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Anti-Gag Cytolytic T Lymphocytes Specific for an Alternative Translational Reading Frame-Derived Epitope and Resistance Versus Susceptibility to Retrovirus-Induced Murine AIDS in F_1 Mice

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Murine AIDS (MAIDS) develops in susceptible mouse strains after infection with the LP-BM5 murine leukemia virus complex that contains causative defective, and ecotropic helper, retroviruses. We previously demonstrated that the MAIDS-resistant H-2^d strains BALB/cByJ and C57BL/KsJ generate MHC class I (K^d) restricted virus-specific $CD8^+$ cytolytic T lymphocytes (CTLs) that lyse cells expressing either defective or ecotropic gag proteins. In contrast, the congenic BALB.B and closely related C57BL/6J MAIDS-susceptible H-2^b strains were unable to serve as a source of gag-specific CTLs (Schwarz and Green, 1994), suggesting that anti-gag CTLs might provide a basis for resistance to MAIDS. Although its susceptibility to MAIDS was unknown, the (BALB/c × C57BL/6J) F_1 (CBY6F_1) strain could also produce H-2^d-, but not H-2^b-, restricted, anti-gag CTLs (Schwarz and Green, 1994). Because of this correlation between anti-gag CTLs and resistance to MAIDS, it was important to provide more direct evidence in support of CTL-mediated protection and to determine both the fine specificity of CByB6F_1 anti-gag CTLs, in comparison with the resistant C57BL/Ks and BALB/c strains, and the susceptibility of this F_1 strain to LP-BM5-induced MAIDS. We report here that no symptoms of MAIDS were observed in CBY6F_1 (H-$2^{d\times b}$) mice. For F_2 mice, in contrast to the high susceptibility of H-$2^{b/b}$ mice, 77% of H-$2^{d/d}$ and 81% of H-$2^{b/d}$ F_2 mice did not exhibit MAIDS after LP-BM5 infection. These results are in contrast to other published studies that concluded that susceptibility, rather than resistance, is dominant in F_1 (resistant × susceptible or susceptible × resistant) mice. We also show that CBY6F_1 anti-gag CTLs exhibit a fine specificity shared by the MAIDS-resistant BALB/c and C57BL/Ks strains, that is, the immunodominant gag epitope, SYNTGRFPPL, encoded by an alternative open reading frame. Together with our direct demonstration here that *in vivo* monoclonal antibody (mAb) depletion of $CD8^+$ T cells converts genetically resistant mice to MAIDS susceptibility, these data on the ability to mount anti-ORF2/SYNTGRFPPL, gag-specific CTL responses strongly suggest that CTLs are a primary factor in determining MAIDS resistance. Accordingly, given the K^d-restricted nature of the CTLs, the main genetic determinant of resistance appeared to be the codominant expression of the resistant H-2^d haplotype. Interestingly, however, 19% of H-$2^{d/b}$ and 23% of the H-$2^{d/d}$ F_2 mice had at least one clinical aspect of MAIDS, suggesting that a non-MHC genetic determinant(s) can negatively influence T-cell protection and thus disease outcome

Key Words: cytolytic T lymphocytes; antigens; peptides; epitopes; antigen presentation; MAIDS; rodent; immunodeficiency disease; mRNA; MHC.

INTRODUCTION

The LP-BM5 isolate of murine retroviruses induces a profound and progressive immunodeficiency in certain inbred strains of mice (Mosier *et al.,* 1985; Aziz *et al.,* 1989; Jolicoeur, 1991; Morse *et al.,* 1992). After infection, there is an early hypergammaglobulinemia and a generalized enlargement of lymphoid organs, including the spleen and lymph nodes, probably due to an inappropriate activation and resultant proliferation of both B and T lymphocytes. Ultimately, T and B lymphocytes are rendered hyporesponsive to either antigenic or mitogenic challenge. As a consequence, LP-BM5-infected, immunosuppressed mice die after exposure to environmental pathogens that cause only minor infections in healthy mice (Buller *et al.,* 1987). Because some of these disease features resemble those of humans infected with HIV, this mouse model is referred to as murine AIDS (MAIDS).

Although three classes of murine retroviruses have been isolated from the LP-BM5 mixture, neither the BM5 ecotropic (BM5) nor mink cell cytopathic focus-inducing (MCF) replication-competent viruses, alone or in combination, are able to cause MAIDS (Aziz *et al.,* 1989; Chattopadhyay *et al.,* 1989). Rather, the BM5 and MCF viruses serve as helper viruses for the transmission of a replication-defective virus, which is the proximal etiological agent of MAIDS (Jolicoeur, 1991; Chattopadhyay *et al.,* 1989, 1991). Large deletions within the *pol* and *env* genes of this defective virus result in the production of only a modified gag protein (Huang and Jolicoeur, 1990). With the exception of a continuous stretch of about 25 amino

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acids (AA) in the COOH-terminus of p15 and 25 AA in the N-terminus of p12, the gag polyprotein encoded by the defective virus shares significant homology with other murine retroviruses, including the BM5 ecotropic helper virus.

We have previously demonstrated that MAIDS-resistant, BALB/cByJ (BALB/c) and C57BL/KsJ (C57BL/Ks) (H-2^d) mice generate MHC class I-restricted gag-specific $CD8^+$ cytolytic T lymphocytes (CTLs), which lyse cells expressing either defective (dG) or ecotropic (eG) gag proteins, as encoded by recombinant Sindbis virus or vaccinia (Vac) virus expression. In contrast to these MAIDS-resistant strains, the congenic BALB.B (H-2^b) and closely related C57BL/6J (H-2^b) MAIDS-susceptible strains were unable to serve as a source of gag-specific CTLs (Schwarz and Green, 1994), suggesting that anti-gag CTLs might play a key role in resistance to LP-BM5-induced MAIDS. Furthermore, the (BALB/c × C57BL/6J) F_1 hybrid ($CBY6F_1$) strain could also be stimulated to produce a population of H-2^d-, but not H-2^b-, restricted, anti-gag CTLs (Schwarz and Green, 1994). Using human 143B or murine MC57 (H-2^b) cells coinfected with dG- or eG-Vac-expressing viruses and recombinant vaccinia viruses expressing different H-2^d class I molecules, it was shown that the anti-gag CTLs recognized a viral epitope(s) presented in the context of H-$2K^d$ (Schwarz and Green, 1994). In addition, we subsequently demonstrated that the fine specificity of BALB/c-derived, anti-gag CTLs was accounted for by a uniquely produced epitope encoded in an alternative translational open reading frame (ORF). Relative to the primary ORF (ORF1) that encodes the structural defective or ecotropic viral gag protein, the CTLs were specific for a minimal peptide determinant defined as the ORF2-generated SYNTGRFPPL epitope (Mayrand *et al.,* 1998; Mayrand and Green, 1998).

The ability of the $CBY6F_1$ mice to generate gag-specific CTLs was of particular interest because previous studies with several F_1 (MAIDS-resistant × MAIDS-susceptible or MAIDS-susceptible × MAIDS-resistant) strains have led to the conclusion that susceptibility, rather than resistance, is dominant (Makino *et al.,* 1990; Huang *et al.,* 1992). By way of contrast, Hammelin-Bourassa *et al.* (1989) demonstrated that F_1 progeny of the susceptible, C57BL/6 × resistant, A/J strains were more resistant than susceptible to LP-BM5 virus-induced MAIDS, and Makino *et al.* (1991) reported that 55% of the F_1 progeny of resistant, RIIIS × susceptible, B10.RIII strains were MAIDS resistant. Taken together, these latter two studies suggest that in certain F_1 strains, MAIDS resistance may be dominant over MAIDS susceptibility, whereas the opposite may be true for other F_1 strains.

