CD19 Signaling Pathways Play a Major Role for Murine AIDS Induction and Progression

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CD19 Signaling Pathways Play a Major Role for Murine AIDS Induction and Progression

Sonja M. Knoetig, Ted A. Torrey, Zohreh Naghashfar, Tom McCarty, and Herbert C. Morse III

Infection of genetically susceptible mice with the LP-BM5 mixture of murine leukemia viruses including an etiologic defective virus (BM5def) causes an immunodeficiency syndrome called murine AIDS (MAIDS). The disease is characterized by interactions between B cells and CD4+ T cells resulting in polyclonal activation of both cell types. It is known that BM5def is expressed at highest levels in B cells and that B cells serve as viral APC. The CD19-CD21 complex and CD22 on the surface of B cells play critical roles as regulators of B cell responses to a variety of stimuli, influencing cell activation, differentiation, and survival. CD19 integrates positive signals induced by B cell receptor ligation by interacting with the protooncogene Vav, which leads to subsequent tyrosine phosphorylation of this molecule. In contrast, CD22 negatively regulates Vav phosphorylation. To analyze the role of CD19, CD21, Vav, and CD22 in MAIDS, we infected mice deficient in CD19, CD21 (CR2), Vav-1, or CD22 with LP-BM5 murine leukemia viruses. Infected CR2−/− mice developed MAIDS with a time course and severity indistinguishable from that of wild-type mice. In contrast, CD19 as well as Vav-1 deficiency restricted viral replication and suppressed the development of typical signs of MAIDS including splenomegaly, lymphadenopathy, and hypergammaglobulinemia. Finally, CD22 deficiency was found to accelerate MAIDS development. These results provide novel insights into the B cell signaling pathways required for normal induction and progression of MAIDS. The Journal of Immunology, 2002, 169: 5607–5614.

Mice infected with the LP-BM5 mixture of murine leukemia viruses (MuLV)2 develop a syndrome of lymphopenia and immunodeficiency, termed murine AIDS (MAIDS) (1). MAIDS is characterized by a rapid and persistent proliferation of B and CD4+ T cells, hypergammaglobulinemia, phenotypic abnormalities of lymphocyte subsets, and increasingly severe defects of cellular and humoral immunity (2). Later in the course of disease, malignant transformation of both T and B cells may occur (1, 3, 4).

Induction of MAIDS requires expression of the MA and p12 portions (5) of the gag gene of replication-defective viruses designated BM5def (6, 7) or Du5H (8). MAIDS can be induced by helper-defective viral infection (9), but in disease induced by the LP-BM5 virus mixture, nonpathogenic, replication-competent ecotropic, and mink cell focus-forming (MCF) viruses serve as helpers for cell-to-cell transmission of the defective genome and are known to accelerate development of disease (5, 7).

Following infection with helper-free virus, BM5def is expressed most prominently in mature B cells (10). However, in mixed virus inoculation, infection of B cells is required for efficient infection of T cells and macrophages, because animals lacking functional mature B cells are resistant to MAIDS (11, 12). This conclusion was based on studies in mice depleted of B cells from birth by administration of a rabbit Ab to IgM or mice with a B cell deficiency due to a targeted mutation of the membrane exon of the IgM H chain gene. Consequently, these studies did not elucidate specific B cell signaling pathways involved in MAIDS pathogenesis.

Signaling through the B cell receptor (BCR) directly regulates B cell development, activation, and differentiation. Nevertheless, it is important that B cells have appropriately established thresholds for initiation of transmembrane signals to allow the generation of physiologically relevant responses to foreign or self Ag. Potential response regulators on the surface of B cells include CD19, which forms a complex with CD21, CD81, and Leu13 (13). Ligation of the CD19 complex initiates a cascade of biologic responses that can modulate signal transduction through the B cell-Ag receptor complex and other cell surface receptors. Recent studies with mice that lack CD19 or CD21 as the result of gene knockouts (KO) suggest that the CD19-CD21 complex serves as a general response regulator that governs humoral immune responses. Decreased humoral immune responses in these mice correlate with a general lack of secondary follicles and germinal center (GC) formation within lymphoid tissues (14–16). Phosphorylated CD19 interacts with Vav, leading to subsequent phosphorylation of Vav by activated tyrosine kinases (17). Vav is a member of the large family of guanine nucleotide exchange factors. These proteins can stimulate signaling reactions via the c-Jun NH2-terminal kinase and p38 mitogen-activated protein kinases (18). Another function of Rho family GTPases is participation in phosphoinositols (4, 5) bisphosphate hydrolysis by stimulating phosphatidylinositol 4-phosphate 5-kinase that synthesizes phosphoinositols, which generate more substrate for phospholipase C2. In agreement with these observations, Vav has been shown to be important for BCR-induced proliferation, T helper-dependent IgG class switching, and Ab responses to T cell-dependent Ag (20).

