JOURNAL OF VIROLOGY, Feb. 2008, p. 1438–1447 Vol. 82, No. 3
0022-538X/08/$08.00+0 doi:10.1128/JVI.01991-07
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Replication of Beta- and Gammaretroviruses Is Restricted in I/LnJ Mice via the Same Genetic Mechanism▽

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Received 10 September 2007/Accepted 19 November 2007

Mice of the I/LnJ inbred strain are unique in their ability to mount a robust and sustained humoral immune response capable of neutralizing infection with a betaretrovirus, mouse mammary tumor virus (MMTV). Virus-neutralizing antibodies (Abs) coat MMTV virions secreted by infected cells, preventing virus spread and hence the formation of mammary tumors. To investigate whether I/LnJ mice resist infection with other retroviruses besides MMTV, the animals were infected with murine leukemia virus (MuLV), a gammaretrovirus. MuLV-infected I/LnJ mice produced virus-neutralizing Abs that block virus transmission and virally induced disease. Generation of virus-neutralizing Abs required gamma interferon but was independent of interleukin-12. This unique mechanism of retrovirus resistance is governed by a single recessive gene, *v*irus *i*nfectivity *c*ontroller *1* (*vic1*), mapped to chromosome 17. In addition to controlling the antivirus humoral immune response, *vic1* is also required for an antiviral cytotoxic response. Both types of responses were maintained in mice of the susceptible genetic background but congenic for the I/LnJ *vic1* locus. Although the *vic1*-mediated resistance to MuLV resembles the mechanism of retroviral recovery controlled by the *r*esistance to *F*riend *v*irus *3* (*rfv3*) gene, the *rfv3* gene has been mapped to chromosome 15 and confers resistance to MuLV but not to MMTV. Thus, we have identified a unique virus resistance mechanism that controls immunity against two distinct retroviruses.

Since the discovery of human immunodeficiency virus (HIV), there has been a great deal of interest in antiretroviral immunity. This interest is especially intense because of the failure to develop a successful vaccine against HIV. The vast majority of untreated HIV-infected individuals exhibit evidence of ongoing viral replication and progressive $CD4^+$ T-cell depletion. In contrast, 5 to 15% of all HIV-infected individuals, known as long-term nonprogressors, remain clinically stable and do not develop AIDS for more than 15 years (27, 31). Furthermore, about 1% of all HIV-infected individuals, referred to as elite controllers, are able to maintain viral loads below the limit of detection for more than 25 years following infection (10). Studies on the limited numbers of long-term nonprogressors and elite controllers suggested that host factors, including humoral and cytotoxic antivirus immune responses, could potentially underlie the mechanism of retrovirus resistance in humans (10). This suggests that there are genetic variations in the human population that result in the differential ability of these individuals to mount profound and sustained virus-specific immune responses.

There are obvious difficulties in studying the genetics of resistance to viral infections in humans. In contrast, animal models are very useful for studying the genetics of virus immune mechanisms that are capable of preventing virus spread and of alleviating virally induced disease. Previous studies conducted by our laboratory have revealed that inbred I/LnJ mice have the unique ability to mount a prolonged neutralizing immune response against mouse mammary tumor virus (MMTV) that completely blocks virus transmission and protects the mice from MMTV-induced mammary tumors (14, 33).

MMTV is a betaretrovirus that is passed though the milk of lactating female mice to their newborn pups (9). Antigen-presenting cells are the initial targets for MMTV, whereas mammary epithelial cells are the major targets for the virus (9, 42). The formation of mammary tumors involves multiple rounds of reinfection and reintegration until the retrovirus is inserted into the host genome upstream of a protooncogene by chance (28). I/LnJ mice fostered by viremic females become MMTV infected but produce antivirus neutralizing antibodies (Abs) that coat virus particles and interfere with subsequent virus entry (5, 33). This humoral immune response is apparent as early as 4 weeks after infection and is sustained through the life span of the animal (33). As a result, MMTV-infected I/LnJ mice resist virally induced mammary tumors and secrete virions that are coated with Abs. These Abs cause neutralization of the virions and prevent the transmission of infectious virus to their offspring (33). Gamma interferon (IFN-γ)-producing $CD4^+$ T cells are absolutely required for the production of Abs against MMTV because I/LnJ-derived splenocytes lacking $CD4^+$ T cells are not capable of conferring Ab production to susceptible MMTV-infected I/LnJ IFN-$\gamma^{-/-}$ mice upon transfer (5). In contrast, I/LnJ-derived splenocytes lacking either IFN-γ-producing natural killer (NK) cells or $CD8^+$ T cells are sufficient to transfer the ability to produce antivirus Abs to MMTV-infected IFN-$\gamma^{-/-}$ I/LnJ mice (5). Therefore, IFN-γ-secreting $CD4^+$ T_H1 T cells are indispensable for the production of virus-neutralizing Abs and cannot be replaced by other IFN-γ-producing cells.

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▽ Published ahead of print on 5 December 2007.

In this study, we investigated whether this unique antivirus immune response is applied to other genera of retroviruses. Murine leukemia virus (MuLV) is a gammaretrovirus that is transmitted as an exogenous or an endogenous virus (9) and infects cells of the lymphoid origin (35). Exogenous MuLV is passed through the blood of infected animals and primarily infects cells of lymphoid origin (35). Susceptible mice develop severe splenomegaly and subsequently succumb to leukemia (35). Here we sought to determine whether the potent antivirus neutralizing response identified in MMTV-infected I/LnJ mice is also mounted in MuLV-infected I/LnJ mice and if the same genetic locus is responsible for immunity against these two distinct retroviruses.

MATERIALS AND METHODS

Mice. All of the mice used in this study were bred and maintained at the animal facility of The Jackson Laboratory or the University of Chicago. I/LnJ and BALB/cJ mice were purchased from The Jackson Laboratory. C3H/HeN MMTV-free mice were originally purchased from the National Cancer Institute Frederick Cancer Research Facility, Frederick, MD, and maintained in our colony for more than 10 years. I/LnJ mice were crossed to B6.129P2(B6)-$B2m^{tm1Unc}$ (21) mice and to B6.129-$Il12b^{tm1Jm}$ mice (24) for 10 repetitive generations, and heterozygous N_{10} mice were intercrossed to generate I/LnJ mice with a targeted mutation of β2-microglobulin (β2M) (I/LnJ $\beta 2M^{-/-}$ mice) or interleukin-12β (IL-12β) (I/LnJ IL-$12\beta^{-/-}$ mice), respectively. I/LnJ IFN-$\gamma^{-/-}$ mice were previously described (33). These studies have been reviewed and approved by the Animal Care and Use Committees at The Jackson Laboratory and at the University of Chicago.

To generate the C3H/HeN$^{vic1I/LnJ}$ congenic line, we first crossed C3H/HeN females to I/LnJ males to produce F_1 females. F_1 females were then backcrossed to C3H/HeN males and the resulting N_2 females were genotyped with the markers *D17Mit24* at 37 Mb and the self-made marker (5′AGTTAGAACATC CACTGATGTTCA3′, 5′ATTTGATCAGTTATCTTCTCAACT3′) at 60 Mb that flanked the I/LnJ locus containing the *vic1* gene (see Fig. 5A). Females inheriting the I/LnJ *vic1* allele were backcrossed to C3H/HeN males to produce N_3 offspring. At each generation, only those offspring that had received the I/LnJ allele of the *vic1* locus were selected for the next round of backcrossing. Two 10th-generation carriers of the I/LnJ allele of the *vic1* locus were intercrossed, and offspring homozygous for both I/LnJ *vic1* alleles were selected to continue the line through brother-sister matings in the following generations.

To generate the BALB/cJ$^{vic1I/LnJ}$ line, we crossed BALB/cJ females to I/LnJ males to produce F_1 females. F_1 females then were backcrossed to BALB/cJ males, and the resulting N_2 females were genotyped with the markers *D17Mit24* at 37 Mb and *D17Mit185* at 68.3 Mb that flanked the I/LnJ locus containing the *vic1* gene (see Fig. 5A). Females carrying the I/LnJ *vic1* allele were backcrossed to BALB/cJ at each generation as described above.

