5-1992

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Jennifer G. Erbe
Dartmouth College

Kathy A. Green
Dartmouth College

Karen M. Crassi
Dartmouth College

Herbert C. Morse
National Institute of Allergy and Infectious Diseases

W R. Green
Dartmouth College

JOURNAL OF VIROLOGY, May 1992, p. 3251–3256
0022-538X/92/053251-06$02.00/0

Vol. 66, No. 5

Cytolytic T Lymphocytes Specific for Tumors and Infected Cells from Mice with a Retrovirus-Induced Immunodeficiency Syndrome

JENNIFER G. ERBE,[1] KATHY A. GREEN,[1] KAREN M. CRASSI,[1] HERBERT C. MORSE III,[2] AND WILLIAM R. GREEN[1]*

Department of Microbiology and Norris Cotton Cancer Center, Dartmouth Medical School, Hanover, New Hampshire 03756,[1] and Laboratory of Immunopathology, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892[2]

Received 3 October 1991/Accepted 12 February 1992

LP-BM5 retrovirus complex-infected C57BL/6 mice develop immunodeficiency, somewhat analogous to AIDS, termed murine AIDS (MAIDS). After secondary stimulation with syngeneic B-cell lymphomas from LP-BM5-infected mice, C57BL/6 mice produced vigorous CD8$^+$ cytotoxic T lymphocytes specific for MAIDS-associated tumors. An anti-LP-BM5 specificity was suggested because spleen and lymph node cells from LP-BM5-infected mice served as target cells in competition assays, and cells from LP-BM5, but not ecotropic, virus-infected mice functioned as secondary in vitro stimulators to generate cytotoxic T lymphocytes to MAIDS tumors.

Recently, several laboratories have described a generalized immunodeficiency syndrome in susceptible mouse strains following infection by a murine retrovirus isolate termed LP-BM5 (20, 24, 25), that originated from the Duplan-Latarjet isolate (19) of murine leukemia virus (MuLV). The LP-BM5 isolate is a complex mixture of MuLV including ecotropic, mink cell focus-inducing, and defective viruses; currently it is generally agreed that the defective genome is the proximal agent causing the immunodeficiency (1, 3, 11, 15). Although this murine immunodeficiency is not initiated by a lentivirus, the syndrome bears a number of characteristics similar to those of AIDS and has been termed murine AIDS (MAIDS). Among the similarities are severe immunodeficiency of both T- and B-cell responses, dependence of the initiation of disease on the presence of CD4$^+$ T cells, hypergammaglobulinemia, lymphadenopathy, increased susceptibility to progressive infection by environmental pathogens that cause only inapparent infections without mortality in normal individuals (2, 12, 17, 18, 20, 24–27, 35), and an increased incidence of tumors, particularly terminal B-cell lineage lymphomas. As has been suggested for cytotoxic T-lymphocyte (CTL) responses to human immunodeficiency virus (30, 31, 33, 34), the development or, conversely, the lack of the generation of CTLs to LP-BM5 MuLV-infected cells might play a critical role in the initiation and maintenance of MAIDS. In the present study we address the question of whether the prototype MAIDS-susceptible C57BL/6 (B6) strain can generate CTLs specific to LP-BM5 MuLV-induced tumors and infected cells.

The B6-1710 and B6-1153 tumors, established as biologically cloned cell lines from lymphomas from infected B6 mice which had features of MAIDS, are of the B-cell lineage: B220$^+$, CD5$^+$, Lyb-8$^+$, Ly-17$^+$, ThB$^+$ (17), and Ia$^+$ (Table 1). As to the presence and expression of the virus components of the LP-BM5 MuLV complex, assessed via virologic and molecular probing techniques (references 4 and 16 and Tables 1 and 2), B6-1710 produced infectious ecotropic MuLV, but neither tumor produced mink cell focus-inducing virus. Both tumors carried integrated copies of the defective genome detectable by Southern blot analysis and, following cocultivation with cells producing ecotropic MuLV, produced virus capable of inducing MAIDS. Much higher levels of mRNA encoded by the defective virus were expressed, however, by B6-1710 than by B6-1153. Immunoprecipitation studies of metabolically labeled cells showed that both MAIDS-associated tumors expressed the Pr60^{gag} precursor characteristic of the defective genome (3, 14, 16). As detected by flow cytometry, B6-1710 and B6-1153 readily expressed both the D^b and K^b class I major histocompatibility complex (MHC) antigens, and as detected by broadly reactive goat antiserum, they expressed gp70. Unlike B6-1710 and control AKR.H-2^{b}SL1 cells, B6-1153 did not express readily detectable levels of *gag*-related antigens. A panel of monoclonal antibodies to distinct *env*-related determinants, which readily stained the AKR/Gross/Akv$^+$ AKR.H-2^{b}SL1 tumor (8), failed to bind to B6-1153 or B6-1710, with the exception of those monoclonal antibodies defining the p15(E)^b and p15(E)^c epitopes broadly distributed among MuLV (28, 32).

