AGE-DEPENDENT PRODUCTION OF IgA AND IgM AUTOANTIBODIES AGAINST IgG2a IN A COLONY OF 129/Sv MICE*

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Although much of the basic immunological work has been done with mice, little is known about anti-IgG autoantibodies in this species. Dresser (1, 2) has reported the occurrence, in CBA mice, of anti-IgG antibody (Ab)[1] detected by a hemolytic-plaque assay after stimulation with endotoxin or immunization against sheep erythrocytes. IgM rheumatoid factor has also been described in various strains of mice with a systemic lupus erythematosus-like disease (3). Recently, we have tried to induce anti-IgG in mice of the 129/Sv strain by inoculating autologous IgG. To our surprise, we found that the sera of all the animals had, before any inoculation, anti-IgG detectable by agglutination of particles coated with autologous IgG. The possibilities to investigate the mechanism of production and the biological role of this kind of Ab prompted us to undertake a study of the nature and specificity of the mouse anti-IgG.

Materials and Methods

Mice. 129/Sv and C57BL/6 mice were maintained in specific pathogen-free conditions in our Institute by Dr. G. Warnier. The original animals were obtained from Dr. J. L. Guenet, Institut Pasteur, Paris. BALB/c, AKR, and DBA/2 mice were purchased from Iffa Credo (Les Oncins, Lyon, France), and C57BL/10J, A/J, CE/J, RIII/J, SeA/GnJ, SJL/J, and LP/J mice from The Jackson Laboratory (Bar Harbor, Maine).

Myelomas. The myelomas were obtained from the National Institutes of Health, Bethesda, Md. and transplanted in our laboratory.

Igs. IgG was isolated from mouse, human, rat, goat, cow, and rabbit sera by precipitation with ammonium sulfate, followed by ion-exchange chromatography on DEAE-cellulose and, in the case of mouse IgG, by gel filtration on Ultrogel AcA 44 (LKB, Bzomma, Sweden) to remove contaminating transferrin. Mouse IgG was also prepared by affinity chromatography on protein A-Sepharose (Pharmacia, Fine Chemicals, Uppsala, Sweden). These latter preparations were slightly contaminated with IgA and IgM.

The purification of the various myeloma IgG subclasses was completed by passage through columns of Sepharose-conjugated rabbit Ab against the contaminating subclasses. Final purity was checked by double diffusion using specific anti-subclass antisera obtained from Bionetics (Litton, Bionetics Laboratory Products, Md.). The MOPC 315 IgA myeloma was isolated by affinity chromatography on a N-ε-dinitrophenyl lysine-Sepharose column. Normal C57Bl/6 IgM was purified from ascitic fluid (4) by gel filtration on Ultrogel AcA 22, followed by preparative Pevikon-block electrophoresis to separate IgM from α_2-macroglobulin.

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[1] *Abbreviations used in this paper:* Ab, antibody; BSA, bovine serum albumin; GBS, 0.1 M glycine-NaOH buffer, pH 9.2, containing 0.17 M NaCl; PACIA, particle-counting immunoassay; PBS, phosphate-buffered saline; PBS-Tween, PBS containing 0.01% Tween 20; RIA, radioimmunoassay.

Experiments were also done with polyclonal IgG1 and IgG2a separated by chromatography on a protein A-Sepharose column eluted with 0.1 M citrate buffers of decreasing pH as described by Ey et al. (5). To remove contaminants, the IgG1-rich fraction was passed through a rabbit anti-IgG2a-Sepharose column, and the IgG2a-rich fraction through rabbit anti-IgG1- and rabbit anti-IgG3-Sepharose columns.

To reduce heat-aggregated IgG, the proteins (10 mg/ml) in 0.05 M Tris buffer, pH 8.2, were incubated with 3 mM dithiothreitol at 37°C for 45 min, and then alkylated with 6 mM iodoacetamide. Reduction and alkylation were checked by acrylamide gel electrophoresis in 8 M urea and 0.1 M formic acid. Fab and Fc fragments of 129/Sv IgG were prepared by papain digestion (6) and purified by DEAE-cellulose chromatography.

Preparation of IgG-Coated Latex. Polystyrene particles (0.8-micron diameter) were a gift from Rhône-Poulenc (Courbevoie, France). The particles were coated by mixing 25 μl of a 10-mg/ml solution of 129/Sv IgG in phosphate-buffered saline (PBS) with 425 μl of 1/5-diluted 0.1 M glycine buffer, pH 9.2, containing 0.17 M NaCl (GBS) and 50 μl of a 10% suspension. After a 45-min incubation at room temperature and one washing with 1 ml of 1/5-diluted GBS, the particles were resuspended in 1 ml of GBS containing 1% bovine serum albumin (BSA). The preparation constituted a stock suspension of IgG-coated latex particles (0.5% wt/vol).

