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Contrasting Effects from a Single Major Histocompatibility Complex Class II Molecule (*H-2E*) in Recovery from Friend Virus Leukemia

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Resistance to erythroleukemia induced by infection with the Friend virus complex (FV) has been mapped to several genes residing both within and outside the murine major histocompatibility complex (MHC). MHC genes located in the *A*, *D*, and *Qa/Tla* regions of the murine *H-2* complex have been shown to affect disease resistance through their capacity to regulate various aspects of the host immune response to viral antigens. This study establishes *H-2E* as the fourth MHC locus controlling immunological resistance to FV. Our investigation into the role of *H-2E* molecules revealed two distinct and opposite effects on recovery from Friend disease. *H-2*$^{b/b}$ mice normally lack a functional *E* gene product and are resistant to high doses of FV. The expression of *H-2E* molecules in *H-2* recombinant or transgenic mice of this genotype resulted in a significant decrease in spontaneous recovery from FV-induced leukemia. In contrast, *H-2E* expression also appeared to influence recovery from Friend disease in a positive manner, since blocking these molecules with anti-E antibodies in vivo significantly decreased recovery from Friend disease. The data indicate that the positive effects of *H-2E* molecules derive from their function as restriction elements for helper T-cell recognition of the viral envelope glycoprotein, and we postulate that the negative effects are due to *H-2E*-dependent deletions in the T-cell repertoire during development.

Infection with Friend virus complex (FV), which is composed of the replication-competent Friend murine leukemia helper virus (F-MuLV) and an acutely transforming, replication-defective, spleen focus-forming virus, causes a rapid and usually fatal erythroleukemia associated with severe immunosuppression in susceptible mice (14, 25, 35). Successful infection is characterized by the appearance of splenomegaly within 14 to 20 days, which may persist until death of the host or remit spontaneously with subsequent development of resistance to rechallenge, depending on the immunological characteristics of the host. Several genes governing immunologic resistance to FV have already been mapped, and several are located within regions of the major histocompatibility complex (MHC), designated *H-2* (the revised *H-2* nomenclature proposed by Klein et al. (15) is used in this report) in the mouse (3, 16, 21). The *H-2D* region exerts the strongest effect on host recovery, probably acting through regulation of cytotoxic T-lymphocyte responses to viral antigens (7, 22, 33). *Rfv-2* has been mapped to the *Qa-Tla* region at the right end of the *H-2* complex and influences resistance by a mechanism not yet defined (4, 21). *Rfv-3* regulates the production of virus neutralizing and anti-leukemia cell antibodies but maps outside the murine MHC (3, 5, 9). Recent evidence has also documented a role for class II MHC-encoded gene products in the regulation of T-cell responses to the F-MuLV envelope glycoprotein. Analysis of in vitro proliferative responses using anti-class II monoclonal antibodies (MAbs) and genetically defined antigen-presenting cells identified *H-2A*-encoded glycoproteins as restriction elements for T-cell recognition of viral envelope antigens (18). The relevance of *A* gene products to in vivo resistance was subsequently demonstrated by a reduced rate of FV recovery in *H-2A* mutant bm12 mice compared with normal mice (20). At each of the MHC-linked loci governing FV-specific immunity, alleles of the *H-2*^{b} haplotype are associated with resistance, while alleles expressed in the *H-2*^{k} or *H-2*^{d} haplotype are associated with susceptibility.

This study examines the role of *H-2E* gene products in host immunity to FV infection. *H-2*$^{b/b}$ mice do not express *E* molecules because they lack a functional *Ea* gene (17). However, in heterozygous *H-2*$^{a/b}$ mice, *E* molecules were implicated in the presentation of viral envelope glycoproteins to *H-2*$^{a/b}$ T cells by blocking of in vitro virus-specific T-cell proliferative responses with anti-E MAbs (18). Attempts to measure the influence of E glycoproteins in vivo by comparison of FV recovery rates in various inbred strains are confounded by their close linkage to resistance alleles in the *A*, *D*, and *Qa/Tla* regions of the murine MHC. However, in the current work, the existence of a mouse strain with two recombinations within the MHC region allowed the study of *E*-positive mice on the background of the MHC *H-2*^{b} haplotype. In addition, we monitored the frequency of recovery in *H-2*$^{b/b}$ mice which contained an *Ea*^{k} transgene and expressed an Ea^{k}Eb^b heterodimeric glycoprotein. We also utilized in vivo treatments with MAbs recognizing host *E* molecules in *H-2*$^{a/b}$ mice to monitor effects on disease progression. Results of all approaches indicate a role for *H-2E* in the host response to FV, identifying a fourth MHC locus controlling immunological resistance to this retrovirus.