Another critical signal transduction element modulating BCR responses is CD22 (21). Following BCR cross-linking, the Src

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2 Abbreviations used in this paper: MuLV, murine leukemia virus; BCR, B cell receptor; CR2, CD21/CD81; GC, germinal center; GFP, green fluorescent protein; HPRT, hypoxanthine phosphoribosyltransferase; KO, knockout; MCF, mink cell focus-forming; MAIDS, murine AIDS; p.i., postinfection.
homology protein 1 tyrosine phosphatase associates with tyrosine-phosphorylated CD22, which induces Src homology protein 1 phosphatase activity (22–24). This results in suppression of mitogen-activated protein kinase activation and counterregulation of CD19 effects (25). B cells from CD22-deficient mice have the phenotype of chronically activated cells and are hyperresponsive to BCR ligation, exhibiting increased Ca\(^{2+}\) flux and proliferation (26). This indicates that the normal role of CD22 is to negatively regulate receptor signaling, although some indications of positive regulation have also been reported (26).

In the present study, we examined the role of BCR response regulators in MAIDS by infection of CD21−, CD19−, Vav−/−, and CD22−/− mice. The results show that CD21 is not required for MAIDS induction and that LP-BM5 infection can induce GC formation and isotype switching to IgG and even IgE in CD21-deficient animals. Second, we provide evidence that the CD19-Vav signaling pathway is required for LP-BM5 replication and subsequent development of lymphadenopathy and immunodeficiency. Finally, we show that CD22 deficiency accelerates disease progression, suggesting a negative regulatory role for this molecule in MAIDS.

Materials and Methods

**Mice**

C57BL/6 (B6) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). B6.CD19−/− (CD19−−) mice were originally generated by R. Rickert (University of Cologne, Cologne, Germany) (14). B6.Vav−/− (Vav−−) mice (27) were a gift from J. Rivera (National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD). B6.Cr2− (hereafter referred to as CR2−−) mice (16) were obtained from M. Carroll (Harvard Medical School, Boston, MA). The murine Cr2 locus encodes both mCR1 and mCR2 (complement receptor 1 and 2); disruption of this locus renders mice deficient in both mCR1 and mCR2 (CD35 and CD21). B6.CD22−/− mice (26) were generated by T. Tedder (Duke University Medical Center, Durham, NC). Mice were inoculated i.p. with 0.1 ml of LP-BM5 mixed virus pools at 6–10 wk of age.

**Virus and virus assays**

BM5eco and LP-BM5 virus stocks were prepared from the G6 clone of chronically infected SC-1 cells as described previously (6). LP-BM5 virus stocks contain a mixture of nonpathogenic ecotropic and MCF MuLV and stocks contain a mixture of nonpathogenic ecotropic and MCF MuLV and LP-BM5 virus stocks were prepared from the G6 clone of BM5def. At selected time points after infection, 105 infected cells were incubated with 8 Ci [3H]thymidine (PerkinElmer, NEN, Boston, MA) and harvested 6 h later for assessment of thymidine incorporation by scintillation counting (Betaplate system; PerkinElmer Wallac, Boston, MA).

**Measurement of serum Ig levels**

Serum IgE levels were measured by ELISA using Ab and standards purchased from BD PharMingen according to their recommendations. Briefly, 96-well microtiter plates (Corning, Corning, NY) were coated with 2 μg/ml of the 02111D anti-IgE Ab overnight. After blocking the plates with BSA and incubation with the samples, biotinylated anti-mouse IgE Ab (02122D) was added, followed by incubation with streptavidin-HRP. Plates were developed with ABTS peroxidase substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD).

For ELISA determinations of serum IgG1, IgG3, and IgM, affinity-purified goat anti-mouse IgG1, IgG3, or IgM Ab (Southern Biotechnology Associates, Birmingham, AL) were diluted to 5 μg/ml to coat 96-well microtiter plates overnight at 4°C. The plates were then blocked with BSA, and sera were plated and allowed to incubate for 2 h at 37°C. The plates were washed, and HRP-labeled goat anti-mouse IgG1, IgG3, or IgM (Southern Biotechnology Associates) was then added. After 2 h at 37°C, plates were developed with ABTS peroxidase substrate.

