Retrovirus-Induced Murine Acquired Immunodeficiency Syndrome: Natural History of Infection and Differing Susceptibility of Inbred Mouse Strains

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C57BL mice (*Fv-1^b*) develop a severe immunodeficiency disease following inoculation as adults with LP-BM5 murine leukemia virus (MuLV), a derivative of Duplan-Laterjet virus which contains B-tropic ecotropic and mink cell focus-inducing MuLVs and a putative defective genome which may be the proximal cause of disease. The stages of development of this disease were defined for C57BL mice on the basis of lymphadenopathy and splenomegaly; histopathological changes consistent with B-cell activation; and alterations in expression of cell surface antigens affected by proliferation of T cells, B cells, and macrophages. By using this disease profile as a standard, the response of adult mice of various inbred strains and selected F₁ hybrids was compared. We show that although the strains which are highly sensitive are of the *Fv-1^b* genotype (i.e., permissive for B-tropic MuLVs), certain *Fv-1^b* strains, e.g., BALB/c and A/J, are resistant to murine acquired immunodeficiency syndrome, whereas certain *Fv-1ⁿ* strains (permissive for N-tropic MuLVs but restrictive for B-tropic MuLVs), notably P/N, BDP, and AKR, show moderate sensitivity and (C57BL/6 × CBA/N)F₁ mice (*Fv-1^{n/b}* and thus dually restrictive) are of relatively high susceptibility. The results of virus recovery tests suggest that apparently anomalous sensitivity, based on predicted *Fv-1* restriction, may reflect MuLV induction and/or mutation to provide a helper virus for which the host is permissive.

Adult C57BL mice respond to infection with LP-BM5 murine leukemia virus (MuLV) by developing a syndrome referred to as murine acquired immunodeficiency syndrome (MAIDS), characterized by stimulation of T cells, polyclonal B-cell proliferation, immunodeficiency, and, in some cases, B-cell lymphomas (6, 13, 19-21, 29). The original source of the MuLV was the Duplan-Laterjet strain of radiation leukemia virus, derived from a nonthymic lymphoma developing in an X-irradiated C57BL/6 (B6) mouse (14). Virus preparations currently used for inducing MAIDS are uncloned and represent variable mixtures of C-type B-tropic retroviruses, including ecotropic and mink cell focus-inducing (MCF) MuLVs (1, 21). Although an ecotropic MuLV pseudotype of an LP-BM5-derived MCF MuLV was once thought to induce disease (21), further studies indicate that biologically cloned isolates of neither of these components, tested individually or in various combinations, can induce MAIDS (S. K. Chattopadhyay, H. C. Morse III, M. Makino, S. K. Ruscetti, and J. W. Hartley, submitted for publication), and recent evidence implicates a defective virus in the induction of disease (S. K. Chattopadhyay, M. Makino, J. W. Hartley, and H. C. Morse III, Proc. 6th International Workshop on Immune-Deficient Animals in Biomedical Research, in press).

The dramatic effect on virus infection and spread imposed by permissive or restrictive alleles at the *Fv-1* locus has long been recognized as a major determinant of sensitivity to induction of disease in mice by ecotropic and MCF MuLVs. In preliminary studies of the range of sensitivity of inbred mouse strains to MAIDS, it was found, unexpectedly, that BALB/c mice, which, like C57BL, are *Fv-1^b* and thus

permissive for infection with B-tropic MuLVs (8, 22), did not develop MAIDS following inoculation as adults with LP-BM5 MuLV. In addition, many (B6 × CBA/N)F₁ mice (*Fv-1^{n/b}* *Rmcf^{rs}*) did develop disease, although they carry not only the *Fv-1ⁿ* allele, dominantly restrictive for B-tropic MuLV (22, 24), but also the dominant allele *Rmcf^r*, which inhibits MCF MuLV infection (10). To investigate these findings further, we tested a variety of inbred mouse strains and selected F₁ hybrids for susceptibility to development of MAIDS. To facilitate comparisons between strains, we defined the stages of progression of disease for the highly sensitive strain B6 by gross, histopathological, and flow-microfluorometric (FACS) examinations.

