Genetic Control of Antinuclear Antibodies in Mice Infected with Rauscher Leukemia Virus

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SUMMARY

The incidence of antinuclear antibodies after Rauscher leukemia virus inoculation was found to be significantly higher in C57BL/6 than in BALB/c mice and still greater in their F1 hybrids. The relationships among antinuclear antibody incidence, erythroblastic disease, Rauscher leukemia virus production, and the H-2 genotypes were studied in the F1 generation and backcrosses using different virus inocula. The results observed suggest that (a) at least two genes are involved in the control of susceptibility to Rauscher leukemia virus-induced erythroblastosis, one of them probably being H-2 linked, and that (b) a non-H-2-linked gene seems to control, at the same time, induction of antinuclear antibodies, focus-forming virus production in the spleen, and susceptibility to the disease. It can be concluded that C-type viruses play an active role in antinuclear antibody induction.

INTRODUCTION

We reported in previously published papers (4, 5) that mice infected with Graffi virus or RLV produce antinuclear antibodies. Croken et al. (9) confirmed this observation by the inoculation of Scripps leukemia virus. By comparing the incidence of antinuclear antibodies induced by RLV or other factors such as hydralazine, polyinosinate-polycytidylylate, or complexes of denatured DNA and methylated bovine serum albumin in the same strains, we found recently that antinuclear antibody incidence was considerably higher in C57BL/6 than in BALB/c mice, and still greater in their F1 hybrids. The F1 generation and backcrosses were obtained from our own colonies. The Fv-1" genotype (20) of these lines was controlled by XC testing (22) using B- and N-tropic MuLV.

Viruses. RLV originally obtained from Dr. F. J. Rauscher (National Cancer Institute, Bethesda, Md.) was regularly maintained by in vivo acellular transmission in BALB/c mice according to a routine method (6). The NB tropism of the virus was controlled in vitro by XC plaque formation, using the C3H cell line (21) as N-type cells and the original 3T3 cell lines of Todaro and Green (26) as B-type cells. Aliquots of the same virus preparation stored in liquid nitrogen were used in all experiments. The titer was 10^6 plaque-forming units/ml in the XC test and a 50% lethal dose of 10^3.5/ml as calculated by in vivo assay in BALB/c.

Erythroblastic disease was induced by i.p. inoculation of 0.1 ml of the RLV suspension at various dilutions and was assessed by splenic palpation 28 days later. The titer of RLV in recipient spleens at that time was established by i.p. inoculation of 10-fold dilutions of individual spleen acellular extracts in 1-month-old BALB/c mice. After 28 days all mice were killed, and individual spleen weights were recorded and compared to controls.

Antinuclear Antibodies. Antinuclear antibodies were routinely sought 2 months after viral inoculation by an indirect immunofluorescence antibody technique on liver sections, as previously described (3). The titer of antinuclear antibodies appearing after MuLV inoculation was established previously (5); it reached 1/10 in BALB/c x C57BL/6 F1 hybrids 2 months after viral inoculation. Antinuclear antibodies showed a predominant antinucleoprotein activity, associated with a weaker anti-DNA specificity.

H-2 Typing. H-2 typing was routinely performed by in vitro cytotoxicity assays using rabbit complement and trypan blue exclusion tests. Anti-H-2S typing serum was a BALB/c anti-C57BL/6 spleen cell twice absorbed in vitro with B10.D2 spleen cells. Anti-H-2D typing serum was a C57BL/6 anti-BALB/c similarly absorbed with BALB/b spleen cells.

MATERIALS AND METHODS

Mice. BALB/c and C57BL/6 mice maintained by single-line, brother-to-sister matings, along with their F1 hybrids and backcrosses, were obtained from our own colonies. The Fv-1" genotype (20) of these lines was controlled by XC testing (22) using B- and N-tropic MuLV.

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RESULTS

Antinuclear Antibody Incidence after RLV Inoculation. As shown in Table 1, the incidence of antinuclear antibodies 2 months after RLV inoculation was higher in C57BL/6 than in BALB/c mice and still higher in the F1 hybrids. The
discrepancy between BALB/c and C57BL/6 was also found when another oncornavirus, the Moloney leukemia virus, was used (results not shown). However, in the latter case, C57BL/6 and F, showed the same incidence of antinuclear antibodies, suggesting that the erythroblastic component present in the RLV complex could have been responsible for the additive induction of antinuclear antibodies in F, hybrids. It was therefore decided to study the relationship between antinuclear antibody induction and erythroblastic disease in RLV-infected mice.