Because the $CBY6F_1$ mouse strain can generate gag-specific $CD8^+$ CTL responses apparently similar to those characterized in the MAIDS-resistant BALB/c and C57BL/Ks strains and unlike the MAIDS-susceptible C57BL/6 and BALB.B strains, it was important to determine the susceptibility of this F_1 strain to LP-BM5-induced MAIDS. If anti-gag CTLs are critical for resistance, it would be paradoxical if $CBY6F_1$ mice would possess a MAIDS-susceptible phenotype, as suggested for the majority of the (resistant × susceptible) F_1 strains cited above. If, indeed, CBY6 F_1 mice demonstrate susceptibility to LP-BM5-induced MAIDS, however, differences in the fine specificity of the gag-specific CTLs generated in the BALB/c and C57BL/Ks versus $CBY6F_1$ strains could account for the resistance versus susceptibility among these mouse strains. Alternatively, perhaps anti-gag CTLs are not the prime factor in determining resistance. To further investigate these issues, experiments were conducted in the present study to address three issues: (1) Is there direct evidence that $CD8^+$ CTLs confer resistance to MAIDS? (2) Do gag-specific CTLs derived from H-2^d mouse strains other than BALB/c (i.e., C57BL/Ks and $CBY6F_1$) share the ORF2-derived, SYNTGRFPPL specificity of the BALB/c-derived anti-gag CTLs? (3) Are $CBY6F_1$ mice, like many other (resistant × susceptible) F_1 strains, susceptible to LP-BM5-induced MAIDS?

RESULTS

In vivo depletion of $CD8^+$ CTLs converts resistant mice to susceptibility to LP-BM5 murine leukemia virus-induced MAIDS

To directly assess the contribution of $CD8^+$ CTLs to MAIDS resistance, prototypical resistant adult BALB/cByJ mice were depleted *in vivo* of $CD8^+$ T cells, and a portion of these were concurrently infected with LP-BM5 virus (as detailed in Materials and Methods). Postinfection, weekly injections of purified anti-CD8 ascites were administered to ensure that CD8 depletion was maintained. Confirmation of chronic $CD8^+$ T-cell depletion throughout the experiment was achieved via weekly flow cytometric analyses of splenocytes from a control group of mice that received only anti-CD8 treatment: >95% $CD8^+$ cells were eliminated, compared with naive age/gender-matched untreated mice (data not shown). This untreated mouse group was also subdivided into uninfected versus LP-BM5-infected control subsets. Six weeks after LP-BM5 infection, the mice were sacrificed and evaluated for several parameters indicative of MAIDS: spleen hyperplasia, elevated serum IgG_{2a} and IgM levels, and diminished mitogenic responses [ConA and lipopolysaccharide (LPS)]. LP-BM5-infected C57BL/6 mice, included in parallel as positive controls for the ability of the LP-BM5 virus preparation used in this experiment to induce MAIDS, demonstrated characteristically severe splenic hyperplasia, hypergammaglobulinemia, and inhibited mitogenic responses as a measure of immunosuppression (data not shown).

As depicted in Fig. 1, anti-CD8 treatment converted LP-BM5-infected BALB/cByJ-resistant mice to clear

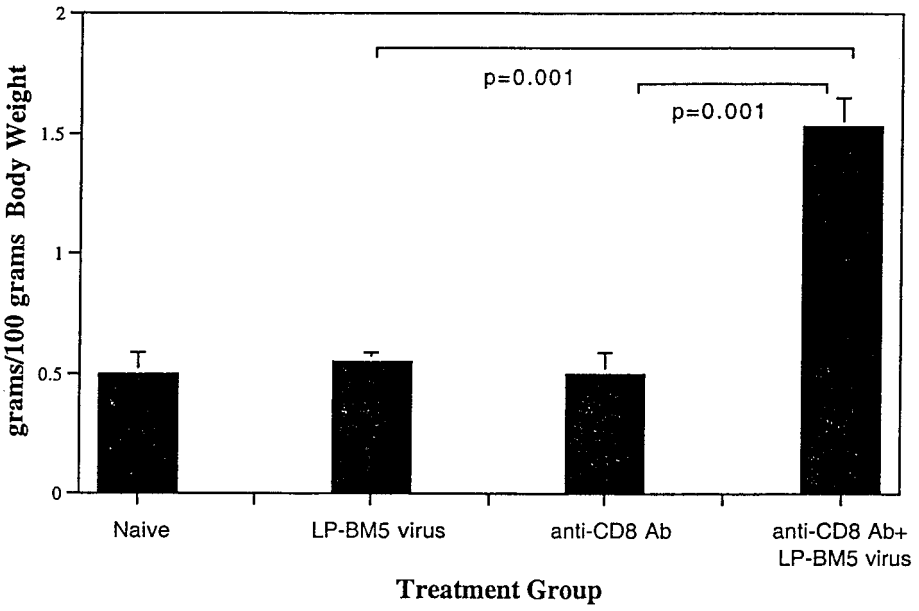

FIG. 1. Resistance to LP-BM5 virus-induced MAIDS requires $CD8^+$ T cells. BALB/c adult mice were either untreated, infected with LP-BM5 virus, chronically depleted of $CD8^+$ T cells by *in vivo* mAb infusion, or both virus infected and depleted of $CD8^+$ T cells (see Materials and Methods). At 6 weeks postinfection, the mice were sacrificed, and spleen weight was determined as an index of MAIDS susceptibility. Student's *t* test-determined *P* values of <0.05 were accepted as statistically significant differences among treatment groups.

MAIDS susceptibility, based on these multiple disease criteria. For example, splenic hyperplasia was obvious. CD8-depleted and LP-BM5-infected mice exhibited an average spleen weight of 1.53 ± 0.12 g/100 g body weight (BW) compared with naive mice or mice treated with either only anti-CD8 (0.50 ± 0.09 g/100 g BW; $P = 0.001$) or only LP-BM5 virus (0.55 ± 0.04 g/100 g BW; $P = 0.001$). These results were corroborated by both serum IgG_{2a} and IgM levels (data not shown). Specifically, anti-CD8-treated/LP-BM5-infected mice were hypergammaglobulinemic with average serum IgG_{2a} levels of 558 μg/ml compared with either noninfected, anti-CD8-treated mice (209 μg/ml; $P = 0.006$) or nondepleted, LP-BM5-infected mice (245 μg/ml; $P = 0.013$). Additionally, anti-CD8-treated/LP-BM5-infected mice had average serum IgM levels of 511 ± 26 μg/ml compared with noninfected, anti-CD8-treated mice (146 ± 27 μg/ml; $P = 0.001$) or nondepleted, LP-BM5-infected mice (171 ± 9.3 μg/ml; $P = 0.001$). Similarly indicative of MAIDS were the immunodeficiency parameters examined. Thus the ability of anti-CD8-treated/LP-BM5-infected mice to generate T-cell and B-cell mitogen responses was substantially diminished compared with both uninfected anti-CD8-treated mice ($P = 0.001$ for ConA and $P = 0.003$ for LPS) and nondepleted, LP-BM5-infected mice ($P = 0.003$ for ConA and $P = 0.02$ for LPS). Collectively, these data demonstrate that $CD8^+$ T cells are required for resistance to LP-BM5 virus-induced MAIDS, consistent with a direct role for BALB/cByJ-derived, gag-/SYNTGRFPPL-specific CTLs in the resistant phenotype.

