

GENETIC CONTROL OF T CELL RESPONSIVENESS TO THE FRIEND MURINE LEUKEMIA VIRUS ENVELOPE ANTIGEN

Identification of Class II Loci of the H-2 as Immune Response Genes

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Genetic control of cellular and humoral immune responses to pathogenic viruses has been studied using several different types of viruses and a few host species (reviewed in references 1-5). Most of these studies, however, focused on the recognition of viral antigens on infected target cells by cytotoxic T cells, and little is known about how the induction of immune responses to pathogenic viruses is genetically controlled (4, 5). Of course, immune response (Ir)¹ gene phenomena have been described and their mechanisms have been intensively analysed using synthetic and natural antigens of limited numbers of antigenic determinants (reviewed in references 6 and 7). Consequently, it has been considered likely that Ir gene phenomena control host immune responses to viral antigens through the mechanisms quite similar to what have been observed with synthetic peptides (5, 8). In fact, the presence of viral antigen-specific, H-2I region-restricted helper T cells has been demonstrated in mouse models of influenza virus (4, 9), Sendai virus (5), reovirus (10), and herpes simplex virus (11, 12) infections. However, direct demonstration of Ir gene-controlled responder/non-responder phenotypes to viral antigens has been rarely presented (13), except in the humoral immune responses of inbred mice to human hepatitis B virus surface antigen (14, 15). In the present study we have investigated cellular mechanisms for the influence of H-2 on the induction of protective immunity against murine retrovirus infection and identified H-2-linked Ir genes controlling the T cell responsiveness to the viral envelope glycoprotein.

Friend murine leukemia virus complex (FV) is a retrovirus complex composed of replication competent Friend murine leukemia helper virus (F-MuLV) and defective Friend spleen focus-forming virus (SFFV) (16). FV causes extremely rapid progression of an erythroleukemia associated with severe immunosuppression when injected to immunocompetent adult mice or newborn mice of susceptible strains (17, 18). This FV-induced leukemia usually results in massive splenomegaly occurring 1-2

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¹ *Abbreviations used in this paper:* HA, hemagglutinin; Ir, immune response gene; F-MuLV, Friend murine leukemia helper virus; FV, Friend virus complex; PBBS, phosphate-buffered balanced salt solution; PEC, peritoneal exudate cells; RML, Rocky Mountain Laboratories; R-MuLV, Rauscher murine leukemia virus; SFFU, spleen focus-forming unit.

wk after virus inoculation and in death of infected animals within 1–3 mo. However, in certain mouse strains, spontaneous recovery from leukemic splenomegaly occurs 2–4 wk after virus inoculation and recovered mice show long-lasting resistance to FV (17, 19). The production of antiviral antibodies is required for the recovery and is associated with the clearance of viremia (20, 21). In addition, we have detected both cytotoxic effector cells and proliferative L3T4⁺ cells specific for FV-induced leukemia cells in FV-infected mice (22–24). In FV-infected mice, a gene(s) in H-2K or I region influences the ability to generate leukemia cell-specific proliferative T cells, whereas the H-2D locus appears to control the time course of development of this T cell response (24). More recently we studied protective immunization of mice against FV with a recombinant vaccinia virus that expressed the *env* gene of F-MuLV (25, 26). By using several H-2 recombinant mouse strains, a gene or genes in H-2K or I region was shown to control the development of protective immunity after immunization with the vaccinia-F-MuLV *env* (26). Moreover, this protective immunity was functionally mediated by priming of helper T cells (25, 26).

These results strongly suggest that a gene(s) in H-2K or I region controls T cell responsiveness to the F-MuLV *env* gene products. In this paper we provide direct evidence that H-2I region (class II loci) gene products expressed on APCs function as the restriction elements for the T cell recognition of F-MuLV envelope protein.

Materials and Methods

Animals. C57BL/10SnJ, B10.A/SgSnJ, A.BY/SnJ, A/WySnJ, B10.MBR/SxEG, and B6.C-H-2^{bm12}/KhEg mice were purchased from The Jackson Laboratory, Bar Harbor, ME. B10.A(1R)/Sg, B10.A(4R)/Sg, B10.A(5R)/Sg, and B10.A(18R)/Sg mice were kindly provided by Dr. Jack Stimpfling (McLaughlin Research Institute, Great Falls, MT) from his colony. To eliminate possible effects of the *Rfv-3* gene (21, 27) on T cell and/or APC functions, all mice used in this study were F₁ hybrids with the *Rfv-3*^{+/+} genotype. F₁ hybrid mice were bred at the Rocky Mountain Laboratories (RML), Hamilton, MT. H-2 alleles and parameters of anti-FV immune responses of the H-2 congenic and recombinant mice used in this study are summarized in Table I. Both male and female mice 2–6 mo old were used; however, all sources of APC and T cells were age and sex matched in each experiment.

Purified Viruses. Sucrose density purified virions of Rauscher murine leukemia virus (R-MuLV) from JLS-V9 leukemia cell line (No. 1089 and 1090) were kindly provided by the Biological Carcinogenesis Program, National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD. Virions of an infectious molecular clone of F-MuLV, FB-29 (29), were purified from culture supernatant of chronically infected, Fischer rat embryo (FRE) cells or *Mus dunni* cells by using a previously described Percoll density gradient method (30). Purity of the virions was confirmed by SDS-PAGE. FV and Rauscher virus complex are biologically quite similar (16) and F-MuLV and R-MuLV are immunologically highly crossreactive sharing a large number of antigenic determinants (31, 32). Therefore, we used both of these virus strains to test F-MuLV-specific T cell responses. Purified virions were dialyzed against Dulbecco's PBS without the divalent cations (D-PBS⁻), and were inactivated by UV irradiation (33). Inactivation of plaque-forming activity was determined by a focal immunofluorescence assay (34) on live *Mus dunni* cells.

Preparation of TNP-KLH. TNP haptens were conjugated to KLH (Calbiochem-Behring Corp., La Jolla, CA) by a standard method (35). The preparation used in this study was conjugated with 7.7 mol TNP/10⁵ g KLH.

Immunization of Mice. Mice were immunized in the hind foot pads with 100 µg of the virions or TNP-KLH emulsified in CFA (Difco Laboratories, Inc., Detroit, MI). The recombinant vaccinia virus expressing the entire *env* gene from the F-MuLV clone 57 (25) and the control recombinant vaccinia virus expressing the influenza hemagglutinin (HA) gene (36) were kindly provided by Dr. Bernard Moss, Laboratory of Viral Diseases, National Institute of Allergy

TABLE I
*Alleles at H-2 Loci and Parameters of Anti-FV Immune Response
of the Mouse Strains Used in This Study*