MATERIALS AND METHODS

Mice. Strains AL/N, AKR/N, BALB/cAnN, CBA/N, C57BL/6N (B6), C57BL/10N, DBA/2N, FVB/N, I/StN, NFS/N, and P/N were obtained from the Division of Research Resources, National Institutes of Health; strains A/J, BDP/J, C57BL/6J, C57BL/KsJ, C57BR/cdJ, C57L/J, LG/J, RIIS/J, and SEC/1ReJ were supplied by The Jackson Laboratory, Bar Harbor, Maine. Strains B10.F, B10.F(13R), and B10.P(10R) were bred in our laboratories from mice kindly provided by J. Stimpfling, McLaughlin Research Institute, Great Falls, Mont. The F₁ hybrids of B6 and CBA/N were also bred by us. Mice were inoculated intraperitoneally with LP-BM5 MuLV, using 0.1 or 1.0 ml for adults (1 to 4 months of age) or 0.02 ml for neonates (0 to 7 days of age), and were monitored for disease by observation and palpation of the peripheral lymph nodes and spleen at intervals of about 1 week.

Virus and virus assays. Virus pools, derived from chronically infected SC-1 cultures as previously described (1),

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were assayed for ecotropic MuLV by XC plaque tests (27) in SC-1 cells (7), for MCF MuLV by focus induction tests in mink lung cells, directly or by the SC-1 UV-Mink assay (3, 9), and for their ability to induce MAIDS in adult B6 mice. Virus pools used contained $10^{3.7}$ to $10^{5.6}$ XC PFU per ml and $10^{2.8}$ to $10^{4.7}$ MCF focus-forming units per ml. Virus recovery from mice was determined by infectious-center assays of mitomycin C-treated spleen cells (2, 3).

***Fv-1* and *Rmcf* typing.** With the following exceptions, genotypes in Table 2 are based on published information (10, 22, 28). Previously described procedures (10, 18, 22) were used to type P/N (*Fv-1ⁿ Rmcfⁿ*) and C57BL/Ks, RIIS, and FVB (*Fv-1^b Rmcfⁿ*) and to type ecotropic and MCF MuLV isolates for N/B tropism. BDP, previously typed as *Fv-1^b* (28), was found to be *Fv-1ⁿ Rmcf^s*.

Histopathology. Samples of spleen, lymph nodes, thymus, lungs, liver, and kidneys were fixed in Tellyesniczky solution (10% neutral Formalin and 5% glacial acetic acid in 70% ethanol), sectioned, and stained with hematoxylin and eosin.

FACS. FACS assays of single-cell suspensions prepared from spleens were performed on a FACS 440 by established techniques (4, 11). Antibodies used are detailed elsewhere (4, 11, 19) and included reagents to Thy-1, kappa light chain, immunoglobulin (IgG), Ly-5(B220), Ly-17, and Mac-1.

RESULTS

Although significant enlargement of spleen and lymph nodes may appear by 3 or 4 weeks after inoculation of susceptible mice with LP-BM5 MuLV, animals often survive for extended periods with extremely large peripheral lymph nodes and with spleen weights estimated to be in excess of 0.75 g. Death occurs, often suddenly, from several possible causes including respiratory failure due to infiltration of lungs by lymphoid cells and/or compression of air passages by enlarged mediastinal nodes, lymphoid infiltration of the kidneys, and, in a proportion of advanced cases, aggressive infiltration of the central nervous system (13). Because of the unpredictable time of death, mortality data were not found to be useful for comparing patterns of disease induction. To establish criteria which would encompass time of onset, severity of response, and rate of progression, we evaluated the sensitive B6 strain during the first 3 months postinoculation for the following parameters: spleen weight, degree of lymphadenopathy, histopathological changes, and FACS profiles of splenic cell populations for cell size and reactivity with antibodies to cell surface antigens.

Gross and histopathological measures of disease progression. Early in the course of infection (2 to 3 weeks), histopathological changes were consistent with a severe immunological reaction, perhaps at least in part in response to infection with LP-BM5 ecotropic virus (M. Makino and H. C. Morse III, unpublished observations). In the spleen there was an increase in the size of follicles, although their spherical shape and location within periaarterial lymphoid sheaths (PALS) were maintained (Fig. 1a and b). This increase was caused by a progressive replacement of the normal population of small lymphocytes with immunoblasts, plasmacytoid cells, and plasma cells. For markedly reactive spleens, weights several times normal were encountered, but the histological criterion for identifying the transition from the reactive state to the early stage of MAIDS was marked distortion of PALS, often in an hourglass shape, due to further proliferation of immunoblasts and plasma cells, resulting in partial or complete loss of the mantle zone (Fig. 1c to e). In advanced disease, spleens frequently weighed 1 g or

more and were filled with nodular masses of lymphoid cells. There was a concomitant increase in the number of immunoblasts, plasmacytoid cells, and plasma cells throughout the deep cortex and medulla of lymph nodes (Fig. 1f) and in the thymic medullae. Virtually all nodes, but especially the subcervical and parathymic nodes, were enlarged and often congested.

