RHEUMATOID FACTORS IN 129XB RECOMBINANT INBRED STRAINS

Igh-1–Linked Control of Allotypic and Isotypic Specificities

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The interactions between rheumatoid factors (RF)[1] and homologous IgG have now been studied extensively in man and mouse, and molecular localization of a number of antigenic determinants recognized by these RF has even been achieved (1–9). However, the low affinity of RF-IgG interactions and the intriguing binding of certain human RF to DNA-histone complexes (10–13) have raised the possibility that RF might actually be produced in response to foreign antigens fortuitously cross-reactive with autologous IgG. Similar cross-reactions could be responsible for the unusually high frequency of RF-secreting clones observed in populations of polyclonally activated murine B lymphocytes (14–15).

The narrow specificity of murine RF for subclass- and occasionally for allotype-specific structures, however, is difficult to explain on this basis and rather suggests that mouse RF represent genuine anti-IgG autoantibodies.

To test the latter hypothesis, we took advantage of the allotypic specificity of certain RF spontaneously produced by mice of the 129/Sv strain. These mice often have high titers of autoantibodies specific for IgG2a, the majority of which, however, fail to react with IgG2a when the latter is encoded by the Igh-1^b allele (16). The development of recombinant inbred (RI) strains between 129/Sv and C57BL/6 mice, which carry the Igh-1^a and Igh-1^b alleles, respectively, provided us with the opportunity to test the involvement of autologous IgG in the induction of RF by comparing RF allotypic specificities with autologous allotypes (17). In addition, the influence of Igh-1–linked genes on immunoglobulin class and isotypic specificity of RF could also be examined with these strains, since C57BL/6 mice essentially have IgM anti-IgG1 RF while 129/Sv almost exclusively produce IgG2a-specific RF that predominantly belong to the IgA class.

Materials and Methods

Mice. 129/Sv, C57Bl/6, and 129XB RI were derived from the breeding stocks of the Institut Pasteur, Paris, France. Animals were tested for RF production when >20 wk old.

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[1] *Abbreviations used in this paper:* RF, rheumatoid factor(s); RI, recombinant inbred; RIA, radioimmunoassay.

Only female mice were used in the present study.

Proteins. IgG was isolated from ascites fluid or serum by affinity chromatography on Protein A Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) occasionally followed by immunoabsorption with rabbit antibodies made specific for mouse IgG subclasses and covalently coupled to aminated Sepharose (18). IgG preparations used for solid phase coating in radioimmunoassay (RIA) were further purified by ultracentrifugation over a sucrose gradient to remove any contaminating IgM or IgA. IgA and IgM were purified by gel filtration on AcA22 (LKB Produkter, Bromma, Sweden) and Pevikon-block electrophoresis (Serva, Heidelberg, Federal Republic of Germany). The origin and characteristics of the monoclonal proteins used here are indicated in Table I.

Allotype Typing. The a and b allotypic forms of IgG2a were detected as described previously (19).

RF Determinations. RF levels and specificities were determined either by solid phase RIA or by agglutination reactions as described previously (7, 19). The only modification introduced in the present experiments was the use of monoclonal IgA and IgM RF to calibrate the assays.

Determination of Immunoglobulin Concentrations. IgA and IgG subclasses were measured by a solid-phase competition RIA using ^{125}I-labeled monoclonal IgA (TEPC15), IgG1 (108B7), IgG2a (1103G4), IgG2b (308A8), and IgG3 (FLOPC21) and polyvinyl wells coated with class- or subclass-specific rabbit antibodies. The rabbit antibodies routinely used to measure IgG2a in our laboratory failed to detect IgG2a of the b allotype. The latter was assayed using wells coated with BALB/c antibodies directed against C57BL/6 IgG2a and a ^{125}I-labeled IgG2a^b monoclonal protein (A7708F1). IgM levels were determined by a radioimmunometric assay with ^{125}I-labeled affinity-purified rabbit antibodies against mouse IgM. The degree of cross-reaction observed in these assays was <3%.

