Induction of Murine Acquired Immunodeficiency Syndrome (MAIDS) in Allophenic Mice Generated from Strains Susceptible and Resistant to Disease

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Summary

To examine whether a retroviral disease can be controlled in animals in which cells from a resistant strain coexist in a state of immunological tolerance with cells from a susceptible strain, allophenic mice were constructed and infected with LP-BM5 murine leukemia viruses which induce a fatal disorder, termed murine acquired immunodeficiency syndrome (MAIDS), characterized by lymphoproliferation and immunodeficiency in susceptible inbred strains of mice. We found that in two different strain combinations, resistance to MAIDS was contingent on the presence in individual animals of >50% of lymphocytes of resistant strain origin and correlated with reduction or elimination of retrovirus. In contrast, animals harboring substantial, but less than predominant, numbers of genetically resistant lymphocytes developed disease and died within the same time frame as susceptible control mice with uncontained proliferation of retrovirus.

Can a retroviral infection be controlled or eradicated by the presence of immune cells that resist infection? Current thought on therapies for human retroviral disease considers the possibility that rendering at least part of a patient's immune system resistant to infection will prove beneficial (1, 2). To gain insight into the ability of resistant cells to alter the outcome of retroviral disease, allophenic mice were constructed from inbred strains of mice susceptible and resistant to the syndrome of lymphoproliferation and immunodeficiency induced by LP-BM5 murine leukemia viruses (MuLV)[1] termed murine acquired immunodeficiency syndrome (MAIDS) (3–5). Induction of MAIDS is dependent on expression of a replication defective virus, BM5def, that encodes a unique Gag polyprotein (4, 6).

Allophenic mice (designated strain A↔strain B) bear cells of two different genotypes, with individual cells deriving from and expressing the characteristics of one donor strain or the other, but not of both, as in an F_1 animal. We used as the susceptible donor the prototypic MAIDS-sensitive strain, C57BL/6 (B6). The 129/SvJ (129) and A/J strains were selected as resistant donors because they failed to exhibit any signs of disease for ≥38 wk after infection.

These studies establish that MAIDS can be controlled by the presence of immune system cells that resist disease, and suggest thresholds for the percentage of cells of resistant genotype required to prevent progressive disease. Critically, assessment of retroviral burden over the course of disease reveals that although infection is initially established in allophenics with significant numbers of cells of susceptible genotype, those mice with a preponderance of cells of resistant genotype are able to contain the viral burden and remain healthy.

Materials and Methods

Generation of Allophenic Mice and Determination of Lymphoid Chimerism. The allophenic mice were constructed either by embryo fusion at the four to eight cell stage of development in the case of B6↔A/J allophenics, or by injection of embryonic stem cells into a blastocyst in the case of the B6↔129 allophenics (7–9).

Percent chimerism of B6↔129 allophenics was determined from tail blood samples obtained before infection. PBL were obtained from heparinized blood, and isolated on Lympholyte-M (Cedarlane Labs. Ltd., Hornby, Ontario, Canada) cushions, stained with fluorescent antibodies to the cell surface antigen Ly-9.1

[1] *Abbreviations used in this paper:* BM5def, BM5 defective; HPRT, hypoxanthine phosphoribosyl transferase; MAIDS, murine acquired immunodeficiency syndrome; MCF, mink cell focus-forming; MuLV, murine leukemia virus.

(PharMingen, San Diego, CA), and analyzed on a cell sorter (FACS® 440; Becton Dickinson, San Jose, CA). B6 mice do not express Ly-9.1 (10, 11).

Percent chimerism of B6↔A/J allophenics was determined from tail blood samples by subtracting the percent of H-2D^b-expressing cells from total cells. H2-D^b, expressed on cells of B6 but not A/J origin, was detected with a biotin-labeled mAb (PharMingen) and APC-avidin (Southern Biotechnology Assoc., Birmingham, AL) by flow cytometry.

To assess the stability of chimerism, four allophenics were left uninfected. Chimerism was assessed at weeks 0 and 24 in three mice and at weeks 0, 8, 15, and 24 in a fourth. Chimerism was remarkably stable, varying by at most 6% from the initial evaluation.

To assess whether chimerism in PBL reflected that of other lymphoid compartments, we compared chimerism in PBL with that in spleen. We found <10% variation between the two. Indeed, a close correlation in chimerism between spleen and peripheral blood of allophenics has been previously described (12). We therefore used peripheral blood to assess chimerism because it minimized both trauma to the mice and the possibility that the course of disease would be altered by experimental manipulation.

