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In Murine AIDS, B Cells Are Early Targets of Defective Virus and Are Required for Efficient Infection and Expression of Defective Virus in T Cells and Macrophages

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Previous studies showed that B cells and CD4$^+$ T cells are required for induction of a murine retrovirus-induced immunodeficiency syndrome, murine AIDS. Using B6 mice deficient in mature B cells as a result of a knockout of the transmembrane exon of the immunoglobulin M gene, we found that spleen and other tissues from murine AIDS virus-infected mice did not express the defective virus (BM5def) required for induction of disease, even though helper viruses were readily detectable and BM5def proviral DNA was present. This indicates that the B-lineage cells are the primary targets for infection and expression of the defective virus and that in the absence of mature B cells, there is inefficient infection of T cells and macrophages.

Infection of susceptible strains of mice, such as C57BL/6 (B6), with the LP-BM5 mixture of murine leukemia viruses (MuLVs) induces a syndrome of severe immunodeficiency and progressive lymphoproliferation termed murine AIDS (MAIDS) (11, 17). The immunodeficiency involves defects in T cells, B cells, and macrophages (17), while B cells are the predominant proliferative population (10, 13). The disease is a consequence of complex host-virus interactions that are only partially understood. Induction of MAIDS requires the presence of CD4$^+$ T cells (20) and B cells (2) and expression of the MA and p12 portions (18) of the *gag* gene of replication-defective viruses designated BM5def (3, 4) or Du5H (1). MAIDS can be induced by helper-free defective virus (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 (4, 18).

At present, the extent to which abnormalities of various cell types can be ascribed to direct effects of BM5def on infected cells is not known. It has been suggested that B cells are the major targets for infection by defective viruses in mice infected helper free (10), although studies of mice infected with the virus mixture showed that peritoneal macrophages (5) and T cells (14, 19), as well as B cells (4, 8, 13, 19), can serve as targets for infection and expression of BM5def.

In the present study, we evaluated the targets for BM5def following mixed-virus infection by first determining the levels of defective virus mRNA in various spleen cell subsets at different times after infection. For these analyses, B cells, T cells (CD4$^+$ plus CD8$^+$), and macrophages were purified from spleens by positive selection by using magnetic cell separation techniques (16) followed by single-cell sorting. Flow cytometry (fluorescence-activated cell sorter) (FACS) studies of purified cells showed that 98% or more expressed the selected phenotype (data not shown). Semiquantitative reverse transcription-PCR (RT-PCR) analyses of these preparations (Fig. 1) revealed that at 1 week postinfection, both T cells and B cells expressed defective virus, with B cells expressing higher levels than T cells. The demonstration that different subsets of splenic cells are infected at this time extends the findings of early studies showing infection of unseparated spleen cells at 1 week by Northern (RNA) analyses of BM5def expression (6). Considerably higher levels of expression were detected with mRNA from cells of mice infected for 2 weeks or more. At each time point cells were examined, the levels of expression were lower for T cells than for B cells, while expression in macrophages was closer to the levels in B cells than to those in T cells. These studies showed that splenic as well as peritoneal Mac-1$^+$ cells (5) are targets for expression of BM5def and that macrophages from spleens expressed levels of defective virus approximating those in B cells.

The studies that revealed a requirement for B cells in MAIDS had utilized mice made B cell deficient by treatment from birth with anti-immunoglobulin M (IgM) antibodies (2). This treatment regimen is difficult to maintain long-term and precludes the use of B-cell reconstitution experiments to further dissect the role of B cells in disease. To overcome these problems, we studied mice with a B-cell deficiency due to a targeted mutation of the membrane exon of the IgM heavy chain gene (12). B-cell development in homozygous mutant mice is arrested at the pre-B-cell stage of maturation, and mature B cells are undetectable (12).

Mice derived from the fifth- or sixth-generation backcross to normal B6 mice were intercrossed to yield homozygous mutant (B6 · μMT$^{-/-}$) and control (B6 · μMT$^{+/-}$) mice. Infected heterozygous mice developed MAIDS with a time course like that for normal B6 mice (data not shown), indicating that residual genetic contributions to this strain derived from MAIDS-resistant 129 mice (17) did not interfere with induction of disease, at least in mice heterozygous for the mutation. Normal infected B6 mice were used as controls in the remaining experiments. In contrast to B6 mice, homozygous mutant mice infected for up to 15 weeks did not develop any signs of MAIDS, including lymphadenopathy, splenomegaly, histopathologic abnormalities, or changes in cell surface marker expression detected by FACS (Table 1 and data not shown).