Infiltration of the lungs, kidneys, and liver occurred in advanced disease, seen first as perivascular cuffs of immunoblasts and plasmacytoid cells (Fig. 1g). As the disease progressed, extensive replacement of normal parenchyma was seen in the lungs, kidneys, and, to a lesser extent, liver. In very advanced cases, the central nervous system may be severely infiltrated by a similar process, initially involving the choroid plexus (13).

FACS analysis of disease progression. Spleen cells from normal B6 mice or B6 mice infected 2 to 12 weeks previously with LP-BM5 MuLV were reacted with a series of fluorescein isothiocyanate-labeled antibodies to cell surface antigens and examined by FACS (Fig. 2 and data not shown). At 2 weeks after infection, dual-parameter analyses revealed the appearance of a small population of large, dull kappa⁺ cells that increased progressively in size over the next 10 weeks. An expanding population of cells with increased forward-angle light scatter (8% in normal spleens, 46% in 12-week spleen [Fig. 2]) included cells staining at low intensities with antibody to the kappa light chain and IgG (Fig. 2) and also Ly-5(B220) and ThB (data not shown) and cells staining at normal intensity with monoclonal anti-Mac-1 antibody (data not shown). The frequencies of Mac-1⁺ cells increased from 5% in normal spleens to 21% in mice infected for 12 weeks. Note the time-dependent positional shifts in the populations of kappa⁻ and IgG⁻ cells that suggest the presence of few cells that are truly surface immunoglobulin negative. These changes probably reflect the presence of cytophilic immunoglobulin (19) and autoantibodies to cell surface antigens (D. M. Klinman and H. C. Morse III, J. Immunol., in press). Similar changes in cell populations have been described for lymph nodes of mice with MAIDS (29).

Stages of MAIDS. Similar stages of disease, from early to advanced, were definable by each of several criteria presented here and in other studies of MAIDS pathogenesis (19, 21; Klinman and Morse, in press). Table 1 compares the onset and progression of MAIDS in terms of gross findings, FACS profiles, and histopathology, defining a reactive, presumably prodromal phase and three stages of the disease. These stages are equivalent to those designating progressive changes in the humoral immune system which have been described for MAIDS (Klinman and Morse, in press). In applying these criteria for diagnosis, two points must be recognized. First, the time frame for disease progression given for B6 in Fig. 2 applies only to highly sensitive strains; a much more extended course is generally seen in less susceptible strains. Second, even among sensitive strains, individual mice with stage 3 disease may vary greatly in the degree of extralymphoid infiltration and FACS analysis of cell subsets in the spleen (19).

Strain distribution of sensitivity to MAIDS and relation to *Fv-1* genotype. The development of disease in a variety of inbred mouse strains inoculated with LP-BM5 MuLV was determined by gross and histopathological examination of mice killed that had obvious disease or those in groups terminated at either 6 to 7 or 8 to 20 months after infection (Table 2). All mice of the highly susceptible strains, listed first, developed stage 2 or 3 disease usually within 2 to 4 months. Incidence curves for B6, C57BL/10, and I/St mice

FIG. 1. Spleen sections from B6 mice inoculated with LP-BM5 virus 21 days previously. (a) Enlarged PALS, typical of ++ reactive response. Magnification, $\times 132$. (b) Detail of the PALS within the rectangle in panel a, showing the moderately enlarged follicle. Magnification, $\times 330$. (c) Much greater enlargement of the PALS and compression of surrounding red pulp, typical of stage 1 disease. Magnification, $\times 132$. (d) Detail of PALS within rectangle in panel c showing the almost complete replacement of the normal population of small lymphocytes by cells derived from the greatly enlarged follicle. Magnification, $\times 330$. (e) Extensive enlargement of PALS and almost complete obliteration of the red pulp, typical of stage 2 or 3 disease. Magnification, $\times 132$. (f) Immunoblasts (large arrows), plasmacytoid cells (arrowheads), and plasma cells (small arrows) within PALS. Magnification, $\times 1,980$. (g) Solid infiltration of cells as in panel f, in lung tissue of a B6 mouse 13 weeks postinoculation; bronchiole at upper right. Magnification, $\times 660$. (h) Spleen from (CBA/N $\times$ B6) F_1 mouse inoculated 13 months previously. The central arteriole (arrow) is surrounded by fibrous tissue devoid of lymphocytes. Sinusoids (arrowheads) define the boundary of the original PALS. Magnification, $\times 660$.

