STUDIES ON HUMAN ANTIBODIES

VI. Selective Variations in Subgroup Composition and Genetic Markers*

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(Received for publication 5 December 1967)

Previous studies of isolated human antibodies have revealed relative homogeneity when compared with whole γ-globulin of the donor. Such isolated antibodies may be of a single class (γG, γM, or γA) and may show a predominance of a single light chain type (kappa or lambda) (1). They may show selective exclusion or predominance of individual Gm or Inv genetic factors present in donor sera (2), and may show relatively more homogeneity of electrophoretic mobility of isolated chains as compared to whole γ-globulin (3). Human γG-immunoglobulin has been shown to contain four subgroups based on antigenic differences in the heavy polypeptide chains (4–6). Approximate percentages of normal γG and frequency of occurrence of myeloma proteins of each subgroup are: γG1—65%, γG2—23%, γG3—8% (7), and γG4—4%.[1] It has been shown that individual Gm genetic antigens are found only in molecules of a single subgroup (8, 9). With the recent addition of Gm(n) as a genetic marker for the majority of γG2-molecules (10) and Gm(g) as an allele for Gm(b) among γG3-molecules (11), virtually all γG-molecules may be positively identified by heavy chain subgroup and more than 85% by genotype.

Immunization with dextran, levan, and blood group substances has been shown to elicit antibody responses which may persist for many years (12). Previous studies have demonstrated that isolated anti-dextrans are usually heterogeneous as to class of immunoglobulin (2) light chain type (1), and in amino acid composition (13, 14). In addition, heterogeneity of antibody specificity for the type of linkage of the repeating glucosyl units (15–17) and

* Aided by grants from the Public Health Service (AM 09792-03), the National Science Foundation (GB 3675), and the General Research Support Grant of the United States Public Health Service.

[1] The nomenclature used for the heavy chain subgroups of γG-globulins are those recently adopted by a subcommittee of the World Health Organization: γG1 (We or γ_{2b}); γG2 (Ne or γ_{2a}); γG3 (Vi or γ_{2c}); γG4 (Ge or γ_{2d}).

heterogeneity of combining site size, based on variable affinity for oligosaccharides of the isomaltose series, have been demonstrated (18–20).

The subfractionation of anti-dextran based on these variations in specificity (19, 20) offers a possible means of obtaining more homogeneous antibodies. This report summarizes the quantitative characterization of several such antibodies as to heavy chain subgroup, genotype, and light chain type. The findings indicate that some isolated antibodies, or purified subfractions, may be very homogeneous and consist of a single heavy chain subgroup, contain Gm antigens from only one of two alternative alleles, and contain antigens of a single light chain type. They thus closely resemble myeloma proteins.

TABLE I

Type of Dextran Used for Immunization and Glycosidic Specificity of Human Anti-Dextrans Studied (15–17)

Subject	Dextran used for immunization	Specificities for glycosidic linkage
1	Native dextran B1255	α-(1 → 6)
20	Clinical Swedish dextran OP155	α-(1 → 6)
J. Ge.	Native dextran B512	α-(1 → 6)
Fo.	NRRL* B512	α-(1 → 6)
R.G.M.	NRRL B1424	α-(1 → 6) + α-(1 → 2)
333	NRRL B1299 S-3	α-(1 → 6) + α-(1 → 2)
332	NRRL B1299 S-3	α-(1 → 6) + α-(1 → 2)
30	NRRL B512	α-(1 → 6)

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Materials and Methods

45 fractions of isolated antibodies from 13 normal persons were prepared and of these 34 were in sufficient concentration for study in multiple systems.

Human Antisera.—Anti-dextrans or subfractions from eight donors were studied (Table I). Varying antibody specificities related to type of glucosyl linkage (15–17) were obtained, depending upon the dextran preparation used for immunization. Teichoic acid antibodies from these and one additional donor (Da) immunized with teichoic acid Copenhagen (21) were also studied. Four persons were immunized with porcine blood group A substance (Ortho 63-2622, Wh, R.G., 6460). Antibodies to dextran, levan, diphtheria toxoid, and alum-precipitated tetanus toxoid were obtained from a single individual (subject 1) (13).

