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Major Histocompatibility Complex Class II-Regulated Immunity to Murine Leukemia Virus Protects against Early T- but Not Late B-Cell Lymphomas

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We studied the relative importance of class I and class II major histocompatibility complex (MHC) immunoregulation in the control of T- and B-cell lymphomas induced by murine leukemia virus. Previously, we have described a mink cell focus-inducing (MCF) murine leukemia virus, MCF 1233, which induces not only lymphoblastic T-cell lymphomas but also follicle center cell or lymphoblastic B-cell lymphomas. We now report that the outcome of neonatal infection with MCF 1233 in *H-2*-congenic C57BL/10 and C57BL/6 mice is decisively influenced by the *H-2 I-A* locus. A total of 64% of *H-2 I-A*^{k,d} mice [B10.BR, B10.D2, B10.A(2R), B10.A(4R), and B10.MBR] developed T-cell lymphomas after MCF 1233 infection (mean latency, 37 weeks). In contrast, *H-2 I-A*^{b} [B10, B10.A(5R), B6], *H-2 I-A*$^{b/k}$ [(B10.A × B10)F$_1$ and (B10 × B10.A)F$_1$], and *H-2 I-A*^{bm12} (bm12) mice were resistant against T-cell lymphomagenesis, but 65% of these *H-2 I-A*$^{b,\ b/k,\ bm12}$ animals developed B-cell lymphomas (mean latency, 71 weeks). Animals of T-cell lymphoma-susceptible strains that escaped from T-cell lymphomagenesis developed B-cell lymphomas with similar frequency as animals of T-cell lymphoma-resistant strains, but with a shorter latency. *H-2* class II-determined regulation of antiviral immunity was reflected in the presence of high titers of antiviral envelope antibodies in T-cell lymphoma-resistant B-cell lymphoma-susceptible *H-2 I-A*$^{b,\ b/k,\ bm12}$ mice, whereas in T-cell lymphoma-susceptible *H-2 I-A*^{k,d} mice no antiviral antibodies were found. At week 4 after neonatal MCF 1233 infection, a high percentage of thymocytes were virally infected in both T-cell lymphoma-susceptible and -resistant mice. However, T-cell lymphoma-resistant animals cleared the thymic infection between weeks 4 and 10 of age, coinciding with a sharp rise in serum levels of antiviral antibodies. We conclude that the pleiotropic effects of MCF 1233 infection in *H-2*-congenic mice result from MHC class II *I-A*-determined T-cell response differences.

Lymphoma induction by slowly transforming murine leukemia viruses (MuLV) results from a complex series of oncogenic events in virus-infected cells, often including activation of cellular oncogenes by retroviral insertion into host cell DNA (1, 15). Multiple genes of the host influence tumor susceptibility, some of which are encoded by the major histocompatibility complex (MHC) (reviewed in reference 60). It is well established that MHC-restricted T-cell responses decisively influence the outcome of virus infection (13, 63). Immune response regulation by the MHC reflects the natural functions of class I and II MHC molecules as restriction elements for antigen-specific T-cell responses (reviewed in references 25 and 48).

MuLV-induced lymphomagenesis has been a model of MHC-disease association since first reported by Lilly and colleagues many years ago (28). Several immune mechanisms have been postulated to explain the MHC influence on lymphoma susceptibility, including regulation of antibody response against virus-induced tumor cells (12, 47, 55), proliferative T-cell responses (4), and regulation of cytotoxic T-cell (T_c) reactivity (20). T_c effectiveness may also be influenced by another postulated mechanism, namely, MHC allelic differences in class I MHC expression on virus-infected cells (32). The relative importance of class I MHC regulation for resistance to lymphomagenesis, reflected in T_c response differences, versus class II MHC regulation, reflected in T-helper cell (T_h) response differences (and thus differences in antibody response), in MuLV-infected mice has not been studied in detail. Moreover, studies concerning MHC influence on murine lymphomagenesis have been largely focused on T-cell lymphomagenesis, while in humans the development of B-cell lymphomas has been linked to disturbed immunoregulation (6, 14, 29, 30, 41, 42). In the mouse, besides mineral oil-induced plasmacytomas (44) and Abelson MuLV-induced pre-B-cell lymphomas (46), the only B-cell lymphomas studied in some detail have been those associated with graft-versus-host reaction. The pathogenesis of these graft-versus-host-induced B-cell lymphomas depends on MHC-directed T-cell reactivity (19, 38).

Previously, we have described the characteristics of a mink cell focus-inducing (MCF) MuLV, MCF 1233, which causes a broad spectrum of different lymphoid neoplasms (59). In a limited study, the MHC complex of the mouse (*H-2* complex) appeared to influence the frequency and the type of lymphomas induced (58). We now report that infection with MCF 1233 results in the development of both T- and B-cell lymphomas histologically similar to human non-Hodgkin's lymphomas. We observed a dramatic effect of the class II-encoding *H-2 I-A* region on lymphomagenesis by this

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virus. On one hand, we document a strong influence of the *I-A* locus on susceptibility to early T-cell lymphomas associated with a weak *I-A*-restricted T_h response. On the other hand, strong *I-A*-restricted T-cell reactivity does not prevent the development of late-arising B-cell tumors.

MATERIALS AND METHODS

Mice. The mutant mouse strains B6.C-H-2^{bm1} (bm1), B6.C-H-2^{bm12} (bm12), and B6.C-H-2^{bm14} (bm14) were purchased from the animal department of the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB), Amsterdam. B10.MBR mice came from the Max Planck Institut, Tübingen, Federal Republic of Germany (courtesy of J. Klein). All other strains were obtained from OLAC Ltd., Bicester, United Kingdom. Mice were inoculated with MCF 1233 intraperitoneally (i.p.) within 24 h after birth.

Virus. The origin and characteristics of the MCF 1233 virus were described previously (59). Briefly, this dualtropic B-tropic MuLV was biologically cloned from a T-cell tumor. This T-cell tumor arose in a B10.A mouse after milk transmission of a naturally occurring B-tropic ecotropic MuLV (31). MCF 1233 was propagated in SC-1 mouse cells, and aliquots of tissue culture supernatant were stored at −80°C. A single batch of virus-containing supernatant was used. A 0.1-ml sample of culture supernatant containing 7×10^4 focus-forming units (FFU) of virus was injected per mouse. The titer of MuLV was determined by the percentage immunofluorescence assay as described previously (59).

Characterization of lymphomas. For evaluation of tumor development, mice were observed twice a week. Moribund mice were sacrificed by cervical dislocation. Macroscopically enlarged and/or affected lymphoid organs were removed and handled in three ways. For histologic evaluation, part of the tumor was fixed in Formalin (10% in phosphate-buffered saline). Invariably, specimens of liver and spleen were fixed in Formalin independent of the macroscopic aspect. Part of the tumor was cryopreserved as a viable cell suspension in liquid nitrogen in 10% dimethyl sulfoxide as described previously (2). The remainder of the tumor was stored in liquid nitrogen. Mice still alive at week 104 (end of the follow-up period) were sacrificed and autopsied regardless their health.

