MONOCLONAL ANTI-IgG AUTOANTIBODIES DERIVED FROM LIPOPOLYSACCHARIDE-ACTIVATED SPLEEN CELLS OF 129/Sv MICE*

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Anti-IgG autoantibodies directed against the constant region of the IgG molecule are regularly found in a number of diseases. They are often collectively called rheumatoid factors (RF),[1] although their specificities tend to vary from one disease to another. In rheumatoid arthritis, for example, reactions have been described with a subclass-specific antigen called Ga, with many different Gm allotypic markers, and with heterologous IgG (1–3). On the contrary, in subacute bacterial endocarditis, anti-IgG autoantibodies are generally useless for Gm-typing and show little, if any, reaction with heterologous IgG (3). Whether these differences correspond to the existence of specific stimuli that vary from one disease to another, or whether they reflect intrinsic differences in the anti-IgG repertoire of individuals that are prone to develop these diseases is not known.

Similar specificity differences have been observed for the anti-IgG autoantibodies spontaneously produced by certain mouse strains in some colonies. These anti-IgG autoantibodies exhibit a restricted specificity for certain mouse IgG subclasses that varies from one strain to another; most bind preferentially to IgG2a but some react better with IgG1 (4, 5). In this case, genetic studies have demonstrated (6) an allotype-linked control of the levels of these autoantibodies and suggested a similar type of control for their specificities. Yet, like for human RF, the actual origin of the specificity differences between mouse anti-IgG autoantibodies remains unknown. In this context, it was of interest to find out whether the narrow specificity of some mouse RF is due either to the existence of highly selective stimuli that trigger only a small number of RF-secreting clones, or to a relative oligoclonality of the mouse RF repertoire.

As a first step in this investigation, we have attempted to identify at the clonal level the RF specificities encoded in the repertoire of a strain, namely the 129/Sv, that's characterized by a spontaneous production of IgG2a-specific RF. We have therefore analyzed the specificities of the monoclonal RF secreted by hybridomas derived from 129/Sv spleen cells that had been polyclonally activated in vivo with a bacterial lipopolysaccharide (LPS). The results demonstrate that RF spontaneously produced by the 129/Sv mice represents only a minor component of a highly diversified

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[1] *Abbreviations used in this paper:* Igh-C, constant region of the Ig heavy chain; Igh-V, variable region of the Ig heavy chain; LPS, lipopolysaccharide; RF, rheumatoid factor.

repertoire. They also show that a large proportion of the B lymphocytes activated by LPS are committed to the secretion of anti-IgG autoantibodies.

Materials and Methods

Mice. 129/Sv mice were maintained in the specific pathogen-free colony of our institute by Dr. Guy Warnier. These mice were originally derived from breeders obtained from Dr. J.-L. Guénet, Institut Pasteur, Paris, France.

Polyclonal Stimulation with LPS. Mice were inoculated intraperitoneally with 50 μg LPS from *Escherichia coli* 055:B5 (Difco Laboratories, Detroit, Mich.) in 200 μl sterile saline. Their spleen cells were used for hybridization 3–4 d later.

Cell Fusion and Culture. Hybrid clones were obtained by fusing LPS-activated spleen cells and SP2/0-Ag-14 myeloma cells (7) at a 10:1 ratio with 30% polyethylene glycol, 1,000 mol wt (BDH, Poole, England), essentially as described by Claflin and Williams (8). The only modification was the use of a thymocyte-conditioned medium to clone the cells. This medium was obtained by culturing 2×10^6 thymocytes from 4-wk-old 129/Sv mice in 1 ml of Dulbecco's modified Eagle's medium supplemented with 30% fetal bovine serum, L-glutamine (1.5×10^{-3} M), L-asparagine (0.24×10^{-3} M), L-arginine (0.55×10^{-3} M), and 2-mercaptoethanol (5×10^{-5} M). After 24 h, the cells were centrifuged and the medium kept frozen at −30°C. Thymocyte-conditioned medium was also used to support the growth of selected clones in mass cultures. The myeloma cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum.

Radioimmunoassays. Polypropylene wells (Removawell; Dynatech, Kloten, Switzerland) were coated by overnight incubation at room temperature with 25 μl of a 10 μg/ml protein solution in diluted glycine-buffered saline (0.02 M glycine, 0.03 M NaCl, pH 9.2). After washing in saline containing 0.1 ml Tween 20 (Technicon Chemicals, Orcq, Belgium) per liter (NaCl-Tween), they were incubated overnight at 37°C either with control culture medium or with medium from hybrid cell cultures. After further washing with NaCl-Tween, they were incubated at 37°C for 4 h with ^{125}I-labeled affinity-purified goat antibodies specific for mouse IgM or IgA. Bound radioactivity was counted after a last series of four washes in NaCl-Tween. The specifically bound radioactivity was calculated by subtracting the counts per minute associated with wells incubated with control medium. As the data obtained in direct binding assays were of the all or none type, they were expressed as specifically bound radioactivity. For competition experiments, we calculated the exact amount of RF bound per well by reference to a standard curve constructed with known quantities of ^{125}I-labeled IgM (MOPC 104E) bound to wells coated with anti-mouse IgM antibodies.