Studies of Infected Mice. Mice were judged to have MAIDS on the basis of progressive lymphadenopathy and splenomegaly and changes in cellular populations and cell surface antigens characteristic of MAIDS: the development of a B220 dull population of cells, excessive numbers of CD11b (Mac-1)$^+$ cells, and the emergence of a CD4$^+$, Thy 1.2$^-$ population of cells (13). Because spleen biopsies were performed, spleen weights at death are not presented, as they would not provide an accurate measure of lymphoproliferation.

Virus recovery was determined by infectious center assays (14–16) using XC cell plaque assays in SC-1 cells for ecotropic MuLV, and immunofluorescent focus induction using monoclonal antibody 514 (17) in *Mus dunni* cells (18) for mink cell focus-forming (MCF) MuLV.

mRNA for BM5 defective (BM5def) was detected by reverse transcriptase-PCR using primers and the probe described previously (19, 20). PCR products were analyzed by Southern blot hybridization with fluorescein-labeled probes. To control for quantitative and qualitative variability of cDNAs, the housekeeping gene hypoxanthine phosphoribosyl transferase (HPRT) was transcribed and amplified for all samples. To determine the relative expression of BM5def in different samples, we established standard curves for HPRT and BM5def by titration of cDNA samples prepared from spleens of mice infected with LP-BM5 virus that had the highest signals for BM5def or HPRT expression in initial screening. Amplification of HPRT for 24 cycles and of BM5def for 23 cycles gave linear correlations between the amounts of template cDNAs and PCR products (Fig.1). The intensity of hybridizing bands was determined by densitometric scanning (Hewlett-Packard Scanner; Hewlett-Packard Co., Palo Alto, CA) (21). Data represent differences in transcript levels normalized to HPRT expression and compared to a positive control (cDNA prepared from RNA of B6 spleen 4 wk after inoculation with LP-BM5 MuLV). Relative values are presented as follows: ++++ = 100% of control; +++ = 70–90%; ++ = 40–60%; + = 10–30%; trace = <10%; − = undetectable.

For mice studied at more than one time point, the first sample was from a hemisplenectomy, the second sample was from the residual spleen, and the third sample was from lymph node.

Statistical Analysis. The statistical significance of the association between ≤50% lymphocytes of resistant strain origin and development of disease, and >50% lymphocytes of resistant strain origin and resistance to disease was assessed by Fisher's Exact Test (StactXact3 software; CYTEL Software Corporation, Cambridge, MA).