Isolation and Preparation of Antibody.—Sera were cleared by prolonged centrifugation at 4°C. Antibodies to teichoic acid, diphtheria toxoid, tetanus toxoid, and A substance were allowed to precipitate for 7 days with respective antigens at the point of maximum precipitation, as determined previously by quantitative precipitin curves (22). Precipitates were washed three times in cold phosphate-buffered saline, pH 7.2. Washed antigen-antibody precipitates of teichoic acid, diphtheria toxoid, A substance, and tetanus toxoid were dissolved in excess of the respective antigen. The sequential precipitation of α-(1 → 6)- and α-(1 → 2)-specific anti-dextrans from subjects 332, 333, and R.G.M. has been described in detail previously (14, 16). Dextran–anti-dextran precipitates with α-(1 → 6) specificity were treated with dextranase (23), while the subsequent α-(1 → 2)-specific precipitates were dissolved in

antigen excess. Residual precipitates were partially dissolved by the addition of 1 M acetate buffer, pH 3.8, for 1 hr at 37°C and then supernatants were analyzed. Oligosaccharides of the isomaltose series were used to elute antibodies preferentially on the basis of heterogeneity in size of combining site (20). In subject 1, an isomaltohexaose (IM6) elution was followed by an isomaltotriose (IM3) elution with 60% total recovery of antibody nitrogen. In subject 20, the reverse order of elution was carried out and antibody recovery was 62%. In subjects 20 and J. Ge., sequential elution of anti-dextran using oligosaccharides of increasing size [glucose, methyl-α-D-glucoside, and isomaltose (IM2) to isomaltohexaose (IM6)] was carried out. The first two elutions (glucose and methyl-α-D-glucoside) represented 28% yield of total antibody nitrogen in subject 20, and 42% in subject J. Ge. Purified anti-levan of subject 1 was obtained by absorption on levan gel and elution of the antibody. For removal of a total of 816 μg of anti-levan nitrogen in 204 ml of serum from subject 1, 50 mg of levan gel were packed into a column. After the serum had been passed through the column, quantitative precipitation showed removal of anti-levan. Antibody was eluted from the column with 0.1 M sodium acetate buffer, pH 3.8, and the fractions containing eluted antibody were pooled, concentrated by ultrafiltration, and dialyzed versus phosphate-buffered saline, pH 7.2. Total antibody recovery was 61%.

Myeloma and Macroglobulin Proteins.—Purified immunoglobulins were prepared from patients with an established diagnosis of multiple myeloma or macroglobulinemia. Proteins were isolated and heavy or light chains or enzymatic digestion fragments were prepared as previously described (7).

Antisera.—Antisera specific for γG-, γA-, γM-, kappa, lambda, γG-heavy chain subgroups, and genetic factors were prepared by the immunization of rabbits, cynomolgus or rhesus monkeys, or baboons with isolated whole immunoglobulins, polypeptide chains, or enzymatic digestion fragments. The antisera were absorbed with isolated Bence Jones proteins, myeloma proteins or digestion fragments, fraction II, isolated normal γG or selected sera in order to obtain the light chain type or heavy chain class, subgroup, or Gm specificity sought as has been described in detail (1, 4, 7, 10, 24). The baboon antisera were prepared through the cooperation of Dr. J. Moor-Jankowski and the Laboratory for Experimental Medicine and Surgery in Primates.

Precipitin Analysis.—Isolated antibodies were qualitatively characterized by microimmunoelectrophoresis or Ouchterlony double diffusion. For semiquantitative experiments serial two-fold dilutions of isolated antibody were reacted with specific antisera in double diffusion in agar (1) and compared with immunoglobulin solutions of known concentration. Quantitation of γG-, γA-, γM-, kappa, lambda, γG2-, γG3-, and γG4-subgroups was carried out by radial immunodiffusion (25, 26) modified as previously described for the γG3-subgroup (7). The isolated immunoglobulins used as standards were tested in high concentration on radial diffusion plates for concentrations of contaminant immunoglobulins and were found to contain less than 1% of proteins of other classes, subgroups, or of the opposite light chain type. Protein concentrations of standard solutions were determined by the Folin-Lowry technique and Kjeldahl or ninhydrin nitrogen determination (22).

