A NEW MOUSE IMMUNOGLOBULIN: IgG3*

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A library of murine myeloma tumors was catalogued (1) with respect to the immunoglobulins they produced. Of 161 sera screened, 19 were of the IgG1 class, 10 of the IgG2a class, 11 of the IgG2b class, and 120 of the IgA class.[1] In addition, we found one tumor (J606) which produced an immunoglobulin untypable by any of our anti-class sera and which possessed anti-levan activity. The purpose of the present study was to characterize this new immunoglobulin with respect to certain structural and biologic properties.

Materials and Methods

Antisera.—Rabbit antisera specific for mouse immunoglobulins were prepared by injection of 1–5 mg of the appropriate myeloma protein in complete Freund's adjuvant every 2 wk. The resultant antisera were made specific by absorption with myeloma proteins of a different class or subclass. Rabbit anti-J606 Fc antiserum was prepared by absorbing with J606 Fab prepared by papain digestion.

Antigenic Analysis.—Immunodiffusion, immunoelectrophoresis, and radial immunodiffusion were performed as previously described (2). The immunoelectrophoresis modification described by Osserman (3) was used to identify the J606-like component in normal mouse serum.

Isolation.—(a) The J606 protein was purified from the serum by starch block electrophoresis (4). The purity of the isolated protein was determined by immunoelectrophoretic analysis at a concentration of 10–20 mg/ml, using an antiserum prepared against whole mouse serum. (b) The J606 protein was also purified by bringing the serum to 30% of saturation with a solution of saturated ammonium sulfate, dialyzing against 0.9% NaCl buffered with 0.01 M potassium phosphate buffer (PBS),[2] pH 7.4, reprecipitating with 30% ammonium sulfate, and

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[1] Hirst, J., and M. Cohn. Unpublished results.

[2] *Abbreviations used in this paper:* BGG, bovine gamma globulin; NMS, normal mouse serum; PBS, potassium phosphate buffer; PCA, passive cutaneous anaphylaxis; RPCA; reversed PCA; v̄, partial specific volume.

finally dialyzing against 0.02 M potassium phosphate buffer, pH 8.0. The J606 protein, which precipitates under these conditions, was redissolved in PBS.

Reduction and Alkylation.—Proteins were partially reduced and alkylated as previously described (5) using 0.2 M 2-mercaptoethanol and 0.3 M recrystallyzed iodoacetamide. For peptide mapping, alkylation was done with ^{14}C-labeled iodoacetamide. For molecular weights and peptide mapping, the proteins were also completely reduced and alkylated in 8 M urea: 0.1 M tris (hydroxymethyl) aminomethane (Tris), pH 8, again using 0.2 M 2-mercaptoethanol and 0.3 M iodoacetamide. H and L chain separation was accomplished by G-100 gel filtration in 1 M acetic acid. Molecular weight determinations of fully reduced and alkylated heavy and light chains of J606 protein were carried out according to the method described by Andrews (6) using a Sephadex G-200 column equilibrated with 8 M urea and 0.05 M propionic acid.

Enzymatic Digestion.—Papain digestion (7) was carried out using 1% w/w papain for 45 min at 37°C. Digestion was stopped by the addition of a five-fold molar excess of iodoacetamide over the cysteine present in the reaction mixture. The resultant Fc fragment was insoluble and was purified by repeated washing with cold PBS at pH 7.0. The Fab was purified by starch block electrophoresis. For peptide mapping, trypsin digestion was carried out on completely reduced and alkylated H and L chains using 1% w/w of TPCK-trypsin (Worthington Biochemical Corp., Freehold, N. J.) for 16 hr at 37°C in 0.1 M ammonium bicarbonate, pH 8. The reaction was stopped by lyophilization of the reaction mixture.

Peptide Mapping and Amino Acid Analyses.—Peptide mapping, elution of peptides, and analyses of peptides for amino acid composition were carried out as previously described (8). Amino acid analyses were performed using a Beckman Model 120C amino acid analyzer equipped with a 12 cm "short" column which was capable of resolving glucosamine, galactosamine, and tryptophan. Carboxy-terminal amino acids were determined by digestion with carboxypeptidases A or B (9) and by hydrazinolysis (10). Both α- and γ-chains were used as controls.

Ultracentrifugation.—Analytical ultracentrifugation was carried out on isolated J606 preparations at different protein concentrations using a Beckman model E analytical ultracentrifuge equipped with schlieren and interference optics. The sedimentation coefficient of the protein was determined by standard procedures (11). The molecular weight of freshly isolated J606 protein was determined by sedimentation equilibrium according to the method described by Yphantis (12). The partial specific volume ($\bar{v}$) of the J606 was calculated to be 0.735 on the basis of its amino acid composition (13).

