Resistance to Friend Murine Leukemia Virus Infection Conferred by the Fv-4 Gene Is Recessive but Appears Dominant from the Effect of the Immune System

FENGMIN ZHANG,1,2 LAMIN TA YA,1 YASUMASA IWATANI,1 KYOKO HIGO,1 YASUNORI SUZUKI,1 MASAKAZU TANAKA,1 TOMOMI NAKAHARA,1 TAKEHI ONO,1 HIROYUKI SAKAI,1 KAGEMASA KURIBAYASHI,3 AND AKINORI ISHIMOTO1*

Laboratory of Gene Analysis, Department of Viral Oncology, Institute for Virus Research, Kyoto University, Sakyo-ku, Kyoto 606-8507,1 and Department of Bioregulation, School of Medicine, Mie University, Tsu, Mie 514-8507,2 Japan, and Department of Microbiology, Harbin Medical University, Harbin 150086, People’s Republic of China3

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Fv-4 is a mouse gene that dominantly confers resistance to infection with Friend murine leukemia virus (F-MuLV) (S. Suzuki, Jpn. J. Exp. Med. 45:473–478, 1975). However, the resistance caused by Fv-4 is recessive in nude mice, which suggests that immunological effects play important roles in this resistance in vivo (K. Higo, Y. Kubo, Y. Iwatani, T. Ono, M. Maeda, H. Hiai, T. Masuda, K. Kuribayashi, F. Zhang, T. Lamin, A. Adachi, and A. Ishimoto, J. Virol. 71:750–754, 1997). To determine the immunological effect on the resistance in vivo, we infected immunologically immature newborn mice homozygous (Fv-4+/+) and heterozygous (Fv-4+/−) for Fv-4. Although the Fv-4+/+ mice showed complete resistance to F-MuLV whether infected neonatally or as adolescents, the Fv-4+/− mice showed high sensitivity to viral proliferation and disease induction when infected as newborns but complete resistance when infected as adolescent mice. To confirm the immunological effect on the resistance in adolescent mice with the Fv-4+/+ and Fv-4+/− genotypes, we examined the effect of an immunosuppressant drug, FK506, on the resistance. The mice with the Fv-4+/+ genotype treated with FK506 still showed resistance, but the mice with the Fv-4+/− genotype became highly sensitive to F-MuLV infection. Flow cytometric analysis to detect the Fv-4 gene product showed that the Fv-4 gene product was expressed on the cells from newborn and adolescent mice. The Fv-4 gene product was also detected on the cells from the FK506-treated mice as well as on those from untreated mice. However, a quantitative difference in the gene product between the cells with the Fv-4+/+ and Fv-4+/− genotypes was detected by indirect staining for flow cytometry. These results show that the resistance to F-MuLV infection conferred by the Fv-4 gene is originally recessive, but it looks dominant in adolescent mice mainly because of the effect of the immune system.

Susceptibility to retroviral infection is influenced by the genetic background of the host (2, 3, 4). Among many genes influencing Friend murine leukaemia virus (F-MuLV) infection (5), Fv-1, Fv-2, and Fv-4 have been extensively studied. Fv-4 was molecularly cloned much earlier than the others (1, 18, 19, 20, 26). However, much about the Fv-4 gene is poorly understood. Fv-4 is located on chromosome 12, and resistance to exogenous infection with ecotropic MuLV is reported to be inherited as a simple Mendelian dominant gene (12, 25, 31). We previously reported that transplantation of bone marrow cells from BALB/Fv-4 mice, i.e., Fv-4 congenic BALB/c mice (Fv-4+/+) established by breeding wild-type mice carrying the Fv-4 resistance gene with susceptible (Fv-4−/−) BALB/c mice (done by Odaka et al. [25]), can prevent the development of immune AIDS in susceptible mice infected with the murine AIDS virus (22). The resistant mice were found to have a glycoprotein equivalent to the exogenous ecotropic MuLV envelope on their lymphocytes, while mice with the Fv-4−/− genotype did not (10, 11, 14, 32), and sequence analysis characterized the Fv-4 gene as a truncated MuLV sequence containing the 3′ portion of the pol region, the entire env gene, and the 3′ long terminal repeat (9, 13), so that viral interference by competitive blocking of the virus receptor became a prime candidate for the Fv-4 restriction mechanism. However, there is no direct evidence that the Fv-4 gene product binds directly to the receptor. Despite the complete expression of Fv-4 resistance in vivo described above, cells carrying the Fv-4 gene are partially permissive to virus infection in vitro (22, 30, 31), and some reports have suggested a more complex mechanism of Fv-4-mediated resistance in vivo (7).