Of many antisera showing γG-subgroup specificity, only a few were suitable for quantitative radial immunodiffusion. After absorption, these antisera showed complete fusion without spur formation with all myeloma proteins of the subgroup and with normal sera. They showed no cross-precipitation or inhibition of precipitation by proteins of other subgroups except as previously described for the γG2- and γG3-subgroups (10), and contained no antibodies to other serum proteins. The prior diffusion of known immunoglobulins of other classes and subgroups was shown not to affect the precipitin ring size of standard solutions subsequently applied to the same test well. For each γG-subgroup quantitation, from two to seven purified standard proteins of varying genetic type and light chain type were employed and gave similar results as did at least two different antisera for each system. The minimum concentra-

tions of γG-subgroup proteins detectable were as follows: γG1: 20–30 μg/ml; γG2: 10 μg/ml; γG3:20 μg/ml; γG4: 10 μg/ml.

Gm Typing.—Gm typing was carried out using standard hemagglutination-inhibition techniques. In addition, typing for Gm(n) and Gm(g) was carried out using precipitation techniques in an Ouchterlony system (7, 10). A radial diffusion system for Gm typing for Gm(n) and Gm(g) using 5 μl samples of sera, isolated myeloma proteins, and some of the isolated antibodies was developed. Complete agreement between the microtechnique, the Ouchterlony precipitin technique, and hemagglutination-inhibition techniques for Gm(n) and Gm(g) were shown with a panel of normal sera and isolated myeloma proteins in concentrations as low as 40–50 μg/ml. A baboon antiserum made against a Gm(g+) myeloma protein (7) or a monkey antiserum against Gm(n+) isolated heavy chains absorbed to yield desired subgroup and genetic specificity (10) were incorporated into radial diffusion plates prepared as previously described (7). With plates of 1 mm uniform thickness, wells 2.8 mm in diameter were filled with 5 μl of sample. Only sera positive for these Gm antigens produced precipitin rings. Gm(n) typing was also carried out using human red blood cells coated with a Gm(n+) myeloma protein using bis-diazotized benzidine (27).

RESULTS

Quantitation of γG–Heavy Chain Subgroups.—The chief finding of interest was that the anti-levan isolated from donor 1 and isolated anti-dextrans from six of eight donors were composed predominantly or exclusively of γG2-heavy chains (Table II). In the table, the quantitative data by radial immunodiffusion are expressed as percentages of the total antibody protein by nitrogen determination; the heavy chain subgroup quantitations are expressed as percentages of total γG. For example, the quantitative values for the most homogeneous antibody, anti-levan isolated from subject 1 were: antibody total protein-0.32: γG1-0.0, γG2-0.31, γG3-0.00, γG4-0.00, total γG-0.32, γA-0.0, γM-0.0, kappa-0.30, lambda-0.00 mg/ml. All of the isolated anti-dextrans from subjects 1, 20, J.Ge., and 30 and anti-dextrans of α-(1 → 6) specificity from subjects 333 and 332 were primarily γG2-subgroup proteins. Anti-dextran from subject Fo., also of α-(1 → 6) specificity, contained an equal mixture of γG1- and γG2-molecules. Only in subject R.G.M. were anti-dextrans of α-(1 → 6) specificity composed primarily of molecules of the major or γG1-subgroup. Anti-dextrans of predominantly α-(1 → 2) specificity from subjects 333 and 332 were primarily γG2, and those from donor R.G.M. were primarily γG1 in parallel with the findings for the anti-dextrans of α-(1 → 6) specificity from the same individuals. Fig. 1 illustrates the radial diffusion quantitation of γG, γG2, γG3, and γG4 for three anti-dextrans isolated as oligosaccharide eluates from subject 20, with each of the isolates exhibiting exclusively γG2-subgroup heavy chains. From the sensitivity of the detection technique and total protein concentration, one can estimate the γG1, γG3, and γG4 content of many of the preparations to be less than 5% of the total antibody protein despite the fact that the mean percentage for these three subgroups totals 75–80% of pooled γG. For example, in the glucose eluate of isolated anti-dextran from subject 20, less than 20/1800's (sensitivity of detection vs. total

TABLE II

Percentages of γG-Heavy Chain Subgroups, Classes of Immunoglobulins, and Light Chain Types in Isolated Human Antibodies as Determined by Radial Immunodiffusion Using Specific Antisera