**RNA purification, reverse transcription, Southern blotting, and real-time PCR**

Mouse spleen samples were stored at −70°C in TRIzol Reagent (Invitrogen, Carlsbad, CA) until further processing. RNA was extracted according to the manufacturer’s directions. RNA (1 μg) was reverse transcribed using MuLV reverse transcriptase (Invitrogen) according to the recommendations of the manufacturer. The cDNA solution was diluted to 10 μl and 10 μl were used for specific amplification by PCR. The primers and probe used for BM5def were 5′-CCTTTCCTTTATGCAGACT-3′ (sense), 5′-ACCAGGGGGGAATACCTCG-3′ (antisense), and 5′-CCTGCCC AAGGGCACAGGT-3′ (probe). The primers and probe for hypoxanthine phosphoribosyltransferase (HPRT) were 5′-GTGGTATACCGGCA AGATCTGTG-3′ (sense), 5′-GATCCAACTCTGCGCATCTTACGGC-3′ (antisense), and 5′-GTGGTGGATGCTGCCCTGAC-3′ (probe). Amplifications were done for 22 cycles (BM5def) and 24 cycles (HPRT). The PCR products were separated on 1.5% agarose gels, blotted onto a nylon membrane (Hybond-N+; Amersham Pharmacia Biotech, Buckinghamshire, U.K.), and hybridized with the fluorescein-labeled probe using the Gene Images 3′-oligo labeling and ECF signal amplification system (Amersham Pharmacia Biotech).

For real-time PCR, 1 μl of diluted cDNA was amplified using Sybr Green PCR Master Mix (PE Applied Biosystems, Foster City, CA) and 0.33 μM each of sense and antisense primers. The primers used for BM5def were 5′-CCTCTGTAAGTGCGGTTCTC-3′ (sense) and 5′-CG GCCGCCCTTCCTCTAATC-3′ (antisense); the primers for HPRT were 5′-GGGAGCGCATCATGATGTG-3′ (sense) and 5′-TCCAGAGTCCGCAAAGAAC-3′ (antisense). All PCR were performed on an ABI PRISM 7700 sequence detector system (PE Applied Biosystems). Serial logarithmic dilutions of a pool of cDNA obtained from three B6 mice 12 wk postinfection (p.i.) with LP-BM5 were prepared in triplicate to generate standard curves. The fluorescence signals of each well were collected every 7 s, and threshold cycles were set by determining the point at which the fluorescence intensity increased in a linear manner. The ratio between the BM5def and HPRT content in the samples was defined as the normalization factor. Relative BM5def mRNA quantities were determined by dividing the interpolation-derived values from the standard curve by the normalization factor.

**Virus-binding assays**

Virus binding was detected as previously described (33, 34) with modifications. To reduce virus interference with non-B cells, spleen cells were treated with ACK lysis buffer (Biofluids, Rockville, MD) to lyse RBC and subsequently depleted of CD43+ and CD44+ cells using anti-CD43 and anti-CD44 paramagnetic beads (Miltenyi Biotech, Auburn, CA) and column purification according to the manufacturer’s protocol (VarioMACS; Miltenyi Biotech). Cells (1 × 106) were pelleted and resuspended in 100 μl of BM5eco virus supernatant plus 8 μg/ml polybrene. Cells were incubated at 37°C for 40 min with gentle agitation to maintain cell suspension. Following incubation with virus, cells were washed twice with 2 ml of ice-cold PBS-5% FBS. Cells were then incubated for 30 min on ice with PE-conjugated anti-B220 (BD PharMingen) and biotinylated mAb 83A25, which recognizes ectropic viral envelope gp70. Cells were then washed and incubated with FITC-conjugated avidin (Zymed Laboratories, San Francisco, CA) for 30 min at 4°C. After another wash, cells were analyzed by flow cytometry. The human lymphoblastoid cell line WIL-2.NS (American Type Culture Collection, Rockville, MD), which lacks ectropic virus receptor, was assayed for nonspecific viral binding using the same assay described as performed without B220 staining.
**In vitro infection and integration**

