INCIDENCE AND SPECIFICITIES OF IgA AND IgM ANTII-IgG AUTOANTIBODIES IN VARIOUS MOUSE STRAINS AND COLONIES

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We reported previously that 129/Sv mice from our specific pathogen-free (SPF) colony produce IgM and IgA autoantibodies (autoAb) specific for the Fc region of IgG2a. Their titers increase with age until ~20 wk. These antibodies preferentially bind IgG2a that has been aggregated by heating, by adsorption on polystyrene, or by binding to antigen. Their specificity is highly restricted as no reaction is detected with IgG from other species or with mouse immunoglobulins other than IgG2a. In addition, their binding to IgG2a shows an allotypic restriction as they fail to react with IgG2a of the Ig-1b allotype (1).

In this work, we examined whether mice from other strains also produced anti-IgG autoAb upon aging, and whether this production was influenced by the environment. Our results indicated that environmental factors were responsible for the induction of anti-IgG but that, in a given colony, major differences existed between anti-IgG responses of individual strains.

In the second part of this work we compared the specificities of the anti-IgG detected in the various strains with the specificity of the 129/Sv anti-IgG. We observed that in most strains IgA anti-IgG was specific for IgG2a whereas, in general, IgM anti-IgG reacted not only with all mouse IgG classes and subclasses but also with IgG from other species.

Materials and Methods

Mice. All mice from our institute (International Institute of Cellular and Molecular Pathology, Université Catholique de Louvain, Brussels, Belgium) (ICP) were maintained in SPF conditions by Dr. G. Warner. These mice were derived from breeders obtained from Dr. J. L. Guenet, Institut Pasteur (IP), Paris, France, who also kindly provided adult SPF C57BL/6, 129/Sv, and DBA/2, and germ-free DBA/2. The other SPF mice were purchased from Iffa Credo (IC), Les Oncins, Lyon, France, and from The Jackson Laboratory (JL), Bar Harbor, Maine. It was previously shown that the anti-IgG titer of 129/Sv mice from ICP increases with age until ~20 wk. Accordingly, all mice of this study were >20 wk old. Animals from JL were retired breeders, animals from ICP and IP were virgin mice, those from IC were of both kinds. The strains from ICP, i.e., C57BL/6, BALB/c, DBA/2, C3H/He, and 129/Sv, were checked by Microbiological Associates, Walkersville, Md. for antibodies against common murine viruses. Hemagglutination inhibition failed to reveal any reaction against

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Abbreviations used in this paper: Ab, antibody; agg. U, agglutination unit; autoAb, autoantibody; IC, Iffa Credo, ICP, International Institute of Cellular and Molecular Pathology; IP, Institut Pasteur de Paris; JL, The Jackson Laboratory; RIA, radioimmunoassay; SPF, specific pathogen-free.
pneumonia virus, reovirus type 3, encephalomyelitis virus (GDV II), K virus, polyoma virus, mouse hepatitis virus, and ectromelia virus. No antibodies against Sendai virus, mouse adenovirus, mouse hepatitis virus, and lymphocytic choriomeningitis virus were detected by complement fixation. However, the serum from C3H/He mice had an anticomplementary activity.

**Immunoglobulins.** The myelomas were obtained from the National Institutes of Health, Bethesda, Md. and transplanted in our laboratory. Immunoglobulins were isolated as described previously (1). IgG preparations used to coat polystyrene wells (Removawell; Dynatech, Kloten, Switzerland) for the radioimmunoassay (RIA) were separated from contaminating IgA and IgM by ultracentrifugation on a linear sucrose gradient.

**Techniques.** The preparation of IgG-coated latex, the agglutination measurement by the particle-counting technique, and the preparation of Ig-Sepharose (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) or Ig-polystyrene conjugates for absorption experiments were as in reference 1. The agglutinating activity was expressed in agglutination units (agg. U)/μl corresponding to the inverse of the volume of serum (in μl) required to reduce the number of particles by 50%. IgA and IgM anti-IgG were measured by RIA as described previously (1) with two modifications: polystyrene wells were coated with 50 μl of a 30-μg/ml solution of IgG instead of 150 μl of a 10-μg/ml solution, and mouse serum was incubated with the IgG-coated wells for 24 h instead of 4 h.