MATERIALS AND METHODS

Animals. C57BL/10SnJ, B10.A/SgSnJ, B10.A(2R)/SgSnJ, and A.BY/SnJ mice were purchased from the Jackson Laboratory, Bar Harbor, Maine. B10.A(18R) mice were kindly provided by M. Oldstone, Scripps Clinic and Research Foun-

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dation, La Jolla, Calif. Transgenic C57BL/10 mice carrying the Ea^k gene (2) exhibit tissue-specific expression of a heterodimeric Ea^kEb^b molecule. B10.AF(35R) mice were obtained from the McLaughlin Research Institute, Great Falls, Mont. The B10.AF(35R) line derived its *Eb* gene from B10.A(5R) and its *Ea* gene from a recombination between B10.A(5R) and B10.F(13R) (37). The resulting hybrid gene product has antigenic properties very similar to those of Ea^kEb^b (12). F_1 mice were bred at the Rocky Mountain Laboratories, Hamilton, Mont. Mice of both sexes were introduced into experiments at 8 to 16 weeks of age and maintained according to guidelines defined by the Rocky Mountain Laboratories Animal Care and Use Committee.

MAbs. MAbs used in this study included 14-4-4S (Ea specific) (27), 17-3-3S (Eb specific) (27), 10-3.6 ($A^{k,r,f,s}$ specific) (26), and 25-9-17S (A^b specific) (28). Antibodies were purified from culture supernatants by passage over protein A-Sepharose (Pharmacia, Piscataway, N.J.) and stored frozen until use. The specificity and activity of each reagent have been documented elsewhere (29, 31). Purified 14-4-4S MAb conjugated to fluorescein isothiocyanate (30) was used to monitor cell surface *H-2E* expression on peripheral blood lymphocytes of F_1 progeny derived from Ea^k transgenic parents. Fluorescence-activated cell sorting analyses clearly distinguished nonexpressors from animals having 15 to 25% Ea^k-positive cells.

FV stocks. The B-tropic strain of FV (FV-B) (7) was used in all mouse inoculations. FV stocks used for animal infection consisted of a 20% (wt/vol) homogenate of spleens from FV-infected BALB/cByJ mice prepared in 0.2 M phosphate-buffered saline containing 2 mM EDTA. The infectivity titer, expressed as spleen focus-forming units (SFFU), was determined in a spleen focus assay as previously described (7). Purified F-MuLV used in T-cell proliferation assays was prepared from culture supernatants of *Mus dunni* cells chronically infected with the FB-29 molecular clone of F-MuLV (34), using a previously described Percoll density gradient technique (23). Inactivation of virus infectivity prior to addition to in vitro cultures was accomplished by UV irradiation as previously described (18). Recombinant vaccinia viruses expressing the F-MuLV envelope gene (10) and the F-MuLV *gag* gene (19) have been described previously.

FV infection and spleen palpation. Animals were infected with FV by intravenous (i.v.) injection of virus stock diluted in phosphate-buffered saline containing 2% fetal bovine serum. Mice were examined every 10 to 14 days by spleen palpation under ether anesthesia. Leukemic mice had obvious (4- to 20-fold) spleen enlargement (7). The F_1 mouse strains used in this work all have the $Rfv\text{-}3^{r/s}$ genotype; therefore, after FV infection they spontaneously recover from viremia and have markedly reduced virus expression in spleen, bone marrow, and liver despite the continued persistence of gross enlargement of these organs due to the presence of erythroleukemia cells (6, 9). Thus, measurements of blood, plasma, or spleen virus or viral proteins are not valid in evaluating recovery from leukemia. In contrast, weekly or biweekly spleen palpation under anesthesia has been found to be an extremely accurate and sensitive technique for detection of FV leukemia. All mice with splenomegaly of more than four times normal spleen size (>0.4 g) persisting longer than 6 weeks after infection die of leukemia within the following 1 to 2 months (3, 7).