To generate the BALB/cJ$^{rfv3I/LnJ}$ line, we used a strategy similar to that described above, except that we genotyped mice with different markers, specifically, *D15Mit5* at 43.3 Mb and *D15Mit193* at 97.7 Mb, that are located upstream and downstream of the *rfv3* locus (see Fig. 5A) (39).

Infection with MMTV. Mice were MMTV infected either via fostering by viremic females or via intraperitoneal (i.p.) injection of biologically active milk-borne MMTV virions into 3- to 4-week-old mice as previously described (33). Two distinct MMTV variants, MMTV(C3H) and MMTV(LA), were used for infection. MMTV(C3H) persists in C3H/HeN mice and consists of a single virus variant with a Vβ14-specific superantigen (Sag) (7, 25). MMTV(LA) is a naturally occurring virus of BALB/cJ mice which consists of three different exogenous MMTVs, BALB2, BALBLA, and BALB14, with Vβ2-, Vβ6-, and Vβ14-specific Sags, respectively (15, 30). The Vβ6-specific Sag encoded by BALBLA can be presented by both the I-E and I-A molecules of major histocompatibility complex (MHC) class II and thus is capable of efficiently infecting I-E-negative mice, like C57BL/6J mice (32). The deletion of Sag-cognate T cells was used as a readout for successful MMTV infection of mice. Infection with either MMTV(C3H) or MMTV(LA) resulted in the production of Abs that were capable of cross-neutralizing both viruses (33). Thus, in our experiments, MMTV(C3H) and MMTV(LA) were used interchangeably.

Infection with MuLV. Moloney MuLV (Mo-MuLV) was a gift from H. Fan, University of California at Irvine. Mo-MuLV was propagated in NIH 3T3 mouse fibroblasts. Rauscher-like MuLV (RL-MuLV), a mixture consisting of NB-tropic ecotropic and mink lung cell focus-forming viruses, was described previously (19) and was propagated in SC-1 embryonic mouse fibroblasts (ATCC, Manassas, VA).

Both virus isolates were tested for mouse hepatitis virus, mouse thymic virus, mouse parvovirus, pneumonia virus of the mouse, polyomavirus, reovirus type 3, enzootic diarrhea of infant mice, Sendai virus, *Mycoplasma pulmonis*, *M. arthritidis*, cilium-associated respiratory bacillus, ectromelia virus, *Encephalitozoon cuniculi*, Theiler's virus, Hantaan virus (Korean hemorrhagic virus), lymphocytic choriomeningitis virus, lactic dehydrogenase enzyme, minute virus of the mouse, mouse adenovirus, and mouse cytomegalovirus by the Diagnostic Laboratory at The Jackson Laboratory and were found to be negative for all the pathogens.

Tests for ecotropic virus were conducted by using the XC plaque assay (36) with titers expressed in PFU. Experimental 3- to 4-week-old mice were inoculated i.p. with supernatant from chronically infected cells containing 8×10^3 to 10×10^3 PFU of RL-MuLV or Mo-MuLV. Mice inoculated with the same volume of supernatant harvested from uninfected cells were used as control mice.

Immunization. RL-MuLV virions were isolated from supernatants of RL-MuLV-infected SC-1 cells via centrifugation at $95{,}000 \times g$. The same-density fraction isolated from cultures of uninfected SC-1 cells was used as a control. Preimmune sera were collected from 2-month-old BALB/cJ, I/LnJ, or I/LnJ IFN-γ-deficient mice, which were then immunized with 1% Triton X-100-treated RL-MuLV virion proteins (100 μg/mouse) in complete Freund's adjuvant (CFA) by subcutaneous injection into two hind footpads and four locations in the back. Mice were challenged 3 weeks later with the same dose of antigen in incomplete Freund's adjuvant. Control mice were immunized with virus-free protein fraction treated with 1% Triton X-100. Serum samples were collected 10 days after the last challenge and tested for reactivity against RL-MuLV virion proteins by ELISA. Alkaline phosphatase (AP)-labeled goat anti-mouse polyisotypic or immunoglobulin G2a (IgG2a)-specific immunoglobulin Abs were used in the second step (Southern Biotech, Birmingham, AL). All sera were used at a 5×10^{-3} dilution. Readings obtained with sera from mice immunized with the virus-free fraction were subtracted.

Western blot analysis. MMTV(C3H) or MMTV(LA) virions were purified from milk-filled stomachs of newborn pups fostered on viremic mothers as previously described (33). Mo-MuLV virions were isolated from supernatants of Mo-MuLV-infected NIH 3T3 cells via centrifugation at $95{,}000 \times g$. RL-MuLV virions were isolated as described above. MMTV and MuLV virion proteins were electrophoresed on 10% polyacrylamide gels under reducing conditions in the presence of 1% sodium dodecyl sulfate, transferred to nitrocellulose membranes, immunoblotted with sera from either MMTV- or MuLV-infected and uninfected mice, and detected with Western blot detection reagents (Amersham Pharmacia Biotech, Inc., Piscataway, NJ). Goat polyclonal serum against Friend MuLV (F-MuLV) gp70 (2) was used as a positive control for the MuLV-specific Western blot assay. Anti-Env and anti-Gag MMTV monoclonal Abs were used as positive controls for the MMTV-specific Western blot assay (33). Either anti-mouse IgG2a-specific, IgG1, or polyisotypic immunoglobulin Abs coupled to horseradish peroxidase (HRP) were used at the second step (Southern Biotech, Birmingham, AL). A 1×10^{-2} dilution of serum samples was used.

Cocultivation assay (infectious-center assay). SC-1 cells were plated at 2×10^5/60-mm dish the day before infection. On the day of infection, the medium was changed and 10^6 mitomycin C-treated single-cell suspensions from the spleens of infected mice were added, followed by the XC PFU assay.

Flow cytometry. Mononuclear peripheral-blood lymphocytes were stained with fluorescein isothiocyanate-coupled monoclonal Abs against the $V\beta14^+$ [for MMTV(C3H)] or $V\beta6^+$ T-cell receptor chain [for MMTV(LA)] (BD Biosciences, San Diego, CA). Anti-CD4 Abs coupled to phycoerythrin (Invitrogen, Carlsbad, CA) were used in the second dimension. Leukocytes were recovered from heparinized blood samples by centrifugation through a Ficoll-Hypaque cushion. Peripheral-blood lymphocytes were analyzed with a FACScan or FACScanto (Becton Dickinson, Mountain View, CA) flow cytometer and the CELLQuest software program.

ELISA. An enzyme-linked immunosorbent assay (ELISA) to detect anti-MMTV Abs in 10- to 12-week-old neonatally infected mice or in mice injected with the virus 10 to 12 weeks following infection was performed as previously described (33). An ELISA was also used to detect anti-MuLV Abs in MuLV-infected (10 to 12 weeks after infection) or immunized mice. Accordingly, virions isolated from RL-MuLV-infected SC-1 cells were treated with 0.2% Triton X-100 and were bound to plastic in borate-buffered saline overnight, followed by incubation with mouse serum samples at 4°C for 1 h. Goat anti-mouse polyisotypic or IgG2a-, IgG1-, or IgM-specific Abs coupled to AP were used at the second step. Ovalbumin (2%) was used as a blocking reagent. All sera were preabsorbed with 20% fetal calf serum in phosphate-buffered saline with 0.05%