Identification of the peptide epitope recognized by gag-specific CTLs derived from the $CBY6F_1$ and C57BL/Ks mouse strains

To test the possibility that the $CBY6F_1$ strain recognized the same gag-associated CTL specificity as the MAIDS-resistant BALB/c strain, a series of experiments were carried out using, as a source of antigen, defective or ecotropic gag-expressing Sindbis and vaccinia virus recombinants and the synthetic, ORF2-derived peptide that represents the minimal immunodominant epitope identified in BALB/c mice. As shown in the representative experiment of Table 1, $CBY6F_1$ mice primed and restimulated with dG-Vac, expressing AA 1–532 of the defective gag protein (def-gag), efficiently lysed P815B target cells either infected with a Sindbis virus expressing either def-gag AA 208–240 or AA 1–342, or pulsed with the ORF2-derived SYNTGRFPPL peptide (61%, 60%, and 58% specific lysis at an effector-to-target (E/T) ratio of 100:1, respectively) compared with target cells infected with a recombinant Sindbis virus expressing def-gag AA 208–225 or noninfected/untreated P815B (mock) target cells (10% or −1% specific lysis, respectively). Because of the uniform activity against targets capable of expressing the ORF2-defined CTL epitope, including synthetic peptide pulsed P815B target cells, but not target cells infected with SIN:dG(208–225), which does not encode the ORF2 epitope, these results were consistent with SYNTGRFPPL as the major specificity of the F_1 anti-gag CTL. Similar to dG-Vac *in vitro* restimulation, spleen cells from these same dG-Vac primed but not

TABLE 1

CBY6F$_1$ Mice Generate Anti-Gag CTLs with Specificity for the ORF2-Derived SYNTGRFPPL Epitope[a]

Effector cell stimulation			Target cell % specific lysis for P815B cells				
Primary *in vivo*	Secondary *in vitro*	E/T ratio	Mock	SIN:dG(208–225)[b]	SIN:dG(208–240)[b]	SIN:dG(1–342)[b]	ORF2 SYNTGRFPPL
dG-Vac	dG-Vac[c]	100:1	−1	10	**61**	**60**	**58**
		20:1	−3	5	**34**	**31**	**38**
	K^d-Vac[c]	100:1	−1	38	19	17	ND[d]
		20:1	−3	20	7	8	ND
	None	100:1	0	−6	5	4	4
	SIN:dG(208–225) J774.2 cells	100:1	1	22	19	17	19
	SIN:dG(208–240) J774.2 cells	100:1	1	18	**69**	**63**	**67**
		20:1	−1	10	**34**	**34**	**26**
	SIN:dG(1–342) J774.2 cells	100:1	−1	9	**32**	**32**	**42**
		20:1	−2	5	**10**	**11**	**15**

[a] ^{51}Cr release assay of CTLs, generated by priming CBY6F$_1$ mice i.p. with dG-Vac virus (2×10^7 pfu) followed by *in vitro* restimulation of primed spleen cells with J774.A2 tumor cells infected with various recombinant Sindbis or vaccinia viruses, as indicated. P815 target cells were similarly infected or pulsed with 100 pg/ml synthetic (ORF2) peptide epitope. Spontaneous release values for target cells were 16% or lower.

[b] SIN:dG(208–240) and SIN:dG(1–342), versus SIN:dG(208–225) are Sindbis virus constructs encoding regions of the defective gag protein that do versus do not encode for the ORF2-derived SYNTGRFPPL epitope.

[c] dG-Vac is a recombinant vaccinia virus construct encoding for the entire defective gag protein, whereas K^d-Vac is a control that encodes for the K^d MHC class I protein.

[d] ND, not determined.

nonimmunized CBY6F$_1$ mice (data not shown) were successfully restimulated with recombinant Sindbis viruses expressing def-gag AA 208–240 or 1–342 but not 208–225. These CTLs caused substantial lysis above background of the same set of ORF2 epitope-encoding recombinant Sindbis virus-infected P815B target cells and those target cells pulsed with SYNTGRFPPL peptide (Table 1). Reverse transcription–polymerase chain reaction (RT–PCR) demonstrated that all minigene RNA products were expressed in the Sindbis-infected target cells used in this experiment, and maintenance of an ORF was confirmed by sequencing of the viral plasmids (data not shown). Collectively, these results regarding anti-gag CTL fine specificity confirmed in two additional experiments using dG-Vac *in vivo* priming were consistent with the hypothesis that the SYNTGRFPPL peptide acts as the immunodominant CTL epitope in F$_1$ mice.

A CBY6F$_1$ CTL specificity for SYNTGRFPPL was further supported by experiments demonstrating that CTLs were also generated from mice primed with eG-Vac, but not naive mice, after restimulation of primed spleen cells *in vitro* with ORF2-derived SYNTGRFPPL peptide. These CTLs efficiently lysed P815B target cells either infected with SIN:dG(208–240) or pulsed with the SYNTGRFPPL peptide compared with little or no lysis of mock infected target cells or those infected with SIN:dG(208–225) (Fig. 2). RT–PCR was again used to verify expression of all recombinant Sindbis-encoded minigene RNA products in these P815B target cells (data not shown). The lack of lysis of NK-susceptible Yac-1 target cells underscored the specificity of the response. Two repeat experiments employing eG-Vac priming demonstrated similar results as those depicted in Fig. 2, suggesting that like the BALB/c strain, the CBY6F$_1$ mice recognized the ORF2-derived SYNTGRFPPL epitope of either def- or eco-gag origin.

To evaluate the *in vivo* immunogenicity of ORF2-derived SYNTGRFPPL epitope under conditions where its expression was not driven by a recombinant virus expression system (i.e., Sindbis or vaccinia virus), CBY6F$_1$ mice were primed by infection with the LP-BM5 retroviral complex and evaluated for their ability to generate secondary anti-gag, and specifically anti-SYNTGRFPPL, CTL responses. LP-BM5 primed spleen cells restimulated with the SYNTGRFPPL peptide efficiently lysed target cells infected with either SIN:dG(208–240) or SIN:dG(1–342) (69% or 82% specific lysis, respectively) compared with mock or SIN:dG(208–225)-infected targets (24% or 20% specific lysis, respectively) at a 100:1 E/T ratio (Table 2). These data provided additional evidence that SYNTGRFPPL is the immunodominant peptide recognized by CBY6F$_1$-derived gag-specific CTL. RT–PCR was again used to verify expression of all minigene messages in the Sindbis-infected P815B target cells, and maintenance of the insert ORFs was confirmed by sequencing the viral construct (data not shown). These results demonstrated that similar to recombinant Sindbis- or vaccinia vector-mediated expression, *in vivo* LP-BM5 retrovirus infection

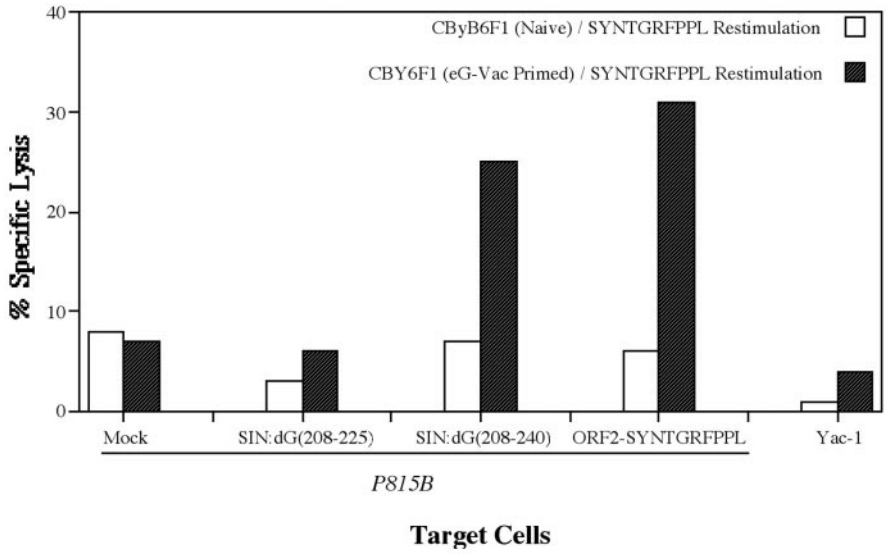

FIG. 2. Recognition of the ORF2-derived SYNTGRFPPL by gag-specific CTLs generated in the CBY6F$_1$ strain. Gag-specific CTLs were generated by eG-Vac priming CBY6F$_1$ mice, followed by *in vitro* restimulation with synthetic, ORF2-derived SYNTGRFPPL peptide. Lytic activity was measured using ^{51}Cr-radiolabeled target cells: P815B, either infected with the indicated def-gag-expressing Sindbis recombinants (see Table 1) or pulsed with 100 pg/ml ORF2-derived SYNTGRFPPL peptide, or Yac-1, as a target cell highly susceptible to lysis by NK cells. Percent specific lysis at an E/T ratio of 100:1 is shown, although other E/T ratios were used with a similar pattern of results. Spontaneous release values for target cells were 28% or lower.