IgG Preparations

MOUSE IgG1. The following three preparations were used: MOPC 31c, a BALB/c myeloma protein, H108B7, a 129/Sv hybridoma obtained from unstimulated 129/Sv spleen cells, and polyclonal IgG1 isolated from the serum of C57Bl/6 mice. All preparations were purified by elution from protein A-Sepharose with 0.1 M citrate buffer at pH 6.0. As checked by double immunodiffusion in agarose gel, these three preparations were free of other subclasses.

MOUSE IgG2a. To test the potential allotypic specificity of anti-IgG hybridomas, we used two IgG2a^a molecules and one IgG2a^b preparation. These were, respectively, MOPC173, a BALB/c IgG2a myeloma, H 1103G4, a 129/Sv hybridoma derived from LPS-activated 129/Sv spleen cells, and polyclonal IgG2a isolated from the serum of SJL/J mice, which have the IgG2a^b allotype. These preparations were obtained by elution from protein A-Sepharose with a 0.1 M citrate buffer, pH 5.0. Whereas no contamination with other subclasses could be detected by double immunodiffusion in the monoclonal IgG2a proteins, IgG1 and some IgG3 were found in the polyclonal IgG2a of SJL mice. The contaminating IgG1 was removed by passage through a rabbit anti-mouse IgG1-Sepharose column. No attempts were made to remove the traces of IgG3 detected in this preparation.

MOUSE IgG2b. We used H308A8, an IgG2b monoclonal protein secreted by a hybridoma derived from LPS-activated 129/Sv spleen cells. This protein was found to be devoid of contaminating subclasses after elution from protein A-Sepharose at pH 3.0.

MOUSE IgG3. We used the BALB/c myeloma protein, FLOPC21, which was isolated by elution at pH 5.0 from protein A-Sepharose and subsequent passage through a rabbit anti-IgG2a-Sepharose column. All BALB/c myeloma cells used in this study were generously provided by Dr. M. Potter (National Institutes of Health, Bethesda, Md.).

RAT IgG SUBCLASSES. Purified rat IgG subclasses were kindly provided by Dr. Hervé Bazin (Experimental Immunology Unit, Université Catholique de Louvain, Brussels, Belgium).

OTHER IgG PREPARATIONS. Bovine IgG1 and IgG2 and chicken immunoglobulins were gifts of Dr. J. P. Vaerman, and human IgG subclasses were provided by Dr. D. Delacroix, both from this laboratory. Goat IgG1 and IgG2 were isolated as described in Delacroix and Vaerman (9) with a slight modification. In brief, goat IgG purified by chromatography on DEAE-cellulose was passed through a protein A-Sepharose column in a 0.1 M phosphate buffer, pH 8. Pure IgG1 was recovered in the flowthrough. After washing with 0.1 M phosphate, pH 6.5, the IgG2 was eluted in 0.1 M citrate buffer, pH 5.9. Purity was checked by double immunodiffusion with subclass-specific rabbit antisera. Rabbit IgG was isolated by precipitation with 50% saturated ammonium sulfate followed by chromatography on DEAE-cellulose.

Heat Aggregation of Immunoglobulins. The various IgG subclasses were dissolved in phosphate-buffered saline (pH 7.5) at a concentration of 5 mg/ml. They were then heated until they became opalescent. The time and temperature needed for aggregation to occur varied widely from one subclass to another, ranging from 10 min at 63°C for mouse IgG3 to ~1 h at 75°C for mouse IgG1.

Fab and Fc Fragments. H108B7 (100 mg) was digested with 1 mg papain (Boehringer, Mannheim, West Germany) at 37°C for 24 h in a 0.1 M phosphate buffer, pH 8, containing 0.001 M EDTA and 0.02 M cysteine. The reaction was stopped by addition of a slight excess of iodoacetamide. The mixture was then submitted to gel filtration on AcA 44 Ultrogel (LKB, Bromma, Sweden). This produced two peaks, the first containing pure Fc fragments, and the second consisting of Fab slightly contaminated with Fc fragments. The Fab fragments were further purified by passage through a protein A column that selectively retained the contaminating Fc fragments.