Strain	H-2 haplotype	Alleles at H-2 loci*							Recovery from splenomegaly [†]		Protective immunity after vaccinia- F-MuLV <i>env</i> [‡]
		K	A _β	A _α	E _β	E _α	S	D	High dose	Low dose	
(C57BL/10 × A.BY)F ₁	<i>b/b</i>	<i>b/b</i>	<i>b/b</i>	<i>b/b</i>	<i>b/b</i>	<i>-/-</i>	<i>b/b</i>	<i>b/b</i>	+	+	+
(B10.A × A.BY)F ₁	<i>a/b</i>	<i>k/b</i>	<i>k/b</i>	<i>k/b</i>	<i>k/b</i>	<i>k/-</i>	<i>d/b</i>	<i>d/b</i>	-	+	+
(B10.A × A/WySn)F ₁	<i>a/a</i>	<i>k/k</i>	<i>k/k</i>	<i>k/k</i>	<i>k/k</i>	<i>k/k</i>	<i>d/d</i>	<i>d/d</i>	-	-	-
(B10.A(1R) × A/WySn)F ₁	<i>h1/a</i>	<i>k/k</i>	<i>k/k</i>	<i>k/k</i>	<i>k/k</i>	<i>k/k</i>	<i>d/d</i>	<i>b/d</i>	-	ND	-
(B10.A(4R) × A/WySn)F ₁	<i>h4/a</i>	<i>k/k</i>	<i>k/k</i>	<i>k/k</i>	<i>k/k</i>	<i>-/k</i>	<i>b/d</i>	<i>b/d</i>	-	ND	-
(B10.A(5R) × A/WySn)F ₁	<i>i5/a</i>	<i>b/k</i>	<i>b/k</i>	<i>b/k</i>	<i>b/k</i>	<i>k/k</i>	<i>d/d</i>	<i>d/d</i>	-	ND	+
(B10.A(18R) × A/WySn)F ₁	<i>i18/a</i>	<i>b/k</i>	<i>b/k</i>	<i>b/k</i>	<i>b/k</i>	<i>-/k</i>	<i>b/d</i>	<i>d/d</i>	-	ND	+
(B10.MBR × A.BY)F ₁	<i>bq1/b</i>	<i>b/b</i>	<i>k/b</i>	<i>k/b</i>	<i>k/b</i>	<i>k/-</i>	<i>k/b</i>	<i>q/b</i>	ND	ND	ND
(B10.MBR × A/WySn)F ₁	<i>bq1/a</i>	<i>b/k</i>	<i>k/k</i>	<i>k/k</i>	<i>k/k</i>	<i>k/k</i>	<i>k/d</i>	<i>q/d</i>	ND	ND	ND
(B6.C- <i>H-2^{bm12}</i> × A/WySn)F ₁	<i>bm12/a</i>	<i>b/k</i>	<i>bm12/k</i>	<i>b/k</i>	<i>b/k</i>	<i>-/k</i>	<i>b/d</i>	<i>b/d</i>	ND	ND	ND

* Based on references 8 and 28. In each strain of mice underlined loci are sharing the same allele with the *H-2^b* haplotype. Previously used term of I region corresponds to A_β-E_α loci.

[†] Heterozygous *H-2^{ab}* (B10.A × A.BY)F₁ mice did not recover from splenomegaly if 1,500 SFFU of FV were inoculated. However, if given 150 or 15 SFFU of FV, a significant number of the same strain of mice recovered (17, 19).

[‡] Mice were immunized with the vaccinia-F-MuLV *env* recombinant vaccine. 30 d later, they were challenged with i.v. inoculation of 1,500 SFFU of FV (25, 26).

^{||} *E_α* gene of the *H-2^b* haplotype is defective. Therefore, (C57BL/10 × A.BY)F₁ mice do not express any E_αE_β complex. However, (B10.A × A.BY)F₁, (B10.A(5R) × A/WySn)F₁, (B10.A(18R) × A/WySn)F₁, (B10.MBR × A.BY)F₁, and (B6.C- *H-2^{bm12}* × A/WySn)F₁ express two types of I-E molecules: E_α^kE_β^k and E_α^kE_β^b. All other mouse strains in this table express only one type of I-E molecule: E_α^kE_β^k. See reference 28 for detailed explanation.

and Infectious Diseases, Bethesda, MD. Mice were immunized by exposing a needle scratch at the base of the tail to 10⁷ plaque forming units (PFU) of recombinant vaccinia virus in 1 μl.

Preparation of APCs. Peritoneal exudate cells (PEC) were elicited by intraperitoneal injection of 0.5–1.0 ml 2.5% oyster glycogen (Type II; Sigma Chemical Co., St. Louis, MO) in D-PBS⁻ (37). PEC were harvested 3–4 d after the injection and were irradiated (2,000 rad) in a ¹³⁷Cs γ-irradiator. Irradiated PEC were washed once, suspended at 10⁷ cells/ml in prewarmed phosphate buffered balanced salt solution (PBBS) containing 10% FCS of a lot selected for low background stimulation of DNA synthesis (HyClone Laboratories, Inc., Logan, UT) and were pulsed with an antigen at 37°C for 1 h. Antigen-pulsed PEC were washed once with cold D-PBS⁻ and three times with cold PBBS. Control unpulsed PEC were prepared by exactly the same procedure except that D-PBS⁻ was added to prewarmed PBBS instead of an antigen.

The APC activity of glycogen-elicited PEC was confirmed by comparison with that of spleen adherent cells analyzing stimulator cell dose-response curves, and the glycogen-elicited PEC were usually better as APC than spleen adherent cells were. Therefore, we used these PEC as APC in all the following experiments.

Antigen dose-response curves of T cell proliferative response were analyzed in preliminary experiments for TNP-KLH and purified virions. In both cases, resultant dose-response curves were rather flat throughout the concentration range from 10 to 100 μg/ml, and the highest magnitude of proliferation was observed when PEC were pulsed with 100 μg/ml of antigen. Therefore, this concentration was used to pulse PEC throughout further experiments.

T Lymphocyte Proliferative Assay. Nylon wool-passed T cells were prepared by a standard technique (35, 38) from the pooled spleens and lymph nodes of two to three mice from immunized and unimmunized control groups. The average composition of the nylon wool-passed

T cell preparation determined by immunofluorescence was >90% Thy-1.2⁺ and <3% surface Ig⁺. 2–5 × 10⁵ T cells (exact numbers are described for each experiment in Results section) were cultured with various numbers of antigen-pulsed or unpulsed APC in 200 μl Click's medium (39) supplemented with 50 μg/ml gentamicin sulfate and 7.5% heat-inactivated FCS of the selected lot in a well of round-bottomed 96-well plastic tissue culture plates (Linbro No. 76-042-05; Flow Laboratories, McLean, VA). Cells were pulsed with 1 μCi/well [³H]thymidine (6.7 Ci/mol; New England Nuclear, Boston, MA) for a final ~20 h, harvested on glass fiber filters, and the incorporation of [³H]thymidine into DNA was measured with a liquid scintillation counter. In several preliminary experiments kinetics of the T cell proliferation under the stimulation of antigen-pulsed PEC was analyzed, and a peak in incorporation of the thymidine was always observed when it was added 3 d after the start of the culture. Therefore, in all further experiments, thymidine incorporation was measured after 3 d of culture. The average amount of [³H]thymidine incorporation was calculated for each number of APC from the results of four parallel wells and the magnitude of antigen-specific T cell proliferative response was expressed by Δcpm values using the following formula: Δcpm = (average incorporation of [³H]thymidine under the stimulation of antigen-pulsed APC) – (average incorporation of [³H]thymidine under the stimulation of unpulsed APC).

mAb to Class I and Class II MHC Antigens and Blocking of T Cell Proliferation. Mouse ascites fluids containing the following mAbs were purchased from Organon Teknika Corporation, Durham, NC: anti-I-A^{b,d} (Ia.m8) IgM from clone 28-16-8S (40), and anti-K^{b,f} (H-2.m83) IgM from clone 28-13-3S (40). Low molecular weight, nonspecific inhibitory substances were removed from the ascites by ultrafiltration (41). Purified IgG of the following mAbs from culture supernatant of hybridoma cells were kindly provided by Dr. Linda L. Perry, Laboratory of Persistent Viral Diseases, RML: anti-I-A^{k,l,r,s} (Ia.m27) IgG2a from clone 10-3.6 (42), anti-I-A^{b,d,p,q,v} (Ia.m25) IgG2a from clone 25-9-17S (40), anti-I-E^{k,d,p,r} (Ia.m7) IgG2a from clone 14-4-4S (43), and anti-K^{k,p,q,r} (H-2.m93) IgG2a from clone 11-4.1 (42). To block antigen-specific T cell proliferative responses, various dilutions of the mAbs or control Click's medium were added to the culture and were present throughout the culture period (41).

