The Journal of Experimental Medicine

Unique Resistance of I/LnJ Mice to a Retrovirus Is Due to Sustained Interferon γ–dependent Production of Virus-neutralizing Antibodies

Alexandra Purdy, Laure Case, Melody Duvall, Max Overstrom-Coleman, Nilah Monnier, Alexander Chervonsky, and Tatyana Golovkina

The Jackson Laboratory, Bar Harbor, ME 04609

Abstract

Selection of immune escape variants impairs the ability of the immune system to sustain an efficient antiviral response and to control retroviral infections. Like other retroviruses, mouse mammary tumor virus (MMTV) is not efficiently eliminated by the immune system of susceptible mice. In contrast, MMTV-infected I/LnJ mice are capable of producing IgG2a virus-neutralizing antibodies, sustain this response throughout their life, and secrete antibody-coated virions into the milk, thereby preventing infection of their progeny. Antibodies were produced in response to several MMTV variants and were cross-reactive to them. Resistance to MMTV infection was recessive and was dependent on interferon (IFN)-γ production, because I/LnJ mice with targeted deletion of the INF-γ gene failed to produce any virus-neutralizing antibodies. These findings reveal a novel mechanism of resistance to retroviral infection that is based on a robust and sustained IFN-γ–dependent humoral immune response.

Key words: IFN-γ • virus-neutralizing antibodies • retrovirus • infection • resistance

Introduction

Exogenous mouse mammary tumor virus (MMTV)* is transmitted through the milk of infected females to suckling newborns, where it initially infects cells of the immune system (1). The MMTV genome encodes a superantigen (Sag; reference 2) that is presented by MHC class II molecules expressed on infected B cells and is recognized by TCRs expressed on cognate T cells (3, 4). Different Sags stimulate distinct subsets of T cells (each with a particular TCR Vβ chain), causing them to proliferate and allowing them to become infected with the virus (5). After the initial proliferation of T cells, steady deletion of the cognate T cells is observed in all infected mice (6). From the cells of the immune system, the virus passes to the mammary tissue and causes mammary carcinomas as a result of insertional mutagenesis (7).

Three mechanisms of resistance to MMTV infection in mice have previously been discovered. The first was mapped to the MHC locus, H2 (8). Inbred mice of *b*, *f*, *q*, and *s* H2 haplotypes do not express I-E MHC class II molecules because of mutations in either the H2-Ea or H2-Eb gene (9, 10). As I-E H2 molecules are better suited for Sag presentation, mice with the I-E-negative H2 haplotypes are relatively resistant to MMTV infection and MMTV-induced mammary tumors (11–13).

The second mechanism of resistance to MMTV was found in mice that carry endogenous proviruses, which encode Sags similar to Sag of exogenous virus. Viral Sags expressed by endogenous *Mtvs* cause negative selection of T cells bearing cognate Vβ elements in the thymus and in the periphery (14–16). As a result, mice exposed to an exogenous MMTV that encodes a Sag with the same specificity as the endogenous *Mtvs* are resistant to MMTV infection and do not develop mammary gland tumors (5, 17).

Previously we have identified a third mechanism of resistance to MMTV inherited by I/LnJ mice (18). We showed that resistance to MMTV(C3H)-induced mammary tumors in mice from this strain is due to impaired mammary gland infection and is not related to the MHC locus or presence of endogenous *Mtv*s (18). Here we found that production of avirulent virions by infected I/LnJ cells underlies the impaired mammary gland infection and sought to identify the mechanism involved.

A. Purdy, L. Case, and M. Duvall contributed equally to this work.

Address correspondence to Dr. T. Golovkina, The Jackson Laboratory, 600 Main St., Bar Harbor, ME 04609. Phone: 207-288-6287; Fax: 207-288-6078; E-mail: tvg@aretha.jax.org

**Abbreviations used in this paper:* AP, alkaline phosphatase; MMTV, mouse mammary tumor virus; MuLV, murine leukemia virus; Sag, superantigen.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/2003/01/233/11 $8.00
Volume 197, Number 2, January 20, 2003 233–243
http://www.jem.org/cgi/doi/10.1084/jem.20021499

Materials and Methods

Mice. All mice used in this study were bred and maintained at the animal facility of The Jackson Laboratory. I/LnJ, C3.JK-*H2*^{j} H2-T18^{b}/Sn (C3.JK), and BALB/cJ mice were purchased from The Jackson Laboratory. I/LnJ mice were crossed to B6.129S7-*Ifng*^{tm1Ts} (19) for 10 repetitive generations, and heterozygous N10 mice were intercrossed to generate I/LnJ mice with targeted mutation of IFN-γ (I/LnJ IFN-γ KO). C3H/HeN MMTV^{+} mice infected with the MMTV(C3H) virus variant were purchased from the National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD. The MMTV(LA) virus variant (20) was passed on BALB/cJ mice.

Antibodies and Flow Cytometry. Mononuclear peripheral blood lymphocytes were stained with FITC-coupled monoclonal antibodies against the Vβ2, Vβ6, and Vβ14 TCR chains (BD Biosciences). Anti-CD4 antibodies coupled to PE (Life Technologies) 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 using a FACScan™ (Becton Dickinson) flow cytometer and the CELLQuest™ software program.

RNase T_1 Protection Assays. RNaseT_1 protection assays were performed as described previously (21), using probes specific for BALB2, BALBLA, and BALB14 viral transcripts (20). 40 μg of total RNA isolated from the lactating mammary glands and 5 μg of RNA isolated from milk were used.

Production of Cytokines by Activated T Cells. Anti-CD3 antibodies (5 μg/ml) were attached to 6-well plates for 1.5 h at 37°C in BBS buffer. Wells were washed with PBS two times. 9×10^6 cells isolated from the lymph nodes pooled from three mice were added per a well and incubated for 24 h in the CO_2 incubator. RNA isolated from activated cells was subjected to RNase protection analysis with Riboquant probes using the manufacturer's protocol (BD Biosciences). 20 μg of splenic RNA and 5 μg of lymph node RNA (before and after activation) were loaded on a 6% urea/acrylamide gel.

Mammary Gland Tumorigenesis. Mammary gland tumor incidence in MMTV(LA)-infected BALB/cJ and I/LnJ mice was monitored by weekly palpation of the animals. Tumor-bearing mice were killed and DNA isolated from a portion of each tumor was subjected to Southern blot analysis as described previously (22). All of the tumors contained new MMTV integrants, indicating that the tumors were caused by the virus (unpublished data).

Production of Monoclonal Antibodies against MMTV Virion Proteins. Endogenous *Mtv*-free male mice, a gift of Dr. Marrack (National Jewish Medical and Research Center, Denver, CO; reference 23), were immunized with 1% Triton X-100-treated MMTV(LA) virions (100 μg of proteins) in CFA by subcutaneous injection in two hind footpads and four locations in the back. Mice were challenged 3 wk later with the same doses of virion proteins in IFA. 72 h before fusion mice were challenged with the same amounts of proteins by intravenous injection. Fusion was done according to standard protocol. Antibodies were characterized by ELISA and Western blot analysis.

ELISA with MMTV Virion Proteins. Either MMTV(C3H) isolated from the milk of C3H/HeN MMTV^{+} mice or MMTV(LA) isolated from the milk of BALB/cLA mice as described (20, 23) was resuspended in PBS with 0.2% Triton X-100 and bound to the plate at 0.5 μg/ml in BBS buffer overnight at 4°C. Nonspecific binding was blocked by 1% bovine serum albumin for 2 h at 37°C. Secondary antibodies against different mouse immunoglobulins classes conjugated with alkaline phosphatase (AP) diluted in the ELISA buffer (PBS, 0.05% Tween 20, and 0.1% sodium azide) were used at the second step. Background obtained with the secondary antibody alone (goat anti–mouse immunoglobulins coupled to AP) was subtracted. To test for antivirus antibodies in mouse sera, serum samples were diluted in the ELISA buffer and incubated with virion proteins bound to plastic before adding the secondary antibodies.

Western Blot Analysis. Viral particles were isolated from BALB/cLA milk. Viral proteins were electrophoresed on 10% polyacrylamide gels under reducing conditions in the presence of 1% sodium dodecyl sulfate. The proteins were then transferred to nitrocellulose membranes, treated with different serum samples or anti-MMTV protein monoclonal antibodies at the first step and with conjugates coupled to horseradish peroxidase (Upstate Biotechnology) at the second step, and detected with Western Blot Detection Reagents (Amersham Biosciences).