B6/J or B6/N (B6) mice were immunized with the B6-1710 tumor cell line as a homogeneous source of cells containing and expressing both the ecotropic and defective viruses in the LP-BM5 MuLV mixture. Primed spleen cells from B6-1710-immunized mice were restimulated in vitro with irradiated homologous tumor cells and tested for cytolytic activity in standard 4-h ^{51}Cr release assays. This sensitization scheme led to the development of vigorously cytolytic activity to B6-1710 targets (Table 3). This activity appeared specific in that *H-2^{b+}* tumor targets induced by AKR/Gross MuLV were not lysed at levels significantly above background (no in vitro restimulation), nor were these AKR/Gross MuLV-induced tumors able to stimulate B6-1710-primed cells to exhibit substantial (>10% over background) lysis of B6-1710 or the other targets. Consistent with a lack of major antigenic cross-reactivity between this LP-BM5 MuLV-induced tumor and the AKR/Gross MuLV-induced tumors, anti-AKR/Gross virus CTL induced following immunization with (BALB)B.GV, which readily lysed the *H-2^b*-compatible, susceptible targets E♂G2 and AKR.H-2^{b}SL1 but not cl.18-5 as we have shown previously (8), were

* Corresponding author.

TABLE 1. Characteristics of MAIDS B-lineage lymphoma lines

Cell surface antigens[b] and antibodies	AKR.H-2^{b}SL1[a]			B6-1710			B6-1153		
	% Cells positive	TMFI	ΔTMFI	% Cells positive	TMFI	ΔTMFI	% Cells positive	TMFI	ΔTMFI
MHC									
Expt 1									
Anti-DNP	5.0	6	NA	4.8	14	NA	5.6	7	NA
Anti-D^b	99.5	89	83	98.7	107	93	99.7	93	86
Anti-K^b	100.0	381	375	99.7	321	307	100.0	490	483
Expt 2									
Anti-ornithine decarboxylase	—	—	—	6.9	29	NA	3.0	12	NA
Anti-I-A^b	—	—	—	99.6	474	445	92.7	380	368[c]
Viral antigens									
Expt 3									
Anti-HuIgD	16.1	15	NA	—	—	—	1.0	6	NA
Anti-CD8	12.4	14	NA	28.8[d]	54[d]	NA	0.8	5	NA
Anti-gp70^a	95.5	163	149	—	—	—	1.0	8	3
Anti-gp70^b	99.0	439	425	25.3[d]	54[d]	0[d]	1.3	9	4
Anti-gp70^c	99.1	154	139	—	—	—	1.2	11	5
Anti-gp70^d	99.5	421	407	—	—	—	0.3	8	3
Expt 4									
Anti-HuCD8	8.1	8	NA	—	—	—	1.2	1	NA
Anti-p15(E)^a	55.0	27	19	—	—	—	2.4	2	1
Expt 5									
Anti-HuFcR$_{II}$	17.5	21	NA	11.3	43	NA	3.6	8	NA
Anti-p15(E)^b	99.8	125	104	82.3	115	82	4.0	6	0
Anti-p15(E)^c	100.0	387	366	96.1	178	135	3.3	7	0
Expt 6									
Anti-MAC-2	4.2	2	NA	6.7	7	NA	0.5	1	NA
Anti-gp70^f	99.6	116	114	10.8	15	8	0.3	1	0
Anti-gp70^g	94.8	49	47	11.3	11	4	0.5	1	0
Anti-gp70^h	99.2	142	140	11.1	18	11	0.8	1	0
NGS	5.5	20	NA	6.7	32	NA	14.6	8	NA
Anti-AKR gp69-71	99.8	320	300	99.2	337	305	98.5	157	149
Anti-p10	95.8	130	110	94.1	215	183	4.7	6	0
Anti-p12	95.7	130	110	63.8	100	68	7.6	4	0
Anti-p15	82.1	69	49	56.0	96	64	5.8	6	0
Anti-p30	93.5	129	109	80.5	127	95	12.6	7	0

[a] TMFI, total mean fluorescence intensity. ΔTMFI was calculated by subtracting the TMFI from that of the isotype-matched antibody control. —, not determined; NA, not applicable.

[b] As determined by flow cytometric analysis. DNP, dinitrophenol; NGS, normal goat serum.

[c] Initial isolate of B6-1153 was Ia$^-$ (17).