Virologic studies showed that the frequencies of spleen cells

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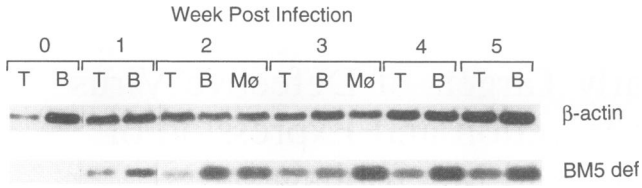

FIG. 1. Semiquantitative RT-PCR analyses of β-actin and BM5def transcripts in a purified population of spleen cells from B6 mice infected with LP-BM5 MuLV for 1 to 5 weeks. The β-actin gene was used as a housekeeping-gene control for the quality of the RNA and cDNA prepared from it. The primers for amplification of the β-actin gene were 5′-CCTTCTACAATGAGC-3′ (sense) and 5′-ACGTCA CACTTCATG-3′ (antisense), and the probe was 5′-CATGGATGC CACAGGATTCCA-3′. The primers used for BM5def were 5′-CC TTTTCCTTTCTCGACACT-3′ (sense) and 5′-ACCAGGGGGGG AATACCTCG-3′ (antisense), and the probe was CTCTGCCAAA GGGACCAGTT. The lengths of amplified products for β-actin and BM5def were 594 and 245 bp, respectively. The electrophoresed amplified products were transferred to nylon membrane and detected by enhanced chemiluminescence (Amersham, Arlington Heights, Ill.) with fluorescein-dUTP-labelled oligonucleotide probes. At the level of exposure used to analyze BM5def expression for all samples but the one for the 1-week time point, there was no detectable signal for either the 1-week T-cell or the 1-week B-cell preparation. The comparison of expression in these cell subsets from mice infected for 1 week was obtained from a longer exposure of the same blot. Mø, macrophage.

producing infectious ecotropic and MCF viruses were generally higher for B6 mice than for B6 · μMT$^{-/-}$ mice (Table 1). These results differed from those of earlier studies of anti-IgM-treated mice, which revealed comparable expression of both classes of helper virus in spleens of control and B-cell-deficient animals (2). The basis for the differences in helper virus recovery from mice rendered B cell deficient by genetic manipulation versus injection of anti-IgM antibodies is not

TABLE 1. Infection of B6 and B6 · μMT$^{-/-}$ mice with LP-BM5 MuLV[a]

Strain	Wk post-infection	Spleen wt (mg)	Stage of disease[b]	Virus recovery (PFU or FFU[c] [$\log_{10}$/10^7 cells])	
				Ecotropic	MCF
B6	1	85	ND[d]	<1.0	<1.0
	3	160	ND	3.1	<1.0
	6	250	ND	ND	ND
	9	695	1	5.0	4.4
	15	1,000	1	5.0	ND
B6 · μMT$^{-/-}$	1	30	ND	<1–2.0	<1.0
	3	36	ND	<1.0	<1.0
	6	46	ND	4.4	3.5
	9	45	N	4.0	2.7
	15	45	N	4.0	ND

[a] Mice were infected with the LP-BM5 virus mixture and killed at the indicated times after infection. Spleen weights were determined, and single-cell suspensions were prepared for (i) FACS analyses, (ii) infectious center tests of ecotropic and MCF viruses using mitomycin-treated cells, and (iii) mixing with RNAzol for later RT-PCR studies. Portions of spleen and other tissues were fixed, sectioned, and stained for histopathologic studies. Numbers indicate the mean values for two to three mice per group.

[b] Histopathologic criteria for staging progression of MAIDS have been presented elsewhere (7). Briefly, N indicates no change from normal; R indicates reaction to infection but with changes insufficient to be MAIDS; and stages 1, 2, and 3 indicate changes characteristic of MAIDS of increasing severity.

[c] FFU, focus-forming units.

[d] ND, not done.

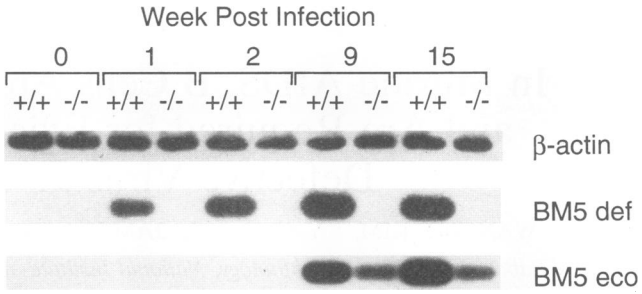

FIG. 2. Semiquantitative RT-PCR analyses of β-actin, BM5def, and BM5eco transcripts in spleens of B6 and B6 · μMT$^{-/-}$ mice infected for 1 to 15 weeks. The primers used for BM5eco were 5′-GGCCTAGAATATGCCGCTC-3′ (sense) and 5′-TGTAGTCCT GGTCGTGGATG-3′ (antisense), and the probe was GTCCTGTT AGGAGTAGGG. The length of amplified product was 318 bp. Samples were prepared from spleens of B6 (+/+) or mutant (−/−) mice at the indicated times postinfection.

certain. The monoclonal antibody-treated mice have macrophages filled with immune complexes and are undergoing chronic antigenic stimulation with the rabbit anti-IgM antibody used for B-cell suppression. The knockout mice simply lack B cells and experience less insult to other components of the immune system. These distinctions between the models may account for the differences in helper virus expression.