Immunization. Preimmune sera were collected from 2-mo-old BALB/cJ and I/LnJ mice, which were then immunized with MMTV(LA) virion proteins isolated from 100 μl of milk in CFA by subcutaneous injection in two hind footpads and four locations in the back. Mice were challenged 3 wk later with the same dose of antigen in IFA. Serum samples were collected 10 d later and the reactivity of pre and postimmune serum samples to MMTV(LA) virion proteins was compared by ELISA. AP-labeled goat anti–mouse immunoglobulin antibodies were used in the second step (Sigma-Aldrich).

MMTV Infection. Biologically active MMTV virions were isolated from the milk of MMTV(LA)-infected BALB/cJ females as described previously (22, 24). Proteins from virions isolated from 100 μl of milk were injected intraperitoneally into 3- to 4-wk-old I/LnJ and BALB/cJ females as described previously (24).

Virus-Neutralization Assay. The in vitro neutralization procedure was performed at room temperature as published previously (25). Briefly, purified MMTV(LA) or MMTV(C3H) virions (virions isolated from ~100 μl of milk in 20 μl of PBS) were incubated with 200 μl of serum from MMTV(LA)-, MMTV(C3H)-infected or uninfected I/LnJ mice diluted in PBS (1:20) at room temperature. After 2 h, the resulting 220 μl were injected directly into the mammary glands of control BALB/cJ mice. For the in vivo neutralization experiment, newborn BALB/cJ mice suckling on viremic BALB/cLA females were injected intraperitoneally with 50 μl of serum from either uninfected or MMTV(LA)-infected I/LnJ mice (diluted 1/10 in PBS) every other day for 9 d (5 injections). Mice were bred, and RNA isolated from their lactating mammary glands after the first pregnancy was subjected to RNase T_1 protection analysis with MMTV-specific probes.

Results

The Mammary Gland Cells of I/LnJ Mice Become Infected with MMTV(LA), but Viruses Shed into Milk Are Not Infectious. In our previous studies we showed that in I/LnJ mice exposed to the exogenous MMTV(C3H), infection did not progress to the mammary glands (18). Another MMTV, MMTV(LA) originated from BALB/cJ mice, consists of three different exogenous MMTVs: BALB2, BALBLA, and BALB14, with Vβ2-, Vβ6-, and Vβ14-specific Sags, respectively (20, 26). These MMTV(LA) viruses are produced at much higher titers than are MMTV(C3H) viruses (20). To determine whether infection with MMTV(LA) progresses to to the mammary glands, I/LnJ mice were fostered by BALB/cJ

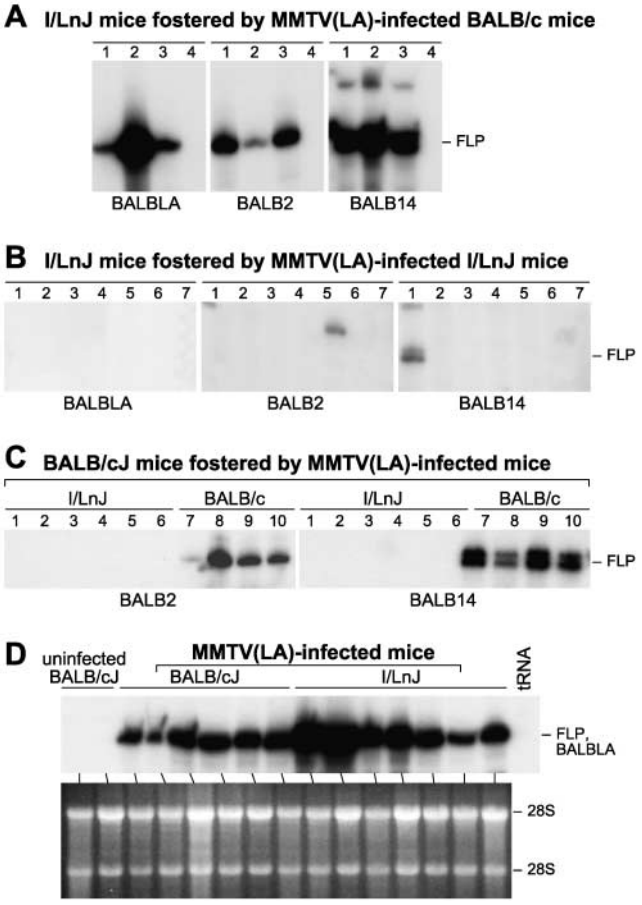


Figure 1. Virions produced by MMTV(LA)-infected I/LnJ mice are not infectious. (A) Mammary glands of I/LnJ mice fostered by MMTV(LA)-infected (BALB/cLA) females become infected and shed viruses into the milk. I/LnJ females fostered on BALB/cLA milk were bred, and RNA isolated from their milk was subjected to RNase T_1 protection analysis with probes specific for BALB2, BALBLA, and BALB14. Lanes 1–3: milk RNA from infected I/LnJ mice; lane 4, milk RNA from uninfected I/LnJ mice. Three different MMTV-infected I/LnJ mice of 220 tested are shown. (B) Viruses produced by MMTV(LA)-infected I/LnJ mice cannot infect their offspring. Female offspring from MMTV(LA)-infected I/LnJ mice were bred, and RNA isolated from their milk was subjected to RNase T_1 protection analysis. Lanes 1–7: milk RNA from individual infected females fostered by four different MMTV-infected I/LnJ females. 50 different I/LnJ mice fostered by 15 distinct MMTV-infected I/LnJ females were tested. (C) Viruses produced by MMTV(LA)-infected I/LnJ mice are not infectious even in susceptible BALB/cJ mice. BALB/cJ females were fostered by MMTV(LA)-infected I/LnJ or BALB/cJ mice. RNA isolated from their milk after the first pregnancy was subjected to RNase T_1 protection analysis with BALB2, BALBLA, and BALB14-specific probes (BALB2 and BALB14 shown). 1–6, 6 of 15 tested different BALB/cJ females fostered by 5 distinct MMTV-infected I/LnJ females shown. 7–10, BALB/cJ mice fostered by BALB/cLA females. Results shown in A, B, and C were from the same RNase T_1 protection experiment using equal amounts of RNA, and thus, can be compared quantitatively. FLP, full-length protection. (D) MMTV(LA)-infected BALB/cJ and I/LnJ mice produce equal amounts of virus into their milk. Top panel, RNA (5 μg) isolated from equal amounts of milk of mice indicated was subjected to RNaseT_1 protection analysis with probes specific for BALB2, BALBLA and BALB14 sags (only BALBLA is shown). The amount of radioactivity per viral RNA-specific fragment was quantified using a PhosphorImager. Bottom panel, the same RNA samples (15 μg) were separated on an agarose gel to verify RNA integrity.

mice infected with MMTV(LA) and frequencies of Sag-cognate T cells in the periphery were analyzed at 14 wk of age. Whereas uninfected I/LnJ mice have 9.6 ± 0.7% of $CD4^+/V\beta14^+$ and 6.5 ± 0.25% of $CD4^+/V\beta2^+$ T cells (n = 12), MMTV(LA)-infected 14-wk-old I/LnJ mice have 0.89 ± 0.6% of $CD4^+/V\beta14^+$ T cells and 0.64 ± 0.21% of $CD4^+/V\beta2^+$ T cells (n = 12). We did not analyze a subset of $CD4^+/V\beta6^+$ T cells affected by a third virus, MMTV(LA), present in the mixture, because I/LnJ mice inherit *Mtv*7 and thus, do not have $CD4^+/V\beta6^+$ T cells (18). Infected I/LnJ females were bred, tested for the presence of virus in their milk, and monitored for mammary tumors. Mammary glands of all fostered I/LnJ mice became infected, as they secreted all three MMTVs into their milk (Fig. 1 A, lanes 1–3). Quantitative analysis of viral RNA isolated from the milk of infected mice ruled out differences in virus titers between infected BALB/cJ and I/LnJ mice (Fig. 1 D). Thus, in contrast to MMTV(C3H), infection with a stronger MMTV(LA) virus variant progressed to the mammary glands of I/LnJ mice.

Although mammary glands of fostered I/LnJ mice became MMTV infected, they were resistant to mammary tumor development. Whereas 80% of MMTV(LA)-infected BALB/cJ females (16/20) developed mammary tumors by 1 yr of age, whereas none of 24 I/LnJ females infected with the same viruses had developed tumors even at 1.5 yr of age.

To determine whether the virus secreted by infected I/LnJ mice was passed to their offspring, we produced second-generation females and tested their milk for virus presence. Almost all animals either had eliminated the viruses (Fig. 1 B, lanes 2, 3, 4, 6, and 7) or produced them at significantly reduced titers compared with their mothers (Fig. 1 B, lanes 1 and 5). Thus, after only one passage through I/LnJ mice MMTV(LA) was severely attenuated.