Results

Allotypic Specificity of Anti-IgG2a RF in 129XB Strains. It was previously shown, in solid phase RIA with IgG2a^a- and IgG2a^b-coated polyvinyl wells, that ~80% of the monoclonal anti-IgG2a RF derived from 129/Sv spleen cells fail to react with IgG2a of the b allotype (7). This allotypic specificity was confirmed in agglutination-inhibition experiments with heat-aggregated IgG2a of either allotype and IgG2a^a-coated polystyrene particles. The latter approach was used here to determine the allotypic specificity of RF in the serum of 129/Sv, C57BL/6,

TABLE I

*Isotype and Origin of Monoclonal Proteins**

Protein	Isotype	Specificity	Strain of origin
108B7	IgG1, κ	—	129/Sv
A6204G12	IgG1, κ	Anti-DNP	129/Sv
MOPC173	IgG2a^a, κ	—	BALB/c
1103G4	IgG2a^a, κ	—	129/Sv
A6202F4	IgG2a^a, κ	Anti-DNP	129/Sv
A7708F11	IgG2a^b, κ	—	C57Bl/6
A7708F1	IgG2a^b, κ	—	C57Bl/6
308A8	IgG2b, κ	—	129/Sv
FLOPC21	IgG3, κ	—	BALB/c
A2901D2	IgM, κ	Anti-IgG2a	129/Sv
A2902C1	IgA, κ	Anti-IgG2a	129/Sv

* With the exception of MOPC173 and FLOPC21, which were kindly provided by Dr. M. Potter (National Institutes of Health, Bethesda, MD), all these proteins were produced by hybridomas derived in our laboratory by fusion of spleen cells with SP2/0-Ag-14 cells (20).

and 129XB RI mice. Appropriate dilutions of these sera were preincubated for 1 h at 37°C with increasing concentrations of heat-aggregated IgG2a monoclonal proteins: two of the a allotype, MOPC173 and A6202F4, and two of the b allotype, A7708F1 and A7708F11. Polystyrene particles coated with a third monoclonal IgG2a^a protein, 1103G4, were then added and, after 1 h more at 37°C, residual agglutinating activities were measured by instrumentally counting nonagglutinated particles. With this technique it was possible to determine the specificity of RF even in low-titered sera. For 129/Sv mice, which carry the Igh-1^a allele, complete inhibitions were easily obtained with IgG2a^a but not with IgG2a^b aggregates. In contrast, for C57BL/6 mice, which carry the Igh-1^b allele, both IgG2a^a and IgG2a^b were potent competitors, which indicates that most C57BL/6 RF reacted with IgG2a^a-coated particles via structures that are shared by both IgG2a allotypes. When the same assay was used to determine allotypic specificities in 129XB RI strains, it was found that Igh-1^b mice invariably had RF of the C57BL/6-type, whereas five of six Igh-1^a strains produced RF with the same allotypic specificity as 129/Sv mice (Fig. 1 and Table II). Attempts were also made to find out whether Igh-1^b strains produced IgG2a^b-specific RF in addition to the already detected nonallotypic anti-IgG2a autoantibodies. Unfortunately, these efforts were frustrated by our failure to produce stable suspensions of particles coated with IgG2a^b molecules.

We therefore tried to determine allotypic specificities by solid phase RIA with IgG2a^a- and IgG2a^b-coated polyvinyl wells. The levels of IgA and IgM antibodies reactive with such wells are shown in Fig. 2 together with other RIA data. These experiments confirmed the allotypic specificity of anti-IgG2a autoantibodies in Igh-1^a strains with high RF titers. For example, only 1% of the IgG2a-specific