Subject	Antibody	Method of isolation	Antibody nitrogen × 6.25	H chain subgroup				Class of immunoglobulin			L chain type	
				Per cent of total γG				Per cent			Per cent	
				γG1	γG2	γG3	γG4	γG	γA	γM	κ	λ
			mg/ml									
1	Dextran	IM6 eluate	0.41	0	100	0	(0)*	100	0	0	97	3
	"	IM3 eluate	0.26	0	100	0	0	89	11	0	15	85
	"	N236 ppt‡	0.32	0	100	0	0	75	9	16	79	11
	Levan	Acid eluate	0.32	0	100	0	0	100	0	0	100	0
	Diphtheria toxoid	Ppt§	0.49	(62)	38	0	0	28	18	55	56	44
	Tetanus	Ppt§	0.13	(81)	19	0	0	64	18	18	62	38
20	Dextran	IM3 eluate	0.16	0	100	0	0	92	8	0	30	70
	"	IM6 eluate	0.31	0	100	0	0	86	14	0	68	32
	"	Glucose eluate	1.82	(21)	79	0	0	95	5	0	42	58
	"	Methyl-α-D-glucoside eluate	0.37	(18)	82	0	0	88	12	0	11	89
	"	OP155 ppt‡		(50)	50	0	0	100	0	0	21	79
J.Ge.	Dextran	Glucose eluate	0.63	(3)	97	0	0	100	0	0	79	21
	"	Methyl-α-D-glucoside eluate	2.86	(8)	92	0	0				92	8
	"	N236 ppt‡	0.08	(33)	67	0	0	82	18	0	65	35
Fo.	Dextran	B512 ppt‡	0.27	50	50	0	0	68	32	0	75	25
	Teichoic acid	Ppt‖	0.33	(20)	80	0	0	42	0	58	100	0
R.G.M.	Dextran	N1424 ppt‡	0.19	(100)	0							
	"	N279 ppt‡	0.42	(75)	25	0		79	6	15	83	17
	"	Remaining ppt ‖¶		(50)	33	2	15	83	13	4	28	72
	Teichoic acid	Ppt‖	0.07	(57)	43	0	0	100	0	0		
333	Dextran	B1299 S-3 ppt‡	0.14	(75)	25	0	0	100	0	0	43	57
	"	N279 ppt‡	0.38	(0)	100	0	(0)	94	6	0	86	14
	"	Remaining ppt ‖¶	0.26	(0)	100	0	(0)	62	38	0		
	Teichoic acid	Ppt‖	0.12	(25)	75	0	0	100	0	0	40	60
332	Dextran	B1299 S-3 ppt‡	0.13	(81)	19	0	0	100	0	0		
	"	N279 ppt‡	0.19	0	(100)	0	(0)	74	26	0		
	"	Remaining ppt ‖¶	0.30	(0)	100	(0)	(0)	50	37	13	20	80
	Teichoic acid	Ppt‖	0.12	0	80	20	0	67	33	0		
30	Dextran	OP155‡	0.16	(14)	86	0	0	100	0	0	40	60
Ortho 63-2622	A substance	γG-eluate§	0.41	(70)	30	0	0	77	14	10	20	80
Wh	A substance	Ppt‖		(50)	(25)	(25)	(0)	90	10	0		
R.G.	A substance	γG-eluate§	1.06	(60)	(40)	0	0	95	5	0	86	14
6460	A substance	Ppt‖		(57)	(29)	(14)	(0)	95	5	0		
Da	B teichoic	Ppt§	0.12	(50)	50	0	0	83	17	0	36	64

* ( ), estimated percentages.

‡ Dextranase eluate.

§ Antigen excess.

‖ Acid eluate.

¶ Predominate specificity for α-(1 → 2) linkages.

γG in μg/ml) was γG3-subgroup, or less than 0.8% instead of the normal percentage of 8%, a selective exclusion of this subgroup of at least 10-fold magnitude. The γG3- and γG4-proteins were clearly reduced below the normal mean percentages in all specimens showing a total protein concentration of greater than 0.2 mg/ml total γG.

