Superantigen-reactive CD4+ T Cells Are Required to Stimulate B Cells after Infection with Mouse Mammary Tumor Virus

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Summary

Superantigens are defined by their ability to stimulate a large fraction of T cells via interaction with the T cell receptor (TCR) Vβ domain. Endogenous superantigens, classically termed minor lymphocyte-stimulating (Mls) antigens, were recently identified as products of open reading frames (ORF) in integrated proviral copies of mouse mammary tumor virus (MMTV). We have described an infectious MMTV homologue of the classical endogenous superantigen Mls-1* (Mtv-7). The ORF molecules of both the endogenous Mtv-7 and the infectious MMTV(SW) interact with T cells expressing the TCR Vβ6, 7, 8.1, and 9 domains. Furthermore, the COOH termini of their ORF molecules, thought to confer TCR specificity, are very similar. Since successful transport of MMTV from the site of infection in the gut to the mammary gland depends on a functional immune system, we were interested in determining the early events after and requirements for MMTV infection. We show that MMTV(SW) infection induces a massive response of Vβ6 + CD4+ T cells, which interact with the viral ORF. Concomitantly, we observed a B cell response and differentiation that depends on both the presence and stimulation of the superantigen-reactive T cells. Furthermore, we show that B cells are the main target of the initial MMTV infection as judged by the presence of the reverse-transcribed viral genome and ORF transcripts. Thus, we suggest that MMTV infection of B cells leads to ORF-mediated B-T cell interaction, which maintains and possibly amplifies viral infection.

Minor lymphocyte-stimulating (Mls) antigens are encoded by an open reading frame (ORF)1 in the 3' LTR of endogenous mouse mammary tumor virus (MMTV) (1-3). They stimulate a large proportion of T cells due to the fact that T cell reactivity to Mls antigens is determined by the usage of the V segment of the TCR β chain (4, 5). The classical (and strongest) Mls determinant, Mls-1*, which interacts with T cells expressing Vβ6, 7, 8.1, and 9 elements (4-7), is encoded by the ORF of the Mtv-7 proviral locus (8). We have recently found an exogenous (infectious) MMTV, MMTV(SW), with properties of Mls-1* (9). The ORF molecules of Mtv-7 and MMTV(SW) display an almost identical amino acid sequence, particularly in COOH termini implied to confer TCR Vβ specificity. In fact, both endogenous Mtv-7 and infectious MMTV(SW) interact with the same TCR Vβ elements. In an MMTV-infected host, ORF-reactive T cells are deleted from both the thymic and peripheral T cell pool, although at a considerably slower rate than after expression of the endogenous Mtv-7 (Mls-1*) locus. Furthermore, injection of adult mice with MMTV(SW) leads to expansion of Vβ6+ CD4+ T cells similar to that observed after injection of Mls-1*-expressing B cells (9). A functional immune system seems to be a prerequisite for successful transport of MMTV to the mammary gland after neonatal infection through the gut mucosa. Thus, thymectomy, absence of T cells (in nude mice), or deletion of the ORF-reactive T cells prevent virus transmission to the next generation (10-12).

While very much attention has been focused on the fate of T cells interacting with Mls, the effects of T-B cell interaction on the Mls-presenting B cell have received less attention. Generally, it was found that Mls-reactive T cell clones provide polyclonal B cell help in vitro (13-15), whereas in vivo terminal differentiation of a minor fraction of the injected Mls-1* B cells was found (16, 17). Since B cells efficiently present ORF molecules from endogenous Mtvs, which are then recognized by ORF-specific T cells, it has been speculated that the ORF from infectious MTVs, like

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1 Abbreviations used in this paper: MMTV, mouse mammary tumor virus; ORF, open reading frame.
Materials and Methods

Mice. BALB/c and BALB/c nu/nu mice were purchased from HO Harlan OLAC Ltd. (Bicester, UK). Mls-1(+ Mtv-7) congenic BALB/c (BALB.D2) mice were bred from breeding pairs originally obtained from H. Festenstein (London Hospital Medical College) (19). MMTV(SW)-infected BALB/c mice were maintained from mice originally purchased from IFFA CREDO (L'Arbresle, France) (9). All experiments female mice 6-10 wk of age were used.

