B Cell Response After MMTV Infection: Extrafollicular Plasmablasts Represent the Main Infected Population and Can Transmit Viral Infection

Carlos Ardanín, Pilar Martín, Isabel Ferrero, Iñigo Azcoitia, Fabienne Anjuère, Heidi Diggelmann, Frédéric Luthi, Sanjiv Luther and Hans Acha-Orbea

*From January 1, 2022 to May 15, 2022. Cites 34 articles, 16 of which you can access for free at:
http://www.jimmunol.org/content/162/5/2538.full#ref-list-1

Why The JI? Submit online.

• Rapid Reviews! 30 days* from submission to initial decision
• No Triage! Every submission reviewed by practicing scientists
• Fast Publication! 4 weeks from acceptance to publication

*average

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

The Journal of Immunology is published twice each month by The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 1999 by The American Association of Immunologists All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
B Cell Response After MMTV Infection: Extrafollicular Plasmablasts Represent the Main Infected Population and Can Transmit Viral Infection

Carlos Ardavín, Pilar Martín, Isabel Ferrero, Inigo Azcoitia, Fabienne Anjüere, Heidi Diggelmann, Frédéric Luthi, Sanjiv Luther, and Hans Acha-Orbea

The immune response to mouse mammary tumor virus (MMTV) relies on the presentation of an MMTV-encoded superantigen by infected B cells to superantigen-specific T cells. The initial extrafollicular B cell differentiation involved the generation of B cells expressing low levels of B220. These B220low B cells corresponded to plasmablasts that expressed high levels of CD43 and syndecan-1 and were CD62 ligand and IgD−. Viral DNA was detected nearly exclusively in these B220low B cells by PCR, and retroviral type-A particles were observed in their cytoplasm by electron microscopy. An MMTV transmission to the offspring was also achieved after transfer of B220low CD62 ligand−CD43+ plasmablasts into noninfected females. These data suggest that B220low plasmablasts, representing the bulk of infected B cells, are capable of sustaining viral replication and may be involved in the transmission of MMTV. The Journal of Immunology, 1999, 162: 2538–2545.

MOuse mammary tumor virus (MMTV) is a B-type retrovirus that, upon infection of the mammary gland epithelial cells, promotes the development of mammary carcinomas by insertional activation of host protooncogenes (reviewed in Ref. 1).

MMTV is transmitted through milk to newborn mice during the first 2 wk of life by initially infecting B cells in the Peyer’s patches (2). Efficient MMTV infection is dependent upon the presentation of an MMTV-encoded superantigen (SAg) by the initial pool of infected B cells to SAg-reactive T cells (3). The resulting SAg-specific Th cell response, mediated by the cross-linking of B cell MHC class II molecules with TCR Vβ elements, leads to the amplification of the infected cells by inducing a strong B cell proliferation and differentiation response (4). Immunohistochemical analysis of the draining lymph node after infection with MMTV has demonstrated that MMTV-SAg drives extrafollicular and follicular B cell differentiation, following a very similar pattern to that described for conventional Ags (5).

The follicular phase of a B cell response comprises a complex series of events occurring during the germinall center reaction, implying the somatic hypermutation of Ig genes and the selection of high-affinity mutants (6). In contrast, extrafollicular B cell differentiation involves an early Ag-specific proliferation and differentiation of B cells taking place within the so-called extrafollicular foci and leading to the generation of a first wave of plasmablasts (7). These extrafollicular plasmablasts appear to display low levels of B220 and to express the proteoglycan molecule syndecan-1 (CD138) (8, 9). Interestingly, although plasma cells have been considered to be short-lived cells, two recent reports have demonstrated that a substantial fraction of them can survive for periods of >1 yr (10, 11).