[d] Staining of B6-1710 was performed with an immunoglobulin G3-specific fluorescein isothiocyanate-conjugated antibody. The data listed for the staining of AKR.H-2^{b}SL1 and B6-1153 used the fluorescein isothiocyanate-conjugated goat anti-mouse polyvalent antibody, but a similar pattern of reactivity was also obtained with the immunoglobulin G3-specific secondary antibody.

unable to lyse B6-1710 tumor targets (Table 3). A number of additional syngeneic tumor targets, including E♀K1, several B-cell lineage lines and hybridomas, and those induced by the Friend-Moloney-Rauscher group of MuLV (MBL2 and RBL5), and the allogeneic targets P815 (*H-2^d*) and YAC-1 (*H-2^a*) were not recognized when employed in direct lytic or competitive inhibition cytolytic assays (data not shown), indicating that the lysis of B6-1710 was specific and not due to natural killer or promiscuous killer activities.

Somewhat surprisingly, a second MAIDS-associated tumor of B6 origin, B6-1153, was a poor target for effector cells raised against B6-1710, relative to the homologous B6-1710 targets, the level of lysis ranging from low but significant to questionably positive (Fig. 1A; see also Table 4 below). That B6-1153 cells were immunogenic and were susceptible to lysis was shown by generating cytolytic effectors against this MAIDS-derived tumor by homologous secondary stimulation (Fig. 1D and Table 4). Extensive lysis of B6-1710 targets by these killer cells was also observed, consistent with the

TABLE 2. Virologic characterization of MAIDS B-lineage lymphoma lines

Virus content and expression	B6-1710	B6-1153
Infectious MuLV[a]		
Ecotropic	1.8	—
MCF	—	—
Defective MuLV[b]		
DNA	+	+
mRNA	≥10×	1×
Pr60^{gag}	+	+

[a] The initial isolate of B6-1710 produced infectious mink cell focus-inducing (MCF) MuLV (4, 17). The value indicates the number of focus-forming units ($\log_{10}$) per 10^7 cells; —, no detectable virus.

[b] DNA analysis: +, 2 or more integrated copies of defective MuLV genome detected by Southern blot hybridization. For mRNA analysis, values indicate relative levels of expression on Northern blot hybridization. Protein analysis: +, presence of Pr60^{gag} encoded by the defective virus (3). It was not possible to compare relative levels expressed in the two lines by immunoprecipitation of metabolically labeled cells. Primary data on the content and expression of the defective virus genome in these lines have been presented elsewhere (4, 16).

TABLE 3. Generation of effector cells cytolytic to the B6-1710 MAIDS-associated tumor: lack of cross-reactivity with anti-AKR/Gross MuLV CTLs[a]

Effector cell stimulator		Effector/target ratio	% Specific lysis of:			
			B6 strain:		AKR.H-2^b strain:	
In vivo	In vitro		B6-1710	E♂G2	SL1	cl.18-5
B6-1710	None	20:1	8	4	5	0
	B6-1710	20:1	79	4	4	−1
		4:1	37	1	1	−1
	E♂G2	20:1	17	15	8	−1
		4:1	5	6	4	−2
	AKR.H-2^{b}SL1	20:1	18	6	7	1
		4:1	5	4	3	−1
	cl.18-5	20:1	16	3	6	−1
(BALB)B.GV	None	20:1	2	2	2	−2
	E♂G2	20:1	0	68	72	−1
		4:1	−2	57	54	−2
	AKR.H-2^{b}SL1	20:1	−1	64	72	−2
		4:1	−2	36	35	−2
	cl.18-5	20:1	−2	9	4	−1
	B6-1710	20:1	−1	5	2	−1

[a] C57BL/6 mice were immunized with 10^6 B6-1710 or (BALB)B.GV tumor cells 10 days before spleen cells from these mice were removed for secondary in vitro stimulation. The values of spontaneous release from the target cells ranged from 8 to 12%, with the exception of B6-1710, whose value was 25%.

recognition of common, presumably LP-BM5 MuLV-associated, antigens on the two MAIDS tumors.

To characterize the type of effector cells responsible for the lysis of the MAIDS tumor lines, responder cells primed in vivo and restimulated in vitro with the homologous tumor were treated with various monoclonal antibodies plus complement just prior to the cytolytic assay. In this way the cell surface phenotype of both of the cytolytic effectors raised against B6-1710 and against B6-1153 was shown to be Thy-1$^+$ Ly-2(CD8)$^+$ Ly-4(CD4)$^-$, indicating that the effectors were conventional CD8$^+$ CTLs (data not shown).