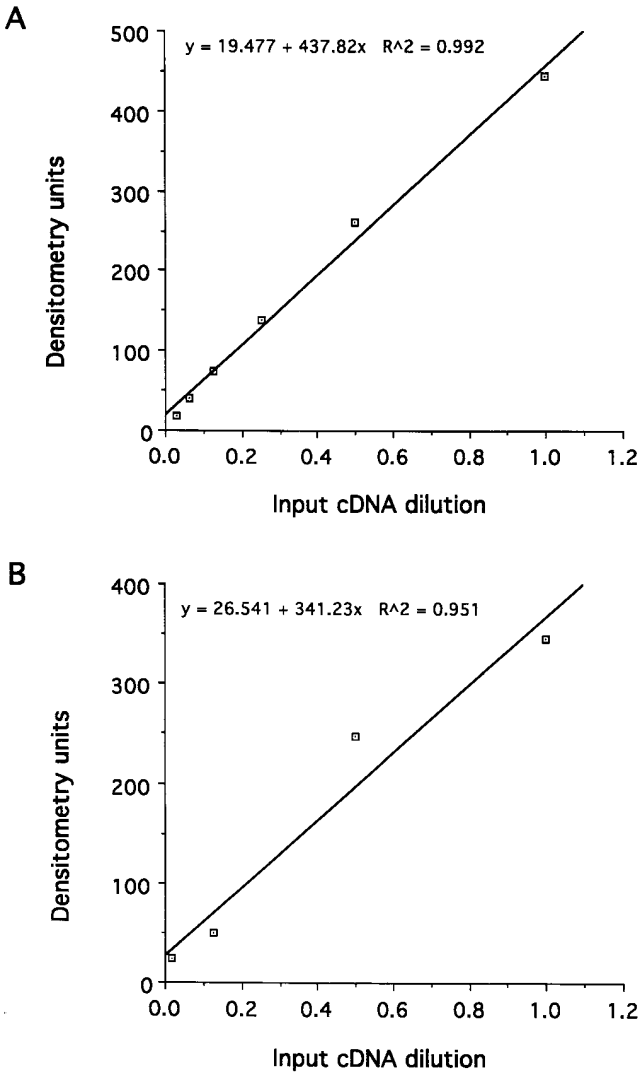

Figure 1. Correlation between input cDNA and HPRT or BM5def RT-PCR signals. RNA isolated from the spleens of LP-BM5 infected mice was reverse transcribed and twofold serial dilutions of the undiluted cDNAs were amplified for 24 cycles for HPRT(*A*), and for 23 cycles for BM5def(*B*). The PCR products were Southern blotted and detected by hybridization with fluorescein-labeled probes. Signal strength was analyzed by densitometry.

Results

Studies of Time to Disease or Death in Unmanipulated B6↔29 Mice Infected with LP-BM5 MuLV. We first asked whether induction of MAIDS could be controlled by the presence of cells from the 129 strain of inbred mice. 129 mice are of the Fv-1^{nr} (22) genotype and therefore restrict replication of the B-tropic helper viruses in LP-BM5 MuLV. Furthermore, they are also resistant to MAIDS development following infection with BM5def pseudotyped