Antibodies. The following mAbs were used in this study: 14.2 (rat IgM anti-Vβ14) (20); 44.22 (rat IgG anti-Vβ8) (21); GK1.5 (anti-CD4) (22); F23.1 (mouse IgG2a anti-Vβ8.1-3) (23); Lyt-2 (33.6.7) (anti-CD8) (24); 3.168.8.1 (rat IgM anti-CD8) (25); polyclonal rabbit F(ab)2, anti–mouse Ig.

Reconstitution of Athymic BALB/c nu/nu Mice. BALB/c nu/nu mice were reconstituted with either Vβ6 + CD4 + or Vβ14 + CD4 + T cells as described (26). Briefly, BALB/c lymph node cells were enriched in these T cell subsets by elimination of CD8 + T cells through complement lysis using the mAb 3.168.8.1 (anti-CD8). B cells and Vδ8 + T cells were removed by incubation with F23.1 (anti-Vβ8.1-3) followed by goat anti–mouse Ig coupled to magnetic beads (Dynabeads; Dynal A.S., Oslo, Norway) and elimination on a magnet. The cells were then stained directly by fluorescence-labeled mAb 44.22 (anti-Vβ6) and biotinylated mAb 14.2 (anti-Vβ14) followed by streptavidin–PE. Vβ6 + and Vβ14 + cells were subsequently sorted using a FACStar Plus® (Becton Dickinson & Co., Mountain View, CA). Sorted cell populations, which were >97% pure Vβ6 + CD4 + or Vβ14 + CD4 + T cells, were washed and counted. 10⁵ cells were injected intravenously per BALB/c nu/nu mouse. Reconstitution of recipient animals was ascertained 4 wk later by analysis of the percentage of the transferred cells among total blood lymphocytes. Mice were tail bled and leukocytes were recovered from heparinized blood samples by centrifugation through a Ficoll (Pharmacia, Uppsala, Sweden) cushion.

Milk Collection, Virus Purification, and Virus Titration. Milk was aspirated from MMTV(SW)-infected or noninfected, lactating BALB/c females after injection of 0.5 IU sytoxcin/oxytocine (Sandoz, Basel, Switzerland), pooled, and stored at −70°C. A pool of milk was diluted 1:2 with PBS and centrifuged at 600 g for 10 min to skim. The skimmed milk serum was then diluted five times with PBS, 0.1 M EDTA, pH 7.4, centrifuged at 15,000 g for 1 h, and the virus pellet was resuspended in PBS. Remaining casein aggregates were removed by a quick spin at 600 g. The virus titers in these preparations were determined using a sandwich ELISA specific for MMTV gp52 as described before (9). Purified MMTV was diluted at 10⁵ particles/25 μl and stored in aliquots at −70°C. Control milk not containing MMTV particles was treated and diluted accordingly.

Injections and Sampling. Purified MMTV (10⁵ particles) or a virus-free control milk preparation were injected into the hind footpads of recipient mice. After the appropriate time the popliteal, paraaortic, and inguinal lymph nodes from the injected and noninjected footpads were isolated and pooled.

Flow Microfluorometry. Lymph node cells (10⁶) were stained with anti-TCR Vβ-specific monoclonal hybridoma supernatants followed by fluoresceinated anti–rat or anti–mouse IgM or IgG antibodies. B cells were detected using a polyclonal rabbit F(ab)2, anti–mouse Ig directly labeled with fluorescein. PE-coupled anti-CD4 (GR1.5) (Becton Dickinson & Co.) was used in the second dimension. Biotinylated anti-CD8 (Lyt-2) plus streptavidin-PE Texas red (Tandem; Southern Biotechnology Associates, Inc., Birmingham, AL) was used in the third dimension.

Leukocytes were recovered from heparinized blood samples by centrifugation through a Ficoll (Pharmacia) cushion. These cells were stained in one step with a mixture of FITC-labeled anti–TCR Vβ antibody and PE-coupled anti-CD4. For DNA analysis 3 × 10⁵ cells were stained with FITC-conjugated antibodies as above, washed in PBS, and fixed in 70% ethanol overnight as described in (27). Cells were centrifuged at 3,000 rpm for 5 min and stained at room temperature in 1 ml PBS containing 1 mg/ml glucose, 100 Kuntz U/ml RNase A, and 50 μg/ml propidium iodide. The cells were analyzed using the Doublet Discrimination Module (Becton Dickinson & Co.). Analysis was performed on a FACScan® cell analyzer using Lysys II for data evaluation (Becton Dickinson & Co.).