Previous reports have demonstrated that very few B cells are infected in the first days after MMTV injection. It was estimated that in the draining popliteal lymph node (PO-LN), ~1 of 10,000 B cells were infected 48 h after injection of the Swiss strain of MMTV, MMTV(SW), into the footpad of BALB/c mice (12). However, between days 2 and 6 (i.e., during the extrafollicular phase of the B cell response), the number of infected B cells increases >1000-fold by cell division-mediated expansion of the initial number of infected cells. This SAg-mediated B cell response has been demonstrated to be required for a successful MMTV infection of the mammary gland (3, 13). However, the mechanism of virus transmission to the mammary gland is still largely unknown, because the data dealing with the capacity of different lymphocyte subsets to transmit MMTV infection after transfer into appropriate hosts remain controversial (reviewed in Ref. 14). In addition, it has been reported that under physiological conditions, large quantities of new virus particles are not produced during the first phase of infection, but only by the infected epithelial cells of the mammary and salivary glands under the control of the endocrine system by pregnancy hormones (reviewed in Ref. 15). Finally, the characterization of the cell type responsible for virus transfer to the mammary gland is further complicated by virus spread from the initially infected B cells to T cells (16). Therefore, to get new insights on the transmission of the virus to the mammary gland, we have extensively analyzed the B cell response induced upon infection by MMTV(SW). Our results support the hypothesis that the MMTV(SW)-infected plasmablasts differentiated during the extrafollicular B cell response, which can be...
identified and therefore traced during the infection process on the basis of its phenotypic profile and which may be involved in MMTV transmission to the mammary gland and to the offspring.

Materials and Methods

**Mice**

BALB/c mice were purchased from Harlan Olac (Bicester, U.K.). In all experiments, 8- to 10-wk-old mice were used.

**Antibodies**

The following Abs were used in this study: FD11-54-3 (anti-MHC class II, biotin-conjugated; Ref. 17), H1-2F3 (anti-CD69, conjugated; Ref. 19), Mel-14 (anti-CD62 ligand (CD62L), biotin-conjugated; Ref. 21), CD44 (anti-CD44, biotin-conjugated); Ref. 22), GL1 (anti-B7-2, biotin-conjugated; Pharmingen), RA3-6B2 (anti-IgM, biotin-conjugated; Phar-Mingen), LO-MM (anti-IgM, biotin-conjugated; Caltag, San Francisco, CA), RA3-6B2 (anti-CD22, FITC-conjugated; Caltag), H129.19 (anti-CD4, phycoerythrin (PE)-conjugated; Boehringer Mannheim, Mannheim, Germany), 53-6.7 (anti-CD8, PE-conjugated; Boehringer Mannheim), and M5-114 (anti-MHC class II, PE-conjugated; Boehringer Mannheim).

**Flow cytometry**

The kinetics of B cell and T cell activation as well as the percentage of Vß6+1 B cells were analyzed after triple-staining with biotinylated anti-CD69, followed by streptavidin (SA)-tricolor (Caltag, San Francisco, CA), PE-conjugated anti-CD3 and anti-CD8, and fluoresceinated anti-Vß6. CD4+ and CD8+ cells were separated in the FL2 channel using appropriate dilutions of the two mAbs. Analysis of the B220+ and B220+CD62L B cells was performed after triple-staining with biotinylated mAbs against CD69, IgM, IgG2a, IgD, CD62L, CD43, syndecan-1, LFA-1, and CD44, followed by SA-triclor (Caltag), PE-conjugated anti-MHC class II, and fluoresceinated anti-B220. Analysis was performed on a FACSscan (Becton Dickinson, Mountain View, CA) cell analyzer, using Lysys II software for data evaluation.

**Cell cycle analysis**

The cell cycle was analyzed on FACS-sorted MHC class II+ B220+ or MHC class II+ B220+CD62L+ B cells from the draining PO-LN after double staining with biotinylated anti-MHC class II followed by SA-PE (Caltag) and fluoresceinated anti-B220. After reanalysis, the sorted cell populations had a purity of >99% (data not shown).

**Polymerase chain reaction**

DNA of 50,000 FACS-sorted MHC class II+ B220+ or MHC class II+ B220+CD62L+ B cells (purified from the draining PO-LN, as assessed by the analysis of the percentage of cycling cells and of B and T blast cells (data not shown). As previously reported, B cells underwent a biphasic activation: an initial transient T cell-independent B cell activation, occurring within the first 24 h, was followed by a second B cell activation phase starting 2 days after infection and reaching a peak by day 4 (24). This second B cell activation phase was accompanied by a strong B cell proliferative response that amplified the initial number of infected B cells and required the presentation by the infected cells of an MMTV(SW)-encoded SAg to the SAg-reactive Th cells (3).

