Genetic Control of Natural Immunity to Ecotropic Mouse Leukemia Viruses: Immune Response Genes

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Humoral immune response to ecotropic leukemia viruses in AKR and C57BL/6 mice was controlled by a gene that mapped in linkage group IX. Mice of the AKR strain had an immune nonresponsive allele of this gene, whereas mice of the C57BL/6 strain had an immune responsive allele. Antibody against murine leukemia virus (MuLV) reacted primarily with p15 protein of the viral envelope. It was concluded that the failure to find antibody production in AKR mice was the result of a genetic immunological defect, rather than the result of immunological tolerance that was induced by the persistent viremia of endogenous MuLV.

Mice from a wide variety of inbred strains (4, 7) and mice of feral origins (9) naturally form antibody against murine leukemia virus (MuLV). These antibodies against ecotropic MuLV bind to the virion but do not neutralize viral infectivity (1, 3). Although this immune response to MuLV is widespread in mice, quantitative differences exist amongst inbred strains (4, 7). This variation in immune response appears to be the result of several modifying factors, one of which is the immune stimulation caused by the production of endogenous MuLV (8).

In the preceding report (8) we described that antibody production in genetic crosses between AKR and C57L mice was influenced by the production of endogenous MuLV. Mice of both the AKR and C57L strains produced low titers of anti-MuLV antibody, whereas F1 hybrids produced high levels of antibody. In the C57L × (AKR × C57L)F1 backcross (which segregated for the production of infectious MuLV) it was observed that immune response was correlated with the production of infectious MuLV. Mice that produced infectious MuLV also produced anti-MuLV antibodies, whereas mice that did not produce anti-MuLV antibody were of the virus-free phenotype. As a result of these findings, it was proposed that the failure of C57L mice to naturally produce anti-MuLV antibodies was due to genetic repression of immunogenic endogenous MuLV in mice of this strain. Since AKR mice continuously produced high levels of immunogenic MuLV, but did not immunologically respond to the virus, it was proposed that mice of this strain had a genetic defect in their immune response to antigens of MuLV.

In this report I describe studies demonstrating that the immune response of mice to endogenous ecotropic MuLV is under genetic control. In crosses between C57BL/6 and AKR mice I have found evidence for an immune response (Ir) gene that controls humoral immunity to MuLV and is linked to the Ss marker in linkage group IX. This Ir gene controls immune response to the viral protein p15. Mice of the C57BL/6 strain are immune responsive to p15, whereas mice of the AKR strain are not immune responsive to the same protein.

MATERIALS AND METHODS

RIP Assay. See the accompanying manuscript (8) for details on radioimmune precipitation (RIP) assays used for the detection of antibodies against ecotropic MuLV.

Typing for Ss serum protein. Sera from individual mice were typed for Ss serum protein by immunodiffusion tests (12) with an antisera prepared in rabbits against highly purified Ss protein (kindly provided by D. Shreffler, University of Michigan). Immunodiffusion tests were performed in preformed 1% agar plates (Hyland Laboratories, Inc.); after addition of reagents the plates were incubated at room temperature overnight in a humidified container.

Mice. C57BL/6 and AKR mice were purchased from Jackson Laboratories. F1 hybrid and backcross mice were bred at McArdle Laboratory. Backcross mice were caged according to sex and chronological order of birth; viral status of the mice was not a factor in caging.

RESULTS

Antibodies against MuLV in the sera of AKR, C57BL/6, and F1 hybrid mice. The re-
results of RIP assays with the sera of parental C57BL/6 and AKR mice, as well as with sera from F1 hybrids, are presented in Fig. 1. Mice of both parental strains, AKR and C57BL/6, contained low titers of antibodies against MuLV. In most instances, sera of mice of these strains failed to show detectable reactions at a dilution of 1:20. In contrast, F1 hybrid mice consistently had high titers of antibody against MuLV. Antibody titers of F1 hybrid mice were generally greater than 1:640.

It was inferred from these findings that the AKR genome contributed the immunogenic virus to the F1 hybrid, whereas the C57BL/6 genome contributed a dominant Ir gene(s). To test this hypothesis, a genetic backcross [(AKR × C57BL/6)F1 × AKR] was prepared between the F1 hybrid and mice of the parental AKR strain. Mice of this backcross were uniformly positive for infectious MuLV (immunogen) but were found to segregate for C57BL/6 Ir genes. [Eleven mice of the (AKR × C57BL/6)F1 × AKR backcross were typed for infectious ecotropic MuLV by the XC plaque assay with tail biopsies. All 11 mice contained >10^9 plaque-forming units (PFU)/ml of infectious MuLV.]

