dextran	0	0	1	2	3	3	3	
1	"	A substance	0	0	1	2	3	3	3	
30	"	Dextran	0	0	1	2	3	3	3	
Lu.	"	Teichoic acid	0	1	2	3	3	3	3	
Bo.	"	" "	1	2	3	3	3	3	3	
1	"	" "	1	2	3	3	3	3	3	
54	"	Dextran	—	3	3	3	3	3	3	
220	"	"	—	3	3	3	3	3	3	
1	"	"	—	3	3	3	3	3	3	
1	"	Levan	3	3	3	3	3	3	3	
Al.	Gm(a−)	Whole γ-globulin	3	3	3	3	3	3	3	
20	"	Dextran	3	3	3	3	3	3	3	
173	"	Teichoic acid	3	3	3	3	3	3	3	
Da.	"	" "	3	3	3	3	3	3	3	
216	"	Dextran	3	3	3	3	3	3	3	
Fo.	"	"	3	3	3	3	3	3	3	
Fo.	"	Teichoic acid	3	3	3	3	3	3	3	
Fr.	"	" "	3	3	3	3	3	3	3	
98	"	Dextran	3	3	3	3	3	3	3	
98	"	Teichoic acid	3	3	3	3	3	3	3	
205	"	Dextran	3	3	3	3	3	3	3	
205	"	Teichoic acid	—	3	3	3	3	3	3	
To.	"	A substance	3	3	3	3	3	3	3	
176	"	Dextran	3	3	3	3	3	3	3	
176	"	Teichoic acid	3	3	3	3	3	3	3	
201	"	Dextran	—	3	3	3	3	3	3	

* Not available for testing.

The patterns of partial inhibition of the Gm test systems by antiteichoic acid antibodies from Bo., 1, and Fr. probably represent small concentrations of those Gm factors in the antibody solutions. This is supported by the observation that no antibody isolated from an individual whose phenotype was negative

TABLE II

Inhibition of Gm(b) Typing System by Isolated Antibodies

Donor	Donor γ-globulin phenotype	Antibody	Agglutination of test system at various concentrations of inhibitor (antibody), mg/ml							
			1.0	0.5	0.25	0.12	0.06	0.03	0.015	0
Al.	Gm(b+)	Whole γ-globulin	0	0	0	0	1	2	2	2
Th.	"	Nuclei	0	0	0	0	0	1	2	
Wh.	"	A substance	—	0	0	0	0	1	2	
Da.	"	Teichoic acid	0	0	0	0	1	2	2	
1	"	Tetanus toxoid	0	0	0	0	1	2	2	
49	"	Dextran	0	0	0	1	2	2	2	
216	"	"	0	0	0	1	2	2	2	
Fa.	"	Teichoic acid	—	0	0	1	2	2	2	
Fo.	"	Dextran	0	0	0	1	2	2	2	
To.	"	A substance	0	0	0	1	2	2	2	
176	"	Dextran	0	0	0	1	2	2	2	
20	"	"	0	0	1	2	2	2	2	
Fo.	"	Teichoic acid	0	1	2	2	2	2	2	
98	"	Dextran	0	1	2	2	2	2	2	
Bo.	"	Teichoic acid	1	2	2	2	2	2	2	
Fr.	"	" "	1	2	2	2	2	2	2	
An.	"	" "	2	2	2	2	2	2	2	
173	"	" "	2	2	2	2	2	2	2	
Ca.	"	Dextran	2	2	2	2	2	2	2	
54	"	"	—	2	2	2	2	2	2	
1	"	"	—	2	2	2	2	2	2	
1	"	A substance	2	2	2	2	2	2	2	
1	"	Levan	2	2	2	2	2	2	2	
1	"	Teichoic acid	2	2	2	2	2	2	2	
220	"	Dextran	—	2	2	2	2	2	2	
98	"	Teichoic acid	2	2	2	2	2	2	2	
205	"	Dextran	2	2	2	2	2	2	2	
205	"	Teichoic acid	—	2	2	2	2	2	2	
Lu.	"	" "	2	2	2	2	2	2	2	
176	"	" "	2	2	2	2	2	2	2	
201	"	Dextran	—	2	2	2	2	2	2	
Wi.	Gm(b−)	Whole γ-globulin	2	2	2	2	2	2	2	
186	"	Dextran	—	2	2	2	2	2	2	
Ma.	"	"	2	2	2	2	2	2	2	
30	"	"	2	2	2	2	2	2	2	