FIG. 2. FACS profiles of spleen cells from uninfected B6 mice (week 0) or mice infected with LP-BM5 MuLV for 2 to 12 weeks by using monoclonal antibodies to kappa and IgG and analyses of forward-angle light scatter. Single-parameter profiles plot cell number versus fluorescence intensity over four decades, with each decade represented by 125 channels. Dual-parameter analyses are presented as 64×64 contour plots, with each of four decades of fluorescence represented by 16 channels. The same cells were also tested with antibodies specific for Ly-5(B220), ThB, Mac-1, and Ly-17 (not shown). In relation to FACS diagnoses listed in Table 1, a diagnosis of + corresponds to patterns like those shown for 2 weeks postinfection that exhibit a small population of dull kappa⁺, dull Ly-5(B220)⁺, dull ThB⁺ blast cells, a slightly increased frequency of IgG⁺ and Mac-1⁺ cells, and increased Ly-17 expression. The 4-week panels correspond to a diagnosis of ++ in having expanded populations of dull kappa⁺, dull Ly-5(B220)⁺, dull ThB⁺, IgG⁺, and Mac-1⁺ cells and a much reduced population of bright kappa⁺ cells of normal size. The proportion of bright Ly-17⁺ cells was increased. Profiles from weeks 8 and 12 correspond to FACS diagnoses of +++ and are characterized by the virtual absence of kappa⁺ cells of normal size and staining intensity, greatly reduced frequencies of dull or bright Ly-5(B220)⁺ or ThB⁺ cells, marked increases in IgG⁺ and Mac-1⁺ cells, and often reduced populations of bright Ly-17⁺ cells.

TABLE 1. Stages of MAIDS as determined by various diagnostic measures

Stage of disease	Range of spleen wt (mg)	Lymphadenopathy ^a	FACS diagnosis ^b	Histopathology
Reactive (R)	90–500 ^c	– to tr	(+)	+ to +++ R (Fig. 1a, b)
1	190–250	tr to +	+	+ (Fig. 1c, d)
2	200–450	++	++ to +++	++ to +++ (Fig. 1e–g)
3	500–2,000	+++	+++	+++ (Fig. 1e–g)

^a –, Normal. tr, Trace (<20 mg). +, Subcervical lymph nodes were 20 to 180 mg; visible enlargement of other peripheral, mediastinal, and/or peritoneal nodes. ++, Subcervical lymph nodes were 190 to 340 mg; comparable increase in other nodes. +++, Subcervical lymph nodes were 350 to 1,500 mg; comparable increase in other nodes.

^b See Fig. 2 legend. (+) designates FACS profiles and frequencies of cell populations that differ slightly from normal and resemble changes seen in mice inoculated with LP-BM5 ecotropic MuLV (29).

^c High spleen weights (300 to 500 mg) in the reactive (R) category were found in old mice of resistant or partially resistant strains.

TABLE 2. Development of MAIDS in various strains of mice inoculated with LP-BM5 MuLVs