Cyanogen Bromide Cleavage.—Cyanogen bromide cleavage was carried out in 70% v/v formic acid for 24 hr at room temperature (14).

Starch Gel Electrophoresis.—Vertical starch gel electrophoresis was performed either by employing a 0.05 M glycine buffer, pH 8.8 (15), or an acid urea-formate buffer, pH 3.0 (16).

Acrylamide Gel Electrophoresis.—The procedure of Sulitzeanu et al. was used (17). Gels were run at an acrylamide concentration of 4.5%.

Skin Fixation.—Reversed passive cutaneous anaphylaxis was performed in guinea pigs according to the method of Ovary (18). Anaphylactic shock was examined by injecting BALB/c mice with 100 µg J606 protein intravenously and challenging with an intravenous injection of 100 µg levan B523 24 hr later. The passive cutaneous anaphylaxis (PCA) test was carried out on BALB/c mice injected intradermally with 1, 10, and 100 µg of J606 protein and challenging with an intravenous injection of 10 or 20 µg levan B523 plus Evans Blue after 3½, 4, and 4½ hr. The test was read 30–50 min after the injection of the levan.

Complement Binding.—The capacity of unaggregated immunoglobulins to bind the activated first component of complement C$\bar{1}$ was performed using a modification of the C$\bar{1}$ fixation test.[3]

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[3] Augener, W., and H. M. Grey. Relationship between immunoglobulin aggregation and C$\bar{1}$ binding. Manuscript in preparation.

Chemicals.—The levans used in this study were kindly provided by Doctors A. Jeanes and E. A. Kabat. Their characteristics are listed in Table I. Iodinated A protein was prepared with ^{131}I using a modification of the chloramine-T method (19). Radioactive levan was prepared by iodination of the p-hydroxy-benzyl-levan with ^{131}I, again using the chloramine-T method. The p-hydroxy-benzyl-levan was prepared by conjugating levan B1662 with α-bromo-p-nitrotoluene and subsequently reducing, diazotizing, and hydrolyzing the nitro group according to the method of Siskind et al. (20).

RESULTS

Immunologic Characterization.—The J606 protein was first identified as an immunoglobulin showing a reaction on an Ouchterlony diffusion slide with a

TABLE I

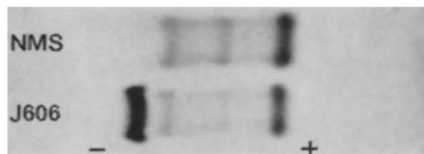| Levan	Source	Linkage	Donor
NRRL B-523; Fraction M (3664–46)	*Leuconostoc mesenteroides*	Beta 2–6	EAK, AJ
NRRL B-512; Fraction A (P.P. 2, 3801-30)	*L. mesenteroides*	Beta 2–6	EAK, AJ
NRRL B-1662; Fraction S (4100–77R)	*Bacillus sp.*	Beta 2–6	AJ
Inulin	Rye grass	Beta 2–1	Sigma
Aerobacter levan batch 19	Aerobacter	—	AJ

Fig. 1. Cellulose acetate electrophoresis of serum from a mouse carrying the J606 tumor and normal mouse serum (NMS). A homogenous peak in the $\gamma 2$ region is evident in the J606 serum.

beta 2–6–linked levan. No other myeloma immunoglobulin in our collection showed this reaction. Fig. 1 shows the electrophoretic appearance of the J606 protein in the serum of mice bearing the J606 tumor. The J606 purified protein showed one major band on acrylamide gel electrophoresis (Fig. 2). The purified protein retained anti-levan activity. Diluting the protein revealed that the band actually consisted of a series of two or three closely running bands of decreasing intensity. All the bands were removed following precipitation of the protein with the levan (Fig. 2).