Ig Estimation. Levels of IgM and IgG subclasses in culture supernatants were determined separately for each subclass by ELISA as described (28). Values were expressed (in μg/ml) by reference to a standard curve obtained with a mouse IgG reference serum (64-901; Miles Scientific, Naperville, IL). Detection limit of the individual tests was 10 ng/ml. Total Ig levels correspond to the addition of the values for IgM and each of the IgG subclasses.

Polymerase Chain Reaction. DNA was isolated from total lymph node cells or FACS*-sorted B and T cells by digestion in Tris/EDTA/SDS, pH 8.0, plus protease K (100 μg/ml) at 52°C overnight. DNA was purified and resuspended at a DNA equivalent of 10⁶ cells per 50 μl. 20% was used per PCR reaction, corresponding to ~1 μg of genomic DNA. Cellular RNA was isolated from total lymph node cells or FACS*-sorted B and T cells after the guanidinium isothiocyanate/acid phenol procedure (29). Purified RNA was resuspended at the RNA equivalent of 10⁶ cells/2 μl. The reverse transcription was performed in 1 × PCR buffer with 2 μl RNA solution as described elsewhere (30). Viral cDNA was obtained as described before (9).

Oligonucleotides to amplify all Mtv ORFs were chosen on the basis of high degree of conservation between the sequenced ORF molecules, resulting in a 898-bp PCR product: 5′ oligo, TCG-TGCAGGAGGCTCTCAC (Vβ84); 3′ oligo, GTGTCGACCGACCAAAGTGAGGAAAAACCTTG (Vβ71). To specifically detect MMTV(SW)/Mtv-7 ORF COOH terminus was used in combination with Vβ84, yielding a 766-bp PCR product. 3′ oligo, GGCCACCCCATGAGTATATTTCC (Vβ83). A primer in the 5′ noncoding region of the 5′ splicing site for subgenomic mRNAs (31) was designed to specifically amplify spliced ORF mRNA: 5′ oligo, CAGGGAGCTGCAGTCTAAC; 3′ oligo, GGAACCGCTGCAGATTATAGGA. DNA or cDNA was boiled for 3 min in the presence of the

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A B Cell Response.

Results

Acute Infection of BALB/c Mice with MMTV(SW) Induces a B Cell Response. After footpad injection of adult BALB/c mice with 10⁹ MMTV(SW) particles purified from mouse milk, we observed an approximately fourfold size increase of the local (popliteal, paraaortic, and inguinal) lymph nodes. As noted previously (9) this size increase was partially due to an increase of Mls-1a-reactive VB6 + CD4 + T cells from 10.9 ± 0.5% of CD4 in animals injected with an MMTV-free milk preparation to 24.4 ± 2.6% at day 6 after MMTV(SW) infection. In contrast non-Mls-1a-reactive T cells such as VB14 + CD4 + did not specifically increase, discounting virus-dependent proliferation of the B cell responses were virus dependent since the MMTV-free milk preparation did not induce a detectable response (Fig. 1, A and B).

Using a combination of cell surface labeling and DNA content analysis, we determined the percentage of cells in cycle. Between days 4 and 6 after MMTV(SW) infection a minor fraction of B cells (2%) was found in S + G2 + M phase compared with 0.8% in noninfected control lymph nodes. Slightly more (4%) CD4 + T cells are in cycle at day 4 (compared with 0.5% in controls). Analysis of MMTV(SW) ORF-responsive T cells indicated an enrichment of cycling cells (8% among VB6 + T cells).

Titration of the injected MMTV(SW) dose from 10⁹ to 10⁶ virus particles indicates that the B cell response is high as long as there is a significant CD4 + VB6 + T cell response (Fig. 2). This suggests that a critical virus dose is required to induce a detectable local immune response.

The B Cell Response to MMTV(SW) Depends on the Presence of Mls-1a-reactive T Cells. BALB.D2 mice are (Mtv-7) Mls-1a-congenic BALB/c mice. Therefore, they delete >95% of the T cells that react with Mls-1a within the first 10 d after birth (32). After injection of exogenous MMTV(SW) into BALB.D2 mice the B cell response was profoundly diminished compared with the response in control BALB/c mice. Both BALB/c and BALB.D2 mice, however, responded equally well to MMTV(C3H), an exogenous MMTV that encodes an ORF molecule interacting with T cells expressing the VB14 and VB15 TCRs (2) (Table 1).