Mouse strain and genotype ^a				Age at inoculation	Total no. inoculated	Incidence, severity, and latency of disease at ^b :			
						2-7 mo		8-20 mo	
						No. with MAIDS/no. observed	No. with stage 2-3 MAIDS	No. with MAIDS/no. observed	No. with stage 2-3 MAIDS
<i>b/b</i>	C57BL/6	<i>b</i>	<i>s/s</i>	Adult	44	43/44	43		
	C57BL/10	<i>b</i>		Adult	34	34/34	34		
	B10.F	<i>p</i>		Adult	9	9/9	9		
	B10.F(13R)	<i>bu</i>		Adult	3	3/3	3		
	B10.P(10R)	<i>kpl</i>		Adult	4	4/4	4		
	I/St	<i>j</i>	<i>s/s</i>	Adult	15	15/15	15		
<i>b/b</i>	C57BL/Ks	<i>d</i>	<i>s/s</i>	Adult	3			0/3	
	BALB/c	<i>d</i>	<i>s/s</i>	Adult	18	0/10		0/8	
	BALB/c			7 days	13	8/13	8		
	LG	<i>d</i>		Adult	11	0/6		0/5	
	SEC/1Re	<i>d</i>		Adult	9	2/6	0	1/3*	1
	A	<i>a</i>	<i>s/s</i>	Adult	17	0/6		2/11*	0
	AL	<i>a</i>		Adult	9	0/6		2/3*	2
	RIIIS	<i>r</i>	<i>s/s</i>	Adult	11	0/6		0/5	
	FVB		<i>s/s</i>	Adult	5	0/5			
<i>n/n</i>	C57L	<i>b</i>	<i>s/s</i>	Adult	8			2/8*	2
	P/N	<i>p</i>	<i>s/s</i>	Adult	7	1/2 ^c	1	2/5	1
	P/N			3 days	2	1/1	1	1/1	1
	BDP	<i>p</i>	<i>s/s</i>	Adult	12	11/12 ^d	10		
	AKR	<i>k</i>	<i>s/s</i>	Adult	5	0/5 ^e			
	AKR			0 days	15	0/2		9/13 ^f	3
	C57BR/cd	<i>k</i>	<i>s/s</i>	Adult	7			1/7*	1 ^g
	CBA/N	<i>k</i>	<i>r/r</i>	Adult	12	0/8		0/4	
	DBA/2	<i>d</i>	<i>r/r</i>	Adult	4			1/4*	0
	NFS	<i>sq4</i>	<i>s/s</i>	Adult	6	0/6			
<i>n/b</i>	(B6 × CBA/N)F ₁	<i>b/k</i>	<i>s/r</i>	Adult	44	29/29	28	13/15	12
	(CBA/N × B6)F ₁	<i>b/k</i>	<i>s/r</i>	Adult	16	11/12 ^c	11	3/4	3

^a Blank spaces, Not determined. For *Rmcf*, *s* denotes sensitivity and *r* denotes resistance.

^b Asterisks next to entries represent mice diagnosed 1 year or longer after inoculation.

^c Denominator includes one lymphoblastic lymphoma at 7 months, probably spontaneous.

^d Denominator includes one thymic lymphoma at 6 months, probably spontaneous.

^e Denominator includes four thymic lymphomas at 5 to 7 months, probably spontaneous.

^f Denominator includes two thymic lymphomas at 9 months, probably spontaneous.

^g Immunoblastic lymphoma with myelogenous component, provisionally classified as LP-BM5 related.

are presented in Fig. 3 and illustrate the close similarity of response in these strains. These and the other highly sensitive strains are *Fv-1^b*, as would be predicted from the B-tropism of LP-BM5 MuLVs, but a number of other strains of this genotype are at most minimally sensitive (Table 2). Strains carrying the *n* allele at *Fv-1* and thus restrictive for replication of LP-BM5 MuLVs tended to be resistant, but some showed a clear capability of developing MAIDS, although generally with reduced efficiency compared with sensitive *Fv-1^b* strains.

Resistance in *Fv-1^b* strains. Mice of strain BALB/c were highly resistant after inoculation as adults, but were found to be moderately sensitive when inoculated at 1 week of age, 8 of 13 mice developing stage 2 or 3 disease within 6 months (Table 2). To determine whether differences in susceptibility were related to differences in replication of LP-BM5 MuLVs, spleen cells from individual B6 mice inoculated as adults and BALB/c mice inoculated as adults or neonates, were assayed in infectious-center tests. Efficient replication of ecotropic MuLV was correlated with induction of disease, virus recovery being greatly reduced in adult-inoculated BALB/c mice when compared with B6 or neonatally inocu-

lated BALB/c mice (Table 3). Parallel quantitative data for MCF MuLV were not obtained in this experiment, but in a detailed study to be reported elsewhere (M. Makino et al., in preparation), tests for MCF MuLV at comparable time points revealed little or none in spleens of adult-infected BALB/c mice, whereas 10² to 10³ FFU/10⁷ cells were detected in BALB/c mice inoculated as neonates or B6 mice inoculated as adults. Since many MCF MuLVs require ecotropic MuLV for efficient replication in vivo, poor recovery from adult-inoculated BALB/c mice probably reflects low levels of ecotropic helper virus (Table 3). Whether reduced MCF MuLV replication directly affects pathogenesis of MAIDS is not yet clear.

