B and T Cells Are Required for Mouse Mammary Tumor Virus Spread Within the Mammary Gland

Tatyana V. Golovkina, Jaquelin P. Dudley and Susan R. Ross

*J Immunol* 1998; 161:2375-2382; ;
http://www.jimmunol.org/content/161/5/2375

**References**

This article cites 25 articles, 13 of which you can access for free at:
http://www.jimmunol.org/content/161/5/2375.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
B and T Cells Are Required for Mouse Mammary Tumor Virus Spread Within the Mammary Gland

Tatyana V. Golovkina, Jaquelin P. Dudley, and Susan R. Ross

Mouse mammary tumor virus (MMTV) is an infectious retrovirus transmitted through milk from mother to newborns. MMTV encodes a superantigen (SAg) whose activity is indispensable for the virus life cycle, since a genetically engineered virus with a mutation in the sag gene neither amplified in cells of the immune system of suckling pups nor infected their mammary glands. When wild-type MMTV was injected directly into the mammary glands of uninfected pubescent mice, their lymphoid as well as mammary gland cells became virus infected. To test whether this infection of lymphoid cells was dependent on SAg activity and required for virus spread within the mammary gland, we performed mammary gland injections of wild-type MMTV(C3H) into two strains of transgenic mice that lacked SAg-cognate, Vβ14+ T cells. Neither the MTV-ORF or LEL strains showed infection of their mammary glands. Moreover, no MMTV infection of their peripheral lymphocytes was detected. Similar experiments with mice lacking B cells (μ-chain knockouts) showed no detectable virus spread in the mammary glands or lymphoid tissues. These data suggest that SAg activity and MMTV-infected lymphocytes are required, not only for initial steps of viral infection, but also for virus spread within the mammary gland. Virus spread at late times in infection determines whether MMTV induces mammary tumors. The Journal of Immunology, 1998, 161: 2375–2382.

Received for publication January 22, 1998. Accepted for publication April 30, 1998.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This work was supported by United States Public Health Service Grants CA65795 to T.V.G. and CA52646 to S.R.R. and J.P.D.

Abbreviations used in this paper: MMTV, mouse mammary tumor virus; LTR, long terminal repeat; SAg, superantigen.

Copyright © 1998 by The American Association of Immunologists
SAG EXPRESSION PREVENTS MMTV SPREAD WITHIN THE MAMMARY GLAND

PRO/Cla transgenic mice containing the genetically engineered MMTV provirus with the mutated sag gene were described elsewhere (15). To avoid viral recombination between the Mtv1 endogenous provirus in C3H/HeN mice and the transgene, the HYB PRO/Cla transgene was bred onto the BALB/c background (Mtv6, -8, and -9) for two generations. Transgenic females null for Mtv1 were identified by Southern blot analysis and showed no deletion of Vβ14 T cells (not shown), in contrast to the HYB PRO/Cla females in the C3H/HeN (Mtv1') background (15). The MTV-ORF no. 16 and LEL no. 1 transgenic mice were previously described (7, 16).

Homozygous IGH6/B6L6 (H-2b) females (μ-chain knockout mouse) (22) purchased from The Jackson Laboratory (Bar Harbor, ME) were crossed with C3H/HeN MMTV-(-H-2b) males (National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD) to generate F1 progeny. The F1 progeny were intercrossed, and F2 generation female mice were typed for surface IgM expression and the MHC class II H-2k haplotype. C3H/HeN and LEL no. 1 transgenic mice were previously described (7, 16).

Mammary gland tumor xenografting

Mammary gland tumor incidence in the MMTV(C3H)-injected LEL transgenic mice and their nontransgenic littermates was monitored by weekly palpation of the animals. Tumor-bearing mice were sacrificed and DNA isolated from a portion of each tumor was subjected to Southern blot analysis as described previously (17). All of the tumors contained new MMTV integrants, indicating that the tumors were caused by the virus (not shown).