Spleen cells (10^6/ml) were cocultured with ecotropic MuLV-green fluorescent protein (GFP)-producing NIH 3T3 cells (35) in the presence of 2 μg/ml polybrene in RPMI 1640 supplemented with 10% FCS and 50 mM 2-ME. For B cell stimulation, LPS (Sigma-Aldrich, St. Louis, MO) at a concentration of 20 μg/ml, anti-IgM (Jackson Immunoresearch Laboratories, West Grove, PA) at a concentration of 15 μg/ml, or PMA (20 ng/ml; Sigma-Aldrich) and ionomycin (250 ng/ml; Sigma-Aldrich) were added. Cells were harvested after 48 or 72 h, after plating, and infection was analyzed by flow cytometry using determination of green fluorescence. To exclude contaminating producer cells from the analysis, cells were stained with B220-PE Ab as a B cell marker.

**In vivo BrdU labeling**

BrdU (Sigma-Aldrich) was added to the drinking water of wild-type and CD19- and Vav-1-deficient mice at a concentration of 1 mg/ml 6 days before harvesting spleens. The incorporation of BrdU was determined by flow cytometry using BrdU Flow kit (BD Pharmingen). Splenocytes were labeled with PE-conjugated anti-B220 to detect B cells, washed, fixed, and stained for incorporated BrdU using FITC-conjugated anti-BrdU Ab according to the manufacturer’s instruction.

**Results**

**MAIDS-associated lymphoproliferative disease and histologic changes in wild-type and CR2-, CD22-, CD19-, and Vav-1-deficient mice**

At 4, 8, and 12 wk p.i., mice were screened for MAIDS-associated splenomegaly, lymphadenopathy, and histopathologic changes by criteria previously described. Spleens and lymph nodes of infected B6 and CR2-deficient mice were found severalfold enlarged compared with those of uninfected mice (Table I). In contrast, the CD19-deficient mice displayed only slight splenomegaly or lymphadenopathy after infection with LP-BM5 (Table I). The histopathological picture in splenic sections ranged from no lesions to appearance of follicular hyperplasia and development of a few small active GC. To determine whether MAIDS induction is impeded because of the reduced number of B cells in CD19-deficient mice (14) or as a result of the disrupted signaling pathway initiated by CD19 ligation, we infected Vav-1-deficient mice with the disease-inducing virus mixture. Signaling through CD19 induces formation of a CD19-Vav-phosphatidylinositol 3 kinase complex that activates the Vav-mitogen-activated protein kinase pathway. Vav-1-deficient mice have normal numbers of conventional B cells (36). When infected with the MAIDS-inducing virus mixture, Vav-1-deficient mice showed somewhat increased spleen and lymph node sizes with spleen weights doubling at 12 wk p.i. This was accompanied by mild immunopathologic changes characterized by the appearance of proliferating immunoblasts and plasma cells in splenic follicles (Table I).

CD22 is another critical cell surface molecule capable of modulating signals through the BCR as well as BCR-independent signals (26). Notably, infected CD22-deficient mice displayed a rapid onset of splenomegaly and lymphadenopathy; at 4 wk p.i., spleen and lymph node weights were ~2-fold higher than those of infected B6 mice (Table I). However, this hyperreactivity was not observed at later times, indicating an acceleration in disease progression rather than an increased susceptibility.

**Phenotypic changes in spleen cells from wild-type and CR2-, CD19-, Vav-1-, and CD22-KO mice**

Table I. Development of MAIDS in wild-type, CR2-, CD19-, Vav-1, and CD22-KO mice*

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Weeks After Infection</th>
<th>Tissue Weight (mg)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Spleen</td>
<td>Lymph Nodes</td>
</tr>
<tr>
<td>B6</td>
<td>0</td>
<td>80</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>155 (80)</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>453 (80)</td>
<td>207</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1150 (103)</td>
<td>630</td>
</tr>
<tr>
<td>CR2-/-</td>
<td>0</td>
<td>83</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>223 (80)</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>593 (83)</td>
<td>243</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>933 (90)</td>
<td>540</td>
</tr>
<tr>
<td>CD19-/-</td>
<td>0</td>
<td>85</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>87 (80)</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>120 (90)</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>150 (107)</td>
<td>47</td>
</tr>
<tr>
<td>Vav-/-</td>
<td>0</td>
<td>70</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>88 (67)</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>110 (70)</td>
<td>18</td>
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<tr>
<td></td>
<td>12</td>
<td>145 (70)</td>
<td>20</td>
</tr>
<tr>
<td>CD22-/-</td>
<td>0</td>
<td>80</td>
<td>&lt;10</td>
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<tr>
<td></td>
<td>4</td>
<td>309 (83)</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>406 (85)</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1010 (90)</td>
<td>763</td>
</tr>
</tbody>
</table>

*Data are for three mice per data point and are representative of two experiments involving three mice compared at each time point.