Although some splenomegaly might have been induced by inoculation of anti-MHC MAbs in these experiments, no such splenomegaly was ever noted in control uninfected mice. Furthermore, the massive splenomegaly usually observed (spleen size, 1 to 2 g) persisted throughout the observation period up to 95 days postinfection, whereas the final injection of anti-MHC MAbs was usually given 10 days after infection. For all of these reasons, we feel confident that the splenomegaly observed was due to FV-induced leukemia.

TABLE 1. Influence of the Ea^k transgene on recovery from FV

Expt	Virus dose (SFFU)	No. of leukemic mice/total (% leukemic)[a]	
		E negative	*E* positive
1	1,000	0/25 (0)	14/46 (30.4)[b]
2	2,000	6/49 (12.2)	26/64 (40.6)[b]

[a] Mice were derived from male A.BY and transgenic female C57BL/10 mice hemizygous for the Ea^k transgene. Because $H\text{-}2^b$ mice lack a functional Ea^b gene, Ea^k-negative mice are unable to make an EaEb dimeric protein molecule and thus are negative for expression of cell surface E protein. In contrast, Ea^k-positive mice can make an Ea^kEb^b heterodimer protein molecule and are positive for cell surface E protein expression. Progeny were tested for *H-2E* expression and infected with the indicated dose of FV. Animals were evaluated every 10 to 14 days by spleen palpation. Data shown are for 95 days postinfection, and there were no significant changes between 50 and 95 days.

[b] Significantly different from the value for *E*-negative littermates ($P < 0.001$) by chi-square analysis.

Immunizations. To elicit delayed-type hypersensitivity (DTH) responses to viral antigens, purified UV-inactivated F-MuLV was emulsified with an equal volume of complete Freund's adjuvant and injected subcutaneously in a 0.1-ml volume (100 μg of viral protein per mouse). Immunization with recombinant vaccinia virus expressing F-MuLV *env* or *gag* gene was accomplished by tail scratch with 10^7 PFU in 1 μl.

Virus neutralization assay. Titers of F-MuLV neutralizing activity were determined in sera collected by retro-orbital bleeding of individual mice by inhibition of plaque-forming activity in the focal infectivity assay (32). Activity ascribable to the immunoglobulin G (IgG) fraction of serum immunoglobulin was determined by pretreatment of samples with 0.1 M 2-mercaptoethanol for 30 min. Neutralizing IgM titers were determined by subtraction of IgG values from results obtained with untreated serum samples.

T-cell proliferation assays. Assays were performed as described previously (18, 29). Lymph node T cells collected from four to five mice per treatment group were cultured at 4×10^5 per well in RPMI 1640 containing 10% fetal calf serum, 100 U of penicillin per ml, 5×10^{-5} M 2-mercaptoethanol, and 1 mM L-glutamine. Peritoneal exudate cells from syngeneic normal mice injected intraperitoneally with 0.5 ml of 2.5% oyster glycogen (Sigma Chemical Co., St. Louis, Mo.) 3 days earlier were used as a source of antigen-presenting cells. Antigen-presenting cells (10^7/ml) were pulsed with 100 μg of purified F-MuLV or ovalbumin (Sigma) per ml for 2 h at 37°C, washed three times, diluted in the same culture medium, and cocultured in graded concentrations with responder T cells in a final volume of 200 μl. Three days later, cultures were pulsed with 1 μCi of [^{3}H]thymidine per well and harvested with a Titertek cell harvester (Skatron Inc., Sterling, Va.) 16 to 24 h later. Results are presented as the means of triplicate wells. Variation was generally less than 10% of the mean.

DTH assays. DTH to F-MuLV antigens was measured by footpad challenge of animals primed with recombinant vaccinia viruses by tail scratch. Animals were challenged with 10^6 irradiated (10,000 rads) Y57-2C FV leukemia cells (8) in the right hind footpad. Twenty-four hours later, swelling was measured with an engineer's micrometer, and values obtained with the uninjected footpad were subtracted from the swelling response of the injected footpad. Specific swelling was determined by comparison with the response of unimmunized animals.