for a Gm factor showed any inhibition of the test system for that factor. These test systems can detect the Gm or Inv(a) factors if they represent no less than about 5 per cent of the protein concentration at 1 mg/ml or about 10 per cent at 0.5 mg/ml. It appears probable that with a more sensitive technique, certain of the antibodies which gave no detectable inhibition would give evidence of

small amounts of the genetic factors, when obtained from phenotypically positive donors.

Table IV allows comparison between the Gm(a), Gm(b), and Inv(a) phenotype of each donor and the ability of antibodies isolated from them to inhibit the various test systems. Inhibition capacity was graded as (−) for no de-

TABLE III

Inhibition of Inv(a) Typing System by Isolated Antibodies

Donor	Donor γ-globulin phenotype	Antibody	Agglutination of test system at various concentrations of inhibitor (antibody), mg/ml							
			1.0	0.5	0.25	0.12	0.06	0.03	0.015	0
Al.	Inv(a+)	Whole γ-globulin	0	0	0	0	0	1	2	3
Fo.	"	Dextran	0	0	0	0	1	2	3	
Fa.	"	Teichoic acid	—	0	1	2	3	3	3	
98	"	Dextran	0	0	1	2	3	3	3	
Th.	"	Nuclei	0	—	—	—	—	—	—	
Da.	"	Teichoic acid	0	1	2	3	3	3	3	
Fr.	"	" "	3	3	3	3	3	3	3	
54	"	Dextran	—	3	3	3	3	3	3	
220	"	"	—	3	3	3	3	3	3	
Wi	Inv(a−)	Whole γ-globulin	3	3	3	3	3	3	3	
An.	"	Teichoic acid	3	3	3	3	3	3	3	
20	"	Dextran	3	3	3	3	3	3	3	
Bo.	"	Teichoic acid	3	3	3	3	3	3	3	
49	"	Dextran	3	3	3	3	3	3	3	
186	"	"	—	3	3	3	3	3	3	
1	"	"	—	3	3	3	3	3	3	
205	"	"	3	3	3	3	3	3	3	
205	"	Teichoic acid	—	3	3	3	3	3	3	
Lu.	"	" "	3	3	3	3	3	3	3	
Ma.	"	Dextran	3	3	3	3	3	3	3	
30	"	"	3	3	3	3	3	3	3	
176	"	"	3	3	3	3	3	3	3	
201	"	"	3	3	3	3	3	3	3	

tectable inhibition through 5+ for inhibition at a higher dilution than isolated γ-globulin of that phenotype. It is apparent that the presence of detectable quantities of the various genetic factors in isolated antibodies was independent of the antibody specificity. Among the antiteichoic acid antibodies, An. inhibited Gm(a) but not Gm(b), Da. inhibited Gm(b) but not Gm(a) and 1 and 205 inhibited in neither system. In titrations of the Inv(a) factor in antibodies isolated from Inv(a+) donors, Fa. antiteichoic acid and Fo. antidextran inhibited the test system, while Fr. antiteichoic acid and 54 antidextran did not. Among antidextran antibodies, Ca. and Ma. inhibited Gm(a) but not Gm(b),

20 inhibited Gm(b) but not Gm(a), and 1, 220, and 54 inhibited in neither system.