To determine if the B-cell deficiency also affected expression of BM5def, we used a semiquantitative RT-PCR analysis of viral transcripts. Strikingly, spleens (Fig. 2), lymph nodes, and thymuses (data not shown) from mutant mice failed to exhibit transcripts for the defective virus when examined at any of a series of time points from 1 to 15 weeks postinfection, while increasing levels of expression were detected in spleens of nonmutant B6 mice (Fig. 2). In agreement with the data from the infectious center tests, the levels of ecotropic virus expression were lower in spleens of B6 · μMT$^{-/-}$ mice than in spleens of nonmutant controls.

DNA PCR analyses of BM5def proviral sequences (Fig. 3) showed the defective genome to be present at high levels in spleens of B6 mice infected for as little as 1 week but to be below the levels of detection in spleens of mutant mice until 6 weeks postinfection. On prolonged exposures of the same blot, signals of BM5def-related sequences were found at extremely low levels at the earlier time points (data not shown).

Although unlikely, it was formally possible that mutant mice might carry strain 129 genes linked to the mutant Ig heavy-chain locus that could inhibit spread and expression of defective virus and induction of disease when present in the homozygous state in B6 · μMT$^{-/-}$ mice. To examine this

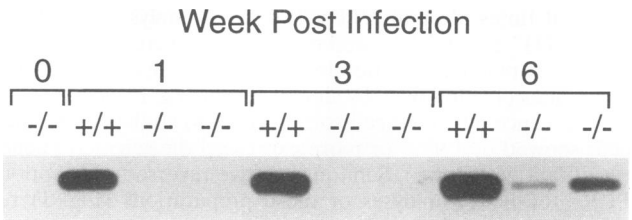

FIG. 3. DNA PCR analyses of BM5def proviral sequences in spleens of B6 (+/+) and B6 · μMT$^{-/-}$ (−/−) mice infected for 1, 3, or 6 weeks. Splenic DNA from an uninfected mutant is shown in the first lane.

possibility, a B6 · μMT$^{-/-}$ mouse was reconstituted intravenously with 10^8 spleen cells from a B6 *nu/nu* mouse and infected with LP-BM5 MuLV 3 days later. When autopsied at 10 weeks postinfection, the mouse had a 550-mg spleen and generalized lymphadenopathy. FACS and histopathologic studies of spleen cells were consistent with the diagnosis of stage 1 MAIDS, and RT-PCR analyses revealed high levels of BM5def transcripts (data not shown).

These findings indicate that the absence of MAIDS-related T-cell abnormalities in B-cell-deficient mice is related to inefficient infection and expression of the etiologic defective virus in cells other than B cells. Since B-cell differentiation in the mutant mice appears to proceed normally through the pre-B-cell stage, the crucial targets for infection are most likely among the more mature cells in this lineage. The demonstration that disease develops in B6 · μMT$^{-/-}$ mice reconstituted with nude spleen cells is consistent with this suggestion. It is also possible, however, that pre-B cells in the B6 · μMT$^{-/-}$ mice become infected but then die as they undergo abortive differentiation to mature B cells, resulting in the failure to detect expression of BM5def.

In view of the substantial levels of helper virus expression, it is quite remarkable that non-B cells in the mutants do not express the defective virus, especially since infection of both T cells and macrophages is readily detectable in nonmutant mice by 1 week postinfection (Fig. 1) and by DNA PCR in the mutant mice by 6 weeks (Fig. 3). Lack of T-cell and macrophage activation in the infected mutant mice may limit potential targets for productive infection, since integration of murine retroviruses is dependent on cell proliferation (15). This seems unlikely, however, since no BM5def transcripts were detected in mutant mouse thymuses, lymphoid compartments with high cell turnover, while high levels were present in the thymuses of B6 mice infected for 3 weeks (data not shown).

The demonstration that the mutant mice can be reconstituted with normal B cells should provide an opportunity to determine if specific subsets of B cells are particularly important to induction of MAIDS. It was reported that CD5$^+$ (B-1a) cells are expanded in infected B6 mice and that they express BM5def at higher levels than do conventional B cells (8). Selective B-cell reconstitution experiments using B6 · μMT$^{-/-}$ mice should provide an opportunity to further explore this issue.

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