leads to the physiological production of immunogenic levels of the SYNTGRFPPL peptide sufficient to prime SYNTGRFPPL-specific precursor CTLs in CBY6F$_1$ mice, as we had previously shown in MAIDS-resistant BALB/c mice (Mayrand *et al.,* 1998). Similar results were obtained in two repeat experiments. Collectively, these data confirmed that gag-specific CTLs derived from CBY6F$_1$ mice recognize the same ORF2-derived SYNTGRFPPL peptide as the MAIDS-resistant BALB/c strain.

Because, as mentioned earlier, a population of gag-specific CTLs derived from the MAIDS-resistant C57BL/Ks mouse strain also recognizes a K^d-presented epitope located within the gag protein of the defective virus, a similar series of experiments were performed to determine the CTL fine specificity of this strain. Similar to BALB/c (Mayrand *et al.,* 1998) and CBY6F$_1$ (Table 2) mice, LP-BM5 primed and SYNTGRFPPL peptide or SIN:dG(208–240) restimulated C57BL/Ks effector cells demonstrated substantial lysis (above background levels) of only those P815B target cells infected with recombinant Sindbis viruses expressing the ORF2-SYNTGRFPPL epitope, not mock or SIN:dG(208–225)-infected target cells or NK-susceptible Yac-1 targets (data not shown). These findings were supported by data from both a repeat LP-BM5 priming experiment in which a similar pattern of results was obtained, although the lysis values were somewhat lower, and three experiments in which anti-ORF2/SYNTGRFPPL C57BL/Ks CTLs were produced after appropriate *in vitro* restimulation of dG-Vac primed mice. Taken together, these results strongly suggested that the gag-specific CTLs derived from MAIDS-resistant C57BL/Ks mice recognize the same ORF2-generated

TABLE 2

CBY6F$_1$ Mice Primed with LP-BM5 Virus Generate Anti-Gag CTLs with Specificity for the ORF2-Derived SYNTGRFPPL Epitope[a]

Effector cell stimulation		E/T ratio	Target cell % specific lysis for P815B cells				
Primary *in vivo*	Secondary *in vitro*		Mock	SIN:dG(208–225)	SIN:dG(208–240)	SIN:dG(1–342)	Yac-1[b]
LP-BM5 virus	None	100:1	30	14	31	32	9
		20:1	18	12	21	30	3
	ORF2-SYNTGRFPPL	100:1	24	20	**69**	**82**	7
		20:1	16	11	**51**	**61**	4

[a] CTLs were generated by priming CBY6F$_1$ mice i.p. with LP-BM5 virus (1.8×10^5 pfu, followed by *in vitro* restimulation of primed spleen cells with 10 μg/ml synthetic ORF2 peptide epitope as indicated. Spontaneous release values for ^{51}Cr-labeled target cells, including those infected with various recombinant Sindbis viruses, as described in Table 1, were 11% or lower.

[b] Yac-1 is a target cell highly susceptible to lysis by NK cells.

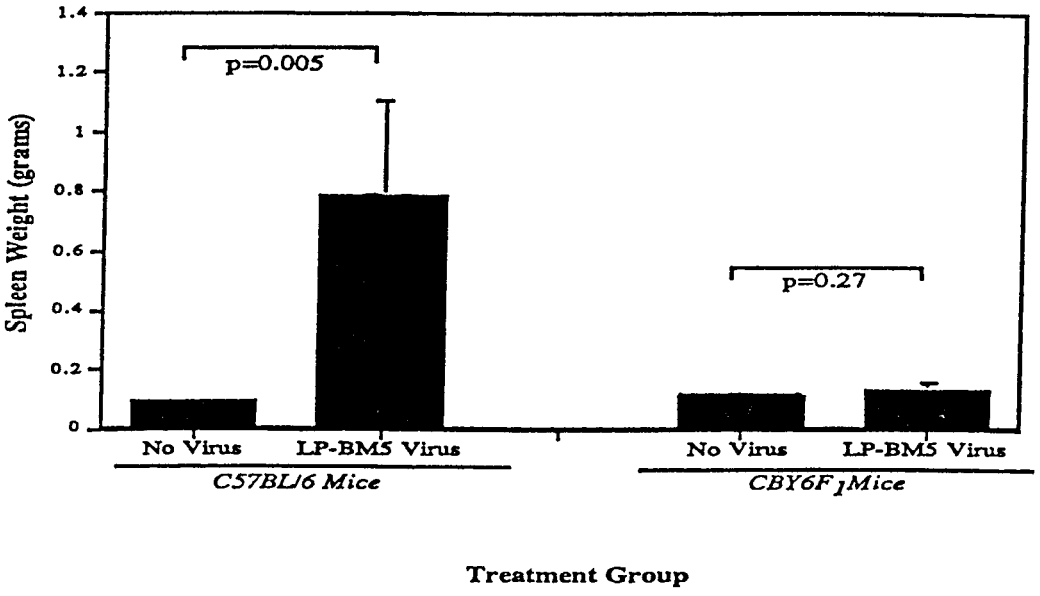

FIG. 3. Determination of the MAIDS phenotype of CBY6F$_1$ mouse via spleen hyperplasia. Adult (5 weeks old) CBY6F$_1$ or C57BL/6 mice were infected i.p. with LP-BM5 virus (1.8×10^5 pfu). At 15 weeks post-virus delivery, spleen weight was determined as a measurement of MAIDS susceptibility or resistance. All mice ($n = 30$) were age matched at the time of LP-BM5 delivery. Data depicted represents the group mean ± SD. Student's t test-determined P values <0.05 were accepted as statistically significant differences among treatment groups.

SYNTGRFPPL peptide as those derived from the BALB/c and CBY6F$_1$ strains.

Characterization of the MAIDS phenotype of the CBY6F$_1$ mouse strain

As discussed, according to the published literature (Makino *et al.,* 1990; Huang *et al.,* 1992), susceptibility to MAIDS, rather than resistance, is dominant, at least in the F$_1$ strains used in those studies. Although the CBY6F$_1$ strain might thus be expected to also be susceptible to LP-BM5-induced MAIDS, it seemed curious to us that only two of the three H-2^d-positive mice strains that were shown to be capable of generating gag-specific/anti-SYNTGRFPPL responses would be resistant to MAIDS. To directly address the issue of MAIDS resistance versus susceptibility, the disease phenotype of the CBY6F$_1$ strain was assessed via intraperitoneal delivery of LP-BM5 virus to adult mice, followed by standard measurements of the hallmark MAIDS features: splenomegaly and hypergammaglobulinemia. As depicted in Fig. 3, when uninfected versus LP-BM5 virus-infected CBY6F$_1$ mice were compared, no significant differences were observed ($P = 0.27$) with respect to spleen weight at 15 weeks postinfection. By contrast and as expected, susceptible C57BL/6 mice infected with LP-BM5 had significant splenomegaly compared with noninfected C57BL/6 mice ($P = 0.005$). Additionally, visual observation of the lymph node sizes indicated no LP-BM5-induced enlargement in the CBY6F$_1$ mice, whereas massive enlargement of lymph nodes was observed in infected C57BL/6 mice. These data suggested that there was no LP-BM5 virus-induced hyperplasia of lymphoid organs in CBY6F$_1$ mice at a time when B6 mice were found to be MAIDS susceptible.