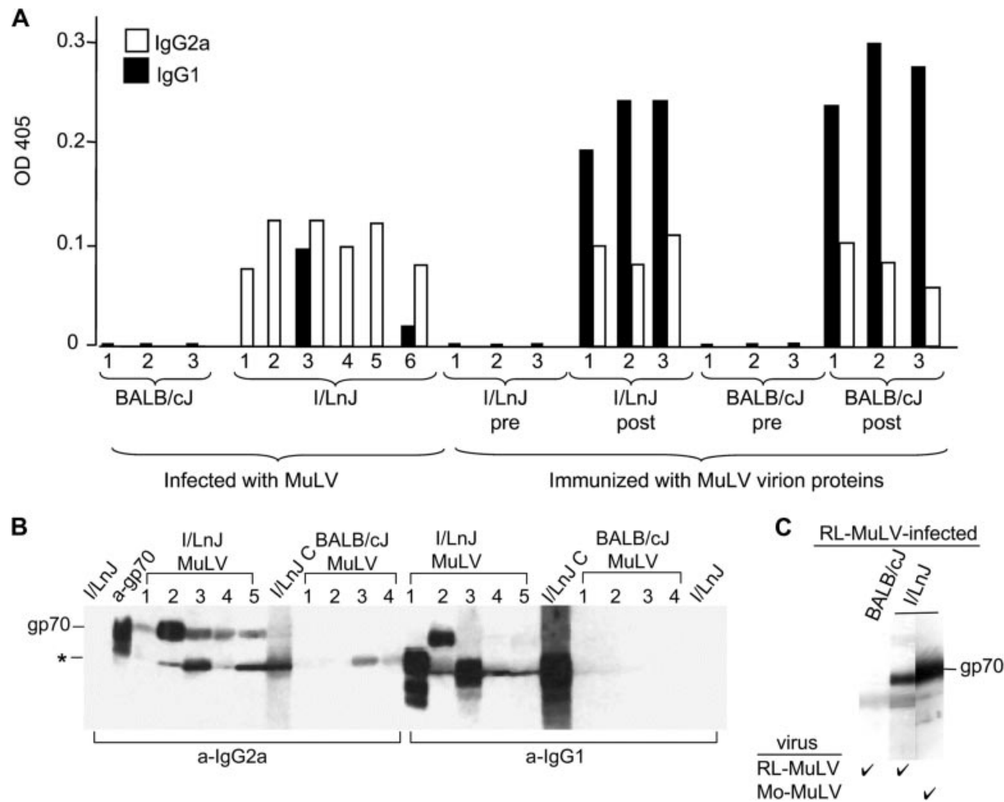

FIG. 1. MuLV-infected I/LnJ mice produce antivirus Abs. (A) Production of IgG2a-specific antivirus Abs is a specialized response against MuLV infection. Three- to 4-week-old I/LnJ and BALB/cJ males were either infected with RL-MuLV or immunized with RL-MuLV virion proteins. Sera collected from all groups of mice were tested for reactivity against RL-MuLV virion proteins in an ELISA 10 weeks after infection or 5 weeks after immunization. pre, preimmune sera; post, immune sera. Each number represents an individual animal. Anti-mouse IgG2a- or IgG1-specific Abs coupled to AP were used at the second step. (B) MuLV-infected I/LnJ mice produce Abs against virion proteins. Western blot assay with RL-MuLV virion proteins and sera from RL-MuLV-infected (I/LnJ MuLV, BALB/cJ MuLV) mice 12 weeks after infection. I/LnJ, pooled sera of five uninfected I/LnJ mice; I/LnJ C, sera from three I/LnJ mice that received an injection of virus-free tissue culture supernatants from uninfected SC-1 cells. Five individual I/LnJ sera (1 to 5) and four individual BALB/cJ sera (1 to 4) were used. a-gp70, goat polyclonal serum against F-MuLV gp70. Anti-mouse IgG2a- or IgG1-specific Abs coupled to HRP were used at the second step. *, nonspecific band due to reactivity against calf serum proteins. Numbers correspond to individual mice. (C) Anti-RL-MuLV Abs cross-react with Mo-MuLV virion proteins. Western blot assay of RL-MuLV or Mo-MuLV virion proteins and sera from RL-MuLV-infected I/LnJ and BALB/cJ mice 12 weeks after infection. Anti-mouse IgG2a-specific Abs coupled to HRP were used at the second step. OD 405, optical density at 405 nm.

Tween 20 overnight at +4°C to remove reactivity against calf serum proteins. All sera were used at a 5×10^{-3} dilution. Readings obtained with sera from mice injected with virus-free medium were subtracted.

Virus neutralization assay. Serum samples of MuLV-infected I/LnJ and BALB/cJ mice were treated at 56°C for 10 min and incubated with the virus at room temperature for 2 h. The samples were then filtered through 0.22-μm filters and plated for XC plaque assay. Sera from mice injected with virus-free medium were used as a control. A 1×10^{-1} dilution of sera in phosphate-buffered saline was used in the neutralizing procedure.

RESULTS

I/LnJ mice infected with MuLV produce neutralizing antivirus Abs. Previously, we showed that MMTV-infected I/LnJ mice produced antivirus neutralizing Abs of predominantly the IgG2a isotype (33). The biased shift to the IgG2a-specific Ab response was a result of IFN-γ produced in response to MMTV infection (33). We sought to examine whether this unique antivirus response was mounted upon infection with a different retrovirus, specifically, MuLV. Accordingly, I/LnJ and susceptible BALB/cJ mice were challenged by an i.p. injection of RL-MuLV (19, 40). RL-MuLV is a virus mixture that consists of ecotropic MuLV and mink lung cell focus-forming viruses (19). Upon infection of susceptible mice, RL-MuLV causes an increase in hematopoiesis due to the activation of pluripotent hematopoietic stem cells that results in splenomegaly (19).

Two months after MuLV was introduced, animals were bled and serum samples were tested for Abs interacting with MuLV virion proteins by ELISA and Western blot assays. As Fig. 1 illustrates, all MuLV-infected I/LnJ mice showed production of the IgG2a-specific Abs that recognize MuLV virion proteins. Moreover, the production of the IgG2a antivirus Abs required infection because mice immunized with MuLV virion proteins produced Abs without a shift toward the IgG2a antivirus Ab isotype (Fig. 1A). In addition, both BALB/cJ and I/LnJ mice responded similarly to viral antigens upon immunization (Fig. 1A). Western blot analysis with purified MuLV virions has shown that the *env* gene product (gp70SU) is a major target recognized by these Abs (Fig. 1B).

To determine whether anti-MuLV Abs were virus neutralizing, we performed a virus neutralization experiment, fol-

TABLE 1. MuLV-infected I/LnJ mice produce virus-neutralizing antibodies[a]

Serum source	Mean no. of PFU/ml ± SEM (no. of mice, virus)	% Inhibition
Uninfected BALB/cJ mice	$1.0 \times 10^6 \pm 0.10 \times 10^6$ (3, RL-MuLV)	
Uninfected BALB/cJ mice	$1.0 \times 10^6 \pm 0.03 \times 10^6$ (3, Mo-MuLV)	
Uninfected I/LnJ mice	$0.8 \times 10^6 \pm 0.02 \times 10^6$ (3, RL-MuLV)	
Uninfected I/LnJ mice	$0.9 \times 10^6 \pm 0.07 \times 10^6$ (4, Mo-MuLV)	
RL-MuLV-infected BALB/cJ mice	$1.0 \times 10^6 \pm 0.20 \times 10^6$ (4, RL-MuLV)	0
RL-MuLV-infected BALB/cJ mice	$1.2 \times 10^6 \pm 0.25 \times 10^6$ (3, Mo-MuLV)	0
Mo-MuLV-infected BALB/cJ mice	$1.1 \times 10^6 \pm 0.10 \times 10^6$ (3, RL-MuLV)	0
Mo-MuLV-infected BALB/cJ mice	$1.0 \times 10^6 \pm 0.04 \times 10^6$ (3, Mo-MuLV)	0
RL-MuLV-infected I/LnJ mice	$5.4 \times 10^3 \pm 6.40 \times 10^3$ (6, RL-MuLV)	99.3
RL-MuLV-infected I/LnJ mice	$4.9 \times 10^3 \pm 3.60 \times 10^3$ (3, Mo-MuLV)	99.5
Mo-MuLV-infected I/LnJ mice	$5.0 \times 10^3 \pm 1.60 \times 10^3$ (5, RL-MuLV)	99.4
Mo-MuLV-infected I/LnJ mice	$4.0 \times 10^3 \pm 2.80 \times 10^3$ (3, Mo-MuLV)	99.6

[a] Serum samples of RL-MuLV- or Mo-MuLV-infected I/LnJ and BALB/cJ mice incubated with supernatants from MuLV-infected tissue cultures indicated in parentheses were subjected to an XC plaque assay. Uninfected BALB/cJ and I/LnJ mice were inoculated with virus-free tissue culture medium. No plaques were produced with MuLV-infected BALB/cJ or I/LnJ serum samples not incubated with the virus. Mice were analyzed at 12 weeks postinfection.

lowed by an XC plaque test (36). A 1×10^{-1} dilution of sera from RL-MuLV-infected I/LnJ mice was capable of neutralizing 99.5% of the RL-MuLV virus (Table 1). I/LnJ mice also produced virus-neutralizing Abs upon infection with ecotropic Mo-MuLV (Table 1). The anti-RL-MuLV and anti-Mo-MuLV Abs were cross-reactive as they recognized determinants of gp70Env that are important for virus infectivity and are common between the two viruses (Fig. 1C and Table 1).