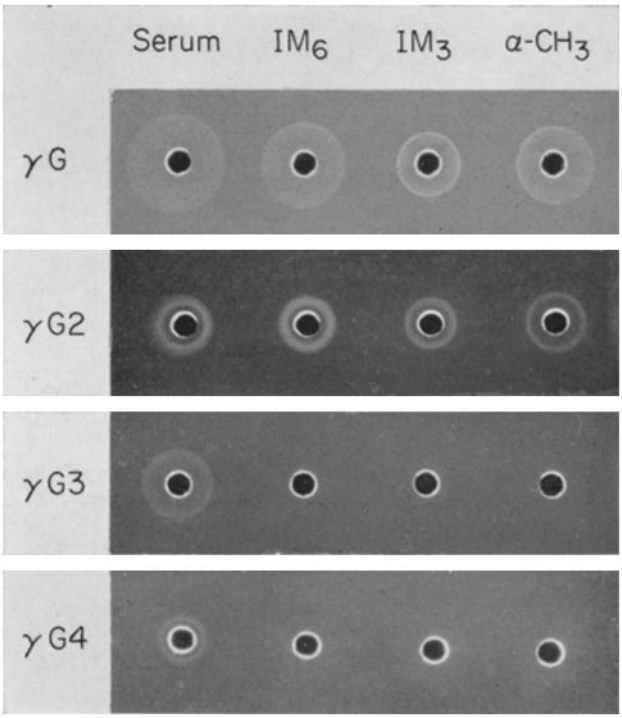

FIG. 1. Radial immunodiffusion analysis of serum and subfractionated anti-dextrans from subject 20 is shown. The oligosaccharide IM6, IM3, and methyl-α-D-glucoside (α-CH_3) eluates contained γG2-heavy chain subgroup molecules, but lacked γG3 and γG4. The whole serum was diluted 1:3.5 for analysis of γG2, and 1:10 for analysis of total γG.

The predominance of γG2-subgroup in many of the preparations was supported both by the inability to detect γG1-, γG3-, and γG4-molecules, the agreement between the antibody protein by nitrogen determination and the γG and γG2 quantitations by radial immunodiffusion. In only 1 of 23 isolated anti-dextrans from the eight donors could γG3- and γG4-molecules be detected (subject R.G.M.).

Three of the antibodies to teichoic acid (subjects Fo., 333, and 332) contained predominantly γG2-molecules as well (Table II). Two other isolated antibodies to teichoic acid contained similar ratios of subgroup molecules to

those found in whole γ-globulin. This was also the case for the isolated diphtheria and tetanus anti-toxins from subject 1. Some of these antibodies were only available at low protein concentrations and low concentrations of γG3- and γG4-molecules could not be ruled out.

Of the four antibodies specific for A substance, γG1 predominated in all, γG2 was present in each in approximately the usual percentage. The identification of γG1-molecules in one of the antibodies to A substance and lack of γG1 in two anti-dextrans and one anti-levan are shown in Fig. 2. In two antibody preparations, γG3 could be detected (subjects Wh. and 6460) but γG4 was not detected.

In most of the isolated antibodies, γA- and γM-molecules were absent or in concentrations of less than 10–20% of the total antibody protein (Table II).

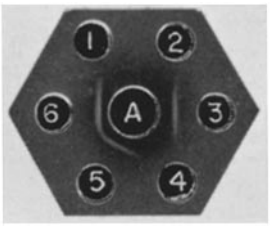

FIG. 2. The characterization of isolated human antibodies for γG1-subgroup molecules is shown. The central well contains an absorbed rabbit antiserum specific for γG1. Wells 1 and 2 contain IM3 eluates of anti-dextrans isolated from subjects 20 and 1; well 4 contains anti-levan isolated from subject 1. Well 3 contains fraction II, and well 6 an isolated γG1-subgroup myeloma protein. Isolated antibody to A substance from subject R.G.M., shown in well 5, shows a complete fusion of the precipitin line with that of the γG1–myeloma protein. The anti-dextrans and anti-levan fail to react. All materials were tested at 0.5 mg/ml protein concentration.