Results

Influence of the H-2 Haplotype of APC on Proliferative Response of T Cells Primed with R-MuLV. Since homozygous *H-2^{a/a}* mice failed to develop protective immunity against FV after immunization with the vaccinia-F-MuLV *env* recombinant (26) and T cells from FV-infected *H-2^{a/a}* mice did not show significant proliferative response under the stimulation of FV-induced erythroleukemia cells (24), it was postulated that T cells in *H-2^{a/a}* mice could not be primed with the F-MuLV antigens. To test whether T cells could recognize the viral antigens in the context of H-2^a MHC molecules, we first examined proliferative response of nylon wool-purified T cells from immunized *H-2^{a/b}* mice under the stimulation of *H-2^{a/a}* and *H-2^{b/b}* PEC. Because of the availability of a large quantity of purified virions, R-MuLV, which is highly crossreactive with F-MuLV, was used to immunize mice along with CFA. As shown in Fig. 1, *H-2^{b/b}* PEC pulsed with UV-irradiated R-MuLV induced three to four times higher peak proliferative response of R-MuLV-primed *H-2^{a/b}* T cells than pulsed *H-2^{a/a}* PEC did. This difference in APC activity to R-MuLV-primed *H-2^{a/b}* T cells between *H-2^{a/a}* and *H-2^{b/b}* PEC was not because of the difference in kinetics of T cell proliferation because both under the stimulation of *H-2^{a/a}* PEC and under the stimulation of *H-2^{b/b}* PEC the peak of thymidine incorporation was observed when [³H]thymidine was added on day 3 (data not shown). R-MuLV-specific T cell proliferative responses were analyzed repeatedly from 17 to 31 d after priming of the *H-2^{a/b}* mice and peak Δcpm values under the stimulation of *H-2^{b/b}* or *H-2^{a/b}* PEC were always three to seven times higher than those under the stimulation of *H-2^{a/a}*

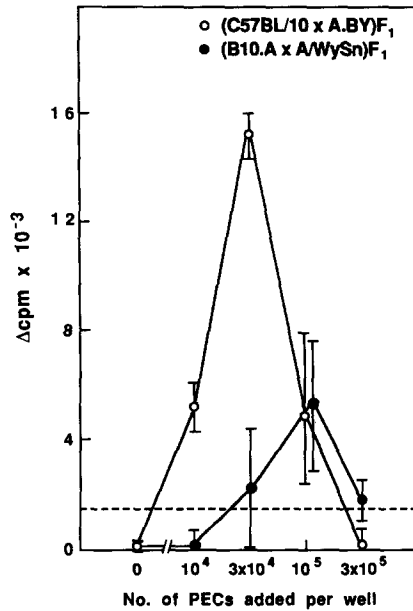


FIGURE 1. Proliferative responses of the T cells from $H-2^{a/b}$ mice immunized with the purified whole virions of R-MuLV in CFA. Responder T cells were prepared 31 d after immunization and were cultured with indicated numbers of PEC harvested from (C57BL/10 × A.BY)F₁ ($H-2^{b/b}$) or (B10.A × A/WySn)F₁ ($H-2^{a/a}$) mice. Dashed line shows the maximum level of proliferative response exhibited by T cells from unprimed control $H-2^{a/b}$ mice under the stimulation of antigen-pulsed PEC (mean + SD). Each Δcpm value shows mean ± SD.

PECs (Table II). Therefore, the observed difference in APC activity between $H-2^{a/a}$ and $H-2^{b/b}$ PEC was not caused by a difference in the time course of priming between $H-2^a$ -restricted and $H-2^b$ -restricted T cell populations. Thus, these results clearly showed that the PEC having the $H-2^b$ haplotype were more efficient than those having only the $H-2^a$ haplotype to present the viral antigens to R-MuLV-primed $H-2^{a/b}$ T cells. In addition, there was no significant difference between the APC activity of heterozygous $H-2^{a/b}$ PECs and that of homozygous $H-2^{b/b}$ PEC to R-MuLV-primed T cells, confirming the dominance of $H-2^b$ haplotype.

TABLE II
Proliferative Response of T Cells from the (B10.A × A.BY)F₁ Mice
Immunized with R-MuLV in CFA under the Stimulation of APC
That Have Different H-2 Haplotypes

Exp.	Days after priming	No. of PEC per well*	[³ H]Thymidine incorporation by 2 × 10 ⁵ T cells (Δcpm) under the stimulation of		
			$H-2^{b/b}$ PEC	$H-2^{a/b}$ PEC	$H-2^{a/a}$ PEC
1	17	10 ⁵	6,460 ± 1,876	6,764 ± 744 ‡	1,632 ± 788
2	19	3 × 10 ⁴	7,608 ± 811	ND	2,508 ± 1,084
3	24	3 × 10 ⁴	8,014 ± 1,007	7,582 ± 996	2,744 ± 1,156
4	31	3 × 10 ⁴	15,377 ± 1,706	ND	2,248 ± 2,637

Stimulator cell dose-response curves were analyzed in all of these experiments and the data are represented by peak proliferative response. No significant proliferative response of T cells from unimmunized control mice under the stimulation of R-MuLV-pulsed PEC was observed in any of these experiments.

* PEC were pulsed with UV-irradiated R-MuLV.

‡ Δcpm values in boxes are significantly higher than those under the stimulation of $H-2^{a/a}$ PEC in each experiment ($p < 0.05$ by Student's t test).

Proliferative Response of T Cells Primed with TNP-KLH. To eliminate the possibility that the above-described difference in the R-MuLV-primed T cell proliferative response under the stimulation of $H-2^{a/a}$ and $H-2^{b/b}$ PEC was a result of general functional impairment of $H-2^{a/a}$ PEC, we tested the proliferative response of $H-2^{a/b}$ T cells primed with TNP-KLH as a control antigen. As shown representatively in Fig. 2, there was no general impairment in APC activity of $H-2^{a/a}$ PEC, compared with that of $H-2^{b/b}$ PEC, that could explain the three to sevenfold difference in R-MuLV-specific T cell proliferative response.