The sharing of unique, presumably LP-BM5 MuLV-related, CTL-defined antigens by B6-1710 and B6-1153 could be seen more easily by employing the heterologous MAIDS tumor as a secondary in vitro stimulator cell. In this way it was clear that B6-1710 cells expressed an antigen(s) to which the B6-1153-immunized responder cells had been primed, resulting in CTLs with nearly comparable activities against the two targets (Fig. 1C and Table 4). Conversely, B6-1153 tumor stimulator cells also presented a recall antigen(s) to B6-1710-primed responders (Fig. 1B and Table 4). In this case, however, the lysis of heterologous B6-1710 targets was always substantially greater than that of B6-1153 targets. Collectively, these data suggest that CD8$^+$ CTLs can be raised to determinants unique to MAIDS-derived tumors and are most consistent with the existence of at least two CTL-defined antigen systems: (i) an immunodominant antigen(s) expressed on B6-1710 but either absent or (more likely) functionally present to a substantially lesser extent on B6-1153 (Fig. 1A and B) and (ii) an antigen(s) expressed on both B6-1153 and B6-1710 tumor cells at roughly similar functional levels (Fig. 1C). This view was strongly supported by the results with additional target cells (Fig. 1). Panel D is further consistent with the close relationship of the two MAIDS-associated tumors but also indicates that secondary restimulation with B6-1153 additionally results in a lesser degree of cross-reactive lysis of a number of the other tumors, particularly E♂G2 and RBL5, with even some recognition of allogeneic targets such as YAC-1 and P815. Further phenotyping experiments utilizing additional antibodies, including anti-NK1.1, were unsuccessful in allowing the cells that lysed the non-MAIDS tumors to be characterized as a unique effector cell subset.

We next addressed the question of whether cells from B6 mice infected with LP-BM5 MuLV expressed CTL-defined viral antigens comparable to those defined on the B6-1710 and B6-1153 tumors. Spleen cells from mice 5 to 22 weeks postinfection were employed as secondary in vitro stimulator cells in an attempt to generate LP-BM5 MuLV-specific CTL from MAIDS tumor-primed responder cells, based on our previous observation that the use of normal cells as secondary in vitro stimulators provided the most sensitive way to detect AKR/Gross/Akv CTL-defined MuLV determinants in the AKR.H-2^b congenic and AKXL recombinant inbred strains (7, 9). Lymph node (data not shown) and spleen cells from LP-BM5-infected mice, but not uninfected mice or those infected with the ecotropic virus component of the LP-BM5 mixture, stimulated substantial levels of cytolytic activity from both B6-1710- and B6-1153-primed responder populations (Table 4). Infected spleen cell-restimulated CTL activity was directed only to the MAIDS tumors; a variety of other tumor cell lines, including E♂G2, E♀K1, AKR.H-2^{b}SL1, cl.18-5, EL4(S), MBL2, RBL5, P815, YAC-1, and AKR SL8, were not lysed (data not shown). The specificity of effector cells raised by restimulation with preparations of LP-BM5 MuLV-infected spleen cells was further characterized by competitive-inhibition ^{51}Cr release assays (data not shown). Both effector cells generated after initial in vivo priming with B6-1710 or B6-1153 tumor cells recognized spleen and lymph node cells from LP-BM5-infected mice relative to those from uninfected mice.

The studies presented here provide the first evidence, to our knowledge, that specific CTL responses can be generated to LP-BM5 MuLV-derived cells and that these responses can be elicited by a mouse strain that is susceptible to MAIDS, B6. The specificity of such CTLs for unique MuLV-related determinants, rather than tumor-specific antigens, was suggested by the patterns of tumor target lysis (Tables 1 to 3; Fig. 1), flow cytometric analysis of the expression of cell surface viral antigens (Table 1), and the ability to elicit CTL responses specifically effective against MAIDS tumors following in vitro restimulation with spleen cells (Table 4) and lymph node cells from LP-BM5 MuLV-infected B6 mice. These results were thus consistent with the possibility that if the production of antiviral CTLs occurs or can be achieved in vivo, the responses may be effective against infected "normal" cells.

If CTLs are produced in vivo, the destruction of infected cells by antiviral CTLs might play a role in the MAIDS disease process in a deleterious way, as has been suggested for AIDS, by eliminating regulatory Th cells or altering Th subset distribution and function and thereby contributing to the development of immunodeficiency (5, 6, 13, 22, 23, 29). Along these lines, it should be noted that the MHC seems to control the incidence of MAIDS (10, 21); specifically, the major determinant of genetic resistance or susceptibility to MAIDS in crosses between susceptible *H-2^b* and resistant *H-2^d* mice mapped to *H-2D*, and rather surprisingly, susceptibility was found to be a dominant trait (21). These results are thus consistent with the possibility that class I MHC-restricted recognition of virus-infected cells by CD8$^+$ CTLs is involved in disease pathogenesis. Alternatively, antiviral