CD4 + VB6 + T Cells Are Sufficient to Stimulate a B Cell Response to MMTV(SW). Nude mice lack a thymus and are therefore not able to generate a functional T cell compartment; however, they can be efficiently reconstituted with T cells (26). We reconstituted BALB/c nu/nu mice with either 10⁹ CD4 + VB6 + or CD4 + VB14 + T cells isolated by cell sorting from lymph nodes of normal BALB/c mice. Greater than 90% of the CD4 + T cells in transferred nude mice expressed the selected phenotype, whereas contamination with the alternative subset was <1%. Nude mice reconstituted playing 9.9 ± 1.2% of CD4 + T cells in control animals and 8.6 ± 1.5% at day 6 after infection. Absolute cell counts for the different CD4 + T cell subsets over time are shown in Fig. 1 A. The T cell response in the local lymph nodes was paralleled by a specific increase of B cells from 20.0 ± 2.3% of total lymph node cells in noninfected animals to 32.7 ± 3.0% at day 6 after infection (Fig. 1 B). Both B and T cell responses were virus dependent since the MMTV-free milk preparation did not induce a detectable response (Fig. 1, A and B).

Figure 1. Local immune response to MMTV(SW). 10⁹ MMTV(SW) particles were injected into one hind footpad of recipient BALB/c mice. At appropriate time points after infection the local lymph nodes (popliteal, paraaortic, and inguinal) were isolated, pooled, and analyzed for lymphocyte subsets using flow microfluorometry. (A) Absolute cell counts in local lymph nodes for VB6 + CD4 + T cells (▲) and VB14 + CD4 + T cells (○) after MMTV(SW) infection and for VB6 + CD4 + T cells (▲) in mice injected with a MMTV-free milk preparation. Data represent mean values from two to four animals per time point. (B) B cell response upon MMTV(SW) injection (O) and upon injection of the control milk preparation (●). Data represent mean values derived from the same animals as above.

Figure 2. Effect of MMTV(SW) dose on T and B cell responses. Response of VB6 + T cells indicated as percentage of CD4 + T cells (○) and concomitant B cell response (●) (indicated as absolute cell counts in popliteal, paraaortic, and inguinal lymph nodes) upon titration of the MMTV(SW) dose. Data represent mean values of two animals at day 4 after injection of MMTV(SW).
Table 1. B Cell Response to MMTV in Mls-1+ Congenic Mice

| Virus injection | BALB/c | BALB.D2 |
|-----------------|--------|---------|
|                 | n      | B cells* | n      | B cells* |
| None            | 9      | 0.7 ± 0.5| 7      | 1.1 ± 0.7|
| MMTV free       | 3      | 0.7 ± 0.3| 2      | 0.9/0.6 |
| MMTV (C3H)      | 3      | 3.4 ± 1.3| 3      | 3.5 ± 1.2|
| MMTV (SW)       | 4      | 6.9 ± 1.4| 4      | 2.2 ± 1.1|

* Absolute numbers (× 10⁶) (± SD) of B cells in popliteal, paraaortic, and inguinal lymph nodes 6 d after injection of 10⁹ MMTV particles or an MMTV-free milk preparation.

Table 2. Immune Response Induced by MMTV in Either Vβ6+ CD4+ or Vβ14+ CD4+ T Cell-reconstituted BALB/c nu/nu Mice

| Injection      | No cells | Vβ14+ CD4+ T cells | Vβ6+ CD4+ T cells |
|----------------|----------|--------------------|-------------------|
|                | B cells* | CD4+*              | B cells           | Vβ14+ CD4+*          | B cells | Vβ6+ CD4+*          |
| None           | 2.3 ± 1.4| 0.03 ± 0.02        |                  |                   |
| MMTV (C3H)     | 2.6 ± 1.3| 0.02 ± 0.02        | 2.9 ± 0.8        | 0.3 ± 0.1          | 2.1 ± 0.4| 0.3 ± 0.1          |
| MMTV (SW)      | 3.4 ± 0.5| 0.02 ± 0.02        | 9.9 ± 3.9        | 0.6 ± 0.2          | 3.9 ± 1.8| 0.3 ± 0.1          |