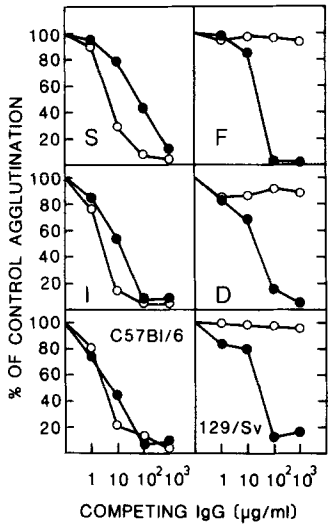

FIGURE 1. Allotypic specificity of anti-IgG2a autoantibodies in 129/Sv, C57BL/6, and 129XB RI strains. Inhibition of the agglutination of IgG2a^a (1103G4)-coated polystyrene particles in the presence of heat-aggregated IgG2a^a (A6202F4, ●) and IgG2a^b (A7708F1, ○). Sera diluted so as to obtain control agglutinations of ~80% were preincubated with the aggregates for 1 h at 37°C. 129/Sv and 129XB RI strains F and D carry the Igh-1^a allele; C57BL/6 and 129XB RI strains S and I the Igh-1^b allele.

TABLE II

Allotypic Specificity of Anti-IgG2a RF in 129/Sv, C57Bl/6, and 129XB RI Strains

Strain*	Igh-1 allele	Concentration (μg/ml) of heat-aggregated IgG2a required to inhibit by 50% agglutinations of IgG2a^a-coated polystyrene particles:‡			
		IgG2a^a		IgG2a^b	
		MOPC173	A6202F4	A7708F11	A7708F1
129/Sv	a	18	28	>1,000	>1,000
C57Bl/6	b	2.3	3	3	21
129XBD	a	12	22	>1,000	>1,000
129XBF	a	17	25	>1,000	>1,000
129XBG	a	0.2	20	5	9
129XBK	a	2.1	4	>1,000	>1,000
129XBQ	a	2.4	5	>1,000	>1,000
129XBR	a	8.1	11	>1,000	>1,000
129XBH	b	1.7	0.6	3	8
129XBI	b	0.1	10	3	6
129XBN	b	1.6	6	1	8
129XBS	b	0.4	6	4	31
129XBT	b	3.9	32	3	4

* Pooled sera of 5–10 female mice of each strain were diluted so as to agglutinate a 0.05% (vol/vol) suspension of polystyrene particles coated with IgG2a^a monoclonal protein 1103G4 by 70–80% after 1 h incubation at 37°C.

‡ Appropriately diluted sera were preincubated for 1 h at 37°C with increasing concentrations (1, 10, 100, and 1,000 μg/ml) of heat-aggregated IgG2a before addition of polystyrene particles. The concentrations required to inhibit agglutinations by 50% were calculated by interpolation on graphs like those presented in Fig. 1.

RF of 129XBR mice bound to wells coated with IgG2a^b. However, for a number of strains and especially for Igh-1^b strains, it was not possible to make significant comparisons between the amounts of IgG2a^a- and IgG2a^b-reactive antibodies because the levels of anti-IgG2a RF were too low. Nevertheless, in some, like the 129XBI, it seemed that IgG2a-specific RF reacted better with IgG2a^b than with IgG2a^a. To find out whether this small difference could reflect the existence of a subset of anti-IgG2a antibodies that would specifically react with IgG2a^b allotypic markers, competition RIA were carried out with various heat-aggregated immunoglobulins. These experiments confirmed the previously described isotypic specificity of mouse RF (7), and demonstrated that the binding of a significant proportion of IgG2a-specific RF of 129XBI mice to IgG2a^b-coated wells could be inhibited by IgG2a^b aggregates only (Table III). It thus appeared that strain 129XBI produced two kinds of IgG2a-specific RF:one reactive with isotype-specific structures shared by IgG2a^a and IgG2a^b and one specific for the allotypic markers of IgG2a^b.