(i) Immunofluorescence studies. After thawing, tumor cell suspensions were incubated at 37°C for 2 to 3 h in Iscove modified Dulbecco medium supplemented with 10% heat-inactivated fetal calf serum, penicillin (100 IU/ml), streptomycin (100 μg/ml), glutamine (2 mM), and 2-mercaptoethanol (20 μM). To remove nonviable cells and erythrocytes, we centrifuged 2 ml of cell suspension (containing a maximum of 10^8 cells) for 10 min at 1,000 × *g* on a 4-ml cushion of Ficoll-Hypaque (1.079 g/cm^3). Consistently more than 90% of tumor cells prepared in this way were viable. After washing, the cells were suspended in phosphate-buffered saline with 2% bovine serum albumin and 0.1% sodium azide. We used monoclonal antibodies directed against T200 (30G12), Thy-1 (59 AD2.2), Lyt-1 (53-7.3), Lyt-2 (53-6.7) (26), L3T4 (H129.19) (43), B220 (RA3-2C2 and RA3-6B2) (9), mac-1 (MI/70.15) (51), and *I-A* (17/227R7) and *I-E* (13/18) (27). As second antibodies, the following fluorescein isothiocyanate (FITC)-labeled antisera were used: rabbit anti-rat immunoglobulin (1:40; Organon Teknika, Malvern, Pa.) and goat anti-mouse immunoglobulin (1:20; CLB, Amsterdam).

Criteria used to determine the lineage of the lymphomas analyzed this way were as follows (52). Lymphomas were phenotypically classified as T-cell tumors if >50% of the cells stained positively for Thy-1 and/or L3T4 and/or Lyt-2 antigens. If >50% of the cells stained with fluorescein-labeled goat anti-mouse immunoglobulin or >50% of the tumor cells expressed the B220 antigen or both, lymphomas were classified as B-cell tumors. If 30 to 50% of the cells expressed Thy-1 antigen and in addition 30 to 50% of the cells expressed surface immunoglobulins or B220 antigen or both, tumors were phenotypically considered mixtures of T and B cells. Lymphomas lacking expression of Thy-1, L3T4, Lyt-2, surface immunoglobulin, and/or B220 were phenotypically classified as null-cell tumors.

TABLE 1. Classification criteria for MCF 1233-induced lymphomas

Histology[a]	Phenotype[b]	Genotype[c]	Classification
FCC	B	B[d]	B
	Null	B[d]	B
	T + B mix	B[d]	B
	NT[e]	B[d]	B
LB	T	T[d]	T
	B	B[d]	B
	Null	T, B	According to genotype
	T + B mix	T, B	According to genotype
	NT	T, B	According to genotype
ND[f]	All categories	T, B, hyperplasia[g]	According to genotype
Hyperplasia	All categories	T, B, hyperplasia	According to genotype

[a] Pattengale and Taylor classification (39); FCC, follicle center cell lymphoma; LB, lymphoblastic lymphoma.
[b] Criteria mentioned in Materials and Methods.
[c] Determined by TCRbeta-chain, immunoglobulin heavy-chain, and immunoglobulin light-chain kappa gene configuration.
[d] Genotypic analysis performed in a majority but not all cases.
[e] NT, Not tested.
[f] ND, Not done; 139 of the 260 tumors described (53%) have not been characterized histologically.
[g] Hyperplasia criteria at genotypic analysis: no (oligo)clonal TCRbeta-chain, immunoglobulin heavy-chain, or immunoglobulin light-chain kappa gene rearrangements detected.

(ii) Pathologic evaluation. Formalin-fixed sections were screened for tumor involvement. The histologic classification criteria of Pattengale and Taylor (39) were used to determine lymphoma type.

(iii) DNA analysis. Genotypic characterization of the lymphoma DNAs involved analysis of T-cell receptor (TCR) beta-chain gene and immunoglobulin kappa light-chain gene configurations. In selected cases, immunoglobulin heavy-chain region gene configuration was determined as well. A detailed description of this procedure has been given elsewhere (52). Table 1 summarizes final classification criteria for MCF 1233-induced lymphomas, as determined in a previous study (52).

Measurement of anti-viral envelope antibodies. Blood was obtained by orbital puncture from 12-week-old mice which had been inoculated with MCF 1233 and observed for tumor development. If thymectomy was performed, mice were bled at autopsy. Antibodies to MCF 1233 envelope antigens were detected by precipitation of either intact ^{3}H-labeled MCF 1233 virus or intact ^{3}H-labeled 12N4Cl165 virus, an ecotropic AKR-type virus which shares many epitopes with MCF 1233 (59). Anti-viral envelope antibodies showed broad cross-reactivity to these two viruses, as confirmed for a limited panel of sera in our study (data not shown). Details of

TABLE 2. *H-2 I-A* influence on the outcome of MCF 1233 infection

Strain	*H-2* type					No. neonatally infected with MCF 1233	No.[a] evaluable at wk 104	No. of tumors (wk 0–104) (%)	No. of classified tumors (%)[b]			Mean latency of tumors in weeks (SEM)		
	K	*I-A*	*I-E*	*D*	*TL*				Total	T	B	Total	T	B
B10	*b*	*b*	*b*	*b*	*b*	31	23	15 (65)	9	3 (33)	6 (66)	73 (6)	45 (2)	82 (7)
B10.A(5R)	*b*	*b*	*k*	*d*	*a*	38	34	20 (59)	17	4 (24)	13 (76)	68 (4)	57 (8)	73 (5)
B10.A(4R)	*k*	*k*	*b*	*b*	*b*	63	42	40 (95)	33	25 (76)	8 (24)	48 (2)	42 (2)	60 (5)
B10.A(2R)	*k*	*k*	*k*	*b*	*b*	27	17	16 (94)	15	10 (67)	5 (33)	44 (5)	35 (3)	67 (12)
B10.A	*k*	*k*	*k*	*d*	*a*	37	29	27 (93)	23	15 (65)	8 (35)	42 (4)	35 (6)	49 (5)
B10.MBR	*b*	*k*	*k*	*q*	*a*	26	22	22 (100)	21	18 (86)	3 (14)	31 (2)	28 (2)	42 (2)
B10.BR	*k*	*k*	*k*	*k*	*b*	31	23	21 (91)	15	13 (87)	2 (13)	41 (4)	34 (2)	76 (28)
B10.D2	*d*	*d*	*d*	*d*	*c*	34	20	20 (100)	18	13 (72)	5 (28)	51 (4)	47 (5)	62 (11)
(B10.A × B10)F_1	*b/k*	*b/k*	*b/k*	*b/d*	*b/a*	22	19	17 (89)	15	2 (13)	13 (87)	72 (4)	58 (5)	74 (4)
(B10 × B10.A)F_1	*b/k*	*b/k*	*b/k*	*b/d*	*b/a*	34	27	27 (100)	25	3 (12)	22 (88)	64 (4)	27 (4)	69 (3)
B6	*b*	*b*	*b*	*b*	*b*	23	17	15 (88)	10	1 (10)	9 (90)	69 (6)	69	67 (7)
bm12	*b*	*bm12*	*b*	*b*	*b*	41	27	20 (74)	14	3 (21)	11 (79)	80 (4)	97 (4)	72 (5)

[a] Animals for which the cause of death was unknown and animals which died from causes other than lymphoma were withdrawn from the study.