To investigate whether MMTV(LA) produced by infected I/LnJ mice was modified in such a way that it was no longer infectious in susceptible mice, we examined MMTV-susceptible BALB/cJ and C3H/HeN mice that were foster-nursed by the same MMTV(LA)-infected I/LnJ females. All fostered BALB/cJ and C3H/HeN mice either did not produce virus or produced virus at much reduced titers as determined by RNase T_1 analysis with MMTV(LA)-specific probes (Fig. 1 C, data shown for BALB/cJ mice only). Thus, although I/LnJ mice became infected with MMTV(LA), the viruses they produced were modified such that they lost infectivity.

MMTV-infected I/LnJ Mice Produce Antibodies against MMTV. The results of described experiments suggest that MMTV proteins required for virus infectivity were either modified or blocked by anti-virus neutralizing antibodies produced by infected I/LnJ mice. To test this hypothesis, virus was isolated from the milk of MMTV(LA)-infected I/LnJ and BALB/cJ females and subjected to an ELISA to test for the presence of immunoglobulins coating the viral particles. Virus isolated from the milk of infected I/LnJ females was coated with immunoglobulins of the IgG2a isotype, while virus isolated from infected BALB/cJ milk was not (Fig. 2 A, left and right graph).

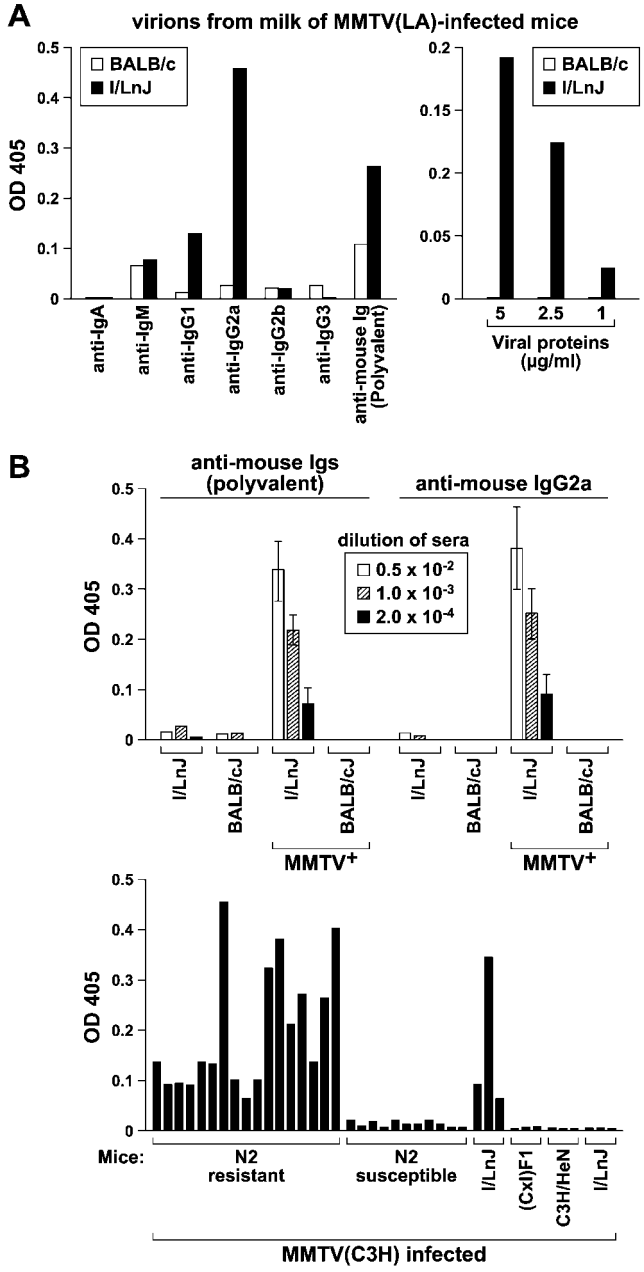


Figure 2. MMTV-infected I/LnJ mice produce IgG2a-specific antibodies against MMTV. (A) Viral particles isolated from the milk of I/LnJ mice fostered by BALB/cLA mothers are coated with antibodies of the IgG2a isotype. Left graph, virions were purified from the milk of MMTV(LA)-infected I/LnJ and BALB/cJ mice, bound to plastic, and analyzed for coating immunoglobulins by ELISA. The reaction was developed with goat anti–mouse isotype-specific immunoglobulins coupled to AP. Right graph, purified anti-gp52 mAbs of the IgG1 isotype were bound to plastic at 3 µg/ml followed by incubation with virions collected from milk of MMTV(LA)-infected BALB/cJ and I/LnJ mice. ELISA was developed with anti–mouse IgG2a-specific immunoglobulins coupled to AP. (B) IgG2a-specific antibodies reactive against MMTV virion proteins are present in the sera of MMTV-infected I/LnJ mice and resistant N_2 mice. Top graph, the ELISA was performed with MMTV(LA) virion proteins isolated from 8-wk-old BALB/cLA mice and serum samples from age-matched uninfected or MMTV(LA)-infected I/LnJ and BALB/cJ mice and developed with either goat anti–mouse polyvalent immunoglobulins or goat anti-mouse IgG2a-specific immunoglobulins coupled to AP. Bottom graph, serum samples from MMTV(C3H)-infected resistant and susceptible N_2 mice, as well as from MMTV(C3H)-infected I/LnJ, C3H/HeN, and F_1 mice were tested for reactivity against MMTV virion proteins by ELISA. Anti–mouse IgG2a antibodies coupled to AP were used at the second step. Sera were diluted 10^{-3}.

Next we sought to determine whether anti-MMTV antibodies were present in the sera of MMTV(LA)-infected I/LnJ mice. Serum samples were collected from I/LnJ and BALB/cJ mice that were either infected with MMTV(LA) through foster nursing or were virus-free. Again we used ELISA to test for the reactivity of different sera samples to MMTV(LA) virion proteins bound to the plate. In contrast to BALB/cLA mice, sera of MMTV(LA)-infected I/LnJ mice contained antibodies reactive to the MMTV virion proteins, and these antibodies belonged to the IgG2a isotype (Fig. 2 B, top graph).

Unlike MMTV(LA)-infected I/LnJ mice, I/LnJ mice infected with MMTV(C3H) showed impaired mammary gland infection, whereas hybrid F_1 mice obtained from crosses between susceptible C3H/HeN females and resistant I/LnJ males were susceptible to infection (18). However, when the F_1 females were backcrossed to I/LnJ males, an N_2 generation was produced in which 50% of the females were mammary gland infected, whereas the other 50% of the females showed impaired mammary gland infection (18). Furthermore, all susceptible N_2 females, but not resistant N_2 females, developed mammary tumors (18). When serum samples from MMTV(C3H)-infected resistant and susceptible N_2 females were tested in an ELISA for their reactivity against MMTV virion proteins it appeared that all sera from the resistant N_2 females contained anti-MMTV antibodies of the IgG2a isotype (Fig. 2 B, bottom graph). In contrast, no MMTV(C3H)-infected susceptible N_2 mice showed production of anti-MMTV antibodies (Fig. 2 B, bottom graph). Thus, resistance to mammary gland infection and subsequent mammary tumor development cosegregated with production of virus-neutralizing antibodies and are controlled by a single recessive gene.

Such an efficient development of anti-virus antibodies in I/LnJ mice was unexpected, as these animals were exposed to MMTV as neonates and, thus, were anticipated to be tolerant to the viral antigens. Previously we showed that newly integrated MMTV proviruses were detected in thymi of I/LnJ mice exposed to the virus as neonates suggesting that they were infected (18). Furthermore, Sag-cognate T cells were deleted from both $CD4^+$ and $CD8^+$ single-positive (SP) thymocytes subsets. The normal percentage of $V\beta14^+$ T cells among SP $CD4^+$ and $CD8^+$ T cells in thymi of uninfected I/LnJ mice is 11 ± 0.7, $n = 3$ and 4.0 ± 0.4, $n = 3$, respectively. This percentage declined to 6.1 ± 0.9 and 1.2 ± 0.9, $n = 5$, respectively, in thymi of infected 14-wk-old I/LnJ mice. Thus, although MMTV infection of the thymus in I/LnJ mice results in deletion of Sag-reactive T cells, it does not lead to tolerance to other viral proteins.