The retrovirus resistance mechanism requires IFN-γ produced in an IL-12-independent fashion. We showed that IFN-γ is indispensable to the MMTV resistance mechanism inherited by I/LnJ mice (33) as I/LnJ mice with a targeted mutation of IFN-γ failed to initiate any virus-specific humoral immune response (33). Knowing that the MMTV resistance mechanism in I/LnJ mice is controlled by IFN-γ, we sought to determine whether IFN-γ also controls antivirus Ab production in MuLV-infected I/LnJ mice.

IFN-$\gamma^{-/-}$ and IFN-$\gamma^{+/+}$ I/LnJ mice were infected with RL-MuLV and tested for anti-MuLV Abs starting at 3 weeks postinfection. In contrast to IFN-$\gamma^{+/+}$ I/LnJ mice, none of the MuLV-infected IFN-$\gamma^{-/-}$ I/LnJ mice produced antivirus Abs of any isotype (Fig. 2A; shown are mice at 12 weeks postinfection). To confirm that the animals were infected and to ensure that they were unable to produce the virus-neutralizing immune response shortly after infection, we compared the titers of infectious virus produced by spleen cells of RL-MuLV-infected IFN-$\gamma^{-/-}$ I/LnJ mice at different time points following infection. At 2 weeks after infection, IFN-$\gamma^{+/+}$ I/LnJ mice exhibited 240 ± 30 PFU/1×10^6 cells ($n = 5$), while age-matched, virus-infected IFN-$\gamma^{-/-}$ I/LnJ mice showed 400 ± 20 PFU/1×10^6 cells ($n = 5$). At 7 weeks after infection, no infectious virions were detected in the spleens of infected IFN-$\gamma^{+/+}$ mice, whereas the virus titer in IFN-$\gamma^{-/-}$ mice reached 2×10^3 PFU/1×10^6 cells. The immunization of IFN-$\gamma^{-/-}$ I/LnJ mice with viral antigens in CFA resulted in the production of all but the IgG2a-specific Abs (IFN-$\gamma^{-/-}$ mice lack this Ab isotype) (Fig. 2B). Thus, IFN-$\gamma^{-/-}$ I/LnJ mice were capable of forming germinal centers and mounting an antivirus Ab response upon immunization. However, they produced no Abs in response to natural retroviral infection and, unlike IFN-$\gamma^{+/+}$ I/LnJ mice, were unable to resist infection with MuLV.

IFN-γ production is primarily controlled by IL-12, a proinflammatory cytokine produced by dendritic cells and phagocytes in response to various pathogens via a TYK2/JAK2/STAT4-dependent mechanism (24). IL-12 consists of two

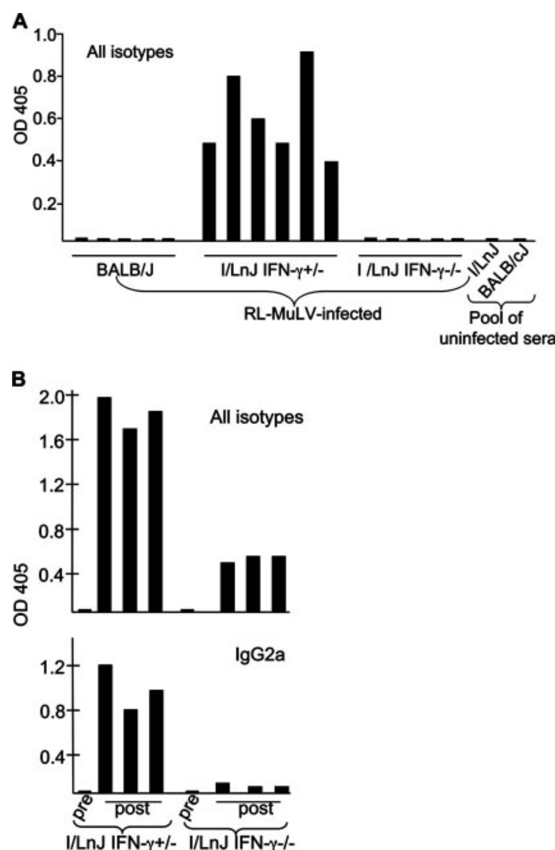

FIG. 2. IFN-γ-deficient I/LnJ mice mount an antivirus Ab response upon immunization but not in response to natural infection. (A) MuLV-infected IFN-$\gamma^{-/-}$ I/LnJ mice are not capable of producing antivirus Abs. Serum samples from MuLV-infected IFN-$\gamma^{-/-}$ and IFN-$\gamma^{+/-}$ I/LnJ mice were tested for reactivity against MuLV virion proteins by ELISA at 12 weeks after infection. Anti-mouse polyvalent Abs coupled to AP were used at the second step. Each bar represents an individual mouse. Sera from five uninfected BALB/cJ and three uninfected I/LnJ mice were used as controls. Sera from all of the animals were also screened for anti-MuLV Abs 3 weeks following infection, and no antivirus Abs were detected by an MuLV-specific ELISA (not shown). (B) IFN-$\gamma^{-/-}$ I/LnJ mice produce antivirus Abs when immunized with viral antigens in CFA. Three-month-old IFN-$\gamma^{+/+}$ and IFN-$\gamma^{-/-}$ I/LnJ mice were immunized with MuLV virion proteins, and their preimmune and immune sera were tested in an ELISA for reactivity against virion proteins. Polyvalent or IgG2a-specific anti-mouse Abs coupled to AP were used at the second step. pre, pooled sera collected from all mice prior to immunization; post, immune serum samples. Each bar represents an individual mouse. OD 405, optical density at 405 nm.

subunits, p35 (α subunit) and p40 (β subunit), both of which are required for the biological activity of the cytokine (41). Mice deficient in IL-12β do not make biologically active IL-12 and are highly susceptible to infection with many pathogens (24, 29).

To test whether IL-12 contributes to the resistance mechanism in I/LnJ mice by stimulating IFN-γ production, we generated IL-12$\beta^{-/-}$ I/LnJ mice by crossing B6.129-*Il12b*^{tm1Jm} mice (24) to I/LnJ mice and tested their offspring for the ability to produce virus-neutralizing Abs following infection with MMTV, as well as with RL-MuLV. Unexpectedly, IL-12$\beta^{-/-}$ I/LnJ mice infected with either MMTV or MuLV retained the ability to make virus-neutralizing Abs of the IgG2a isotype and produced them at titers similar to those of IL-12$^{+/+}$ mice (Fig. 3). In addition, all MuLV-infected IL-12$^{-/-}$ I/LnJ mice showed no infectious MuLV produced by their splenocytes, as determined by a plaque assay (Table 2). Therefore, we concluded that IL-12 is dispensable in the pathway leading to IFN-γ-mediated production of antiretrovirus Abs in infected I/LnJ mice.