As an independent measurement of MAIDS induction, serum Ig levels were determined at 6 and 15 weeks after LP-BM5 delivery. As reported in the literature, the serum Ig levels in highly susceptible C57BL/6 mice achieved maximal plateau levels at 4–8 weeks after LP-BM5 infection (Pattengale *et al.,* 1982). Hence, the 6-week serum samples are shown to demonstrate that although the LP-BM5-infected C57BL/6 mice had serum IgG$_{2a}$ and IgM levels significantly higher ($P = 0.01$ and 0.001, respectively) than noninfected C57BL/6 mice, no significant differences were detected between LP-BM5-infected and naive CBY6F$_1$ mice (IgG$_{2a}$, $P = 0.28$; IgM, $P = 0.47$; Figs. 4A and 4B, respectively). With disease progression, the serum Ig levels in MAIDS mice began to decline, most likely as a result of the functional inactivation of the B cells as immunodeficiency develops. As a result, the elevation in serum Ig levels from the 15-week postvirus serum samples was less pronounced in the LP-BM5-infected C57BL/6 mice but still highly significant (IgG$_{2a}$, $P = 0.01$; IgM, $P = 0.003$; data not shown). In contrast, because as late as 15 weeks after LP-BM5 virus infection, CBY6F$_1$ mice had serum IgG$_{2a}$ ($P = 0.30$) and IgM ($P = 0.42$) levels comparable to naive mice, we concluded that LP-BM5 infection of adult CBY6F$_1$ mice does not cause hypergammaglobulinemia.

Results from an additional independent experiment using the same F$_1$ strain ($n = 15$) confirmed the lack of elevation of serum Ig levels in CBY6F$_1$ mice infected with

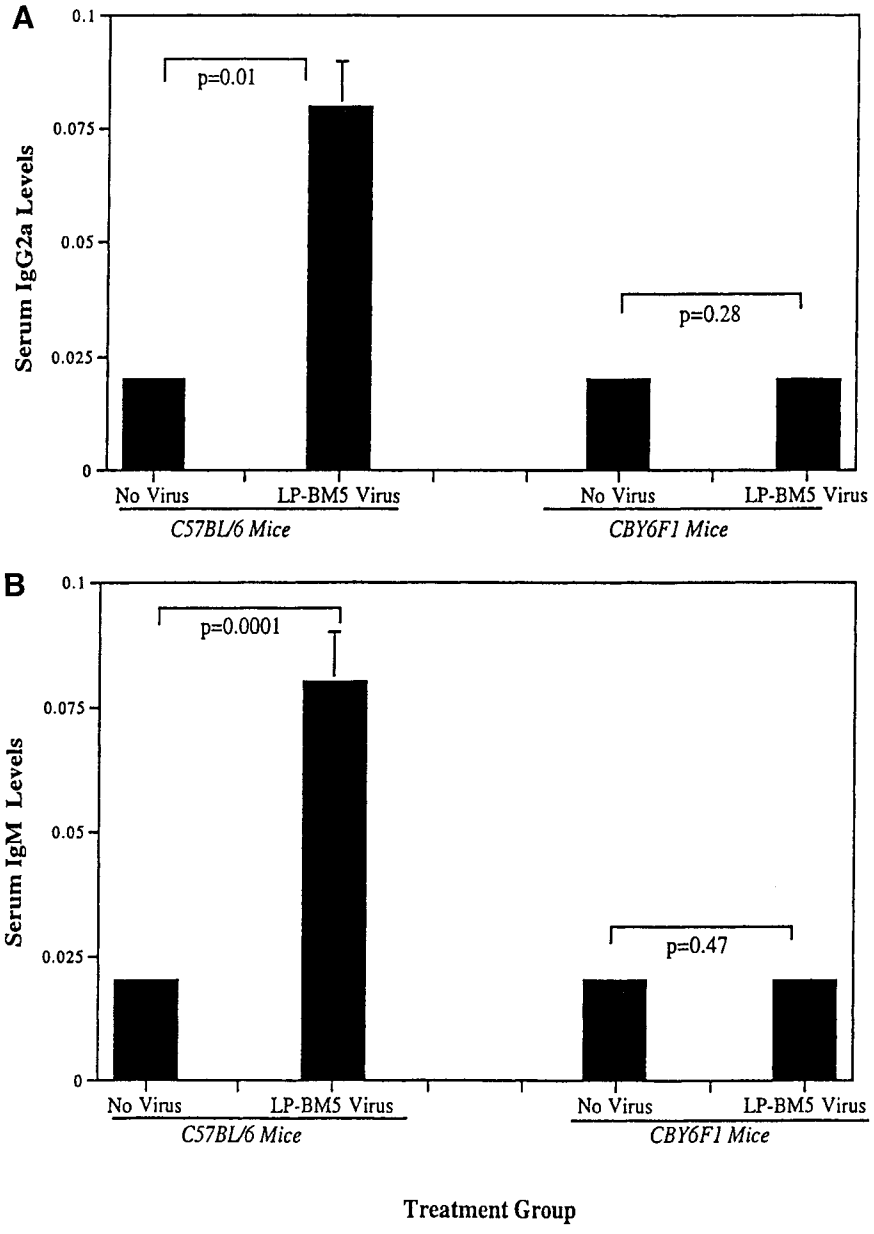

FIG. 4. Determination of the MAIDS phenotype of CBY6F$_1$ mouse via serum Ig levels after LP-BM5 infection. Adult (5 weeks old) CBY6F$_1$ or C57BL/6 mice were infected i.p. with LP-BM5 virus (1.8×10^5 pfu). At 6 weeks post-virus delivery, mice were bled and an ELISA was used to determine the relative levels of serum IgG$_{2a}$ (A) and IgM (B). All mice ($n = 30$) were age matched at the time of LP-BM5 delivery. Data depicted represent the group mean ± SD. Student's *t* test-determined *P* values <0.05 were accepted as statistically significant differences among treatment groups.

the LP-BM5 virus (data not shown). Because measurements of spleen hyperplasia and hypergammaglobulinemia are routinely used as prototypical disease features to determine MAIDS susceptibility or resistance (Pattengale *et al.,* 1982), these data demonstrating both a lack of splenomegaly and normal serum Ig levels as late as 15 weeks postvirus suggested that contrary to other F$_1$ strains that have been reported on (Makino *et al.,* 1990, 1991; Huang *et al.,* 1992), resistance is dominant over susceptibility in CBY6F$_1$ mice.