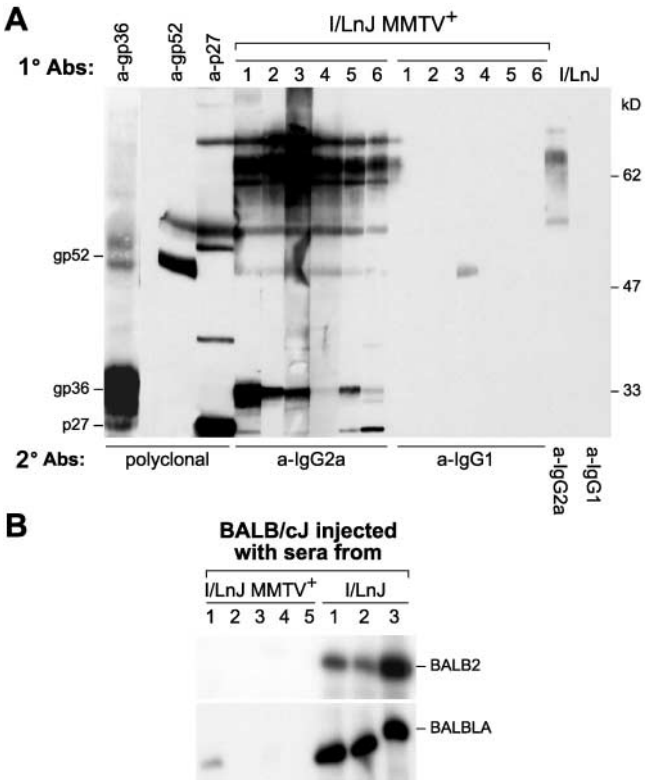


Figure 3. Antibodies produced by MMTV-infected I/LnJ mice recognize major virion proteins and neutralize the virus in vivo. (A) Antibodies produced by MMTV-infected I/LnJ mice recognize major virion proteins. Western blot analysis of MMTV virion proteins with sera of MMTV(LA)-infected I/LnJ mice. MMTV(LA) purified from the milk of BALB/cLA females was run on a 10% denaturing acrylamide gel, blotted to nitrocellulose, and incubated with mouse monoclonal antibodies against gp52 (a-gp52), p27 (a-p27), gp36 (a-gp36), or sera (10^{-2} dilution) from infected I/LnJ mice (I/LnJ MMTV$^+$) or uninfected I/LnJ mice (I/LnJ). Blots were developed with anti–mouse polyclonal, or anti–mouse IgG2a-specific or IgG1-specific antibodies coupled to horseradish peroxidase. 1–6, individual mice of 30 tested. (B) Sera from MMTV-infected I/LnJ mice protect susceptible mice from MMTV infection. BALB/cJ mice fostered by viremic BALB/cLA females were injected as newborns with sera from uninfected or infected I/LnJ mice. RNA isolated from lactating mammary glands of these animals was screened for viral transcripts in an MMTV-specific RNase T_1 protection analysis (BALB2 and BALB14 probes shown).

Antibodies Produced by Infected I/LnJ Mice React with Major Virion Proteins and Are Capable of Neutralizing MMTV. To determine which viral proteins are recognized by anti-MMTV antibodies, lysates of MMTV(LA) viral particles purified from the milk of BALB/cLA females were separated by denaturing PAGE, transferred to nitrocellulose, and incubated with sera from either infected or uninfected I/LnJ mice. The blots were then developed with conjugated secondary antibodies against either IgG2a or IgG1 mouse immunoglobulins. There are three major proteins within the MMTV virion: gp52, the surface (SU) protein product of the *env* gene; gp36, the transmembrane (TM) product of the *env* gene; and p27, the product of the *gag* gene. A pool of sera from three uninfected I/LnJ mice showed no reactivity against any of these MMTV proteins (Fig. 3 A). In contrast, all six MMTV(LA) infected I/LnJ mice tested produced anti-gp52 and anti-gp36 antibodies as well as antibodies against p27 (Fig. 3 A). Most of the reactive antibodies were of the IgG2a isotype (Fig. 3 A). Thus, MMTV-infected I/LnJ mice are capable of making antibodies against internal and surface viral proteins.

Antibodies produced by MMTV-infected I/LnJ mice were tested for their ability to neutralizing virus in two types of assays. First, sera from 3- to 4-mo-old MMTV(LA)-infected I/LnJ and BALB/cJ mice were used in an in vitro neutralization assay. Sera from age-matched uninfected I/LnJ and BALB/cJ mice were used as controls. Purified MMTV(LA) virions incubated with different sera were injected directly into the mammary glands of previously uninfected BALB/cJ mice. All successfully infected mice show deletion of Sag-cognate T cells (6, 27). The deletion of Sag-cognate T cells measured 8 wk after infection was used as indicator of virus infectivity. Uninfected control BALB/cJ mice have 9.2 ± 0.8% of peripheral CD4$^+$Vβ14$^+$ T cells, $n = 10$. BALB/cJ mice injected with virus preincubated with infected BALB/cJ serum, uninfected BALB/cJ serum, or uninfected I/LnJ serum became infected with MMTV, showing a diminution in the percentage of CD4$^+$Vβ14$^+$ to 2.4 ± 0.6%, $n = 5$, to 2.1 ± 0.7%, $n = 5$, and to 2.8 ± 1.2, $n = 5$, respectively. Only BALB/cJ mice injected with MMTV virions preincubated with MMTV(LA)-infected I/LnJ serum did not show deletion of CD4$^+$Vβ14$^+$ (10.5 ± 0.4%, $n = 5$), suggesting that they were virus-free. Furthermore, the same BALB/cJ mice did not show mammary gland infection as determined by RNase T_1 protection analysis (unpublished data). We have also performed experiments with MMTV(C3H) virions preincubated with MMTV(LA)-infected I/LnJ sera and with MMTV(LA) virions preincubated with MMTV(C3H)-infected I/LnJ sera and obtained similar results (unpublished data). Thus, MMTV pretreatment with serum from infected I/LnJ mice in vitro results in virus neutralization and block of infection.

Second, in order to determine whether antibodies produced by infected I/LnJ mice were capable of neutralizing virus in vivo, newborn mice infected with MMTV were used. Two groups of newborn BALB/cJ mice suckling on viremic mothers were injected with sera from MMTV-infected or uninfected I/LnJ mice. Pubescent mice were bred and RNA isolated from their lactating mammary glands was subjected to an MMTV-specific RNase T_1 protection assay (Fig. 3 B). Only mice injected with sera from infected I/LnJ mice were protected from MMTV, as no MMTV(LA)-specific transcripts were detected in their mammary glands. Thus, antibody-containing sera of MMTV(LA)-infected I/LnJ mice neutralize virus infectivity in susceptible mice in vitro and in vivo.

The Kinetics of Anti-MMTV Antibody Production and a Class Switch to IgG2a in Naturally Infected I/LnJ and BALB/cJ Mice. By 8 wk of age naturally infected I/LnJ mice produce virus-specific antibodies of the IgG2a isotype (Fig. 2 B). To determine when this antibody response is

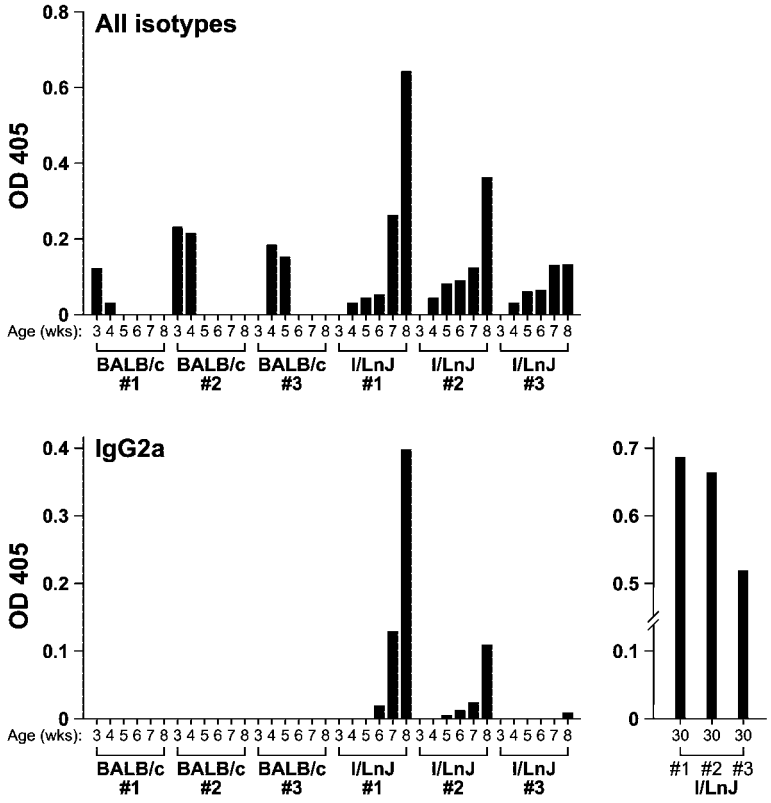


Figure 4. Kinetics of anti-MMTV antibody appearance in neonatally infected I/LnJ and BALB/cJ mice. Mice of each strain were bled weekly from 3 to 8 wk of age, and the presence of antibodies against MMTV virion proteins in their sera was detected by an ELISA (shown for three individual mice). A 2.5×10^{-2} dilution of sera was used in this experiment. Average readings obtained with sera from age-matched uninfected mice were subtracted. 1–3, individual mice at 8 wk of age. Bottom right graph, the same #1–3 I/LnJ mice at 30 wk. Importantly, anti-MMTV antibody production steadily increased in infected I/LnJ mice.