Essential role of cytotoxic responses in the protection against retrovirally induced disease. Even though we demonstrated that virus-neutralizing Abs are required for controlling retrovirus replication in infected I/LnJ mice (5), the question of whether cytotoxic responses also contribute to the retrovirus resistance in these animals remained. The cytotoxic T-cell response plays a role in both the recovery from F-MuLV and in modulating MMTV replication in mice from resistant strains (20, 34). Mice with a targeted deletion of β2M do not express MHC class I molecules and thus lack functional CD8^{+} CTLs (21). Furthermore, β2M-deficient mice have functionally suppressed NK cells (18, 22), lack β2M-associated nonclassical MHC molecules, including CD1 (26), and thus do not generate CD1-restricited NK T cells (3). Therefore, mice with a targeted mutation of β2M fail to produce CTL-, NK-, and NK T-cell-mediated cytotoxic responses. To investigate whether these cytotoxic responses are required for retrovirus resistance in I/LnJ mice, we tested β2M$^{-/-}$ I/LnJ mice for their ability to resist retroviral infection and to transmit infectious virions.

β2M$^{-/-}$ and β2M$^{+/+}$ I/LnJ mice were infected with RL-MuLV and monitored for antivirus Ab production, virus replication, and disease development. Even though β2M$^{-/-}$ II/LnJ mice produced antivirus Abs similarly to their β2M$^{+/+}$ littermates (Fig. 3B), they still developed splenomegaly and their spleen cells secreted infectious virus (Table 2). However, the titers of the infectious virus secreted by β2M$^{-/-}$ I/LnJ splenocytes were significantly lower ($P < 0.0001$) compared to the titers produced by β2M$^{+/+}$ virus-susceptible BALB/cJ splenocytes (Table 2). These data established that antivirus Abs alone are insufficient in eliminating infectious virus and that the Abs have to operate in concert with antivirus cytotoxic responses in order to confer full protection against MuLV in I/LnJ mice.

To determine whether antivirus Abs alone are sufficient in restricting virus transmission in the MMTV system, β2M$^{-/-}$ and β2M$^{+/+}$ I/LnJ mice were infected with MMTV via fostering by viremic mothers and examined for the presence of infectious virus. MMTV encodes a Sag which plays an important role in the virus life cycle (1). It is presented by MHC class II molecules and stimulates cognate T cells to divide. Activated T cells undergo deletion, an event which is commonly used to

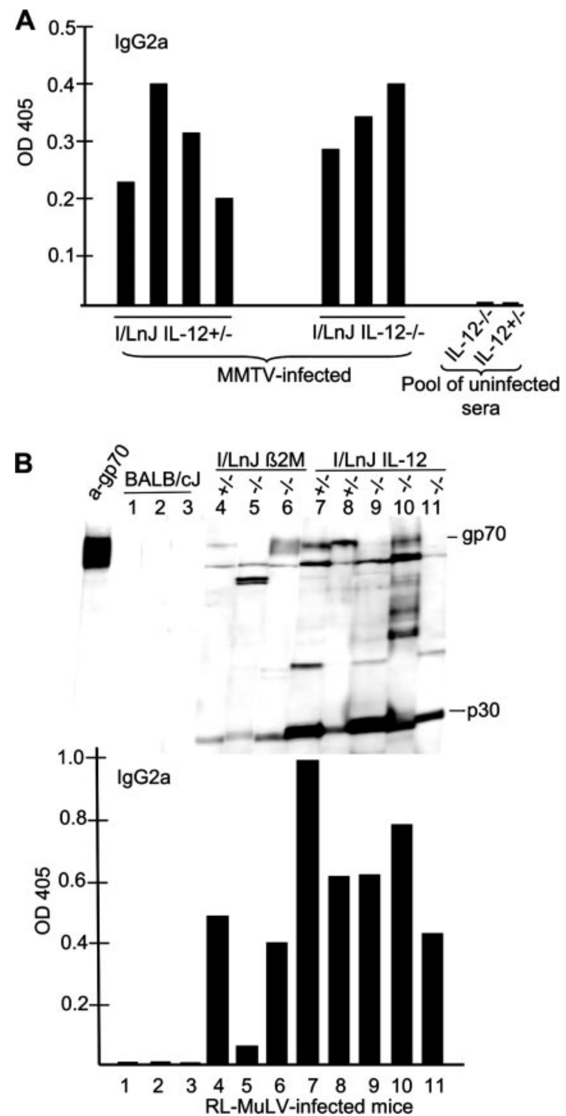

FIG. 3. β2M- and IL-12-deficient I/LnJ mice generate retrovirus-neutralizing Abs. (A) IL-12 is not required for antivirus Ab production in MMTV-infected I/LnJ mice. Serum samples from IL-12$\beta^{-/-}$ and IL-12$\beta^{+/-}$ I/LnJ mice infected with MMTV as neonates were tested for reactivity against MMTV virion proteins by ELISA 12 weeks after infection. Anti-mouse IgG2a-specific Abs coupled to AP were used at the second step. Each bar represents an individual mouse. Sera from five uninfected BALB/cJ and three uninfected I/LnJ mice were used as controls. (B) β2M$^{-/-}$ and IL-12$^{-/-}$ I/LnJ mice produce anti-MuLV Abs upon infection. Serum samples from β2M$^{-/-}$ and IL-12$^{-/-}$ I/LnJ mice, as well as their heterozygous littermates, were infected with RL-MuLV and tested for reactivity against MuLV virion proteins by Western blot assay (top) and by ELISA (bottom) 12 weeks after infection. gp70, surface glycoprotein, product of the *env* gene. p30, capsid protein, product of the *gag* gene. Anti-mouse IgG2a-specific Abs were used at the second step. Numbers indicate individual mice. a-gp70, polyclonal serum against F-MuLV gp70. The high optical density reading in the ELISA and the low reactivity in the Western blot assay observed for mouse 4 may be due to Abs that recognize native (nondenatured) antigenic determinants. OD 405, optical density at 405 nm.

determine whether animals are MMTV infected (25). After one virus passage, animals from all groups became MMTV infected, showing both deletion of Sag-cognate T cells and integrated proviruses in the cells of the lymphoid system and

TABLE 2. Comparison of the susceptibilities of IL-12- and β2M-deficient I/LnJ mice to infection with MuLV[a]

Mouse strain	Genotype	Mean spleen wt (g) ± SEM (no. of mice)	Mean no. of PFU/10^5 cells ± SEM
I/LnJ	IL-$12^{+/-}$	0.08 ± 0.008 (3)	0
I/LnJ	IL-$12^{-/-}$	0.08 ± 0.01 (3)	0
I/LnJ	β$2M^{+/-}$	0.08 ± 0.005 (3)	0
I/LnJ	β$2M^{-/-}$	0.22 ± 0.083 (3)	83 ± 35
I/LnJ	WT[b]	0.08 ± 0.005 (7)	0
BALB/cJ	WT	0.24 ± 0.009 (9)	1,350 ± 120

[a] Spleen cells from RL-MuLV-infected mice with the indicated genotypes were subjected to an infectious-center assay 10 weeks after infection.
[b] WT, wild type.

mammary glands (data not shown). Animals from all groups produced anti-MMTV Abs at comparable titers (Fig. 4).

To test whether anti-MMTV Abs produced by infected β$2M^{-/-}$ I/LnJ mice were capable of neutralizing the virus, we fostered susceptible BALB/cJ mice on their milk and looked for evidence of infection. In contrast to virus produced by MMTV(LA)-infected BALB/cJ mice, virus produced by MMTV-infected β$2M^{-/-}$ I/LnJ mice was fully neutralized, as susceptible BALB/cJ mice fostered on their milk remained uninfected. Uninfected BALB/cJ mice have 11.8% ± 0.5% peripheral $CD4^+$ Vβ6^+ T cells (n = 10). Whereas 12-week-old BALB/cJ mice fostered by MMTV(LA)-infected BALB/cJ females had 1.2% ± 0.2% peripheral $CD4^+$ Vβ6^+ T cells (n = 8), age-matched BALB/cJ mice fostered by MMTV(LA)-infected β$2M^{-/-}$ I/LnJ mice exhibited 10.9% ± 0.3% $CD4^+$ Vβ6^+ T cells (n = 5), indicating that they were virus free. These data are consistent with our previous results showing that virus-neutralizing Abs are the sole factor preventing virus spread in MMTV-infected I/LnJ mice (5). However, we cannot exclude the possibility that virus-specific cytotoxic immune responses play a role in protecting mice from MMTV-induced mammary tumors since this trait was not examined in this study.