Genomic DNA isolation, PCR, and Southern blot analysis

High m.w. DNA (0.25 μg) isolated from spleens, thymi, or Peyer’s patches was amplified by PCR. Amplification of newly integrated copies of exogenous MMTV(C3H) and HYB PRO/Cla viruses in nontransgenic mice was accomplished using the followingLTR-specific primers: 5'-AATTCCGGAGAATCTGACCTTGCCATC-3' (nt 268–289) and 5'-TAACTGTCTATTTTGACCTTCCAGCTG-3' (nt 923–988) (19). Semi quantitative PCR was carried out as previously described (18). Briefly, 35 cycles of 1 min at 55°C, 1 min at 72°C, and 1 min at 94°C gave linear DNA amplification, whereas after 35 cycles, the amplification had plateaued (non-quantitative conditions). After PCR amplification, 1 μl (1/40 of the volume, semi-quantitative conditions) or 20 μl (½ of the volume, non-quantitative conditions) of each reaction mixture were incubated with MfeI restriction enzyme (New England Biolabs, Beverly, MA), as indicated in the figure legend, and the resulting products were analyzed on 1.5% agarose gels. These primers also amplify some endogenous MMTVs under non-quantitative PCR conditions. To amplify the MMTV(C3H)-integrated proviruses in the MTV-ORF and LEL transgenic mice, different primers were used, since the primers described above amplified both the transgenecises. In this case, a forward primer specific for the MMTV(C3H) LTR (5'-GACAGTGACTGACATAGAAGACTC-3', nt 898–923) and a gag-specific MMTV BR6 reverse primer (5'-CTTCAGCTCTTCCTCTCG TAGGGGAGAC-3', nt 1613–1859) were used. Amplification with this primer set was carried out as follows: 35 cycles of 1 min at 94°C, 1 min at 72°C, and 1 min at 94°C. After PCR amplification, 1 μl of each reaction (1/40 part of the volume) was run on 1.5% agarose gel. Southern blots of the PCR products were hybridized with LTR- (20) or gag-specific probes. The gag-specific probe was cloned as a 2-kb PstI-Xhol fragment from the HYB MMTV plasmid (21).

Results

A functional MMTV SAg is required to establish infection in vivo

MMTV can infect only the mammary glands of mice with both functional B cells and SAg-cognate T cells (5, 7, 8). Although these immune system cells are important for MMTV transmission, an absolute requirement for SAg in the MMTV life cycle has not yet been shown. We introduced a transgene, HYB PRO/Cla, with a framelabel deletion at amino acid 113 in the sag gene into C3H/HeN mice. This framelabel deletion caused premature termination of this protein (15). Because this termination occurred upstream of the SAg hypervariable region that determines TCR specificity, no deletion of Vβ14 T cells should occur in these mice. However, this mutation was corrected in transgenic females by retroviral recombination with the endogenous Mtv1 virus present in C3H/HeN mouse strain, leading to the generation of a Vβ14-deleting, milk-transmitted virus (15). To prevent this recombination, the transgene was crossed into BALB/c mice, which lack Mtv1. The Mtv1 HYB PRO/Cla transgenic animals generated from these crosses expressed the transgene, shed virus in their milk (Fig. 1A, lanes 4 and 5, respectively) (15), and showed no deletion of their SAg-cognate Vβ14 T cells (data not shown).

To determine whether the mutated MMTV produced by the Mtv1 HYB PRO/Cla transgenic females was infectious, these mice were bred and offspring generated. None of the nontransgenic Mtv1 HYB PRO/Cla-nursed offspring acquired virus, since no transgene-specific RNA was detected in either their mammary glands or milk (Fig. 1A, lanes 2 and 1, respectively). In contrast, mice nursed on a Mtv1 HYB PRO/Cla transgenic mice acquired infectious virus (Fig. 1A, lanes 5 and 6), as previously shown (15). These results indicated that a SAg-minus MMTV could not infect mammary gland cells.

The primary targets for MMTV during milk-borne infection are lymphocytes. To determine whether SAg was also required for infection of lymphoid cells, we examined the relative levels of viral DNA present in the lymphoid organs of nontransgenic mice nursed on the Mtv1 HYB PRO/Cla-transgenic mothers using PCR analysis (see Materials and Methods). After 31 cycles of PCR amplification, no exogenous MMTV DNA was detected in DNA isolated from the spleens, thymi, or Peyer’s patches of four different 3-mo-old Mtv1 HYB PRO/Cla-infected mice (mice 1– 4, Fig. 1B, top panel), in contrast to mice nursed on Mtv1 HYB PRO/Cla-infected mice (E.C., Fig. 1B). However, after 35 cycles, viral DNA was detectable in these organs (Fig. 2B, bottom panel), indicating that there was low level infection of lymphoid cells. Thus, the SAg-mutated virus did not amplify in the lymphoid cells and this was responsible for our failure to detect MMTV infection in the mammary gland.