It should be noted that antidextran antibodies from 1, 220, and 54 failed to inhibit any of the genetic typing systems in this study, though serum from two

TABLE IV

Phenotypes of Donor γ-Globulin and Isolated Antibodies

Donor	Antibody	Serum Gm phenotype	Antibody Gm phenotype	Serum Inv(a) phenotype	Antibody Inv(a) phenotype	Content of	
						β_{2A}	19S γ
						per cent	*per cent*
1	Dextran*	$a_{4+}b_{4+}$	a_b_	a_	a_	0	0
	Levan*		"			0	0
	Teichoic acid‡		"			Trace§	5–15
	A substance*		$a_{1+}b_{-}$			0	10–20
	Tetanus toxoid‡		$a_{2+}b_{4+}$			0	0
Wh.	A substance*	"	$a_{5+}b_{5+}$	"		5–15	0
Ca.	Dextran*	"	$a_{1+}b_{-}$	"		0	5–15
49	"	"	$a_{2+}b_{3+}$	"	"	5–15	5–15
An.	Teichoic acid*	"	$a_{4+}b_{-}$	"	"	Trace	Trace
Bo.	" "	"	$a_{1+}b_{1+}$	"	"		
Lu.	" "	"	$a_{1+}b_{-}$	"	"		
220	Dextran*	"	a_b_	a_{4+}	"	5–15	0
Th.	Nuclei‖	"	$a_{3+}b_{5+}$	"	$a_{>1+}$	5–15	0
54	Dextran*	"	a_b_	"	a_	0	15–25
Fa.	Teichoic acid*	"	$a_{2+}b_{3+}$	"	a_{1+}	0	Trace
30	Dextran*	$a_{4+}b_{-}$	$a_{1+}b_{-}$	a_	a_	0	0
Ma.	"	"	$a_{3+}b_{-}$	"	"		
186	"	"	$a_{2+}b_{-}$	"	"	0	0
To.	A substance*	$a_{-}b_{4+}$	$a_{-}b_{3+}$	"		Trace	0
20	Dextran*	"	$a_{-}b_{2+}$	"	"	0	0
216	"	"	$a_{-}b_{3+}$	"		Trace	0
176	"	"	"	"	"	"	0
	Teichoic acid‡		a_b_			0	0
205	Dextran*	"	"	"	"	0	0
	Teichoic acid*		"		"		
201	Dextran*	"	"	"	"	0	0
Fo.	"	"	$a_{-}b_{3+}$	a_{4+}	a_{3+}	Trace	0
	Teichoic acid‡		$a_{-}b_{1+}$				
98	Dextran*	"	"	"	a_{1+}		
	Teichoic acid‡		a_b_			"	0
173	" " ‡	"	"	"		"	0
Fr.	" " *	"	$a_{-}b_{1+}$	"	a_	5–15	Trace
Da.	" " *	"	$a_{-}b_{4+}$	"	a_{1+}		

* Dissolved in excess antigen.

‡ Papain-digested.

§ Less than 5 per cent.

‖ DNAase eluate.

of these individuals contained all three genetic factors, and serum from the other donor had two. These antibodies were isolated by procedures identical with those used to prepare antidextran antibodies found to contain Gm or Inv(a) factors, and all were predominantly 7S γ-globulin by immunoglobulin analysis.

Results of semiquantitative estimation of 19S and β_{2A} content of 26 of the 33 antibodies used in this study are also shown in Table IV. Strong precipitin bands were seen in each instance between the isolated antibody and an antiserum specific for the fast fragment of 7S γ-globulin. In only one instance (antidextran from 49) did the approximate content of 19S and β_{2A} γ-globulin exceed 20 per cent of the total antibody protein. The remaining antibodies were estimated to contain less than 20 per cent 19S or β_{2A} γ-globulin, and a number had no detectable amounts of these immunoglobulins. Several isolated antibodies were excluded from this study because they contained more than 30 per cent 19S and β_{2A} γ-globulin.