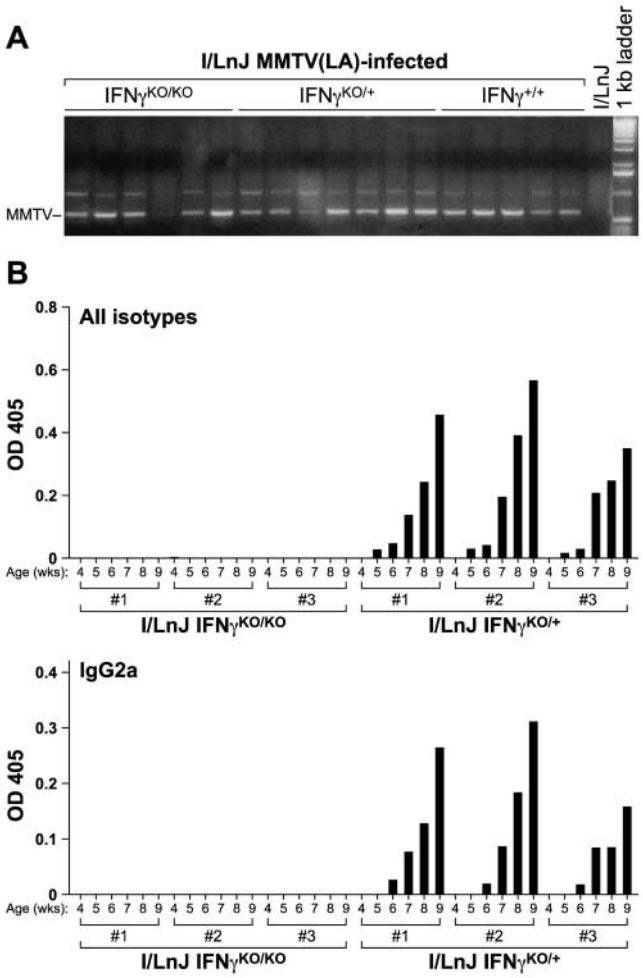
initiated and whether it is unique to I/LnJ mice, susceptible BALB/cJ mice and resistant I/LnJ mice were fostered on viremic BALB/cJ females and presence of antibodies against MMTV in their sera was analyzed by ELISA at different time point. Two different secondary antibodies were used to detect antibodies present in the sera by ELISA: anti–mouse Ig nonisotype specific and IgG2a-specific antibodies (Fig. 4). These experiments revealed that the initial production of anti-MMTV antibodies could be detected in both infected I/LnJ and BALB/cJ mice from 3 to 6 wk after birth. However, antibodies were no longer observed in BALB/cJ mice after they reached 6 to 7 wk of age. In addition, the temporal production of anti-MMTV antibodies in BALB/cJ mice did not include a specific increase in antibodies of the IgG2a isotype, as no such antibodies were detected in BALB/cJ sera. Thus, susceptible BALB/cJ mice were incapable of mounting a long-lasting antibody response against virus and did not class-switch to the IgG2a isotype. In contrast, in I/LnJ mice, after a wave of poly-isotypic early response, a steady and increasing production of anti-MMTV antibodies of the IgG2a isotype began at ~6 wk of age (Fig. 4).

INF-γ Is Required for Anti-virus Antibody Production in I/LnJ Mice. Neonatally MMTV-infected I/LnJ mice produce anti-virus neutralizing antibodies of the IgG2a isotype and sustain this production throughout their lifetime. Class switching to the IgG2a isotype is induced primarily by IFN-γ (28). IFN-γ is a pleiotropic cytokine that plays a central role in promoting innate and adaptive mechanisms of host defense (29).

To demonstrate that IFN-γ is involved in resistance of I/LnJ mice to retroviral infection, mice with targeted mutation if IFN-γ (30) were backcrossed to I/LnJ mice for 10 generations. To investigate whether IFN-γ KO I/LnJ mice are susceptible to MMTV infection, they were fostered by viremic females along with their heterozygous littermates, and kinetics of antivirus antibody production was studied. All animals became MMTV infected since they demonstrated deletion of peripheral Sag-cognate $CD4^+/V\beta14^+$ T cells. MMTV(LA)-infected 8-wk-old IFN-γ KO/KO, KO/+, and +/+ mice had $0.8 \pm 0.3\%$ ($n = 5$), $0.87 \pm 0.4\%$ ($n = 8$), and $0.8 \pm 0.5\%$ ($n = 4$) of $CD4^+/V\beta14^+$ T cells, respectively, whereas uninfected mice of the same genotypes had $9.5 \pm 07\%$ ($n = 5$), $9.3 \pm 0.5\%$ ($n = 5$), and $9.7 \pm 0.2\%$ ($n = 6$) of $CD4^+/V\beta14^+$ T cells, respectively. Furthermore, all animals contained newly integrated MMTV proviruses within the lymphoid system and the virus load did not differ between mice of three different genotypes (Fig. 5 A). These data suggest that IFN-γ is not required for MMTV infection and its absence does not increase the virus load.

To determine whether IFN-γ was necessary for anti-MMTV antibodies production, infected IFN-γ–deficient and IFN-γ–sufficient I/LnJ mice were bled and their sera were tested for reactivity against MMTV in ELISA. None of MMTV-infected IFN-γ–deficient mice produced anti-MMTV antibodies, whereas initial polyvalent response followed by the class switch to IgG2a isotype was apparent in IFN-γ–sufficient mice (Fig. 5 B).

To investigate whether failure to produce anti-virus antibodies by IFN-γ–deficient mice results in successful



C

Mice (n)	Fostered by	CD4/Vβ2 (%)	CD4/Vβ14 (%)
BALB/cJ (12)	BALB/cJ	7.6 ± 0.32	10.7 ± 0.40
BALB/cJ (15)	I/LnJ IFNγ$^{KO/KO}$ MMTV$^+$	2.3 ± 0.19	1.2 ± 0.27
BALB/cJ (15)	I/LnJ IFNγ$^{KO/+}$ MMTV$^+$	7.6 ± 0.57	10.3 ± 0.98

Figure 5. IFN-γ is required for antivirus antibody production in I/LnJ mice. (A) IFN-γ is not required for initial infection and its absence does not lead to an increase in virus load. PCR was performed with BALB14 LTR-specific primers under semiquantitative conditions (reference 20) with 0.1 mg of DNA isolated from spleens of infected IFN-γ–sufficient or IFN-γ–deficient I/LnJ mice nursed by BALB/cLA females. All mice analyzed were 3-mo-old. I/LnJ, splenic DNA from an uninfected I/LnJ mouse. MMTV, PCR product of predicted 590 bp. Densitometry was done on the Nighthawk gel documentation system (PDI). (B) MMTV-infected IFN-γ–deficient I/LnJ mice are not capable of making anti-MMTV antibodies. Serum samples from MMTV(LA)-infected IFN-γ KO/KO, KO/+, and +/+ (unpublished data) I/LnJ mice were tested for reactivity against MMTV virion proteins by ELISA at different time points. Anti–mouse IgG2a antibodies or anti-mouse polyvalent antibodies coupled to AP were used at the second step. Sera were diluted 2.5×10^{-2}. IFN-γ +/+ mice showed the same phenotype as IFN-γ KO/+ mice (unpublished data). (C) IFN-γ deficiency results in production of infectious virions by I/LnJ mice. BALB/cJ females were fostered by MMTV(LA)-infected IFN-γ–deficient or IFN-γ–sufficient I/LnJ mice. Mice were bled at 8 wk of age and their peripheral blood lymphocytes were analyzed for the frequencies of Sag-cognate CD4$^+$ T cells (Vβ2$^+$ and Vβ14$^+$). Three IFN-γ KO/KO, three IFN-γ KO/+, and two IFN-γ +/+ MMTV(LA)-infected I/LnJ foster mothers were used for these experiments.