Proliferative Response of F-MuLV Envelope-specific T Cells under the Stimulation of $H-2^{a/a}$ and $H-2^{b/b}$ PEC. Purified R-MuLV virions used as immunogens in the previous experiments contain envelope, core, and polymerase proteins. However, earlier studies (25, 26) indicated that the F-MuLV envelope protein by itself could induce protective immunity against FV. Furthermore, H-2 haplotype had a significant influence on induction of the protective immunity with the vaccinia-F-MuLV *env* vaccine (26). Therefore, to analyze the influence of the H-2 on APC control of the F-MuLV envelope-specific T cell response, we immunized mice with the recombinant vaccinia virus that expressed the entire F-MuLV *env* gene, but not the *gag* or *pol* genes. T cells from the $H-2^{a/b}$ mice immunized with the vaccinia-F-MuLV *env* proliferated under the stimulation of $H-2^{b/b}$ PEC pulsed with UV-irradiated F-MuLV (FB-29) or R-MuLV, and there was no significant difference in the stimulator cell dose-response curves (Fig. 3, A and B) or in the antigen dose-response curves (not shown) obtained using these two virus strains, confirming the crossreactivity of the viruses at the T cell level. Importantly, while $H-2^{b/b}$ PEC induced significant proliferative responses of F-MuLV envelope-specific T cells, only marginal proliferative responses were observed when the T cells were stimulated with $H-2^{a/a}$ PEC (Fig. 3). The T cells from the $H-2^{a/b}$ mice immunized with the control vaccinia-influenza HA did not

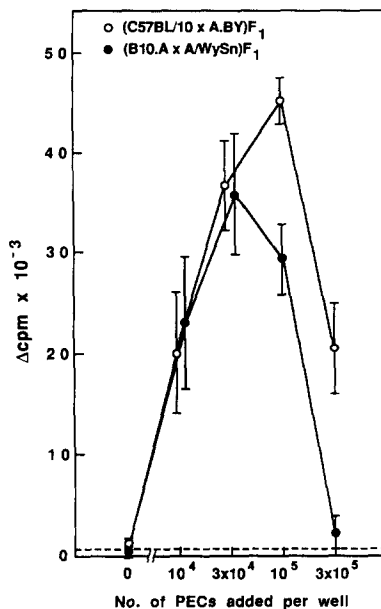


FIGURE 2. Proliferative responses of TNP-KLH-primed, $H-2^{a/b}$ T cells under the stimulation of antigen-pulsed, $H-2^{a/a}$ or $H-2^{b/b}$ PEC. Experiments were done repeatedly from 17 to 40 d after immunization, and this representative figure shows the result on day 17. Each Δcpm value shows mean \pm SD. Dashed line shows the maximum level of proliferative response exhibited by control unprimed T cells under the stimulation of TNP-KLH-pulsed PEC (mean \pm SD).

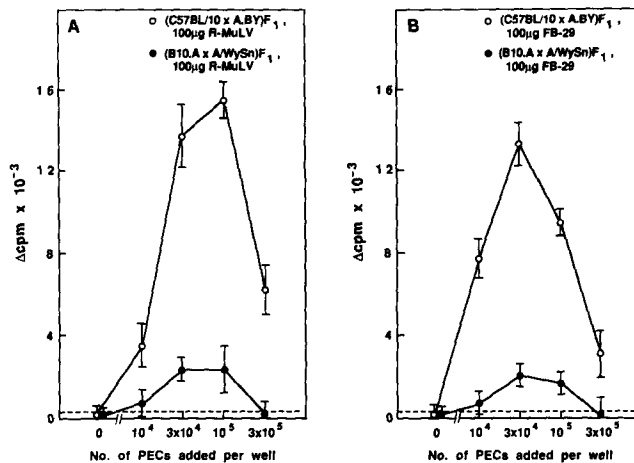


FIGURE 3. Proliferative responses of F-MuLV envelope-specific $H-2^{a/b}$ T cells under the stimulation of antigen-pulsed, $H-2^{a/a}$ or $H-2^{b/b}$ PEC. Experiments were done repeatedly from 16 to 32 d after immunization and all the results were similar to this representative figure. 4×10^5 T cells were cultured in a well of 96-well culture plates in this particular experiment. PEC were pulsed either with UV-irradiated R-MuLV (A) or with UV-irradiated F-MuLV (FB-29) (B). Each Δ cpm value shows mean \pm SD. Dashed lines show maximum levels of proliferative response exhibited by T cells from the $H-2^{a/b}$ mice immunized with the control vaccinia-influenza-HA vaccine under the stimulation of R-MuLV- or F-MuLV-pulsed PEC (mean + SD).

proliferate under the stimulation of PEC pulsed with purified F-MuLV or R-MuLV, showing the lack of cross-stimulation of vaccinia virus-specific T cells with the purified MuLVs. Thus, this result suggested that F-MuLV envelope-specific T cells from $H-2^{a/b}$ mice recognized the envelope protein in the context of $H-2^b$ MHC products but the same T cells failed to recognize the antigen in the context of $H-2^a$ MHC products.

Mapping of the Ir Gene for F-MuLV Envelope Protein in A_β , A_α , or E_β Loci of the H-2. To determine the gene locus (or loci) within the H-2 that influences the APC activity to F-MuLV envelope-specific T cells, we first tested several H-2 recombinant mouse strains as the sources of PEC. As shown in Fig. 4, the PEC from (B10.A(5R) \times A/WySn) F_1 or (B10.A(18R) \times A/WySn) F_1 mice having the b allele at least at the K, A_β , A_α , and E_β loci of the H-2 stimulated the proliferative response of the F-MuLV envelope-specific $H-2^{a/b}$ T cells. However, the PECs from (B10.A(1R) \times A/WySn) F_1 or (B10.A(4R) \times A/WySn) F_1 mice having only the k allele at these loci did not evoke significant proliferative response even when they had the b allele at the E_α , S, and/or D loci of the H-2.

To distinguish the possible influence of the b allele at the K locus from that of the b allele at the A_β , A_α , and/or E_β loci, we next used the B10.MBR strain of mice that has a recombination between the K and A_β loci of the H-2 (see Table I). When PEC from (B10.MBR \times A.BY) F_1 and (B10.MBR \times A/WySn) F_1 mice were used to stimulate $H-2^{a/b}$ T cells primed specifically for F-MuLV envelope protein, significantly higher envelope-specific proliferative responses were observed under the stimulation of (B10.MBR \times A.BY) F_1 ($K^{b/b}$, $I-A^{k/b}$) PEC (Table III). Thus, the PEC having the b allele both at the K and I-A loci were more efficient at presenting the antigen to F-MuLV envelope-specific T cells than were the PEC having the b allele only at the K locus. However, the Δ cpm values obtained under the stimulation of

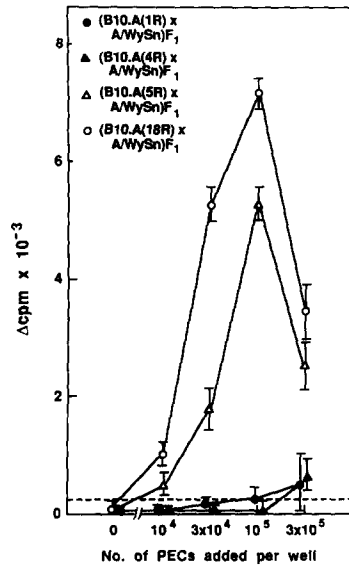


FIGURE 4. Proliferative responses of F-MuLV envelope-specific, $H-2^{a/b}$ T cells under the stimulation of antigen-pulsed PEC from four H-2 recombinant mouse strains. Experiments were done repeatedly from 21 to 32 d after immunization and all the results were similar to this representative figure. T cells were prepared from $(B10.A \times A.BY)F_1$ mice immunized with the vaccinia-F-MuLV *env* vaccine, and 2×10^5 T cells per well were cultured with PEC pulsed with UV-irradiated R-MuLV. For H-2 alleles of the sources of PEC, see Table I. Each Δ cpm value shows mean \pm SD. Dashed line shows the maximum level of proliferative response exhibited by control vaccinia-influenza-HA-primed T cells under the stimulation of R-MuLV-pulsed PEC (mean + SD).