RF Isotype and Isotypic Specificity in 129XB Strains. The Igh-1 genotypes of 129XB RI strains also affected the immunoglobulin class as well as the isotypic specificity of RF, although here their influence was more variable than on allotypic specificities. When IgA and IgM autoantibodies were separately measured by solid phase RIA, it appeared that IgA anti-IgG2a autoantibodies

TABLE III
Isotypic and Allotypic Specificities of RF in 129XBI Mice

Inhibitors	IgM* bound to wells coated with		
	IgG1	IgG2a^a	IgG2a^b
—	12,400	6,800	9,200
IgG1	1,100	6,100	11,000
IgG2a^a	13,200	1,500	9,700
IgG2a^b	11,800	1,900	1,800

* Serum pooled from Igh-1^b 129XBI females was diluted 1/50 in Tris-buffered saline containing 5% fetal bovine serum and incubated with 100 μg/ml (final concentration) of heat-aggregated IgG1 (A6204G12), IgG2a^a (MOPC173), and IgG2a^b (A7708F11). After 2 h at 37°C samples were transferred to wells coated with IgG1 (108B7), IgG2a^a (1103G4), and IgG2a^b (A7708F11) and left for 1 h at 37°C. After washing, the plates were further incubated at 37°C for 2 h with ^{125}I-labeled affinity-purified rabbit anti-mouse IgM antibodies.
Figures correspond to the mean of triplicate measurements and represent specifically bound cpm obtained after subtraction of the radioactivity of wells coated with IgG but not incubated with mouse serum.

represented the predominant RF species in most Igh-1^a strains whereas, in Igh-1^b strains, most RF belonged to the IgM class, were specific for IgG1, and showed no allotypic specificity (Fig. 2). Conversely, IgA anti-IgG2a was virtually absent from Igh-1^b sera and very little IgM anti-IgG1 could be detected in Igh-1^a strains. IgA anti-IgG1 and IgM anti-IgG2a levels were generally low and showed little variation from one strain to another, except for the very high IgM anti-IgG2a titers found in Igh-1^a strain 129XBF. From the data presented in Fig. 2 it was possible to calculate ratios of IgG2a- over IgG1-specific RF taking into account both IgA and IgM antibodies. The differences between Igh-1^a and Igh-1^b mice which were barely significant for strains with low RF titers became quite considerable for those with high titers (Fig. 3). This clearly illustrated the influence of the Igh-1 genotype on RF isotypic specificity. In contrast to their strong influence on the specificity and immunoglobulin class of RF, Igh-1–linked genes had no clear effect on total RF levels despite considerable variations of the latter from one strain to another (Table IV).

Immunoglobulin Levels in 129XB Strains. To find out whether strains with high RF titers would have higher Ig levels as well, we measured the serum concentration of IgA, IgM, and IgG subclasses. While no significant correlation was observed between the concentrations of any Ig and the levels of RF in individual strains, it was found that IgA and IgG2a concentrations were significantly higher in Igh-1^a than in Igh-1^b strains ($p < 0.05$ by Mann-Whitney rank test) (Fig. 4).

Discussion

The role of autologous IgG in the induction of human RF has been difficult to establish partly because of the many cross-reactions of RF with heterologous IgG. The binding of certain human RF to antigens apparently unrelated to IgG has even led to the idea that the reaction of RF with IgG was due to fortuitous cross-reactions (10–13). In the mouse, RF display a much narrower specificity: they usually react with only one IgG subclass (7, 21–23) and in some cases they