To determine if normal mouse serum contained a protein similar to J606, a rabbit antiserum specific for J606 Fc fragment was prepared. When this rabbit antiserum was allowed to react with normal mouse serum following immuno-

electrophoresis a distinct cathodal arc was observed which bore antigenic determinants in common with the J606 Fc fragment (Fig. 3). When compared with the electrophoretic mobility of mouse IgG1 and IgG2, the J606 component
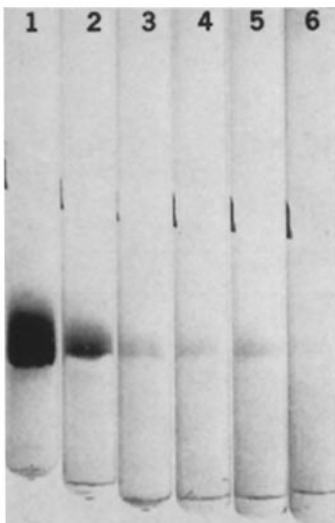

Fig. 2. Acrylamide gel electrophoresis of the J606 protein purified by method (b) (see Materials and Methods). 1. 150 μg of J606 protein; 2. 50 μg of J606 protein; 3. 10 μg of J606 protein; 4. 5 μg of J606 protein; 5. supernatant after precipitation of 500 μg of J606 protein with 40 μg of levan B512; 6; supernatant after precipitation of 500 μg of J606 protein with 20 μg of levan B512.
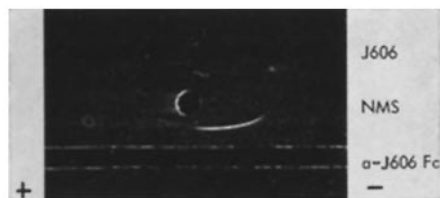

Fig. 3. J606 in normal mouse serum. Immunoelectrophoresis of NMS reacted against rabbit anti-J606 Fc antiserum (lower trough). Purified J606 protein at a concentration of 2 mg/ml is in the upper trough. A line of continuity was formed between the J606 protein and the protein present in NMS.

in normal mouse serum moved in the same position as the mouse IgG2 (Fig. 4). Radial immunodiffusion analysis indicated that the concentration of the J606 protein in normal BALB/c mouse serum was 0.1–0.2 mg/ml.

The rabbit anti-J606 Fc was also used in Ouchterlony analysis against myeloma proteins representative of the five other mouse immunoglobulins (IgG1, IgG2a, IgG2b, IgA, and IgM). No precipitin reaction was seen (Fig. 5) nor

was there any inhibition of the J606 anti-J606 line with any of the proteins tested. The protein did, however, show a reaction with antiserum directed toward the kappa light chain.

The possibility was entertained that J606 represented the homologue to either IgD or IgE, two human immunoglobulin classes which have not been previously identified in the mouse. Immunodiffusion analysis was performed using the J606 protein and rabbit antisera specific for human IgD and IgE.
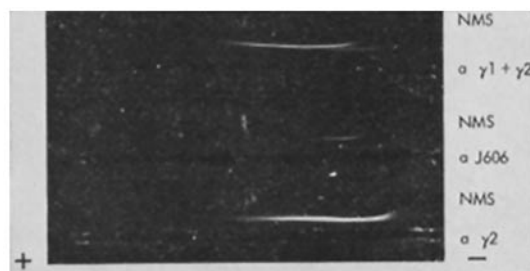

FIG. 4. Electrophoretic mobility of J606-like protein in NMS. Immunoelectrophoresis of NMS subsequently reacted against antiserum directed toward IgG1 + IgG2, IgG2, and J606 Fc. The J606-like protein in normal serum has a mobility similar to that of IgG2.
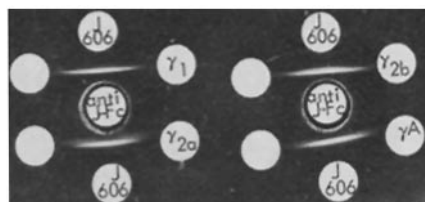

FIG. 5. Ouchterlony analysis of anti-J606 Fc reacted against IgG1, IgG2a, IgG2b, and IgA at a concentration of 1 mg/ml. Lines of precipitation are only seen with the J606 protein and extend sharply and undeflected toward the wells containing the other immunoglobulins indicating a lack of cross-reaction with these proteins. There was also no reaction with an IgM protein (not shown).

J606 did not precipitate with either antiserum nor did it inhibit the precipitation or IgD or IgE with their respective antisera.

Physical and Chemical Studies

(a) *Papain Digestion.*—As shown in Fig. 6, papain digested the J606 protein into two fragments which, when compared by immunodifusion, were non–cross-reacting. The slower migrating fragment reacted with a kappa chain antiserum and showed anti-levan activity (see below) and was thereby identified as the Fab fragment. The faster migrating fragment (Fc) was almost totally insoluble

at neutral pH. At a concentration of 5 mg/ml in PBS at pH7, less than 5% of the Fc fragment was soluble.