$(B10.MBR \times A/WySn)F_1$ PEC fluctuated from marginal to significant levels, possibly because of incomplete depletion of APC from the responder T cell preparations in some experiments and augmentation of the envelope-specific proliferative responses by mixed lymphocyte reaction caused by the incompatibility at the D locus. In fact, the background proliferative responses under the stimulation of unpulsed, $(B10.MBR \times A.BY)F_1$ or $(B10.MBR \times A/WySn)F_1$ PEC were always twice as high as those under the stimulation of syngeneic $H-2^{a/b}$ PEC. To eliminate the difficulties caused by the D-locus incompatibility and possible contamination of APC to

TABLE III
Proliferative Response of F-MuLV Envelope-specific, $H-2^{a/b}$ T Cells under the Stimulation of $(B10.MBR \times A.BY)F_1$ or $(B10.MBR \times A/WySn)F_1$ PEC Pulsed with UV-irradiated F-MuLV (FB-29)

Immunization of $(B10.A \times A.BY)F_1$ mice in vivo*	Days after final immunization	No. of PEC added per well†	[³ H]Thymidine incorporation by 5×10^5 T cells (Δ cpm value) under the stimulation of PEC from	
			$(B10.MBR \times A.BY)F_1$	$(B10.MBR \times A/WySn)F_1$
Once	16	10^5	$4,958 \pm 2,309$	$1,902 \pm 1,307$
Once	17	3×10^4	$23,050 \pm 2,277^{\S}$	$13,711 \pm 1,373$
Once	28	10^5	$23,534 \pm 1,375^{\S}$	$7,106 \pm 473$
Once	29	10^5	$16,358 \pm 877^{\S}$	$4,909 \pm 952$
Twice	10	10^5	$40,773 \pm 2,196^{\S}$	$2,084 \pm 1,229$
Twice	13	3×10^4	$24,146 \pm 3,207^{\S}$	$14,799 \pm 1,713$

* $(B10.A \times A.BY)F_1$ ($H-2^{a/b}$) mice were immunized with the vaccinia-F-MuLV *env* recombinant vaccine. In some experiments mice were boosted 3 mo later by repeating the same immunization. The spleen and lymph node cells from two immunized mice were pooled to prepare T cells in each experiment.

† Stimulator cell dose-response curves were analyzed in each of these experiments and the results are represented here by peak Δ cpm values.

§ These Δ cpm values are significantly higher than the other value of the same experiment ($p < 0.05$ by Student's *t* test).

T cell preparations, syngeneic T cell proliferative responses were analyzed using both immune T cells and PEC from (B10.MBR \times A.BY) F_1 and (B10.MBR \times A/WySn) F_1 mice. As shown in Fig. 5, F-MuLV envelope-specific T cells from immunized (B10.MBR \times A.BY) F_1 mice proliferated strongly under the stimulation of antigen-pulsed, syngeneic PEC, while T cells from (B10.MBR \times A/WySn) F_1 mice immunized with the same vaccinia-F-MuLV *env* vaccine were almost totally nonresponsive to the stimulation with syngeneic PEC pulsed with F-MuLV. Thus, T cells were not primed with F-MuLV envelope protein in (B10.MBR \times A/WySn) F_1 mice that are $K^{b/k}$ but $I-A^{k/k}$. These results strongly indicated that proliferative T cells recognized the F-MuLV envelope protein in the context of $I-A^b$, hybrid $I-A^{k/b}$, and/or hybrid $I-E^{k/b}$ molecules, but not in the context of K^b , K^k , $I-A^k$, or $I-E^k$ molecules.

Blocking of T Cell Proliferation by Anti- $I-A^b$ and Anti- $I-E^k$ mAbs. To demonstrate directly the MHC molecules that function as the restriction elements in the recognition of F-MuLV envelope, we examined blocking of T cell proliferative responses with several anti-H-2 mAbs. Fig. 6 shows representative results of repeated antibody dose-response analyses. Under the stimulation of $H-2^{b/b}$ PEC (Fig. 6A) both of the two anti- $I-A^b$ mAbs strongly inhibited the F-MuLV envelope-specific T cell proliferative response, whereas the anti- $I-E^k$ and anti- K^b mAbs did not. When $H-2^{a/b}$ PEC were used to stimulate the envelope-specific T cells (Fig. 6B), both the anti- $I-A^b$ mAbs, again, significantly inhibited the proliferative response. However, more importantly, the anti- $I-A^k$ mAb did not block the proliferative response at all (*arrow*), and neither the anti- K^b nor the anti- K^k mAb inhibited the envelope-specific T cell proliferation. Interestingly, anti- $I-E^k$ mAb 14-4-4S exhibited an intermediate inhibitory effect on the envelope-specific T cell proliferative response (*arrow*).

The lack of inhibition of the F-MuLV envelope-specific T cell proliferative re-

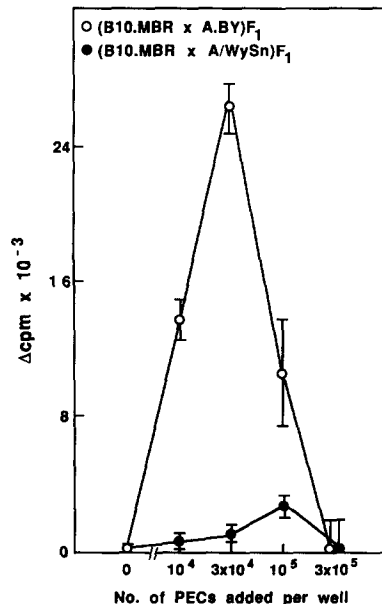


FIG. 5. Proliferative responses of F-MuLV envelope-specific T cells from (B10.MBR \times A.BY) F_1 and (B10.MBR \times A/WySn) F_1 mice under the stimulation of syngeneic PEC. Mice were immunized with the vaccinia-F-MuLV *env* recombinant vaccine and immune T cells were prepared from 16 to 29 d after the immunization. PEC were pulsed with UV-irradiated F-MuLV (FB-29), and indicated numbers were cultured with 5×10^5 T cells per well. The results of repeated experiments were completely consistent with this representative figure. Each Δ cpm value shows mean \pm SD.