[b] Classification criteria as listed in Table 1. Tumors for which insufficient material was available (15%) remained unclassified. In addition, one histiocytic B10 lymphoma and one lymphocytic B6 lymphoma, both lacking clonal TCRbeta-chain or immunoglobulin gene rearrangements or both, were considered to be unclassified, as were histiologically and/or genotypically biclonal (T plus B) lymphomas [B10.A, $n = 1$; B10.A(4R), $n = 1$; (B10.A × B10)F_1, $n = 2$].

the radioimmunoprecipitation assay have been described previously (53). The titer against MuLV was determined as the serum dilution that precipitates 50% of the maximal virus precipitation. Six aliquoted standard sera served as the control for day-to-day variations.

Viral infection of lymphoid organs. Cell suspensions of thymus and spleen were prepared by gently pressing the organs through nylon gauze. Bone marrow cell suspensions were obtained by flushing femurs. Cells were pelleted and suspended in phosphate-buffered saline with 2% bovine serum albumin and 0.1% sodium azide. Erythrocytes were removed from splenocytes by NH_4Cl treatment. Cells were incubated with an FITC-conjugated goat antiserum against Tween-ether-disrupted Moloney MuLV (1:40 dilution, extensively absorbed to normal mouse spleen cells [a gift from J. Gruber]), which reacts with all known structural proteins of both MCF virus and ecotropic MuLV. In addition, cells were incubated with monoclonal antibody HY13 (tissue culture supernatant, 1:1 dilution) reactive to gp-70–p15(E) complex–$pr80^{env}$ viral antigens of a broad panel of MCF viruses (8) (a gift from M. W. Cloyd, National Institutes of Health, Bethesda, Md.). As the second antibody, FITC-conjugated goat anti-mouse immunoglobulin (1:20 dilution; CLB, Amsterdam) was used.

FACS/Epics analysis. All cell populations were at least 75% viable as determined by trypan blue uptake and low-angle light scatter at the time of immunofluorescence analysis. Nonviable cells and erythrocytes, generally those below channel 70, were gated out. Fluorescence was analyzed on an Epics C (Coulter Electronics, Inc., Hialeah, Fla.) or FACS IV (Becton Dickinson and Co., Paramus, N.J.).

Statistical analysis. Tumor survival index curves were made by the actuarial life survival method. Mean antibody titers are given as geometric mean titers. Statistics on differences in thymic infection grade between *H-2* congenic strains were performed by the Mann-Whitney U test.

RESULTS

Intra-*H-2* mapping of susceptibility to lymphomas induced by MCF 1233. We set out to map the loci within the *H-2* complex that influence the outcome of MCF 1233 infection. To this end, we chose a panel of *H-2*-congenic C57BL/10 strains, the *H-2* haplotypes of which are listed in Table 2. These strains develop spontaneous lymphomas at a very low frequency (10 to 15% at week 104) (31; unpublished data). All mice were neonatally inoculated (i.p.) with MCF 1233 and observed for symptoms of disease twice a week. At autopsy of moribund mice, most tumors were found as generalized lymphomas involving more than one of the following organs: thymus, spleen, lymph nodes (mesenteric, thoracic, axillary, and/or inguinal), Peyer's patches, liver, and kidney. The course of MCF 1233 lymphomagenesis is illustrated in Fig. 1 and summarized in detail in Table 2.

B10 ($H\text{-}2^b$) mice were relatively resistant to tumor induction by MCF 1233 compared with B10.D2 ($H\text{-}2^d$) and B10.BR ($H\text{-}2^k$) mice (Fig. 1a). These two tumor-susceptible strains developed lymphomas from about week 28 of age to a cumulative death rate at the end of the study (week 104) of 90 to 100%. In contrast, the earliest tumors in resistant B10 ($H\text{-}2^b$) mice arose about week 40, and at week 104, 35% of these mice were still tumor free.

To allow a precise mapping of the locus (loci) within the *H-2* complex that determines the susceptibility to MCF 1233-induced lymphomagenesis, we infected a broad panel of intra-*H-2* recombinant C57BL/10 mouse strains with MCF 1233 (Fig. 1b; Table 2). These intra-*H-2* recombinant strains behaved identically to either the prototype resistant B10 strain [e.g., B10.A(5R)] or the prototype susceptible B10.D2 and B10.BR strains [e.g., B10.A, B10.A(2R), and B10.A(4R)].

The data allow the unambiguous conclusion that the class II-encoding *H-2 I-A* region has a major influence on the course of MCF 1233-induced lymphomagenesis. C57BL/10 *H-2* $I\text{-}A^b$ mice were relatively tumor resistant, while *H-2* $I\text{-}A^k$ and *H-2* $I\text{-}A^d$ mice were tumor susceptible. We did not observe any influence of the *I-E* locus on lymphoma susceptibility; both B10 ($I\text{-}A^b$, $I\text{-}E^b$) and B10.A(5R) ($I\text{-}A^b$, $I\text{-}E^k$) mice were lymphoma resistant, whereas both B10.A(2R) ($I\text{-}A^k$, $I\text{-}E^k$) and B10.A(4R) ($I\text{-}A^k$, $I\text{-}E^b$) mice were susceptible (Fig. 1b; Table 2).

Apart from the *I-A* locus, the *D* region appeared to exert an additional influence on tumor susceptibility, because