To determine the immunoglobulin levels, polystyrene wells were coated by overnight incubation with 150 μl of a 0.3-μg/ml solution of either 129/Sv IgG, MOPC 104E IgM, or TEPC 15 IgA in 20 mM glycine-NaOH buffer, pH 9.2, containing 30 mM NaCl. The sera were diluted in phosphate-buffered saline containing 5% fetal calf serum. These dilutions were 1/10,000, 1/2,000, and 1/400 for the determinations of IgG, IgM, and IgA, respectively. Sequentially, 100 μl of these diluted samples and 50 μl of 128I-labeled purified goat or rabbit antibodies against mouse IgG, IgM, or IgA were added to the appropriate wells and incubated overnight at 37°C in a water-saturated atmosphere. After washing with 0.9% NaCl containing 0.01% Tween A20 (Technicon Instruments Corp., Tarrytown, N. Y.), the wells were counted for bound radioactive antibody (Ab). Standards were purified 129/Sv IgG, MOPC 104E IgM, and TEPC 15 IgA.

**Results**

**Incidence of Anti-Ig.** The sera of five mouse strains, BALB/c, C57BL/6, C3H (sublines C3H/HeJ at JL and ICP, C3H/Ico at IC), DBA/2, and 129/Sv from four colonies were tested for their ability to agglutinate latex particles coated with mouse IgG. Despite the great dispersion of individual agglutination titers, the results showed that the colony had a strong influence on the production of anti-IgG and that strains differed markedly in their response to the same environment.

**Influence of Colony (Fig. 1).** Most animals from ICP and IP produced large amounts of anti-IgG. It should be noted that these colonies are closely related as the ICP progenitors came from IP. The incidence of anti-IgG was much lower in animals from JL, where only some animals produced moderate quantities of anti-IgG. It was nearly undetectable in animals from IC, where most animals were negative. This influence of environment on the production of anti-IgG was best illustrated by the 129/Sv mice, which produced ~100-times more anti-IgG in animals from ICP and IP than in animals from JL. Evidence for the involvement of an infectious agent was provided by the fact that germ-free DBA/2 from IP (courtesy of Dr. J. L. Guenet) were negative (<0.2 agg. U/μl) in contrast to their SPF relatives.

**Influence of Strain (Fig. 1).** Major differences in the anti-IgG titers were observed between mouse strains in the same environment. In animals from ICP, 129/Sv and the endotoxin-resistant C3H/He were the highest producers followed by DBA/2 and C57BL/6. Nearly all the animals of these strains were positive (>0.2 agg. U/μl). In
Fig. 1. Agglutinating activities of sera from individual mice of various strains and colonies. C3H mice from JL and ICP belong to the endotoxin-resistant subline C3H/He, whereas C3H mice from IC were of the subline C3H/Ico. The horizontal bars correspond to the mean of the log values. The horizontal broken line is the lower limit of detection.

Contrast, most of the BALB/c mice remained negative. In animals from JL, 5 strains of the 23 tested were consistently positive: AKR/J, CBA/J, C57L/J, NZB/BlNJ, and 129/J. A few strains had ~50% positive animals: BALB/cJ (6/14), RF/J (8/15), C57BR/cdJ (6/17), C57BL/6J (8/13), and C3H/HeJ (6/14). All the other strains (A/J, C57BL/10J, C58/J, CE/J, DBA/J, LP/J, P/J, RIII/J, SEA/GnJ, SJL/J, SWR/J, 129/Sv, and DBA/2J) were negative. With the exception of NZB/BlNJ, none of the positive strains is known to develop systemic autoimmune disorders. This study was repeated on two groups of animals obtained at a 6-mo interval from JL. As no significant differences were found between the groups, the results of typical strains are presented together (Fig. 2).

Nature of Anti-IgG. Our previous work with the 129/Sv mice from ICP had shown that IgA and, to a lesser extent, IgM contributed to the total anti-IgG activity measured by agglutination. To determine the distribution of IgA and IgM anti-IgG in the strains of this study, we pooled the sera according to strain and origin and
tested them by a solid-phase RIA using 129/Sv IgG-coated polystyrene wells (Figs. 3 and 4).