* Absolute cell counts (× 10⁶) (± SD) in popliteal, paraaortic, and inguinal lymph nodes from three to five mice determined between days 4 and six after injection of 10⁹ MMTV particles. Statistically significant increases are underlined.
Figure 3. Ig secretion by MMTV(SW)-infected lymph node cells. Lymph node cells were isolated 6 d after infection with MMTV(SW), and 5 x 10^6 cells were cultured for 3 d in 200 μl of complete DMEM supplemented with 5% FCS without any further stimulation. Total Ig (IgG + IgM) levels in supernatants were subsequently determined using isotype-specific sandwich ELISAs as described in Materials and Methods. Supernatants were derived from: (A) infected and noninfected BALB/c or BALB.D2 mice; and (B) infected and noninfected BALB/c nu/nu (nu/nu) controls or BALB/c nu/nu mice reconstituted with either Vβ14+ CD4+ T cells (Vβ14/nu) or Vβ6+ CD4+ T cells (Vβ6/nu).

Figure 4. Isotype pattern of Ig production by MMTV(SW)-infected lymph node cells. Lymph node cells from MMTV(SW)-infected BALB/c, BALB.D2, and Vβ14/nu or Vβ6/nu reconstituted nude mice were cultured as described above. Ig subclass distribution was determined using isotype-specific sandwich ELISAs as described in Materials and Methods.

Figure 5. Analysis of MMTV(SW) infection by PCR. (A) Lymph node cells from draining (popliteal and paraaortic) or nondraining (axillary) lymph nodes (LN) were isolated at the indicated time points after injection of MMTV(SW) into the footpad of BALB/c mice. Lymphocyte DNA was isolated and subjected to PCR analysis specific for MMTV(SW) (and Mtv-7) ORF DNA yielding a 766-bp PCR product. As a control we amplified all Mtv ORFs present in BALB/c mice, including endogenous Mtv's 6, 8, and 9, yielding a 898-bp PCR product. (B) At day 6 after infection lymph node cells were separated into B and T cells (both populations were >99% pure) using FACS®. Isolated DNA was then subjected to PCR specific for MMTV(SW) ORF DNA or all Mtv ORFs as described above.
Figure 6. Analysis of MMTV(SW) ORF mRNA expression by PCR. (A) Specificity of the PCR for spliced ORF mRNA. A primer at the 5’ splicing site for subgenomic mRNAs together with the MMTV(SW) (and M-MuLV) ORF-specific primer described in Fig. 5 were tested on genomic DNA from BALB/c and BALB.D2 mice and on the cDNA derived from the MMTV(SW) genome in which the splice site and the ORF are separated by ~8 kb of gag-pol-env sequences. The quality of these preparations was confirmed by primers that amplify a 766-bp stretch within the MMTV(SW) or M-MuLV ORF, which do not require a splicing event. The quality of the BALB/c DNA was confirmed with primers that amplify all endogenous Mtv’s (see Fig. 5 B). (B) Lymph node cells from draining (popliteal and paraaortic) lymph nodes were isolated at day 6 after injection of MMTV(SW) into the footpad of BALB/c mice. Lymph node cells were then separated into B and T cells using FACS to a purity of >99.5%. Lymph node cells from noninfected BALB/c mice were used as a control. After RNA isolation, the prepared cDNA was subjected to PCR analysis specific for spliced MMTV(SW) ORF mRNA. The observed PCR products were 1.0 and 1.8 kb. The quality of the cDNAs was confirmed by control PCR specific for β-actin mRNA.

Discussion

Infection with MMTV occurs vertically after transmission from mother to offspring by the milk. Several lines of evidence suggest that the immune system and particularly T cells play a crucial role for MMTV infection and transport to the mammary gland. However, early MMTV infection events, including the role of the superantigen activity in viral infection and that of the different lymphocyte subsets as putative intermediate virus hosts, are still poorly understood.