Agglutination Tests. All murine sera were decomplemented before the agglutination test by heating at 56°C for 30 min. The agglutination of IgG-coated particles was measured by the particle counting immunoassay (PACIA) (7). In this method, the residual nonagglutinated particles are counted by a blood-cell counter (AutoCounter from Technicon Instruments Corp., Tarrytown, N. Y.). Equal volumes (25 μl) of the agglutinator and a 0.05% (wt/vol) latex suspension (1/10-diluted stock suspension) were incubated for 30 min in a shaking water bath (320 cycles/min) at 37°C. The mixture was then diluted 100 times with GBS and the tubes placed in the sampler of a Technicon AutoAnalyzer. Before their passage through the cell of the AutoCounter, the samples were automatically diluted 8.7 times with GBS containing 0.1% Tween 20 (Atlas Chemical Industries, Inc., Wilmington, Del.). The rate of sampling was 60 samples/h with a sample per wash ratio of 1.

Expression of Agglutinating Activity. The agglutinating activity of heated mouse serum was titrated by making serial dilutions in GBS containing 1% BSA. The inverse of the volume (in microliters) of mouse serum required to reduce the number of particles by 50% was taken to be the number of agglutination units.

Radioimmunoassays. The amount of anti-IgG was measured by two radioimmunoassays (RIA), one based on the measurement of the amounts of labeled goat Ab fixed by the autoAb that is bound to the insolubilized murine IgG, and the other on the determination of Igs in the eluate of a mouse IgG immunosorbent. The first procedure, which was applied to IgA and IgM autoAb, was easier, but not suitable, for the determination of IgG autoAb because of the IgG used to coat the tubes.

The various steps of the first RIA were as follows.

1. Tube Coating. 3-ml polystyrene tubes were coated with IgG from 129/Sv mice by overnight incubation at room temperature with 200 μl of a solution of 10 μg/ml of 129/Sv IgG in 1/5-diluted GBS. The unbound proteins were removed by one washing with 1 ml of PBS containing 0.01% Tween 20 (PBS-Tween).

2. Binding of Anti-IgG. The 129/Sv serum (100 μl), at appropriate dilutions in PBS containing 5% fetal calf serum, was incubated in the coated tubes for 3 h at 37°C, and then washed twice with 1 ml of PBS-Tween.

3. Determination of IgA and IgM Anti-IgG Retained on the Tube Walls. The tubes were incubated for 3 h at 37°C with 200 μl of PBS containing purified ^{125}I-labeled goat Ab against murine IgA or IgM, and 5% fetal calf serum. The tubes were then washed twice with 1 ml and once with 2 ml of PBS-Tween and counted for radioactivity. The goat Ab had been eluted from columns of IgA- or IgM-Sepharose by 0.05 M citrate buffer, pH 2.6, containing 1 M sodium chloride. The Ab were labeled with ^{125}I by the chloramine T method.

4. Blank Corrections. The results were corrected by subtracting the radioactivity of two blanks. The first consisted of murine IgG-coated tubes which had been incubated with fetal calf serum in place of mouse serum and then with the radioactive goat Ab. The second consisted of fetal calf serum-coated tubes which had been incubated with mouse serum followed by radioactive Ab.

5. Calibration Curves. They were obtained by measuring the binding of the radioactive goat Ab to tubes which had been coated with increasing amounts of monoclonal IgA (MOPC 315) or IgM (MOPC 104E). The quantity of IgA or IgM effectively present on the tube walls had been calculated from the results obtained in a coating experiment performed with ^{125}I-labeled monoclonal IgA or IgM.

In the second RIA, where the anti-IgG were determined in the eluates, the steps before the elution were similar to those of the former test except that larger volumes of reagents were used, i.e. 3 ml of 129/Sv IgG (20 μg/ml) to coat 6-ml tubes, 3 ml of the tested mouse sera and 3.5 ml of PBS-Tween for the various washings. For elution, the tubes were shaken at room temperature for 30 min with 3 ml of a solution of BSA (1 mg/ml) adjusted to pH 2.8 with HCl and containing 0.1 mg/ml of horse spleen ferritin used as a carrier for the following precipitation step. The concentrations of IgA, IgM, and IgG were determined in the 3-ml eluates by the method of Salmon et al. (8) after neutralization of pH with 0.25 ml of 0.45 M Tris, pH 10.4. As the sensitivity of the assay for IgA and IgM was not sufficient, the eluates were concentrated by precipitation with ammonium sulfate at 52% saturation.

Coating of Polystyrene Tubes with Various Mouse Ig Classes and Subclasses. Polystyrene tubes were coated by overnight incubation with 200 μl of 1/5-diluted GBS containing various myeloma proteins from BALB/c mice. The unbound proteins were removed by washings with PBS containing 0.01% Tween 20. Each monoclonal Ig was labeled with ^{125}I to check its adsorption on the tube walls. Increasing concentrations were used to determine the saturation point of the tubes. This was reached at about 100 μg/ml for all Igs except IgG2a, which required a concentration of 250 μg/ml. The accessibility for Ab to the Fc-antigenic determinants of the various IgG subclasses on the tube walls was shown by the absorption of specific antisera. Their initial and residual activities were tested by hemagglutination of tanned sheep erythrocytes coated with murine IgG. The absorption by each insolubilized subclass caused at least a twofold specific decrease of the dilution titer of the corresponding antiserum. Further control was provided by Dr. R. Milne from our laboratory. He checked the binding of ^{125}I-labeled purified rabbit Ab against murine Fab fragments. He observed similar binding for all Ig classes and subclasses.