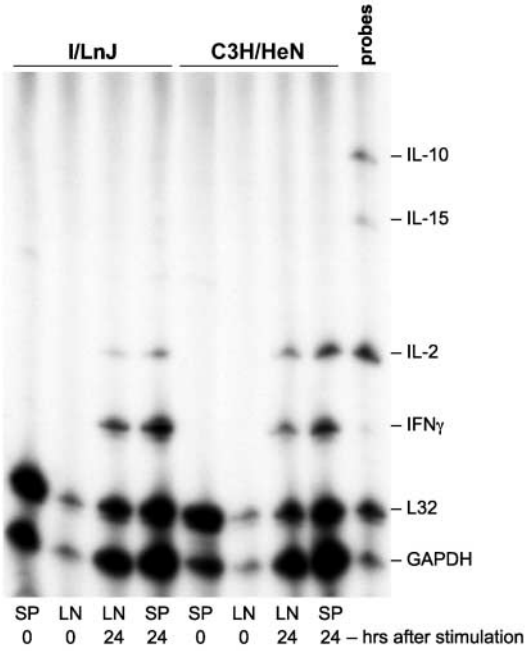


Figure 6. Resistant I/LnJ mice and susceptible C3H/HeN mice do not differ in IFN-γ levels triggered by conventional stimuli. RNA isolated from anti-CD3 activated age- and sex-matched I/LnJ (pool of three mice) and C3H/HeN (pool of three mice) splenocytes was subjected to RNase protection analysis with Riboquant probes. This experiment was repeated two more times with the same results. SP, spleen; LN, peripheral lymph nodes; L32 and GAPDH, housekeeping genes.

virus transmission, we fostered susceptible BALB/cJ mice by MMTV-infected IFN-γ–deficient and IFN-γ–sufficient I/LnJ mice and analyzed the frequencies of Sag-cognate T cells in the periphery of 8-wk-old mice (Fig. 5 C). All BALB/cJ mice fostered on IFN-γ–sufficient I/LnJ mice did not become MMTV infected and had normal frequencies of Sag-cognate T cells. In contrast, BALB/cJ mice fostered by IFN-γ–deficient I/LnJ mice became MMTV infected, as they showed deletion of Sag-cognate T cells (Fig. 5 C). Importantly, the steady state level of IFN-γ in the sera of I/LnJ mice was no different from that in the sera of C3H/HeN and BALB/cJ mice and was below the detectable 30 pg/ml. Similarly, the level of IFN-γ produced by T cells in response to anti-CD3 antibodies was similar in susceptible and resistant mice (Fig. 6). Thus, antiretroviral humoral immune response in I/LnJ mice is determined by the IFN-γ produced specifically in response to viral infection.

Antivirus Antibodies of the IgG2a Isotype Are a Specialized Response to Retroviral Infection. MMTV infection in I/LnJ mice results in production of antivirus antibodies of the IgG2a isotype (Fig. 2). It is possible that all antibody responses in I/LnJ mice were skewed toward the IgG2a isotype or it is also possible that this resulted from the activation of a specific pathway in response to retroviral infection. Immunization of I/LnJ mice with ovalbumin resulted in production of antibodies of multiple isotypes and did not differ from a response in BALB/cJ mice (unpublished data). Moreover, when both I/LnJ and control BALB/cJ mice were immunized with MMTV virion pro-

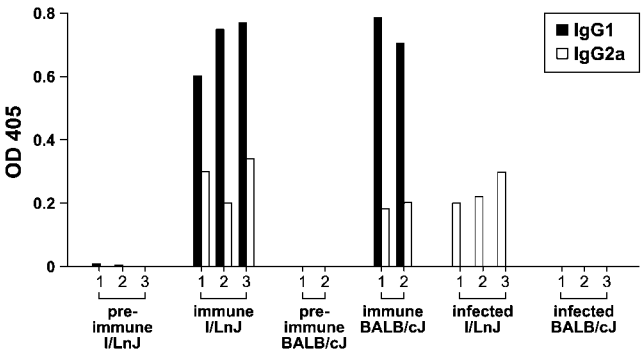


Figure 7. Infection with MMTV is required to induce an IgG2a-biased virus-neutralizing immune response. Adult I/LnJ and BALB/cJ mice were immunized with MMTV(LA) virion proteins and their pre- and postimmune sera were tested 30 d later in an ELISA for reactivity against virion proteins. Another group of age-matched and sex-matched mice was injected intraperitoneally with the same amount of biologically active MMTV(LA), and their sera were tested 30 d later for anti-MMTV antibodies. All sera were used at 10^{-2} dilution. Goat anti–mouse IgG2a- and IgG1-specific antibodies were used at the second step. Data from individual mice are shown. Additional mice were analyzed by Western blot. Whereas 7/7 and 2/7 infected adult I/LnJ mice showed production of MMTV-specific antibodies of the IgG2a and IgG1 isotype, respectively, all immunized I/LnJ mice produced anti-MMTV antibodies of both the IgG2a and IgG1 isotype. In contrast, 0/10 infected adult BALB/cJ mice showed production of any anti-MMTV antibodies, while 5/5 of immunized BALB/cJ mice produced anti-MMTV antibodies of both the IgG2a and IgG1 isotype.

teins in CFA their responses were remarkably similar (Fig. 7). Neither I/LnJ mice nor BALB/cJ mice immunized with virion proteins in CFA show a bias in production of anti-virus antibodies toward the IgG2a isotype (Fig. 7). As a control, a group of I/LnJ mice and a group of BALB/cJ mice were injected intraperitoneally with biologically active MMTV(LA). While MMTV(LA)-infected I/LnJ mice produced antivirus antibodies of only the IgG2a isotype, MMTV-infected BALB/cJ mice failed to produce antivirus antibodies (Fig. 7). Thus, the immune response in I/LnJ mice is not skewed in general toward the IgG2a isotype.

After immunization with MMTV proteins in CFA, even susceptible BALB/cJ mice were capable of producing virus-neutralizing antibodies, because MMTV virions preincubated with sera from immunized BALB/cJ mice completely neutralized the virus in in vitro experiment. Uninfected control BALB/cJ mice had 9.2 ± 0.8%, $n = 10$ of $CD4^+V\beta14^+$ T cells. BALB/cJ mice injected with MMTV virions preincubated with sera from either I/LnJ or BALB/cJ mice immunized with MMTV(LA) virion proteins did not show deletion of $CD4^+V\beta14^+$ T cells (9.6 ± 0.37% for 5 I/LnJ sera and 9.6 ± 0.7 for 5 BALB/cJ sera), suggesting that they were virus-free. In contrast, sera from MMTV(LA)-infected BALB/cJ mice did not neutralize the virus (2.79 ± 1% of $CD4^+V\beta14^+$ T cells, $n = 5$). As expected, control sera from infected I/LnJ mice completely blocked infection (9.8 ± 0.4% of $CD4^+/V\beta14^+$ T cells, $n = 5$). Thus, immunization with MMTV virion proteins in adjuvant, but not a natural infection, stimulates production of virus-neutralizing antibodies in susceptible BALB/cJ mice. In contrast, resistant I/LnJ mice have a unique ability to produce neutralizing antibodies upon infection without exogenous adjuvant.

Discussion

Humoral immune responses play an important role in antiviral defense. Antiviral antibodies prevent the spread of viral infections through two main mechanisms: (a) block of interactions of viral surface proteins (Env) with their receptors; (b) facilitation of virus uptake into phagocytic cells through interaction with the Fc receptors or through the complement pathway. Although for many viral infections antibody production is a key to clearance (31), retroviruses such as HIV are able to escape humoral immune responses despite the fact that anti-HIV antibodies are being produced (32). Retroviral infections persist and involve rapid mutations of genes that encode antigenic proteins, leading to selection of immune escape variants. In order for a humoral immune response against a retrovirus to be capable of blocking virus transmission, it must be robust and sustained. I/LnJ mice are unique in meeting these requirements: they efficiently generate anti-MMTV neutralizing antibodies, they are able to sustain this response throughout their lifespan (Fig. 4) and they produce antibodies against multiple viral proteins that cross-react with different MMTV variants (Fig. 3).

Our interest was drawn to I/LnJ mice because of their remarkable resistance to MMTV-induced mammary tumors (11, 18) and we searched for the mechanism underlying this resistance. We found that MMTV-infected I/LnJ mice produced antibodies against the virus that were able to (a) neutralize MMTV in vitro; (b) abort MMTV infection when injected in vivo (Fig. 3 C); and (c) coat virions secreted into milk (Fig. 2 A), thereby preventing further virus transmission. Antibodies were produced in response to four MMTV variants: MMTV(C3H), BALB2, BALB14, and BALBLA, and were cross-reactive to them. It is possible that the antibodies recognize protein determinants that viruses cannot change without losing infectivity.