Studies on the susceptibility of nude mice to MuLV infection suggested that immunological effects play important roles in the resistance to F-MuLV infection caused by Fv-4 in nude mice (7, 16). To better understand the role of the immune system in the resistance, we investigated the gene dosage effect of the Fv-4 gene product under immune-deficient conditions. In this study, two inbred strains of mice with the Fv-4 resistance gene, FRG and BALB/Fv-4 mice, were used. The FRG mouse, formerly called the G mouse and supplied by Chugai Pharmaceutical Co. Ltd., is the first laboratory strain shown to be resistant to F-MuLV by virtue of carrying the Fv-4 gene (28, 29).

Newborn (younger-than-24-h-old) and adolescent (4-week-old) BALB/c, BALB/Fv-4, FRG, (BALB/c × BALB/Fv-4)F2, and (BALB/c × FRG)F2 mice were infected with 0.1 and 0.5 ml, respectively, of NB-tropic F-MuLV (about 5.2 log focus-forming units [FFU]/0.2 ml by the UV-XC test [27] on SC-1 cells [6]) intraperitoneally to compare the susceptibilities of mice with immature and mature immune systems to F-MuLV infection (Table 1). Among the adolescent mice, only the BALB/c mice without Fv-4 that were inoculated with the virus
TABLE 1. Susceptibility of mice with the Fv-4 gene to F-MuLV

<table>
<thead>
<tr>
<th>Strain</th>
<th>Fv-4 genotype</th>
<th>No. of mice with splenomegaly (&gt;200 mg)/total</th>
<th>Virus titer (log FFU/0.2 ml) at postinoculation day:</th>
<th>No. of mice with splenomegaly (&gt;350 mg)/total</th>
<th>Virus titer (log FFU/0.2 ml) at postinoculation day:</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>-/-</td>
<td>7/7</td>
<td>4.2</td>
<td>10/10</td>
<td>5.2</td>
</tr>
<tr>
<td>BALB/Fv-4</td>
<td>r/r</td>
<td>0/14</td>
<td>Negative</td>
<td>0/10</td>
<td>Negative</td>
</tr>
<tr>
<td>(BALB/c × BALB/Fv-4)F1</td>
<td>r/-</td>
<td>9/9</td>
<td>3.4</td>
<td>0/13</td>
<td>Negative</td>
</tr>
<tr>
<td>FRG</td>
<td>r/r</td>
<td>0/7</td>
<td>Negative</td>
<td>0/10</td>
<td>Negative</td>
</tr>
<tr>
<td>(BALB/c × FRG)F1</td>
<td>r/-</td>
<td>12/12</td>
<td>4.0</td>
<td>0/19</td>
<td>Negative</td>
</tr>
</tbody>
</table>

a Mice were killed 3 weeks after infection.

b Titer of 5% spleen homogenate detected by the UV-XC test (27) on SC-1 cells (6).

FIG. 1. Fv-4 expression on thymic cells from 4-week-old BALB/c, (BALB/c × BALB/Fv-4)F1, and BALB/Fv-4 mice detected by flow cytometry with direct (A to C) and indirect (D and E) staining. After the Fc receptor was blocked with rat anti-mouse CD16-CD32 monoclonal antibody, cells were treated with FITC-conjugated anti-Fv-4 antibody (A). As a blocking test, cells were blocked with serum from normal BALB/c mice (B) or with anti-Fv-4 BALB/c serum (C) and were stained with FITC-conjugated anti-Fv-4 antibody. For indirect staining, cells were first treated with normal BALB/c serum (D) or anti-Fv-4 BALB/c serum (E) and were then stained with FITC-conjugated goat anti-mouse IgG antibody.
developed the disease and were susceptible to viral proliferation. No proliferation of virus was detected 10 or 25 days after inoculation in the mice with the $Fv-4^{r/r}$ genotype or the $Fv-4^{r/2}$ genotype when they were inoculated as adolescents. However, (BALB/c $\times$ BALB/Fv-4)$F_1$ and (BALB/c $\times$ FRG)$F_1$ mice with the $Fv-4^{r/2}$ genotype developed the disease within 10 days when inoculated neonatally. Viral proliferation was also detected in the spleens of the mice with $Fv-4^{r/2}$ genotype 10 days after inoculation. Many mice with the $Fv-4^{2/2}$ and $Fv-4^{r/2}$ genotypes inoculated neonatally started to die at around 2 weeks after infection. It was thus confirmed that the F-MuLV resistance phenotype of BALB/Fv-4 and FRG mice appeared to be inherited as a dominant trait when the mice were infected as adolescents but as a recessive trait when they were infected neonatally.