DISCUSSION

Studies of isolated myeloma proteins and Waldenström macroglobulins have shown these proteins to be homogeneous in their content of the genetic γ-globulin factors and of the group I and group II determinants. These findings suggested that a clone of γ-globulin–producing cells releases molecules which have only one of two possible allelic genetic factors, and either group I or group II determinants (9, 23). In addition, a significant percentage of clones are thought to produce γ-globulin carrying genetic determinants for which there are as yet no identifying test systems (Gm and Inv negative). Assigning the frequency of various genetic factors among monoclonal γ-globulins would allow a calculation of percentage distribution of the various clones of γ-globulin–producing cells, based on their genetic type. Currently available data, however, are not adequate to allow an accurate appraisal of this distribution for all factors. There is reasonable evidence that over 80 per cent of clones in a Gm(b) homozygous individual produce "Gm negative" 7S γ-globulin (23, 24). In the case of Gm(a) homozygous individuals the information is more limited, but it is probable that up to 40 per cent of the γ-globulin–producing clones in such an individual produce Gm-negative γ-globulin (24).

The number of isolated antibodies from Gm-positive donors which failed to inhibit either the Gm or the Inv(a) system would undoubtedly be reduced by more sensitive techniques. Within the limits of the methods used, however, a higher per cent of the antibodies from Gm(a+) donors clearly inhibited the Gm(a) system than was found for inhibition of the Gm(b) system by antibodies from Gm(b+) individuals. This is in general agreement with the prediction from the myeloma data that a Gm-positive individual has a greater number of Gm(a) than Gm(b) γ-globulin producing clones (25, 9).

Unfortunately, the Inv(b) testing system now available has proven too variable to allow conclusions on the distribution of this factor in isolated antibodies. To offset this deficiency antibodies were obtained from as many Inv(a+) individuals as possible. This factor also showed selective distribution similar to that found with the Gm(a) and Gm(b) factors. Current information on the distribution of the Inv(b) factor among myeloma proteins and in whole γ-globulin of the normal population gives no reason to suspect that the Inv(b) factor would be distributed in a different fashion than the Inv(a) in these antibodies, except perhaps in frequency.

Consideration should be given to the possibility that some of the deficiencies in the genetic factors in the isolated antibodies might be due to alterations in the antibody during the course of preparation, particularly in view of the variety of methods utilized. However, this explanation appears remote, at least for most of the antibodies studied, because for each of the methods utilized positive results were obtained with certain antibodies. In addition, and perhaps of more importance, is that some of the antibodies from Gm(a+b+) sera served as their own controls, giving differential inhibition in the Gm(a) and Gm(b) systems. For example, An. antiteichoic acid strongly inhibited the Gm(a) system but failed to inhibit the Gm(b) system. Antitetanus toxoid from donor 1 on the other hand, inhibited the Gm(b) system to a significantly greater degree than the Gm(a) system.

It is interesting to note that 5 antibodies isolated from a single donor, 1, had a variety of inhibitory patterns. Three of the antibodies (antidextran, antilevan, and antiteichoic acid) failed to inhibit either Gm system, 1 (anti-A substance) inhibited the Gm(a) but not the Gm(b) system, and the 5th antibody (antitetanus toxoid) inhibited both systems, but to a significantly greater degree in the Gm(b) titration.

Since the β_{2A} and 19S classes of immunoglobulins lack Gm determinants (1), it was important to carry out analyses for these immunoglobulins on the various isolated antibodies. Antigenic analysis by semiquantitative techniques indicated definite β_{2A} and 19S material in many instances. The highest concentration was in antidextran from 49, in which as much as 30 per cent of the antibody protein was 19S and β_{2A} γ-globulin. This antibody which showed partial inhibition in both Gm systems might well have been a stronger inhibitor as a pure 7S fraction. The remainder showed less than 20 per cent of these other immunoglobulins which was insufficient to affect the inhibition titers significantly. A number of the antibodies which lacked detectable Gm determinants showed only the presence of 7S γ-globulin (antilevan and antiteichoic acid from 1, antiteichoic acid from 176, and antidextrans from 201 and 205).