**Immunodeficiency in CR2- and CD22-deficient mice but not in CD19- and Vav-1-KO animals**

Immunodeficiency of increasing severity is a second prominent feature of MAIDS (38, 39). At 4, 8, and 12 wk p.i., spleen cells were prepared from wild-type and KO mice and stimulated for 48 h with LPS, Con A, and PMA/monomycin. As soon as 4 wk p.i., cells from infected B6, CR2-/-, and CD22-/- mice showed decreased proliferative response to mitogenic stimulation compared with cells from uninfected control mice (Fig. 2A). It was notable that cells from infected CD22-deficient mice were significantly increased, and the size of the CD11b+ population was expanded. Furthermore, an expanded subpopulation of CD4+Thy-1- cells characteristic of MAIDS could be detected (37). At 12 wk p.i., spleen cells from infected B6, CR2-/-, and CD22-/- mice showed identical profiles for all markers tested (Fig. 1B and data not shown). In contrast to the marked changes in B6, CR2-/-, and CD22-/- mice induced by LP-BM5 infection, the phenotype of spleen cells from infected CD19-/- and Vav-1-/- mice was very similar to that of uninfected mice at 4 and 12 wk p.i., with no evidence of B cell activation or differentiation (Fig. 1B and data not shown). To evaluate further B cell activation markers, we screened B6 and CD19-/- B cells for CD80 and CD86 as well as for MHC class II expression at 8 wk p.i. As expected, B cells from B6 mice carried increased levels of these molecules, whereas B cells from CD19-/- mice showed no significant changes in expression (data not shown).
less responsive to all mitogens tested at this early time point ($p < 0.001$; Fig. 2A). In contrast, mitogenic responses of infected Vav-1- and CD19-deficient mice were not affected. At 12 wk p.i., spleen cells from infected B6 and CR2- and CD22-deficient mice were almost totally nonresponsive to both B and T cell mitogens (Fig. 2B). In contrast, cells from infected CD19- and Vav-1-deficient mice showed no or moderate proliferative defects ($p < 0.05$; Fig. 2B). It should be noted that the intrinsic ability of CD19- spleen cells to proliferate in response to LPS was reduced in all experiments (two experiments per time point); the same was true for the ability of Vav-1-deficient spleen cells to respond to Con A.

LP-BM5 infection induces polyclonal Ig production, including switch to IgE in CR2- and CD22-deficient mice

Elevated levels of polyclonal Ab in serum are a well-known feature of MAIDS; however, the absence of CR2, CD19, or Vav results in severely impaired humoral responses to T cell-dependent Ag (14, 16, 20). Therefore, we measured serum Ab levels by ELISA in groups of control and BM5-infected B6 and KO mice at 8 wk p.i. (Fig. 3). Increased levels of IgE, IgM, IgG1, and IgG3 were detected in LP-BM5-infected B6 and CR2- and CD22-deficient mice. When comparing Ig levels in infected CD19$^{-/-}$ and Vav-1$^{-/-}$ mice vs uninfected controls, only Vav$^{-/-}$ mice showed slightly increased IgE and IgM levels. Thus, the polyclonal B cell response that leads to accumulation of serum Ig does not occur in CD19-deficient mice and only marginally in Vav-1-deficient mice. Furthermore, these results suggest that MAIDS infection can induce class switching in the absence of CR2.

Suppression of viral replication in CD19- and Vav-1-deficient mice

To examine whether the mild disease course in CD19- and Vav-1-deficient mice is due to impaired virus replication in these mice, we used RT-PCR followed by Southern blotting of amplified transcripts to detect levels of BM5def mRNA in spleen samples at 12 wk p.i. Of interest, BM5def transcripts in spleen cells from CD19-deficient mice were below the limits of detection, and Vav-1$^{-/-}$ mice showed clearly reduced levels compared with infected B6 mice (Fig. 4A). Real-time PCR confirmed these results; no BM5def transcripts were detected in spleen cells from CD19$^{-/-}$ mice, and BM5def levels in Vav-1-deficient animals ranged from 30 to 64% of those found in infected wild-type mice 12 wk p.i. (data not shown).