Results

Agglutinating Activity as a Function of Age and Sex. Serum samples were collected from male and female 129/Sv mice of various ages. The ability of each serum to agglutinate latex beads coated with IgG from 129/Sv mice was tested with the PACIA system. The samples had been heated at 56°C for 30 min to destroy the labile agglutinating factor described by Cambiaso et al. (9). The heat-resistant agglutinating activity increased about 10-fold between 10 and 20 wk (Fig. 1). In general, animals older than 20 wk had ≅ 40–50 agg. U/μl. No significant difference was observed between males and females.

Size of the Agglutinating Factor. The size of the agglutinator in the serum of 30-wk-old 129/Sv mice was analyzed by ultracentrifugation in an isokinetic sucrose gradient (10). At pH 7.2, most of the agglutinating activity was distributed as a polydisperse material with a sedimentation coefficient exceeding 11 S. At pH 2.0, 77% of the total activity was recovered, after neutralization of pH, as a 11 S peak, 19% as a broad fraction in the 20 S region, and 4% in the 7–11 S region. This profile suggested that the agglutinators could be IgM and dimeric IgA anti-IgG.

Nature of the Agglutinating Factors. To further characterize the nature of the agglutinators, we tested the ability of insolubilized antisera against mouse Ig to remove the agglutinating activity of 129/Sv serum. Samples of a serum pool from 129/Sv mice of 30 wk or more, were incubated with increasing volumes of various Sepharose conjugates. These included: goat IgG anti-murine IgA, goat IgG anti-murine IgM, rabbit IgG anti-murine IgG, and, as controls, goat IgG or rabbit IgG. After centrifu-

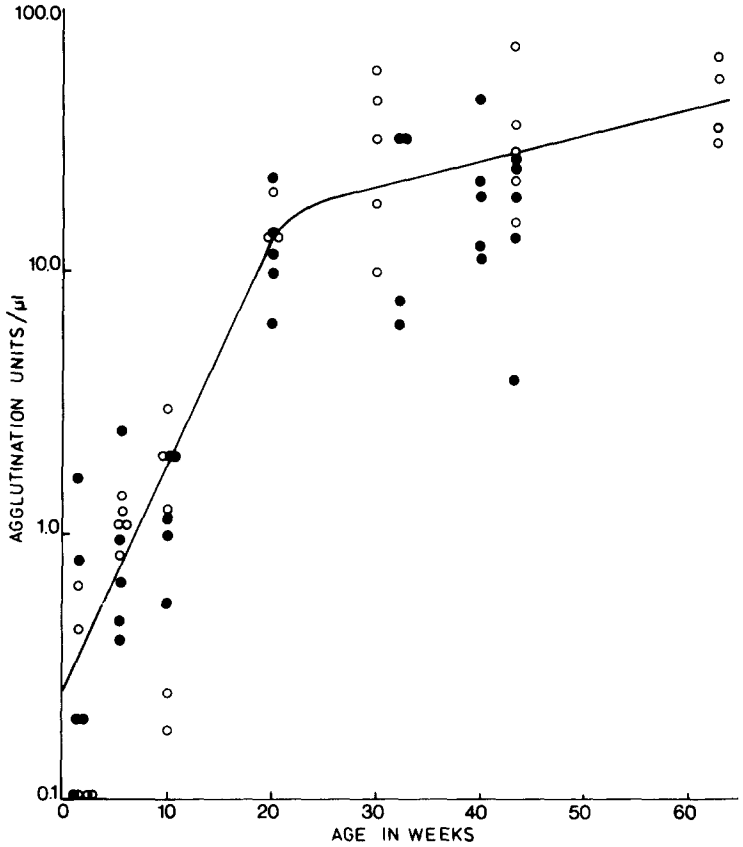

FIG. 1. Agglutinating activities of sera from individual 129/Sv mice, as a function of age, towards autologous IgG-coated latex. ●, males; ○, females.

gation, we measured the residual agglutinating activity in the supernates.

To check the efficiency of the immunoadsorbents we also determined, by RIA, the residual Ig. 50% of IgG and >95% of IgM and IgA were removed. Whereas the removal of IgA resulted in a nearly complete loss (≅ 95%) of the agglutinator, the agglutinating activity was only moderately (≅ 20%) reduced by the anti-IgM-Sepharose, and not at all by the anti-IgG-Sepharose.

A further argument for the IgM and polymeric IgA nature of the agglutinator was given by its loss of activity after rupture of disulfide bridges by reduction. Agglutination was decreased by 80% after treatment of the mouse serum with 2 mM dithiothreitol for 45 min at 37°C.