Latex Agglutination Tests. These were carried out with the Technicon PACIA system (Technicon Instruments Corp., International Division, Geneva, Switzerland) as described previously (6). Polystyrene particles (0.8 μm Diam) were a gift from Rhône-Poulenc (Courbevoie, France). The particles were coated by incubating 250 μg protein and 50 μl of a 10% particle suspension in a total volume of 500 μl of 0.02 M glycine and 0.03 M NaCl pH 9.2 for 45 min at room temperature. After one washing with the same buffer, the particles were resuspended in 1 ml of 0.1 M glycine and 0.15 M NaCl pH 9.2 containing 1% bovine serum albumin. Before use, this suspension was further diluted 10 times in the same buffer.

Results

Hybridomas Secreting Anti-IgG Autoantibodies. Four hybridizations were carried out with spleen cells from 20-wk-old 129/Sv mice that had received an intraperitoneal injection of 50 μg LPS 3–4 d before. For each hybridization, the spleen cells collected from three mice were pooled. After hybridization, the cells were cloned by limiting dilution in 3,000 microtiter wells. 1 wk later, clones of hybrid cells were observed in 500–1,000 wells. When the clones covered at least 20% of the surface of the well, they were tested for the production of IgM anti-IgG autoantibody. The four hybridizations yielded 8, 5, 32, an 23 stable hybrid cell lines, respectively, all secreting anti-IgG autoantibodies. Typical screening data are shown in Table I.

The frequency of anti-IgG secreting clones was ~3% of the total number of hybridomas. From the Poisson distribution, it follows that only ~1.5% of the wells with anti-IgG activity contained more than one anti-IgG secreting clone. Therefore, anti-IgG secreting clones were expanded without further subcloning and their culture medium used directly for the specificity assays.

One hybridization performed with spleen cells from 129/Sv mice not stimulated

TABLE I

Detection of Hybridomas Secreting IgM Anti-Mouse IgG Autoantibodies

Hybridomas	IgM bound to wells coated with	
	BSA*	129/Sv IgG
1413 A3	48 ± 29	520 ± 24
1415 C7	30 ± 31	2,500 ± 13
1415 D9	43 ± 39	2,280 ± 12
1303 A6	7 ± 12	1,750 ± 25

The binding of IgM antibodies to polypropylene wells coated with 10 μg/ml BSA or 129/Sv IgG was determined as described under Materials and Methods. The values represent specifically bound counts per minute and correspond to the mean of three determinations ± 1 SD. The nonspecifically bound radioactivity was 200 and 750 cpm for BSA and IgG-coated wells, respectively.

* Bovine serum albumin.

TABLE II

*Binding of Monoclonal 129/Sv Anti-IgG Autoantibodies to Mouse IgG Subclasses**

Hybridomas	Antibody bound to wells coated with			
	IgG1	IgG2a	IgG2b	IgG3
1301 G3	3,055 ± 244	0	0	0
1305 B4	3,627 ± 1,068	94 ± 20	0	0
1307 A6	2,773 ± 46	0	0	0
1310 B1	3,040 ± 178	0	0	0
1309 G1	1,925 ± 114	0	0	0
1312 A3	1,496 ± 540	0	0	0
1408 G11	2,659 ± 453	0	0	0
1402 H9	1,179 ± 303	0	0	0
1408 F8	1,856 ± 1,000	0	0	0
307 A11	5,870 ± 621	4,439 ± 526	0	0
1302 G1	3,361 ± 140	1,549 ± 819	0	0
1415 C7	3,877 ± 671	1,157 ± 263	0	0
1415 D9	4,716 ± 756	1,531 ± 188	0	0
1303 A6	2,733 ± 891	891 ± 384	0	589 ± 123
1305 A9	0	2,606 ± 430	0	0
102 C6	0	2,638 ± 81	1,036 ± 30	0

* Polypropylene wells were coated with 10 μg/ml of the following IgG preparations: H108 B7, a 129/Sv monoclonal IgG1; MOPC 173, a BALB/c IgG2a myeloma protein; H308A8, a 129/Sv monoclonal IgG2b; and FLOPC 21, a BALB/c IgG3 myeloma protein. The values represent specifically bound counts per minute, obtained after substraction of the nonspecific binding to wells incubated with control medium. The latter amounted to ~600 cpm in all cases. Each value represents the mean of three determinations ± 1 SD. Binding of monoclonal antibodies to these wells was determined by ^{125}I-labeled affinity-purified goat anti-mouse IgM antibodies in all cases except for 102 C6, which was revealed with goat anti-mouse IgA antibodies.

with LPS yielded 160 hybrid clones, only 1 of which secreted an anti-IgG autoantibody. This autoantibody, of the IgA class, was included in the subsequent specificity studies.

