The Genetic Control of Serum IgA Level in Axenic and Holoxenic Mice. The Effect of Prednisolone (37511)

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(Introduced by I. Gresser)

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Previous studies of the production of IgA in axenic mice[2] have established the following points: (a) In C3H and CBA/J axenic mouse strains, serum IgA is either not detectable, or only found in very small amounts. By the age of 3 mo, C57BL/6 and Ha/ICR axenic mice have very low levels of serum IgA which increase slightly with age (1, 2). (b) Cecal IgA is found in all four axenic strains (2, 3). Like the secretory IgA of all other mammals, it carries more antigenic determinants than serum IgA. (c) Immunization with a wide variety of substances is ineffective in modifying cecal or serum IgA levels. However immunization with ferritin or sterilized intestinal contents leads to the transitory appearance of small amounts of serum IgA. (d) Serum IgA levels are unchanged in CBA/J mice, but significantly increased in Ha/IRC mice after administration of glucocorticoids. The questions raised by these findings are whether the low level of the IgA serum in axenic mice and its increase after a corticosteroid treatment are under genetic control and are linked with the H2 histocompatibility locus. The aim of the present work was to extend previous observations to other strains of mice and to study IgA production in hybrids of serum IgA producer and nonproducer mice.

Materials and Methods. This work was carried out on the following inbred mouse strains: C3H, SJL/J, CBA/J, BALB/c, C57BL/6, AKR, the randombred mice Ha/ICR and various hybrid mice. Male and female, axenic and holoxenic animals from 3 to 5 mo old were used. All mice were fed on the same sterilized diet. Blood samples were taken from the orbital sinuses with heparinized pipettes and were immediately centrifuged. Plasma samples were stored at −20° until assayed.

A dose of 20 mg of prednisolone/100 g of food was incorporated in the diet of the mice and administered over 14 days. Animals were bled 7 days before the beginning of treatment, on the last day of treatment, and in some cases, 10 days after the end of treatment. Axenic C3H and Ha/IRC mice were not bled before treatment but blood samples were taken from control mice reared in the same isolator. One half of both the treated and control mice were bled on the last day of treatment, while the other half of each group was bled 10 days later.

IgA titers were measured individually, using a semiquantitative method of double immunodiffusion in agar (4). The plasma samples were tested with a mono-specific rabbit anti-IgA serum. Titers are expressed as the logarithm to the base 2 of the reciprocal of the highest dilution showing a distinct precipitation line. Plasma samples which produced on precipitation line when undiluted are assigned a titer of 0. The mean titers are the arithmetic means of the titers of plasma from groups of 6–12 mice. Statistical comparisons of the mean titers were made using the Student's t test and the Mann and Whitney U test. The computations were carried out on an Olivetti computer Programma 101. The IgA levels of some of

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[2] Axenic: germfree; holoxenic: conventional; neoholoxenic: born axenic, but reared in a normal environment.

TABLE I. Serum IgA Titers in Axenic Mice.[a]

Strains	H2	Time in relation to prednisolone treatment				
		Day:	—7		14	24
C3H	k		0		0	0
CBA/J	k		0		0	ND
AKR	k		0.16 ± 0.40		1.00 ± 0.70	ND
SJL/J	s		0.83 ± 0.60	—[b]	2.60 ± 0.99	1.00 ± 0.00
C57BL/6	b		2.00 ± 0.52	—[b]	3.14 ± 0.69	ND
BALB/c	d		2.12 ± 1.07		ND	ND
Ha/ICR randombred			0.66 ± 0.54	—[b]	1.50 ± 0.92	0.50 ± 0.92

[a] Mean titers ± SD. By the quantitative method of double immunodiffusion and by single radial immunodiffusion one has shown that 0 titer correspond to sera which contain less than 0.010 mg/ml of IgA.

[b] Only significant comparisons are shown: $p < 0.05$.

the plasma samples were also quantified by single radial immunodiffusion. A reference serum with a known concentration of IgA was used as the standard.[3]

Results. Axenic and holoxenic inbred strains. The serum IgA titers found in axenic mice strongly suggest the existence of two distinct categories of mice: producer strains and nonproducer strains. No IgA was detectable in axenic C3H, CBA/J, or AKR serum whereas BALB/c, SJL/J, C57BL/6 and Ha/IRC axenic mice of similar age had IgA titers varying between 0.6 and 2.0. Holoxenic mice of both categories had significantly higher titers of between 4.8 and 9.4 (Tables I and II).