The resistance to retroviruses in I/LnJ mice is controlled by a major locus mapped to chromosome 17. We have previously reported that the mechanism of resistance to MMTV infection in I/LnJ mice is recessive and is controlled by a single gene, *vic1* (*v*irus *i*nfectivity *c*ontroller *1*) (14, 33). To map the *vic1* gene, we performed a genome-wide screen by simple sequence length polymorphism analysis of DNA from 150 susceptible and 150 resistant N_2 mice obtained from crosses between susceptible (C3H/HeN × I/LnJ)F_1 and resistant I/LnJ mice with markers positioned every 20 to 40 Mb throughout all of the chromosomes. This analysis aimed at identifying an association between the resistant phenotype and an I/LnJ-derived genotype. Map Manager software version QTb2968k was used to identify linkage of the *vic1* gene to particular chromosomes. *vic1* was mapped to an ~23-Mb region of chromosome 17 between marker *D17Mit24* and a self-made marker at position 60 Mb (Fig. 5A) since the percentage of resistant N_2 mice homozygous for the I/LnJ alleles within the indicated intervals peaked between these markers (not shown).

To prove that the *vic1* gene is necessary and sufficient to make susceptible mice produce antivirus Abs and to fine map the *vic1* gene, we generated a congenic mouse strain by transferring $vic1^{I/LnJ}$ from resistant I/LnJ mice onto the susceptible C3H/HeN background (the C3H/$HeN^{vic1I/LnJ}$ line) and screened these congenic mice for the ability to produce virus-neutralizing Abs. Accordingly, C3H/$HeN^{vic1I/LnJ}$ mice were MMTV infected via i.p. injection and tested for antivirus Ab production 8 to 10 weeks after infection. C3H/$HeN^{vic1I/LnJ}$ congenic mice produced IgG2a-specific anti-MMTV Abs which recognize major MMTV virion proteins (Fig. 5C) similar to MMTV-infected I/LnJ mice (33) (Fig. 5B).

Next, we sought to determine whether the same locus controls susceptibility to infection with MuLV. Since C3H/HeN mice are resistant to MuLV via an unknown mechanism (L. K. Case and T. V. Golovkina, unpublished data), C3H/$HeN^{vic1I/LnJ}$ mice were not suitable for this test. Therefore, we produced another congenic mouse strain by transferring the I/LnJ *vic1* locus onto the MuLV-susceptible BALB/cJ background (the BALB/$cJ^{vic1I/LnJ}$ line) (Fig. 5A). Congenic BALB/$cJ^{vic1I/LnJ}$ mice and their wild-type littermates were infected with RL-MuLV and screened for antivirus Abs 8 to 10 weeks after infection. All virus-infected BALB/$cJ^{vic1I/LnJ}$ animals produced the IgG2a virus-specific Abs recognizing a major virus protein (gp70Env) (Fig. 5D). Some sera also recognized p30Gag (not shown).

We showed that the virus-specific humoral and cytotoxic immune responses are both required to confer full protection from virally induced disease in MuLV-infected I/LnJ mice (Table 2). To determine whether the production of Abs and the antivirus cytotoxic response are both controlled by the same locus, we followed disease progression in RL-MuLV-infected BALB/$cJ^{vic1I/LnJ}$ congenic mice by monitoring splenomegaly. Whereas none of the Ab-producing BALB/$cJ^{vic1I/LnJ}$ mice developed splenomegaly, all non-Ab-producing wild-type littermates had significantly enlarged spleens (Table 3). To compare the virus titers in spleens between Ab-producing and non-Ab-producing animals, we performed infectious-center assays (8). While infected non-Ab producers had 1,156 ± 365 PFU/10^5 spleen cells, no infectious virus was detected in the spleens of infected Ab producers (Table 3). BALB/$cJ^{vic1/LnJ}$

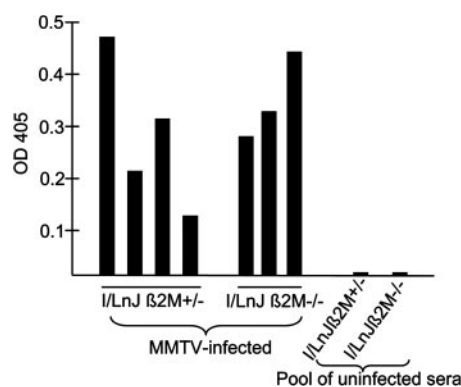

FIG. 4. MMTV-infected β2M-deficient I/LnJ mice produce antivirus Abs of the IgG2a isotype. Serum samples from β$2M^{-/-}$ and β$2M^{+/-}$ I/LnJ mice infected with MMTV as neonates were tested for reactivity against MMTV virion proteins by ELISA 12 weeks after infection. Anti-mouse IgG2a Abs coupled to AP were used at the second step. OD 405, optical density at 405 nm.