MMTV spread within the mammary gland requires B cells

Infected lymphoid cells are required for the primary infection of mammary gland cells during milk-borne transmission, but it is not known whether they play a role in MMTV spread within the mammary gland. Although mice naturally acquire virus through the milk, MMTV can also be introduced by injection into the mammary gland of subacutely infected mice. To determine whether MMTV infection into the mammary gland resulted in systemic infection of lymphoid cells, we performed PCR analysis on DNA isolated from spleen and Peyer’s patches of mice infected in this manner, using
MMTV(C3H) LTR-specific primers. We found that both tissues from the infected mice acquired new proviral copies of MMTV (Fig. 2), indicating that lymphoid cells were infected after virus injection into the mammary gland.

B cells are believed to be the first cells infected during milk-borne MMTV transmission to offspring (3, 8). To determine whether they were needed for MMTV spread within the mammary gland, we injected virus into mice that lacked B cells due to the targeted mutagenesis of their Ig mu-chain gene (strain IGH6/BL6 (22)). This gene disruption was available only in C57BL/6 mice, which are H-2b and do not express the MHC class II I-E molecule. Because of this, most MMTV SAsgs, including that encoded by MMTV(C3H), are presented inefficiently and these mice are less susceptible to MMTV(C3H) infection (23). IGH6/BL6 mice were crossed with C3H/HeN mice, which are H-2k, an MHC haplotype that efficiently presents the MMTV(C3H) SAg (24, 25). F1-(IgM1/H-2k1) and F2-generation female mice (IgM1H-2k1, IgM1H-2k2, IgM2H-2k1, IgM2H-2k2; see Fig. 3) derived from these crosses received mammary gland injections of MMTV at 3 wk of age. Four weeks after injection, immune response to the virus was assessed by determination of the percentage of peripheral CD41/Vb141 T cells. Both the F1- and F2-generation IgM1H-2k1 mice were infected, since they deleted about 30% of their Vb14 SAgs-reactive T cells (Table I). In contrast, none of the H-2k- or IgM- mice showed deletion (Table I),

**FIGURE 1.** SAg activity is required for the milk-borne infection by MMTV. A, RNase protection assays of mammary gland and milk RNAs isolated from HYB PRO/Cla mice and their nontransgenic progeny. RNA isolated from the lactating mammary glands (LMG) (lanes 2, 4, 6, 7, and 8) or milk (lanes 1, 3, and 5) of HYB PRO/Cla Mtv transgenic females (lane 4, LMG; lane 3, milk) or their nontransgenic offspring (lane 2, LMG; lane 1, milk) were subjected to RNase T1 protection assays. Identical results were obtained with four other nontransgenic offspring analyzed (data not shown). A probe specific for the MMTV(C3H) LTR was used (17). Lane 8, RNA isolated from the LMG of a C3H/HeN MMTV1 female; lane 7, RNA isolated from the LMG of a C3H/HeN MMTV2 female; lanes 6 and 5, RNA isolated from the LMG (lane 6) or milk (lane 5) of a nontransgenic C3H/HeN offspring nursed on a C3H/HeN HYB PRO/Cla Mtv1 mouse. Abbreviations: p, probe; MMTV(C3H), full protection. B, Transmission of a SAg-minus MMTV results in limited lymphocyte infection. PCR followed by MfeI digestion was carried out with MMTV(C3H)-specific primers under semi-quantitative (top panel) or non-quantitative conditions (bottom panel) (see Materials and Methods) using DNA isolated from spleens and Peyer’s patches of nontransgenic mice (mice 1–4) nursed on HYB PRO/Cla Mtv1 mice. MfeI digestion carried out with MMTV(C3H)-specific primers under semi-quantitative (top panel) or non-quantitative conditions (bottom panel) (see Materials and Methods) using DNA isolated from spleens and Peyer’s patches of nontransgenic mice (mice 1–4) nursed on HYB PRO/Cla Mtv1 mice.
indicating that these mice were not MMTV infected. However, the absence of cognate T cell deletion in the B cell-deficient and H-2<sup>k</sup>- (I-E<sup>−</sup>) mice could also be due to inefficient SAg presentation, because of the lack of B cells or appropriate MHC haplotype. To determine whether the mice were infected, we isolated DNA from the spleens of the injected mice and performed PCR specific for MMTV(C3H) proviral DNA. As shown in Figure 4, only the IgM<sup>−</sup>H-2<sup>k</sup>− (lanes A–J) but not the IgM<sup>−</sup>H-2<sup>k</sup>− (lanes K–N), IgM<sup>−</sup>H-2<sup>k</sup>− (lane O), or IgM<sup>−</sup>H-2<sup>k</sup>− (lane P–R) mice contained MMTV(C3H) proviruses in their splenocytes. Therefore, lack of deletion of SAg-reactive T cells in IgM<sup>−</sup> or H-2<sup>k</sup>− mice directly correlated with the lack of infection.