Specificity of Monoclonal Anti-IgG Autoantibodies for Mouse IgG Subclasses. The specificities of 68 monoclonal anti-IgG autoantibodies were first tested by measuring their ability to bind to wells coated with various mouse IgG subclasses. The majority of

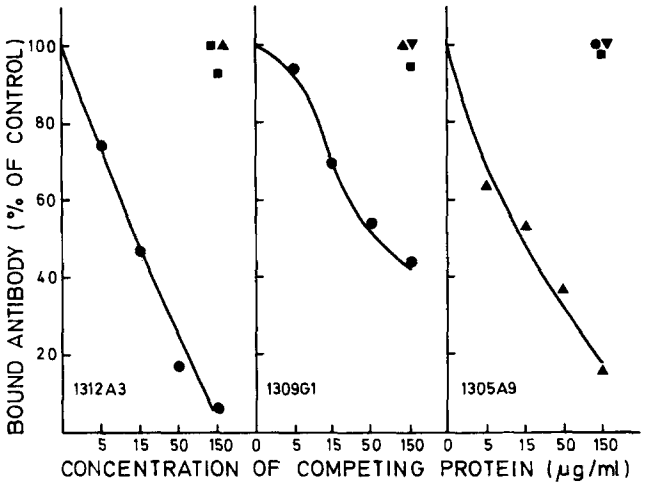

FIG. 1. Reaction of monoclonal anti-IgG autoantibodies with heat-aggregated mouse IgG subclasses. The binding of monoclonal antibodies 1312A3 and 1309G1 to wells coated with IgG1 (H108B7) was measured in the presence of various concentrations of heat-aggregated IgG1 (H108B7, ●), IgG2a (MOPC 173, ▲), IgG2b (H308A8, ▼), and IgG3 (FLOPC21, ■). A similar experiment was carried out with antibody 1305A9 in wells coated with IgG2a (MOPC173).

TABLE III

Allotypic Specificity of Monoclonal 129/Sv Anti-IgG Autoantibodies

Hybridomas	Antibody* bound to wells coated with				
	IgG1[a]	IgG1[b]	IgG2a[a]		IgG2a[b]
	H108B7	Polyclonal‡	MOPC173	1103G4	Polyclonal§
Anti-IgG1 clones					
1301 G3	6,518	6,536			
1307 A6	5,209	5,572			
1408 F8	3,787	2,702			
1408 G4	5,239	4,069			
Anti-IgG2a clones					
1305 A9			1,100	1,390	0
102 C6			1,288	2,077	1,386

* Measured as in Table II.

‡ Isolated from the serum of C57Bl/6 mice.

§ Isolated from the serum of SJL/J mice.

these clones (58 of 68) reacted exclusively with IgG1. This preferential binding was observed with two different IgG1 preparations: MOPC 31C, a BALB/c myeloma protein, and H108B7, a 129/Sv hybridoma of unknown specificity. A second group of eight clones reacted mainly with IgG1 but cross-reacted significantly with IgG2a. One monoclonal antibody bound to IgG1, IgG2a, and IgG3, and one (1305A9) reacted only with IgG2a. The monoclonal anti-IgG autoantibody derived from unstimulated 129/Sv spleen cells (102C6) bound preferentially to IgG2a but cross-reacted with IgG2b (Table II).

Similar specificity patterns were obtained in agglutination assays using polystyrene particles coated with 129/Sv IgG1 and IgG2a. These data, which were confirmed by

TABLE IV

*Relative Binding of Monoclonal Anti-IgG Autoantibodies to Heterologous IgG Subclasses**

Specificity for mouse IgG	Rat				Cow		Goat		Rabbit	Human			
	IgG1	IgG2a	IgG2b	IgG2c	IgG1	IgG2	IgG1	IgG2	IgG	IgG1	IgG2	IgG3	IgG4
Anti-IgG1													
1301G3	2.98	0	0.06	1.98	0	3.03	0	1.69	2.99	0	0.02	0	0.10
1304H2	2.63	0.02	0.09	1.98	0	3.03	0	1.39	2.18	0	0	0	0.03
1312A3	2.09	0	0.10	1.82	0	2.17	0	1.21	2.18	0.02	0.01	0	0
1408G11	2.12	0.05	0.10	1.46	0.02	3.14	0.04	1.51	1.64	0.02	0.01	0	0
1312B12	1.94	0.02	0.40	1.96	0	1.90	0.01	1.06	2.06	0.01	0.01	0	0
1314C10	2.39	0	0.84	1.86	0.01	3.42	0.10	1.32	2.33	0.05	0	0.03	0
1415B12	2.70	0.50	0.07	1.46	0	3.79	0.02	1.75	2.99	0.02	0.02	0.03	0
1408F8	4.04	3.99	2.18	5.65	0.10	7.54	0.23	2.96	4.02	0.11	0.03	0.08	0.02
1307A6	1.67	1.17	0.17	1.06	0.98	2.34	0.44	0.83	1.36	0.02	0.02	0.01	0
1309G1	10.53	0.69	0.27	5.29	0.04	4.41	1.10	5.07	1.41	5.25	3.32	2.19	1.14
Anti-(IgG1 and IgG2a)													
307A11	3.75	0.01	1.11	4.13	0	4.43	0	2.37	3.97	0	0	0	0
302G1	2.56	0	0.49	2.32	0	2.65	0	1.35	2.56	0	0	0	0.03
1415C7	2.31	0.04	0.62	2.09	0	2.52	0.02	1.22	2.09	0.01	0.01	0.02	0
1415D9	2.48	0	0.52	2.08	0	2.50	0	1.32	2.36	0	0	0	0.02
Anti-IgG2a													
1305A9	0	0	0	0	0	0	—	—	0	—‡	—	—	—
Anti-(IgG2a and IgG2b)													
102C6	0	0	0	0	0	0	—	—	0	—‡	—	—	—