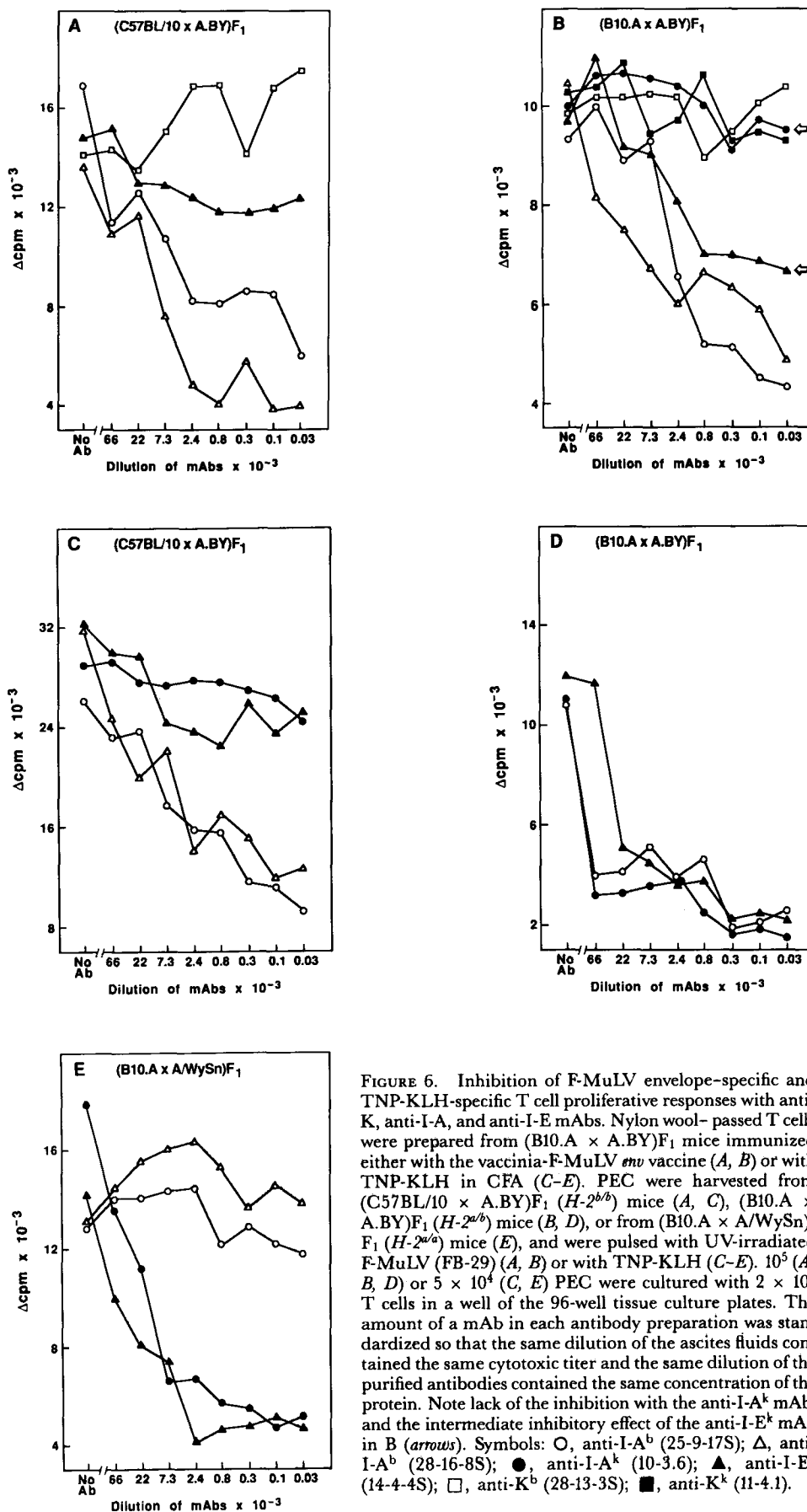


FIGURE 6. Inhibition of F-MuLV envelope-specific and TNP-KLH-specific T cell proliferative responses with anti-K, anti-I-A, and anti-I-E mAbs. Nylon wool-passed T cells were prepared from (B10.A \times A.BY)_{F1} mice immunized either with the vaccinia-F-MuLV *env* vaccine (A, B) or with TNP-KLH in CFA (C-E). PEC were harvested from (C57BL/10 \times A.BY)_{F1} (*H-2^{b/b}*) mice (A, C), (B10.A \times A.BY)_{F1} (*H-2^{a/b}*) mice (B, D), or from (B10.A \times A/WySn)_{F1} (*H-2^{a/a}*) mice (E), and were pulsed with UV-irradiated F-MuLV (FB-29) (A, B) or with TNP-KLH (C-E). 10^5 (A, B, D) or 5×10^4 (C, E) PEC were cultured with 2×10^5 T cells in a well of the 96-well tissue culture plates. The amount of a mAb in each antibody preparation was standardized so that the same dilution of the ascites fluids contained the same cytotoxic titer and the same dilution of the purified antibodies contained the same concentration of the protein. Note lack of the inhibition with the anti-I-A^k mAb, and the intermediate inhibitory effect of the anti-I-E^k mAb in B (arrows). Symbols: \circ , anti-I-A^b (25-9-17S); Δ , anti-I-A^b (28-16-8S); \bullet , anti-I-A^k (10-3.6); \blacktriangle , anti-I-E^k (14-4-4S); \square , anti-K^b (28-13-3S); \blacksquare , anti-K^k (11-4.1).

sponse under the stimulation of $H-2^{a/b}$ PECs with the anti-I-A^k mAb was not because we used an antibody of inappropriate specificity or inadequate affinity. Fig. 6, C-E show the results of control experiments in which the inhibition of TNP-KLH-specific T cell proliferative response was analyzed. As shown in Fig. 6, C-E, the anti-I-A and anti-I-E mAbs used in these experiments had appropriate haplotype specificity and adequate affinity to inhibit T cell proliferative responses. Moreover, all the anti-I-A and anti-I-E mAbs used inhibited the T cell proliferative response through their binding to APC, but not by the binding to T cells, because only the mAbs directed to MHC antigens expressed on PEC, but not the mAbs directed to the MHC molecules that are expressed on T cells but absent on PEC, inhibited the proliferation (Fig. 6, compare C and E). Thus, these results directly indicate that F-MuLV envelope-specific T cells from $H-2^{a/b}$ mice recognize the envelope protein in association with I-A^b, hybrid I-A^{k/b}, and/or hybrid I-E^{k/b} class II MHC molecules expressed on APC.

bm12 Mutation Abolished T Cell Responsiveness to F-MuLV Envelope Protein. Both of the anti-I-A^b mAbs that inhibited the F-MuLV envelope-specific T cell proliferative responses under the stimulation of $H-2^{a/b}$ PEC in previous section do not react with the mutant I-A molecule of B6.C- $H-2^{bm12}$ (bm12) mice (44). This mutation occurred in the β_1 exon of the $A\beta^b$ gene (45, 46). Therefore, it was of interest to test whether the presence of wild-type $A\beta^b$ chain was essential for the recognition of F-MuLV envelope glycoprotein by T cells. We hence immunized both (B10.A \times A.BY)F₁ ($H-2^{a/b}$) and (bm12 \times A/WySn)F₁ ($H-2^{bm12/a}$) mice with the vaccinia-F-MuLV *env* vaccine, and tested the envelope-specific T cell proliferative responses under the stimulation of PECs elicited from these two strains of mice. T cells from immunized (B10.A \times A.BY)F₁ mice, again, proliferated strongly under the stimulation of syngeneic PEC pulsed with F-MuLV (Fig. 7). However, T cells from immunized (bm12 \times A/WySn)F₁ mice did not show any significant envelope-specific proliferative responses both under the stimulation of syngeneic PEC and under the stimulation of $H-2^{a/b}$ PEC (Fig. 7, squares). Therefore, T cells were not primed with F-MuLV envelope protein in $H-2^{bm12/a}$ mice.