These results, which are considered in light of our correlation between MAIDS resistance and the ability to mount anti-ORF2/SYNTGRFPPL, gag-specific CTL responses in the context of H-2^d, but not H-2^b, further implied that the primary genetic factor in determining MAIDS resistance versus susceptibility might be the codominant expression of the resistant H-2^d haplotype. To extend the analysis of the induction of MAIDS in mice expressing the H-2^b and/or H-2^d haplotypes, particularly given the disparity of the findings for F$_1$ strains in the literature versus our demonstration of the MAIDS-resistant phenotype of CBY6F$_1$ mice (Figs. 3 and 4), additional experiments were conducted using F$_2$ mice obtained from the intercrossing of CBY6F$_1$ mice. The resulting F$_2$

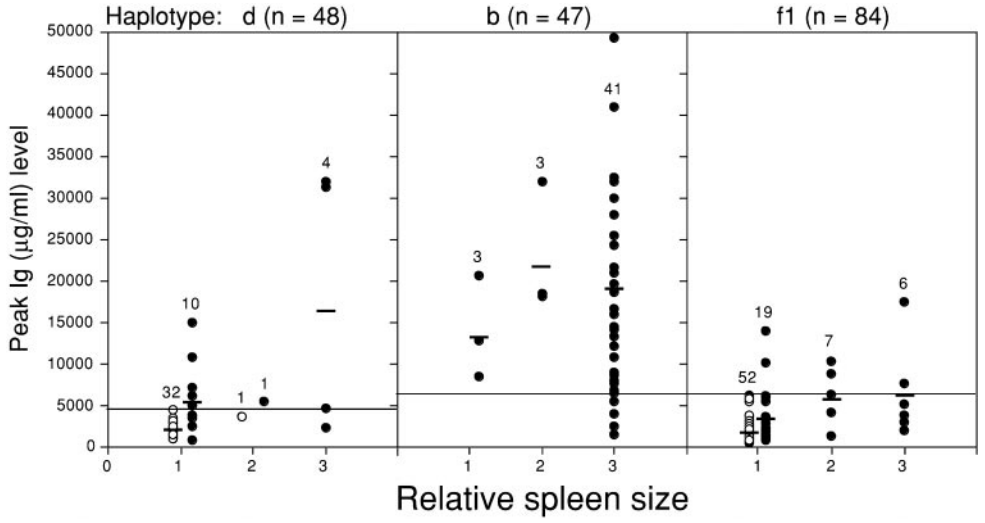

FIG. 5. The absence of splenomegaly and hyper-Ig levels after LP-BM5 injection correlates with the presence of the H-2^{d} allele, indicating protection from MAIDS was conferred by H-2^{d}. CBY6F$_1$ (H-2$^{d\times b}$) were crossed to generate H-2$^{b/b}$, H-2$^{d/d}$, and H-2$^{d/b}$ intercross progeny. These F$_2$ mice were genotyped and then infected with 0.5 ml of LP-BM5 mixture at 6 weeks of age. Mice were serially bled at 3- to 4-week intervals, and total Ig levels were determined by ELISA. At the time of sacrifice (when mice were moribund or at 27 weeks), all mice were tested for the presence of defective gag by PCR. Spleen size and/or spleen cells counts were determined, and a numerical score of 1, 2, or 3 was assigned (see Materials and Methods). Data are plotted as the spleen size versus highest Ig level at any point during the study, and the mean values are indicated for all data groups. ○, Defective gag-negative mice. ●, Defective gag-positive mice. H-2$^{b/b}$ mice were all positive for defective virus (47 mice), whereas H-2$^{d/d}$ mice were found to be virus positive (15 mice) and negative (33 mice), and H-2$^{b/d}$ mice were found to be both virus positive (32 mice) and negative (52 mice). Values directly above data points refer to the numbers of mice in each group. For reference, bars are present at the highest Ig levels for virus-negative H-2$^{d/d}$ mice and H-2$^{b/d}$ mice; the Ig values for virus-negative H-2$^{b/d}$ mice are used as the uninfected Ig value for H-2$^{b/b}$ mice.

mice should allow independent segregation of non H-2 genetic traits possibly influencing the development of MAIDS from the proposed "resistant" (H-2$^{d/d}$ and H-2$^{b/d}$) and "susceptible" (H-2$^{b/b}$) H-2 genotypes. When H-2$^{d/d}$ or H-2$^{b/d}$ mice were compared with H-2$^{b/b}$ F$_2$ mice with respect to serum Ig levels at various time points after infection with the LP-BM5 virus, both H-2$^{d/d}$ and H-2$^{b/d}$ F$_2$ mice demonstrated roughly comparable serum Ig levels, substantially lower than those of the H-2$^{b/b}$ F$_2$ mice, which had strongly elevated levels during the 3- to 12-week postinfection period (data not shown). This lack of obvious hypergammaglobulinemia was observed for both H-2$^{d/d}$ and H-2$^{b/d}$ F$_2$ mice and was essentially independent of whether the mice were scored as LP-BM5 defective virus positive or negative by PCR assessment of viral DNA. The defective virus-positive F$_2$ mice of the H-2$^{d/d}$ genotype, which is associated with MAIDS resistance (Makino *et al.,* 1990), had somewhat higher levels of Ig at the 10- to 16-week time period than the defective virus-positive F$_2$ H-2$^{b/d}$ mice. However, this elevation in Ig levels in defective virus-positive H-2$^{d/d}$ mice was transient, in that it was not sustained at time points extending out to 24 weeks postinfection. By contrast, all H-2$^{b/b}$ F$_2$ mice were defective virus positive and presented with hyper-Ig (data not shown), similar to that previously demonstrated for the LP-BM5 infected, prototypic MAIDS-susceptible B6 mice.

Further analysis of these same LP-BM5 virus-injected F$_2$ mice is depicted in Fig. 5. F$_2$ mice are grouped on the basis of their highest serum Ig levels, MHC genotype, spleen weights, and defective virus status. When all mice were analyzed in this manner, several distinct differences emerged. First, with respect to serum Ig levels after infection with the LP-BM5 virus, most H-2$^{d/d}$ (77%) and H-2$^{b/d}$ F$_2$ (81%) mice demonstrated "normal" serum Ig levels relative to the significantly elevated serum Ig levels of H-2$^{b/b}$ F$_2$ mice. Specifically, 91% of H-2$^{b/b}$ F$_2$ mice had serum Ig levels greater than 5953 μg/ml, which is the highest value of defective virus-negative H-2$^{b/d}$ mice (Fig. 5). Second, although only 23% of H-2$^{d/d}$ and 19% of H-2$^{b/d}$ F$_2$ mice demonstrated at least one aspect of disease (spleen hyperplasia and/or elevated Ig levels), 100% of H-2$^{b/b}$ F$_2$ mice displayed at least one aspect of disease (Fig. 5). Third, these results demonstrate that the development of MAIDS in the "resistant" H-2$^{d/d}$ F$_2$ mice was similar to that in H-2$^{b/d}$ F$_2$ mice. Thus we found that H-2$^{b/d}$ mice, whether they be F$_1$ or F$_2$ intercrosses, presented with a MAIDS-resistant phenotype.

DISCUSSION

Our finding that CBY6F$_1$ (H-2$^{d\times b}$) and both H-2$^{d/d}$ and H-2$^{b/d}$ F$_2$ mice possess a MAIDS-resistant phenotype, compared with H-2$^{b/b}$ F$_2$ mice, was of particular interest in view of the studies by Makino *et al.* (1990) and Huang *et al.* (1992), which both concluded that susceptibility, rather than resistance, is dominant in F$_1$ (resistant × susceptible or susceptible × resistant) mice. Makino *et*

al. (1990) used F_1 hybrids of the MAIDS-susceptible, (C57BL/6 or C57BL/10) strain crossed with a highly resistant (B10.D2 or A/J) or moderately resistant (B10.BR or B10.A) strain in the evaluation of the dominance of susceptibility versus resistance to LP-BM5 virus-induced MAIDS. Although limited by the number of animals evaluated, this study concluded that all F_1 strains examined demonstrated some signs of MAIDS (i.e., splenomegaly and histopathology) by 10 weeks postinfection. Interestingly, most F_1 strains examined by Makino *et al.* (1990) demonstrated a significantly delayed onset of disease. Although disease onset occurred at 3–4 weeks in prototypic, MAIDS-susceptible C57BL/6 mice, MAIDS onset was not clearly identified until 10–15 weeks postinfection in the F_1 mice (Makino *et al.,* 1990). In keeping with the notion of F_1 MAIDS susceptibility but with kinetics similar to those of B6 mice, Huang *et al.* (1992) demonstrated MAIDS onset as early as 4–5 weeks in F_1 progeny of susceptible C57BL/6N females crossed with males from the resistant LG/J strain. Taken together, the data from these two studies suggested that although disease onset may be delayed in some F_1 strains, MAIDS susceptibility appears to be dominant over resistance.