Incidence of Erythroblastic Disease after RLV Inoculation. Table 2 shows that 28 days after RLV inoculation a clear-cut discrepancy appeared between BALB/c mice, which were highly susceptible to erythroblastic disease even at low doses of the inoculum, and C57BL/6 mice, which were highly resistant even at high doses. The susceptibility appeared to be intermediate in hybrids, which were sensitive at high doses but relatively resistant at low doses. These results exclude the possibility of a 1-gene control with dominant resistance on susceptibility. They could be due to the interaction of 2 or more independent genes or to a unique codominant gene with an allele for resistance in C57BL/6 and an allele for sensitivity in BALB/c. The results in backcrosses differed significantly from those expected for 1 codominant gene since 28% sensitive mice were observed in C57BL/6 x (C57BL/6 x BALB/c) given injections of high RLV doses versus 43.5% expected (p < 0.05), and 63% sensitive were found in BALB/c x (C57BL/6 x BALB/c) receiving low-RLV inocula versus 48.5% expected (p < 0.01). Therefore, erythroblastic disease induced by RLV was controlled by at least 2 genes.

Correlation between Antinuclear Antibodies and Erythroblastic Disease. Tables 3 and 4 illustrate the relationship between the appearances of antinuclear antibodies and erythroblastic disease after RLV inoculation at low and high doses, respectively. Two different backcross populations were chosen for these experiments, to take advantage of the intermediate incidence of the disease in these cases. When low doses of the inoculum were given, BALB/c x (C57BL/6 x BALB/c) appeared as the most discriminating subpopulation since 63% showed evidence of erythroblastic disease (Table 2). Table 3 indicates that the incidence of antinuclear antibodies was identical in mice with overt erythroblastic disease (10%) and in mice free of any pathological symptoms (11%). At high doses of the inoculum, BALB/
with F1 hybrids (Table 1) ruled out this possibility. Second, the gene that controls susceptibility to RLV-induced erythroblastosis is linked to the antinuclear antibody-susceptibility gene; however, in this case BALB/c mice should have demonstrated a very high level of antinuclear antibodies. Third, one of the genes involved in susceptibility to RLV influences the antinuclear antibody incidence. According to this hypothesis, the effects of this gene are detectable in C57BL/6 × (C57BL/6 × BALB/c) backcrosses but not in BALB/c × (C57BL/6 × BALB/c) backcrosses. Therefore, it could be dominant for susceptibility with the allele for resistance in C57BL/6 and that in BALB/c.

**Correlation among RLV Titters in the Spleens of RLV-infected Mice, Erythroblastic Disease, and H-2 Antigens.**

The titer of RLV in the spleens of RLV-infected mice was tested individually in 10 BALB/c × (C57BL/6 × BALB/c) mice with erythroblastosis disease and comparatively in 10 BALB/c × (C57BL/6 × BALB/c) mice free of any disease. All of them had been inoculated 28 days before with 0.1 × 10^{-3} RLV i.p. The RLV titer by spleen equivalent was 10^{-3} (mean titer differing 10-fold) in mice with erythroblastosis disease. When the H-2 phenotypes were recorded, 9 of 10 mice with overt disease were found homozygous H-2^{a/b}, whereas 9 of 10 intact animals showed a heterozygous H-2^{a/b} phenotype. This suggests that low doses of inoculum an H-2-linked gene may play some role in the appearance of the disease. However, this gene would not be involved in antinuclear antibody induction, since at such doses the same antinuclear antibody incidence was found in BALB/c × (C57BL/6 × BALB/c) with or without erythroblastosis disease (Table 3).

**Table 4**

| No. with antinuclear antibodies | No. | | |
|-----|-----|-----|
| B6 (B6 × C) with erythroblastosis disease 1 mo. after viral inoculation | 12 (6M, 6F)^a | 10 (84) (6M, 6F) |
| B6 (B6 × C) without erythroblastosis disease 1 mo. after viral inoculation | 32 (18M, 14F) | 10 (31) (4M, 6F) |
| Total | 44 | 20 (45) |

^a Numbers in parentheses, percentage.

^b Numbers with letters in parentheses, number of mice of each sex.