(b) *Ultracentrifugal Analysis.*—When the J606 protein, which was pure as judged by antigenic analysis, was analyzed in the ultracentrifuge by sedimentation velocity at neutral pH, an asymetric peak with a shelf of rapidly sedimenting material was obtained (upper pattern, Fig. 7). One explanation for this heterogeneity was that the J606 protein had a tendency to aggregate. To
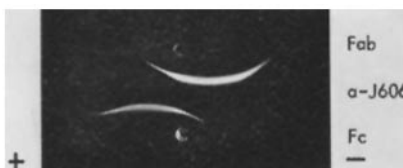

Fig. 6. Papain fragments of J606 protein. Immunoelectrophoresis of the isolated Fab and Fc fragments from a papain digest of the J606 protein. The center trough contains an unabsorbed rabbit anti-J606 antiserum.
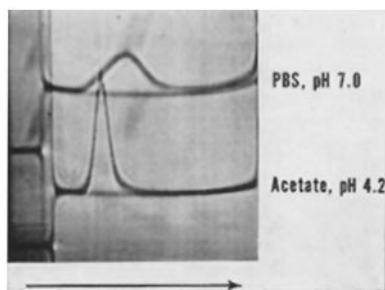

Fig. 7. Ultracentrifuge pattern of J606 protein at a concentration of 5 mg/ml. Upper pattern was obtained when protein was dissolved in PBS, pH 7. The sedimentation coefficient obtained under these conditions was 10.5S. Lower pattern was obtained with protein dissolved in 0.1 M acetate buffer, pH 4.2.

evaluate this possibility, a sedimentation velocity experiment was performed at pH 4.2. Under these conditions a single homogeneous peak was obtained with a sedimentation coefficient of 5.8S (lower pattern, Fig. 7).

The formation of noncovalently bound aggregates at neutral pH was further confirmed by studying the concentration dependency of the sedimentation coefficient. Fig. 8 illustrates the s vs. c plot. At pH 4.2 a linear plot was obtained with an extrapolated $s_{20, w}^0$ value of 6.3S. In contrast to this, at neutral pH a curvilinear plot was obtained. This anomalous behavior is typical of a concentration-dependent associating system and is further proof that the J606 protein formed noncovalent aggregates with itself.

Molecular weight determinations were performed on the intact protein and

Fab fragment at pH 4.0 by using sedimentation equilibrium. These data are shown in Table II and indicate that the Fab fragment and intact protein have molecular weights similar to those of γG: 150,000 for the intact protein and 47,000 for the Fab fragment.
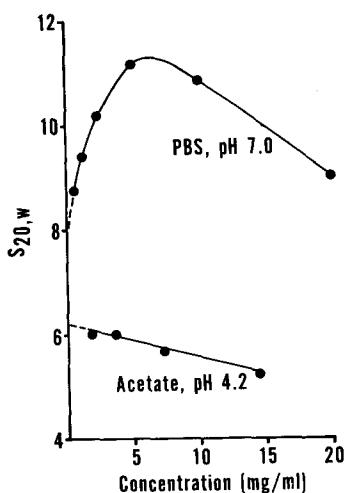

FIG. 8. s vs. c plot of J606 myeloma protein in PBS, pH 7, and in 0.1 M acetate buffer, pH 4.2.

TABLE II

Sedimentation Equilibrium Data for J606 Protein and Fab Fragment

Protein	No. of deter-mina-tions	Protein Concentration	$\bar{v}$	rpm	Average mol. wt.
		(mg/ml)			
J606 intact protein	3	0.2–0.8	0.735	16,200	149,500
					(148,000–152,000)
J606 Fab	2	0.3–0.6	0.730	24,630	47,200
					(45,500– 48,900)

Samples in 0.22 M acetate buffer, pH 4.0, were run at 20°C in a Spinco Model E Ultracentrifuge equipped with Rayleigh interference optics. Molecular weights were calculated from the plot of ln$c$ vs. r^2. The values presented are averages of determinations shown. The ranges of values is indicated in parentheses. Solvent density was 1.0021. Partial specific volume was calculated from amino acid and sugar composition.

After partial reduction and alkylation, J606 protein could be separated into heavy and light chains by gel filtration on G-100 Sephadex in 1 M acetic acid. The light chain peak contained 23% of the total OD 280 absorbing material. Molecular weights of the completely reduced and alkylated heavy and light chains were obtained by gel filtration on G-200 in 8 M urea– 0.05 M propionic

acid. Reduced and alkylated ^{131}I-labeled human γG was used as a marker. The H and L chains of J606 eluted in identical positions to their human γG counterparts and therefore had the same mol wt (H = 50,000; L = 23,000). These mol wt are in accord with the mol wt of 150,000 obtained for the intact protein assuming that J606 has a molecular formula of H_2L_2.