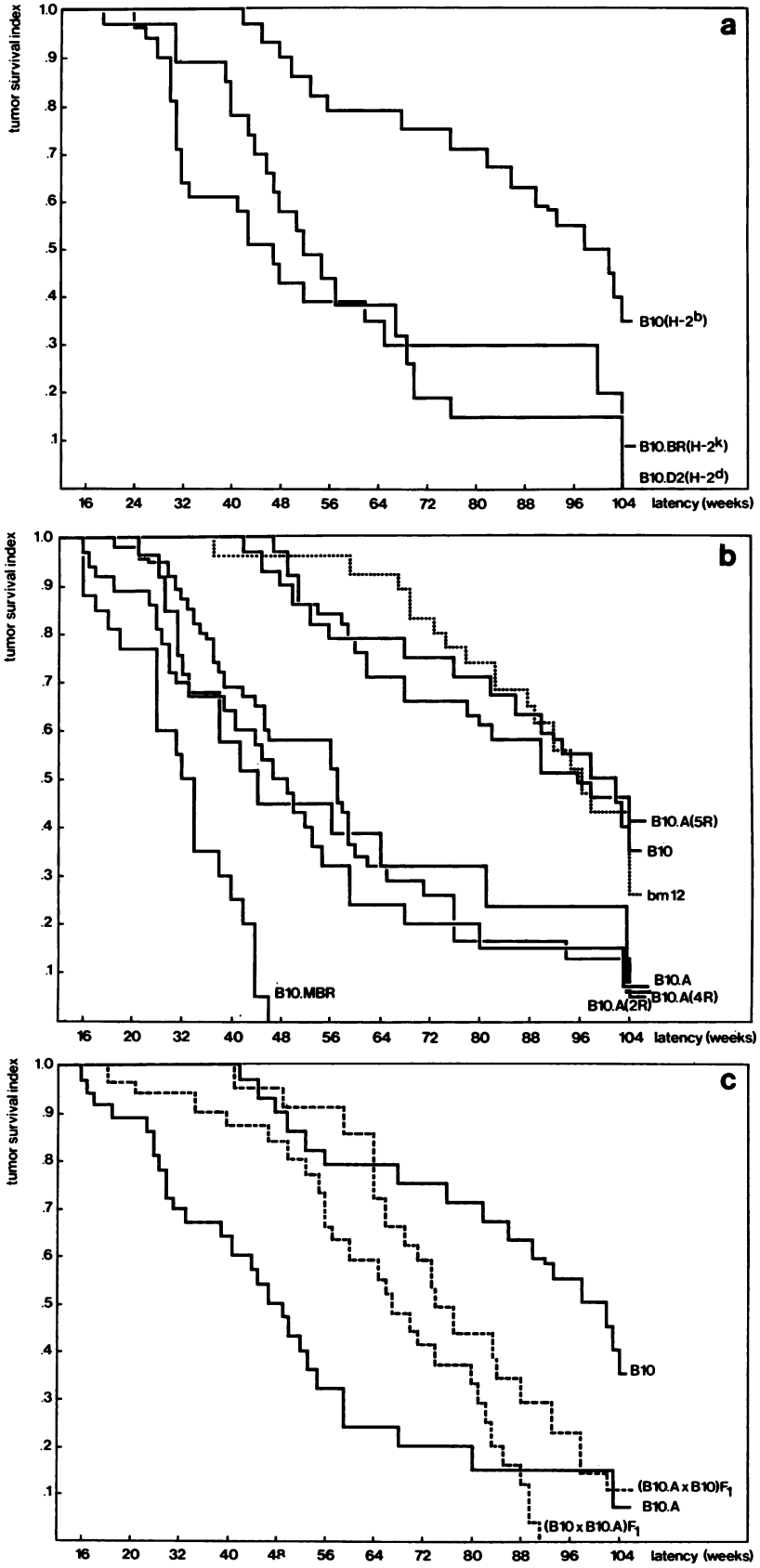

FIG. 1. Tumor survival index curves of MCF 1233-infected *H-2*-congenic C57BL mice. (a) B10, B10.D2, and B10.BR mice; (b) various intra-*H-2* recombinant C57BL/10 mice and *H-2 I-A*^{b} mutant bm12 mice; (c) B10, B10.A, (B10.A × B10)F_1, and (B10 × B10.A)F_1 mice. Newborn animals were inoculated i.p. with 7×10^4 FFU of MCF 1233. Tumor survival index is given by the actuarial life table method. Mice which died from causes other than tumor or which had no known cause of death were censored from the time point of death. More details are given in Table 2.

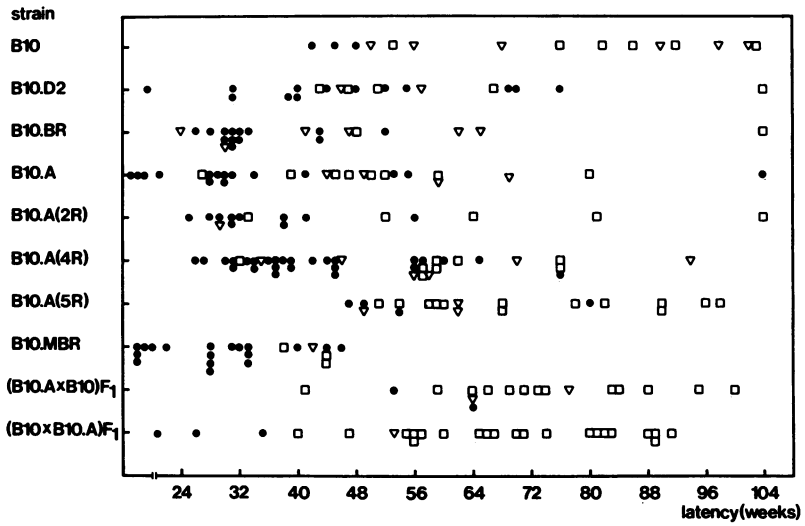

FIG. 2. Correlation between latency and type of MCF 1233-induced tumors in various *H-2*-congenic C57BL/10 strains. Depicted are lymphomas as listed in Table 1. Symbols: ●, T-cell lymphoma; □, B-cell lymphoma; ▽, unclassified lymphoma. Classification criteria are as in Table 1.

B10.MBR mice (K^b, $I\text{-}A^k$, $I\text{-}E^k$, D^q) were extremely sensitive to lymphoma development. In B10.MBR mice, lymphomas arose earlier than in mice which have D^b, D^d, or D^k alleles in combination with $I\text{-}A^k$. We found no correlation between *TL* haplotype and lymphoma susceptibility (Table 2).

The (B10.A × B10)F_1 and (B10 × B10.A)F_1 hybrids (Fig. 1c; Table 2) were relatively resistant to early tumor induction by MCF 1233 similar to the B10 parent, but ultimately developed lymphomas at a higher percentage than the B10 strain.

Given the important role of *H-2* class II *I-A* molecules in the course of MCF 1233 infection, it was of interest to determine whether the *H-2* $I\text{-}A^b$ mutant strain bm12 would show a difference from other *H-2* $I\text{-}A^b$ mice. Mice of the C57BL/6 strain (B6, $H\text{-}2^b$), of which the bm12 mutant strain was derived, had the same pattern of disease upon MCF 1233 infection as C57BL/10 (B10, $H\text{-}2^b$) mice (Table 2). bm12 mice showed similar resistance to early lymphoma development as B10, B10.A(5R), and B6 mice (Fig. 1b, Table 2). Therefore, the bm12 mutation in the $I\text{-}A^b$ molecule does not affect its role as an immune response gene in MCF 1233 infection. In addition, a small number of C57BL/6 class I $H\text{-}2K^b$ mutant bm1 mice and class I $H\text{-}2D^b$ mutant bm14 mice were infected with MCF 1233. These mice generally showed the same pattern of disease upon MCF 1233 infection as other *H-2* $I\text{-}A^b$ mice (data not shown).