Only the antibodies to diphtheria toxoid, the anti-dextrans and anti–teichoic acid antibodies of subjects Fo. and 332, and anti-dextran from subject 333 showed higher percentages of γA- or γM-molecules. Of interest is the persistence of γA- and γM-molecules in one of the antibodies to dextran isolated from subject 1 despite an interval of 13 yr from the last immunization to collection of specimens for these isolations. The shortest intervals between immunization and the collection of specimens were in subjects J.Ge., Fo., 333, and 332 with intervals of 6–8, 6, 3, and 8 months respectively.

Quantitation of Light Chain Types.—Quantitation was also carried out employing antisera specific for either kappa or lambda light chain determinants both by immunoprecipitation of serially diluted isolated antibodies and by radial immunodiffusion. Two different antisera were used for each light chain type. Because of the predominance of γG-molecules in the isolated antibodies, purified γG–myeloma proteins were employed as standards. The limit of sensi-

tivity of the radial immunodiffusion quantitations was 10 μg/ml for both the kappa and lambda systems. Marked variations in ratio of kappa to lambda were seen (Table II). At one extreme, anti-levan isolated from subject 1 contained exclusively kappa determinants, and as little as 10/320's of the isolated antibody should have shown detectable lambda determinants based upon sensitivity of the system for detecting lambda proteins vs. the total μg/ml of γG present. At the opposite extreme, the methyl-α-D-glucoside eluate of anti-dextran from subject 20 showed 89% lambda and 11% kappa determinants.

Marked variation in kappa:lambda ratio was seen among the subfractions of anti-dextran. Purified anti-dextran isolated from single donors by specificity for glycosidic linkage, or by heterogeneity of affinity for oligosaccharides,

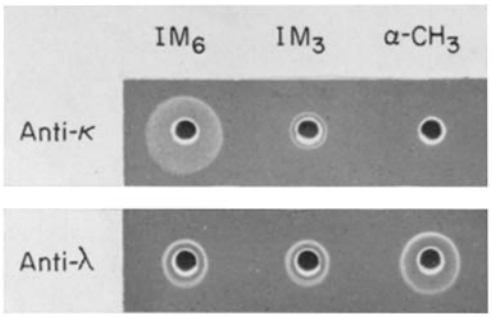

FIG. 3. Quantitation of light chain type kappa and lambda gamma globulins in isolated anti-dextrans from subject 20. The type-specific antiserum is uniformly incorporated in the agar plate. The predominance of kappa molecules in the IM6, and lambda molecules in the IM3 and methyl-α-D-glucoside eluates is shown.

showed variations in kappa:lambda ratios. For example, the IM6 eluates from both subjects 1 and 20 contained predominantly kappa antibodies; the IM3 eluates were predominantly lambda in type. The variability of kappa and lambda light chain determinants in isolated antibodies from subject 20 are illustrated in Fig. 3. The methyl-α-D-glucoside eluate from subject 20 contained 89% lambda-type material; the same eluate from subject J.Ge contained approximately 92% kappa molecules. In subject R.G.M., α-(1 $\rightarrow$ 6)-specific anti-dextran consisted largely of kappa determinants and the subsequent α-(1 $\rightarrow$ 2)-specific fraction had somewhat more lambda-type molecules. The isolated antibodies to diphtheria and tetanus toxoids showed mixtures of light chain determinants in approximately the usual 2:1 ratios found in normal γG-immunoglobulin. Isolated antibodies to A substance showed, in one individual, a predominance of kappa determinants, and in the other a predominance of lambda.

Gm Typing of Selected Isolated Antibodies.—Some of the isolated antibodies, known to be predominantly of the γG2-heavy chain subgroup, were studied

for Gm(n), a genetic antigen recently described, and localized to the Fc portion of molecules of the γG2-subgroup (10). Data from some of the individuals is shown in Table III. The isolated antibodies were successfully typed for Gm(n) using either Ouchterlony double diffusion, radial immunodiffusion as described above, or the hemagglutination-inhibition system using red blood cells coated with γG2-Gm(n+) myeloma proteins. Gm(n+) antibodies were obtained