Differences between the distribution of genetic factors in whole γ-globulin and in isolated antibodies indicate a relative homogeneity of the antibody γ-globulin. This confirms observations made on the distribution of group I and

group II determinants in isolated antibodies (8). Many antibodies in this study were those utilized in the preceding paper in which the electrophoretic distribution of isolated light chains from human antibodies was studied (7). An effort was made to correlate the banding of L chains with the complement of genetic factors found in the antibodies. Antidextran antibodies tended to show sharp banding of the L chains, and these antibodies also showed deletions of Gm and Inv factors by genetic typing. Anti-A Wh. showed marked heterogeneity of L chains and also had a full complement of Gm genetic factors. In other instances this correlation was not evident.

Previous studies on genetic factors present in incomplete anti-Rh antibodies have indicated that these antibodies may lack factors present in the donor's γ-globulin (26, 27). There had been suggestive evidence from these studies of a high percentage of Gm(b) deficiency in anti-Rh antibodies from Gm(b+) individuals, although such interpretations were possibly compromised by difficulties inherent in the test system. The present observations with other antibodies suggest that similar Gm(b) deficiencies in Rh antibodies would be highly likely.

Gell and Kelus reported the absence of one and possibly two allotypes of γ-globulin in an antihapten antibody isolated from a rabbit (28). Recent studies by Rieder and Oudin (29), also in the rabbit, have demonstrated alterations in the normal ratios of various allotypic globulin factors in isolated antibodies against ovalbumin and DNP–bovine γ-globulin. They noted no complete deletions of genetic factors present in the whole γ-globulin of the donor animal. It has been established that those allotypes of rabbit γ-globulin which are controlled by the same genetic locus lie on different γ-globulin molecules (30–32), which is in line with the human work showing that myeloma proteins contain only one allelic factor even in heterozygous individuals.

The present studies indicate that certain isolated human antibodies give results for the distribution of genetic factors similar to those obtained in studies of isolated human myeloma proteins. The pattern of distribution with isolated antibodies could result from the stimulation of only a few of the potential γ-globulin–producing cell types, either because of the limited antigenic specificity of some of the antigens, or because of the minimal doses used for immunization. Antidextran antibodies which in many instances showed marked genetic deletions were directed against a limited number of determinants (10, 33), while many of the other antibodies probably were directed against a considerably larger number of different antigenic groupings, for example antitetanus toxoid. It seems possible that antibodies to a single antigenic determinant produced in heterozygous individuals might, like the myeloma proteins, show only one or the other allelic factors but never both. On the other hand, the weak antigenic stimulus used to immunize many of these individuals may have influenced the number of cells responding and hence resulted in a de-

ficiency of the Gm and Inv determinants. However, it should be mentioned that some of the antibodies showing deletions were present in high concentrations in the original serum. The exact roles of the heterogeneity of antigenic determinants and the strength and duration of antigenic stimulus in limiting the genetic heterogeneity of antibody protein remain to be evaluated.

SUMMARY

Human antibodies against dextran, teichoic acid, blood group A substance, levan, tetanus toxoid, and nuclei were isolated and analyzed for their content of Gm(a), Gm(b), and Inv(a) γ-globulin genetic factors. The majority of these antibodies contained all the genetic factors determined in the donor's whole γ-globulin, but in many antibodies at very different concentrations. In a few instances specific factors could not be detected despite their presence in the individual's whole γ-globulin. Different antibodies isolated from the serum of the same individual showed different relative concentrations of genetic factors. The distribution of genetic factors seen in certain isolated human antibodies appeared to approach the selective occurrence of these factors in myeloma proteins.

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