Antibodies against MuLV in the sera of mice of the (AKR × C57BL/6)F1 × AKR backcross. The results of RIP assays with the sera of 35 mice of the (AKR × C57BL/6)F1 × AKR backcross are presented in Fig. 2. Sera from mice of this genetic cross demonstrated considerable variation in titers of antibody against MuLV. Some mice were of the AKR parental phenotype and contained low titers (<1:20) of antibody, whereas other mice were of the F1 phenotype and contained either intermediate (1:20 to 1:80) or high titers (>1:160) of antibody.

Concordance of antibody titers against MuLV and the presence of Ss protein in sera. Mice of the (AKR × C57BL/6)F1 × AKR backcross were typed for the Ss protein in serum samples by immunodiffusion with a rabbit antiserum prepared against purified Ss protein. Genetic control of the production of Ss protein is closely linked (within one cross-over unit) to the H-2 and Ir-1 loci in linkage group IX (12). Mice of the AKR strain had the Ss1 allele and expressed low levels of Ss protein in their sera; mice of the C57BL/6 strain had the Ss2 allele and expressed high levels of Ss protein in their sera (10). Mice of the (AKR × C57BL/6)F1 × AKR backcross were of either the Ss1 or Ss2 genotypes; sera of mice with the Ss2 allele genotype contained sufficient Ss protein to form a demonstrable precipitate with anti-Ss sera, whereas sera of mice of the Ss1 genotype did not contain sufficient Ss protein to give a precipitin line.

A comparison between the Ss phenotypes and the production of antibody to MuLV in mice of the (AKR × C57BL/6)F1 × AKR backcross is

Fig. 1. RIP assays with sera from AKR, C57BL/6, and F1 hybrid mice. Sera from individual mice (3 to 6 months old) were examined for antibody against MuLV by the RIP assay. Sera from AKR and C57BL/6 mice contained low-titered anti-MuLV antibodies; sera from the F1 hybrids contained anti-MuLV antibodies in higher titer than mice of either of the parental strains.
Fig. 2. Concordance between the production of anti-MuLV antibodies and the Ss phenotype of mice of the (AKR × C57BL/6)F₁ × AKR backcross. Sera of individual mice (3 to 6 months old) were examined in the RIP assay for antibody against MuLV and in an immunodiffusion assay for the presence of Ss serum protein. Mice of the Ss[λ] phenotype produced anti-MuLV antibodies at lower titer than mice of the Ss[σ] phenotype. Differences between these two groups of mice were highly significant (P < 0.001) in the two-sample t test (t = -3.94).

presented in Fig. 2. A strong concordance was observed in the segregation of these two traits. Thus, 9 of the 10 mice with the lowest titers (<1:20) of antibody against MuLV were Ss[λ] (AKR parental) homozygotes, whereas 8 of the 9 mice with the highest titers (titers 1:320 to 1:1,250) of antibody against MuLV were Ss[σ] (F₁ type) heterozygotes.

In some instances, mice of the Ss[λ] genotype had intermediate titers (1:20 to 1:80) of antibody against MuLV. This may have been the result of a second Ir gene that was contributed by the C57BL/6 genotype but segregated independently of the Ss-linked Ir gene.

Identification of MuLV proteins that were immunogenic to mice of the (AKR × C57BL/6)F₁ × AKR backcross. To identify MuLV proteins immunogenic in backcross mice, RIP reactions were performed with detergent-disrupted (0.5% Nonidet P-40) AKR MuLV and the immune complexes were separated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate.

Control preparations of AKR MuLV (Fig. 3) contained seven virion polypeptides (gp70, gp45, p30, p17, p15, p12, and p10). Sera from mice of the (AKR × C57BL/6)F₁ × AKR backcross reacted primarily with viral protein p15 (Fig. 3). Precipitation of p15 from detergent-disrupted MuLV was observed only with sera from mice that had high titers of antibody against intact MuLV in the RIP assay.