To determine whether the $Fv-4$ gene was expressed in newborn as well as in adolescent mice, cells from thymus and spleen were stained directly with fluorescein isothiocyanate (FITC)-conjugated anti-$Fv-4$ immunoglobulin G (IgG) and indirectly with anti-$Fv-4$ mouse serum and FITC-conjugated goat anti-mouse IgG for flow cytometry analysis. The anti-$Fv-4$ IgG was purified from the sera of BALB/c mice ($Fv-4^{r/r}$) immunized with spleen cells from BALB/Fv-4 mice ($Fv-4^{2/2}$) by using a protein A-Sepharose column and labeled with FITC, and FITC-conjugated anti-$Fv-4$ IgG was prepared. FITC-conjugated goat anti-mouse IgG was purchased from MBL Co. Ltd. (Nagoya, Japan).

As shown in Fig. 1, the specificity of the anti-$Fv-4$ antibody was confirmed by staining of thymic cells from adult BALB/c, (BALB/c $\times$ BALB/Fv-4)$F_1$, and BALB/Fv-4 mice, because it was blocked by anti-$Fv-4$ serum but not by normal BALB/c serum in direct staining. Blocking with anti-$Fv-4$ BALB/c serum (Fig. 1C) showed an increased size of the negative peak in heterozygous (BALB/c $\times$ BALB/Fv-4)$F_1$ and homozygous BALB/Fv-4 mice accompanied by loss of population with at least greater than 1 log unit staining intensity compared with blocking with normal serum (Fig. 1B). The expression of the $Fv-4$ gene was clearly detected on thymic cells from newborn BALB/Fv-4 and (BALB/c $\times$ BALB/Fv-4)$F_1$ mice by both direct and indirect methods (Fig. 2) (10). Although only the

FIG. 2. $Fv-4$ expression on thymic cells from newborn BALB/c, (BALB/c $\times$ BALB/Fv-4)$F_1$, and BALB/Fv-4 mice detected by flow cytometry with direct (A) and indirect (B) staining.

FIG. 3. $Fv-4$ expression on thymic cells from FK506-treated mice detected by flow cytometry with direct (A) and indirect (B) staining. The thymic cells were prepared from the mice treated with FK506 every other day for a total of 10 times.
expression of Fv-4 on thymus cells is shown in this paper, fluorescence-activated cell sorter analysis of the fluorescence detected on spleen cells was shown in our previous paper (7). The level of fluorescence detected on the spleen cells was almost the same as that detected on the thymic cells, except for the staining of B cells among spleen cells by FITC-conjugated anti-mouse IgG goat serum (data not shown). However, the indirect staining, which may be low in specificity but high in sensitivity, was interesting. By the indirect staining, some difference between the Fv-4<sup>a</sup> and Fv-4<sup>b</sup> genotypes in the intensity of fluorescence showing Fv-4<sup>a</sup> gene expression was observed on cells from both adolescent (Fig. 1) and newborn (Fig. 2) mice.