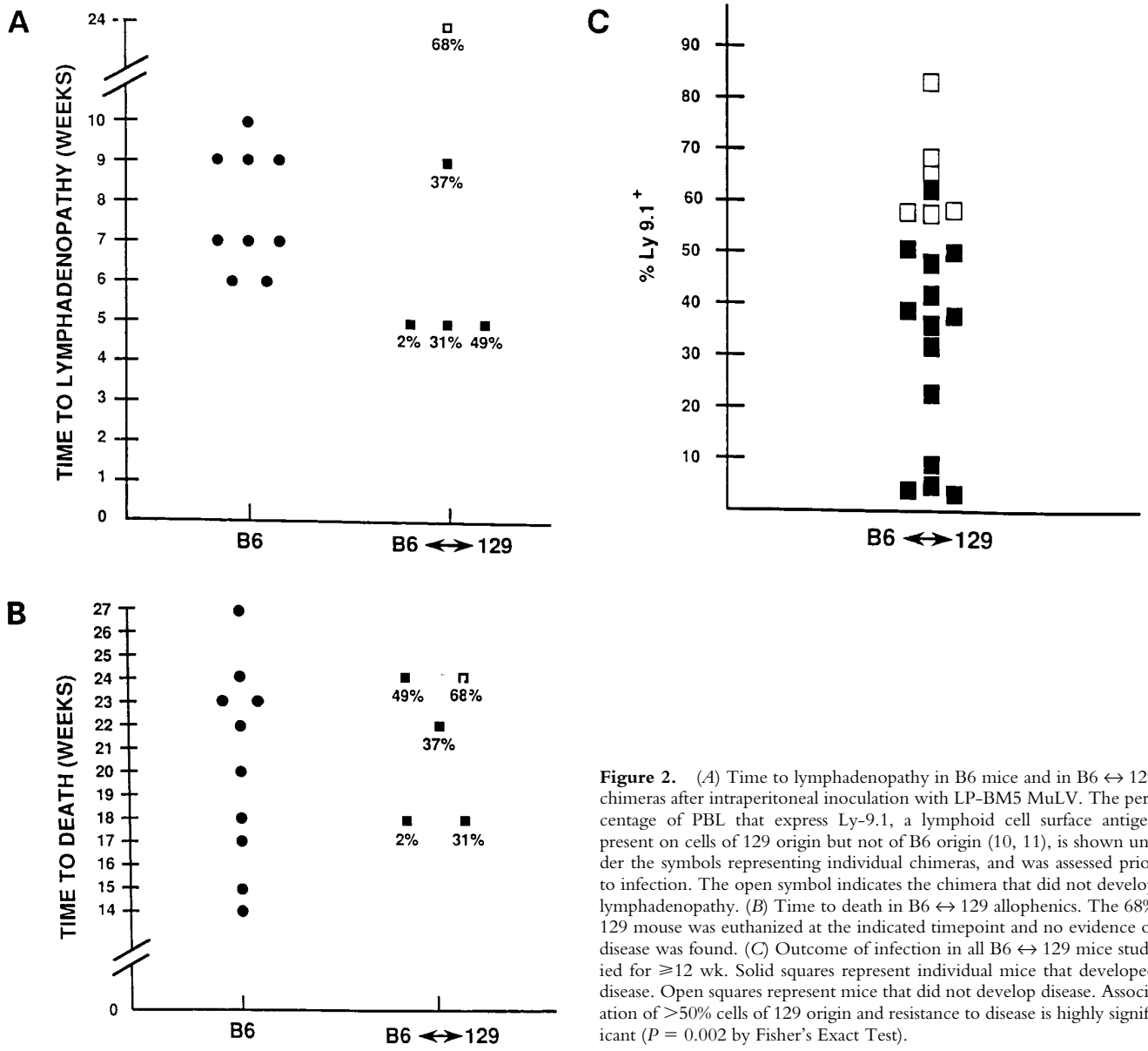

Figure 2. (*A*) Time to lymphadenopathy in B6 mice and in B6 ↔ 129 chimeras after intraperitoneal inoculation with LP-BM5 MuLV. The percentage of PBL that express Ly-9.1, a lymphoid cell surface antigen present on cells of 129 origin but not of B6 origin (10, 11), is shown under the symbols representing individual chimeras, and was assessed prior to infection. The open symbol indicates the chimera that did not develop lymphadenopathy. (*B*) Time to death in B6 ↔ 129 allophenics. The 68% 129 mouse was euthanized at the indicated timepoint and no evidence of disease was found. (*C*) Outcome of infection in all B6 ↔ 129 mice studied for ≥12 wk. Solid squares represent individual mice that developed disease. Open squares represent mice that did not develop disease. Association of >50% cells of 129 origin and resistance to disease is highly significant ($P = 0.002$ by Fisher's Exact Test).

with a N-tropic helper virus (23); the mechanism of this resistance is not known.

The initial study determined time to lymphadenopathy and time to death following infection of unmanipulated B6↔129 allophenics. To meaningfully assess these two clinical endpoints of infection, mice were not subjected to procedures such as splenic biopsy which have an impact on the course of viral disease (Sechler, J.M.G., and A.S. Rosenberg, unpublished observation). Instead, the mice were bled only after development of lymphadenopathy for assessment of cellular populations indicative of MAIDS.

Four mice with <50% PBL of 129 origin developed lymphadenopathy and died in a time course similar to that of fully susceptible B6 mice. Only the allophenic harboring 68% PBL of 129 origin failed to develop disease (Fig. 2, *A* and *B*). 10 additional B6↔129 mice with primarily B6 PBL also developed lymphadenopathy secondary to MAIDS in the same time frame as B6 mice, whereas 6 mice with 57–82% 129 PBL never developed lymphadenopathy and remained healthy (Fig. 2 *C*). Association between development of disease and having ≤50% of lymphocytes of resistant strain origin, and resisting disease and having >50% of lymphocytes of resistant strain origin, was highly significant ($P = 0.0002$ by Fisher's Exact Test).

These data indicate that protection against MAIDS in this strain combination requires that a majority of lymphoid cells be of the resistant strain and that mice with lower frequencies of 129 cells have no survival advantage over inbred susceptible animals. The frequency of cells from the resistant strain required to prevent induction of MAIDS is

Table 1. *Virus Assays and Documentation of MAIDS in B6↔129 Allophenics*

Mouse	Virus Assays				Documentation of MAIDS (time postinfection in wk)
	Weeks after infection	ECO [PFU or FFU ($Log_{10}/10^7$ cells)]	MCF [PFU or FFU ($Log_{10}/10^7$ cells)]	BM5def (RT-PCR)	
B6 ↔ 129					
%LY-9.1$^+$					
46	4	2.0	<1.3	−	19
57	4	1.0	<1.3	−	Killed 05 wk NED
62	4	3.1	1.3	++++	19
82	4	1.0	<1.3	−	Died 10 wk NED*
22	4	4.5	2.0	++++	
	19	4.2	4.0	++	19
59	4	2.5	<1.3	+++	
	19	<1	<1.3	+++	
	35	<1	<1.3	TR	Killed 35 wk NED
59	13	<1	<1.3	+	Killed 13 wk NED
49	24	4.8	NT	NT	24
68	24	<1	NT	NT	Killed 24 wk NED
Controls					
B6	4	3.8	2.5	++++	4
129SvJ	4	<1	<1.3	−	
	19	<1	<1.3	−	>52 wk NED

