Mouse mammary tumor virus (MMTV) is an oncogenic type B retrovirus which infects mainly B lymphocytes (4, 21). In the first hours after encounter with MMTV, a polyclonal T-cell-independent B-cell activation is observed (2). As much as 80% of B cells are activated by MMTV, but only a few of them become infected. The few infected B lymphocytes present a viral superantigen (Sag) bound to major histocompatibility complex (MHC) class II molecules to T cells expressing a specific T-cell receptor Vβ element. Subsequent Sag-specific T-helper-cell responses result in a strong preferential amplification and differentiation of infected B cells (for a review, see reference 21).

In all known retroviral infections, activated or cycling lymphocytes are required for infection to occur. For murine leukemia virus, Rous sarcoma virus, and spleen necrosis virus, the cell cycle of the target cells has been found to be necessary for viral integration and productive infection (3, 12, 14, 26). In addition, retroviral transcription requires an integrated proviral template (8). While nuclear breakdown during mitosis was shown to contribute to the integration of retroviral DNA into the nucleus, human immunodeficiency virus (HIV) has been shown to be independent of mitosis due to its karyophilic core protein (7). However, HIV requires T-lymphocyte activation for infection. In HIV infection, the inefficient reverse transcription in quiescent peripheral blood lymphocytes is caused by low levels of deoxynucleotides and contributes to a cytoplasmatic pool of mostly incomplete viral DNA which can be rescued after mitogenic stimulation (13).

For MMTV infection, there are two main interpretations for the early infection events. Either MMTV-induced activation facilitates infection of naive small resting B cells, or, alternatively, MMTV preferentially infects preactivated B cells. To address this question, we analyzed the effect of polyclonal B-cell stimulation on MMTV infection in mice.

To activate B lymphocytes, 7- to 8-week-old BALB/c mice were injected subcutaneously (s.c.) into the hind footpad with a single dose of either lipopolysaccharide (LPS) from Escherichia coli (Sigma, San Diego, Calif.) or monoclonal antibodies (MAbs) that induce a polyclonal B-cell stimulation via cross-linking of surface immunoglobulin (sIg) or CD40.

We investigated whether mouse mammary tumor virus (MMTV) favors preactivated or naive B cells as targets for efficient infection. We have demonstrated previously that MMTV activates B cells upon infection. Here, we show that polyclonal activation of B cells leads instead to lower infection levels and attenuated superantigen-specific T-cell responses in vivo. This indicates that naive small resting B cells are the major targets of MMTV infection and that the activation induced by MMTV is sufficient to allow efficient infection.

![Figure 1](http://jvi.asm.org/)

**FIG. 1.** Mitogen-induced B-cell activation and proliferation. A single dose of 2.4G2 (28 µg) or LPS (10 µg) was injected s.c. into the hind footpads of BALB/c mice, or mice were left untreated (c). Anti-IgD MAb (50 µg), anti-CD40 (50 µg), anti-IgD plus anti-CD40 (50 µg each), or anti-IgM (10 µg) was injected s.c. into the hind footpad 30 min after injection of 2.4G2 (28 µg). The percentages of CD69+ B cells (A) and BrdU+ B cells (B) among B220+ B cells in the draining POP-LN were analyzed 24 (●) and 48 (■) h later. Each column represents the mean percentage ± standard deviation of B cells from four mice.
BALB/c mice received a single dose of mitogen s.c. (10 to 50 μg) either before, concomitantly with, or after s.c. injection of MMTV(SW), a retrovirus which expresses a Vβ6-specific Sag (10⁸ virus particles [16]). We analyzed the percentage of Sag-reactive Vβ6⁺ T cells in the CD4⁺ T-cell population and the amount of viral DNA in the draining PO-LN at days 3.5 to 4 after infection. This time point allows measuring both reduced or enhanced responses, since maximal stimulation was observed on days 5 to 6. In order to quantify infection levels, 500 ng of DNA of cells extracted from the draining PO-LN was analyzed by PCR. Trace amounts of [α-³²P]dATP were added, and 30 cycles (1 cycle consisting of 5 min at 95°C; 30 cycles with 1 cycle consisting of 1 min at 95°C, 1 min at 55°C, and 1 min at 72°C, and, finally, an extension step for 10 min at 72°C) were used. The Sag sequences of the endogenous proviruses Mtv-6, Mtv-8, and Mtv-9 were amplified as internal standards relative to MMTV(SW) in the same tube with previously described primers [17]. This PCR method is linear for MMTV(SW) in the range of 3 to 95% of Mtv-6 signals. For weaker signals, the PCR slightly overestimates the signals. DNA extracted from lymphocytes derived from uninfected BALB/c mice or from PO-LN of mice 3.5 days after injection of MMTV (3 x 10⁶ to 3 x 10⁷ viral particles) was used as the control. PCR products were separated on a 6% denaturing polyacrylamide gel, which was dried and then exposed to Kodak X-Omat film (Eastman Kodak Company, Rochester, N.Y.). The linear range of the PCR was determined by PhosphorImager analysis. In Fig. 2, the PCR was linear after injection of more than 10⁷ viral particles.

As shown in Fig. 2A, treatment of mice with LPS before or during MMTV challenge strongly reduced the amount of detectable viral DNA in the draining PO-LN cells. In agreement with this, the expansion of MMTV(SW) Sag-specific Vβ6⁺ T cells was relatively low. We confirmed the effect of each antibody treatment by surface staining of several lymphocyte activation markers. In Fig. 1B, we show the percentage of B cells having incorporated 5’-bromo-2’-deoxyuridine (BrdU; Sigma) incorporation. After staining with RA3-3A1 (phycoerythrin-conjugated anti-murine B220; Caltag), fixation in formaldehyde, and DNase treatment, flow cytometry was performed with anti-BrdU–FITC (Becton Dickinson) (for the method used, see reference 28). One group of mice received 3 mg of BrdU intraperitoneally and thereafter 1 mg/ml continuously in the drinking water at the same time as mitogen or antibody, and the mice were analyzed after 24 h. The other group of mice received BrdU 24 h after mitogen or antibody injection during a 24-h pulse. Among the different treatment protocols, anti-CD40 induced the lowest level of cell division, followed by LPS, and the strongest induction of the cell cycle was found with anti-IgD or anti-IgD plus anti-CD40. Anti-FcγRII treatment did not lead to significant activation and proliferation.
CD4+ T cells was impaired in LPS-treated mice (Fig. 2B). The T-cell stimulation by MMTV was intermediate when LPS was injected 48 h before infection, indicating that the effect of LPS was transient and partially reversible after 48 h. One day after infection, LPS- and anti-IgD- or anti-IgD-plus-anti-CD40-treated mice, however, resulted in a strong reduction of the stimulation of Vβ6+ CD4+ T cells compared with infected control mice.

Our studies clearly show that MMTV infection of B cells occurs efficiently without preactivation of the target cells, as has been shown for HIV infection of nonproliferating monocytes and HeLa cells (19, 30). The capability of MMTV to activate its target cell in the early phase of infection might be an efficient strategy of retroviruses to facilitate infection of naive target cells.

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