To determine whether mammary gland cells of these animals were infected, we isolated RNA from their lactating mammary glands. This RNA was subjected to RNase T<sub>1</sub> protection analysis with the probe specific for the LTR of the MMTV(C3H) exogenous virus. All the F<sub>1</sub> and F<sub>2</sub> generation IgM<sup>−</sup>H-2<sup>k</sup>− (shown only for the F<sub>2</sub> generation) were infected, since they expressed high levels of MMTV-specific RNA in their mammary glands (Fig. 5, lanes A–J). In contrast, no MMTV-specific transcripts were detected in either the IgM<sup>−</sup>H-2<sup>k</sup>− (lanes P–R), IgM<sup>−</sup>H-2<sup>k</sup>− (lane O), or IgM<sup>−</sup>H-2<sup>k</sup>− (lanes K–N). After an overnight exposure of the gel in Figure 5, a very faint signal was seen for two of the four IgM<sup>−</sup>H-2<sup>k</sup>− mice (not shown). As was previously reported, H-2<sup>m</sup> mice can be infected at a low level (26). One IgM<sup>−</sup>H-2<sup>k</sup>− mouse also showed no mammary gland expression of MMTV(C3H) (Fig. 5, mouse J); however, this mouse did show lymphocyte infection (Fig. 4, bottom panel, IgM<sup>−</sup>H-2<sup>k</sup>−, mouse J). Thus, in the absence of B cells, there was no virus spread within the immune system or mammary gland.

**FIGURE 2.** Injection of virus directly into the mammary gland results in lymphoid cell infection. PCR followed by MfeI digestion was carried out with MMTV(C3H)-specific primers under non-quantitative conditions using DNA isolated from spleen (SP) and Peyer’s patches (P.P.) of three different C3H/HeN mice. Endogenous MMTVs contain no MfeI site and are visible after digestion with this enzyme. MMTV was injected into the mammary gland of 3-wk-old mice. Mice were sacrificed 5 wk after injection. Abbreviations: N.C.; negative control, DNA isolated from the spleen of the C3H/HeN MMTV− mouse.

**FIGURE 3.** Establishment of B cell-deficient I-E-positive mice. Heterozygous IGH6/BL6 (IgM<sup>+</sup>H-2<sup>k</sup>) females were crossed with C3H/HeN MMTV− (IgM<sup>+</sup>H-2<sup>k</sup>) males to generate F<sub>1</sub> heterozygotes. The F<sub>1</sub> mice were intercrossed and the F<sub>2</sub> generation female mice were typed for surface IgM and MHC class II H-2<sup>k</sup> expression. The mammary glands of the four different groups generated were injected with MMTV at 3 to 4 wk of age.