The envelope-specific proliferative responses of $H-2^{a/b}$ T cells under the stimulation of $H-2^{bm12/a}$ PEC were significantly lower than those under the stimulation of syngeneic PEC (Fig. 7 and Table IV). In three of the five repeated experiments, the Δ cpm values obtained under the stimulation of $H-2^{bm12/a}$ PEC were marginal. However, relatively high level of envelope-specific proliferative responses were observed in two other experiments (Table IV). Thus, the expression of wild-type $A\beta^b$ chain was definitely required to prime T cells with F-MuLV envelope protein in vivo, and antigen-presenting activity of $H-2^{bm12/a}$ PECs to the envelope-specific T cells was, at least, lower than that of $H-2^{a/b}$ PEC, but we could not distinguish whether the lack of T cell priming with F-MuLV envelope protein in $H-2^{bm12/a}$ mice was due to a restricted T cell repertoire or reduced antigen-presenting activity to envelope-specific T cells, or both.

Discussion

In this report we have clearly demonstrated an influence of the H-2 on presentation of F-MuLV envelope protein to specifically primed proliferative T cells. APC having the dominant $H-2^b$ haplotype can present the envelope protein to immune T cells and can induce a T cell proliferative response, while APC having only the

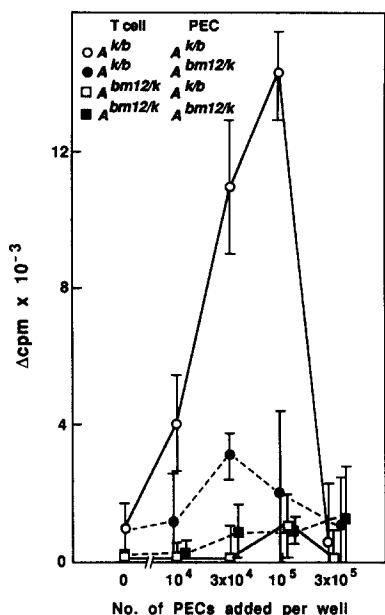


FIGURE 7. Proliferative responses of F-MuLV envelope-specific T cells from (B10.A × A.BY)_{F1} and (bm12 × A/WySn)_{F1} mice under the stimulation of syngeneic and coisogenic PEC. Mice were immunized with the vaccinia-F-MuLV *env* recombinant vaccine as described and immune T cells were prepared from 16 to 20 d after the immunization. PEC were pulsed with UV-irradiated F-MuLV (FB-29), and indicated numbers of PEC were cultured with 5×10^5 T cells per well. The results of three repeated experiments were completely consistent with this representative figure. Each Δ cpm value shows mean \pm SD.

H-2^a haplotype cannot (Fig. 3). This phenomenon is not caused by general functional impairment of APC having the *H-2^{a/a}* genotype because in the control experiments *H-2^{a/a}* APC presented the antigen to TNP-KLH-primed T cells as effectively as *H-2^{b/b}* APC did (Fig. 2). Genetic mapping experiments show that the gene(s) controlling the antigen-presenting activity to F-MuLV envelope-specific T cells is located in K- or I-region loci of the H-2 (Fig. 4). Moreover, T cells in (B10.MBR × A/WySn)_{F1} mice that have the *K^{b/k}*, *I-A^{k/k}* genotype cannot be primed with the envelope antigen (Fig. 5). The most likely explanation for this influence of the H-2 on antigen-presenting activity is that F-MuLV envelope-specific T cells in *H-2^{a/b}*

TABLE IV
Proliferative Responses of F-MuLV Envelope-specific *H-2^{a/b}* T Cells under the Stimulation of Syngeneic *I-A^{k/b}* or Coisogenic *I-A^{bm12/k}* PEC Pulsed with UV-irradiated F-MuLV (FB-29)

Immunization of (B10.A × A.BY) _{F1} mice in vivo*	Days after final immunization	No. of PEC added per well [†]	³ H]Thymidine incorporation by 5×10^5 T cells (Δ cpm value) under the stimulation of PEC from	
			(B10.A × A.BY) _{F1}	(bm12 × A/WySn) _{F1}
Once	16	10^5	$14,305 \pm 1,400^{\S}$	$1,890 \pm 2,563$
Once	17	3×10^4	$14,590 \pm 821^{\S}$	$7,722 \pm 1,536$
Once	19	10^5	$4,666 \pm 1,022^{\S}$	$1,297 \pm 1,119$
Twice	10	3×10^4	$12,133 \pm 1,893^{\S}$	$3,154 \pm 616$
Twice	13	3×10^4	$13,007 \pm 2,306$	$8,179 \pm 2,235$

* The schedule of immunization was the same as that described in Table III.

[†] Stimulator cell dose-response curves were analyzed in each of these experiments and the results are represented here by peak Δ cpm values.

[§] These Δ cpm values are significantly higher than the other value of the same experiment ($p < 0.05$ by Student's *t* test).

mice recognize the envelope protein only in association with I-A^b, hybrid I-A^{k/b}, and/or hybrid I-E^{k/b} molecules.

To demonstrate directly that these class II MHC molecules were actually functioning as restriction elements in the recognition of F-MuLV envelope antigen by T cells, we did blocking experiments with anti-H-2 mAbs (Fig. 6). Both of the two anti-I-A^b mAbs used strongly inhibited the F-MuLV envelope-specific T cell proliferative response under the stimulation of *H-2^{a/b}* or *H-2^{b/b}* PEC, while the anti-K^b, anti-K^k, and, more importantly, anti-I-A^k mAbs did not block even when the T cells were stimulated with heterozygous *H-2^{a/b}* PEC. Since both of the anti-I-A^b mAbs used in this study do not react with the mutant I-A molecule of bm12 mice (44), the presence of wild-type A_β^b chain seems to be essential for the formation of the antigenic determinants recognized with these mAbs. In addition, the antigenic determinant recognized with anti-I-A^k mAb 10-3.6 is located on A_β^k chain (47). Therefore, within the limitation of mAbs used, the results of our blocking experiments, along with the results obtained with the H-2 recombinant mice, indicate that A_α^bA_β^b and possibly A_α^kA_β^b class II molecules are the restriction elements for F-MuLV envelope-specific T cells, while A_α^kA_β^k and A_α^bA_β^k molecules are not.

Importance of A_β^b chain in T cell recognition of F-MuLV envelope protein is further substantiated by the experiments using the mutant bm12 mice. T cells are not primed with the envelope antigen in (bm12 × A/WySn)F₁ mice (Fig. 7), and F-MuLV envelope-specific T cells from *H-2^{a/b}* mice are more efficiently stimulated with syngeneic PEC than with *I-A^{bm12/a}* PEC (Fig. 7 and Table IV). Therefore, A_β^b chain seems to play a critical role in stimulation of F-MuLV envelope-specific proliferative T cells. In addition, although (bm12 × A/WySn)F₁ PEC are thought to express A_α^bA_β^k hybrid class II molecules along with A_α^bA_β^{bm12}, A_α^kA_β^{bm12}, and A_α^kA_β^k molecules, there is no complementation effect by this hybrid I-A molecule to stimulate the envelope-specific T cells. This result further supports the conclusion that A_α^bA_β^k molecule is not involved in T cell recognition of F-MuLV envelope antigen.