To confirm the immunological effects on the susceptibility of mice with the Fv-4 gene to F-MuLV infection, we examined the susceptibility of mice treated with the immuno-suppressant drug FK506. FK506, an antibiotic isolated from the fermentation broth of Streptomyces tukubaenis and reported to be a potent immunosuppressant (8, 15, 21), was supplied by Fuji-sawa Pharmaceutical Co. Ltd. The 4-week-old mice were treated with FK506 at 5.0 mg/kg/day subcutaneously every other day. The mice were infected with 0.5 ml of F-MuLV intraperitoneally on the day after the third administration of FK506. From the next day of virus infection, mice continued to be treated with FK506 every other day for an additional 7 and 10 days. After infection for 16 and 26 days, mice were killed to examine the effects of the immuno-suppressant on resistance to viral replication and disease development (Table 2). Although there were no prominent effects of FK506 on the susceptibility of BALB/c (Fv-4<sup>−/−</sup>) and BALB/Fv-4<sup>b</sup> (Fv-4<sup>b</sup>) mice to F-MuLV infection, (BALB/c × BALB/Fv-4<sup>b</sup>)<sub>F<sub>1</sub></sub> (Fv-4<sup>b</sup>) mice were rendered susceptible to F-MuLV infection by the treatment. Viral proliferation in the spleen was detected in all FK506-treated mice with the Fv-4<sup>b</sup> genotype, even when splenomegaly was not detected. The reason why the spleen weights for the Fv-4<sup>b</sup>-treated virus-infected (BALB/c × BALB/Fv-4<sup>b</sup>)<sub>F<sub>1</sub></sub> (Fv-4<sup>b</sup>) mice are not equivalent to those for virus-infected BALB/c (Fv-4<sup>−/−</sup>) mice may depend on the effect of heterozygously coded Fv-4<sup>a</sup> gene expression. As a prominent side effect of FK506 treatment, growth disorders, such as loss of body weight or interruption of increase of body weight, including spleen weight, was often observed in both infected and uninfected mice. The effect of FK506 on Fv-4<sup>a</sup> gene expression in cells from thymuses and spleens of FK506-treated mice was analyzed by flow cytometry (Fig. 3). Expression of Fv-4<sup>a</sup> was detected on the cells from the mice with the Fv-4<sup>−/−</sup> genotype treated with FK506 every other day for a total of 3 or 10 times, as well as on the cells from untreated mice (Fig. 3). The data obtained by flow cytometry suggested that conversion from resistance to sensitivity does not depend on the decrease of Fv-4<sup>a</sup> gene expression but depends on other factors, such as suppression of the immune reaction by FK506.

Newborn mice are more sensitive than adolescent mice to MuLV in general (5, 16). Although it has been known for F-MuLV infection to develop into erythroid leukemia even in mice infected as adolescents (7), the adolescent (BALB/c × BALB/Fv-4<sup>b</sup>)<sub>F<sub>1</sub></sub> mice were resistant to F-MuLV infection. That flow cytometry for Fv-4<sup>a</sup> gene expression did not detect clear differences between the cells from adolescent and newborn mice suggests that the immunological immaturity of newborn F<sub>1</sub> mice is an important factor in their susceptibility to F-MuLV infection.

It has been reported that the Fv-1 locus has two codominant alleles, Fv-1<sup>α</sup> and Fv-1<sup>β</sup>. Cells carrying the Fv-1<sup>α</sup> allele are susceptible to N-tropic MuLVs, whereas cells carrying the Fv-1<sup>β</sup> allele are susceptible to B-tropic viruses (17, 23). The Fv-2 locus encodes a dominant host factor that confers susceptibility to F-MuLV-induced erythroleukemia (18, 24). Our observations about the susceptibilities of newborn mice and mice treated with the immunosuppressant FK506 to F-MuLV infection suggest that Fv-4<sup>a</sup> is essentially a recessive gene that looks dominant because it acts in cooperation with other factors. The difference in susceptibility between the homozygous (Fv-4<sup>−/−</sup>) and heterozygous (Fv-4<sup>b−/−</sup>) mice under immune-deficient conditions may depend on a quantitative difference in the Fv-4<sup>a</sup> gene product due to the gene dosage effect detected by indirect staining.

Homozygotes (Fv-4<sup>α</sup>) showed complete resistance even under the immune-deficient conditions in vivo, but tissue culture cells from these mice are susceptible to viral infection in vitro. This discrepancy in susceptibility to F-MuLV in vivo and in vitro suggests the presence of other mechanisms influencing resistance conferred by Fv-4<sup>a</sup> in vivo in addition to immune response.

Fengmin Zhang and Lamin Ta Ya contributed equally to this work.
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REFERENCES