Concentrations of IgA, IgM and IgG Anti-IgG. The amounts of IgA and IgM combining with 129/Sv IgG bound to polystyrene tubes were evaluated by measuring the radioactivity of the tubes after reaction with ^{125}I-labeled purified goat anti-IgA or anti-IgM Ab. IgM anti-IgG Ab increased with age from 0.35 μg/ml at 4 wk to 6.0 μg/ml at 10 wk, whereas IgA increased from 0.55 μg/ml at 4 wk, up to 40 μg/ml at 52 wk (Fig. 2).

As it was not possible to detect IgG anti-IgG by this assay, they were determined in the eluates from the murine IgG immunosorbent. No significant amounts of IgG anti-

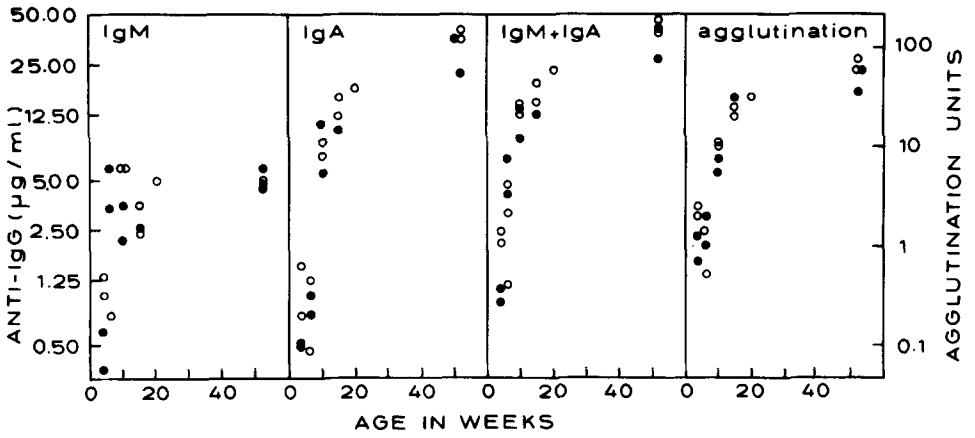

FIG. 2. Agglutinating activity and levels of IgM and IgA anti-IgG determined by RIA in 129/Sv mice as a function of age. Each dot corresponds to a pool of sera from 10 animals. ●, males; ○, females.

IgG (mean, 34 ng/ml; SD, 14.5 ng/ml) were detected, whereas the IgA and IgM anti-IgG detected by this method correlated well with the agglutinating activity of the sera.

To see whether the IgA and IgM retained on the insolubilized IgG corresponded to the agglutinating factors described above, the concentrations of each Ab measured by RIA and the sum of the two were plotted vs. the agglutinating activity of the various serum pools (Fig. 3). A strict linear relationship was found with IgA (Fig. 3 C) and with the sum of IgA and IgM Ab (Fig. 3 C). For IgM Ab, two populations of values had to be considered, i.e. those obtained from animals younger or older than 10 wk. The level of IgM Ab correlated significantly with the agglutinating activity only up to 10 wk (Fig. 3 A).

Specificity of the Anti-IgG AutoAb. The specificity of the anti-IgG of 129/Sv mice was determined by agglutination inhibition caused by (*a*) physically or chemically modified IgG, (*b*) IgG from various species, (*c*) mouse Ig classes or subclasses, and (*d*) IgG from various mouse strains.

Physically or Chemically Altered IgG. Samples of 129/Sv serum were mixed with various amounts of 129/Sv IgG and immediately tested for their agglutinating activity (Fig. 4). Nonaggregated IgG, up to a concentration of 250 μg/ml, failed to inhibit the agglutinator significantly, whereas 4 μg/ml of IgG, aggregated by heating at 63°C for 30 min, reduced the agglutinating activity by 50%.

IgG cross-linked by glutaraldehyde (11) or *bis*diazobenzidine (12), at a concentration of 250 μg/ml in PBS, did not inhibit agglutination. That these IgG preparations did contain aggregates was indicated by their turbidity and, for glutaraldehyde-cross-linked IgG, by their inhibitory capacity toward another agglutinating factor from mouse serum characterized by its heat lability (9).

Reduction and alkylation of IgG makes it unable to react with the Clq factor of complement (13) and with Fc receptors of macrophages (14). In contrast, human rheumatoid factor is able to combine with reduced and alkylated IgG (13). This led us to test the inhibitory activity of heat-aggregated murine IgG after reduction and alkylation. The 129/Sv mouse serum, after incubation with this material, kept most