Results

**Analysis of responding B cells during MMTV infection**

Injection of MMTV(SW) into the footpad of BALB/c mice induced a strong B cell and T cell proliferative response in the draining PO-LN, as assessed by the analysis of the percentage of cycling cells and of B and T blast cells (data not shown). As previously reported, B cells underwent a biphasic activation: an initial transient T cell-independent B cell activation, occurring within the first 24 h, was followed by a second B cell activation phase starting 2 days after infection and reaching a peak by day 4 (24). This second B cell activation phase was accompanied by a strong B cell proliferative response that amplified the initial number of infected B cells and required the presentation by the infected cells of an MMTV(SW)-encoded SAg to the SAg-reactive Th cells (3).

Analysis of the B220 vs MHC class II expression of the B cells during the B cell response revealed the appearance by day 4 of a B cell subpopulation expressing MHC class II levels that were similar to those of control mice, but lower B220 expression (Fig. 1A). This B220+ B cell population, hardly detectable in the
PO-LN of control mice, in which it represented <3% of the total B cells (Fig. 1A), reached a maximum by days 5 and 6, when it constituted ≤40% of the B cell subset, and returned to control levels by day 11 in the PO-LN of MMTV(SW)-infected mice (Fig. 1B). Therefore, the kinetics of the B220low B cells paralleled the B cell activation and proliferation response induced by MMTV(SW). At 6 days after virus injection, the B220low B cell population represented ≤40% of the total lymph node B cells (i.e., 4–5 × 10^6 cells) and contained ≤90% of blast cells, whereas only ~25% blast cells were detected in the B220high B cell population (Fig. 1A). Analysis of the cell cycle of the B220low and B220high B cell subpopulations sorted by FACS (Fig. 2) showed that, although the B220high B cell population had a basal level of cycling cells (3%), the B220low B cells displayed a high percentage of proliferating cells (19% cycling cells). These results indicate that the B220low MHC class II^+ B cell subpopulation contains the vast majority of the dividing B cells during the SAg-mediated B cell response induced by MMTV(SW).

**FIGURE 1.** Analysis of early B cell differentiation after MMTV(SW) injection. A. Dot plots show the B220 vs MHC class II profiles of PO-LN cells from control mice and from mice at day 6 after MMTV(SW) injection. Boxes define B220low (1) and B220high (2) B cells. Histograms represent the B220 expression within the total B cell population in control mice and in MMTV(SW)-injected mice at day 6 and the FSC of B220low and B220high B cells. The percentage of B220low cells within the total B cell population and the percentage of blast cells (FSC >130) are shown for the indicated cell subsets. B. Analysis of B220low B cells during the immune response to MMTV(SW). The percentage of B220low cells within total B cells and the absolute number of B220low B cells per draining PO-LN are shown for the indicated time points after MMTV(SW) injection. The data are representative of four experiments with similar results.

**Analysis of the phenotype of B220low and B220high B cells**

As shown in Fig. 2, the analysis of cell surface marker expression by the B220low and B220high B cells revealed important phenotypic differences between both B cell subpopulations. B220low B cells expressed high levels of the B cell differentiation markers CD43 and syndecan, whereas only a small proportion of B220high B cells expressed these markers. B220low B cells did not express surface IgDs, whereas B220high B cells were IgD^+. Correspondingly, B220low B cells were negative for the homing molecule CD62L (L-selectin), whereas B220high B cells were CD62L^+. In addition, B220low B cells underwent a strong CD44 up-regulation and displayed higher LFA-1 levels than the B220high cells. Finally, ~40% of B220high B cells were positive for the early activation marker CD69, but this marker was down-regulated in the B220low B cells. The phenotype of B220low B cells corresponds to that of plasmablasts, originated by extracellularular differentiation of MMTV-responding B cells, which have switched to downstream
The kinetics of the B cell response, induced by MMTV as shown in Fig. 1, and the phenotypic characteristics of responding B cells described above indicate that B220\textsuperscript{high} B cells that become infected differentiate into B220\textsuperscript{low} plasmablasts during the MMTV(SW)-encoded SAg-mediated B cell response. This early B cell differentiation was accompanied by B220 and CD62L down-regulation and by the up-regulation of CD43 and syndecan-1. Therefore, B220\textsuperscript{plasmablasts} are Ig\textsuperscript{D−} CD62L\textsuperscript{−} CD43\textsuperscript{−} syndecan-1\textsuperscript{−}, whereas most B220\textsuperscript{B cells} are CD62L\textsuperscript{+} Ig\textsuperscript{D+} CD43\textsuperscript{+} syndecan-1\textsuperscript{+}. Interestingly, the B220 vs MHC II profile of PO-LN CD62L\textsuperscript{−} cells and CD43\textsuperscript{+} cells at day 6 after MMTV(SW) injection (Fig. 2B) revealed that \~8–10% of CD62L\textsuperscript{−} CD43\textsuperscript{+} B cells had not down-regulated B220 by day 6, and, therefore, were found within the B220\textsuperscript{B cell subset.}