In direct contrast to these findings, we report here that no symptoms of MAIDS onset were observed in CBY6F_1 (H-$2^{d\times b}$) mice, whereas 77% of H-$2^{d/d}$ and 81% of H-$2^{b/d}$ F_2 mice did not exhibit MAIDS after LP-BM5 infection. These data are consistent with a study by Hammelin-Bourassa *et al.* (1989) that demonstrated F_1 progeny of the susceptible C57BL/6 × resistant A/J strains were more resistant than susceptible to LP-BM5 virus-induced MAIDS. Additionally, Makino *et al.* (1991) reported that only 45% of the F_1 progeny of the resistant RIIIS × susceptible B10.RIII strains were MAIDS susceptible. Given the apparent strain dependence of the dominance of resistance or susceptibility to MAIDS of F_1 mice and the disparate findings reported by various laboratories, a more complete analysis of the inheritance of genes controlling MAIDS resistance versus susceptibility in F_1 and F_2 mice may help to explain the reported differences. Indeed, Makino *et al.* (1990) demonstrated that non-MHC background genes influenced the development of MAIDS. We also find a small subset of resistant H-$2^{d/d}$ F_2 mice somewhat susceptible to MAIDS, consistent with the interpretation that non-*H-2* genes influenced the development of MAIDS.

Our finding that CBY6 F_1 mice generate anti-gag-specific CTLs with a fine specificity shared by the MAIDS-resistant BALB/c and C57BL/Ks strains (Table 1, Fig. 2) supports the argument that (resistant × susceptible) F_1 mice that are capable of responding to defective gag are resistant to LP-BM5-induced MAIDS. The results described in this report should also be considered in light of our previous correlation between MAIDS resistance and the ability to mount such anti-ORF2/SYNTGRFPPL, gag-specific CTL responses in the context of H-2^d, but not H-2^b (Schwarz and Green, 1994; Mayrand *et al.,* 1998; Mayrand and Green, 1998). Taken together, our studies suggest that a primary factor in the determination of MAIDS resistance versus susceptibility most likely is the codominant expression of the resistant H-2^d haplotype, which allows for K^d-restricted $CD8^+$ CTL production. Indeed, our experiments, in which chronic *in vivo* depletion of $CD8^+$ T cells in genetically resistant mice caused them to become MAIDS susceptible (Fig. 1), provide direct linkage of the ability to raise anti-gag CTLs with resistance. These findings are in agreement with the results of Makino *et al.* (1992), which demonstrated that resistant A/J mice were rendered susceptible to MAIDS after *in vivo* depletion of the $CD8^+$ T-cell population. Interestingly, however, 19% of H-$2^{d/b}$ and 23% of the resistant H-$2^{d/d}$ F_2 mice had at least one clinical aspect of MAIDS, suggesting that a non-MHC genetic determinant can negatively influence T-cell protection and thus disease outcome.

MATERIALS AND METHODS

Mice

BALB/cByJ (H-2^d), C57BL/KsJ (H-2^d), C57BL/6J (H-2^b), and CBY6F_1 (H-$2^{d\times b}$) mice (4 weeks old) were obtained from The Jackson Laboratories (Bar Harbor, ME). Additional CBY6F_1 (H-$2^{d\times b}$) mice were produced by breeding BALB/cByJ and C57BL/6J mice at the animal facility of The Scripps Research Institute. CBY6F_2 H-$2^{b/b}$, H-$2^{d/d}$, and H-$2^{d/b}$ mice were generated by inter-cross-breeding of the CBY6F_1 (H-$2^{d\times b}$) mice at the animal facility of The Scripps Research Institute. At 4 weeks of age, mice were MHC typed by a PCR-based assay (see later).

$CD8^+$ T-cell depletion *in vivo*

To test the effect of depletion of $CD8^+$ T cells in a MAIDS-resistant mouse strain, prototypic MAIDS-resistant, adult BALB/cByJ mice were injected i.p. with 0.25 ml (~0.25 μg) purified ascites fluid containing the murine CD8-specific mAb 53-6.72 (IgG_{2a} rat anti-mouse, TIB 105; American Type Culture Collection, Rockville, MD.) on day 7 after LP-BM5 virus infection. Identical weekly injections of anti-CD8-containing ascites were delivered i.p. (up to 2 weeks before sacrifice) to maintain depletion throughout the course of the experiment. Flow cytometric analysis was performed periodically on splenocytes derived from control mice that received the same regimen of anti-CD8 mAb to confirm efficient chronic depletion of $CD8^+$ T cells (i.e., >95% reduction in percent $CD8^+$ cells).

Medium

In addition to L-glutamine (2 mM), penicillin (30 μg/ml), streptomycin (20 mg/ml), and gentamicin (33 μg/ml), medium contained the following additional components:

RPMI-10 (RPMI 1640 and 10% FBS), RPMI-10ME (RPMI-10 plus 50 mM 2-mercaptoethanol), DMEM-10 (Dulbecco's modified Eagle's medium, 10 mM HEPES, and 10% FBS), sensitization media (SM: RPMI-10ME plus 0.1 M nonessential amino acids, 1 mM sodium pyruvate), and SM-rIL2 (SM plus 8 units/ml recombinant interleukin-2). RPMI 1640 containing 10% calf serum was used for conducting ^{51}Cr release assays.

Cell lines

The murine lines J774.2 (macrophage, H-2^d; American Type Culture Collection) passaged in DMEM-10, P815B (mastocytoma, H-2^d, a generous gift from Dr. Jack Bennink, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Bethesda, MD) passaged in RPMI-10ME, and Yac-1 (Moloney murine leukemia virus-induced tumor, H-2^a) passaged in RPMI-10 were used in this series of experiments.

LP-BM5 virus

LP-BM5 virus was prepared in our laboratory as described by Klinken *et al.* (1988). G6 cells, originally provided to us by Drs. Jane Hartley and Herbert Morse as a cloned cell line from SC-1 cells infected with the LP-BM5 virus mixture, were cocultured with noninfected SC-1 cells and cell-free supernatants were harvested, Millipore filtered, and titered. Mice were infected intraperitoneally with 0.25 ml of virus stock that was quantified by an XC plaque assay (Rowe *et al.,* 1970) to contain approximately 7×10^5 ecotropic plaque-forming units (pfu)/ml.