These results suggest that susceptibility to RLV-induced erythroblastosis is controlled by at least 2 independent genes and that 1 of these also plays some role in the production of antinuclear antibodies by RLV-infected mice.

Genetic control of RLV-induced disease has not been extensively studied, but it is probably identical to that of FLV erythroblastosis (12, 13, 17), since both diseases appear as identical pathological entities (2) due to a similar defective SFFV (1, 10) with the same pattern of sensitive and resistant mouse lines. Among the 3 main genes involved in erythroblastosis, susceptibility to Flv-1, which controls the ability to replicate N- or B-tropic viruses (20, 23), could not have been concerned in these experiments, which used B-tropic mice and a B-tropic viral isolate. The experimental conditions reported above support the theory that 2 or more genes control RLV disease. One of these genes could be studied in BALB/c × (C57BL/6 × BALB/c) with low doses of the RLV inoculum. It showed a dominant resistance, with alleles for resistance in C57BL/6 and susceptibility in BALB/c and appeared to be H-2 linked. However, it is not necessarily identical to the H-2-linked Flv-1 described with FLV (7, 8, 15), since at the latter locus susceptibility is dominant. The very weak virus used in our experiments could perhaps explain this discrepancy. In addition, it is possible that the H-2-linked gene could have interacted with the effect of another gene, H-2^{a/b} mice being more resistant than H-2^{a/b} when at the same time heterozygous for this 2nd gene, as previously proposed for FLV (8). This would be in agreement with the 63% incidence of splenomegaly in BALB/c × (C57BL/6 × BALB/c) at low doses of inoculum. Further genetic studies to determine the exact nature of the H-2-linked gene have not been done, since it played no role in the RLV-induced antinuclear antibody incidence which was the main goal of the present experiments. The effects of a 2nd gene could be detected only in mice receiving a high-virus inoculum. It showed a dominant susceptibility, and the results obtained in C57BL/6 × (C57BL/6 × BALB/c) suggest that C57BL/6 bear an allele for resistance and BALB/c an allele for susceptibility. This gene would modify the level of SFFV in the spleen of RLV-infected mice. The same role has been suggested (19) for the Flv-2 gene (16) after FLV inoculation, and both genes evidenced a dominant susceptibility. However, no conclusion about the identity of these genes can be drawn from the above experiments.

Antinuclear antibody induction in C57BL/6 and BALB/c mice is probably controlled by several different genes. One of them is determinant in the induction of anti-DNA antibodies after inoculation of complexes of denatured DNA and methylated bovine serum albumin. It is X linked, C57BL/6 mice bearing the allele for immune response (A. Cannat and B. Varet, unpublished results), and possibly identical to a
similar gene previously described in SJL/J mice (18). In the present experiment the effect of this gene could be detected only in BALB/c × (C57BL/6 × BALB/c) mice, the X chromosome of C57BL/6 being present in all other crosses. However, the level of antinuclear antibodies in BALB/c × (C57BL/6 × BALB/c) did not differ significantly between males and females, suggesting either that the X-linked gene played no role in the RLV-induced antinuclear antibody induction or that its effect was too weak to be detectable under the present experimental conditions.

The clearer results of our experiment reveal the correlation between the appearance of antinuclear antibodies and the susceptibility to the virus-induced erythroblastosis in C57BL/6 × (C57BL/6 × BALB/c) but not in BALB/c × (C57BL/6 × BALB/c) mice. From the data discussed above this suggests that 1 non-H-2-linked gene, possibly identical to the Fv-2, controls antinuclear antibody induction in RLV-infected mice. The mechanism by which it controls the appearance of antinuclear antibodies in RLV-infected mice is not clear. The effect of erythroblastic disease per se can be ruled out since BALB/c × (C57BL/6 × BALB/c) mice with or without disease have the same antinuclear antibody incidence. The observation that the infectious properties of RLV must be maintained for the induction of antinuclear antibodies (4) as well as the fact that the SFFV titer is not detectable in vivo. Biomedicine, 20: 95–101, 1974.

In conclusion, these data emphasize the high degree of complexity in the genetic regulation of antinuclear antibodies induced by C-type viruses. They further suggest that, in the case of antinuclear antibody induction by other agents, such as nononcogenic viruses (25) or drugs (3), a similar interaction between predisposition to antinuclear antibody synthesis and genetically determined "handling" of the inducing agent may be demonstrable.

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REFERENCES


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