TABLE 2. Influence of *H-2E* genotype on recovery from FV in *H-2* recombinant mice

Mouse strain	Selected *H-2* alleles						No. of leukemic mice/total (% leukemic)[a]
	K	*Ab*	*Aa*	*Eb*	*Ea*	*D*	
(B10 × A.BY)F_1	*b/b*	*b/b*	*b/b*	*b/b*	−/−	*b/b*	4/46 (9)
[B10.AF(35R) × A.BY]F_1	*b/b*	*b/b*	*b/b*	*b*[b]/*b*	k/−	*b/b*	11/42 (26)[c]
(B10.A × A.BY)F_1	*k/b*	*k/b*	*k/b*	*k/b*	k/−	*d/b*	33/33

[a] Animals were infected with 1,500 SFFU of FV complex and were evaluated by spleen palpation every 10 to 14 days for 90 days.
[b] The *H-2E* gene of B10.AF(35R) is similar to *H-2E*[b] (see Materials and Methods).
[c] Significantly higher than the value for negative control mice by Fisher's exact test. The two-tailed *P* value was less than 0.05.

Statistical analyses. Data were analyzed by Student's *t* test, Fisher's exact test, or chi-square analysis.

RESULTS

Influence of *Ea*[k] expression on recovery from FV-induced leukemia. To test directly the possible contribution of *H-2E*-encoded glycoproteins in in vivo resistance to FV-induced splenomegaly, we compared recovery rates in *H-2*[b/b] mice expressing or lacking an *Ea*[k] transgene. C57BL/10 female mice hemizygous for the presence of the *Ea*[k] transgene were bred with A.BY males to generate (C57BL/10 × A.BY)F_1 progeny with or without the *Ea*[k] transgene. At 8 weeks of age, progeny were individually typed for cell surface expression of *H-2E*-encoded protein on peripheral blood lymphocytes, and animals of both phenotypes were infected with FV. In two experiments using different doses of FV, *H-2E*-positive mice expressing cell surface *Ea*[k]*Eb*[b] molecules had a significantly higher incidence of leukemia than did *Ea*-negative mice (30 to 40% versus 0 to 12%) (Table 1).

In other experiments, B10.AF(35R) recombinant mice, which express *H-2E* in the background of the *H-2*[b] haplotype, were bred to A.BY mice. The F_1 progeny mice were tested for the ability to recover from infection with 1,500 SFFU of FV. In agreement with the results obtained with *Ea*[k] transgenic mice, the [B10.AF(35R) × A.BY]F_1 mice had a 26% incidence of FV leukemia, compared with a 9% incidence in (C57BL/10 × A.BY)F_1 control mice which lacked the *Ea*[k] gene (Table 2). The mechanism of these weak but statistically significant negative effects on recovery from leukemia was unclear. One likely possibility was that *H-2E* expression might alter selection of the T-cell repertoire during ontogeny by deleting some T cell useful for mediating recovery from FV leukemia.

Effects of anti class II MAbs on recovery from FV-induced splenomegaly. The negative effect of the *Ea*[k] transgene on recovery from FV leukemia was unexpected because previous experiments demonstrated that MAbs specific for Ea or Eb could block FV-specific in vitro T-cell proliferative responses in *H-2*[a/b] mice (18), and this result suggested that *E* molecules might play a positive role in presentation of FV antigens to T cells. To investigate these discrepant findings further, we next carried out studies of the effects of in vivo administration of anti-E MAbs on spontaneous recovery from FV leukemia. Hybrid mice derived from the cross of A.BY (resistant, *H-2*[b]) and B10.A (susceptible, *H-2*[a]) parents usually recover spontaneously from the splenomegaly and leukemia induced by i.v. infection with low, but not high, doses of FV (4, 20). To determine the relative contribution of *E* molecules to recovery from low doses of FV in this hybrid, MAbs specific for the Ea chain were administered intravenously on days 0, 1, 2, 4, 7, and 10 after challenge with 15 SFFU of FV. Both *E*-positive mouse strains, (B10.A × A.BY)F_1 and [B10.AF(35R) × A.BY]F_1, treated with the anti-Ea MAb displayed a markedly reduced ability to recover from FV leukemias compared with untreated controls (Table 3). Similar experiments were done on [B10.A(18R) × A.BY]F_1 mice, which did not express *H-2E*-encoded protein, and the anti-Ea MAb failed to inhibit spontaneous recovery from FV leukemia (Table 3). Thus, the inhibitory effect of the anti-Ea MAb in (B10.A × A.BY)F_1 mice appeared to be specific because it occurred only in the mice expressing a functional *Ea* gene product.