PCR analysis, using primers specific for endogenous as well as exogenous MMTV sequences of the B220\textsuperscript{low} and B220\textsuperscript{high} B cell subpopulations isolated on days 5–7 by FACS, showed that viral DNA could be detected on the B220\textsuperscript{low} but not on the B220\textsuperscript{high} B cells; two to three copies of MMTV(SW) were found per B220\textsuperscript{low} B cell. In fact, as illustrated in Fig. 3, in which the presence of viral DNA is shown for different cell subsets, when total PO-LN cells from control mice or total PO-LN B220\textsuperscript{high} plasmablasts isolated at day 6 after MMTV infection were analyzed, no viral DNA was detected, whereas a strong signal was obtained for day 6 PO-LN B220\textsuperscript{low} plasmablasts. Interestingly, MMTV DNA could be detected in sorted blast B220\textsuperscript{high} B cells but not in small B220\textsuperscript{high} B cells. This is most likely due to the fact that a small proportion of blast CD62L\textsuperscript{−} CD43\textsuperscript{−} \textsuperscript{B cells containing MMTV-infected cells, which had not yet down-regulated B220, was still present at day 6 within the B220\textsuperscript{high} B cell subset (see Fig. 2B). Analysis of the kinetics of the B220\textsuperscript{low} CD62L\textsuperscript{−} CD43\textsuperscript{−} B cell subset revealed that CD62L\textsuperscript{−} CD43\textsuperscript{−} blast cells were no longer present within B220\textsuperscript{high} B cells after day 7 (data not shown), indicating that CD62L\textsuperscript{−} CD43\textsuperscript{−} B cell blasts had down-regulated B220 or had migrated from the lymph node at this time point. Therefore, B220\textsuperscript{low} plasmablasts represent the vast majority of the infected B cell population amplified as a result of the SAg-specific Th cell-mediated B cell proliferation response.

**Systemic immune response to MMTV(SW)**

Analysis of the percentage of cycling cells, Vβ6\textsuperscript{+} cells, and CD69\textsuperscript{+} cells within the B cell and T cell compartments indicated that these parameters did not undergo significant variations in the inguinal and mesenteric lymph nodes, Peyer’s patches, and spleen after MMTV(SW) injection (data not shown), and, therefore, that an immune response against the virus was not mounted in these organs. However, there was a small but significant increase in the percentage of B220\textsuperscript{plasmablasts} after MMTV(SW) injection in the mesenteric lymph node (MS-LN) (Fig. 4) and spleen, but not in the inguinal lymph nodes or in the Peyer’s patches (data not shown).
and of apoptotic cells within B220 low B cells (apoptotic cells, which remained at the control levels found in the B220low plasmablasts in the MS-LN and spleen might be the result cell population, and, therefore, that the increase in the number of suggest that no proliferation occurs in these locations within this B

Comparative analysis of B220 low plasmablasts in the drain-

FIGURE 4. Comparative analysis of B220low plasmablasts in the draining PO-LN and in the nondraining MS-LN. Percentages of B220low B cells among B cells (A and D), of blast cells within B220low B cells (B and E), and of apoptotic cells within B220low B cells (C and F) at the indicated times after MMTV(SW) injection are shown.

shown). Analysis of the percentage of apoptotic cells within the B220low population in the PO-LN during the B cell response to MMTV(SW) (Fig. 4, B and C) revealed an inverted correlation between cycling and apoptotic cells (i.e., the peak of cycling cells coincided with the lowest percentage of apoptotic cells). Interestingly, in the MS-LN and spleen, the B220low plasmablast population did not show variations in the percentage of blast and apoptotic cells, which remained at the control levels found in the PO-LN before and after the B220low B cell response. These data suggest that no proliferation occurs in these locations within this B cell population, and, therefore, that the increase in the number of B220low plasmablasts in the MS-LN and spleen might be the result of the migration of these cells from the responding PO-LN.