<table>
<thead>
<tr>
<th>Mice</th>
<th>Age (in days)</th>
<th>CD4/β14 (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt; IgM&lt;sup&gt;+&lt;/sup&gt;H-2&lt;sup&gt;k&lt;/sup&gt;− MTV&lt;sup&gt;+&lt;/sup&gt;</td>
<td>62</td>
<td>5.9 ± 0.51 (n = 7)</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt; IgM&lt;sup&gt;+&lt;/sup&gt;H-2&lt;sup&gt;k&lt;/sup&gt;− MTV&lt;sup&gt;+&lt;/sup&gt;</td>
<td>58</td>
<td>6.2 ± 0.70 (n = 20)</td>
</tr>
<tr>
<td>F&lt;sub&gt;2&lt;/sub&gt; IgM&lt;sup&gt;+&lt;/sup&gt;H-2&lt;sup&gt;k&lt;/sup&gt;− MTV&lt;sup&gt;+&lt;/sup&gt;</td>
<td>58</td>
<td>8.6 ± 0.30 (n = 3)</td>
</tr>
<tr>
<td>F&lt;sub&gt;2&lt;/sub&gt; IgM&lt;sup&gt;+&lt;/sup&gt;H-2&lt;sup&gt;k&lt;/sup&gt;− MTV&lt;sup&gt;+&lt;/sup&gt;</td>
<td>58</td>
<td>8.7 ± 0.42 (n = 9)</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt; IgM&lt;sup&gt;+&lt;/sup&gt;H-2&lt;sup&gt;k&lt;/sup&gt;− MTV&lt;sup&gt;+&lt;/sup&gt;</td>
<td>58</td>
<td>8.1 (n = 1)</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt; MTV&lt;sup&gt;−&lt;/sup&gt;</td>
<td>8.4 ± 0.20 (n = 3)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>T cells were isolated from the peripheral blood of mice at the ages indicated and analyzed for percentage of CD4<sup>+</sup>/β14<sup>+</sup> as described in Materials and Methods. Values are the mean ± SE.
experiment, a smaller MMTV dose was injected into both LEL (n = 4) and MTV-ORF (n = 6) transgenic mice and nontransgenic (n = 10) littermates (see Materials and Methods) (Fig. 6). After MMTV(C3H) injection directly into the mammary glands of the pubescent mice, we assessed whether the nontransgenic mice were infected by determining their percentage of peripheral CD4⁺/Vβ14⁺ T cells; because the transgenic mice all express the MMTV(C3H) SAg as an endogenous protein, they already lack these T cells (Table II). All nontransgenic mice in both experiments were infected as judged by their deletion of SAg-reactive Vβ14⁺ T cells (Table II).

To determine whether MMTV injection into the mammary gland did result in systemic infection of lymphoid cells, we performed PCR analysis on DNA isolated from spleen and Peyer’s patches of the infected transgenic and nontransgenic mice, using MMTV(C3H) LTR, gag-specific primers. We found that lymphoid tissues from all of the infected nontransgenic mice acquired new proviral copies of MMTV (Fig. 7, LEL n/t and MTV-ORF n/t). In contrast, the lymphoid tissue isolated from the injected transgenic mice showed no evidence of viral infection, except for one LEL transgenic mouse (lane marked LEL p.p.*, Fig. 7). This mouse also developed a mammary tumor.

Two methods were used to determine whether the mammary gland cells of the injected transgenic mice were infected. First, we bred these mice and then examined their nontransgenic offspring for the deletion of Vβ14⁺ T cells. In the first experiment, where a high virus dose was injected, the offspring of five of seven transgenic females showed deletion of their Vβ14⁺ T cells (Table II). These results suggested that MMTV transmission was variable due to low level infection. In support of this, when a lower dose of virus was injected, there was no deletion of this subset of T cells in the nontransgenic offspring of either the LEL or the MTV-ORF transgenic mice (Table II). All offspring of nontransgenic mice that received mammary gland injections of virus showed Vβ14⁺ T cell deletion (Table II).

We also tested directly whether the mammary glands of the injected mice were MMTV infected by isolating RNA from their milk and subjecting it to RNase T₁ protection analysis using a probe specific for MMTV(C3H) transcripts. To ensure that there was ample time for virus spread, all of the mice were analyzed after their fourth pregnancy; we have previously shown, using this assay, that milk-borne infection of mammary gland tissue increases with parity (28). RNA isolated from the milk of the LEL transgenic mice injected with the high virus dose contained little or no detectable MMTV(C3H)-specific RNA, in contrast to the MMTV-injected nontransgenic mice (Fig. 8A), whereas no viral RNA was detected in either the LEL or MTV-ORF mice that received the lower amount of virus (Fig. 8B).

This lower viral load had a dramatic affect on MMTV-induced mammary tumorigenesis. Because MMTV integration next to cellular oncogenes is a stochastic event, the higher the viral load, the more rapidly mice develop mammary tumors (13). Both the transgenic and nontransgenic mice from experiment 1 were continuously bred and monitored for mammary gland tumor incidence. The multiparous nontransgenic mice had a 100% mammary gland tumor incidence by the age of 260 days (Fig. 9). At that time, none
of the transgenic mice developed mammary tumors and only two of seven transgenic mice developed tumors by 300 days (Fig. 9). All the tumors had acquired exogenous MMTV proviruses as detected by Southern blot analysis (not shown).