(c) *Peptide Analysis.*—Peptide mapping was performed on partially reduced and ^{14}C-alkylated H and L chains. The peptide map of light chains revealed a pattern consistent with its being a kappa chain. There was a single radioactive

TABLE III

Fc Glycopeptides of J606 and Different Mammalian IgG

	CHO
	|
	Phe Phe
Human IgG1-4 (22)*	Glu-Glu-Gln-Tyr--Asx-Ser-Thr-Tyr--Arg
	CHO
	|
Rabbit (23)	Glu-Gln-Gln-Phe-Asx-Ser-Thr-Ile-Arg
	CHO
	|
Mouse γ2a (24)	Glx-Asx-(Tyr, Asx, Ser, Thr, Leu)-Arg
	CHO
	|
Mouse γ1 (24)	Glu-Gln (Phe, Asx, Ser, Thr) Phe-Arg
	CHO
	|
J606	(Glu, Glu, Ala, Phe, Asx, Ser, Thr, Tyr, Arg)

* The numbers in parentheses refer to references.

peptide which was eluted and analyzed for amino acid composition. The composition was CMCys, Glu, Asx, which again confirmed that this was a kappa light chain (21). The peptide map of the H chains when compared with the H chain of a γ2b and 1 protein showed a distinctive pattern. It was not possible to clearly demonstrate any peptide that was shared between the J606 H chain and that of the γ2b and γ1 proteins. Because the glycopeptides appear in a characteristic position on the peptide map the spot located in this position was eluted from the map and amino acid analysis performed and compared with a variety of γG glycopeptides. As seen in Table III, all Fc fragment glycopeptides from mammalian γG that have been analyzed have a very characteristic composition and sequence. There is a high degree of homology between the composition of the J606 glycopeptide and that of other γG peptides. On the

other hand, analyses of glycopeptides from mammalian γA, M, D, and E that have been done bear no relationship to the γG or J606 Fc glycopeptide.[4]

A further attempt to evaluate the homology between J606 and other immunoglobulins was made by examining the C-terminal region of this protein. First, an attempt was made to isolate the C-terminal octadecapeptide which is generated following cyanogen bromide cleavage of γG from a variety of mammalian species. However, cyanogen bromide treatment of J606 as well as mouse γ2a, γ2b, and γ1 heavy chains failed to produce a C-terminal octadecapeptide from any of these proteins.
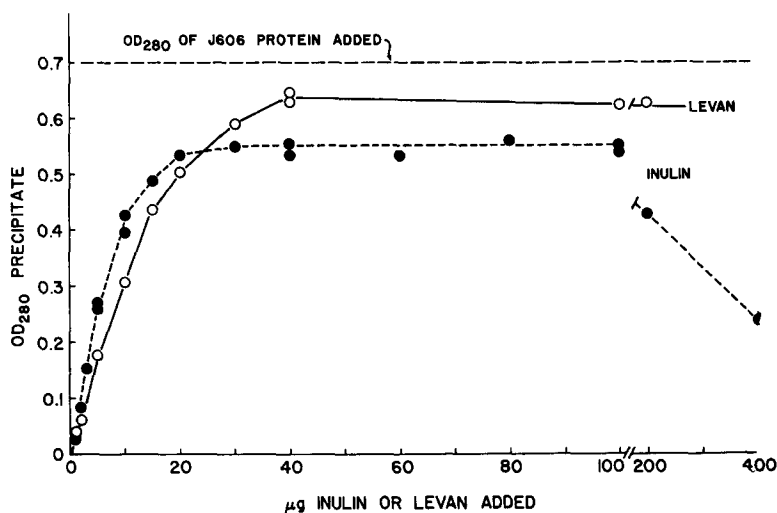

FIG. 9. Precipitation curve of J606 protein purified by Method (b) (see Materials and Methods) versus two levans, B1662 and inulin. To 0.5 ml of PBS containing the levan was added 500 μg of J606 protein in 0.5 ml of PBS with immediate mixing. The tubes were placed at 37°C for 60 min and at 4°C overnight. The tubes were centrifuged at 10,000 g for 15 min and the precipitates washed twice with PBS diluted 1:1 with water. Precipitates were dissolved in 1 ml of 1% acetic acid and the OD$_{280}$ determined.