TABLE III

Gm Types of Donor Sera and Isolated Human Antibodies

Subject	Gm (type of serum)					Antibody method of isolation	Gm (type) of antibody (% antibody protein)*		
	(a)	(g)	(f)	(b)	(n)		(a)	(f)	(n)
1	+	+	+	+	+	Dextran IM6 eluate	−	<1%	−
						Dextran IM3 eluate	−	<1%	<10%
						Levan acid eluate	−	<0.3%	>85%
						Diphtheria toxoid antigen excess	−	<5%	10–40%
20	−	−	+	+	+	Dextran IM3 eluate		<5%	>30%
						Dextran IM6 eluate		<5%	<15%
						Dextran glucose eluate		<1%	10–40%
						Methyl-α-D-glucoside eluate		<2%	>40%
J.Ge.	−	−	+	+	+	Dextran glucose eluate		<4%	+
Fo.	−	−	+	+	+	Dextran B512 dextranase eluate		+	−
30	+	+	−	−	−	Dextran OP155 dextranase eluate	−	−	−

* Estimated from γG concentration at last inhibiting dilution vs. known sensitivities of Gm systems.

only from individuals positive for Gm(n) in whole serum. The IM6 eluate from subject 1 who was known to be heterozygous for the gene complexes Gm^{n+} Gm^{b} Gm^{fy} and Gm^{n-} Gm^{g} Gm^{za} was Gm(n−) whereas all other anti-dextrans and the anti-levan from this donor were Gm(n+) at the same γG2-protein concentrations. The IM6 eluate isolated from subject 20, who was known to be homozygous for Gm(f) at the γG1-locus, and for Gm(b) at the γG3-locus and whose serum was Gm(n+) was repeatedly Gm(n−) in the precipitin system and only a trace of Gm(n) antigen could be detected by hemagglutination inhibition although within the limits of detection his anti-dextrans were exclusively of the γG2-subgroup. Three other isolated anti-dextran frac-

tions from the same individual also shown to be γG2-subgroup molecules were tested at the same protein concentrations and found to be Gm(n+). This donor therefore must have been heterozygous at the γG2-locus and carried both the Gm^{n+} Gm^{b} Gm^{fy} and the Gm^{n-} Gm^{b} Gm^{fy} gene complexes known to exist in Caucasians. The same was probably true of subject Fo., another donor whose serum was Gm(n+). Isolated α-(1 → 6)–specific anti-dextran from this donor contained γG2-protein, 180 μg/ml, but failed to inhibit the Gm(n) system. The Gm(n) system as employed for this study was capable of detecting Gm(n) antigen on γG2-molecules at 8 μg/ml.

DISCUSSION

The most striking finding in this study was the dominance of γG2-subgroup molecules in isolated antibodies to dextran, levan, and teichoic acid despite the fact that this subgroup makes up only 23% of normal γG-globulin. In contrast, other antibodies including anti-A isoagglutinins, anti-tetanus toxoid, anti-diphtheria toxin consisted primarily of the major γG1-subgroup. Anti-Rh antibodies which we have studied are also known to consist primarily of the major γG1-subgroup. At present no explanation for this selective distribution is available. A possible relationship to the carbohydrate nature of these antigens might exist, or it may represent a result of the immunization procedure. The antibodies to dextran and levan were obtained from individuals who had received two subcutaneous injections a number of years previously. The antibodies to teichoic acid probably arose through natural staphylococcal infection. Some suggestion of a relationship to specificity was obtained from the subfractionation of the dextran antibodies. The dextran antibodies of α-(1 → 6) specificity in six of eight donors were predominantly or exclusively γG2-subgroup, and in the seventh and eighth donors (Fo. and R.G.M.), γG2 accounted for 50 and 25% of antibody protein respectively. The dextran antibodies of α-(1 → 2) specificity were similar in heavy chain composition to those of α-(1 → 6) specificity with three individuals studied.

Earlier studies on many of these same antibodies had revealed a number of peculiar features which could not be explained at that time (2). Analyses for the Gm and Inv genetic markers had shown a striking absence of Gm antigens in a number of the anti-dextrans as well as in the anti-levan from subject 1. These studies were carried out with reagents for Gm(a) and Gm(b). Later when Gm(f) became available, the antigen was tested for and also found to be missing. Recently it has been recognized (8) that all of these genetic markers are subgroup specific and are not found in the γG2-subclass proteins, thus explaining their absence in these isolated antibodies. The newly described genetic marker, Gm(n), is the only one available for the γG2-subclass (10), and it was found in a number of the antibodies in the present study. The phenomenon of "allelic exclusion" so characteristic of myeloma proteins (28) and also

demonstrable by fluorescent antibody studies of the products of single cells (29), was apparent in the selective exclusion of Gm(n) in IM6 anti-dextrans of subjects 1 and 20 and not in others, although all their anti-dextran subfractions consisted primarily of γG2-molecules.