The correlation coefficient between the agglutinating activity and the level of IgA plus IgM anti-IgG calculated on 34 samples reached 0.85. However, the RIA detected
significant amounts of anti-IgG in sera with little or no agglutinating activity. Most of these sera contained IgM anti-IgG but no IgA anti-IgG, e.g., sera from C57BL/6 and C3H/He from JL. In contrast, nearly all sera with strong agglutinating activity had both IgM and IgA anti-IgG (Figs. 4 and 5) or IgA alone (see DBA/2 in Fig. 4).

Levels of Immunoglobulins and Anti-IgG. IgG, IgM, and IgA levels were determined
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Table I

Ig and Anti-IgG Levels in Various Mouse Strains from Different Colonies

| Strains   | Colonies | Number of animals* | Agglutination | Anti-IgG | Ig | Anti-IgG/IgM |
|-----------|----------|--------------------|---------------|---------|---|-------------|
|           |          |                    |               | IgA | IgM | IgA | IgG | IgM | IgA anti-IgG/IgG | IgM anti-IgG/IgM |
| C57BL/6   | IC       | 10                 | <0.2          | 0.3  | 0.9 | 84  | 960 | 780 |
|           | JL       | 15                 | 0.2           | 0.2  | 2.9 | 53  | 1,090 | 2,530 |
|           | IP       | 3                  | 8.0           | 4.8  | 41.0 | 255 | 1,700 | 1,260 |
|           | ICP      | 20                 | 3.8           | 2.9  | 9.0 | 312 | 1,900 | 1,750 |
| 129/Sv    | JL       | 15                 | 0.2           | 1.7  | 1.8 | 157 | 11,500 | 1,230 |
|           | IP       | 3                  | 60.0          | 62.2 | 98.6 | 661 | 25,100 | 1,460 |
|           | ICP      | 20                 | 65.0          | 87.2 | 38.8 | 741 | 23,800 | 1,650 |
| DBA/2     | IC       | 10                 | <0.2          | 0.2  | 1.3 | 28  | 5,700 | 850  |
|           | JL       | 15                 | 0.2           | 0.2  | 0.5 | 52  | 7,500 | 1,040 |
|           | IP (germ-free) | 8                | <0.2         | 0.2  | 0.2 | 16  | 390  | 1,620 |
|           | IP (SPF) | 5                  | 17.6          | 22.2 | 0.7 | 144 | 7,700 | 560  |
|           | ICP      | 20                 | 17.6          | 11.2 | 0   | 264 | 13,400 | 650  |
| C3H/HeJ   | IC       | 10                 | <0.2          | 0.1  | 0.2 | 66  | 960  | 1,250 |
|           | JL       | 15                 | 0.3           | 0.3  | 3.7 | 71  | 5,720 | 1,330 |
|           | ICP      | 20                 | 8.0           | 12.9 | 11.0 | 292 | 17,100 | 1,180 |
| BALB/c    | IC       | 10                 | <0.2          | 0.1  | 0.7 | 57  | 3,300 | 330  |
|           | JL       | 15                 | <0.2          | 0.4  | 0.8 | 129 | 4,400 | 1,550 |
|           | ICP      | 20                 | 0.25          | 0.8  | 3.2 | 176 | 11,300 | 1,380 |

* To constitute the serum pools.
‡ Ratios were calculated only for samples containing >2 μg/ml IgA or IgM anti-IgG.

by RIA in the serum pools mentioned in the previous section for the evaluation of anti-IgG (Table I). In a given strain, mice from IP and ICP that produced anti-IgG had a fourfold increase of IgA, a twofold increase of IgG, and no increase of IgM as compared to IC and JL anti-IgG-negative strains. The very high proportion of IgA anti-IgG vs. total IgA should be stressed (Table I). It reached 15% in DBA/2 and ~10% in 129/Sv. These data indicated that mice from IP and ICP were submitted to a persistent immune stimulation resulting in such elevated IgA and IgG levels.