Characterization of lymphomas. We characterized the MCF 1233-induced lymphomas using the same methodology as reported earlier (52), based on histologic typing, cell surface marker analysis, and genotypic analysis (TCR beta-chain gene and immunoglobulin kappa-chain gene configuration). Classification criteria are summarized in Table 1.

(i) Histology. Of 260 lymphomas, 121 (47%) were characterized histologically. The lymphomas fell into two main categories: lymphoblastic tumors and (diffuse or nodular) follicular center cell tumors. The subtype of the follicular center cell tumors (diffuse or nodular; small, large, or mixed cell type) did not correlate with a specific antigenic profile, latency, or strain of origin of the tumor. Besides lymphoblastic and follicular center cell tumors, one histiocytic (B10) and one lymphocytic (B6) lymphoma were found.

(ii) Phenotype. A detailed discussion of the phenotypic lymphoma variants is beyond the scope of this study, but a few points should be made. In general, T-cell lymphomas showed either a double-positive phenotype ($L3T4^+$ $Lyt\text{-}2^+$) or a helper phenotype ($L3T4^+$ $Lyt\text{-}2^-$). Double-negative ($L3T4^-$ $Lyt\text{-}2^-$) or exclusively $Lyt\text{-}2^+$ tumors were rare (data not shown). Within T- or B-cell lymphomas, no specific antigenic profile associated with latency or strain of origin of the tumors was apparent. One interesting exception was the group of early tumors in B10.MBR mice, which coexpressed both T- and B-cell-specific antigens. These Thy-1/B220-positive lymphoblastic lymphomas, which had clonal TCR beta-chain rearrangements, have been described in more detail in a previous report (52). In addition to the B220 antigen, all B-cell lymphomas expressed *H-2* class II (*I-A* and *I-E*) antigens. Most B-cell lymphomas expressed high levels of surface immunoglobulin, which is in agreement with genotypic analysis in which only one pre-B-cell lymphoma was found.

(iii) Clonality. In addition to lineage determination criteria, analysis of the TCR and immunoglobulin gene constellation of the lymphomas yielded information on the clonality of the tumors. A total of 98% of MCF 1233-induced T- and B-cell lymphomas tested for TCR or immunoglobulin gene rearrangements or both (n = 200) were of monoclonal origin (data not shown). Only four lymphomas (2%) contained both clonal T- and B-cell populations.

MHC influence on lymphoma type. Figure 2 shows the results of the lymphoma characterization studies in the various *H-2*-congenic C57BL/10 strains. Table 2 provides a summary of the lymphoma-typing data. It can be seen that in tumor-susceptible *H-2* $I\text{-}A^{k,d}$ mice, the T-cell tumors predominate (75% of the classified lymphomas), while in *H-2* $I\text{-}A^{b,\ b/k,\ bm12}$ mice, mostly B-cell tumors are found (82% of the classified lymphomas). Thus, the *H-2* *I-A* locus not only influences the incidence of lymphomas induced by MCF 1233 but also dramatically affects the lymphoma type. The mean latency of the T-cell tumors in $I\text{-}A^{k,d}$ mice is 37 weeks, and that of B-cell tumors in $I\text{-}A^{b,\ b/k,\ bm12}$ mice is 71 weeks. Within a given C57BL/10 strain, the mean latency of T-cell tumors is always shorter than that of B-cell tumors.

TABLE 3. Development of B-cell lymphomas in MCF 1233-infected *H-2*-congenic C57BL mice

H-2 I-A	No. infected neonatally with MCF 1233	No. evaluable at wk 104[a]	No. of tumors (wk 0–104) (%)[b]				% B lymphomas in T-lymphoma free animals[c]
			Total	Unclassified	T	B[d]	
b, b/k, bm12[e]	189	147	114	24 (21)	16 (14)	74 (65)	59 (74/107)
k, d[f]	218	153	146	21 (14)	94 (64)	31 (21)	81 (31/38)

[a] As in Table 2, footnote *a*.
[b] As in Table 2, footnote *b*.
[c] T-lymphoma-free animals were evaluable animals that did not develop unclassified tumors or T-cell tumors.
[d] Mean latency of B-cell tumors in *H-2 I-A*$^{b,\ b/k,\ bm12}$ mice is 71 weeks, and in *H-2 I-A*$^{k,\ d}$ mice it is 58 weeks ($P < 0.001$; Mann-Whitney U test).
[e] T-lymphoma-resistant strains (*H-2 I-A*$^{b,\ b/k,\ bm12}$: B10, B10.A(5R), (B10 × B10.A)F_1, (B10.A × B10)F_1, B6, bm12.
[f] T-lymphoma-susceptible strains (*H-2 I-A*$^{k,\ d}$): B10.A, B10.A(2R), B10.A(4R), B10.MBR, B10.D2, B10.BR.

The question can be raised whether T-cell tumor-resistant mice (*H-2 I-A*$^{b,b/k,bm12}$) develop more B-cell tumors than mice of T-cell lymphoma-susceptible strains (*H-2 I-A*^{k,d}) which have escaped the development of early T-cell tumors. This question is addressed in Table 3. In this analysis, data from C57BL/10 and C57BL/6 mice are taken together. As most *H-2 I-A*^{k,d} mice died relatively early from T-cell lymphomas, only a small group could be evaluated with respect to late B-cell lymphoma development. It can be concluded that although *H-2 I-A*$^{b,b/k,bm12}$ mice were resistant to the development of T-cell lymphomas, the reverse is not true; *H-2 I-A*^{k,d} mice which escaped T-cell lymphoma development did get B-cell lymphomas. It is of note that the mean latency of B-cell lymphomas was significantly shorter in *H-2 I-A*^{k,d} mice (58 weeks) than in *H-2 I-A*$^{b,b/k,bm12}$ mice (71 weeks) ($P < 0.001$, Mann-Whitney U test).

Anti-viral envelope antibodies. Production of anti-viral envelope antibodies after inoculation with MCF 1233 may contribute to eradication of the infection and prevention of subsequent lymphomagenesis. Virus-precipitating antibodies were assayed at week 12 in the serum of mice neonatally infected with MCF 1233. Tumor development was monitored as well. The results (Fig. 3) showed a marked correlation between high mean antibody titer and relative resistance against early T-cell tumors in *H-2 I-A*$^{b,b/k,bm12}$ mice. The numbers of animals of T-cell lymphoma-resistant *I-A* type (*I-A*$^{b,b/k,bm12}$) with exceptional low antiviral antibody titers (<80) as well as the numbers of animals of susceptible *I-A* type (*I-A*^{k,d}) with high antibody titers (>80) were too low to test the hypothesis that antibodies are directly involved in T-cell lymphoma resistance.