Expression of ecotropic (*ECO*), mink cell focus-forming MCF, and BM5 defective (BM5def) MuLV in splenocytes (except for the third sample, which is from LN) from B6 ↔ 129 chimeras and control mice infected with LP-BM5 MuLV. Relative levels of BM5def transcripts were determined as described in Materials and Methods. ++++ = 100% of control; +++ = 70–90%; ++ = 40–60%; + = 10–30%; trace (*TR*) = <10%; − = undetectable. NT, not tested. Documentation of MAIDS was based on criteria elaborated in Materials and Methods. All animals diagnosed with MAIDS died of progressive disease. NED, no evidence of disease.
*Mouse was found dead of fight wounds.

~60%, as suggested by the susceptible mouse with 62% 129 cells and three resistant mice with 57–59% of PBL from strain 129.

Serial Studies of B6↔129 Mice Infected with LP-BM5 MuLV. To develop an understanding of the relationships among percent chimerism, virus expression, and development of MAIDS, we studied a series of B6↔129 mice by examining splenic biopsies taken soon after infection for the presence of the three viruses that make up the LP-BM5 virus mixture and then observing the mice for development of disease; some mice were biopsied serially (Table 1). Mice were identified at autopsy as having MAIDS or not by gross observations and FACS® analysis of lymphoid tissues. While all allophenics tested 4 wk after inoculation expressed infectious ecotropic MuLV indicating successful infection of all animals, recovery of infectious MCF MuLV was less common and generally correlated with high expression of BM5def.

Interestingly, detection of BM5def at this early timepoint was not predictive of subsequent development of disease (20). First, the two allophenics with 59% PBL of 129 origin in which BM5def was detectable did not develop disease. Second, the allophenic with 46% PBL of 129 origin, in which BM5def was not detected at the 4 wk timepoint, did go on to develop MAIDS as revealed by flow cytometry of PBL 23 wk after inoculation. Since previous experience has shown that mice with fully developed MAIDS uniformly express BM5def at high levels (24; Morawetz, R., and H.C. Morse, unpublished observations), we assume that infection of this mouse early in disease was either at levels below our limit of detection or localized to tissues not assayed.

The B6↔129 mouse with 59% 129 cells studied at three timepoints after infection is of special interest. Tests showed that a moderate proportion of spleen cells obtained at 4 wk after infection produced infectious ecotropic virus, whereas cells recovered at 19 wk from spleen and 35 wk from lymph node produced no detectable virus. In addition, transcripts for BM5def were detected in spleen at 4 and 19 wk but were present in lymph node at only trace levels at