Specificities of IgA and IgM Anti-IgG. IgM anti-IgG induced in mice by endotoxin (2, 3) or by immunization with sheep erythrocytes (4) has a weak specificity, if any, for mouse IgG. In contrast, the anti-IgG that we have detected in the 129/Sv mice of our colony (ICP) reacted only with mouse IgG2a (1). It was therefore interesting to check the respective specificities of the IgM and IgA anti-IgG detected in the other positive strains. Accordingly, the amount of IgA and IgM binding to polystyrene wells coated with various IgG's was measured by RIA. Pilot experiments had shown that IgA- or IgM-coated wells were unable to absorb the agglutinating sera, confirming that the autoAb was directed exclusively against IgG (data not shown).

Species Specificity. The binding of IgA or IgM to insolubilized IgG's from various species was expressed in percentages of the binding to wells coated with 129/Sv IgG.
Clear differences between the specificities of IgA and IgM anti-IgG were observed. Although no significant binding of IgA to IgG from other species was detected, IgM from all strains, except 129/Sv and 129/J, reacted with the IgG of most species (Fig. 5).

Ig Class and Subclass Specificity. Similar experiments were done with polystyrene wells coated with polyclonal IgG1 and IgG2a from 129/Sv and monoclonal BALB/c IgG2b (MOPC 141) and IgG3 (J606). Again, the specificity of IgA was much more restricted than that of IgM (Fig. 6). IgA anti-IgG reacted preferentially with IgG2a in all strains with the exception of C57BL/6 where it bound mainly to IgG1. In 129/J and 129/Sv, IgM shared with IgA the same narrow specificity and reacted only with IgG2a. In all other strains, IgM anti-IgG bound to most of the IgG classes and subclasses with some preference for IgG2a and IgG1.
**Table II**

Binding* of IgA Anti-IgG to Insolubilized IgG from Strains with Different IgG1 and IgG2a Allotypes

| Sources and allotypes of insolubilized IgG | Binding of IgA anti-IgG from various mouse strains |
|-------------------------------------------|---------------------------------------------------|
|                                          | 129/J 129/Sv C3H/He DBA/2 C57BL/6 C57L/J NZB/BinJ |
| 129/Sv a, c,d,e,f,g,h                     | 100 100 100 100 100 100 100 |
| SJL/J b, b                                | 95-93 100 100 100 100 100 100 |
| DBA/2 c, c,d,e,f,g,h                      | 115-97 127-105 101-93 92-90 95-87 96-84 91-87 |
| ARK/J d, c,d,e,f,g,h                      | 95-93 90-80 87-82 89-90 97-89 94-87 92-89 |
| A/J e, c,d,e,f,g,h                        | 116-109 109-95 116-105 89-91 91-85 125 145-107 |
| CE/J f, c,d,e,f,g,h                       | 76-76 67-84 70-80 69-76 142-116 58-74 64-82 |
| RII/J g, c,d,e,f,g,h                      | 118-96 103-87 106-110 86-107 90-95 94-99 106-103 |
| SEA/GnJ h, c,d,e,f,g,h                    | 13 27 13 22 24 16 16 |

*The binding to total IgG adsorbed on the polystyrene wells was expressed in the percentages of the binding to total IgG from 129/Sv mice.

Most results were obtained from two different assays on four samples from the same serum pool.

**Allotypic Specificity.** Because of the lack of specificity of IgM anti-IgG, we restricted our study of allotypic specificity to IgA anti-IgG, using the RIA system. The polystyrene wells were coated with total IgG from various strains representing the various IgG2a (Ig-1) and IgG1 (Ig-4) allotypes (Table II). IgA anti-IgG from 129/J, 129/Sv, C3H/He, DBA/2, C57L/J, and NZB/BinJ bound to all allotypes except Ig-1b. For C57BL/6 mice, which produced IgA anti-IgG directed mainly against IgG1, there was no restriction among the two allotypes of this Ig class, i.e., Ig-4a,c,d,e,f,g,h and Ig-4b. We observed weaker binding of IgA anti-IgG from all strains to the IgG from SEA/GnJ mice, which have the Ig-1b and Ig-4a,c,d,e,f,g,h allotypes. However, as evaluated with 125I-labeled rabbit Ab against mouse Fab, IgG from SEA/GnJ was 30% less adsorbed to polystyrene wells than IgG from other strains. This could explain the apparent weaker reaction of anti-IgG with the SEA/GnJ IgG. Moreover, when tested by an agglutination inhibition test, the SEA/GnJ IgG aggregated by heating or coupling to Sepharose 4B displayed a clear inhibitory activity.