* Wells were coated with 10 μg/ml of the following IgG preparations: IR27, a monoclonal rat IgG1; IR1025, a monoclonal rat IgG2a; IR863, a monoclonal rat IgG2B; IR304, a monoclonal rat IgG2c; polyclonal cow IgG1 and IgG2; polyclonal goat IgG1 and IgG2; polyclonal rabbit IgG; To., a monoclonal human IgG1; Me., a monoclonal human IgG2; Ba., a monoclonal human IgG3; and Ro., a monoclonal human IgG4. The relative binding to heterologous IgG was expressed as the ratio between counts per minute specifically bound to heterologous IgG and counts per minute specifically bound to the autologous IgG subclass. Ratios <0.01 were listed as 0. Cross-reactions critical for the definition of individual anti-IgG specificities were boxed.

‡ 1305A9 and 102C6 have been tested separately on wells coated with a pool of human IgG. No binding was observed.

competition experiments with heat-aggregated IgG subclasses (Fig. 1), demonstrated the existence of five different clonotypes of anti-IgG autoantibodies.

Allotypic Specificity of Monoclonal Anti-IgG Autoantibodies. As reported previously, the anti-IgG2a autoantibodies spontaneously produced by 129/Sv mice, which carry the Igh-C^a allele, fail to react with IgG2a of the b allotype, while the anti-IgG1 detected in the serum of certain C57Bl/6 mice which carry the Igh-C^b allele bind nearly equally well to IgG1 of 129/Sv or C57Bl/6 origin (5). It was interesting, therefore, to find out whether the 129/Sv monoclonal anti-IgG autoantibodies would display any allotypic specificity.

Accordingly, we tested the binding of the 129/Sv monoclonal anti-IgG1 autoantibodies to wells coated with polyclonal IgG1 isolated from C57Bl/6 serum, and found it similar to that observed when the wells were coated with H108B7, the monoclonal 129/Sv IgG1 used in the previous experiments. In contrast, monoclonal anti-IgG2a

autoantibody 1305A9 exhibited a strong allotypic specificity, no reaction being detected with IgG2a isolated from the serum of Igh-C^b mice. This allotypic specificity was not observed for 102C6, the antibody reactive with IgG2a and IgG2b (Table III).

Binding of Monoclonal Anti-IgG Autoantibodies to Heterologous IgG Subclasses. Binding to wells coated with 14 different heterologous IgG preparations was measured after appropriate dilution of the culture medium so as to obtain 80% of the maximum binding to the autologous IgG. Representative results are shown in Table IV. The 34 anti-IgG1 monoclonal autoantibodies tested in these experiments all reacted better with rat IgG1 and IgG2c, bovine and goat IgG2, and rabbit IgG, than with mouse IgG1. Four major clonotypes could be distinguished by the presence or absence of additional cross-reactions. Thus, clonotype I (14 antibodies) cross-reacted only with the IgG mentioned above, whereas clonotype II (11 antibodies) reacted in addition with rat IgG2b, clonotype III (5 antibodies) with rat IgG2a, and clonotype IV (4 antibodies) with rat IgG2a and IgG2b. Three additional specificities were determined by the binding of antibody 1307A6 to bovine and goat IgG1, of antibody 1309G1 to goat IgG1 and human IgG subclasses, and of antibody 1405A9 to chicken immunoglobulins (data not shown). Altogether, seven different anti-IgG1 specificities were identified by these cross-reactions.

The monoclonal antibodies that reacted with mouse IgG1 and IgG2a all had the same cross-reaction pattern with heterologous IgG: in addition to the characteristic cross-reactions of all anti-IgG1 autoantibodies with rat IgG1 and IgG2c, bovine and goat IgG2, and rabbit IgG, they all displayed a strong binding to rat IgG2b. In contrast to the extensive cross-reactions of anti-IgG1 antibodies, no cross-reaction was detected between the two anti-IgG2a autoantibodies (102C6 and 1305A9) and heterologous IgG subclasses.