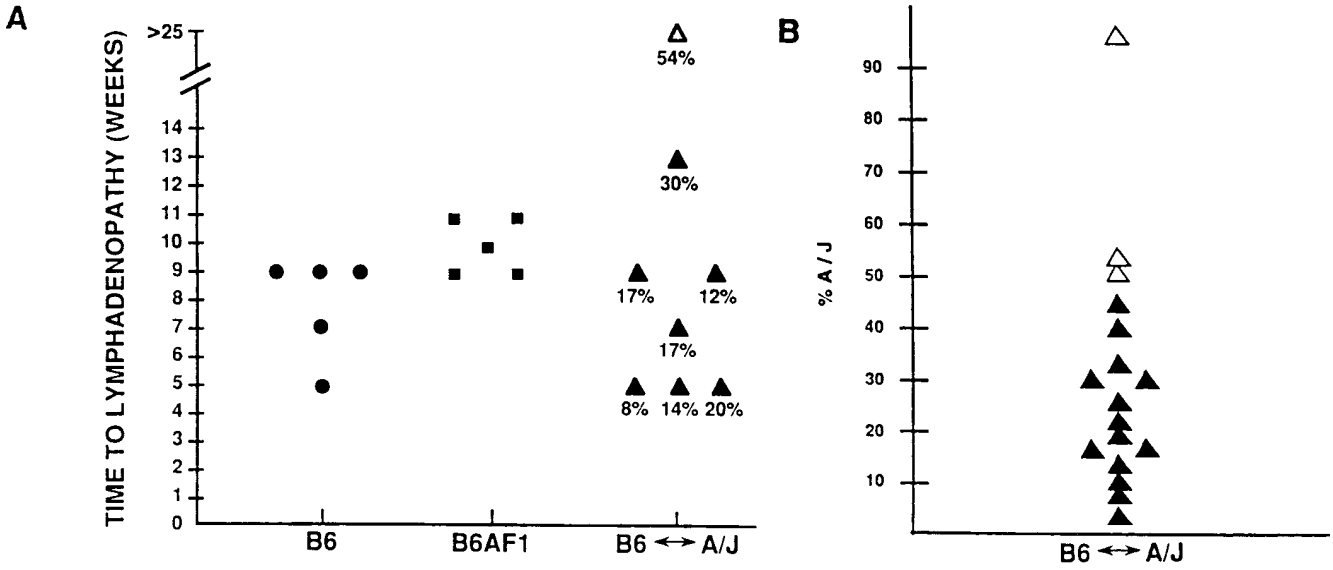

Figure 3. (*A*)Time to lymphadenopathy in B6, B6AF1, and B6 ↔ A/J mice after inoculation with LP-BM5 MuLV. The percentage of A/J cells in PBL of the chimeras is shown beneath the symbols representing individual mice. The open symbol represents the chimera that did not develop lymphadenopathy. (*B*) Outcome of infection in B6 ↔ A/J allophenics studied for ⩾12 wk. Solid triangles represent mice that developed disease. Open triangles represent mice that did not develop disease. Association of >50% cells of A/J origin and resistance to disease is highly significant ($P = 0.0015$ by Fisher's Exact Test).

35 wk. Thus, despite initial infection, the burden of both ecotropic and defective viruses was reduced, and the mouse remained healthy until killed.

Long-term disease-free survival was also associated with undetectable ecotropic virus in the mouse with 68% 129 cells which was sacrificed at 24 wk after inoculation without lymphadenopathy or splenomegaly. This contrasts with the high level expression of helper virus in the mouse with 49% 129 cells which had MAIDS and was moribund when tested at the same time after infection. Prolonged resistance to MAIDS was thus associated with control of ecotropic virus spread and limited expression of BM5def.

Studies of Time to Disease in B6↔A/J Mice Infected with LP-BM5 MuLV. To examine whether similar levels of genotypically resistant cells are required for disease protection if resistance is predicated on an immunologic mechanism, B6↔A/J chimeras were assessed. The disease resistance of A/J mice requires the presence of $CD8^+$ T cells and is presumably immunologically mediated (25). In addition, A/J mice restrict replication of both the ecotropic and BM5def components of the LP-BM5 virus mixture (26, 27).

PBL of B6↔A/J mice ranged in chimerism from 3 to 97% A/J with 14 of 17 mice bearing predominantly B6 PBL (Fig. 3 *B*). Of eight unmanipulated chimeras (Fig. 3 *A*), seven with cells of predominantly B6 origin developed lymphadenopathy within the same time frame as infected B6 and $B6AF_1$ control animals. In contrast, the allophenic with 54% PBL of A/J origin exhibited resistance to MAIDS until sacrifice at 63 wk after infection.

As with the B6↔129 allophenics, an understanding of the relationships among percent chimerism, virus expression, and development of MAIDS was sought. All 14 B6↔A/J mice with <51% A/J PBL developed MAIDS, whereas three mice with >50% A/J cells did not (Fig. 3 *B*). The mouse with 51% A/J cells had splenomegaly at the time of biopsy 4 wk after infection, but did not progress to peripheral lymphadenopathy by the time of a second biopsy 19 wk later, although the spleen was still slightly enlarged and had nodules. Together, these findings suggest that about 50% PB cells of A/J origin are essential to mediate disease resistance. Again, there was a highly significant association between development of disease and having ⩽50% of lymphocytes of resistant strain origin, and resisting disease and having >50% of lymphocytes of resistant strain origin ($P = 0.0015$ by Fisher's Exact Test).

The relationship between virus expression and percent chimerism in B6↔A/J mice was generally consistent with results in the B6↔129 allophenics. In particular, the two MAIDS-resistant mice showed a reduction in the frequency of ecotropic-virus-producing cells between the spleen samples obtained earlier and later in infection. In contrast, the mice with 40 and 44% PBL of A/J origin showed little or no ability to control expression of either helper virus or BM5def (Table 2). Thus, as with resistant B6↔129 chimeras, resistant B6↔A/J chimeras were clearly infected, had an initial burst of virus replication, but were able to contain retroviral burden.