The frequencies of spleen cells producing ecotropic and MCF viruses also differed among the strains (Table II). Cells from B6 mice expressed both classes of MuLV, whereas cells from CD19$^{-/-}$ mice did not harbor any ecotropic MuLV, and Vav-1-deficient mice expressed ecotropic MuLV at much lower levels (Table II). Infectious MCF viruses were also not detected in the

**FIGURE 1.** Phenotype of spleen cells from mice infected with LP-BM5 viruses for 4 (A) or 12 (B) wk. Histograms and dot plots show expression of the indicated molecules and are gated on propidium iodide-negative cells. FSC, Forward angle scatter. MFI, Mean fluorescence intensity.
In summary, replication of all LP-BM5 virus types was clearly noticed in blind-passaged cultures. Levels of MCF-producing cells were 10-fold lower in Vav-1-KO mice compared with B6 mice. However, after passage of infected Mus dunni cells, low to moderate amounts of MCF could be noticed in blind-passaged cultures. Levels of MCF-producing cells were ~10-fold lower in Vav-1-KO mice compared with B6 mice. In summary, replication of all LP-BM5 virus types was clearly suppressed in CD19- and Vav-1-deficient mice.

Virus binding is not affected by CD19 deficiency

To further investigate the nature of viral inhibition in CD19-deficient B cells, we examined the capacity of virus to bind to these cells. B cell-enriched spleen cells obtained from wild-type as well as CD19 KO mice were incubated with ecotropic virus for 40 min and subsequently labeled with mAb 83A25, which recognizes ecotropic viral envelope gp70. A human lymphoblastoid cell line that lacks receptors for ecotropic virus was used as a control for specificity of the assay. As shown in Fig. 4B, virus binding was equivalent in both wild-type and CD19-/- B cells as opposed to minimal binding of ecotropic virus to the human cell line. Thus, absence of CD19 does not influence virus binding to B cells.

Viral integration is not impaired in CD19- and Vav-1-deficient B cells

Another critical step in viral replication is integration of newly synthesized viral DNA into the host genome. To test whether

Table II. Viral recovery in wild-type, CD19-, and Vav-KO mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Virus Inoculated</th>
<th>Ecotropic PFU&lt;sup&gt;c&lt;/sup&gt; (log&lt;sub&gt;10&lt;/sub&gt;/10&lt;sup&gt;7&lt;/sup&gt; cells)</th>
<th>MCF FFU&lt;sup&gt;d&lt;/sup&gt; (log&lt;sub&gt;10&lt;/sub&gt;/10&lt;sup&gt;7&lt;/sup&gt; cells)</th>
<th>Passage&lt;sup&gt;e&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>B6</td>
<td>–</td>
<td>&lt;1</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>3.5</td>
<td>3.1</td>
<td>+</td>
</tr>
<tr>
<td>CD19&lt;sup&gt;–/–&lt;/sup&gt;</td>
<td>–</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>+</td>
</tr>
<tr>
<td>Vav&lt;sup&gt;–/–&lt;/sup&gt;</td>
<td>–</td>
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</tr>
<tr>
<td></td>
<td>+</td>
<td>&lt;1</td>
<td>2.1</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup> Infectious center assays in tissue culture were performed by cocultivation of mitomycin C-treated spleen cells with SC-1 cells for assays of ecotropic MuLV by the XC plaque assay (32) and with Mus dunni cells for assays of mink-infectious viruses by a fluorescent Ab focus-forming test (31).

<sup>b</sup> Mice of the indicated genotype were infected (+) or not (–) with LP-BM5 virus mixture and killed 12 wk p.i.

<sup>c</sup> Titers of ecotropic virus were expressed as log<sub>10</sub> PFU/10<sup>7</sup> cells and titers of mink infectious virus as log<sub>10</sub> infectious forming unit (FFU)/10<sup>7</sup> cells. Numbers indicate the mean for assays of spleen cells from three mice per group.

<sup>d</sup> In MCF virus assays, cultures were subcultured at 9–12 days; –, negative, no foci detected on passage; +, positive, MCF foci detected on passage.
CD19 or Vav-1 deficiency interferes with this process, we cocultured spleen cells from wild-type and CD19- and Vav-1-deficient animals with an ecotropic MuLV-GFP-producing cell line (35). Viral integration was assessed by flow cytometry. Wild-type and CD19- and Vav-1-deficient spleen cells were infectable in culture and expressed GFP after stimulation with anti-IgM, LPS, and PMA/ionomycin (Fig. 5 and data not shown). Although reduced GFP expression could be noted only in Vav-1-deficient B cells after anti-IgM treatment (Fig. 5B), this was due to an impaired proliferative response to anti-IgM as determined by [3H]thymidine uptake (data not shown), which had been shown previously (20). These findings indicate that the reduced viral load in CD19- and Vav-1-deficient animals is not due to defective integration.