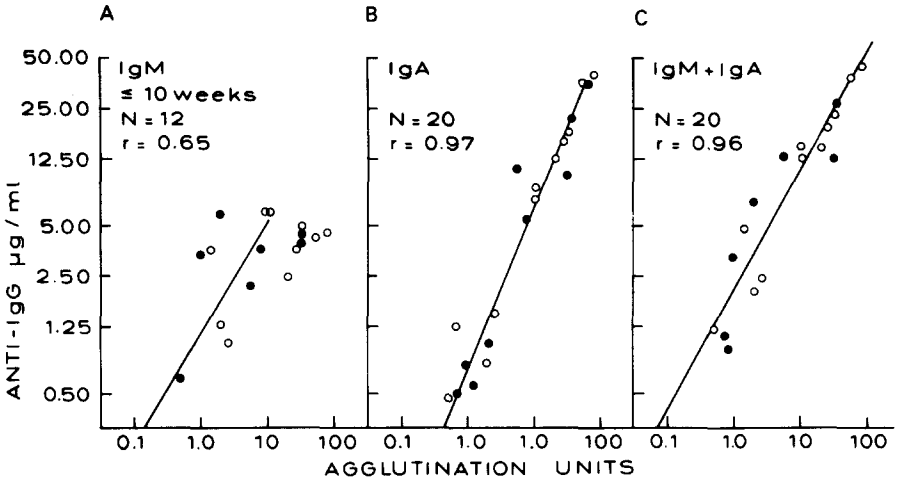

FIG. 3. Correlation between the agglutinating activity of 129/Sv mouse sera and the anti-IgG determined by RIA. Each dot corresponds to a pool of sera from 10 animals. ●, males, ○, females.

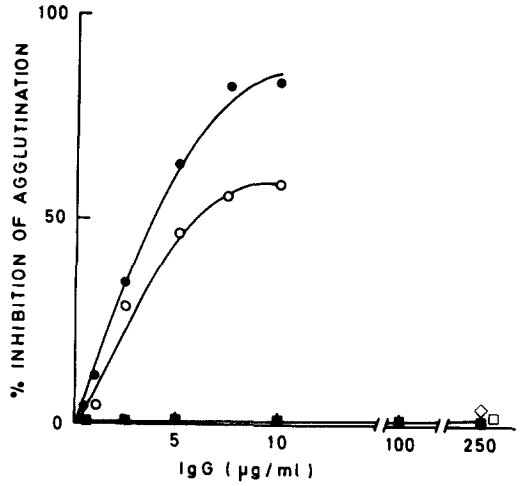

FIG. 4. Inhibition of the agglutinating activity of 129/Sv mouse anti-IgG by various preparations of autologous IgG; heat-aggregated IgG (●, Agg.IgG); reduced and alkylated heat-aggregated IgG (○, Red.alk.agg.IgG); nonaggregated IgG (■, nonagg.IgG); glutaraldehyde (□)- or *bis*diazobenzidine-aggregated IgG (◇). Samples of 25 µl of 129/Sv serum, diluted 1/300 in GBS containing 1% BSA, were mixed with 25 µl of the 129/Sv IgGs at various concentrations and tested for their remaining agglutinating activity.

of its agglutinating activity (Fig. 4). The antigenic determinant recognized by the anti-IgG therefore is different from the reaction site of IgG with Clq or Fc receptors.

IgG from Various Species. Polystyrene tubes were coated with IgG from various species or with BSA by incubation for 2 h with 200 µl of the protein solution (50 µg/ml) in 1/5-diluted GBS. Four tubes were used for each protein. After three washings with PBS, 50 µl of 129/Sv serum, diluted 1/300 with PBS, were incubated in the tubes with rotation at room temperature. After 1 h, the samples were tested for their agglutinating activity. The absorption was expressed by reference to the residual agglutinating activity recovered after incubation with BSA-coated tubes. Marked

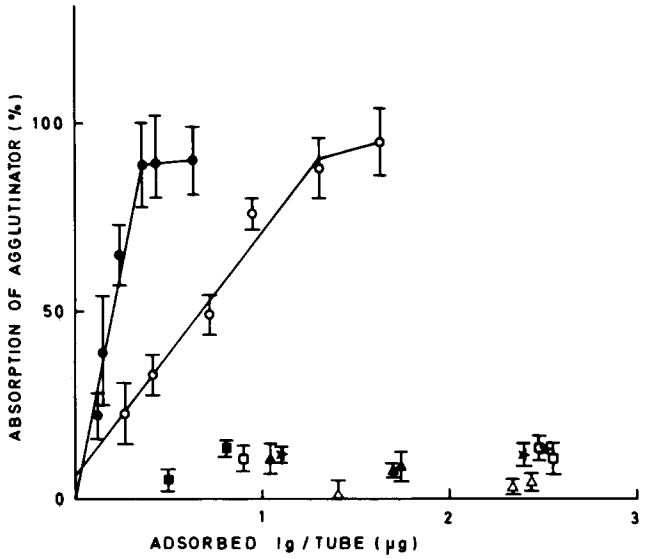

FIG. 5. Absorptions of 129/Sv anti-IgG by various murine Igs on polystyrene tubes. The serum was incubated for 1 h at 37°C in the coated tubes and then tested for its agglutinating activity toward IgG-coated particles. Each dot represents the mean of four determinations.Vertical bars: 1 SD. The amount of Igs bound per tube was measured by using ^{125}I-labeled Igs. ○, IgG; △, IgG_1; ●, IgG_{2a}; ▲, IgG_{2b}; □, IgG_3; ■, IgA; ★, IgM.

absorption was observed only with murine IgG (97%; SE, 1.0), whereas the effect of IgGs from rat (−2.5%; SE, 6.0), rabbit (2%; SE, 10.9), goat (−7%; SE, 8.9), and man (7.75%; SE, 3.5) was negligible.