Sensitivity in *Fv-1ⁿ* strains and *Fv-1^{n/b}* hybrids. Despite the apparent correlation between induction of disease and efficient replication of LP-BM5 ecotropic MuLV seen in B6 compared with BALB/c mice, a surprisingly high degree of sensitivity to MAIDS was displayed by P/N, BDP, AKR, and C57L mice, all of which carry the restrictive *Fv-1ⁿ* allele (Table 2). Compared with the disease pattern in B6 mice, in these strains MAIDS developed later and progressed more slowly and less predictably. A further unexpected finding

FIG. 3. Time course of development of MAIDS. Adult mice of inbred strains C57BL/6, C57BL/10, and I/ST were inoculated with LP-BM5 MuLV and killed for diagnosis when severely ill. The number of mice with stage 2 or 3 disease (Table 1) and the number of mice inoculated are given in parentheses. Progeny of reciprocal crosses of B6 and CBA/N were tested similarly, except that two mice with stage 1 disease (killed at 102 and 513 days) are included.

was that although heterozygous at *Fv-1* and *Rmcf* and thus genetically resistant to infection by B-ecotropic and MCF MuLVs, most progeny of reciprocal matings of B6 and CBA/N developed disease (56 of 60 [93%]) (Fig. 3; Table 2). The majority of diagnoses were of stage 2 or 3 disease, but the average latency was 192 days as compared with 99 days for B6 mice. Some mice developed visible peripheral lymphadenopathy at 2 or 3 months, which later decreased. When such regressor mice were killed at 6 to 13 months postinoculation, their hypoplastic lymph nodes were seen to have lost much of the lymphoid cell population, and all that remained was a hyperplastic stroma (Fig. 1h). Splenic changes were generally those of stage 2 or 3 disease, and occasional infiltration of nonlymphoid tissues was seen. Regression of lymphadenopathy was also frequently noted in strains P/N and BDP/J.

A possible basis for these unexpected sensitivities is shown in Table 4, which presents the characterization of

MuLVs isolated from certain *Fv-1ⁿ* and *Fv-1^{n/b}* mice with MAIDS. For the two inoculated P/N mice tested, the ecotropic MuLVs recovered were N-tropic, as is virus recovered from normal, uninoculated P/N mice, suggesting that an endogenous ecotropic MuLV capable of establishing an efficiently spreading infection could supplant the helper function of the LP-BM5 ecotropic virus. Consistent with this suggestion is the development of MAIDS in 9 of 13 AKR mice inoculated when newborn with LP-BM5 MuLV (Table 2). Virus recovery has not yet been studied, but abundant potential ecotropic MuLV would be available (26). It is of interest that in addition to spleen and lymph node alterations consistent with MAIDS, the thymus, the primary site of spontaneous AKR T-cell lymphoma, was almost always greatly enlarged. Histopathological examination revealed that, in contrast to the predominance of small lymphocytes characteristic of AKR thymic lymphoma, the thymic medullae were filled with the population of immunoblasts, plasmacytoid cells, and plasma cells found in MAIDS (Fig. 1f). In AKR mice inoculated at 1 month of age, only thymic lymphoblastic lymphoma was detected (Table 2), but further studies are required to determine the consistency and basis of this finding.

Virus recovery studies in the hybrids of strains B6 and CBA/N also provide evidence for a new helper virus, of different origin from those probably functioning in strains P/N and AKR. From all five mice with disease, the ecotropic MuLVs recovered were found to have dual N-B host range, as determined by demonstration of equivalent XC plaque titers in *Fv-1ⁿ*, *Fv-1^b*, and *Fv-1^{n/b}* tissue culture cells, even after high-dilution passage through *Fv-1ⁿ* and *Fv-1^b* cells to discriminate mixtures (data not shown). In only one case was B-tropic virus also recovered. Conversion to NB-tropism has been shown to occur on repeated passage of B-tropic viruses from BALB/c mice in *Fv-1* restrictive cells (12, 16) and is associated with point mutations in the p30 region of *gag* (5, 12, 23). Virus with dual host range was not

TABLE 3. Comparison of ecotropic MuLV recovery and sensitivity to MAIDS in B6 and BALB/c mice

Mouse strain	Age when inoculated (wk)	Week (postinoculation) of sacrifice	Diagnosis ^a	Ecotropic MuLV ^b
C57BL/6N	4	4	Stage 2	3.9, 4.1, 4.2
		7	Stage 2-3	4.5, 4.7, 4.8
BALB/cAnN	4	4	+R	0.3, 0.9, 1.0
		7	+R	0.6, 2.1, 2.7
BALB/cAnN	1	26	+R	—, —, 0.3, 1.0
		7	+++R	4.0, 4.3, 4.5
		26	Stage 2-3	3.0, 3.0, 3.8, 4.1

^a Stages are based on gross and histopathological criteria as in Table 1. R, Reactive.