The unique features of the I/LnJ response to MMTV are that such a response is elicited and maintained. We (Fig. 4) and others (33–35) have found that mice susceptible to MMTV infection and MMTV-induced tumors are capable of producing anti-MMTV antibodies. However, the kinetics of this response in I/LnJ is distinct from that in susceptible mice. The initial production of anti-MMTV antibodies in infected BALB/cJ mice started at 3 wk after birth and was terminated at 6 to 7 wk (Fig. 4). In addition, this transient production of anti-MMTV antibodies in BALB/cJ mice did not include a specific increase in antibodies of the IgG2a isotype. In contrast, I/LnJ mice demonstrated a steadily increasing response that was detectable at 4 wk after birth and class switched to the IgG2a isotype at ~7 wk (Fig. 4). This means that both MMTV-susceptible and MMTV-resistant mice did not recognize retroviral antigens as "self" when they were infected as newborns (Fig.

4). However, over time the immune system of susceptible BALB/cJ mice became tolerant to these viral antigens and no longer recognizes them as foreign (Fig. 4). In contrast, in I/LnJ mice, antibody production is sustained throughout their life (Fig. 4). From these data we concluded that the immune system of I/LnJ mice is always aware of retroviral infection.

Infection per se must be a key to production of anti-virus antibodies of exclusively IgG2a isotype in I/LnJ mice, as the production of antibodies of different isotypes against viral proteins were seen in both susceptible and resistant mice when viral proteins were introduced with an adjuvant (Fig. 7). Pathogens (including viruses) are capable of activating APCs through their pathogen-associated molecular patterns (PAMPs), which are recognized by innate immune pattern recognition receptors (PRR; references 36 and 37). Unique molecular features of viruses, such as double-stranded (ds) RNA, can serve as PAMP. Indeed, dsRNA was recently found to be recognized by Toll-like receptor (TLR)3 (38). Recently TLRs and their adaptor proteins were linked to IFN pathways and in some cases were actually induced by IFNs during viral infection (39, 40). In the case of retroviruses it remains unclear what receptors are activated, but I/LnJ mice clearly demonstrate that infection leads to an efficient immune response, implying that MMTV may activate innate immunity.

I/LnJ mice with targeted mutation of IFN-γ were unable to produce any antibodies against the virus (Fig. 5), implicating IFN-γ in the mechanism of resistance inherited by I/LnJ mice. Although we found no evidence of increased IFN-γ production by activated I/LnJ T cells or increased IFN-γ background levels in the sera of I/LnJ mice (Fig. 6), it is plausible that increased IFN-γ production might occur in response to stimuli other than T cell receptor ligation or by other cell types.

IFN-γ is mostly produced by NK cells and certain subpopulations of T cells and activates expression of IFN-γ–responsive genes whose products are engaged in the immune response (41). Other cell types, however, may be also involved in IFN-γ production. Our preliminary data showed that cells of I/LnJ bone marrow origin are capable of conveying the ability to produce anti-MMTV neutralizing antibodies by susceptible mice (unpublished data).

The precise details of the resistance mechanism in I/LnJ mice are yet to be elucidated. Previously we showed that it is controlled by a single gene, virus infectivity controller or *Vic* (18). Our preliminary mapping data position the gene on Chromosome 17 (not MHC-linked; unpublished data). Two alleles of the *Vic* gene of I/LnJ origin are required for antibody production, whereas one allele of the gene from susceptible mice is needed to suppress antibody production. The fact that resistance is recessive suggests that some type of negative regulatory activity is abolished or reduced in I/LnJ mice, allowing these mice to sustain an immune response against retrovirus. For example, it is possible that a negative feed back regulation of IFN-γ signaling is affected, leading to normal (not excessive) but sustained IFN-γ production. One well-known negative regulator of IFN-γ is the cytokine inducible SH2-containing protein 1 (CISH1), which is induced in response to IFN-γ stimulation (42). However, we did not find any differences between resistant I/LnJ mice and susceptible mice in the coding sequences of *Cish1* gene or in up-regulation of *Cish1* mRNA in response to IFN-γ (unpublished data). Initial recognition of MMTV as "nonself" takes place in other strains as well as in I/LnJ mice, but only I/LnJ mice continue to recognize MMTV antigens as "nonself." Thus, susceptible mice become tolerant to MMTV, while resistant I/LnJ mice maintain antiviral immune response, a property for which IFN-γ may be also responsible. One would anticipate that these mice must be more prone to autoimmunity than other strains. This, however, is not the case, as no reports of autoimmunity in I/LnJ mice have been published, and we have not made any such observations in our colony. Moreover, I/LnJ mice as well as other mouse strains have endogenous *Mtv*s (18), but only I/LnJ mice produce anti-viral antibodies when infected with exogenous MMTV. Thus, the mechanism that allows I/LnJ mice to sustain the antiviral response is absolutely dependent on infection with retrovirus.

Another known example of resistance to retroviral infection is resistance to Friend murine leukemia virus (MuLV) conferred by a dominant allele of the *Rfv3* gene. The production of virus-neutralizing antibodies of the IgG2a isotype against Friend MuLV underlies this resistance and results in complete virus clearance (43). Although function of the *Rfv3* gene also remains unknown, the mechanism of action of its encoded product is clearly different from that of the *Vic* gene. The resistant allele of the *Rfv3* gene is dominant, since only one copy is required for the production of anti-F-MuLV neutralizing antibodies (43). In addition, the *Rfv3* gene was mapped to Chromosome 15 (44, 45), whereas the *Vic* gene has been mapped to Chromosome 17.

Thus, we have discovered a novel mechanism of resistance to retroviral infection that results in an efficient antivirus antibody response and blocks virus transmission. Both neutralizing anti-virus antibodies and cytotoxic lymphocytes directed against viral proteins can be detected in humans infected with HIV (46). However, the level at which they control HIV infection is insufficient to prevent the disease from progressing, and virus variants that escape immune recognition are often found in HIV-infected individuals (47). If we knew a way to make the immune response against HIV and other human retroviruses robust and sustained, we would be better able to treat diseases caused by them.

We are thankful to Dr. P. Marrack for $Mtv^{-/-}$ mice and to Dr. S. Ross for helpful discussion, and to Jennifer Smith for the graphic work.

This work was supported by Public Health Service (PHS) grant CA89116 and by an award from the Hillsdale Foundation to T.V. Golovkina, and by a grant from The Jackson Laboratory to T.V. Golovkina. This work was also supported by a grant (CA34196) from the National Cancer Institute to The Jackson Laboratory.