The specificity of inhibition by the anti-Ea MAb was also examined by treating FV-infected (B10.A × A.BY)F_1 mice with various MAbs reactive with other MHC class II glycoproteins. Results shown in Fig. 1 indicated that administration of MAbs directed against the Ea (14-4-4S) or Eb (17-3-3S) chain inhibited recovery completely. Thus, antibody-mediated interference with the normal function of class II *E* caused a loss of resistance to FV-induced disease in vivo. In contrast, treatment with MAbs recognizing the A^k or A^b molecule encoded by the *H-2A* locus inhibited recovery in only 20 to 40% of infected animals, suggesting that in these *H-2*[a/b] mice, *A* molecules may be less effective than *E* molecules in immune-mediated recovery from FV.

Anti-Ea MAb effects on T-cell functions. Since both *A* and *E* molecules are expressed on the same cells, we sought to determine whether an anti-*H-2E* MAb might be interfering with class II function in a general manner such as by depletion of T cells. (A.BY × B10.A)F_1 (*H-2*[a/b]) mice were infected with a recombinant vaccinia virus expressing either the F-MuLV *env* or *gag* gene and treated with saline or with an anti-Ea MAb. On day 12, animals in all groups were challenged in the footpad with *H-2*[b/b] FV leukemia cells, and swelling was measured 24 h later. As indicated in Fig. 2, in vivo treatment with the anti-Ea MAb inhibited DTH responses to F-MuLV

TABLE 3. Leukemia incidence in anti-Ea MAb-treated mice

Mouse strain	Selected *H-2* alleles						No. of leukemic mice/total (% leukemic)[a]	
	K	*Ab*	*Aa*	*Eb*	*Ea*	*D*	No. MAb	MAb treated[b]
[B10.A(18R) × A.BY]F_1	*b/b*	*b/b*	*b/b*	*b/b*	−/−	*d/b*	7/26 (27)	4/30 (13)[c]
(B10.A × A.BY)F_1	*k/b*	*k/b*	*k/b*	*k/b*	*k*/−	*d/b*	3/27 (11)	27/27 (100)[d]
[B10.AF(35R) × A.BY]F_1	*b/b*	*b/b*	*b/b*	*b*[e]/*b*	*k*/−	*b/b*	0/15 (0)	7/15 (47)[d]

[a] Animals were evaluated by spleen palpation every 10 to 14 days until 70 to 95 days postinfection with 50 SFFU of FV.
[b] MAb-treated mice were inoculated i.v. with 120 μg of anti-Ea MAb 14-4-4S on days 0, 1, 2, 4, 7, and 10 postinfection.
[c] No significant difference between values for treated and mock-treated groups.
[d] Significantly higher than the value for saline-treated controls, $P < 0.007$.
[e] The *H-2E* gene of B10.AF(35R) mice is similar to *H-2E*[b] (see Materials and Methods).

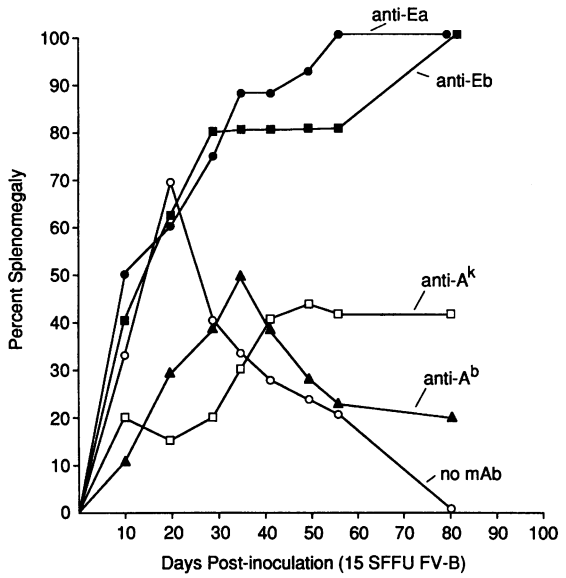

FIG. 1. Incidence of leukemic splenomegaly at various days after FV infection of (B10.A × A.BY)F_1 mice. Mice were inoculated i.v. with 120 μg of the indicated MAb on days 0, 1, 2, 4, 7, and 10 postinfection.