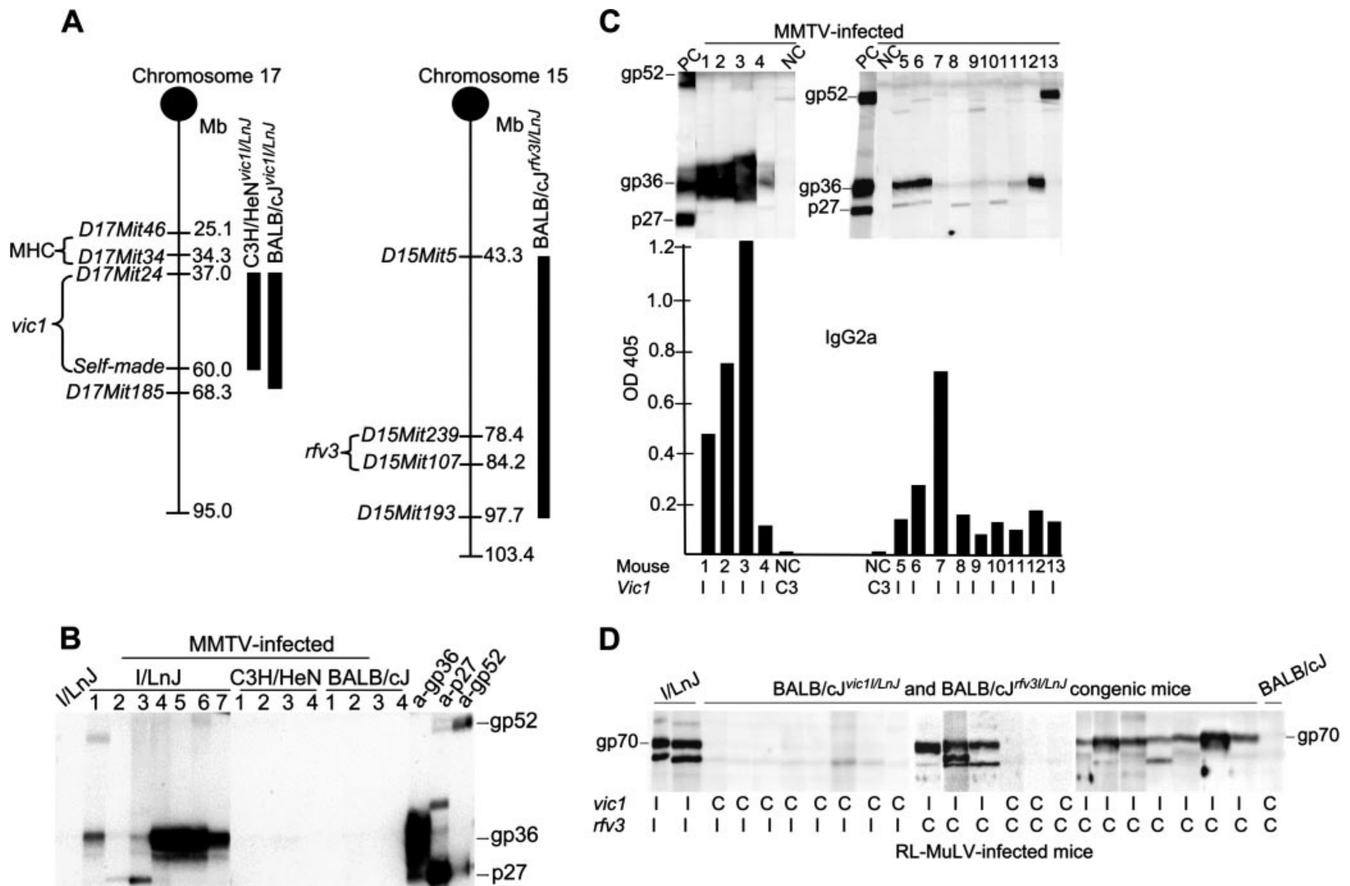

FIG. 5. The I/LnJ *vic1* gene controls resistance to both MMTV and MuLV. (A) Genetic distance and predicted locations of *vic1* on chromosome 17 and *rfv3* on chromosome 15. Regions between markers *D17Mit24* and *D17Mit185* of I/LnJ chromosome 17 and between markers *D15Mit5* and *D15Mit193* of I/LnJ chromosome 15 were transferred to the BALB/cJ genetic background to create the BALB/cJ$^{vicI/LnJ}$ and BALB/cJ$^{rfv3I/LnJ}$ congenic lines, respectively. A region between *D17Mit24* and a self-made marker (at 60 Mb) of the I/LnJ chromosome was transferred onto the C3H//HeN background to generate the C3H/HeN$^{vic1I/LnJ}$ congenic line. The map of *rfv3* was taken from reference 39. (B) MMTV-infected I/LnJ mice produce anti-MMTV Abs which recognize major virion proteins. MMTV(LA) virion proteins separated on an acrylamide gel were incubated with mouse monoclonal Abs against gp52SU (a-gp52), p27CA (a-p27), and gp36TM (a-gp36) (33) or with sera from I/LnJ, BALB/cJ, and C3H/HeN mice injected i.p. with MMTV 12 weeks after infection. Blots were developed with anti-mouse IgG2a-specific Abs coupled to HRP. The numbers at the top indicate individual mice. I/LnJ, pooled sera from five uninfected I/LnJ mice. (C) The I/LnJ *vic1* gene confers production of anti-MMTV Abs in virus-susceptible C3H/HeN mice. Homozygous C3H/HeN$^{vic1I/LnJ}$ congenic mice were infected with MMTV via i.p. injection and tested for antivirus Ab production 10 weeks after infection as described for panel B. The numbers at the top indicate individual mice. PC, positive control, a mixture of monoclonal anti-p27CA, anti-gp36TM, and anti-gp52SU Abs incubated with virion proteins separated on the same gel. I, mice were homozygous for the I/LnJ genome between marker *D17Mit24* and the self-made marker at 60 Mb (panel A). C3, mice were homozygous for the C3H/HeN genome between the same markers. The high optical density reading in the ELISA and the low reactivity in the Western blot assay observed for mouse 7 may be due to Abs recognizing native antigenic determinants. (D) The I/LnJ *vic1* gene, but not the *rfv3* gene, confers the production of anti-MuLV Abs on virus-susceptible BALB/cJ mice. Homozygous BALB/cJ$^{rfv3I/LnJ}$ congenic mice were infected with RL-MuLV and tested for antivirus Ab production 12 weeks after infection. The numbers at the top indicate individual mice. I/LnJ, positive control, sera from RL-MuLV-infected I/LnJ mice. BALB/cJ, negative control, sera from five wild-type BALB/cJ mice infected with RL-MuLV. I, mice were homozygous for the I/LnJ genome between markers *D17Mit24* and *D17Mit185* or markers *D15Mit5* and *D15Mit193* (panel A). C, mice were homozygous for the BALB/cJ genome between the same markers. gp70, surface glycoprotein, product of the *env* gene. OD 405, optical density at 405 nm. NC, negative control.

congenic mice were also capable of producing anti-MMTV Abs when infected with the virus (data not shown).

The recovery to *F*riend *v*irus *3* (*rfv3*) gene, mapped to chromosome 15, influences the ability of resistant mice to mount an MuLV-neutralizing Ab response following infection (6, 17). After F-MuLV infection, mice that are homozygous for the *rfv3* recessive susceptible allele (*rfv*^{s}) (BALB/cJ or A/J, for instance) fail to induce an antivirus neutralizing immune response and succumb to erythroleukemia (6, 17). In contrast, mice with one or two dominant resistant *rfv3* alleles (*rfv*^{r}) (C57BL/6J or C57BL/10J) produce F-MuLV-specific neutralizing Abs and recover from viremia (6, 17). Even though the resistance mechanism controlled by the *vic1* gene is recessive and the gene has been mapped to chromosome 17, *vic1* and *rfv3* bear similarities in their mechanisms of function. To verify that I/LnJ mice inherit the susceptible allele of the *rfv3* gene, we transferred the locus containing the *rfv3* gene from MuLV-resistant I/LnJ mice to MuLV-susceptible BALB/cJ mice (BALB/cJ$^{rfv3I/LnJ}$ congenic line, Fig. 5A) and infected them with RL-MuLV. All homozygous BALB/cJ$^{rfv3I/LnJ}$ mice devel-

TABLE 3. BALB/cJ$^{vic1I/LnJ}$ mice resist MuLV infection[a]

Mouse strain	Genotype at *vic1* locus[b]	Mean spleen wt (g) ± SEM (no. of mice)	Mean no. of PFU/10^5 cells ± SEM
MuLV-infected BALB/cJ	C	0.20 ± 0.012 (5)	1,156 ± 365
MuLV-infected BALB/cJ	I	0.07 ± 0.007 (6)	0
BALB/cJ[c]	C	0.08 ± 0.009 (10)	0
I/LnJ[c]	I	0.08 ± 0.008 (10)	0

[a] Spleen cells from RL-MuLV-infected mice were subjected to an infectious-center assay 12 weeks after infection.
[b] C or I indicates the BALB/cJ or I/LnJ genotype, respectively, at markers *D17Mit24* and *D17Mit185* proximal and distal to the locus containing the *vic1* gene (Fig. 5A).
[c] Uninfected mice.

TABLE 4. *rfv3*^{r} C57BL/6J mice fail to produce anti-MMTV antibodies[a]

Mouse strain (generation)	Virus passage	Mean % of $CD4^+$ $V\beta6^+$ T cells[b] ± SEM (no. of mice)
C57BL/6J	Uninfected	10.4 ± 0.6 (8)
C57BL/6J (G_1)	First	2.7 ± 0.6 (5)
C57BL/6J (G_2)	Second	2.4 ± 0.4 (6)
C57BL/6J (G_4)	Fourth	2.9 ± 0.7 (5)

[a] Percentages of Sag-cognate $CD4^+$ $V\beta6^+$ T cells were analyzed in the peripheral blood of MMTV(LA)-infected C57BL/6J mice of different generations (see explanation in the text). MMTV(LA)-infected I/LnJ mice eliminate the virus by G_2, and thus, the G_2 infected I/LnJ mice have percentages of Sag-cognate T cells indistinguishable from those of uninfected I/LnJ mice (33).
[b] Percentage of $CD4^+$ $V\beta6^+$ T cells among all $CD4^+$ T cells.

oped splenomegaly, produced high titers of infectious virus (Table 3), and did not mount an antivirus neutralizing-Ab response (Fig. 5D). These data established that I/LnJ mice have the susceptible allele of the *rfv3* gene that is unable to support a protective antivirus immune response.