Discussion

Both T and B cells play an essential role in the transmission of milk-borne MMTV, since the mammary glands of mice lacking either of these lymphoid subsets are not efficiently infected (5, 7, 8, 29). B cells are necessary, as initial targets for MMTV infection at minimum, because they are effective SAg-presenting cells for cognate T lymphocytes (8). Similarly, SAg-reactive T cells are required for the amplification and establishment of MMTV infection, at least within the lymphoid compartment (5, 7). Here, we provide proof that a mutant MMTV lacking a functional SAg gene will infect lymphoid cells, but that insufficient amplification of this lymphoid infection blocks viral transmission to the mammary gland.

Although it is now clear how MMTV initiates infection in vivo, how this virus infects mammary gland cells and spreads within this tissue is poorly understood. Expression of integrated MMTV proviruses in mammary cells is regulated by lactogenic hormones. As a result, pregnancy has a dramatic effect on virus load, which also increases with parity (2). The number of infected cells also increases in multiparous animals, showing that virus spread within the mammary gland after the initial infection. Without this spread, tumor induction by MMTV is very inefficient. Because retroviral integration is a stochastic event, increased numbers of integration events will improve the chance of insertion near a given cellular oncogene. For example, MMTV-infected virgin mice have a significantly lower virus load that leads to a decreased mammary tumor incidence and an increased tumor latency (14). In addition, MMTV-induced mammary tumors usually contain large numbers of newly integrated copies of exogenous MMTV, indicating multiple reinfection events (10).

Here we addressed the requirement for lymphoid cells in MMTV infection of and spread within the mammary gland. Not surprisingly, based on experiments by others in which MMTV was injected into peripheral tissue (3), we found that lymphoid as well as mammary cells were efficiently infected after the injection of virus directly into the mammary glands of wild-type, pubescent mice. However, when the same mode was used to introduce MMTV into B cell-deficient mice, we found no infection of either lymphoid cells or mammary tissue. Since the B cell-deficient mice had the MHC H-2b haplotype and thus did not express the I-E type that presents this SAg. Both I-E positive and I-E negative F2 mice were generated. This allowed us also to examine the role of MHC haplotype in virus spread within the mammary gland. Neither I-E+ nor I-E- B cell-deficient animals showed evidence of mammary gland infection. Moreover, I-E- B cell-+ mice also showed no, or very limited, mammary gland infection. This led us to speculate that even in the mammary gland, infected B cells were required to present SAg to cognate T cells for efficient infection.

We therefore tested whether mice lacking such cognate T cells could be infected by mammary gland injection of virus. We found that transgenic mice lacking SAg-reactive T cells showed little or no virus spread and, as a result, did not develop mammary tumors even after MMTV was introduced directly into the mammary gland.

Table II. Percentage of Vβ14+CD4+ T cells in the offspring of LEL and MTV-ORF females that received mammary gland injections of MMTV

<table>
<thead>
<tr>
<th>Expt</th>
<th>Mice</th>
<th>Age (in days)</th>
<th>CD4/Vβ14 (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nontransgenicb</td>
<td>130</td>
<td>4.5 ± 0.89 (n = 6)</td>
</tr>
<tr>
<td></td>
<td>LELb</td>
<td>150</td>
<td>2.5 ± 0.55 (n = 7)</td>
</tr>
<tr>
<td></td>
<td>Nontransgenic offspring</td>
<td>52</td>
<td>4.0 ± 0.15 (n = 5)</td>
</tr>
<tr>
<td></td>
<td>LEL offspring (nontransgenic)</td>
<td>52</td>
<td>4.0 ± 0.13 (n = 5)</td>
</tr>
<tr>
<td></td>
<td>LEL offspring (nontransgenic)</td>
<td>52</td>
<td>7.4 ± 0.21 (n = 2)</td>
</tr>
<tr>
<td>2</td>
<td>Nontransgenicb</td>
<td>152</td>
<td>3.9 ± 1.30 (n = 10)</td>
</tr>
<tr>
<td></td>
<td>MTV-ORFb</td>
<td>130</td>
<td>1.2 ± 0.2 (n = 6)</td>
</tr>
<tr>
<td></td>
<td>LELb</td>
<td>152</td>
<td>1.5 ± 0.2 (n = 4)</td>
</tr>
<tr>
<td></td>
<td>Nontransgenic offspring</td>
<td>52</td>
<td>4.0 ± 0.10 (n = 4)</td>
</tr>
<tr>
<td></td>
<td>MTV-ORF offspring (nontransgenic)</td>
<td>52</td>
<td>7.5 ± 0.50 (n = 4)</td>
</tr>
<tr>
<td></td>
<td>LEL offspring (nontransgenic)</td>
<td>51</td>
<td>7.3 ± 0.40 (n = 4)</td>
</tr>
<tr>
<td></td>
<td>C3H/HeN MTV-</td>
<td></td>
<td>7.3 ± 0.45 (n = 3)</td>
</tr>
</tbody>
</table>