Microscopic study of the PO-LN after MMTV(SW) infection

Fig. 5 shows the histological study of PO-LN from control mice and from mice at days 6 and 11 after injection of MMTV(SW). Although the deep cortical areas and the medullary cords were occupied almost exclusively by small resting lymphocytes in control mice (Fig. 5A), the vast majority of the cells infiltrating these areas were lymphoblasts, plasmablasts, and plasma cells at 6 days after MMTV(SW) injection (Fig. 5B). Moreover, an important number of these cells were observed in the vascular space of the medullary sinuses at this time point (Fig. 5D). Fig. 5C shows that in these same areas at 11 days after injection, macrophages, dead cells, and a few blast cells were observed, indicating that the decline of the B and T cell response was accompanied by a certain degree of cell death and by dead cell removal by phagocytes.

Analysis by electron microscopy of sorted B220low B cells showed that this population was heterogeneous and contained responding B cells in different phases of differentiation (i.e., B lymphoblasts, plasmablasts, and plasma cells (data not shown)) comparable with the cells observed in situ in the deep cortical areas and the medullary cords of the PO-LN 6 days after virus injection (Fig. 5E). Interestingly, some of the B220low cells contained numerous type-A viral particles of 65–75 nm in size in their cytoplasm (Fig. 5F), indicating that viral replication most likely occurs in these cells after MMTV(SW) infection.

Transmission of MMTV to the mammary gland

The data presented in this report, dealing with the detection of MMTV DNA and MMTV type-A particles in B220low B cells, suggest that this B cell population might be involved in virus transmission to the mammary gland, and, therefore, to the offspring. To test this hypothesis, we have analyzed the transmission of MMTV to the offspring of females injected before mating with CD62L−CD43+ plasmablasts, CD62L+CD43−B cells, or total T cells isolated from infected mice. MMTV(SW) SAg presentation in the injected females and infection by MMTV of their offspring were detected by analyzing the deletion of MMTV(SW) SAg-reactive Vβ6+CD4+T cells (Fig. 6). For this assay, the purification of the B220low and B220high B cell subsets has been performed by isolating CD62L−CD43+ and CD62L+CD43−B cells, respectively, because, as shown in Fig. 2B, a small proportion of CD62L−CD43−B cells, which could potentially contain MMTV-infected cells, exists at day 6 within the B220low B cells (see Materials and Methods for details). Females injected with CD62L−CD43+ plasmablasts but not with CD62L+CD43−B cells, T cells, or control total PO-LN cells (data not shown) underwent a strong deletion of Vβ6+CD4+ T cells (Fig. 6A), suggesting that MMTV(SW) SAg-presenting plasmablasts persisted for a long period of time or infected other cells upon injection in an appropriate host. In addition, a strong deletion of Vβ6+CD4+ T cells was observed in the offspring of the females injected with CD62L−CD43+ plasmablasts (Fig. 6B), indicating that the litters were infected with MMTV. No Vβ6+CD4+ T cell deletion was observed in the offspring of females injected with CD62L+CD43−B cells, T cells, or control total PO-LN cells (data not shown) but with CD62L−CD43−B cells, or total T cells by guest on June 8, 2022 http://www.jimmunol.org/ Downloaded from