These results were confirmed by inhibition tests with heat-aggregated IgGs. Whereas the sample of murine-aggregated IgG at 156 μg/ml completely inhibited the agglutination, aggregated IgGs from rat, rabbit, goat, cow, and man inhibited only weakly, even when added at 30-fold higher concentrations.

Mouse Ig Classes and Subclasses. Polystyrene tubes were coated with various myeloma proteins from BALB/c mice corresponding to various classes and subclasses. Particular care was taken to check the adsorption on the tube walls and the accessibility for Ab to the Fc-antigenic determinants (Materials and Methods).

The tubes coated with IgA (MOPC 315), polyclonal IgM from C57Bl/6 mice, IgG1 (MOPC 21), IgG2b (MOPC 141), or IgG3 (J 606), even when they were saturated with the various Igs (2.5 μg/tube), did not absorb any significant amount of agglutinator (Fig. 5). In contrast, all agglutinating activity disappeared after incubation of the mouse serum in the tubes coated with 0.4 μg of monoclonal IgG2a (MOPC 173). These results were confirmed with another series of myeloma proteins, i.e. RPC5 and UPC 10 for IgG2a, MOPC 195 for IgG2b, Y5606 and FLOPC 21 for IgG3, TEPC 15 for IgA, MOPC 104E and TEPC 183 for IgM. The tubes coated with whole IgG (polyclonal IgG from 129/Sv mice) also removed the agglutinator, but they were about four times less active than the IgG2a-coated tubes (Fig. 5).

When heat-aggregated Igs were used for inhibition experiments, IgG2a was also the only Ig able, when polymerized, to react with the agglutinators (Fig. 6). However, in contrast to what was observed with the coated tubes, aggregates of IgG2a were 10 times less inhibitory than those of whole IgG (polyclonal IgG from 129/Sv mice). In

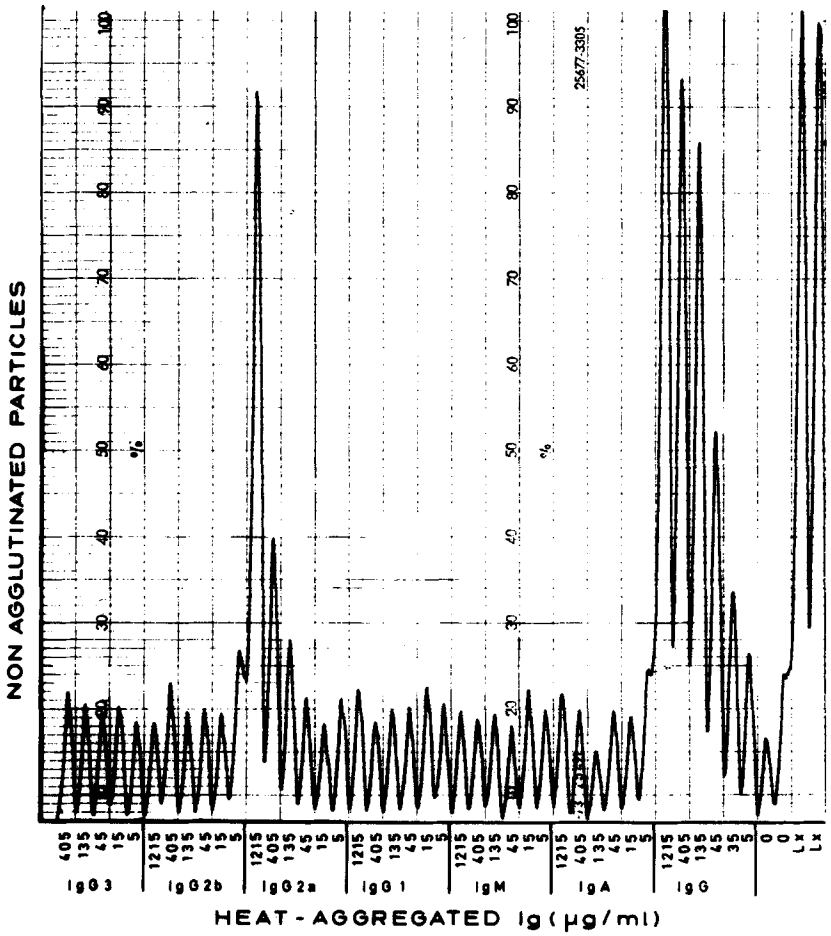

FIG. 6. Inhibitory activity of heat-aggregated murine Igs toward the agglutinator of 129/Sv serum. The peaks on the recorder sheet correspond to the number of non-agglutinated particles. Lx, latex in the absence of agglutinator.

the latter case, the aggregation of IgG2a was presumably favored by the presence of the other IgG subclasses.