To check some of the data obtained in direct binding assays, we tested the ability of various heat-aggregated IgG preparations to inhibit the binding of monoclonal

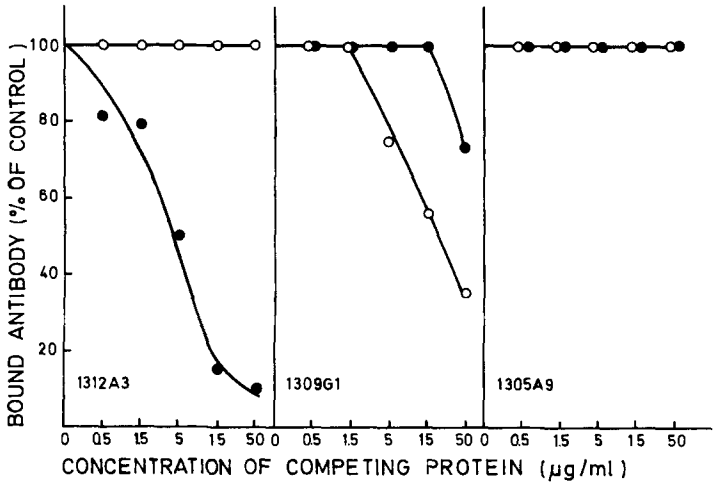

FIG. 2. Reaction of monoclonal anti-IgG autoantibodies with bovine and human IgG aggregated by heating. The binding of monoclonal antibodies 1312A3 and 1309G1 to wells coated with IgG1 (H108B7) was measured in the presence of various concentrations of heat-aggregated bovine IgG2 (●) and human IgG (○). A similar experiment was carried out with antibody 1305A9 in wells coated with IgG2a (MOPC173).

TABLE V

Agglutinating Activity of 129/Sv Monoclonal Anti-IgG Autoantibodies toward Polystyrene Particles Coated with Autologous and Heterologous IgG Subclasses

Hybridomas	Agglutination* of particles coated with					
	Mouse IgG1‡	Mouse IgG2a§	Cow IgG1‖	Cow IgG2‖	Goat IgG1‖	Man IgG‖
1301G3	>80	0	0	>80	0	0
1304H2	>80	0	0	>80	0	0
1312A3	>80	0	0	>80	0	0
1408G11	70	0	0	>80	0	0
1307A6	>80	0	>80	>80	>80	0
1309G1	>80	0	0	>80	0	75
307A11	>80	>80	0	>80	0	0
1415C7	>80	>80	0	>80	0	0
141509	>80	>80	0	>80	0	0
1305A9	0	>80	0	14	0	0
102C6	0	78	0	12	0	0

* Measured by the Technicon PACIA system (6) and expressed in percent of agglutinated particles.

‡ H108B7, monoclonal IgG1 of 129/Sv origin.

§ H1103G4, monoclonal IgG2a of 129/Sv origin.

‖ Polyclonal.

antibodies to wells coated with their autologous target. These competition experiments confirmed the cross-reactions observed in direct binding assays. Three representative experiments are shown in Fig. 2.

The results obtained by the radioimmunoassays were checked further by measuring the agglutinating activities of selected anti-IgG hybridomas toward latex particles coated with various IgG preparations. The results of these agglutination experiments were clear-cut and confirmed the cross-reactions observed in the radioimmunoassays, except that of antibody 1309G1 with goat IgG1 (Table V).

Reaction of Monoclonal Anti-IgG1 Autoantibodies with Mouse IgG1 Fab and Fc Fragments. To localize the antigenic sites recognized by the LPS-induced monoclonal anti-IgG1 autoantibodies, we measured their binding to wells coated with Fab and Fc fragments obtained by papain digestion of a monoclonal IgG1, H108B7. Of 48 anti-IgG1 monoclonal antibodies tested, all but 1 failed to react with either fragment, suggesting that they are directed against antigenic determinants located in the hinge region of the IgG1 molecule. The exception was monoclonal 1405E9, which showed a significant reaction with the IgG1 Fc fragments. Incidentally, this was the only monoclonal that bound to chicken IgG.

Discussion

To analyze the mouse repertoire of anti-IgG autoantibodies, we have attempted to derive hybridomas with anti-IgG activity from the spleen cells of 20-wk-old 129/Sv mice that had been inoculated 3 d before with LPS to polyclonally activate their B lymphocytes. We have obtained 68 hybridomas that produce monoclonal IgM antibodies capable of binding to wells coated with autologous IgG.