Identification of the C-terminal residue of the heavy chain was then attempted. Digestion with carboxypeptidase A or B failed to release any amino acid, whereas hydrazinolysis yielded 0.5 mole glycine/mole of H chain. These data are very similar to those obtained for γ heavy chains where hydrazinolysis identifies a glycine C terminal, but due to the presence of a penultimate proline, digestion with carboxypeptidase A fails to release the C-terminal residue.

Amino acid composition of the J606 H chain was in general unremarkable. Glucosamine was the only amino sugar found. It was present in slightly lower

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[4] Grey, H. M., and C. A. Abel. Unpublished data.

amounts (3 moles/mole H chain) than is present in other γG proteins (25), and much lower amounts than reported for the other immunoglobulin classes where the total carbohydrate content is two to four times greater than γG (26). After partial reduction and alkylation there were 3 carboxymethyl cysteines and after complete reduction and alkylation, there were 9–10 carboxymethyl cysteines. The value of three labile cysteines per heavy chain is in keeping with the peptide mapping where three radioactive spots were obtained

TABLE IV

Concentration	Per cent inhibition of precipitation			
	Fructose	Sucrose	Melezitose*	Raffinose‡
0.2 M	19	85	—	—
0.02 M	8	36	8	18
0.002 M	1	—	5	4
0.0002 M	2	0	0	1

* Melezitose = glucose-fructose-glucose.
‡ Raffinose = glucose-glucose-fructose.

TABLE V

Concentration (mg/ml) of Mouse Immunoglobulins in the Newborn*

	J606	γG1	γG2 (a, b)
Newborn	0.24	0.09	0.27
Mother	0.12	0.32	0.94
N/M	2.0	0.28	0.29

* Two litters of colostrum-deprived newborn mice were bled during the first 2 hr of life. Maternal blood was obtained within 6 hr of delivery. Quantitation of immunoglobulin concentrations was done by radial immunodiffusion using monospecific antisera and suitable myeloma proteins as standards.

from the heavy chain that was partially reduced and labeled with ^{14}C iodoacetamide.

Antigen–Antibody Reactions.—A typical precipitation curve of the purified protein *versus* levan B1662 is presented in Fig. 9. Inulin, a beta 1-2–linked levan, is also precipitated by the J606 protein but this reaction is very temperature sensitive, the precipitate being soluble at 37°C, as well as going into solution in antigen excess, unlike the beta 2-6 levan precipitation reaction.

Sucrose, but not fructose, at a concentration about 1 M will dissolve a levan–J606 precipitate. Formation of a J606–levan precipitate can be inhibited by sucrose but only at high concentrations (Table IV) and perhaps by raffinose, but not by fructose or melezitose. Equilibrium dialysis with ^{14}C sucrose at con-

centrations as high as 10^{-4} M of sucrose failed to detect specific binding of the sucrose to the J606 protein.

Biologic Activities.—Several tests of biologic activities associated with immunoglobulins were performed on the J606 protein. The most striking results were obtained when the placental transfer of this immunoglobulin was examined (Table V). Whereas the concentration of IgG1 and IgG2 in the newborn was less than 30% of that of the maternal serum, the concentration of J606 protein in the newborn was twice that of the maternal serum. No detectable IgA or IgM was present in the newborn serum, in keeping with previous observations (27). That the relatively high concentrations of J606 in the newborn were due

TABLE VI

Biologic Properties of J606 Protein Compared to Other Mouse Immunoglobulins

	J606	IgG1	IgG2	IgA
Placental transport*	+++	+	+	0
Serum half-life in days*, ‡	4	4	(2a)5 (2b)2	1
C1̄ binding	0	0	+	0
Cytophilic attachment§	0	0	+	0
Heterologous PCA	0	0	+	0
Homologous PCA‖	0	+	0	0
Reaction with Protein A¶	+(P)**	0	+(I)‡‡	0

* Morphis and Gitlin, manuscript in preparation.

‡ Fahey, J. L., and S. Sell. See reference 28.

§ Measured by Dr. E. R. Unanue as inhibition of rosette formation using mouse IgG2 antibodies to sheep red blood cells.

‖ Kindly performed by Dr. Z. Ovary and Dr. J. J. Munoz.

¶ Kindly performed by Dr. G. Kronvall.

** Precipitation of protein A with J606 protein.

‡‡ Inhibition of precipitation of protein A with human IgG.

to maternal–fetal transport rather than active production by the newborn was indicated by studies of placental transport of radiolabeled J606 protein (Morphis and Gitlin, manuscript in preparation). In this same study Morphis and Gitlin demonstrated that the J606 protein has a relatively long serum half-life of 4 days.