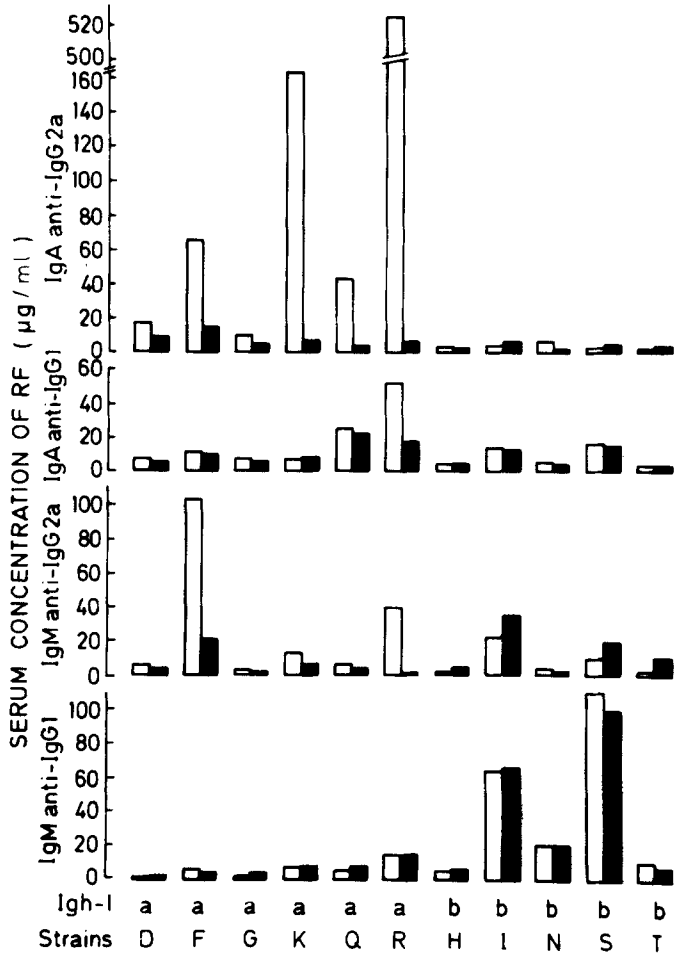

FIGURE 2. Isotypes and specificities of RF in 129/Sv, C57BL/6, and 129XB RI strains. The concentrations of IgA and IgM RF in pooled sera obtained from 5–10 female mice for each strain were determined by solid phase RIA as described in Materials and Methods. Anti-IgG1 autoantibodies were measured with wells coated with a monoclonal IgG1 of 129/Sv origin (108B7, open bars) or with polyclonal IgG1 isolated from the serum of SJL/J mice (closed bars). For anti-IgG2a autoantibodies, four monoclonal proteins were used to coat the wells: two that carried "a" allotypic markers (A6202F4 and 1103G4) and two with "b" allotypic markers (A7708F1 and A7708F11). The results shown here correspond to the mean of the data obtained for either allotype (IgG2a^a, open bars; IgG2a^b, closed bars). Calibration curves for these assays were constructed with monoclonal IgA and IgM RF of 129/Sv origin: A2902C1 and A2901D2, respectively.

exclusively recognize allotypic markers (7).

We have taken advantage of the latter property to assess the role of autologous IgG in the induction of mouse RF. We therefore analyzed the allotypic specificity of the RF produced by 11 recombinant inbred strains that were derived from strains expressing different allotypes, namely 129/Sv (Igh-1^a) and C57BL/6 (Igh-1^b). As previously reported, 129/Sv mice spontaneously produce large amounts of RF specific for IgG2a, ~80% of which fail to react with IgG2a of the b allotype

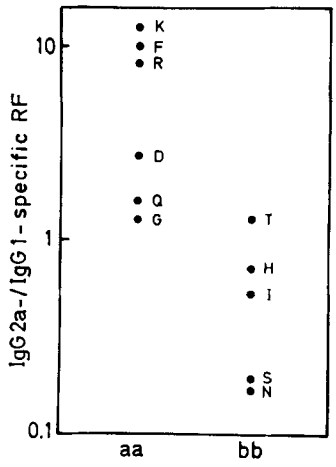

FIGURE 3. Isotypic specificity of RF as a function of Igh-1 genotype. The ratios of IgG2a- over IgG1-specific RF were calculated by cumulating the concentrations of IgA and IgM antibodies reactive with IgG1 or IgG2a of isologous allotypes. The letters identify corresponding 129XB RI strains.