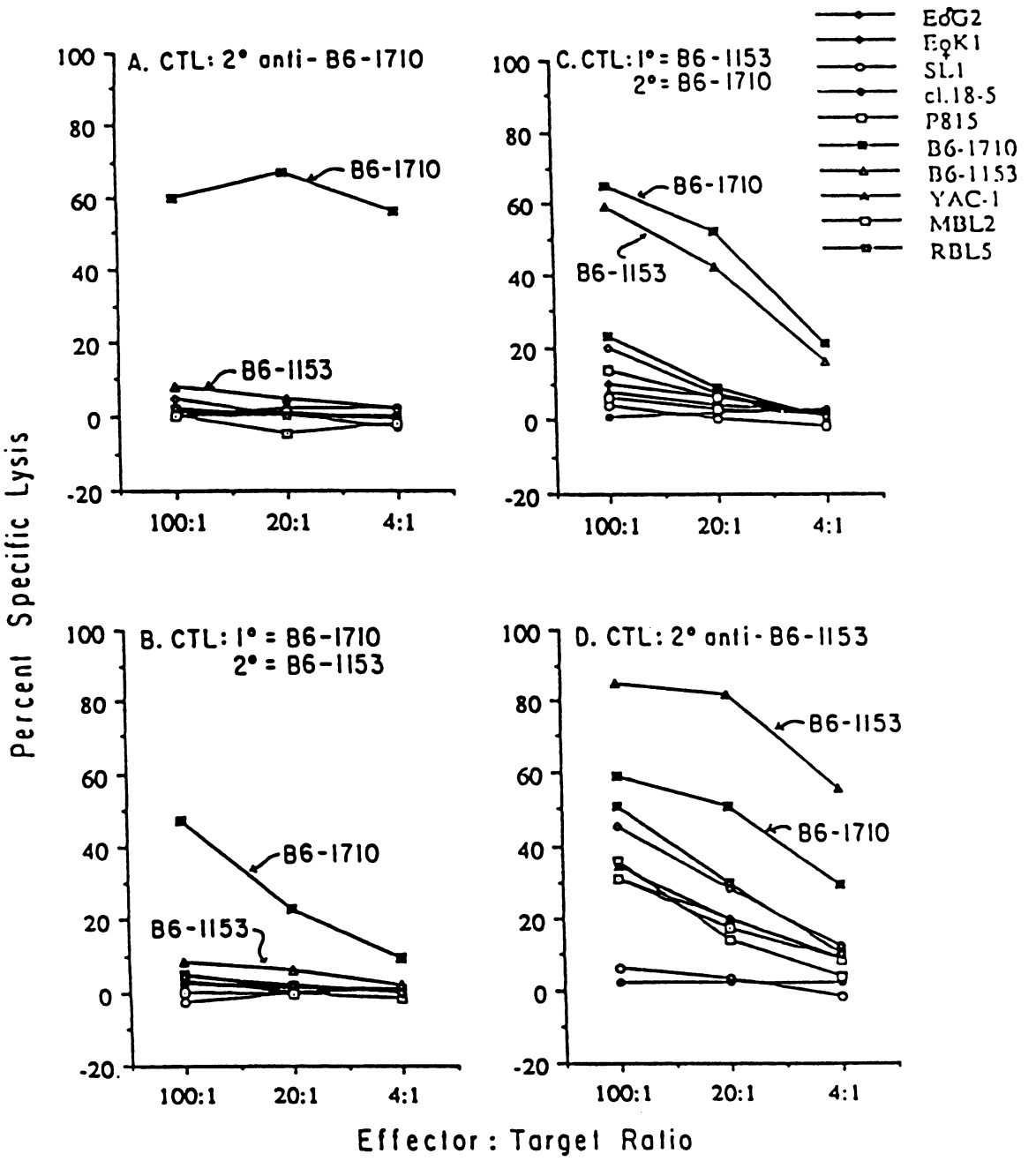

FIG. 1. Summary of target cell specificities of CTLs raised against MAIDS-associated tumors by the direct lytic ^{51}Cr release assay. C57BL/6 mice were immunized with 10^6 B6-1710 (A and B) or 10^6 B6-1153 (C and D) tumor cells 10 days before spleen cells were removed for secondary in vitro stimulation with either irradiated B6-1710 (A and C) or B6-1153 (B and D) stimulator cells.

CTL responses might be viewed as helpful in protection from, or slowing of the progression of, the immunodeficiency if these responses could be enhanced before the immunodeficiency has become full-blown (see below).

Flow cytometric analyses of viral antigen expression showed that only B6-1710 expressed *gag*-related specificities at readily detectable levels (Table 1). Whether the presences on B6-1710 of both serologically defined *gag*-encoded products and a dominant CTL-defined epitope are related is uncertain. Because of the novel sequences defined for the p12- and p15-encoding regions of the *gag* gene of the defective virus (1, 3, 14) and our finding that LP-BM5-infected but not LP-BM5-derived ecotropic virus-infected spleen cells stimulate CTL responses to the MAIDS tumors (Table 4), it is tempting to speculate, however, that the antiviral CTLs directed to the dominant CTL epitope preferentially expressed by B6-1710 may be specific for the defective *gag* product, which exists as a unique 60-kDa precursor. The observations that the B6-1153 tumor, although carrying integrated copies of the defective genome,