Discussion

One approach to treatment of human retroviral disease involves reconstructing the immune system with lymphocytes that cannot be infected to mediate critical immune functions. Unfortunately, animal models to test such an ap-

Table 2. *Virus Assays and Documentation of MAIDS in B6↔A/J Allophenics*

Mouse	Virus Assays				Documentation of MAIDS (time postinfection in wk)
	Weeks after infection	ECO	MCF	BM5def (RT-PCR)	
		[PFU or FFU ($Log_{10}/10^7$ cells)]			
B6 ↔ A/J					
%A/J					
3	4	4.4	2.3	++++	4
	22	4.5	4.2	NT	
22	4	3.8	<1.3	+++	4
	22	4.9	4.1	NT	
30	12	4.2	3.0	+++	4
	27	3.9	4.3	+++	
30	34	3.7	4.2	++++	6
40	4	3.0	1.3	++++	4
	22	4.5	4.1	NT	
	34	3.3	3.1	++++	
44	4	2.0	1.3	+	8
	22	2.3	4.1	NT	
	34	4.0	4.0	+++	
51	4	4.5	<1.3	++	>52wk NED
	23	2.0	1.3	++	
54	12	1.3	TR	TR	Killed 63 wk NED
	27	<1	TR	TR	
Controls					
B6	4	3.8	2.5	++++	4
A/J	4	<1	<1.3	−	
	22	<1	<1.3	NT	>52 wk NED

Expression of ecotropic (*ECO*), mink cell focus-forming MCF, and BM5def MuLV in splenocytes from B6 ↔ A/J chimeras and control mice infected with LP-BM5 MuLV. Relative levels of BM5def transcripts were determined as described in Materials and Methods and legend for Table 1. Documentation of MAIDS was based on criteria elaborated in Materials and Methods. All animals diagnosed with MAIDS died of progressive disease. NED, no evidence of disease.

proach for HIV or SIV are lacking. Thus, despite limitations as a model of disease induced by HIV, we tested a murine retroviral disease which shares some features of human AIDS. Allophenic mice comprised of cells from MAIDS-resistant and -susceptible strains were studied rather than radiation bone marrow chimeras to avoid problems associated with delayed repopulation of lymphocyte subsets critical for induction and progression of disease. Thus, resistance versus susceptibility was studied in a "best case" scenario in which an intact but chimeric immune system with mutual tolerance was present at the time of infection.

Our results suggest that a critical balance in the proportion of lymphoid cells from the MAIDS-resistant and -sensitive strains determines whether mice infected with LP-BM5 MuLV develop MAIDS or exhibit long-term resistance to disease. Indeed, MAIDS was prevented or controlled only in the chimeras that had a predominance of cells of the resistant genotype at the time of infection; no significant benefit with regard to disease progression or survival was conferred on animals in which lymphoid cells of the resistant genotype were in the minority.

It thus seems possible that a race between the rate of virus spread among cells of susceptible genotype and the rate at which virus-specific effector cells and mechanisms are activated determines the outcome of infection with LP-BM5 MuLV. When the proliferation of virus-infected cells outpaces the activation and expansion of effector mechanisms, the animal develops lymphoproliferation and immunodeficiency. This model predicts that disease develops in chimeras with a large pool of genetically susceptible B cells, shown to be the principal early targets of infection (28, 29), coupled with a smaller pool of genotypically resistant effector cells. In these mice the frequency of virus-specific precursors would be limited, lessening the chance that suffi-

cient virus-specific effectors could be generated to prevent disease. In allophenics with a predominance of cells of resistant genotype, there would be a sufficient level of virus-specific effectors and/or effector mechanisms to contain virus spread and prevent development of disease.

An alternative view of the requirement for a majority of resistant genotype cells to mediate resistance is based on the suggestion that B6 mice may provide a factor such as a cytokine necessary for disease induction (24, 30). Thus, resistant allophenics may not have this factor in an amount sufficient to facilitate disease progression, or the resistant strain may provide a factor such as IL-12 (31) that inhibits disease induction. Indeed, a genetically controlled negative regulatory factor has been implicated in resistance to induction of erythroleukemia by Friend virus complex (32).

We can conclude that the presence of genetically resistant immune system cells in sufficient numbers can confer long-term resistance to retroviral disease, even though viral integration and replication has occurred. This may have important implications for novel approaches to therapy of human retroviral disease.

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