Discussion

Successful infection by MMTV requires the establishment of a T cell-dependent B cell response from a susceptible host, determined by the expression of a SAg encoded by the virus (3, 13). The MMTV-SAg expressed by the infected B cells specifically stimulates CD4+ T cells carrying a particular TCR Vβ-chain and therefore triggers strong T cell help, which drives a B cell response leading to the amplification of the infected B cell pool (4). This SAg-induced T cell-dependent B cell response involves extrafollicular as well as follicular B cell differentiation similar to conventional Ag responses (5, 14, 25). Although this B cell response has been shown to be necessary for an efficient infection, little is known about the mechanism of virus transmission to the mammary gland during the later phases of infection; therefore, the identity of the cells supporting the persistence of the virus is still controversial (reviewed in Ref. 14). In this sense, Tsubura et al. (26) reported 10
years ago that transfer of T cells but not B cells from B-MMTV-infected donors resulted in mammary tumor formation in athymic nude mice. Later it was shown that transfer of both B and T cells from 6-mo-old-infected mice can induce virus transmission to the next generation (16). Recently, it has been found that both B and T cells can release infectious MMTV particles in vitro (27). Finally, Upragarin et al. (28) have proposed that the transfer of MMTV (II-TES14) from originally infected B cells to non-SAg-specific Vβ8 T cells may contribute to stable MMTV infection. In this context, to get new insights on the virus transmission, we have extensively analyzed the B cell response and the phenotype of infected cells after injection of MMTV(SW).

The immune response induced by MMTV(SW) was characterized by the activation and proliferation of B cells and MMTV-SAg reactive Vβ6+ CD4+ Th cells, as reported previously (4). As indicated by the analysis of the total cell number in the draining PO-LN as well as the percentage of cycling cells, activated cells, and Vβ6+ CD4+ T cells, the response peaked at days 6 and 7 after MMTV injection and then declined to control levels after 2 wk (data not shown). T cell help mediated by the SAg-specific Vβ6+ CD4+ cells drives B cell activation, proliferation, and differentiation into Ab-forming cells, which lead to the expansion of the initial number of MMTV-infected B cells (3). The pattern of the B cell response induced by MMTV correlated with the kinetics of Ab-forming cells described in the extrafollicular foci of secondary lymphoid organs (8, 9, 29).

Our results show that the immune response to MMTV involves the differentiation of plasmablasts with a high proliferation rate, characterized phenotypically by high MHC class II levels but low expression of the B cell marker B220. Down-regulation of B220 has been described previously on 4-hydroxy-3-nitrophenyl nuclear protein (NP)-specific APCs (8, 9) and after in vitro activation of B cells (30). These B220+ plasmablasts were first detected by day 4 postinjection, peaked by days 6 and 7 when they represented ≤40% of PO-LN B cells, and became barely detectable by day 9. Interestingly, PCR analysis using MMTV-specific primers has shown that viral DNA can be detected in B220+ B cells but not...
in B220<sup>high</sup>-resting B cells, indicating that the B220<sup>low</sup> plasmablasts represent the bulk of MMTV-infected cells during their proliferative phase.

Cell surface marker analysis of B220<sup>low</sup> plasmablasts has evidenced that these cells display high levels of the adhesion molecules LFA-1 and CD44 and low levels of CD62L (i.e., an activated phenotype) (31, 32). By day 6 after MMTV injection, B220<sup>low</sup> B cells expressed the B cell differentiation markers CD43 and syndecan and were IgD<sup>−</sup>. These data indicate that by day 6, B220<sup>low</sup> B cells represent extrafollicular plasmablasts originated by SAg-mediated differentiation of MMTV-infected B220<sup>high</sup> B cells, which have switched to downstream Ig classes. Interestingly, their phenotypic profile allowed the detection and isolation of MMTV-infected cells and corresponded to that described previously for NP-specific Ab-forming cells of the splenic foci (8, 9) involved in the production of low-affinity Abs during the primary response to NP.

Electron microscopy studies of B220<sup>low</sup> FACS-sorted cells have shown that the B220<sup>low</sup> population is morphologically heterogeneous and includes blast and dividing cells, plasmablasts, and plasma cells. Analysis of the draining PO-LN at day 6 after MMTV injection revealed that most B220<sup>low</sup> plasmablasts were localized in the deep cortical areas and in the medullary cords and sinuses. Due to the lymph flow pattern in the mouse PO-LN (33), B220<sup>low</sup> plasmablasts located within the medullary sinus vascular space will most likely exit the lymph node via the efferent lymphatic and, therefore, could migrate to other peripheral lymphoid organs. Interestingly, groups of viral particles corresponding to the MMTV type-A particles described in mammary tumor epithelial cells (34) were found in the cytoplasm of FACS-sorted B220<sup>low</sup> plasmablasts as well as in cells with similar morphological characteristics observed in sections of the draining PO-LN at day 6. This result suggests that viral replication can take place in these cells during the early phase of viral infection and contrasts with previous reports supporting the assertion that viral replication occurs only in later stages, when the mammary gland epithelial cells are infected. Importantly, although intracytoplasmic A particles are considered to be precursors of the core of the mature MMTV particles, it has been shown that, despite being devoid of the characteristic spikes of MMTV, they are capable of inducing mammary tumors in an appropriate host (35).