Thymic infection. One reason for the observed relative resistance to MCF 1233-induced T-cell lymphomagenesis could be that MCF 1233 infection involves different target cells in the various *H-2*-congenic C57BL/10 strains. We therefore analyzed the viral infection grade of thymocytes, splenocytes, and bone marrow cells after neonatal MCF 1233 inoculation of these strains. The percentage of cells expressing viral antigens, determined by immunofluorescence with the anti-MCF gp70–p15(E) complex–pr80^{env} monoclonal antibody HY13 or the polyvalent FITC-conjugated anti-MuLV serum, was taken as a measure of viral infection. No discrepancies between the percentage of anti-FITC-MuLV or HY13-reactive cells were found. In Fig. 4, the course of viral infection of the thymus is shown for both a T-cell lymphoma-susceptible strain, B10.D2 (*H-2*^{d}), and a resistant strain, B10 (*H-2*^{b}). In addition, (B10 × B10.D2)F_1 animals were tested. The similar grade of infection in these strains at week 4 after neonatal MCF 1233 inoculation clearly indicates that MCF 1233 infected thymocytes of both T-cell tumor-susceptible and -resistant strains with similar efficiency. After week 4, a dramatic difference was observed between B10.D2 and B10 mice. B10 mice cleared their thymuses of viral infection, whereas the B10.D2 thymocytes remained virus infected. Monitoring of the titer of anti-viral envelope antibodies during the first weeks after MCF 1233 infection (Fig. 5) showed that the sharp rise in antibody levels in B10 mice preceded the clearance of virus from the thymus.

In the spleen (Fig. 4), B10.D2 mice had a markedly high percentage of virally infected cells at week 6, probably reflecting immigration of infected thymocytes during weeks

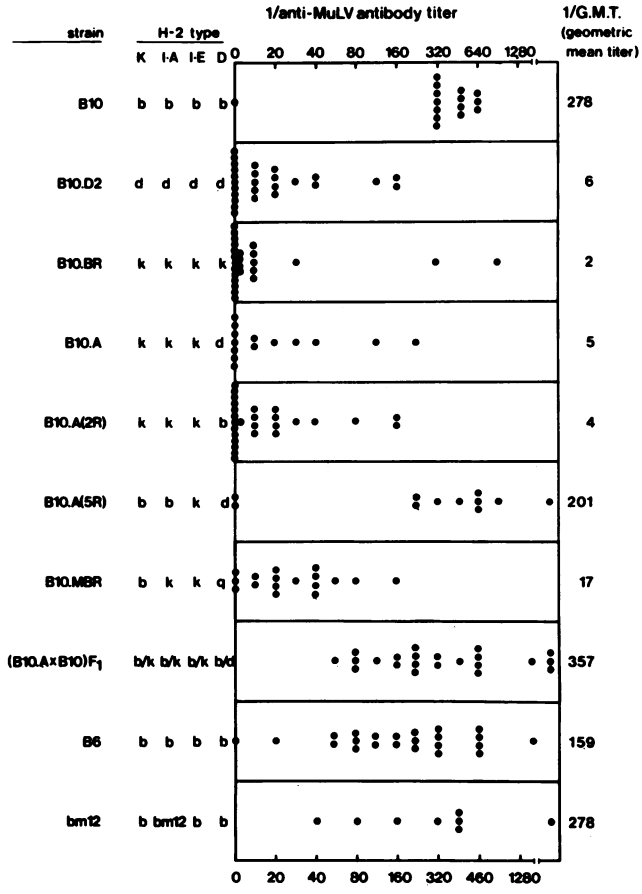

FIG. 3. Anti-MuLV antibody titers in various *H-2*-congenic C57BL mice after neonatal inoculation with MCF 1233. At week 12 of age, sera of MCF 1233-infected mice were tested by a radioimmunoassay for the presence of MuLV-precipitating antibodies. Sera of B10.A(4R) and (B10 × B10.A)F_1 mice were not available. Titer against MuLV is defined as the serum dilution that precipitates 50% of the maximal virus precipitation.

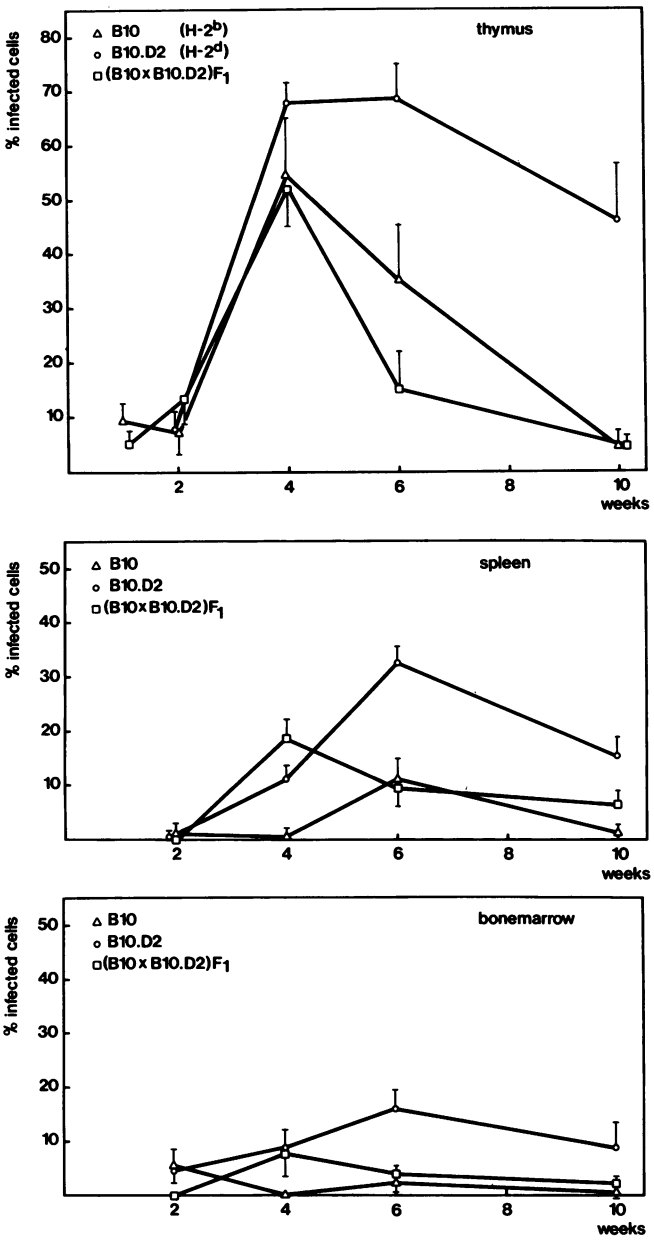

FIG. 4. Kinetics of MCF 1233 infection of thymus, spleen, and bone marrow in a prototype T-cell lymphoma-susceptible strain (B10.D2, *H-2*^{d}), a T-cell lymphoma-resistant strain (B10, *H-2*^{b}), and the (B10 × B10.D2)F_1 hybrid. Newborn mice were inoculated i.p. with 7 × 10^4 FFU of MCF 1233. At various time points, cells of different lymphoid organs were tested in immunofluorescence studies for the expression of viral antigens by their reactivity with a broadly reactive polyclonal goat anti-Moloney MuLV serum. The percentage of viral antigen-expressing cells in MCF 1233-infected mice was measured relative to that of cells of noninfected age-matched control mice. The mean percentage of virus-infected cells is indicated. Bars represent standard error of the mean.