Fig. 3. Polyacrylamide gel electrophoresis of immune precipitates formed between IgG₂a antibodies in mouse sera and Nonidet P-40-disrupted MuLV. The control preparation of AKR MuLV contained seven virion proteins (gp70, gp45, p30, p17, p15, p12, and p10); the AKR virus shown in this figure does not demonstrate the gp70 and gp45 peaks, since these proteins contain a relatively low percentage of the [¹⁴C]leucine in the virion. Sera from mice that had high-titered anti-MuLV antibody in the RIP assay reacted with viral protein p15. In contrast, sera from mice that had low-titered anti-MuLV antibody in the RIP assay did not react with the Nonidet P-40-disrupted MuLV.
DISCUSSION

The production of antibody in mice against p15 protein of endogenous leukemia viruses is genetically controlled by at least one gene (Ir) that maps in linkage group IX. Mice of the AKR strain have an immune nonresponsive allele of this gene, whereas mice of the C57BL/6 strain have an immune responsive allele. Previous studies have shown that Ir genes in linkage group IX also control immune response against exogenous MuLV (6). Gross virus-soluble antigen (2), leukemic cells that contain the X.1 antigen (11), and cell-mediated immunity to Moloney virus-induced leukemias (5). Whether all of these effects on immune response are controlled by a single gene, or by a complex locus, has not been determined. However, since the strain distribution of immune response to Gross virus-soluble antigen, to the MuLV protein p15, and to X.1 antigen does not correlate in mice of different inbred strains, it is likely that these effects are the result of a complex genetic locus.

From the findings presented here and in the accompanying report (8), it is now possible to explain the apparent immune nonresponsiveness of mice of the C57BL/6, C57L, and AKR strains towards endogenous MuLV. Mice of the C57BL/6 and C57L strains are, in fact, capable of an immune response to MuLV, but due to the genetic repression of endogenous leukemia viruses they are not antigenically stimulated to produce antibody. In contrast, mice of the AKR strain produce immunogenic endogenous MuLV but, due to nonresponsive Ir genes, do not develop a secondary immune response to the virus.

In other studies (7) we have shown that mice of the virus-producing C58 and PL strains also do not demonstrate a secondary immune response to MuLV. Since mice of the C58 and PL strains do not produce levels of MuLV higher than that of AKR, we propose that these mice also contain nonresponsive Ir alleles. In fact, I have observed (6a) that the sera from some C58 and PL mice contain high-titered immunoglobulin M (IgM) antibodies against MuLV, although these same sera do not contain IgG antibodies against the virus. These findings are in line with the proposed model of genetic control of the immune response by an Ir gene.

It is of interest to note that mice of the low leukemic I strain produce high titers of antibody against MuLV (7). It would be predicted from these discussions that mice of the I strain would have a more frequent expression of endogenous MuLV than mice of the C57BL/6 and C57L strains. Expression of these endogenous MuLV genomes in mice of the I strain might occur at the level of (i) production of MuLV antigens in the absence of complete virions (e.g., see reference 13) or (ii) the production of poorly infectious XC-defective MuLV (e.g., U. R. Rapp and R. C. Nowinski, Proc. Natl. Acad. Sci., in press). We are presently exploring both of these possibilities.

In summary, immune response of mice to endogenous MuLV appears to be controlled by at least two factors: the production of endogenous MuLV and Ir genes that map within linkage group IX. Antibodies formed naturally to ecotropic MuLV do not neutralize the infectivity of the virion (1, 3), although these antibodies are cytotoxic for cells that produce high titers of MuLV (10). The complexity and independent nature of these factors has obscured the relationship between the production of antibodies to MuLV and the disease susceptibility of inbred mouse strains. It is now possible, however, to determine in genetic crosses whether immune response to the MuLV protein p15 is correlated with the risk of development of spontaneous leukemia.

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ADDENDUM

Recently, we have found that the production of antibody against MuLV in PL mice (another high leukemic strain) also is controlled by an Ir gene in linkage group IX. Seventy-nine mice of the PL × (PL × AKR)F1 and the (PL × AKR)F1 × PL backcrosses were examined concordantly for their production of antibody against MuLV (by the RIP assay) and for their H-2 type (by cytotoxicity assays) on lymph node cells. PL is a strain characterized with a high incidence of leukemia (due to a persistent MuLV viremia), a low production of antibody against MuLV, and the H-2blu genotype; C57L is a strain with a low incidence of leukemia, a low production of antibody against MuLV, and the H-2b genotype. High titers of antibody against MuLV in mice of this backcross were correlated with the H-2blu phenotype (35/36 with RIP titers >20), whereas low titers of antibody against MuLV were correlated with the H-2b genotype (32/43 with RIP titers <20). It was concluded that the production of low titers of antibody against MuLV in PL mice was the result of a genetic nonresponsiveness controlled by the Ir-1 locus in linkage group IX.

LITERATURE CITED