The existence of two categories of axenic mice was confirmed: (a) C3H, CBA/J and AKR mice continued to lack serum IgA after prednisolone treatment. (b) The serum IgA level of SJL/J, C57BL/6 and randombred Ha/ICR mice, was significantly increased after treatment (Table I). However, this increase was transitory and the level returned to its initial value within 10 days of the end of treatment. Such significant modifications were not observed in holoxenic mice. On the contrary holoxenic SJL/J mice had decreased of IgA titers after prednisolone treatment

[3] The reference serum was kindly supplied by Dr. H. Bazin, Department of Experimental Medicine, University of Louvain, Brussels, Belgium.

TABLE II. Serum IgA Titers in Holoxenic Mice.[a]

Strains	H2	Time in relation to prednisolone treatment					
		Day:	—7		14		24
C3H	k	a[b]	4.83 ± 0.93		8.83 ± 6.93		5.50 ± 0.67
		b	5.50 ± 0.98		5.33 ± 0.83		5.17 ± 0.93
CBA/J	k		8.66 ± 0.41		8.50 ± 0.63		ND
AKR	k		6.2 ± 0.75		5.6 ± 0.70		ND
SJL/J	s	a	9.40 ± 1.33	—[c]	5.80 ± 0.73	—[c]	8.80 ± 1.57
		b	7.80 ± 0.57		6.37 ± 1.11		ND (mice dead)
C57BL/6	b		9.17 ± 0.60		8.83 ± 1.00		ND
BALB/c	d		4.75 ± 0.49		ND		ND
Ha/ICR randombred			8.17 ± 0.60		8.00 ± 0.87		7.83 ± 0.79

[a] Mean titers ± SD.

[b] a and b are different groups of mice.

[c] $p < 0.01$.

TABLE III. Serum IgA Titers in Various Hybrid Categories of Mice.[a]

Hybrids	Day:	Time in relation to prednisolone treatment —7	14	24
Axenic				
(CBA/J × C57BL/6)F1		1.00 ± 0.00 —[b]	1.83 ± 0.33	ND
(CBA/J × C57BL/6)F2		1.08 ± 0.27	1.00 ± 0.48	0.62 ± 0.51
Back cross				
(F1 × C57BL/6)		0.16 ± 0.32 —[b]	1.64 ± 0.54 —[a]	0.27 ± 0.53
Holoxenic				
(CBA/J × C57BL/6)F1		5.00 ± 0.71	5.50 ± 0.44	ND
(C3H × SJL/J) F1		5.25 ± 0.89	ND	ND
(C3H × SJL/J) F1		5.50 ± 0.52	ND	ND

[a] Only significant comparisons are shown: $p < 0.01$; [b] $p < 0.001$.

(Table II).

F1 hybrids (CBA/J × C57BL/6). Axenic F1 hybrids had IgA titers intermediate in value between those of the axenic parental strains. However, the IgA titers of neoholoxenic hybrids (5.0) were significantly lower ($p < 0.001$) than those of either parental strain (8.66 and 9.16). F1 hybrids behaved as IgA producer mice: prednisolone treatment produced a significant increase in the serum IgA titers of axenic animals, but was ineffective in holoxenic animals (Table III).

F2 hybrids (CBA × C57BL/6) and back cross (F1 × C57BL/6). Axenic and holoxenic F2 hybrids and back cross mice were bled when 100 days old, and then treated with prednisolone. Before this treatment F2 hybrid mice had serum IgA titers identical to those observed in F1 mice (Tables III and IV). There was no clear cut segregation between phenotypic producers and nonproducers. Back cross mice were mostly nonproducers although some had low levels of serum IgA. Prednisolone treatment had no effect on F2 hybrids but induced an increase in serum IgA titers in back cross mice.

Discussion. The ability to produce a significant level of serum IgA spontaneously from approximately 3 mo of age and to increase this level after corticoid treatment, are two characteristics which seem to be linked. Nonproducer mouse strains, C3H, CBA and AKR, possess neither of these characteristics, whereas producer strains C57BL/6, SJL/J, Ha/IRC possess both. It is only possible to make a distinction between producer and nonproducer strains when they are reared in a germ-free environment; since both types synthesize a large amount of IgA when brought into a nonsterile environment. The difference between them is not caused by a genetic inability of nonproducer to synthesize alpha-chains, but rather by the ability of

TABLE IV. Titer of Serum IgA.[a]

	Day in relation to prednisolone	Titers 0	1	2	3
F2 hybrid	— 7	1	10	3	—
	14	4	4	4	—
	21	4	3	1	—
Back cross	— 7	11	0	1	—
	14	2	1	7	1
	21	10	1	—	—

[a] Number of mice for each titer.

producers to react to an unknown stimulus by producing small amounts of IgA. This reaction may occurr transiently after corticoid treatment. It is probably not a normal immunological reaction.