Table VI summarizes some of the other biologic data available for the J606 protein which has been obtained by us and other investigators. Using a sensitive test for demonstrating binding of C1̄ to immunoglobulins, J606 protein was incapable of binding C1̄. The J606 protein did not inhibit the cytophilic attachment of mouse IgG2 immunoglobulins to macrophages. As yet, in spite of an extensive search, no allotypic markers have been found for this protein (R. Lieberman, unpublished results). We were unable to demonstrate either ana-
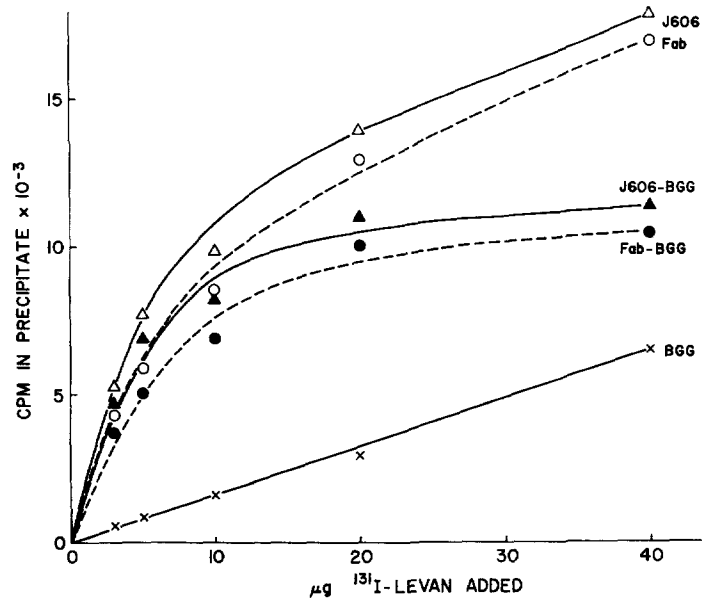
FIG. 10. Precipitation curve of J606 and J606 Fab *versus* [131]I levan. To 0.6 ml of PBS containing 100 µg of bovine gamma globulin (BGG), 100 µg of J606 protein, or 50 µg of J606 Fab + 50 µg of BGG was added the ^{131}I levan (3300 cpm/µg) from a solution of 1 mg/ml. The tubes were mixed and incubated for 10 min at 37°C, then for 60 min at 4°C. 1.4 ml of saturated ammonium sulfate was added with rapid mixing to bring the reaction mixture to 70% saturation with ammonium sulfate. The tubes were then kept at 4°C for an additional 30 min, centrifuged at 10,000 g for 15 min, and the precipitates counted in a gamma well counter.
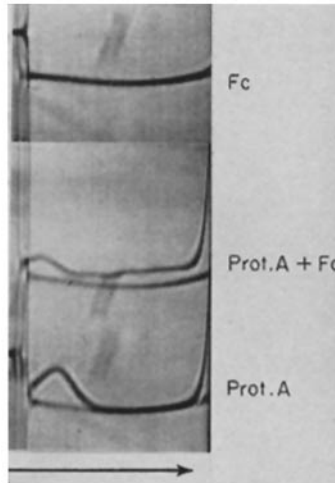
FIG. 11. Ultracentrifuge sedimentation pattern of J606 Fc at 5 mg/ml, the A protein of *Staphylococcus aureus* at 4 mg/ml, and a mixture of the two at the same final concentrations as that of the unmixed proteins. Due to aggregation, no schlieren pattern was obtained with the Fc fragment alone; however, when mixed with protein A, soluble complexes were formed as evidenced by the shelf of rapidly sedimenting material obtained with the mixture which was not observed with either of the unmixed samples.

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phylactic shock or passive cutaneous anaphylaxis in BALB/c mice using the levan–anti-levan system. Additionally, Doctors Z. Ovary and J. Munoz carried out an extensive investigation of both PCA and delayed PCA and reversed PCA (RPCA) reactions in the mouse and were unable to demonstrate any skin-fixing activity by these procedures. J606 also failed to react in RPCA in the guinea pig.