The detection of MMTV DNA and MMTV viral particles in B220<sup>low</sup> plasmablasts reported here suggests that this cell population may be responsible for the transmission of MMTV. In this sense, the increase in B220<sup>low</sup> plasmablasts observed between days 6 and 8 after MMTV injection in the spleen and MS-LN, in which no detectable immune response to MMTV occurred, suggests that the B220<sup>low</sup> plasmablasts found in these lymphoid organs originate in the draining PO-LN, and, therefore, constitute a cell population with migratory capacity. This hypothesis is in agreement with our morphological studies, demonstrating the presence of cells morphologically similar to B220<sup>low</sup> plasmablasts in the medullary sinuses of the draining PO-LN. Interestingly, CD43 expression by B220<sup>low</sup> plasmablasts could be related to their recirculation potential, because this molecule has been demonstrated to participate in lymphocyte homing (36).

The kinetics of V<sub>B</sub>6<sup>+</sup> CD4<sup>+</sup> T cell deletion occurring in females injected with CD62L<sup>−</sup> CD43<sup>−</sup> B cells or T cells isolated from BALB/c mice 6 days after MMTV injection were transferred i.v. into BALB/c females. Deletion of V<sub>B</sub>6<sup>+</sup> CD4<sup>+</sup> T cells was monitored at the indicated times. Data represent the mean ± SD of the values obtained from three females for each experimental condition. B. At 3 months after the transfer of the indicated cell population, the females were crossed with noninfected BALB/c males. Deletion of V<sub>B</sub>6<sup>+</sup> CD4<sup>+</sup> T cells was analyzed in the offspring. Data represent the mean ± SD of the values obtained from the litters of each group of three females used in each experiment.

**FIGURE 6.** Transmission of MMTV by B220<sup>low</sup> plasmablasts. A, CD62L<sup>−</sup> CD43<sup>−</sup> plasmablasts, CD62L<sup>−</sup> CD43<sup>−</sup> B cells, or T cells isolated from BALB/c mice 6 days after MMTV injection were transferred i.v. into BALB/c females. Deletion of V<sub>B</sub>6<sup>+</sup> CD4<sup>+</sup> T cells was monitored at the indicated times. Data represent the mean ± SD of the values obtained from three females for each experimental condition. B. At 3 months after the transfer of the indicated cell population, the females were crossed with noninfected BALB/c males. Deletion of V<sub>B</sub>6<sup>+</sup> CD4<sup>+</sup> T cells was analyzed in the offspring. Data represent the mean ± SD of the values obtained from the litters of each group of three females used in each experiment.
reports examining the issue of the longevity of plasma cells have demonstrated that a substantial fraction of these cells are long-lived, and can survive for periods of >1 yr (10, 11).

Finally, the transfer of CD62L+ CD43+ plasmablasts, but not of CD62L− CD43− B cells or T cells, into noninfected females caused a strong deletion of VB6+ CD4+ T cells in the offspring, demonstrating that MMTV replication occurred in the females after injection of CD62L+ CD43+ plasmablasts and, more importantly, that MMTV was transmitted to the offspring.

In conclusion, our results strongly support the hypothesis that the B220+ plasmablasts generated during the extrafollicular B cell response to MMTV(SW) may play an important role in the transmission of the virus to the mammary gland and the offspring during infection by MMTV.

Acknowledgments

We thank A. Rolink for the anti-CD40 hybridoma FGK45 (Basel Institute for Immunology, Basel, Switzerland) and P. Zaech and C. Knabenhans for their assistance with cell sorting.

References