^b Log₁₀ XC PFU per 10⁷ spleen cells of individual mice. —, No detectable XC plaques.

TABLE 4. Characterization of MuLVs recovered from mice of *Fv-1* incompatible strains which developed MAIDS

Mouse strain	Genotype		Inoculum	Diagnosis ^a	Virus recovery ^b			
	<i>Fv-1</i>	<i>Rmcf</i>			Ecotropic		MCF	
					PFU	Tropism	FFU	Tropism
P/N	<i>n/n</i>	<i>s/s</i>	None	Normal (210)	2.0	N	tr	N
			None	Normal (210)	2.8	nd ^c	Neg	
			LP-BM5 MuLV	Stage 3 (204)	5.5	N	4.1	N
			LP-BM5 MuLV	Stage 1 and B lymphoma* (258)	4.0	N	3.4	N
(B6 × CBA/N)F ₁	<i>n/b</i>	<i>s/r</i>	None	Normal (266)	—		Neg	
			None	Normal (266)	—		Neg	
			LP-BM5 MuLV	Stage 3* (151)	4.4	NB;B	tr	B
			LP-BM5 MuLV	Stage 2 (110)	4.4	NB	Neg	
			LP-BM5 MuLV	Stage 3 (152)	5.1	NB	tr	nd
			LP-BM5 MuLV	Stage 3 (180)	3.9	NB	Neg	
			LP-BM5 MuLV	Stage 1 and pre-B lymphoma* (195)	4.5	NB	Neg	
(CBA/N × B6)F ₁	<i>n/b</i>	<i>s/r</i>						
C57L	<i>n/n</i>	<i>s/s</i>	LP-BM5 MuLV	Stage 3 (231)	—		1.0	N
			LP-BM5 MuLV	Stage 3 (231)	—		1.0	nd

^a Diagnoses were based on gross and histological examination (Table 1) on the day indicated in parentheses; confirmations by FACS analysis are designated by an asterisk. All mice were inoculated as adults, except for a P/N mouse killed 204 days after inoculation as a neonate.

^b Log₁₀ XC PFU or MCF FFU per 10⁷ spleen cells. Tropism refers to N/B host range. —, No detectable XC plaques. In MCF virus assays, negative cultures were subcultured at 9 to 12 days; tr, trace, MCF foci detected on passage; Neg, negative, no foci detected on passage.

^c nd, Not determined.

recovered from LP-BM5 MuLV pools (data not shown), but further experiments are required to determine whether the NB-tropic viruses isolated in this study represent selection of a minority population in the original virus stocks or whether they stem from separate *in vivo* conversions.

The final strain shown in Table 4 is C57L, which lacks endogenous ecotropic MuLV sequences (17). No ecotropic virus was recovered, either before or after development of disease, but N-tropic MCF MuLVs were detected in the mice with MAIDS, albeit at a low level.

DISCUSSION

This study establishes that the natural history of development of MAIDS is through progressive stages with B-cell activation and proliferation as a central feature, accompanied by a gradual decrease in the proportion of small T and B lymphocytes. The expansion of lymphoid tissue is primarily due to B cells in various stages of differentiation from immunoblasts to plasma cells (Fig. 1). The initial source of these cells is the lymphoid follicle, which undergoes characteristic structural changes that appear to be irreversible and progressive, but the rate at which they occur varies with the susceptibility of the strain. Thus, in B6 mice the onset is rapid (a matter of 3 to 4 weeks after inoculation until the initial signs of disease can be detected). Successive stages follow at a fairly uniform rate, most animals having advanced lesions by 2 to 4 months. On the other hand, the less susceptible F₁ progeny of crosses between B6 and CBA/N develop stage 2 or 3 disease in 3 to 10 months, and strains with more limited susceptibility may take a year or more to reach similar stages.