Antigen-Ab complexes were also used to test the specificity of the anti-IgG. IgG1 and IgG2a Ab were isolated from serum of 129/Sv mice immunized against sheep erythrocytes. IgG1 and IgG2a Ab, at subagglutinating dilutions, were used to coat sheep erythrocytes, which, after washing, were incubated in a pool of 129/Sv sera (50, 100, and 200 μl of packed cells in 100 μl of 1/400-diluted serum) for 1 h at 37°C. After centrifugation, the supernates were tested for their residual agglutinating activity. All activity disappeared from the sera incubated with the IgG2a-coated erythrocytes, whereas full activity persisted in the sera having been in contact with IgG1-coated erythrocytes.

IgG from Various Mouse Strains. The specificity of the 129/Sv agglutinator toward IgG from various strains of mice was tested by its reaction with IgG aggregated by heating or adsorbed on polystyrene tubes (Table I). Of 11 strains, 4 had IgG unable

TABLE I

Reaction of 129/Sv Anti-IgG Autoantibody with Murine Igs of Various Allotypic Specificities Expressed by Adsorption on Ig-Coated Polystyrene Tubes or by Inhibition with Heat-Aggregated Igs

Mouse strain	Ig-1 alleles*	Determinants*	Reduction of agglutinating activity after	
			Adsorption on IgG-coated polystyrene tubes	Inhibition by heat-aggregated IgG
			%	%
129/Sv	Ig-1^a	1, 2, 6, 7, 8, 10, 12	100	100
C57BL/6	Ig-1^b	4, 7	0	0
C57BL/10	Ig-1^b	4, 7	0	0
SJL/J	Ig-1^b	4,7	0	0
LP/J	Ig-1^b	4, 7	0	0
DBA/2	Ig-1^c	2, 3, 7	78–95	64
AKR/J	Ig-1^d	1, 2, 5, 7, 12	89	100
A/J	Ig-1^e	1, 2, 5, 6, 7, 8, 12	100	100
CE/J	Ig-1^f	1, 2, 8, 11	100	81
RIII/J	Ig-1^g	2, 3	100	100
SEA/GnJ	Ig-1^h	1, 2, 6, 7, 10, 12	56	100

* Herzenberg listing (15).

to absorb or inhibit the agglutinator. The four negative strains had, according to the classification of Herzenberg et al. (15), the allele Ig-1^b (Table I), characterized by specificities 1.4 and 1.7. All the other strains which had IgG2a reacting with the 129/Sv anti-IgG had either the presence of determinant 1.2 or the absence of 1.4 in common. Hence, the mouse anti-IgG recognizes a determinant linked to the specificity 1.2 or to one antithetic to 1.4.

However, we have observed that the C57Bl/6 IgG, which has the allotype Ig-1^b, showed a peculiar behavior during heat-aggregation or absorption on polystyrene particles. Heating gave a much more opalescent preparation than with IgG of other allotypes. Furthermore, latex suspensions prepared with IgG from C57Bl/6 mice tended to agglutinate spontaneously. It was therefore possible that the determinant recognized by the anti-IgG became inaccessible because of a too strong aggregation or adsorption. For this reason another immunosorbent was tested. We used a C57Bl/6 IgG-Sepharose conjugate and, as a positive control, 129/Sv IgG-conjugate. Whereas the 129/Sv IgG conjugate absorbed 61 or 75% of the agglutinator, C57Bl/6 IgG-conjugate absorbed 0–3%.

Fragments of Murine IgG. We did not succeed in inhibiting the 129/Sv agglutinator by Fc or Fab fragment-coated tubes or by heated Fc fragment. Accordingly, we tested the ability of Sepharose conjugates of IgG fragments to absorb the anti-IgG (Fig. 7). Whereas the IgG-conjugate caused a 90% inhibition of the agglutinating activity, the Fc conjugate caused a 68% inhibition and the Fab conjugate caused 14% inhibition. With another batch of 129/Sv serum, IgG-Sepharose inhibited by 30%, Fc-Sepharose by 23%, and Fab-Sepharose by 3%.

Absence of a General Autoimmune Reaction in 129/Sv Mice. No Ab against native DNA were detected by agglutination of tanned sheep erythrocytes coated with DNA, in