TABLE IV

Total RF Levels and Igh-1 Genotypes in 129XB Strains

Strain*	Igh-1 allele	RF levels‡
		μg/ml
129XBR	a	631
129XBK	a	190
129XBF	a	186
129XBS	b	137
129XBI	b	121
129XBQ	a	79
129XBD	a	30
129XBN	b	28
129XBT	b	23
129XBG	a	23
129XBH	b	17

* Sera were pooled from 5–10 female mice.

‡ Total RF levels correspond to the cumulated concentrations of IgA and IgM autoantibodies specific for isologous IgG1 and IgG2a.

(7, 16). If this allotypic specificity were due to cross-reactions between $IgG2a^a$ and a foreign antigen, one would expect that, whatever their own allotype, different 129XB RI strains would produce RF with the same specificity when they were exposed to the same environmental factors. The data presented here clearly indicate that this was not the case. In every strain where allotype-specific RF were detected they were found to specifically react with isologous IgG2a:$IgG2a^a$-specific RF were detected in most Igh-1^a strains but never in Igh-1^b animals, and conversely the existence of anti-$IgG2a^b$ RF was demonstrated only in strain 129XBI, which carries the Igh-1^b genotype.

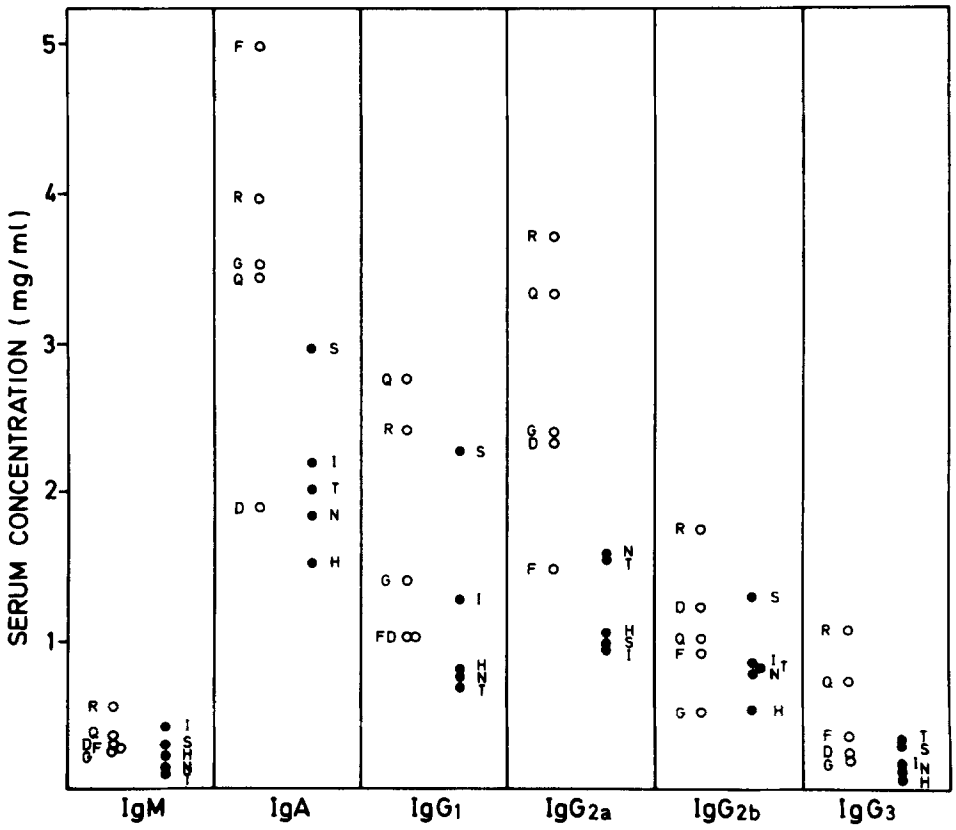

FIGURE 4. Immunoglobulin levels in the serum of 129XB RI strains. Ig levels were measured by RIA in the serum pools used for the RF determinations presented in Fig. 2. Circles and dots correspond to Igh-1^a and Igh-1^b strains, respectively. The letters identify individual 129XB RI strains.