4 to 6, while migrating infected B10 thymocytes are already destroyed by the immune system. In B10 mice, virally infected bone marrow cells were barely detectable throughout the 10 weeks studied, whereas in B10.D2 mice, a small percentage of virally infected cells was found in the bone marrow at weeks 4 to 10.

(B10 × B10.D2)F_1 mice largely followed the pattern of the

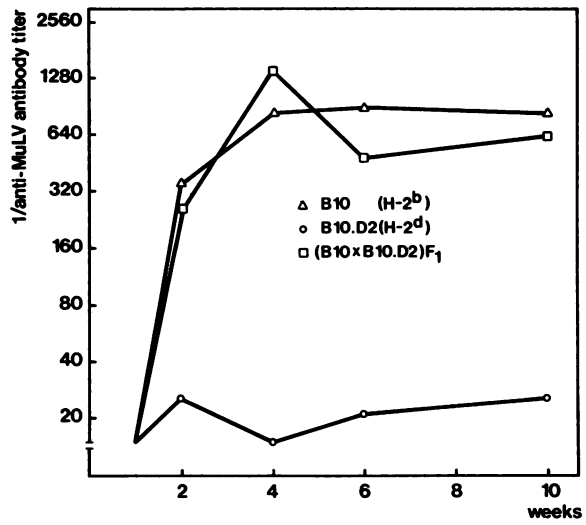

FIG. 5. Kinetics of anti-MuLV antibody response after neonatal MCF 1233 infection of B10.D2, B10, and (B10 × B10.D2)F_1 mice. Viral infection of lymphoid organs in these mice is depicted in Fig. 4. Titers of MuLV-precipitating antibodies were determined by a radioimmunoassay. Curves represent geometric mean titers at various time points after infection.

T-cell lymphoma-resistant B10 parent with respect to viral infection of thymus, spleen, and bone marrow upon MCF 1233 inoculation (Fig. 4), although the virus expression in spleen and bone marrow tended to be somewhat higher at week 4.

Next, we explored whether the same difference in the course of viral infection was seen between other T-cell lymphoma-resistant and -susceptible strains. Therefore, we measured the percentage of viral antigen-expressing thymocytes in various intra-*H-2* recombinant C57BL/10 strains at week 8 after neonatal MCF 1233 infection (Fig. 6). This analysis allowed a more general interpretation of the data obtained with B10 and B10.D2 mice. Mice of T-cell lymphoma-susceptible strains such as B10.A, B10.A(2R), B10.A(4R), and B10.MBR did not clear their thymuses of MCF 1233 infection by week 8. In contrast, most mice belonging to T-cell lymphoma-resistant strains, e.g., B10, B10.A(5R), and (B10 × B10.A)F_1, successfully did so.

DISCUSSION

Despite the fact that MuLV-induced lymphomagenesis was the first documented example of MHC-disease association (28), the mechanisms underlying tumor susceptibility are still poorly understood. Although it is widely assumed that immunoregulation has a decisive influence, only limited data concerning this issue are available.

Here we report that infection of *H-2*-congenic C57BL mice with the MCF 1233 MuLV led to the development of T-cell lymphomas with a relatively short latency if these mice had *H-2 I-A*^{k} or *I-A*^{d} alleles, whereas *H-2 I-A*$^{b,b/k,bm12}$ mice developed B-cell lymphomas with a longer latency. B-cell lymphomas also developed in the few *I-A*^{k} or *I-A*^{d} mice which survived early T-cell lymphomagenesis; however, the latency of these B-cell lymphomas was significantly shorter than in *I-A*$^{b,b/k,bm12}$ mice. Since class II molecules are the restriction elements for T_h reactivity (48), our data indicate a central role for *I-A*-restricted T_h in the outcome of MCF 1233 infection. In our study, strong T_h activity in mice

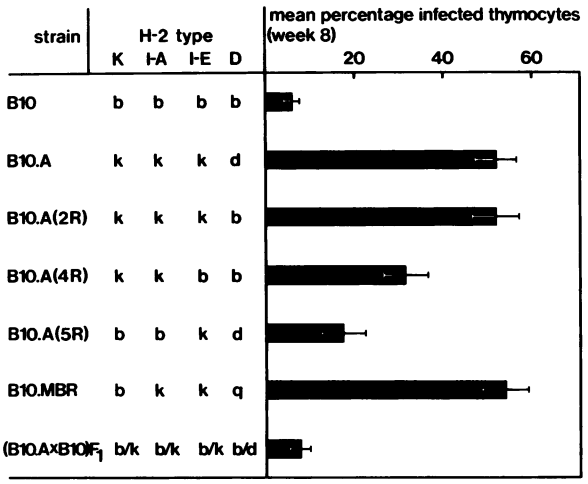

FIG. 6. Thymic infection at week 8 after neonatal MCF 1233 infection of intra-*H-2* recombinant C57BL/10 mice. Newborn mice were inoculated i.p. with 7×10^4 FFU of MCF 1233. At week 8, viral infection of thymocytes was measured by immunofluorescence analysis with the MCF viral envelope-specific monoclonal antibody HY13. Noninfected age-matched thymocytes served as a control. Bars represent standard error of the mean percentage of virally infected cells. Significance of difference with B10: B10.A, $P < 0.001$; B10.A(4R), $P < 0.001$; B10.A(5R), not significant (Mann-Whitney U test).

of the *H-2 I-A*$^{b,b/k,bm12}$ type was reflected in the production of high titers of anti-viral envelope antibodies. A direct causal role of these antibodies (which react with viral envelope antigens) in the protection against MCF 1233 infection is not proven. Several studies indicate that antiviral antibodies protect, albeit partially, against replication of MuLV in vivo (21, 50, 56). In previous studies, we have shown that both titer and subclass of antiviral antibodies produced following various modes of infection with ecotropic MuLV and MCF viruses including MCF 1233 are under *H-2*-linked regulation (53, 54, 58, 62). The antibody response to MuLV is primarily directed at the envelope proteins gp70 and p15E (21). As these viral antigens are present at the cell surface of most tumors induced by AKR- and FMR-type viruses (27), these antibodies could also play an important role in the eradication of (pre)leukemic cells. Preliminary data indicated that whereas MCF 1233-induced T-cell lymphomas of susceptible mice (*H-2 I-A*^{k,d}) express both gp70-p15E and p30 antigens, T-cell lymphomas which arose sporadically in *H-2 I-A*$^{b,b/k,bm12}$ mice do not express p30 and/or gp70-15E viral antigens at the cell surface. This observation underscores the importance of immunosurveillance in MCF 1233-induced lymphomagenesis and is the subject of further study.