The weaker binding of IgA anti-IgG from C57BL/6 to IgG from AKR mice (Table II), which have the allotypes Ig-1b and Ig-4a,c,d,e,f,g,h, was not confirmed by the agglutination inhibition test mentioned above. Therefore, there is presumably no allotypic restriction of the IgA anti-IgG from the C57BL/6 mice.

**Discussion**

Recently, we reported the age-dependent production of anti-IgG autoAb by the 129/Sv mice of our colony (ICP) (1). In this work, we observed a similar anti-IgG production in the C57BL/6, DBA/2, and C3H/He mice from our ICP colony and in the 129/Sv, C57BL/6, and DBA/2 mice from the closely related IP colony. In contrast, no anti-IgG was found in the sera of mice of the same strains but coming from unrelated colonies (IC, JL). These data showed that an environmental factor played a determinant role in this anti-IgG production.

The finding that germ-free mice from IP, in contrast to their SPF relatives, were devoid of anti-IgG, strongly suggested that the environmental factor responsible for the induction of anti-IgG was of infectious origin. This suggestion was further supported by our recent observation (data not shown) that negative animals (DBA/2 and C57BL/6) introduced into ICP became positive within 3 mo. The latter experiment rules out the possibility of a direct influence of food, as foreign animals...
which were kept separately from the local colony, but received the same sterilized food, did not produce anti-IgG after 3 mo of this diet.

The putative chronic infection that led to anti-IgG production at ICP and IP probably also provided the immune stimulus responsible for the increased total IgA and IgG levels of these animals. The predominant IgA increase observed in our anti-IgG positive mice is suggestive of a mucosal localization of the causative infectious agent. So far, we have failed to identify this infectious agent; we know however that it is apparently nonpathogenic, and contaminates SPF mice without changing their intestinal flora, as shown by Dr. G. Wauters of this University, who found no consistent difference in the intestinal bacteria of positive and negative mice. Moreover, examination of the various positive mice for Ab against most common mouse viruses failed to disclose any sign of systemic viral infection (Materials and Methods). These results exclude that the anti-IgG production at ICP could result from a massive infection by a pathogenic bacterium or by a common mouse virus. This suggests that a single microorganism provided the stimulus that led to increased IgA and IgG levels and finally to anti-IgG production.

It should be noted that the titers of anti-IgG, measured by its agglutinating activity, showed great individual variations. These can hardly be attributed to differences in the immune response within the same strain. Differences in the degree of contamination seem more probable.

In contrast to ICP and IP, where nearly all strains consistently produced anti-IgG, no positive animals were detected in animals from IC, whereas in animals from JL, only 5 strains out of 23 had high levels of anti-IgG. Among these, only NZB/BinJ are known to develop a systemic autoimmune disease. The presence of anti-IgG in C57L/J, CBA/J, and 129/J from JL as well as in C57BL/6, DBA/2, C3H/He, and 129/Sv from IP and ICP, all strains which do not develop autoimmune diseases, indicates that under the appropriate stimulus, most strains are able to produce anti-IgG. We have no indication, that the same or a related agent was responsible for the anti-IgG production in animals from JL and ICP.

Besides the large differences between colonies in the incidence of anti-IgG-positive strains, major differences were observed between the amounts of anti-IgG produced by various strains from ICP. Among the five ICP strains, the 129/Sv strain was certainly the highest producer of anti-IgG, the concentration of which exceeded 100 μg per ml of serum. In contrast, in BALB/c mice which also had anti-IgG, the total concentration of IgA and IgM anti-IgG reached only 3 μg/ml. As transmission of the causative agent was apparently easy when the animals were kept together, this low level was probably a result of genetic factors conditioning the susceptibility to contamination or the ability to produce anti-IgG.