Not all anti-IgG2a autoantibodies were allotype-specific: many reacted with structures that are not affected by allotypic variation. Such isotype-specific anti-IgG2a RF predominated in most Igh-1^b strains and in one Igh-1^a strain, namely 129XBG. The latter further differed from other Igh-1^a strains by its very low titers of anti-IgG2a RF, a characteristic shared by most Igh-1^b strains. Although this observation might be purely coincidental, it raises the possibility that allotype-specific RF are only produced in the course of strong RF responses. Anyhow, the lack of allotypic specificity of the RF produced by 129XBG mice demonstrates that, although necessary, the presence of IgG2a^a is not sufficient to trigger RF responses directed against "a" allotypic markers. In this context, it is of interest to note that, despite their Igh-1^a genotype, 129XBG mice immunized with α (1 $\rightarrow$ 6) dextran produce antibodies with idiotypes characteristic of the C57BL/6 strain.[2] This would suggest that, in the presence of a given allotypic form of IgG2a, the fine specificity of anti-IgG2a RF is controlled by genes similar to those that govern idiotype expression.

The predominant isotypic specificity of RF also varied as a function of the Igh-

[2] Yvars, F., D. Holmberg, A. Coutinho, and P. A. Cazenave. Manuscript in preparation.

1 genotype of the strain: in strains with high RF levels, the ratio of IgG2a- over IgG1-specific RF was ~20 times larger in Igh-1^a than in Igh-1^b strains. It was recently demonstrated that virtually all mouse RF are directed against subclass-related structures and that RF specific for the three major IgG subclasses (γ1, γ2a, and γ2b) exist in the mouse repertoire (7). The present observation that, depending on their Igh-1 genotype, 129XB RI strains preferentially activate either γ1- or γ2a-specific RF strengthens the idea that subclass-specificity may be of importance for the in vivo activation of mouse RF. In this context, it is of interest that Igh-1^a strains, which make RF specific for IgG2a, have a selective increase of serum IgG2a levels when compared with Igh-1^b strains.

Summary

To examine the role of autologous IgG in the induction of murine rheumatoid factors (RF) we have analyzed the allotypic specificity of anti-IgG2a RF in recombinant inbred strains derived from 129/Sv (Igh-1^a) and C57BL/6 (Igh-1^b) mice.

In five of six Igh-1^a strains, anti-IgG2a RF reacted with IgG2a^a but failed to react with IgG2a^b. In contrast, isotype-specific RF, which reacted equally well with a and b allotypes of IgG2a, represented the major RF species in one Igh-1^a and all five Igh-1^b strains tested. An additional form of RF specific for IgG2a^b and not reactive with IgG2a^a was detected in one Igh-1^b strain. RF specific for a given allotype was thus only found in the presence of that allotype, which strongly suggests the involvement of autologous IgG in the induction of mouse RF synthesis.

The specificity of RF was apparently further controlled by genes linked to but different from the Igh-C locus, as indicated by the absence of IgG2a^a-specific RF in one of the 6 Igh-1^a strains tested. Because this strain, 129XBG, has been shown to express idiotypic markers characteristic of Igh-1^b mice, it is likely that the genes, which in the presence of a given allotype induce the production of isotype rather than allotype-specific RF, are identical to those that control the expression of idiotypes.

Evidence was also obtained to indicate that Igh-1-linked genes influence the isotypic specificity and the isotype of RF itself: IgA anti-IgG2a predominated in Igh-1^a strains and IgM anti-IgG1 in Igh-1^b strains. Interestingly enough, total IgA and IgG2a levels also were higher in Igh-1^a than in Igh-1^b strains.

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