In addition to antiviral antibodies and antibody-dependent cellular effector mechanisms, other T-cell-mediated effector mechanisms might play a protective role against MCF 1233-induced T-cell lymphomagenesis. The production of gamma interferon may cause enhanced expression of *H-2* class I antigens on virus-infected cells, thereby probably increasing their susceptibility to cytotoxic T lymphocytes. Increased expression of class I *H-2D* antigens on virus-infected thymocytes has been correlated with *H-2*-linked resistance to radiation leukemia virus tumors (32). However, MCF 1233-infected thymocytes show, in general, moderate to high class I expression without strain-specific features (data not shown). Thus, in our hands, *H-2*-dependent clearance of MCF 1233-infected thymocytes was not correlated with the amount of class I molecules present at the cell surface.

The role of T_c activity in the eradication of MCF 1233-infected cells remains unexplored in our study. Virus-specific T_c may be important in both *I-A* responder and *I-A* nonresponder strains (20). At present, it is not known to what extent helper-dependent T_c contribute to the T-cell lymphoma resistance in *I-A*$^{b,b/k,bm12}$ mice. T_h-independent activation of Lyt-2$^+$ T_c has recently been documented by several groups (3, 5, 23). This may provide *I-A*^{k,d} strains some, albeit incomplete, protection against early T-cell lymphoma development. The exceptional T-cell lymphoma susceptibility of B10.MBR mice (K^b, *I-A*^{k}, *I-E*^{k}, D^q) suggests an additional role for *H-2D*-restricted helper-independent T_c reactivity in *I-A* nonresponder strains. The possibility that B10.MBR mice possess extra endogenous ecotropic viral integrations, as has been suggested for B10.AKM mice (11), was excluded by Southern blotting experiments in which we showed that B10.MBR mice, like other *H-2*-congenic C57BL/10 mice, have a single endogenous ecotropic provirus.

The (B10.A × B10)F_1 and (B10 × B10.A)F_1 hybrids responded to MCF 1233 infection as their T-cell lymphoma-resistant B-cell lymphoma-susceptible B10 parent, although the frequency of B-cell lymphomas in the F_1 crosses was higher at week 104. These data indicate dominance of the *I-A* responder gene in resistance against T-cell lymphomagenesis. The higher B-cell lymphoma incidence in (B10 × B10.A)F_1 and (B10.A × B.10)F_1 hybrids remains unexplained, but fits with the finding that in the (B10 × B10.D2)F_1 mice, also a cross between a resistant and a susceptible parent, virus expression in spleen and bone marrow about week 4 tended to be somewhat higher than in the resistant parent (Fig. 4). It is noteworthy that Britt and Chesebro (4) report differences in kinetics of the virus-specific proliferative T-cell response to Friend leukemia virus between *H-2D*$^{b/b}$ and *H-2D*$^{b/d}$ mice, which may also be due to *I-A* heterozygosity in these hybrids.

The course of MCF 1233 infection in the *H-2 I-A*^{b} mutant mouse bm12 was similar to the course in its parent strain B6 and *H-2 I-A*^{b} C57BL/10 mice. Obviously, the bm12 mutation in the class II *I-A*^{b} molecule does not influence the capacity to generate a good class II-restricted anti-MCF 1233 response.

The induction of both T- and B-cell lymphomas by MCF 1233 is puzzling. If the phenotype of a tumor is taken to indicate the phenotype of the target cell in which the initial transformation event took place (7), then it would be likely that different target cells are involved in lymphoma induction by MCF 1233 in the various *H-2*-congenic C57BL strains. However, as indicated by the similar grade of thymocyte infection at week 4 in all these strains, T cells are affected in all strains. We assume, however, that the persistence of viral infection in mice lacking T_h reactivity offers the opportunity to the nonacute transforming MCF 1233 virus to exhibit its oncogenic properties. It may be assumed that the critical period in this process lies within the first few weeks after infection (35, 36). This explains the observation that only minor differences in the kinetics of clearance of infected thymocytes [B10.A(4R) versus B10.A(5R); Fig. 6] result in either T-cell lymphoma susceptibility or T-cell lymphoma resistance. Preliminary results suggest that as early as week 10 after neonatal MCF 1233 infection, clonal T-cell populations emerge within the thymuses of T-cell lymphoma-susceptible mice (data not shown).

The most unexpected and novel aspect of our study is the

late development of B-cell lymphomas in mice which obviously mount a good *I-A*-restricted T_h response against MCF 1233. Apart from plasmacytomas (44) and pre-B-cell lymphomas (46), murine B-cell lymphomas have been documented only incidentally (16–18, 24, 34, 40, 45), and generally these reports do not consider causative immunopathogenic mechanisms. Paradoxically, human B-cell lymphomas form one of the most intriguing examples of tumorigenesis in which immunoregulatory mechanisms may play an important role (14, 30, 42). The recent observation that B-cell non-Hodgkin's lymphoma is a serious manifestation of human immunodeficiency virus infection (41, 57) makes it extremely worthwhile to obtain more insight into the immunopathogenesis of B-cell lymphomas. Although there are several lines of evidence that human B cells, at least in vitro, can be infected by human immunodeficiency virus (33), in B-cell non-Hodgkin's lymphoma associated with human immunodeficiency virus no viral integrations have been found (41).

In mice, MHC-linked immunoregulatory aspects of B-cell lymphomagenesis have only been studied for graft-versus-host-induced B-cell lymphomas. The induction of graft-versus-host lymphomas requires injection of parental strain T cells into F_1 hybrid recipients differing from the donor at class I and class II antigens or at class II antigens only (19, 38). Our data clearly showed that *H-2 I-A*$^{b,b/k,bm12}$ mice are not protected against the development of late B-cell tumors. This result is surprising, because class II MHC-positive viral antigen-bearing B-cell tumors are a prime target for class II-restricted virus-specific T cells. Previous results have shown that DNAs of both MCF 1233-induced T- and B-cell lymphomas contain many ecotropic and MCF viral integrations (61), but cell surface viral antigen expression has not yet been studied. Proviral insertion near known oncogenes (e.g., c-*myc* and *bcl-2*) seems to be a rare event in the B-cell tumors (unpublished observations), in contrast to previous findings in MCF 1233-induced T-cell tumors in which viral integration near c-*myc* and *pim-1* is frequent (10, 49). Moreover, karyotypic analysis of MCF 1233-induced B-cell tumors indicates extensive chromosomal aberrations, whereas in the majority of early T-cell lymphomas, trisomy 15 is the only abnormality found (W. L. E. Vasmel et al., manuscript in preparation). Various chromosomal aberrations acting in concert (1) may be held responsible for B-cell tumorigenesis after a very long latency.

We conclude that the outcome of neonatal MCF 1233 MuLV infection of C57BL mice is determined by MHC class II *I-A*-regulated immune reactivity. Immune response differences lead to the development of T- or B-cell lymphomas, in which completely different pathogenic mechanisms seem to be involved. Thus, this murine lymphoma system provides an interesting model to study MHC-disease association and can deepen our insights into the complex events leading to T- or B-cell pathology after infection with lymphotropic retroviruses.

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