We show here that B cells are the primary target for MMTV infection since ORF transcripts and the bulk of reverse-transcribed viral genome are present in B cells. Since we show that the mature virus particle does not contain ORF transcripts and it is believed that ORF protein is not present in the mature virion (35), it is likely that after infection, de novo ORF protein expression and presentation in the context of MHC class II antigens (36) by infected B cells leads to stimulation of ORF-reactive CD4+ T cells. B cells are then stimulated by these activated CD4+ T cells. This sequence of events is supported by the fact that cycling T cells are observed before cycling B cells in the local response. The hypothesis that MMTV(SW)-infected B cells present the ORF molecule to T cells, thereby inducing direct B-T cell interaction, is consistent with several lines of evidence. (a) CD4+ T cells respond in vitro to ORF molecules from endogenous Mtv’s presented by B cells in the context of MHC class II molecules. The interaction can be blocked by antibodies to MHC class II, CD4, or the TCR (for review see reference 37). (b) B cells are polyclonally inducible using Mls as the target antigen (15). Direct Th–B cell interaction was required for the initial B cell activation step, whereas activation of bystander B cells not expressing the Mls determinant has been shown by some (14) but not by others (13).

After MMTV infection the presence of ORF-reactive CD4+ T cells was both necessary and sufficient to induce a response among B cells. This B cell stimulation may lead to an increased probability of reinfection and/or may indicate that a rare subset of infected B cells is amplified. This latter point is consistent with the data showing a low percentage of proliferating B cells. In any case an activated B cell should provide an appropriate milieu to transcribe the MMTV genome, as has been demonstrated for germline-transmitted, endogenous Mtv’s (38).

After accumulation in the local lymph nodes some B cells undergo differentiation and secrete antibodies. The Ig subclass distribution in culture supernatants of lymphocytes from infected animals implies a T cell– and ORF-dependent Ig production. This is supported by the finding that infected BALB/c mice do not produce significant amounts of IgG and that efficient IgG production in nude mice can be reconstituted by MMTV ORF-reactive Th cells. A predominant IgG2a secretion by infected BALB/c lymph node cells suggests that IFN-γ-producing Th type I cells (39) are induced upon MMTV infection. In fact IFN-γ production has been demonstrated in Mls-specific Th cell clones (40, 41) and in secondary in vitro Mls responses (H. R. MacDonald, unpublished results). In this respect it is interesting to note that
the transiently elevated serum IgG2a levels found after neonatal infection of Mls-1a-bearing B cells could be reversed by anti-IFN-γ antibodies (17).

The IgG2a subclass of antibodies has generally been found as specific (antiviral) as well as nonspecific (polyclonal) antibodies elicited in mice by viral infections (42). After MMTV(SW) infection anti-MMTV antibodies were found at low but comparable titers in supernatants of both infected BALB/c and BALB.D2 mice (not shown), indicating that the viral superantigen probably does not play a role in the MMTV-specific antibody response. However, the massive IgG2a response of BALB/c or Vβ6+ CD4+ reconstituted nude mice is dependent on the viral superantigen since BALB.D2, nude, or Vβ14+ CD4+ reconstituted nude mice secrete only low amounts of IgG2a antibodies. Thus, polyclonal IgG2a production depends on the presence of both the superantigen and CD4+ T cells reactive with it.

Recently Golovkina et al. (12) showed that deletion of superantigen-reactive T cells induced by transgenic expression of a superantigen led to reduced transport of the corresponding exogenous MMTV to the mammary gland. These data have been interpreted by others to mean that superantigen-reactive T cells are the primary target for MMTV infection (43). However, our experiments show that B cells are the primary target of infection and that the absence of superantigen-reactive T cells does not allow efficient stimulation of presumably infected B cells. Similarly, earlier data published by Tsibura et al. (11) indicate that T cells but not B cells from infected mice can transfer MMTV infection to nude mice. Again our experiments suggest that the absence of T cells in nude mice does not allow efficient stimulation of infected B cells. Thus, infected B cells alone may not be able to transfer MMTV to the mammary gland in nude mice. With regard to the latter study it should be noted that we assayed for T cell infection very early (6 d) after local infection. However, Tsibura et al. (11) reported MMTV transport to the mammary gland of nude mice after transfer of T cells isolated 6–7 mo after infection. Infection of T cells may therefore be a late event in MMTV transmission.

Continuous deletion of ORF-reactive CD4+ T cells in infected mice (9) may reflect an ongoing T cell stimulation due to persistence of MMTV(SW)-infected B cells. Thus, MMTV ORF-induced, T cell-dependent B cell stimulation would assure the maintenance and possibly the amplification of the virus and increase the probability of infecting the mammary gland. The absence of superantigen-reactive T cells may deprive the intermediate virus host and thus the virus from this amplification step and therefore lead to an inefficient infection.

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