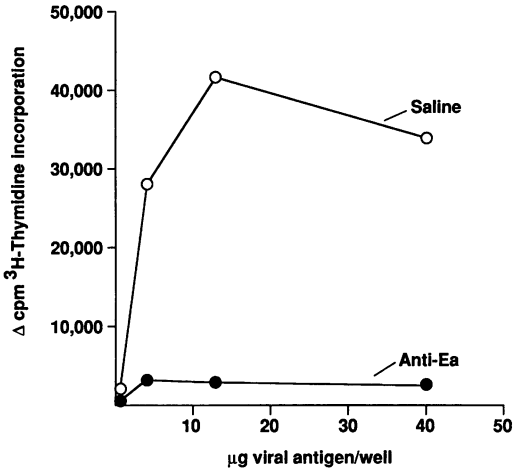

FIG. 3. Effect of anti-Ea MAb treatment on T-cell proliferative responses to UV-inactivated FV particles. Mice were immunized with F-MuLV in complete Freund's adjuvant and were either treated with the anti-Ea MAb as described for Fig. 2 or not treated. On day 17, lymph node T cells were analyzed by in vitro T-cell proliferation assays.

env-encoded antigens but had no effect on the response to F-MuLV *gag*-encoded antigens. These results indicated that host *H-2E*-restricted T cells were specific for epitopes on the viral envelope glycoprotein and produced no global immune suppressive effects. In addition, these results indicate that the antiviral *gag* response is not restricted by *Ea* molecules.

T-cell proliferative responses to F-MuLV antigens were also assessed in vitro. (A.BY × B10.A)F_1 mice were immunized with F-MuLV particles emulsified in complete Freund's adjuvant and were treated with saline or with the anti-Ea MAb. On day 17, lymph node T cells from mice of each group were harvested and cocultured with antigen-presenting cells pulsed with UV-inactivated F-MuLV particles. When responses were measured 4 days later, it was noted that T cells from control animals mounted a strong antiviral proliferative response, whereas T cells from mice treated with the anti-Ea MAb failed to mount a detectable response (Fig. 3). Similar results were obtained for cells from treated and untreated F_1 mice infected with live FV (data not shown), although in vitro responses to this immunogen were generally lower than responses to the adjuvant emulsion.

To determine the effect of the anti-Ea MAb on host antibody responses to F-MuLV, groups of control and anti-Ea-treated (A.BY × B10.A)F_1 mice were bled at 78 days postinfection, and their sera were tested individually for levels of IgM and IgG neutralizing antibodies. IgM $\log_2$ neutralization titers were comparable in both groups of animals (4.3 ± 2.0 [control] and 6.0 ± 0 [MAb-treated group]) but neutralizing antibodies of the IgG class were significantly depressed and at the lower limit of detection in anti-Ea-treated mice (1.0 ± 0 versus 6.2 ± 1.1 for the control group). These results indicate that treatment with the anti-Ea MAb blocks switching of the humoral antiviral envelope antibody response from the IgM to the IgG class of immunoglobulin, possibly because of MAb effects on the priming of viral envelope-specific helper T cells.

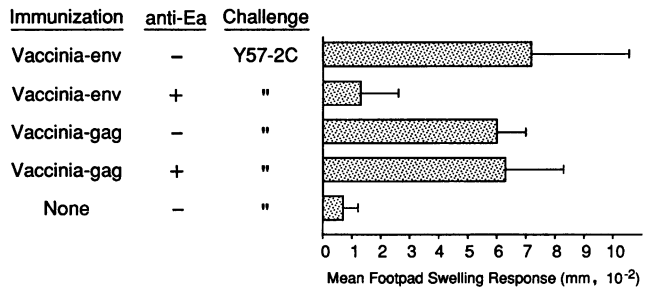

FIG. 2. Effect of anti-Ea MAb treatment on DTH reactivity to F-MuLV envelope and *gag* antigens. Mice were immunized with a recombinant vaccinia virus expressing the F-MuLV *env* or *gag* gene as described in Materials and Methods. Mice were inoculated i.v. with 120 μg of anti-Ea MAb 14-4-4S on days 0, 1, 2, 4, 7, and 10 after immunization.

DISCUSSION

The present data indicate that *H-2E*-encoded proteins appear to have two opposing effects on recovery from FV leukemia. In recombinant or transgenic $H\text{-}2^{b/b}$ mice, E protein expression lowered the incidence of recovery from leukemia. In contrast, in adult mice expressing the E protein, treatment with anti-Ea or anti-Eb MAbs strongly inhibited recovery from leukemia, suggesting that E protein expression could be helpful to the recovery process. In vivo treatment with anti-Ea MAbs appeared to act by blocking the *H-2E*-restricted T-cell responses to FV envelope and immunoglobulin class switching of virus neutralizing antibodies. These results suggested that *H-2E*-encoded molecules serve as restriction elements for helper T-cell recognition of viral envelope antigens in vivo. Recent experiments have identified CD4-positive T-cell clones reactive with two unique peptides located in the F-MuLV envelope protein (13). Interestingly, the N-terminal epitope was restricted by the $H\text{-}2A^b$ allele and the C-terminal epitope was restricted by an *H-2E*-encoded heterodimeric Ea^kEb^b molecule, also present in the Ea^k-positive mice used in this study.