In vivo proliferation

Integration was reduced in Vav-1-deficient B cells in vitro, most likely due to impaired proliferation after BCR engagement. This is in accordance with previous studies that suggested that retroviral integration might depend on cell division because viral integration complexes enter the nucleus only after the nuclear envelope is transiently disassembled during mitosis (40). Therefore, impaired in vivo B cell turnover might be a limiting factor for LP-BM5 replication in CD19- and Vav-1-deficient mice. To analyze in vivo B cell proliferation in B6 and CD19- and Vav-1-KO animals, BrdU was added to the drinking water. We then surface labeled the splenic B cell pool with PE-anti-B220 and counterstained with FITC-anti-BrdU to detect cells that had divided during the 6-day in vivo labeling. On average, 12.9% of wild-type B cells, 15.6% of CD19−/− B cells, and 11.3% of B cells from Vav-1-deficient mice were positive for BrdU (Fig. 6). Thus, in vivo B cell proliferation is not impaired in CD19- or Vav-1-deficient animals.

Discussion

Although the presence of B cells and B cell-T cell interactions have been shown to be critical for development of MAIDS (11, 12, 41, 42), little is known about functional features of B cells that would favor or inhibit MAIDS progression. Possible candidates for affecting MAIDS development are the B cell surface molecules CD19 and associated CR2. The role of these receptors in B cell activation and differentiation has been demonstrated in vitro as well as in vivo (14, 16, 43, 44). For this reason, we looked at responses of CD19- and CR2-deficient mice to LP-BM5 infection. CR2−/− and wild-type mice were found to have equivalent levels of lymphadenopathy and splenomegaly. FACS and histopathologic studies revealed that mice of both genotypes had comparably advanced disease at all time points tested. Surprisingly, LP-BM5-infected CR2−/− mice generated GC reactions and Ig of all isotypes tested, with the magnitude of their responses being equivalent to that of B6 mice. This could be attributable to a strong and persistent antigenic stimulus provided by LP-BM5, because it has been shown that CR2−/− B cells respond to abundant Ag/adjuvant with GC that are quantitatively and qualitatively comparable to those of wild-type mice (45). It should be noted that the CR2−/− mice used in this study were found to express low levels of a hypomorphic CD21/35 molecule (46); however, it has not been established whether expression of this molecule is functionally relevant.

In CD19−/− mice, all of the defining features of MAIDS were nearly absent due to impaired replication of LP-BM5 MuLV. It is unlikely that reduced B cell numbers in these mice were the cause for restricted viral load, because Vav-1-deficient mice, which have normal B cell numbers, showed reduced viral load and mild disease course as well. Furthermore, impaired viral replication and limited MAIDS progression are probably not due to the lack of marginal zone B cells, which are virtually absent in CD19-deficient mice. In general, histological studies do not provide evidence for an involvement of marginal zone cells in MAIDS-related pathology, because progressively expanding GC B cells are the dominating cell type in splenic sections from LP-BM5-infected mice (47).