This represents ~3% of the total number of hybrids generated in four hybridizations, a frequency not wholly unexpected in view of the observation by Dresser (10) that in old CBA mice most of the plaque-forming cells that secrete IgM after stimulation with endotoxin have RF activity. Also, a high proportion (4%) of the monoclonal IgM occurring in Waldenström's macroglobulinemia have been found to exhibit RF activity (11, 12). This latter finding was taken to suggest that autoantibody-synthesizing clones might be more likely to undergo neoplastic transformation. Such an explanation is obviously irrelevant with respect to the frequency of anti-IgG secreting hybridomas obtained after in vivo stimulation with LPS. Provided LPS-activated spleen cells accurately reflect the total B lymphocyte population, one has therefore to admit that ~1 of 30 B lymphocytes is committed to the production of autoantibodies directed against the constant part of the IgG molecule. This would support previous suggestions (10, 13) that RF might play a physiological role in normal immune responses. It will therefore be of interest to find out whether hybridomas with anti-IgG activity occur at the same frequency in mice that, unlike the 129/Sv, do not spontaneously produce large amounts of anti-IgG autoantibodies.

Besides their frequency, the anti-IgG clones obtained here were characterized by their narrow specificity for individual mouse IgG subclasses. The majority (59 of 68)

TABLE VI

Reactivity Patterns of Monoclonal Anti-IgG Autoantibodies of 129/Sv Mice

IgG preparations	Anti-IgG clonotypes (prototype clone)										
	I (1301G3)	II (1314C10)	III (1415B12)	IV (1408F8)	V (1307A6)	VI (1309G1)	VII (1405A9)	VIII (1415C7)	IX (1303A6)	X (1305A9)	XI (102C6)
Mouse											
IgG1	+	+	+	+	+	+	+	+	+	–	–
IgG2a	–	–	–	–	–	–	–	+	+	+	+
IgG2b	–	–	–	–	–	–	–	–	–	–	+
IgG3	–	–	–	–	–	–	–	–	+	–	–
IgG1 Fc	–	–	–	–	–	–	+	–	–	NT*	NT
Rat											
IgG1	+	+	+	+	+	+	+	+	+	–	–
IgG2a	–	–	+	+	+	+	–	–	–	–	–
IgG2b	–	+	–	+	–	–	–	+	–	–	–
IgG2c	+	+	+	+	+	+	+	+	+	–	–
Cow											
IgG1	–	–	–	–	+	–	–	–	–	–	–
IgG2	+	+	+	+	+	+	+	+	+	–	–
Goat											
IgG1	–	–	–	–	+	(+)‡	–	–	–	–	–
IgG2	+	+	+	+	+	+	+	+	+	–	–
Rabbit											
IgG	+	+	+	+	+	+	+	+	+	–	–
Human											
IgG1	–	–	–	–	–	+	–	–	–	–	–
IgG2	–	–	–	–	–	+	–	–	–	–	–
IgG3	–	–	–	–	–	+	–	–	–	–	–
IgG4	–	–	–	–	–	+	–	–	–	–	–
Chicken											
IgG	–	–	–	–	–	–	+	–	–	–	–

* Not tested.

‡ Positive in radioimmunoassay only.

were indeed specific for a single mouse IgG subclass. Most reacted exclusively with IgG1, only two were specific for IgG2a, and none was found to react specifically with either IgG2b or IgG3. This predominance of anti-IgG1 clones was totally unexpected in view of the spontaneous production of anti-IgG2a by the 129/Sv mice used in these experiments. One could argue that the low anti-IgG2a frequency was precisely due to the continuous removal of anti-IgG2a precursors from the pool of cells responsive to LPS. This explanation, however, seems unlikely, as we observed the same anti-IgG1 predominance in RF-negative 129/Sv mice (data not shown). The significance of these anti-IgG autoantibodies remains, at present, totally mysterious, and any speculation about their potential role has to await more information on their capacity to be activated in vivo. It is however intriguing that all the anti-IgG autoantibodies detected in our experiments were primarily directed against IgG1 or IgG2a, which together represent ~80% of the mouse immunoglobulins.

The narrow specificity of these anti-IgG autoantibodies for individual mouse IgG subclasses contrasted sharply with the extensive cross-reactions of all anti-IgG1 clones with rat IgG1 and IgG2c, bovine and goat IgG2, and rabbit IgG; this finding is reminiscent of some human RF that react better with rabbit than with human IgG (14). The antigenic similarity of these IgG subclasses and mouse IgG1 had not been recognized with conventional xenoantisera. A likely explanation for this observation is that these reactions involve highly conserved structures that are also present on the IgG of the immunized animal. Monoclonal anti-IgG autoantibodies might thus be of interest in the study of phylogenic relationships between immunoglobulins.