As noted in earlier studies of immunoglobulin isotype expression (21), immunoglobulin heavy and light chain gene rearrangements (13), and expressed immunoglobulin repertoires (Klinman and Morse, *in press*), the initial phase of B-cell proliferation in strains of high or more limited susceptibility is initially polyclonal. Polyclonality may give way to oligoclonality and then monoclonal disease as MAIDS progresses, but these changes cannot be distinguished on the

basis of cytology (13) (Fig. 1) or size of the spleen or nodes (Table 1). It is only in cases of long-term expanded B-cell proliferation that most nodes contain a high proportion of immunoblasts, as seen in stage 3 disease.

Inbred mouse strains segregate into three or more patterns of clinical response to adult infection with LP-BM5 MuLV preparations. As compared with the prototypic response of B6, it is possible to identify highly sensitive strains, all of which are *Fv-1^b*; highly resistant strains, comprising both *Fv-1ⁿ* and *Fv-1^b*; and, also including both *Fv-1* genotypes, strains of an intermediate phenotype characterized by disease of low frequency and variable latency.

Dominant restrictive alleles at both *Fv-1* and *Rmcf* have been shown to have major modifying effects on MuLV-associated spontaneous and virus-induced lymphoid disease (15, 25). At first inspection, it may appear that sensitivity to MAIDS shows some independence from controls provided by restriction of virus replication mediated by these loci. Several lines of evidence suggest, however, that to establish infection leading to MAIDS, efficient replication of a helper MuLV is required. First, as reported in work to be presented elsewhere (Chattopadhyay et al., *in press*), development of MAIDS is strongly associated with the presence of a 4.8-kilobase genome, which contains MCF and/or xenotropic MuLV *env* sequences and which is dependent on helper MuLV for replication. Second, the increased sensitivity to MAIDS of neonatally inoculated BALB/c mice as compared with adults (Table 2) was strongly correlated with increased efficiency of ecotropic MuLV replication (Table 3). Third, in the three instances of sensitivity to MAIDS displayed in genetic backgrounds inhibitory to B-tropic MuLV, we recovered MuLVs with host ranges allowing unrestricted replication (Table 4). N-tropic ecotropic MuLV, presumably of endogenous origin, was recovered from P/N mice. From hybrids of B6 and CBA/N mice, NB-tropic ecotropic MuLVs were recovered with surprisingly high frequency, presumably representing mutants of LP-BM5 B-ecotropic MuLV that were in the original virus stocks or that were newly generated. For C57L mice, from which no ecotropic

and only small amounts of MCF MuLV were recovered, one can speculate that C57L (*H-2^b*) has an innate, possibly *H-2*-based susceptibility to MAIDS and that if a defective genome is rescued by even a rather poorly replicating N-tropic helper virus, disease will eventually ensue. In AKR mice, another example of a *Fv-1ⁿ* strain with susceptibility to LP-BM5 MuLV-induced disease, high levels of N-tropic ecotropic virus are expressed shortly after birth (26) and N-tropic recombinant viruses of the MCF class are generated in the thymus by 6 months of age (9). Taken together, these observations suggest that since *Fv-1*-controlled restriction is not absolute (22), the initial establishment of a defective genome in a small number of cells could be revealed by later rescue with an unrestricted helper MuLV.

An important role for replication-competent MCF MuLV in induction of the generality of MAIDS cases appears unlikely. Neither mixtures of ecotropic MuLV and biologically cloned LP-BM5 MCF MuLVs nor harvests of cocultivations of ecotropic and MCF MuLVs have induced MAIDS in B6 mice (Chattopadhyay et al., submitted). Also, the presence of the MCF MuLV-restrictive *r* allele of *Rmcf* is ineffective in preventing disease (Table 1; Fig. 2). However, roles for MCF MuLV in modifying host immune responses or in helper virus activity have not been ruled out.

The basis of resistance in certain *Fv-1^b* strains is not obvious from the data presented here, but the general refractoriness of mice of the *H-2^d* haplotype suggests that genes in the MHC region might be involved. In only one of four *Fv-1^b* *H-2^d* strains did any mice inoculated as adults develop disease (three of nine SEC/1ReJ, with only one classified as having advanced disease [Table 2]). As will be reported elsewhere (Makino et al., in preparation), major associations between *H-2* haplotypes, sensitivity or resistance to MAIDS, and replication of ecotropic and MCF MuLVs have been identified.

The differences in strain sensitivity identified in this study will provide useful biological systems for examining the complex interplay between viruses and the immune system in the pathogenesis of MAIDS.

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