In this study, administration of anti-A^b or anti-A^k MAbs to

$H\text{-}2^{a/b}$ mice was associated with a lower increase in leukemia incidence compared with that seen after treatment with the anti-Ea or anti-Eb MAb. It would appear that although each of these class II molecules has the capacity to function in T-cell recognition of F-MuLV envelope antigens, their relative roles in host responsiveness vary according to the host MHC haplotype. Thus, in $H\text{-}2^{b/b}$ mice negative for *H-2E* expression, A^b molecules provide the sole restriction elements for presentation of F-MuLV envelope glycoproteins. However, in $H\text{-}2^{a/b}$ mice expressing *E* as well as *A* gene products, *E* molecules may be more effective in antigen presentation and, ultimately, immune-mediated resistance to disease. Alternatively, the weak effect of anti-A MAbs on recovery might also be due to technical differences in the ability of these reagents to block functions in vivo.

A role for class II E gene products in host resistance to FV was also demonstrated through the study of $H\text{-}2^{b/b}$ transgenic animals carrying an Ea^k transgene. Hybrid progeny produced from these animals varied only in the capacity to express an *H-2E* gene product, yet those animals positive for gene expression consistently exhibited a 30% decrease in recovery from FV-induced leukemia. Similar results were observed in $H\text{-}2^{b/b}$ recombinant mice which expressed *E* molecules. These results contrast sharply with the experiments discussed above in which *H-2E* played a positive role in recovery from FV. The negative influence of *H-2E* on FV recovery is possibly due to the thymic deletion of T-cell receptor specificities (2) that are normally required for an effective anti-FV immune response. According to published evidence, expression of *H-2E* in the mice in our studies would cause deletions of several T-cell receptor families, including V beta 3, 5, 5.1, 5.2, 11, 12 and 17 (1, 11, 36). We have confirmed the V beta 5, 5.1, 5.2, and 17 deletions (data not shown); however, we have been unable to directly link any of the deleted families to the anti-FV immune response in resistant animals, as the response is complex and no single family predominates. This complexity may explain why the negative effects of *H-2E* are relatively weak.

It is interesting that $(B10.A \times A.BY)F_1$ mice, which express *H-2E*, and $[B10.A(18R) \times A.BY]F_1$ mice, which do not express *H-2E*, have similar levels of spontaneous recovery from low doses of FV (21) and fail to recover from high doses of FV (4). These mice both express at least one $H\text{-}2A^b$ allele required for recovery from FV and have identical genotypes at other genes also involved in recovery ($H\text{-}2D^{b/d}$, $H\text{-}2Q^{b/c}$, and $Rfv\text{-}3^{r/s}$). Thus, the similarity of the recovery incidence in these two strains differing in *H-2E* expression appears to indicate that in the *H-2E*-expressing mice, the presence of the positive and negative effects of *H-2E* on recovery may counteract each other.

The identification of *H-2E* as a controlling element in the immune response to FV marks the fourth MHC gene to be characterized in this regard. The involvement of *H-2A*, *-D*, *-Qa/Tla* and now *-E* documents the remarkable complexity of genetic control over host resistance to infection that is attributable to genes of the MHC, not to mention the non-MHC gene, *Rfv-3*, which governs generation of humoral antiviral antibodies. The capacity to protect mice by immunization with a vaccinia virus construct expressing the F-MuLV *env* gene also maps to the *H-2* complex (24) and shows a strain distribution shared by responders to the envelope glycoprotein in vitro (18). Given the diversity of human MHC gene products, it is likely that identification of genes governing resistance to human pathogens will not come easily. While such knowledge may not be required for successful disease prevention when intact virus vaccines containing multiple epitopes can be used as in polio and rabies, it may become an important consideration for vaccines based on limited protein sequences. For such subunit vaccines, it is likely that specific host MHC genes will be required for protection, and identification of those genes may be a prerequisite for successful vaccine development.

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