Recombinant viruses

K^d-Vac, a recombinant vaccinia virus encoding the H-2K^d class I molecule, was a generous gift from Dr. Jack Bennink. Additional recombinant vaccinia and Sindbis viruses were generated using established protocols (Earl and Moss, 1993; Hahn *et al.,* 1992). Intermediate shuttle vectors, used to create the indicated recombinant viruses described later, were constructed using established procedures (Sambrook *et al.,* 1989) or manufacturer-specified protocols. The pSC65 shuttle vector and the Western Reserve isolate of vaccinia virus were generously provided by Dr. Bernard Moss (National Institutes of Health, National Institute of Allergy and Infectious Diseases, Bethesda, MD). The vectors for recombinant Sindbis virus generation, pH3′2J1 (shuttle vector) and pTE3′2J:CAT, were generously provided by Dr. Chang Hahn (University of Virginia, Charlottesville, VA). Plasmid maps will be provided on request. The construction of the recombinant vaccinia and Sindbis viruses expressing ecotropic gag (eG), defective gag (dG), or portions of dG have previously been published (Schwarz and Green, 1994; Mayrand *et al.,* 1998). Briefly, for vaccinia, inserts were cloned into the shuttle vector pSC65, and the following recombinant viruses were generated via homologous recombination between pSC65 and the Western Reserve isolate of vaccinia virus: dG-Vac, containing the entire def-gag sequence (AA 1–532 of the p1/27/A1 retrovirus clone), and eG-Vac, containing the entire ecotropic gag sequence (AA 1–536 of the pEco12 retrovirus clone), which were the generous gifts of Dr. Sisir Chattopadhyay, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Bethesda, MD (Chattopadhyay *et al.,* 1991). The construction of recombinant Sindbis viruses has also been described in detail (Schwarz and Green, 1994; Mayrand *et al.,* 1998). Briefly, inserts were cloned into the shuttle vector pH3′2J1, a fragment containing the insert and subgenomic promoter was subcloned into the full-length Sindbis cDNA contained in pTE3′2J:CAT, and infectious Sindbis virus was recovered after LipoFECTIN (GIBCO BRL, Grand Island, NY)-mediated transfection of viral RNA into BHK-21 (cl.13) cells to create the recombinant viruses encoding the following portions of dG AA 1–532: SIN:dG (1–342), SIN:dG (208–240), and SIN:dG (208–225).

Infection of stimulator and target cells

Next 1×10^7 (vaccinia virus) or 5×10^7 (Sindbis virus) pfu was added to 1×10^6 P815B or J774.2 cells in 500 ml of a balanced salt solution containing 0.1% BSA and allowed to adsorb for 1 h at 37°C with agitation. For vaccinia target cells, samples were incubated at 37°C for an additional 3 h. before ^{51}Cr labeling. Sindbis targets were infected in the same manner but were allowed to express viral proteins for a minimum of 6 h before ^{51}Cr labeling. Under these infection conditions, including this 50:1 m.o.i. for Sindbis virus, essentially 100% of target and stimulator cells infected by the recombinant viruses have previously been shown by flow cytometry and other techniques to express the inserted retroviral protein sequence (Schwarz and Green, 1994; unpublished observations).

Synthetic peptides

The ORF2 peptide (SYNTGRFPPL) was synthesized with unmodified NH_2- and COOH-terminal amino acids using fluorenylmethoxycarbonyl (FMOC)-based chemistry by Research Genetics (Huntsville, AL).

Generation of bulk cytolytic effector cells

Six to 10-week-old mice were infected intravenously with 1–2 $\times 10^7$ pfu dG-Vac, 2×10^7 pfu eG-Vac, or 1.8×10^5 pfu LP-BM5 virus. At least 3 weeks postinfection for recombinant vaccinia priming or 7–10 days for LP-BM5 priming, primed mice were sacrificed and splenic leukocytes were recovered. As a source of stimulatory antigen, 1×10^6 P815B or J774.2 cells were irradiated (8000 rads), infected with the appropriate recombinant virus, and added to 4×10^7 to 5×10^7 primed leukocytes in

MLTC cocultures. Vaccinia virus-specific effectors were generated by directly adding vaccinia virus (dG-Vac or K^d-Vac) to responder cells at an m.o.i. of 10:1. Peptide stimulation was achieved by the addition at a final concentration of 10 μg/ml. Our previous studies have shown that even for recombinant vaccinia virus-mediated *in vivo* priming of resistant mice, both *in vivo* priming and *in vitro* restimulation with sources of retroviral epitopes are necessary for easy detection of antiviral CTL activity in both the MAIDS and other murine retroviral systems (Schwarz and Green, 1994; unpublished observations). Cells were cultured in MLTC in RPMI-10 for 6 days at 37°C in a humidified 5% CO_2 atmosphere.

Chromium release assay

Target cells (10^6 to 2×10^6) were resuspended in 100 μl of FBS and labeled with 200 μCi of ^{51}Cr sodium chromate (New England Nuclear, Wilmington, DE) for 45 min at 37°C. After washing, 4×10^3 or 10^4 target cells were combined with serial dilutions of effector cells in 96-well V-bottom plates. After a 4-h incubation at 37°C, 100 μl of cell-free supernatant was collected and counted. Percent specific lysis of tumor cells was determined using the formula $[(a - b)/c] \times 100$, where a is experimental counts per minute released by target cells incubated with effector cells, b is counts per minute released by target cells incubated alone (spontaneous release), and c is counts per minute released by the freeze-thaw of target cells (approximately 80% of total counts per minute incorporated), and <10% variation was seen between experimental replicates.

RT–PCR

Total RNA was recovered from target cells using a CsTFA-based kit from Pharmacia (Piscataway, NJ) and standard procedures previously described (Schwarz and Green, 1994; Mayrand *et al.,* 1998). After ethanol precipitation, each cDNA sample was subjected to 30 cycles of *Taq* polymerase amplification consisting of 45 s denaturation at 94°C, 60 s annealing at 50°C, and 90 s chain elongation at 72°C with the following primers: pH3′-59, 5′-GGAAATAAAGCATCTCTACG-3′, and pH3′+65, 5′-CTAATGTACCAGCCTGATGC-3′.

MHC genotyping

F_2 mice produced by CBY6F_1 (H-$2^{d\times b}$) inter-cross-breeding were typed for their MHC by microsatellite analysis (Dietrich *et al.,* 1992) using a rapid DNA screening protocol developed by Chen and Evans (1993). Tissue was obtained from the ear or the last 0.5 mm of the tail of 4- to 5-week-old mice and digested using Proteinase K digestion, and the DNA was prepared for PCR amplification as described by Chen and Evans (1993). The DNA samples were amplified with primers to D17Mit28 (MAPPAIRS, Research Genetics, Inc., Huntsville, AL), which allows the MHC haplotype determination of mice as H-2^d, H-2^b, and H-$2^{d\times b}$ through the detection of known microsatellites (see Dietrich *et al.,* 1992 and http://www.informatics.jax.org/searches/marker_form.shtml). PCRs were in 25-μl volumes using standard reagents (Perkin–Elmer Cetus, Norwalk, CT) with concentrations of 1.5 mM $MgCl_2$ and primers to 0.4 M. Reactions were carried out for 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. All PCR analyses included reagent only reactions to control for false-positives. The PCR products, 10 μl, were run out on 6% acrylamide gels, and the bands were visualized with ethidium bromide staining. PCR product from the amplification of DNA from the isolated from the tissues of H-2^d, H-2^b, and H-$2^{d\times b}$ mice were run on each gel to serve as a positive size control for distinguishing bands from the PCR product of DNA obtained from the mice to be typed.

Detection of defective gag viral genomes

F_2 mice injected with LP-BM5 virus were evaluated for MAIDS by both clinical (as above) and molecular means. To determine the presence of integrated defective virus. DNA samples were obtained from the spleen and lymph nodes of mice (as described earlier) and assessed for the presence of the p12 region of defective virus by PCR analysis. Primers to the defective p12 region were as described (Ogata *et al.,* 1993). All samples included DNA from noninfected mice and mice infected with pEco12, a cloned ecotropic murine leukemia virus (kindly provided by Dr. S. Chattopadhyay, Laboratory of Immunopathology, National Institute of Allergy and Infectious Diseases), and known MAIDS positive mice as controls. PCRs were in 25-μl volumes using standard reagents (Perkin–Elmer Cetus) with concentrations of 1.5 mM $MgCl_2$ and primers to 0.5 M. Reactions were carried out for 35 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min. PCR products were detected as described above.

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