The immunological origin of a large amount of the serum IgA in holoxenic mice is questionable for the following reasons. (a) Immunization with a wide variety of substances is ineffective in modifying serum IgA levels. However, immunization with ferritin or sterilized intestinal contents leads to a slight transitory increase in serum IgA levels (1, 5, 6). (b) The gut flora is necessary and sufficient to induce serum IgA production. The establishment of a bacterial flora in the gut occurs rapidly and is completed in 24–48 hr, but serum IgA is not detectable for 4–6 wk. (c) Axenic mice readily produce large amounts of serum IgG when stimulated by various antigens; the quantity of IgG_2 is similar to that observed in holoxenic mice (1). It is possible that serum IgA in C57BL/6 and SJL/J mice may be synthesized in the gut wall, and then passed into the blood. According to this hypothesis the passage of IgA or of IgA producing cells from the gut to the blood would be entirely flora-dependent in C3H, CBA/J and AKR mice, but only partially dependent in producer strains. Another possibility is that producer and nonproducer strains, react differently to dietary antigens. The environment of all axenic mice is necessarily uniform. It is therefore difficult to test directly whether the phenotype of an axenic strain is the result of the control of IgA synthesis, or of receptivity to the triggering stimulus. We therefore studied the genetic transmission of "spontaneous" IgA production. It should be noted that this character may be linked to the H2k locus, which is present in the genomes of the three nonproducer strains, C3H, CBA/J and AKR.

Axenic F1 and F2 hybrids of producer and nonproducer strains were found to have intermediate IgA titers. No clear-cut segregation of characters occurred, which suggests that the mechanism is multigenic. The phenotype of back cross mice is rather surprising, but the number of mice studied was probably too low to draw definitive conclusion. F2 hybrids were not susceptible to prednisolone treatment unlike most back cross mice which behaved as producers. Therefore it may be concluded that the two characters, "IgA producer" and "susceptibility to prednisolone treatment," are not genetically linked.

Neoholoxenic hybrids were tested 100 days after their removal from the isolator. They were found to have significantly lower IgA titers than mice of their parental strains, which reached a normal serum IgA titer 65 days after removal. These results were unexpected and are difficult to explain.

The effect of prednisolone emphasizes the existence of the same two categories of axenic mice. After prednisolone treatment IgA remained undetectable in C3H, CBA/J and AKR mice but it was significantly increased in other strains. The serum IgA titers of all holoxenic strains so far studied (except SJL/J), are unaffected by prednisolone treatment. The mechanism which is responsible for the production of small amounts of serum IgA in axenic producer strains, is probably activated by prednisolone. However, its effect remains quantitatively negligible in holoxenic mice, which have a far more active, flora-dependent, IgA production system superimposed. Prednisolone treatment has an early and transitory effect on IgA production. It is therefore not likely to cause either a proliferation of IgA producing cells in the gut wall, or in the mesenteric lymph nodes after seeding. The redistribution of lymphocytes between the gut compartment and the compartment of the spleen, lymph nodes and blood is not documented in this work, but such a redistribution is a possible explanation for the activity of corticoids in producer strains. As its effect does not last long, prednisolone could act on short-lived IgA producing lymphocytes or could induce a traffic of these cells from the gut and even prevent a privileged homing to the gut. Normal traffic could become possible again only when the administration of the drug is discontinued. A specific cytolysis of these cells would be a more likely explanation for the rapid and transient increase of circulating IgA. However cytolytic agents such as cyclophosphamide,

endoxan, or X-ray irradiation do not enhance serum IgA titers (unpublished data). The induction of protein synthesis, or the modification of membrane permeability could also account for the effect of prednisolone.

Summary. There are two distinct categories of axenic mice: IgA producer strains and IgA nonproducer strains. This distinction is only possible in axenic mice, since both types synthesize a large amount of IgA when brought in a nonsterile environment. IgA producer strains have a transient but significant increase of serum IgA after corticoid treatment. Nonproducer strains have the same H2k locus in their genomes. The control of the serum IgA production or of the passage from the gut wall into the blood is multigenic.

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1. Benveniste, J., Lespinats, G., Adam, C., and Salomon, J-C., J. Immunol. **107**, 1647 (1971).

2. Benveniste, J., Lespinats, G., and Salomon, J-C., J. Immunol. **107**, 1656 (1971).

3. Crabbe, P. A., Bazin, H., Eyssen, H., and Heremans, J-F., Int. Arch. Allergy Appl. Immunol. **34**, 362 (1968).

4. Ouchterlony, Ö., Progr. Allergy **5**, 1 (1958).

5. Crabbe, P. A., Nash, D. R., Bazin, H., Eyssen, H., and Heremans, J-F., J. Exp. Med. **130**, 723 (1969).

6. Finerty, J. H., Evans, C. B., and Tobie, J. E., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **29**, 700 (1970).

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