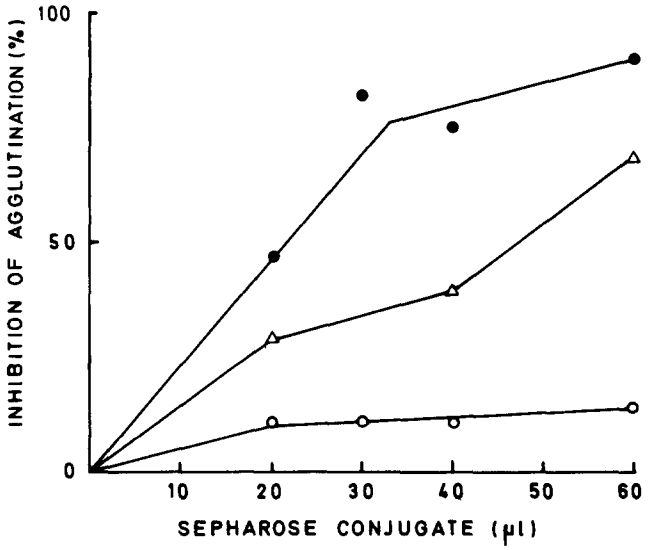

FIG. 7. Inhibition of the agglutinating activity of 129/Sv mouse serum by mouse IgG-, Fc fragment-, or Fab fragment-Sepharose (Seph) conjugates. Samples of 200 µl of 1/200-diluted serum were incubated with increasing volumes of Sepharose in a shaking water bath at 37°C. After centrifugation, the residual agglutinating activity was measured in the supernates. The inhibition was expressed as a percentage of the residual activity after incubation with BSA-Sepharose. The various proteins had been coupled to the Sepharose in a proportion of 2 mg/0.5 ml of packed Sepharose. ●, IgG-Sepharose; △, Fc-Sepharose; ○, Fab-Sepharose.

pools of sera from 129/Sv mice of 4, 10, 20, 29, and 41 wk. The positive control was a serum sample of a patient with systemic lupus erythematosus.

We have also checked whether 10- and 28-wk-old 129/Sv mice had autoAb on their erythrocytes by the Coombs test; no hemagglutination was observed in the presence of rabbit anti-mouse Fab serum, whereas this antiserum caused a clear agglutination of sheep erythrocytes coated with mouse Ab.

Discussion

An autoAb, which is directed against IgG2a and which develops with age, has been detected in a colony of apparently healthy 129/Sv mice by agglutination of murine IgG-coated particles. The anti-IgG was mainly IgM in animals aged <10 wk. Later, the predominant class was IgA without significant contribution of IgG.

In serum, the agglutinator was present as a complex, which was dissociated at acid pH. At pH 2.0, the agglutinator was recovered in an 11 S peak. The latter presumably corresponded to IgA dimers, which represent the major form of IgA in murine serum (16). Quite likely the complex resulted from the association of the autoAb with IgG2a either in monomeric form or bound to an antigen.

The anti-IgG has a very restricted specificity as it fails to react either with murine Ig classes and subclasses other than IgG2a or with IgG from the man, goat, rabbit, rat, and cow. The Ab preferentially reacts with IgG2a when the latter is aggregated or insolubilized. Moreover, it does not react with IgG2a of the Ig-1^b allotype. Further genetic studies are required to see whether the determinants recognized by the anti-IgG are the same as those reacting with alloantisera.

The antigenic determinants recognized by the autoAb seem to be located in the Fc

region as Fc fragments show strong binding and Fab fragments do not. Their determinant is easily masked or altered as shown by the lack of inhibiting activity of 129/Sv IgG aggregated by glutaraldehyde or *bis*diazobenzidine and by the fact that 129/Sv serum did not agglutinate tanned sheep erythrocytes coated with 129/Sv IgG (data not shown).

The heat-stable 129/Sv anti-IgG is clearly different from another thermolabile agglutinating factor also found in 129/Sv mice and described by Cambiaso et al. (13). This factor agglutinates particles coated with heterologous as well as autologous IgG and does not react with reduced and alkylated IgG. Interference by this agglutinating factor was avoided in our experiments by heating all serum samples.

Using a hemolytic-plaque assay, Dresser (1, 2) recently detected cells producing IgM anti-IgG in the spleen of aged CBA mice after immunization with sheep erythrocytes. Some of these Ab react with murine IgG, whereas a different population of Ab is directed against bovine IgG. Further work is necessary to define their relationships with the anti-IgG2a that we have found in 129/Sv mice. The age-dependent production of the anti-IgG could suggest that the loss of suppressor cells with age (17) was responsible for this autoimmune response. However, this hypothesis is not supported by preliminary results of reconstitution experiments.

Recently, we have observed that animals of the 129/Sv strain, but from foreign colonies, did or did not produce this autoAb depending on their origin (J. L. Van Snick and P. L. Masson, manuscript in preparation). It therefore seems that environmental factors are involved in the development of the 129/Sv anti-IgG2a. It must be emphasized that all the tested animals from our 129/Sv colony had the autoAb, that they were kept in specific pathogen-free conditions, and were apparently healthy.

Anti-IgG autoantibodies have been detected, by their ability to agglutinate IgG-coated particles, in a colony of apparently healthy 129/Sv mice. These antibodies belong to the IgA and IgM classes. Their level increases with age, with predominance of IgM in the young and IgA in the adult animals. There is no difference in the level found in males and females. The autoantibodies preferentially react with IgG aggregated by heating, adsorption on polystyrene, coupling to Sepharose, or binding to antigens. The specificity of the autoantibodies is highly restricted. Only mouse IgG is recognized by the autoantibodies as no reaction is detected with human, bovine, rabbit, rat, and goat IgG, and among mouse Ig classes and subclasses, only IgG2a binds the autoantibodies. In addition, in contrast to the other IgG2a allotypes, IgG2a of the Ig-1^b allotype fails to react with the autoantibodies. The relevant antigenic determinants are located in the Fc region and are not destroyed by reduction and alkylation.

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