The mechanisms that cumulatively result in resistance of CD19- and Vav-1-deficient mice to infection with LP-BM5 and MAIDS development appear complex. In vitro infections revealed that virus binding was not affected by the absence of CD19, nor was retroviral integration affected in either CD19- or Vav-1-deficient B cells. These results do not point to an intrinsic B cell defect in these animals, particularly because in vivo B cell turnover was not impaired by the lack of CD19 or Vav-1. However, in vitro infection might represent an optimal system where B cells are adequately stimulated and in close proximity to virus-producing cells. In vivo, initial infection of a number of B cells might occur but be abortive due to unresponsive CD19−/− and Vav-1−/− target cells. The demonstration of dramatically decreased tyrosine phosphorylation of Src-family kinases and multiple downstream effector molecules
in CD19-deficient B cells following BCR ligation suggests that CD19 coordinates the initiation of multiple signaling pathways (48–51), the sum of which seems to be critical to retroviral replication and MAIDS pathogenesis. Furthermore, CD19 has recently been shown to potentiate the signaling initiated through MHC class II cross-linking as well (52). Class II-mediated signals synergize with other signals received via molecules such as CD40. Specifically, class II-dependent signaling enhances, in an Ag-specific manner, the responsiveness of B cells to subsequent T cell help that is provided by the binding of CD40 ligand (CD154) to CD40 (53). Thus, CD19 may be important during MAIDS for potentiating class II signaling to promote B cell responsiveness to subsequent CD40-dependent signals. In agreement with this is the fact that CD40-CD154 interactions are required for MAIDS development, because mice treated with blocking anti-CD154 Abs or mice lacking either CD40 or CD154 are refractory to disease (54–56). More detailed studies revealed that CD40+ B cells and CD154+CD4+ T cells are the cellular subsets which must express these two paired interacting molecules for LP-BM5-induced MAIDS initiation and progression (55). Thus, CD19 on the B cell surface could facilitate the delivery of an optimal signal initiated by Th cells that promotes B cell proliferation, differentiation, and isotype switching after LP-BM5 infection. In addition, ligation of CD19 on murine B cells up-regulates the expression of CD80 and CD86 (57). MAIDS is associated with overexpression of CD80/CD86, and CD80/CD86-CD28 signaling contributes to MAIDS development (58, 59). Loss of CD19 might impair this costimulatory pathway as well. Finally, the scenario seen in the CD19−/− mice might point to a phenomenon that has been recently described for C3H/HeJ mice. This mouse strain lacks Toll-like receptor 4 and therefore has a reduced incidence and increased latency of mouse mammary tumor virus-induced tumors (60). This is due to the fact that murine retroviruses are able to activate B cells via interaction with Toll-like receptor 4, which might initiate in vivo infection pathways (61). Activation of quiescent cells seems to be crucial for the retroviral life cycle, because migration of reverse-transcribed viral DNA into the nucleus and integration into the chromosomes are thought to depend on the nuclear membrane breakdown that occurs during cell division (62–64). The greatly reduced viral load in CD19-deficient mice suggests the hypothesis that CD19 surface receptors might have a major role for LP-BM5-induced B cell activation and infection.

Lack of Vav-1 can partially be compensated by Vav-2 (65), and Vav-1 and Vav-2 have both been implicated in CD19 coreceptor signaling (17, 66). This compensatory effect of Vav-2 might be the reason why Vav-1-deficient mice show some MAIDS symptoms and can sustain limited viral replication, in contrast to CD19-deficient mice, in which virus could be detected only after passage of infected Mus dunni cells; however, the levels of defective virus found in Vav-1-deficient mice should be sufficient to induce more severe disease in these animals. This is an indication that CD19 and Vav-1 are important not only for viral replication but also for initiation of the lymphoproliferative process that develops during MAIDS. Other studies have described similar phenomena; for example, CD40-deficient mice become infected and show virus expression similar to wild-type mice but are refractory to disease due to the disrupted CD40-CD40 ligand interaction (67). For the Vav-1-deficient mice, another mechanism that could contribute to MAIDS restriction has to be considered; defective class switching and GC formation after immunization with T cell-dependent Ags in these mice have been shown to be due to compromised T cell help because Vav is important for establishing signaling thresholds of the TCR as well (68). Based on the hypothesis that MAIDS is a multistep disease in which infection leads to initial T cell activation and subsequent B cell changes (42), Vav-1 deficiency might limit MAIDS on the B cell as well as on the T cell side.

Loss of CD22 altered early LP-BM5 responses, because spleen cells from CD22-deficient mice demonstrated more advanced signs of MAIDS soon after infection than spleen cells from wild-type animals. Differences in signs of MAIDS were greatest at 4 wk p.i., although viral load was not increased at that time point (data not shown). Therefore, the exaggerated response to LP-BM5 infection in CD22-deficient mice is likely to result directly from alterations in the activation state or the signaling capacity of their B cells rather than being due to an increased susceptibility to virus infection. Later in the course of disease, the reduced number of circulating B cells in CD22-deficient animals (26) might limit target and effector cells, which could explain why enhanced disease is not seen in CD22−/− mice at 8 and 12 wk p.i.

This is the first report showing the crucial role of the CD19-Vav signaling pathway in retroviral replication; however, it has been shown before that blocking accessory molecules CD54 and CD11a was effective in suppressing LP-BM5 replication (69). Both studies indicate that blocking the immune response suppresses active retroviral replication and point to a strategy for interfering with retrovirus-associated disease.

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References