Altogether, analyses of the specificities of the monoclonal RF obtained after polyclonal activation of 129/Sv B lymphocytes identified 11 different RF clonotypes (Table VI). This figure is most certainly an underestimation of the actual number of RF specificites encoded in the repertoire of these mice because 6 of 11 clonotypes were represented by single antibodies. In view of this great diversity, it is not surprising that the RF activity detected in the serum of animals injected with LPS shows little specificity for any particular IgG subclass (15). Our data strongly suggest that this is due to the simultaneous activation of a large number of different anti-IgG clones, rather than to an intrinsic lack of specificity of LPS-induced RF.

The RF spontaneously produced by 129/Sv mice, which carry the Igh-C^{aa} genotype, binds preferentially to mouse IgG2a but fails to react with either IgG2a of the b allotype or heterologous IgG subclasses (4). Only 1 of the 11 RF clonotypes identified in the present study (1305A9) displays such a specificity. This clearly demonstrates that in vivo only a minor part of the RF repertoire is activated in these mice. What actually determines the in vivo specificity of RF remains unknown. We have previously shown (6) that the subclass specificity of the RF found in the progeny of various (129/Sv × C57Bl/6) crosses varies according to the Igh-C genotype of these mice, Igh-C^{aa} mice producing mainly anti-IgG2a, and Igh-C^{bb} mice mainly anti-IgG1. However, it was not clear whether this regulation was controlled by the constant region of the Ig heavy chain (Igh-C) locus itself, or by a closely linked locus such as the variable region of the Igh (Igh-V) locus. The present observation that mice, which actively produce anti-IgG2a, have many silent anti-IgG1 clones indicates that the in vivo specificity of RF does not just reflect the repertoire of RF-encoding Igh-V genes associated with a particular Igh-C allele.

The production of autoantibody-secreting hybridomas, as illustrated for RF in the

present work, might be useful to investigate the origin of autoimmune reactions in general. It is indeed theoretically possible to unmask the whole autoimmune repertoire by deriving hybridomas from LPS-activated spleen cells. Comparison of the autoantibodies actually produced in certain diseases to those potentially available from the repertoire might help to determine whether particular autoimmune reactions are induced by specific or polyclonal stimuli.

Summary

In some colonies, 129/Sv mice produce, upon aging, a rheumatoid factor (RF) that is specific for mouse IgG2a but fails to react with IgG2a of the b allotype. It is not known whether this narrow specificity is due to the absence of other RF specificities in the repertoire of these mice or to the selective activation of the production of anti-IgG2a autoantibodies by a specific stimulus. To analyze the RF repertoire of 129/Sv mice, we have derived hybridomas from their spleen cells 3 d after an intraperitoneal injection of lipopolysaccharide. We have obtained 68 hybridomas secreting a monoclonal IgM with RF activity. This represents ~3% of the total number of hybridomas generated in four hybridizations. In addition, one monoclonal IgA RF was derived from unstimulated 129/Sv spleen cells.

The specificities of these monoclonal RF were examined by testing their ability to bind to a panel of homologous and heterologous IgG preparations. The majority of the IgM RF reacted exclusively with a single mouse IgG subclass: 58 with IgG1, and 1 with IgG2a. Eight bound preferentially to IgG1 but cross-reacted to some extent with IgG2a and one was specific for a determinant shared by IgG1, IgG2a, and IgG3. The IgA RF derived from unstimulated spleen cells was primarily directed against IgG2a but cross-reacted somewhat with IgG2b. Identical results were obtained with two different monoclonal IgG1 and IgG2a proteins of the a allotype. No allotypic specificity was found for the anti-IgG1 RF, which all reacted well with IgG1 of the b allotype. In contrast, the IgM anti-IgG2a antibody exhibited such allotypic specificity because it failed to react with IgG2a of the b allotype. When tested on heterologous IgG preparations, all anti-IgG1 RF reacted better with rat IgG1, rat IgG2c, bovine IgG2, goat IgG2, and rabbit IgG than with mouse IgG1, demonstraing a particular homology between these Ig. On the basis of additional cross-reactions with other IgG, including rat IgG2a, rat IgG2b, bovine IgG1, goat IgG1, human IgG, and chicken IgG, seven different anti-IgG1 clonotypes could be identified. However, despite their heterogeneity, nearly all antigenic determinants recognized by anti-IgG1 RF appeared to be located in the hinge region of the molecule. Total lack of binding to IgG1 Fab fragments was indeed observed, and only one antibody reacted with IgG1 Fc fragments. Unlike the anti-IgG1 RF, the IgM and the IgA anti-IgG2a antibodies did not cross-react with any heterologous IgG of the same panel.

Altogether, 11 different RF clonotypes could be distinguished on the basis of their fine specificity. The anti-IgG2a specificity of the RF spontaneously produced by 129/Sv mice is thus not due to the absence of other RF specificities in the repertoire of these mice.

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