TABLE 4. Generation of CTLs directed against B6-1710 and B6-1153 tumors by stimulation with spleen cells from LP-BM5-infected mice[a]

Effector cell stimulator		Effector/target ratio	% Specific lysis of:	
In vivo	In vitro		B6-1710	B6-1153
B6-1710	None	100:1	4	−3
	B6-1710	100:1	78	13
		20:1	57	7
	B6-1153	100:1	40	16
		20:1	16	8
	Normal B6 spleen cells	100:1	4	−2
	LP-BM5-infected B6 spleen cells	100:1	53	19
		20:1	31	13
	LP-BM5 ecotropic component-infected B6 spleen cells	100:1	2	−1
B6-1153	None	100:1	2	5
	B6-1710	100:1	53	45
		20:1	36	24
	B6-1153	100:1	49	78
		20:1	22	51
	Normal B6 spleen cells	100:1	0	5
	LP-BM5-infected B6 Spleen cells	100:1	51	33
		20:1	22	14
	LP-BM5 ecotropic component-infected B6 spleen cells	100:1	−1	5

[a] C57BL/6 mice were immunized with 10^6 B6-1710 or B6-1153 tumor cells 10 days before spleen cells were removed for secondary in vitro stimulation. Values of spontaneous release from the target cells were 15% for B6-1710 and 16% for B6-1153. B6 spleen cells that were used as stimulator cells were obtained from either age-matched normal mice or mice that had been infected with 0.4 ml of either the complete LP-BM5 virus complex or the biologically cloned ecotropic component of LP-BM5. The titers of infectious ecotropic virus in the two preparations, as measured by the X-C plaque test, were approximately equal, being within less than a factor of 2.

appears to express ≤1/10 as much mRNA related to the defective virus as does the B6-1710 tumor (Table 1) and expresses the novel 60-kDa *gag* product in the cytoplasm but not on the cell surface (16), perhaps indicating aberrant turnover or processing, are consistent with speculation of a defective *gag*-encoded dominant CTL specificity. In addition, the pattern of lysis of B6-1710 and B6-1153 targets by CTLs raised via LP-BM5-infected spleen cell restimulation resembled that following B6-1710 stimulation. The mapping of resistance to MAIDS to a class I MHC locus, coupled with the observation that resistance in moderately to highly resistant strains appeared to develop with age (21), suggests a role for an immune response of the CTL type. Additional consistent observations include the finding that tissues from MAIDS-resistant A/J mice do not contain the defective genome, as assessed by Southern blot hybridization, at 7 or 12 weeks postinfection whereas the genome is readily detected in tissues of infected B6 mice as soon as 2 weeks postinfection (4). That the absence of the defective genome in A/J tissues is mediated through the activity of $CD8^+$ T cells is strongly suggested by the observations that mice of this strain treated with anti-CD8 antibodies develop signs of early MAIDS in association with ready detection of the defective genome in the spleen by Southern hybridization (20a). Experiments in which antiviral CTL responses are developed in resistant strains and employed in adoptive transfer schemes will be important in establishing a role for $CD8^+$ CTLs in this clearance of the defective genome.

It should also be pointed out that there is recent evidence that the B6-1710 tumor presents a *gag*-related superantigen to disease-susceptible B6 mice (16). Thus, a strong primary in vitro proliferative response of spleen cells to irradiated B6-1710 tumor cells, compared with a very weak response to B6-1153 stimulator cells, was observed. The response to B6-1710 displayed all the other hallmarks of a superantigen response: predominant $CD4^+$ responder cell type, dependence of recognition on class II MHC, and the preferential expansion of T cells bearing certain V_β T-cell receptor types. These findings lead to the possibility that an overzealous response to a retroviral superantigen presented by infected B-cell lineage antigen-presenting cells is the initiating event for the immunodeficiency and that $CD8^+$ CTL responses directed at these antigen-presenting cells could abrogate the development of the disease.

We thank Janet Hartley for kindly providing the initial virus stocks and infected lines and for her advice; John McMaster, Nicole Benoit, and Kristin Lindgren for technical assistance; Michael Fanger, Bruce Chesebro, and Randolph Noelle for their generous supply of monoclonal antibodies; Hillary White for a critical reading of the manuscript and thoughtful discussions; Robert Rich, Michael Coppola, and David Schwarz for their helpful suggestions; and Ellen Sarrica for her preparation of the manuscript. The flow cytometer was the generous gift of the Fannie E. Rippel Foundation.

This work was supported in part by U.S. Public Health Service grants CA36860 and CA50157 to W.R.G. and by contract NO1-AI-72622 to Microbiologic Associates, Inc., Bethesda, Md. (to H.C.M.). W.R.G. is the recipient of Research Career Development Award KO4 CA01112. The flow cytometer and the irradiation facility are partially supported by the core grant of the Norris Cotton Cancer Center (CA23108).

