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# Rabies Virus Immunity in Genetically Selected High- and Low-Responder Lines of Mice

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The antibody responsiveness to and the specific vaccination effect of rabies virus infection were investigated in high- and low-responder lines of mice produced by two-way selective breedings for quantitative production of antibodies to flagellar (H/f and L/f lines) or somatic (H/s and L/s lines) antigens of salmonellae. After specific immunization, both high lines were more resistant to rabies virus infection than were the low lines, and the protector effect was related to the level of antibody produced, as demonstrated by neutralizing serum activity. The present findings confirm the nonspecific genetic modification of the general antibody responsiveness induced in high- and low-responder lines selected for quantitative antibody production.

Two-way genetic selections of high- and low-responder lines of mice, which have been selected for maximal and minimal antibody production against natural complex immunogens, are of interest in determining genetic and immunological parameters of general and specific immune responsiveness (4, 5, 8, 9).

In these selection experiments homozygosity for the character high or low production of antibodies to selection antigens was attained after a given number of generations. These studies showed that antibody synthesis is a quantitative trait determined by the cumulative effect of independent loci. The number of loci might be related to the complexity of the selection antigens (4-6, 9, 15, 18).

All of the selected high and low lines, in addition to the important quantitative difference in their responses to the selection antigens, show significant distinct high and low responsiveness to nonrelated immunogens, although exceptions have been reported (7, 13, 19).

In the present study, high and low lines of mice obtained by two independent genetic selections for quantitative antibody responsiveness to flagellar (f) or somatic (s) antigens of salmonellae (H/f and L/f and H/s and L/s lines, respectively) were infected with rabies virus (18).

Natural and vaccination-induced resistance and neutralizing antibody synthesis were measured in the four lines.

## MATERIALS AND METHODS

**Mice.** Two-month-old mice of the high- and low-responder lines to flagellar (H/f and L/f lines; $F_{14}$ generation) or somatic (H/s and L/s lines; $F_{17}$ generation) antigens of salmonellae were used (18).

In the virus neutralization tests random-bred albino mice from the outbred colony of the Instituto Biológico were used.

**Specific vaccination effect.** Mice from the four lines received, 2 days apart, two intraperitoneal injections of 0.25 ml of rabies vaccine suspension (10), containing 0.5% mouse brain tissue, from the challenge virus standard strain (12). At 14 days after the first injection, groups of six to eight immunized mice were challenged intracerebrally with $10^{-1}$ to $10^{-5}$ virus dilutions in a volume of 0.03 ml (12). Animals were bled before challenge to determine the antibody titer.

Groups of six to eight nonimmunized mice of the four lines received $10^{-5}$ to $10^{-7}$ virus dilutions intracerebrally.

Animals were observed for the following 21 days.

**Virus neutralization.** Quintuplet dilutions of normal or immune pooled sera were incubated for 90 minutes at 37°C with the same volume of challenge virus standard strain dilutions. Groups of five normal unselected mice received intracerebrally each serum-virus mixture in a volume of 0.03 ml (2). Mortality was followed for 21 days after inoculation.

All sera and virus dilutions were made in distilled water containing 2% normal equine serum, penicillin (1,000 IU/ml), and streptomycin (1.25 mg/ml).

**Statistical analysis.** The 50% lethal doses were determined by the Reed-Muench method (16). The standard error was determined by the Pizzi formula (17).

Probability values were established between control and vaccinated mice and between high- and low-responder lines.

## RESULTS

**Specific vaccination effect.** The effect of vaccination specific to rabies virus infection was

established in high- and low-responder lines obtained after two independent genetic selections for high and low responsiveness to *Salmonella* antigens.

The results obtained in lines H/f, L/f, H/s, and L/s are shown in Table 1. High and low lines of both selections showed differences in the protector effect of immunization after virus inoculation. Both high lines developed a marked resistance to rabies virus infection, although some protection was verified in the low lines.

A difference of 156-fold was observed between lines H/f and L/f in relation to the protective index shown by vaccinated mice. A minor difference was also observed between lines H/s and L/s, which showed an interline difference of 63-fold after specific immunization.

For the natural resistance of the four lines, the results indicate no differences between the 50% lethal doses for H/f and L/f mice or between those for H/s and L/s mice, although some data from the mean survival time suggest that the low lines have a more prolonged survival.

**Neutralizing antibody activity.** Table 2 shows the results of the neutralizing antibody titers of the pooled sera from the four lines after rabies virus immunization. These data are in agreement with the specific vaccination effect data. The antibody titers in both high lines were higher than the titers in the low lines. A 32-fold difference was observed between the antibody titers of lines H/f and L/f, and a 2.5-fold difference was observed between the titers of lines H/s and L/s, although only the difference observed between lines H/f and L/f was highly significant.

The distinct viral concentrations employed in the two separate experiments did not interfere in the conclusion that both high lines had neutralizing antibody titers higher than those of the low lines, but they made impossible to draw a parallel between the two selections.