Submitted: 23 August 2002
Revised: 11 December 2002
Accepted: 11 December 2002

References

1. Acha Orbea, H., and E. Palmer. 1991. Mls–a retrovirus exploits the immune system. *Immunol. Today.* 12:356–361.
2. Choi, Y., J.W. Kappler, and P. Marrack. 1991. A superantigen encoded in the open reading frame of the 3′ long terminal repeat of the mouse mammary tumor virus. *Nature.* 350: 203–207.
3. MacDonald, H.R., R. Schneider, R.K. Lees, R.C. Howe, H. Acha Orbea, H. Festenstein, R.M. Zinkernagel, and H. Hengartner. 1988. T-cell receptor V beta use predicts reactivity and tolerance to Mlsa-encoded antigens. *Nature.* 332: 40–45.
4. MacDonald, H.R., A.L. Glasebrook, R. Schneider, R.K. Lees, H. Pircher, T. Pedrazzini, O. Kanagawa, J.F. Nicolas, R.C. Howe, R.M. Zinkernagel, et al. 1989. T-cell reactivity and tolerance to Mlsa-encoded antigens. *Immunol. Rev.* 107: 89–108.
5. Held, W., G. Waanders, A.N. Shakhov, L. Scarpellino, H. Acha-Orbea, and H.R. MacDonald. 1993. Superantigen-induced immune stimulation amplifies mouse mammary tumor virus infection and allows virus transmission. *Cell.* 74: 529–540.
6. Marrack, P., E. Kushnir, and J. Kappler. 1991. A maternally inherited superantigen encoded by mammary tumor virus. *Nature.* 349:524–526.
7. Peters, G., and C. Dickson. 1987. On the mechanism of carcinogenesis by mouse mammary tumor virus. *In* Cellular and Molecular Biology of Breast Cancer. D. Medina, W. Kidwell, G. Heppner, and E. Anderson, editors. Plenum Publishing Corp., New York. 307–319.
8. Muhlbock, O., and A. Dux. 1981. Histocompatability genes and mammary cancer. Elsevier/North-Holland Biomedical Press, Amsterdam, Netherlands. 545–573.
9. Dembic, Z., M. Ayane, J. Klein, M. Steinmetz, C.O. Benoist, and D.J. Mathis. 1985. Inbred and wild mice carry identical deletions in their E alpha MHC genes. *EMBO J.* 4:127–131.
10. Begovich, A.B., T.H. Vu, and P.P. Jones. 1990. Characterization of the molecular defects in the mouse Ebf and Ebq genes. Implication for the origin of MHC polimorphism. *J. Immunol.* 144:1957–1964.
11. Nandi, S., S. Haslam, and C. Helmich. 1972. Mechanisms of resistance to mammary tumor development in C57BL and I strains of mice. I. Noduligenesis, tumorigenesis, and characteristics of nodules and tumors. *J. Natl. Cancer Inst.* 48:1005–1012.
12. Pucillo, C., R. Cepeda, and R.J. Hodes. 1993. Expression of a MHC class II transgene determines both superantigenicity and susceptibility to mammary tumor virus infection. *J. Exp. Med.* 178:1441–1445.
13. Wrona, T., and J.P. Dudley. 1996. Major histocompatibility complex class II I-E-independent transmission of C3H mouse mammary tumor virus. *J. Virol.* 70:1246–1249.
14. Frankel, W.N., C. Rudy, J.M. Coffin, and B.T. Huber. 1991. Linkage of Mls genes to endogenous mammary tumour viruses of inbred mice. *Nature.* 349:526–528.
15. Woodland, D.L., M.P. Happ, K.J. Gollob, and E. Palmer. 1991. An endogenous retrovirus mediating deletion of alpha beta T cells? *Nature.* 349:529–530.
16. Dyson, P.J., A.M. Knight, S. Fairchild, E. Simpson, and K. Tomonari. 1991. Genes encoding ligands for deletion of V beta 11 T cells cosegregate with mammary tumour virus genomes. *Nature.* 349:531–532.
17. Golovkina, T.V., A.V. Chervonsky, J.P. Dudley, and S.R. Ross. 1992. Transgenic mouse mammary tumor virus superantigen expression prevents viral infection. *Cell.* 69:637–645.
18. Golovkina, T.V. 2000. A novel mechanism of resistance to mouse mammary tumor virus infection. *J. Virol.* 74:2752–2759.
19. Dalton, D.K., S. Pitts-Meek, S. Keshav, I.S. Figari, A. Bradley, and T.A. Stewart. 1993. Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. *Science.* 259:1739–1742.
20. Golovkina, T.V., I. Piazzon, I. Nepomnaschy, V. Buggiano, M. de Olano Vela, and S.R. Ross. 1997. Generation of a tumorigenic milk-borne mouse mammary tumor virus by recombination between endogenous and exogenous viruses. *J. Virol.* 71:3895–3903.
21. Golovkina, T.V., A. Chervonsky, J.C. Prescott, C.A. Janeway, and S.R. Ross. 1994. The mouse mammary tumor virus envelope gene product is required for superantigen presentation to T cells. *J. Exp. Med.* 179:439–446.
22. Golovkina, T.V., A. Jaffe, and S.R. Ross. 1994. Coexpression of exogenous and endogenous mouse mammary tumor virus RNA *in vivo* results in viral recombination and broadens the virus host range. *J. Virol.* 68:5019–5026.
23. Scherer, M.T., L. Ignatowicz, A. Pullen, J. Kappler, and P. Marrack. 1995. The use of mammary tumor virus (Mtv)-negative and single-Mtv mice to evaluate the effects of endogenous viral superantigens on the T cell repertoire. *J. Exp. Med.* 182:1493–1504.
24. Golovkina, T.V., J.P. Dudley, and S.R. Ross. 1998. B and T cells are required for mouse mammary tumor virus spread within the mammary gland. *J. Immunol.* 161:2375–2382.
25. Nandi, S., S. Halsam, and C.D. Aldrich. 1971. Antigenically distinct forms of mammary tumor virus in blood and mammary tissues of mice. *J. Natl. Cancer Inst.* 46:1035–1038.
26. Piazzon, I., A. Goldman, S. Torello, I. Nepomnaschy, A. Deroche, and G. Dran. 1994. Transmission of an Mls-1a-like superantigen to BALB/c mice by foster-nursing on F1 Mls-1bxa mothers. Sex-influenced onset of clonal deletion. *J. Immunol.* 153:1553–1562.
27. Ignatowicz, L., J. Kappler, and P. Marrack. 1992. The effects of chronic infection with a superantigen-producing virus. *J. Exp. Med.* 175:917–923.
28. Snapper, C.M., and W.E. Paul. 1987. Interferon-gamma and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science.* 236:944–947.
29. Farrar, M.A., and R.D. Schreiber. 1993. The molecular cell biology of interferon-gamma and its receptor. *Annu. Rev. Immunol.* 11:571–611.
30. Dalton, D.K., S. Pitts-Meek, S. Keshav, I.S. Figari, A. Bradley, and T.A. Stewart. 1993. Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. *Science.* 259:1739–1742.
31. Ochsenbein, A.F., and R.M. Zinkernagel. 2000. Natural antibodies and complement link innate and acquired immunity. *Immunol. Today.* 21:624–630.
32. Miedema, F., L. Meyaard, M. Koot, M.R. Klein, M.T. Roos, M. Groenink, R.A. Fouchier, A.B. Van't Wout, M. Tersmette, P.T. Schellekens, et al. 1994. Changing virus-host interactions in the course of HIV-1 infection. *Immunol. Rev.* 140:35–72.

33. Altrock, B.W., R.D. Cardiff, and P.B. Blair. 1981. Murine mammary tumor virus seroepidemiology in BALB/cfC3H mice: correlation with tumor development. *J. Natl. Cancer Inst.* 67:163–168.
34. Held, H., A.N. Shakhov, S. Izui, G.A. Waanders, L. Scarpellino, H.R. MacDonald, and H. Acha-Orbea. 1993. Superantigen-reactive $CD4^+$ T cells are required to stimulate B cells after infection with mouse mammary tumor virus. *J. Exp. Med.* 177:359–366.
35. Luther, S.A., I. Maillard, F. Luthi, L. Scarpellino, H. Diggelmann, and H. Acha Orbea. 1997. Early neutralizing antibody response against mouse mammary tumor virus: critical role of viral infection and superantigen-reactive T cells. *J. Immunol.* 159:2807–2814.
36. Janeway, C.A. 1989. Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb. Symp. Quant. Biol.* 54:1–13.
37. Medzhitov, R., and C.A. Janeway, Jr. 1997. Innate immunity: the virtues of a nonclonal system of recognition. *Cell.* 91:295–298.
38. Alexopoulou, L., A.C. Holt, R. Medzhitov, and R.A. Flavell. 2001. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature.* 413:732–738.
39. Toshchakov, V., B.W. Jones, P.Y. Perera, K. Thomas, M.J. Cody, S. Zhang, B.R. Williams, J. Major, T.A. Hamilton, M.J. Fenton, and S.N. Vogel. 2002. TLR4, but not TLR2, mediates IFN-beta-induced STAT1alpha/beta-dependent gene expression in macrophages. *Nat. Immunol.* 3:392–398.
40. Mita, Y., K. Dobashi, Y. Shimizu, T. Nakazawa, and M. Mori. 2001. Toll-like receptor 2 and 4 surface expressions on human monocytes are modulated by interferon-gamma and macrophage colony-stimulating factor. *Immunol. Lett.* 78:97–101.
41. Billiau, A. 1996. Interferon-gamma: biology and role in pathogenesis. *Adv. Immunol.* 62:61–130.
42. Yasukawa, H., A. Sasaki, and A. Yoshimura. 2000. Negative regulation of cytokine signaling pathways. *Annu. Rev. Immunol.* 18:143–164.
43. Chesebro, B., and K. Wehrly. 1979. Identification of a non-*H-2* gene (*Rfv-3*) influencing recovery from viremia and leukemia induced by Friend virus complex. *Proc. Natl. Acad. Sci. USA.* 76:425–429.
44. Hasenkrug, K.J., A. Valenzuela, V.A. Letts, J. Nishio, B. Chesebro, and W.N. Frankel. 1995. Chromosome mapping of *Rfv3*, a host resistance gene to Friend murine retrovirus. *J. Virol.* 69:2617–2620.
45. Super, H.J., K.J. Hasenkrug, S. Simmons, D.M. Brooks, R. Konzek, K.D. Sarge, R.I. Morimoto, N.A. Jenkins, D.J. Gilbert, N.G. Copeland, et al. 1999. Fine mapping of the Friend retrovirus resistance gene, *Rfv3*, on mouse chromosome 15. *J. Virol.* 73:7848–7852.
46. Burton, D.R., and D.C. Montefiori. 1997. The antibody response in HIV-1 infection. *AIDS.* 11 Suppl A:S87–S98.
47. Back, N.K., L. Smit, M. Schutten, P.L. Nara, M. Tersmette, and J. Goudsmit. 1993. Mutations in human immunodeficiency virus type 1 gp41 affect sensitivity to neutralization by gp120 antibodies. *J. Virol.* 67:6897–6902.