REFERENCES

1. **Aziz, D. C., Z. Hanna, and P. Jolicoeur.** 1989. Severe immunodeficiency disease induced by a defective murine leukemia virus. Nature (London) **338:**505–508.
2. **Buller, R. M. L., R. A. Yetter, T. N. Fredrickson, and H. C. Morse III.** 1987. Abrogation of resistance to severe mousepox in C57BL/6 mice infected with LP-BM5 murine leukemia viruses. J. Virol. **61:**383–387.
3. **Chattopadhyay, S. K., H. C. Morse III, M. Makino, S. K. Ruscetti, and J. W. Hartley.** 1989. A defective virus is associated with induction of a murine retrovirus-induced immunodeficiency syndrome, MAIDS. Proc. Natl. Acad. Sci. USA **86:** 3862–3866.
4. **Chattopadhyay, S. K., D. N. Sengupta, T. N. Fredrickson, H. C. Morse III, and J. W. Hartley.** 1991. Characteristics and contributions of defective, ecotropic, and mink cell focus-inducing viruses involved in a retrovirus-induced immunodeficiency syndrome of mice. J. Virol. **65:**4232–4241.
5. **Cheung, S. C., S. K. Chattopadhyay, J. W. Hartley, H. C. Morse III, and P. M. Pitha.** 1991. Aberrant expression of cytokine genes in peritoneal macrophages from mice infected with LP-BM5 MuLV, a murine model of AIDS. J. Immunol. **146:**121–127.
6. **Cheung, S. C., S. K. Chattopadhyay, H. C. Morse III, and P. M. Pitha.** 1991. Expression of defective virus and cytokine genes in murine AIDS. J. Virol. **65:**823–828.
7. **Green, W. R.** 1983. Cell surface expression of cytotoxic T lymphocyte-defined, AKR/Gross leukemia virus-associated tumor antigens by normal AKR.H-2^b splenic B cells. J. Immunol. **131:**3078–3084.
8. **Green, W. R., ar[illegible] M. A. Brown.** 1983. The specificity of H-2 restricted cytotoxic T lymphocytes directed to AKR/Gross leukemia virus-induced tumors. II. Altered gp70 display and production of noninfectious virus particles by an insusceptible variant tumor. Eur. J. Immunol. **13:**871–877.
9. **Green, W. R., and R. F. Graziano.** 1986. CTL-defined retroviral antigens on normal cells; encoding by the Akv-1 proviral locus.

Immunogenetics **23:**106–110.

10. **Hamelin-Bourassa, D., E. Skamene, and F. Gevais.** 1989. Susceptibility to a mouse acquired immunodeficiency syndrome is influenced by the H-2. Immunogenetics **30:**266–272.
11. **Hartley, J. W., T. N. Fredrickson, R. A. Yetter, M. Makino, and H. C. Morse III.** 1989. Retrovirus-induced murine acquired immunodeficiency syndrome: natural history of infection and differing susceptibility of inbred mouse strains. J. Virol. **63:** 1223–1230.
12. **Hass, M., and A. Meshorer.** 1979. Reticulum cell neoplasms induced in C57BL/6 mice by cultured virus grown in stromal hematopoietic cell lines. JNCI **63:**427–436.
13. **Hayakawa, K., and R. R. Hardy.** 1988. Murine CD4+ T cell subsets defined. J. Exp. Med. **168:**1825–1838.
14. **Huang, M., and P. Jolicoeur.** 1990. Characterization of the *gag*/fusion protein encoded by the defective Duplan retrovirus inducing murine acquired immunodeficiency syndrome. J. Virol. **64:**5764–5772.
15. **Huang, M., C. Simard, and P. Jolicoeur.** 1989. Immunodeficiency and clonal growth of target cells induced by helper-free detective retrovirus. Science **246:**1614–1617.
16. **Hugin, A. W., M. S. Vacchio, and H. C. Morse III.** 1991. A virus-encoded "superantigen" in a retrovirus-induced immunodeficiency syndrome of mice. Science **252:**424–427.
17. **Klinken, S. P., T. N. Fredrickson, J. W. Hartley, R. A. Yetter, and H. C. Morse III.** 1988. Evolution of B cell lineage lymphomas in mice with a retrovirus-induced immunodeficiency syndrome, MAIDS. J. Immunol. **140:**1123–1131.
18. **Klinman, D. M., and H. C. Morse III.** 1989. Characteristics of B cell proliferation and activation in murine AIDS. J. Immunol. **142:**1144–1149.
19. **Latarjet, R., and J.-F. Duplan.** 1962. Experiment and discussion on leukaemogenesis by cell-free extracts of radiation-induced leukaemia in mice. Int. J. Radiat. Biol. **5:**339–344.
20. **Legrand, E., R. Daculsi, and J.-F. Duplan.** 1981. Characteristics of the cell populations involved in extrathymic lymphosarcoma induced in C57BL/6 mice by RadLV-RS. Leukemia Res. **5:**223–233.