Since *H-2^{a/a}* APC that express I-E^k as well as I-A^k molecules cannot present the envelope glycoprotein to specifically primed T cells, it is a somewhat unexpected finding that anti-I-E^k mAb 14-4-4S induced intermediate, but statistically significant, inhibition of the envelope-specific T cell proliferative response under the stimulation of *H-2^{a/b}* PEC (Fig. 6 B). However, since this mAb has specificity of Ia.7 (43) and the antigenic determinant for this specificity is expressed on the E_α^k subunit (8), this inhibitory effect is probably mediated by a steric effect caused by binding of the mAb to E_α^k chain of the E_α^kE_β^b complex. Thus, it is likely that, in addition to I-A^b- and hybrid I-A^{k/b}-restricted T cells, some (small) population of F-MuLV envelope-specific T cells in *H-2^{a/b}* mice do recognize the antigen in the context of E_α^kE_β^b. Marginal proliferative responses of *H-2^{a/b}* T cells under the stimulation of *H-2^{bm12/a}* PEC might also be mediated by this hybrid I-E molecule.

The envelope protein of F-MuLV is a large, complex molecule that is composed of 675 amino acids and several carbohydrate chains (48), and it has at least several different antigenic determinants distinguishable with mAbs (31, 32, 49, 50). It is, therefore, quite surprising that the same molecule does not have any T cell determinants that can be recognized in association with H-2^a MHC products. From the results of their extensive analyses on the interaction between protein-derived immunogenic peptides and class II MHC molecules, Buus et al. (51) have calculated

that each class II molecule can bind >50% of immunogenic peptides of 10–17 amino acids and even 10–20% of unbiased (not only immunogenic) small peptides. In this regard, it would be difficult to imagine that none of the peptides derived from such a big molecule as F-MuLV envelope glycoprotein can be bound by I-A^k or I-E^k molecule. On the other hand, since envelope glycoprotein of endogenous murine leukemia viruses is expressed as a differentiation antigen during the normal development of hematopoietic precursor cells and reproductive systems (52, 53), it is possible that T cell repertoire for recognition of the F-MuLV envelope protein is somewhat restricted by self-tolerance to endogenous retroviral antigens. In fact, lack of T cell responsiveness to F-MuLV envelope protein in (bm12 × A/WySn)F₁ mice strongly suggests that T cells recognize a very limited number of epitopes on the envelope antigen. For most of the variety of antigens tested, the immune responsiveness of coisogenic C57BL/6 (B6) and bm12 mice were found to be indistinguishable (summarized in reference 54). H-Y antigen and beef insulin are the only two exceptions where bm12 mice are nonresponders and B6 are responders (54). Interestingly, analyses using heterologous insulins (54, 55) have demonstrated that only one amino acid change between beef and sheep insulins is responsible for the different immune responsiveness between bm12 and B6 mice. Therefore, it is possible that T cell repertoire for recognition of F-MuLV envelope protein is restricted to only one epitope defined by a few amino acid(s). Comparison of T cell responsiveness to endogenous versus exogenous retroviral envelope proteins in *H-2^b* and *H-2^{bm12}* mice might provide further evidence to evaluate this hypothesis.

The Ir gene control of T cell responsiveness to F-MuLV envelope protein described here is completely consistent with our previous results of in vivo immune protection experiments (25, 26). In fact, the mice having the responder *b* allele at I region loci of the H-2 are protectively immunized against FV with the vaccinia-F-MuLV *env* recombinant vaccine, while the mice having only the nonresponder *k* allele in these loci are not protected (26). Since it has previously been shown that the protective immunity against FV is functionally associated with priming of helper T cells with F-MuLV envelope (25, 26), it is now quite appropriate to conclude that the Ir genes for F-MuLV envelope protein do control the induction of helper T cells in vivo. However, the role of the same Ir genes in spontaneous recovery from FV-induced leukemia is still unclear. Our previous studies (17, 19) have demonstrated that the H-2D locus strongly influences spontaneous recovery from FV-induced splenomegaly. This influence appears to act by controlling the time course of the development of Friend leukemia cell-specific T cell proliferative response in FV-infected mice (24). The mechanism by which the H-2D locus influences the dynamics of T cell response is unknown. However, the mice having the *d/b* or *d/d* allele at the D locus do not recover from high-dose inoculation of FV even when they have the responder *b* allele at the H-2I region (Table I). Therefore, the effect of H-2D locus is apparently stronger than that of the Ir genes to F-MuLV envelope. Interestingly, when inoculated with a low dose of FV, *H-2^{a/b}* as well as *H-2^{b/b}* mice recover spontaneously from splenomegaly, while *H-2^{a/a}* mice do not (Table I). We are currently examining whether there is a possible requirement for the responder *b* allele at H-2I for spontaneous recovery of *H-2D^{d/b}* mice from low-dose inoculation of FV.

Finally, one recent study (56) suggested that similar Ir gene phenomenon might

control the T cell responsiveness in humans against human T-lymphotropic virus type-I (HTLV-I). Low responsiveness of T cells against the human retrovirus was associated with the development of leukemia (56). Based on our present results, low responsiveness of T cells to retroviral antigens may also cause lack of or insufficient protective immunity after immunization. Therefore, it will probably be necessary to overcome genetic restrictions of the antiretrovirus immune response when attempting to induce protective immunity against human retrovirus infection. In this regard, our previous study (26) demonstrated that nonresponders to F-MuLV envelope protein could be protected against FV when they were immunized with fixed F-MuLV particles in CFA. Furthermore, a low, but significant, level of T cell proliferative response under the stimulation of *H-2^{a/a}* PEC was observed when the *H-2^{a/b}* mice were immunized with purified R-MuLV virions in CFA (Fig. 1 and Table II). This apparent bypassing of the Ir gene phenomenon might be due to immunization of mice with larger amounts of antigen, use of highly immunostimulatory adjuvant, or simply due to the priming of T cells with the *gag* and/or *pol* gene products. This latter possibility is currently being tested by using recombinant vaccinia viruses that express the F-MuLV *gag* gene.

Summary

T cells primed specifically for the envelope glycoprotein of Friend murine leukemia helper virus (F-MuLV) were prepared by immunizing mice with a recombinant vaccinia virus that expressed the entire *env* gene of F-MuLV. Significant proliferative responses of F-MuLV envelope-specific, *H-2^{a/b}* T cells were observed when the T cells were stimulated with antigen-pulsed peritoneal exudate cells (PEC) having the *b* allele at the K, A β , A α , and E β loci of the H-2. On the other hand, PEC having only the *k* allele at these loci did not induce the envelope-specific T cell proliferation, even when the PEC had the *b* allele at the E α , S, or D loci. F-MuLV envelope-specific proliferation of *H-2^{a/b}* T cells under the stimulation of antigen-pulsed, *H-2^{a/b}* PEC was specifically blocked with anti-I-A^b and anti-I-E^k mAbs but not with anti-K^b, anti-K^k, or anti-I-A^k mAbs. Moreover, (B10.MBR \times A/WySn)F₁ mice that have the *b* allele only at the K locus but not in I-A subregion were nonresponders to the envelope glycoprotein, and the *bm12* mutation at the A β locus completely abolished the T cell responsiveness to this antigen.

These results indicate that proliferative T cells recognize a limited number of epitopes on F-MuLV envelope protein in the context of I-A^b, hybrid I-A^{k/b}, and/or hybrid I-E^{k/b} class II MHC molecules but fail to recognize the same envelope protein in the context of I-A^k or I-E^k molecules. This influence of the H-2I region on T cell recognition of the envelope glycoprotein appeared to control in vivo induction of protective immunity against Friend virus complex after immunization with the vaccinia-F-MuLV *env* vaccine. Thus, these results provide, for the first time, direct evidence for Ir gene-controlled responder/nonresponder phenotypes influencing the immune response to a pathogenic virus of mice.

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