EFFECT OF THE NORMAL MICROFLORA AND AGE AND SEX OF RATS ON THE SERUM IgG_2 LEVEL

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Germfree and ordinary rats, bred in the laboratory, and rats obtained from nurseries were used. IgG_2 was isolated from the serum of noninbred rats and a rabbit antiserum against it obtained. By radial immunodiffusion the quantity of this immunoglobulin was determined in the blood serum of different groups of animals. The IgG_2 content in germfree rats of the Fisher strain was only 19% of that in animals of this strain obtained from the nursery, and in females it was 10-15% higher than in males. The IgG_2 level in newborn rats within a few hours after birth was identical regardless of its concentration in the maternal blood serum. The results may be used as "standards" for various immunological experiments on rats.
KEY WORDS: immunoglobulin, germfree animals, microflora.

The formation of genetically determined inborn factors of immunity, especially immunoglobulins, is stimulated from a very early age by the normal microflora, This has been clearly shown by comparative experiments on ordinary and germfree animals [1, 2, 6, 15]. However, the investigations cited above were only semiquantitative in character. More recently much information has been obtained on different classes of immunoglobulins in many laboratory animals, including rats [3-5, 10, 11, 14, 15]. As regards the quantitative determination of the level of rat immunoglubulins, however, only a few investigations have been undertaken [8, 9, 12] on rats kept under animal house conditions.

The aims of the present investigation were as follows: 1) to determine quantitatively the extent to which the normal microflora influences the serum IgG_2 level in rats; 2) to establish age patterns of the quantitative IgG_2 content in rats in relation to sex; 3) to study correlation between the serum IgG_2 level of lactating females and of their offspring.

EXPERIMENTAL METHOD

Inbred Fisher rats, noninbred OFA rats, and noninbred rats from nurseries were used. Germfree animals were grown and reared in plastic isolators, and fed on a semisynthetic L-474 E12 diet. Antiserum against rat IgG_2 was obtained by McGhee's method [8] and used for quantitative determinations by the simple radial immunodiffusion method [7]. Pooled sera from 100 noninbred mice were used as the standard. The IgG_2 content in it was determined as the pure immunoglobulin G_2 used to obtain the antiserum. Protein was determined by Lowry's method.

EXPERIMENTAL RESULTS

The experiments showed (Table 1) that the IgG_2 level in rats kept in the animal house was 5.4 times higher than in germfree rats of the same strain; this difference was rather greater (8 times) in OFA rats, and greater still in noninbred rats from the nurseries. The IgG_2 level in males was lower by 10-15% than in females kept under the same conditions. This difference was observed in all the different groups of animals ($P=0.01$). In germfree rats kept under ordinary conditions on reaching maturity, the IgG_2 level even after six months did not reach that established in adult rats born in the animal house. In the offspring of these rats, born during the first week at the beginning of conventionalization, the IgG_2 level reached this value after three months, just as it did in young rats obtained from nurseries (Fig. 1). The stimulating role of the microflora was evidently strongest during the first week after birth of the rats.

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TABLE 1. IgG_2 Level in Blood Sera of Rats Kept in Isolators (germfree, Fisher strain; monocontaminated, OFA) under Animal House Conditions (in mg/ml, M ± m)

Fisher				OFA				noninbred	
germfree		ordinary		monocontaminated		ordinary		ordinary	
(33♀)	(23♂)	(35♀)	(25♂)	(23♀)	(24♂)	(28♀)	(26♂)	(212♀)	(227♂)
0,81 ±0,02	0,68 ±0,02	4,41 ±0,02	3,64 ±0,04	0,84 ±0,04	0,72 ±0,04	6,72 ±0,12	5,60 ±0,13	8,40 ±0,17	6,31 ±0,15

Legend. Number of animals used shown in parentheses.

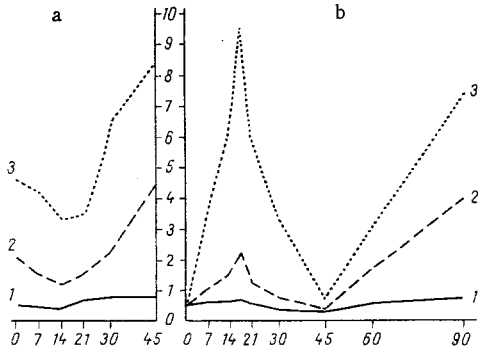

Fig. 1. Changes in serum IgG_2 level in rats (a – lactating females, b – their offspring) depending on age and conditions of keeping). Ordinate, IgG_2 concentration (in mg/ml); abscissa, days of taking samples. 1) Germfree rats. 20 rats of same strain kept in animal house; 3) noninbred rats from nurseries.

The placenta of hemoendothelial type is known to be permeable for antibodies [13]. In that case the immunoglobulin concentration in the maternal blood ought to influence its concentration in the neonatal blood serum before the beginning of milk (colostrum) feeding. The experimental results showed (Fig. 1) that during the first hours of life the IgG_2 level in all the young rats (inbred, noninbred, germfree and ordinary mothers) was the same (0.5-0.6 mg/ml), despite significant differences in its concentration in the mothers. On the subsequent days the initial IgG_2 level in the young rats increased to a maximum on the 17th-19th day after birth, when it was twice to three times higher than in the lactating females and about equal to the level observed in adult rats of the same groups. After the young rats started to feed themselves, after the 17th-19th day of life their blood IgG_2 level began to form rapidly, to reach a minimum after 42-45 days, after which it rose again on account of synthesis of their own IgG_2, so that by the 90th day it was indistinguishable from the adult level (Fig. 1).

During the first day after birth the maternal serum IgG_2 level was significantly lower than the mean values for adult rats. It continued to fall until feeding the young ceased on the 17th-19th day, and during the next 3-4 weeks it regained the mean values for adult rats. This tendency was observed in all groups and the difference was purely quantitative in character (Fig. 1).

The results show that transplacental transmission of IgG_2 in rats is not affected by its level in the blood of pregnant females. During the milk-feeding period, however, the IgG_2 concentration in the lactating females entirely determines its level in their offspring, for this immunoglobulin is evidently readily transmitted by the

alimentary route. Active synthesis of their own immunoglobulin G_2 does not take place in young rats under the age of 42-45 days. It must be noted that the microflora plays a much greater role in determining the serum IgG_2 level of both adult rats and their young than genetic predetermination.

The "standard" data obtained for the IgG_2 levels are essential as a basis for comparison in many types of immunological experiments on rats.

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INCREASED RESISTANCE OF CELLS TO VIRUS CAUSED BY mRNA FOR ANTIVIRAL PROTEIN

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The resistance of L-929 mouse cells to virus after administration of a single dose of homologous and heterologous preparations of messenger RNA for antiviral protein (AVP-mRNA) was studied. If homologous AVP-mRNA was used, inhibition of virus production reached 90-93% and remained steady after passage of the cells for 1.5 months (period of observation). After contact between the cells and heterologous AVP-mRNA inhibition of virus production in the first six passages was about 90%, increasing by the 16th passage to 99.9%. The results indicate a steady increase in the resistance of cells to virus by means of AVP-mRNA, and this could prove to be a new and effective method of nonspecific protection of cells against viruses.

KEY WORDS: interferon; antiviral protein; mRNA; resistance to viruses.

The writers showed previously that mouse cells can produce heterogenic chick interferon for long periods after administration of a single dose of chick interferon mRNA, which has template activity [1].

In the chain of formation and action of interferon, antiviral protein (AVP) is the final product which determines the resistance of the cells to virus [2].

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