20a. **Makino, M., S. K. Chattopadhyay, J. W. Hartley, and H. C. Morse III.** Unpublished data.

21. **Makino, M., H. C. Morse III, T. N. Fredrickson, and J. W. Hartley.** 1990. H-2-associated and background genes influence the development of a murine retrovirus-induced immunodeficiency syndrome, MAIDS. J. Immunol. **144:**4347–4355.
22. **Mizuochi, T., J. Mizuguchi, T. Uchida, K. Ohnishi, M. Nakanishi, Y. Asano, T. Kakiuchi, Y. Fukushima, K. Okuyama, H. C. Morse III, and K. Komuro.** 1990. A selective signaling defect in helper T cells induced by antigen-presenting cells from mice with murine acquired immunodeficiency syndrome. J. Immunol. **144:**313–316.
23. **Morse, H. C., III, R. A. Yetter, C. S. Via, R. R. Hardy, A. Cerny, K. Hayakawa, A. W. Hugin, M. W. Miller, K. L. Holmes, and G. M. Shearer.** 1989. Functional and phenotypic alterations in T cell subsets during the course of MAIDS, a murine retrovirus-induced immunodeficiency syndrome. J. Immunol. **143:**844–850.
24. **Mosier, D. E.** 1986. Animal models for retrovirus-induced immunodeficiency disease. Immunol. Invest. **15:**233–261.
25. **Mosier, D. E., R. A. Yetter, and H. C. Morse III.** 1985. Retroviral induction of acute lymphoproliferative disease and profound immunosuppression in adult C57BL/6 mice. J. Exp. Med. **161:**766–784.
26. **Mosier, D. E., R. A. Yetter, and H. C. Morse III.** 1987. Functional T lymphocytes are required for a murine retrovirus-induced immunodeficiency disease (MAIDS). J. Exp. Med. **165:**1737–1742.
27. **Pattengale, P. K., C. R. Taylor, P. Twomey, S. Hill, J. Jonasson, T. Beardsley, and M. Haas.** 1982. Immunopathology of B cell lymphomas induced in C57BL/6 mice by dualtropic murine leukemia virus (MuLV). Am. J. Pathol. **107:**362–377.
28. **Pinter, A., W. J. Honnen, J.-S. Tung, P. V. O'Donnell, and U. Hammerling.** 1982. Structural domains of endogenous murine leukemia virus gp70s containing specific antigenic determinants defined by monoclonal antibodies. Virology **116:**499–516.
29. **Pitha, P. M., D. Biegel, R. A. Yetter, and H. C. Morse III.** 1988. Abnormal regulation of IFN-α, -β, and -γ expression in MAIDS, a murine retrovirus-induced immunodeficiency syndrome. J. Immunol. **141:**3611–3616.
30. **Plata, F., B. Autran, L. P. Martins, S. Wain-Hobson, M. Raphael, C. Maynard, M. Denis, J. M. Guillan, and P. Debre.** 1987. AIDS virus specific cytotoxic T lymphocytes in lung disorders. Nature (London) **328:**348–351.
31. **Siliciano, R. F., T. Lawton, C. Knoll, R. W. Karr, P. Berman, T. Gregory, and E. L. Reinherz.** 1988. Analysis of host-virus interactions in AIDS with anti-gp120 T cell clones: effect of HIV sequence variation and a mechanism for $CD4^+$ cell depletion. Cell **54:**561–575.
32. **Stone, M. R., and R. C. Nowinski.** 1979. Topological mapping of murine leukemia virus proteins by competition binding assays with monoclonal antibodies. Virology **100:**370–381.
33. **Walker, B. D., S. Chakrabarti, B. Moss, T. J. Paradis, T. Flynn, A. G. Durno, R. S. Blumberg, J. C. Kaplan, M. S. Hirsch, and R. T. Schooley.** 1987. HIV-specific cytotoxic T lymphocytes in seropositive individuals. Nature (London) **328:**345–348.
34. **Walker, B. D., C. Flexner, T. J. Paradis, T. C. Fuller, M. S. Hirsch, R. T. Schooley, and B. Moss.** 1988. HIV-1 reverse transcriptase is a target for cytotoxic T lymphocytes in infected individuals. Science **240:**64–66.
35. **Yetter, R. A., R. M. L. Buller, J. S. Lee, K. L. Elkins, D. E. Mosier, T. N. Fredrickson, and H. C. Morse III.** 1988. $CD4^+$ T cells are required for development of a murine retrovirus-induced immunodeficiency syndrome (MAIDS). J. Exp. Med. **168:**623–635.