The anti-IgG that we have described in adult 129/Sv mice was mainly IgA, whereas IgM prevailed in young animals. In this study, where only adult animals were used, IgA anti-IgG was found in significant amounts only in high producers of anti-IgG. In these animals the proportion of IgA vs. IgM anti-IgG was very different from one group to another; C57BL/6 had mainly IgM anti-IgG, DBA/2 mainly IgA, and 129/Sv both IgM and IgA. Such a variation could be explained by the genetic background of the animals, the nature of the causative agent, or its histological distribution. The genetic factor seems particularly important as the differences found between strains from ICP were similar to those found in strains from at IP.
As reported before for the 129/Sv mice, the IgA anti-IgG from 129/J, NZB/BiJ, C57L/J, DBA/2, and C3H/He had restricted hetero-, iso-, and allotypic specificities. It reacted only with mouse IgG2a, but not with IgG2a of the Ig-1* allotype. C57BL/6 mice also had IgA anti-IgG with a narrow specificity but it was directed against IgG1 and did not show allotypic restriction. In contrast to the specificity of IgA anti-IgG, the antibody activity of IgM anti-IgG was much broader, except in the 129/Sv and 129/J strains, where IgM anti-IgG shared with IgA the same narrow specificity.

Also, IgA anti-IgG had a higher avidity than IgM anti-IgG. This was suggested by the influence of the incubation time on the RIA results. No difference in the amounts of IgA bound to the IgG-coated polystyrene wells was found after 4 or 20 h of incubation at 37°C, whereas the IgM binding increased three times by extending the incubation from 4 to 20 h. This higher avidity of IgA anti-IgG can also be inferred from its higher agglutinating activity. In agglutination-inhibition experiments (data not shown) the specificity of the agglutinator appeared to be that of IgA rather than that of IgM despite a higher concentration of IgM anti-IgG detected by RIA in certain strains. It is probable that this IgM anti-IgG corresponds to the rheumatoid factor described by Dresser (4) in CBA mice, which in our study were devoid of IgA anti-IgG.

Recently, it has been suggested that endotoxin plays a causative role in the production of rheumatoid factor (2, 3). However, endotoxin was apparently not involved in the induction of the anti-IgG that we have described because C3H/He mice from ICP, which are known to be resistant to endotoxin (5), produced significant amounts of anti-IgG.

**Summary**

Mice, >20 wk old, were tested for the presence of anti-IgG autoantibodies by agglutination and radioimmunoassay. IgA and IgM anti-IgG were found in the 129/Sv, C57BL/6, and DBA/2 strains from the local colony at the International Institute of Cellular and Molecular Pathology (ICP), at the Institut Pasteur de Paris (IP), and in the endotoxin-resistant C3H/He strain of ICP. These strains were negative at Ifa Credo (IC) and at The Jackson Laboratory (JL). Among 33 strains from the latter colony, 129/J, AKR/J, CBA/J, C57L/J, and NZB/BiJ were positive. All were specific pathogen-free and, excepting the NZB/BiJ, are not known to develop systemic autoimmune disorders. These differences between colonies suggest an influence of the environment on the production of anti-IgG. Evidence for the role of an infectious agent was provided by the fact that germ-free DBA/2 were negative in contrast to their SPF relatives. Strains which were positive at ICP and IP for anti-IgG had four-times higher serum levels of total IgA and two-times higher levels of total IgG than the corresponding negative strains from IC and JL.

The anti-IgG titers differed markedly from one strain to the other in the same environment; e.g., in mice from ICP, BALB/c mice produced 40-times less anti-IgG than 129/Sv. IgA anti-IgG occurred only in high producers of anti-IgG. In these animals, the proportion of IgA vs. IgM anti-IgG was very different from one group to the other; C57BL/6 had mainly IgM anti-IgG, DBA/2 mainly IgA anti-IgG, and 129/Sv both IgM and IgA anti-IgG.

The IgA anti-IgG from 129/Sv, 129/J, NZB/BiJ, C57L/J, DBA/2, and C3H/He had restricted hetero-, iso-, and allotypic specificities. It reacted only with mouse
IgG2a, but not with the Ig-1\textsuperscript{b} allotype. C57BL/6 also had IgA anti-IgG with a narrow specificity, but directed against IgG1 without allotypic restriction. In contrast to the specificity of IgA anti-IgG, the antibody activity of IgM anti-IgG was much broader, except in the 129/Sv and 129/J strains where IgM anti-IgG shared the same narrow specificity with IgA.

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