Next, we investigated whether *rfv3*^{r} would confer the ability to produce MMTV-neutralizing Abs, the property of the resistant allele of the I/LnJ *vic1* gene (*vic1*^{r}). We have previously shown that C57BL/6J mice that inherit the *rfv3*^{r} gene are susceptible to MMTV(LA) (32). However, we have not followed the virus's fate in infected-mouse pedigrees, leaving the possibility that a potential antivirus immune response may prevent virus transmission. To test whether C57BL/6J mice are able to produce anti-MMTV neutralizing Abs and to eliminate infectious virus, similar to I/LnJ mice, we followed the virus's fate in an MMTV-infected C57BL/6J mouse pedigree. Frequencies of peripheral Sag-cognate $CD4^+$ $V\beta6^+$ T cells in generation 1 (G_1) C57BL/6J mice fostered by viremic BALB/cJ females were analyzed at 3 months of age. All of the animals became infected since they showed deletion of Sag-cognate T cells (Table 4). Two of the fostered C57BL/6J females (virus passage 1) were bred to produce G_2 of the infected-mouse pedigree to follow the virus's fate. G_2 mice (virus passage 2) were bled at 3 months, and their peripheral-blood lymphocytes were analyzed for percentages of Sag-cognate T cells. Similar to G_1 mice, all G_2 animals showed deletion of Sag-cognate T cells (Table 4). Two G_2 females were further bred to produce infected G_3 mice. Two of the G_3 females were used to produce G_4 infected mice (virus passage 4). The percentage of Sag-cognate T cells analyzed in G_4 MMTV(LA)-infected C57BL/6J mice at 3 months of age was reduced, indicating that G_3 MMTV-infected mice passed the virus to their offspring. Animals at each generation were also analyzed for the presence of antivirus Abs, and no virus-reactive Abs were found in their sera (not shown). Therefore, unlike I/LnJ mice, C57BL/6J mice do not produce anti-MMTV Abs, supporting the notion that the function of the *vic1* gene differs from that of the *rfv3* gene and, importantly, that C57BL/6J mice inherit a susceptible allele of the *vic1* gene.

DISCUSSION

We have established that mice of the I/LnJ strain are characterized by the ability to mount a prolonged and highly efficient humoral immune response against retroviruses of different genera, including betaretroviruses (MMTV) (33) and gammaretroviruses (MuLV) (this article). The unique ability to react to exogenous retroviruses is not hampered by the presence of endogenous proviruses, as I/LnJ mice inherit both endogenous MMTVs (23) and MuLVs (not shown).

The antiretrovirus response is entirely dependent on IFN-γ, as MMTV- or MuLV-infected, IFN-$\gamma^{-/-}$ I/LnJ mice were unable to make virus-specific Abs of any isotype (33) (Fig. 2A). Stromnes et al. showed that there was an increased production of MuLV-neutralizing Abs of the IgM isotype in IFN-$\gamma^{-/-}$ C57BL/6J mice at 2 weeks after infection and it remained evident in most of the animals at 4 weeks of age (38). The Ab production gradually declined and did not result in a class switch to the IgG2A isotype (38). MuLV-infected IFN-$\gamma^{-/-}$ I/LnJ mice did not produce antivirus Abs of any isotype starting at 3 weeks after infection (Fig. 2A). Although we did not test sera of MuLV-infected IFN-$\gamma^{-/-}$ I/LnJ mice for antivirus Abs at 2 weeks after infection, it appeared that they were not present or were not neutralizing as the titer of infectious virus produced by infected IFN-$\gamma^{-/-}$ I/LnJ splenocytes were significantly higher compared to infected IFN-$\gamma^{+/+}$ I/LnJ splenocytes (400 ± 20 PFU/10^6 cells [$n = 5$] versus 240 ± 30 PFU/10^6 cells [$n = 5$], $P < 0.0001$) at this time after infection. It is likely that genetic background differences between C57BL/6J and I/LnJ mice contributed to the dissimilarities in the phenotypes produced by the IFN-γ targeted deletion.

The retrovirus resistance mechanism inherited by I/LnJ mice relies on IFN-γ-producing T_H1 $CD4^+$ T cells, as these cells are absolutely necessary for the production of MMTV-neutralizing Abs (5). The differentiation of naive $CD4^+$ T cells into T_H1 cells involves signaling by IFN-γ, IL-12, and the transcription factors T-bet and STAT4 (4). Differentiated T_H1 cells can produce large amounts of IFN-γ in response to signaling through the T-cell receptor or by the combination of cytokines IL-12 and IL-18 (4). These stimuli trigger two independent pathways responsible for inducing IFN-γ (43). Our data indicate that IL-12 is not required for the activation of the antivirus immune response, as IL-12-deficient I/LnJ mice were capable of producing IgG2a-specific virus-neutralizing Abs (Fig. 3). Interestingly, other viruses, such as mouse hepatitis virus (37), lymphocytic choriomeningitis virus (29), vesicular stomatitis virus (29), and F-MuLV (11), are also capable of inducing protective IFN-γ responses in the absence of IL-12. Therefore,

protective T_H1 responses against distinct viruses, including retroviruses, can be induced without the help of IL-12.

Our investigation into the contribution by cytotoxic responses to the retrovirus resistance mechanism revealed that the mechanism is required for full recovery from MuLV-induced disease in I/LnJ mice. Although $\beta2M^{-/-}$ I/LnJ mice produced antivirus neutralizing Abs (Fig. 3), the Abs alone could not entirely control virus replication (Table 2). All virus-infected $\beta2M^{-/-}$ I/LnJ mice developed splenomegaly and secreted infectious viruses, albeit at lower titers compared to susceptible mice (Table 2). The cytotoxic response is also known to control MMTV replication based on the finding that the virus replicates at significantly higher titers in $\beta2M^{-/-}$ than in $\beta2M^{+/+}$ susceptible C3H/HeJ mice (20). Furthermore, the $\beta2M^{-/-}$ C3H/HeJ females developed more tumors per animal compared to the $\beta2M^{+/+}$ C3H/HeJ females (Golovkina, unpublished). Thus, even though the protective role of the cytotoxic response against mammary tumors has not been investigated here, it likely contributes to the tumor resistance in MMTV-infected I/LnJ mice.

I/LnJ is the only known mouse strain that is capable of neutralizing MMTV infection through the production of retrovirus-specific Abs. However, mice from other inbred strains, like C57BL/10J (B10) or C57BL/6J (B6), mount virus-neutralizing Ab responses following infection with F-MuLV and recover from viremia (6, 13). Hasenkrug et al. have shown that the resistant phenotype exhibited by B6 mice is also IFN-γ (38) and $CD4^+$ T cell dependent (16) and is influenced by the production of antivirus CTLs (12, 16). They also showed that the production of anti-MuLV neutralizing Abs is controlled by a single dominant gene, *rfv3*, mapped to chromosome 15 (17). Identification of the *rfv3* gene, however, was never accomplished. Even though the actual effector mechanism of resistance to retroviral infection (production of neutralizing Abs) inherited by I/LnJ mice is similar to the resistance mechanism controlled by *rfv3*, the I/LnJ allele of the *rfv3* gene does not confer the ability of infected congenic BALB/cJ$^{rfv3I/LnJ}$ mice to produce anti-MuLV Abs (Fig. 5D). Similarly, the C57BL/6J allele of *rfv3^r* gene does not grant protection against MMTV (Table 4). At the same time, the I/LnJ *vic1^r* gene, mapped to chromosome 17, confers full protection against both retroviruses MMTV and MuLV on virus-susceptible mice (Fig. 5C and D).

It is clear that genetic variations in the human population result in the differential ability to keep HIV replication under control. Given that retrovirus-infected I/LnJ mice are capable of efficiently restraining the replication of murine retroviruses, our findings may provide an opportunity to relate this unique murine resistance mechanism to retrovirus resistance in humans. The identification of the *vic1* gene will be a major move toward this goal.

ACKNOWLEDGMENTS

We are thankful to members of the laboratory and to Alexander Chervonsky for helpful discussion. We are also thankful to Anna Hall for technical support.

This work was supported by PHS grant CA89116 and by an award from the American Cancer Society to T.V.G. This work was also supported by a grant (CA34196) from the National Cancer Institute to The Jackson Laboratory and by a grant (P30 CA014599) to the University of Chicago.

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