a T cells were isolated from the peripheral blood of mice at the ages indicated, stained, and analyzed for the percentages of CD4+/Vβ14+ T cells as described in Materials and Methods. Values are the mean ± SE.

b Transgenic (LEL or MTV-ORF) and nontransgenic littermates were injected with MMTV(C3H) at 3 wk of age.
the n/t lanes in dose resulted in less infection in the nontransgenic mice as well (compare specific activity and the exposure times were the same. Thus, the lower virus experiments were performed with probes of approximately the same specific activity and the exposure times were the same. The lower virus dose resulted in less infection in the nontransgenic mice as well (compare the n/t lanes in A and B). Abbreviations: p, probe; MMTV(C3H), full protection.

These data suggest that the SAg stimulation of T cells is required for efficient infection of mammary glands and consequent tumorigenesis. Even when infection of the mammary gland cells was achieved, as in the case of the LEL transgenic mice injected with a high virus dose, the virus spread within this tissue was less efficient than that which occurred in mice that had SAg-cognate T cells.

These results demonstrate that infected lymphoid cells play a critical role in infection of the mammary gland. One possibility is that virus spread between mammary gland cells cannot be achieved in the absence of infected lymphoid cells. This may be because cell-cell contact between lymphocytes and mammary cells is the most efficient way to deliver virus. The architecture of the murine mammary gland may also affect how virus spreads. Mammary gland ducts consist of two main cell types, an inner region of ductal cells and an outer monolayer of myoepithelial cells surrounded by a basement membrane (2). At puberty, the mammary epithelial cells proliferate, resulting in a lengthening and branching of the ductal tree until the whole mammary fat pad is filled with cells. The terminal end bud drives ductal morphogenesis and most DNA synthetic activity is associated with these buds, while the ducts are relatively quiescent. The cells that occupy the terminal buds are most likely the targets for MMTV during puberty, since as with most other retroviruses, it is thought to require cell division for its propagation (30). If infected lymphocytes are present near these terminal buds, they could increase infection level of these dividing cells.

Similarly, lymphocytes may be involved in the virus spread that occurs in multiparous mice. Once puberty is over, the mammary cells do not divide in the mouse until pregnancy. MMTV preferentially buds from the alveolar surface of the epithelial cells lining the alveolar lumen (31) so that the virus is released into the milk during lactation. Thus, for MMTV to cause a systemic infection of the mammary gland tissue, it would have to be produced by an infected cell that directed its expression toward subepithelial tissues. B or T cells could fulfill this requirement, since both produce MMTV (9), persist within the tissue, and can be activated by cytokines produced by SAg-activated T cells (32). An additional factor that may come into play is that lymphoid cells can circulate within the tissue, thereby possibly coming into contact with mammary cells at multiple locations.

It is also possible that cytokine production by SAg-stimulated T cells affects the mammary gland tissue directly. For example, cytokines produced by T cells could induce expression of gene products necessary for MMTV infection of mammary gland cells or for mammary tumorigenesis. Since MMTV-infected mice gradually delete SAg-cognate T cells after acquisition of milk-borne virus, this mechanism would require the persistence of such T cells in the mammary gland and not in other peripheral sites. Whether this occurs can be tested by examining the Vβ-repertoire of T cells isolated from the mammary gland and their ability to be activated by SAg.

Taken together, we have established that SAg activation of lymphoid cells is needed not only for the initial stages of MMTV infection, but also for virus spread within the mammary gland. These results demonstrate the importance of different cell types during in vivo virus infections.

Acknowledgments

We thank Kevin Norman for maintaining the mouse colony in excellent condition, members of our laboratories for helpful discussions, and T. Wrona and Dr. Alexander Chervonsky for critically reading the manuscript.

References