It has also been known for a number of years that the anti-dextrans of subject 20 exhibited poor sensitization of intestine and weak PCA (passive cutaneous anaphylaxis) reactions when tested in guinea pigs (30). It is now known that proteins of the γG2-subclass completely lack the capacity for skin fixation for PCA reactions (31) in contrast to other γG-globulins, and the unusual findings with the anti-dextran antibodies are explainable on the basis of high γG2 content.

Analyses for kappa and lambda light chains in the anti-dextrans that were subfractionated were of particular interest. The whole anti-dextran antibody preparations prior to subfractionation contained high concentrations of both kappa and lambda light chains. After subfractionation, however, the IM_6 eluates from two subjects contained a preponderance of kappa chains while the IM_3 eluates were mainly lambda in type. In most other instances, however, no obvious relationship was apparent between antigenic specificity and light chain type. The antibody to levan was of special interest because it showed only kappa chains and no lambda chains were detected by procedures that could determine as little as 1% concentration. It also was entirely γG2 in terms of heavy chain type and inhibited the Gm(n) system equally as well as Gm(n+) myeloma proteins and thus was entirely analagous by these criteria to a myeloma protein of the γG2-Gm(n+) kappa type. This was also one of the antibodies that readily produced "individually specific" rabbit antibodies (32), another well known characteristic of myeloma proteins.

Previous studies (14) have demonstrated clear amino acid composition differences between a number of the antibodies utilized in the present study. Certainly a portion of these relate to kappa and lambda light chain variations as well as heavy chain subgroup composition differences. For example, the large amino acid differences between α-(1 $\rightarrow$ 2) and α-(1 $\rightarrow$ 6) anti-dextrans may well follow the marked difference in kappa:lambda ratios in their light chains. The amino acid composition is probably less affected by the heavy chain subgroups involved because of the similarity of heavy chain subgroup composition in each of three individuals studied and because of marked similarities between the heavy chain subgroups. Only four tryptic peptide differences were observed by fingerprint analysis when the Fc fragments of γG1- and γG2-proteins were compared (33).

The degree of homogeneity observed with these antibodies was progressively increased by subfractionation on the basis of antibody specificity for glycosidic linkage or combining site size, rather than by physicochemical fractionation. The prevalence of γG2-molecules in the antibodies to dextran was apparent

prior to such subfractionation, but subfractionation produced progressive homogeneity both for genetic type of heavy chains and for light chain type as well. The reason for the relatively striking homogeneity in contrast to the marked heterogeneity of most antibodies, even to haptenes, remains obscure. Possibly it relates to the carbohydrate nature of the antigen involved, particularly in view of recent studies showing homogeneous populations of antibodies to bacterial polysaccharides which may be elicited in rabbits (34).

SUMMARY

The composition of various isolated antibodies was determined by quantitative analyses for heavy chain subgroups and light chain types. Certain antibodies such as anti-tetanus toxoid and anti-A isoagglutinins were predominantly of the major γG1-type. However, a high preponderance of molecules of the minor γG2-subgroup was found for antibodies to dextran, levan, and teichoic acid. These findings explain some unusual features previously noted for anti-dextrans such as weak PCA reactions and lack of Gm antigens.

Studies of several isolated antibodies from single heterozygous individuals showed a selective absence of genetic markers in certain antibodies and their presence in others. The "allelic exclusion" principle was clearly evident in the isolated antibodies of two different individuals.

Large differences in the ratio of kappa to lambda light chains were observed for the same type of antibody from different individuals. Subfractionation of dextran antibodies by affinity for specific glycosidic linkage or combining site size produced marked changes in the ratios. The isomaltohexaose eluates of the dextran antibodies from two subjects were primarily kappa and the isomaltotriose eluates were predominantly lambda.

The one anti-levan antibody studied was uniquely homogeneous, consisting exclusively of γG2–heavy chains and kappa light chains. By these criteria as well as others, it closely resembled myeloma proteins.

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