The J606 protein reacted with staphylococcal protein A, a reaction which in humans has been found to be limited to IgG immunoglobulins (29). This interaction is not shown by any of the other mouse immunoglobulins although the IgG2 class is able to inhibit the reaction of the A protein with human gamma globulin (30). In contrast to the reactivity of the J606 Fab fragment with levan (Fig. 10), protein A reactivity was associated with the Fc fragment of J606. Ultracentrifuge analysis indicated this interaction since no complexes

TABLE VII

| | Percent ^{131}I after 50% ammonium sulfate* | |
	Supernatant	Precipitate
Fc + ^{131}I-levan	94	6
Fc + ^{131}I-protein A	59	41
[131]I-levan	99	1
[131]I-protein A	85	15

* Components were mixed together and incubated for 10 min at 37°C. The tubes were then cooled and saturated ammonium sulfate was added to bring the tubes to 50% ammonium sulfate. Then the tubes were allowed to stand at 4°C for 1–2 hr. Subsequently they were centrifuged at 10,000 g for 15 min and the supernatants and precipitates were counted.

were formed upon mixing Fab and protein A, whereas protein A complexed with and solubilized the otherwise insoluble Fc fragment (Fig. 11). The results of several experiments using ^{131}I-labeled Fab and Fc fragments are shown in Table VII and confirm the data obtained with the ultracentrifuge. The association of protein A binding with the Fc fragment rather than with the Fab fragment is in keeping with previous studies performed with human IgG fragments and protein A (29).

DISCUSSION

The present study has characterized certain structural and biologic features of a unique mouse myeloma protein and its normal serum counterpart. We have concluded from these studies that the protein is a member of the IgG class and represents a new subclass of IgG which we propose to call IgG3. The major features of the structure and biologic properties of this protein which led to this conclusion are as follows: (a) The molecular weight of the intact protein and its heavy chain are identical to that of IgG and its heavy chain respectively, these

values being lower than the molecular weights reported for the other major classes of immunoglobulins: IgA, IgM, IgD, and IgE (26). (b) The carbohydrate content of the protein is similar to that of the IgG class and is distinctly lower than that described for the other immunoglobulin classes. The composition of the Fc glycopeptide is typical of the IgG class and again is distinct from the data obtained with other immunoglobulin classes. (c) The C-terminal residue of the heavy chain is glycine which has been reported only in heavy chains of the IgG class. The C-terminal residue of IgM and IgA is tyrosine (9) and that of IgE, lysine.[5] (d) The most striking biologic properties of this protein are also associated with the IgG class; namely, placental transport and reactivity with staphylococcal protein A, although the former can in certain species also be associated with IgM (31).

It is of interest that despite the rather striking structural and biologic features of this protein which identify it with the IgG class, there was no antigenic cross-reactivity between the J606 protein and proteins of other IgG subclasses, i.e., γ2a, γ2b, and γ1. This is in direct contrast to the situation observed in humans where there is extensive cross-reactivity between the four γG subclasses. This, however, is not a unique situation to the J606 protein, since mouse IgG1 cross-reacts poorly, if at all, with IgG2; and in the horse, IgGt, which on sequence analysis is clearly an IgG subclass, cross-reacts little, if at all, with the other IgG subclasses. There is growing evidence that the various IgG subclasses in mammals may have evolved after the major mammalian species diverged from one another (32, 33) so that both structurally and biologically there would be no compelling reason for the subclasses of one mammalian species to be structurally or functionally very closely related to the subclasses of other mammalian species. Following this line of reasoning, the degree of antigenic cross-reactivity between subclasses might give information regarding when, during the course of evolution, the subclasses of a given species emerged. The available data mentioned above would suggest that mouse subclasses diverged from one another earlier than human subclasses and are thereby structurally less similar to one another than the human subclasses. Obviously, amino acid sequence data will give more precise information regarding this point.

SUMMARY

A new subclass of mouse IgG for which we propose the name IgG3 has been shown to have a mol wt of 150,000 consistent with an L_2H_2 structure, and is present in normal mouse serum at a concentration of 0.1–0.2 mg/ml. Its molecular weight, low carbohydrate content, glycopeptide analysis, and C-terminal analysis are all typical of the IgG class. The intact protein had a strong tendency to form noncovalent aggregates with itself which were dissociable in acid. Upon papain digestion an Fab fragment of 47,000 mole wt was generated

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[5] Grey, H. M. and K. Ishizaka. 1970. Unpublished data.

along with an Fc fragment which was insoluble at neutral pH. As for its biology, the protein did not fix complement, was not cytophilic for γG2 receptor sites on macrophages, and did not show passive cutaneous anaphylaxis. It was very efficiently transported across the placenta so that its concentration in the new-born was twice that in the serum of the mother, compared to the concentration of IgG1 and IgG2 proteins which were only present at one-third the concentration of that found in the serum of the mother. The Fc fragment of this protein reacted with and was solubilized by the staphylococcal A protein which also precipitated the intact immunoglobulin. In addition, the myeloma protein which was the prototype for this γG subclass exhibited binding activity for levan which was localized to the Fab fragment.

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