TABLE 2. *Antibody titers in high- and low-responder lines of mice measured by serum neutralization tests*

| Line | $LD_{50}$[a] | Neutralizing serum titer ($log_{10}$) | Ratio of high line to low line | P |
|---|---|---|---|---|
| H/f | 222 | 3.9 ± 0.2 | 32 | $P < 0.001$ |
| L/f | 222 | 2.4 ± 0.2 | | |
| H/s | 19 | 3.9 ± 0.1 | 2.5 | $0.1 < P < 0.2$ |
| L/s | 19 | 3.5 ± 0.3 | | |

[a] $LD_{50}$, 50% lethal dose.

## DISCUSSION

The relationship between genetic background and susceptibility to a given virus or bacterial infection has been demonstrated in mice. The genetic factors affecting the phenotypic expression of susceptibility or resistance to infection may be under either monogenic control or polygenic control which operates at both humoral and cellular levels (1, 3, 4, 14, 18, 20).

An example of a monogenic pattern influencing susceptibility to virus infection is given by the *rgv-1* locus, which is located within the H-2 complex and whose effect has been observed in several viruses that induce leukemia in mice (14).

A two-way genetic selection according to the natural resistance to *Salmonella enteritidis* led, after a certain number of generations, to the attainment of resistant and susceptible lines of mice. The selected lines, when tested for louping ill virus and St. Louis encephalitis virus resistance, presented an inverted behavior, making evident the independent inheritance of the two traits. These lines were equally susceptible to rabies virus infection (20).

Another relevant aspect considered was a possible correlation between antibody production and resistance. Thus, a positive correlation was demonstrated between the natural resistance to *Salmonella typhimurium* and anti-flagellar agglutinin production in the lines of mice selected for *S. enteritidis* infection (11).

More recently, studies on resistance to infections in high- and low-responder lines of mice genetically selected according to the level of

TABLE 1. *Effect of vaccination in high- and low-responder lines of mice from genetic selection to Salmonella antigens*

| Line | $LD_{50}$ ($log_{10}$)[a] | | Protective index[b] | P | Ratio of high line to low line | P |
|---|---|---|---|---|---|---|
| | Nontreated | Vaccinated | | | | |
| H/f | 6.2 ± 0.3 | 2.5 ± 0.5 | 5,000 | <0.001 | 156 | <0.001 |
| L/f | 6.2 ± 0.4 | 4.7 ± 0.4 | 32 | <0.01 | | |
| H/s | 6.8 ± 0.3 | 3.0 ± 0.5 | 6,300 | <0.001 | 63 | <0.001 |
| L/s | 6.0 ± 0.4 | 4.0 ± 0.4 | 100 | <0.001 | | |

[a] $LD_{50}$, 50% lethal dose.

[b] Antilog of the subtraction of the $LD_{50}$ values for control and vaccinated mice.

anti-heterologous erythrocyte production have clearly demonstrated that the low-responder line is more resistant than the high line to *S. typhimurium* and *Yersinia pestis* infections. These interline differences are probably due to the difference in bactericidal activity of macrophages in the two lines. In contrast, the specific vaccination to *Plasmodium berghei* and *Trypanozoma cruzi* induced a better protection in high than in low mice. This effect is related to the antibody level (3).

The present study was undertaken to establish the possible existence of interline differences with respect to response to rabies virus antigens in high and low lines of mice selected for quantitative antibody synthesis against flagellar or somatic *Salmonella* antigens. This was done by determining the effect of specific vaccination and neutralizing antibody activity.

As previously demonstrated, the selective breeding of high and low lines modified the general capacity of antibody production to nonrelated immunogens, in addition to the clear high and low responsiveness to selection antigens (7, 19).

The responsiveness to rabies virus antigens confirmed these observations; i.e., both high-responder lines (H/f and H/s) were more resistant to rabies infection than were the low lines (L/f and L/s) after specific immunization.

The interline difference, in terms of the protective index presented, was 156-fold for H/f and L/f mice and 63-fold for H/s and L/s mice.

In H/f and L/f mice the results of the neutralizing antibody activity confirmed the data referred to above since a highly significant difference (30-fold) in the neutralizing antibody titer was observed. For lines H/s and L/s the interline difference was smaller (2.5-fold) and of low significance.

The distinct behavior of the immunized high and low lines of the two selections with respect to rabies virus reveals an association with the nonspecific genetic control of antibody synthesis which the two selective processes produced. The interline differences of responsiveness to unrelated immunogens were always greater between the H/f and L/f lines than they were between the H/s and L/s lines for the same antigens; this was also true for the rabies virus antigens (19).

These results showed that the nonspecific immune response genes may act on a virus-specific immunity. As for rabies infection, the selected high and low lines provide an excellent biological material for experiments on several infection processes.

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