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Adoptive Transfer of Polyclonal and Cloned Cytolytic T Lymphocytes (CTL) Specific for Mouse AIDS-Associated Tumors Is Effective in Preserving CTL Responses: a Measure of Protection against LP-BM5 Retrovirus-Induced Immunodeficiency

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Cytolytic T lymphocytes (CTL) can be raised against C57BL/6 B-cell lymphomas from mice with LP-BM5 murine leukemia virus-induced AIDS (MAIDS). Adoptive transfer of polyclonal anti-MAIDS tumor CTL or two CTL clones specific for the B6-1710 MAIDS lymphoma caused preservation of major histocompatibility complex-restricted and allogeneic CTL responses, which may be interpreted as indices of protection from LP-BM5 murine leukemia virus-induced immunodeficiency.

An immunodeficiency in which certain mouse strains are inoculated with the LP-BM5 murine leukemia virus (MuLV) isolate has been described (32). LP-BM5, a retrovirus complex that originated from a preparation derived from C57BL mice treated with whole-body irradiation (25), induced nontransplantable reticulum cell neoplasms (18). Because, following further passage, the viral isolate was observed to cause a profound lymphoproliferative disorder and immunodeficiency, it was renamed LP-BM5 (32). LP-BM5 is a mixture of MuLV including ecotropic, recombinant mink cell cytopathic focus-inducing (MCF) and replication-negative defective viruses. The defective genome is the proximal agent causing the immunodeficiency, however, with the ecotropic and perhaps the MCF MuLV serving primarily as helper viruses (1, 5, 17, 19). Because LP-BM5-induced immunodeficiency bears a number of characteristics similar to those of AIDS (4, 22, 23, 26, 30–33, 35, 38), including an increased incidence of terminal B-cell lymphomas, it has been termed mouse AIDS (MAIDS). Inbred mice vary widely in their susceptibilities to MAIDS with C57BL/6 (B6) as the prototypic susceptible strain (29). Genes governing resistance or susceptibility to MAIDS have been mapped to the major histocompatibility complex (MHC) complex (16, 29), most importantly to *H-2D* (29). Given the well-known activity of cytolytic T lymphocytes (CTL) against virus-infected cells, including in MHC class I-linked resistance to MuLV-induced tumorigenesis (for example, see references 3, 6, 7, and 27), we have focussed on CTL responses in the MAIDS system. In keeping with this notion, resistant A-strain mice were rendered susceptible to LP-BM5-induced disease by prior in vivo depletion of $CD8^+$, but not $CD4^+$, T cells (28).

To examine the involvement of specific CTL in MAIDS pathogenesis, B6 mice were stimulated with the syngeneic MAIDS-associated, $CD5^+$ B-cell lymphomas, B6-1710 and B6-1153, selected because of their homogeneity and expression of the defective virus (22) and because in infected mice the defective genome is expressed primarily by $CD5^+$ B cells (20). We reported previously (8) that B6 mice generate highly active secondary $CD8^+$ CTL responses specific for B6-1710 and B6-1153 but not other histocompatible tumors induced by distinctive MuLV, including the AKR/Gross subset we have studied extensively (for examples, see references 14, 15, and 36). Two types of CTL determinants were suggested (8), one shared relatively equally by B6-1710 and B6-1153 and another (immunodominant to the first) present at much higher functional levels on B6-1710 than on B6-1153. The ability to produce B6 anti-MAIDS tumor CTL by secondary stimulation with spleen cells from mice infected with LP-BM5 or a defective viral clone rescued by a molecularly cloned ecotropic helper virus (12), but not ecotropic virus alone (8), suggested (i) the importance of the defective genome in determining the CTL epitopes and (ii) the possibility that anti-MAIDS tumor CTL would be effective against LP-BM5-infected cells in B6 mice with developing MAIDS and thereby could influence the progression of immunodeficiency.

In vivo adoptive transfer of polyclonal anti-MAIDS CTL or tumor-primed effector cells. As a first approach towards testing the potential relevance of anti-MAIDS CTL, polyclonal CTL raised against the B6-1710 and/or B6-1153 tumors were tested for their effect on LP-BM5-induced immunodeficiency in approximately 7-week-old B6 mice. We chose a donor strain histocompatible with and closely related to B6 (*Fv-1*^{b}) but carrying the *n*, rather than the *b*, allele of *Fv-1*, the B6.Fv-1^n congenic. Because both the ecotropic and MCF helper viruses are B-tropic, the B-ecotropic MuLV-restrictive *Fv-1*^{n} product should limit the infection of donor spleen cells by virus originating from the tumor cells used to stimulate CTL production. Indeed, in initial experiments mice receiving B6 anti-MAIDS tumor CTL and LP-BM5 were substantially more able to mount immune responses than fully immunosuppressed mice receiving LP-BM5 MuLV alone, but the extent of preservation of immune reactivity (compared with that of unmanipulated mice) was somewhat limited, apparently due to the transfer of infected donor cells containing LP-BM5, which initiated disease in the B6 recipients. B6.Fv-1^n effector cells highly lytic for the MAIDS tumors could be raised by secondary stimulation of tumor-primed splenic responder cells, including cross-stimulation patterns of lysis of B6-1710 versus B6-1153 target cells (data not shown) indistinguishable from those published for B6 anti-MAIDS tumor CTL (8).

B6.Fv-1^n effectors were next employed in adoptive transfer

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TABLE 1. Effect of adoptive transfer of B6-1710 tumor-primed B6.Fv-1^n spleen cells on LP-BM5 MuLV-induced immunodeficiency in B6 mice

In vivo sequence and inoculation regimen[a]	Stimulation source	E:T ratio[b]	Target cell % specific lysis[c]		
			Anti-AKR/Gross MuLV		Antiallogeneic P815 (*H-2*^{d})
			E♂G2	AKR.H-2^{b}SL1	
Sequence A: day 20, 10^6 B.GV	AKR.H-2^{b}SL1	100:1	87	84	
	AKR.H-2^{b}SL1	20:1	72	75	
	P815	100:1			95
	P815	20:1			93
Sequence B					
Day 0, LP-BM5	AKR.H-2^{b}SL1	100:1	12	14	
	AKR.H-2^{b}SL1	20:1	6	6	
Day 20, 10^6 B.GV	P815	100:1			50
	P815	20:1			34
Sequence C					
Day 0, LP-BM5	AKR.H-2^{b}SL1	100:1	36	41	
	AKR.H-2^{b}SL1	20:1	19	22	
Day 2, 3×10^7 1710-immunized B6.Fv-1^n spleen cells[d]	P815	100:1			78
	P815	20:1			65
Day 20, 10^6 B.GV					

[a] Groups were composed of three C57BL/6 mice each. All injections were given intraperitoneally.
[b] E:T ratio, effector/target ratio.
[c] Percent specific lysis is defined as $[(a - b)/c] \times 100$, where a is experimental counts per minute released, b is spontaneous counts per minute released, and c is freeze-thaw releasable (about 80% of total) counts per minute. The values for percent specific lysis determined on the basis of individual ^{51}Cr-release wells generally showed minimal variation; the values of duplicate wells occasionally differed to a greater extent, but in all such cases the range of the values was ±6% of the mean value.
[d] B6.Fv-1^n mice were immunized with 10^6 B6-1710 tumor cells 10 days prior to removal of their spleens for transfer into B6 mice infected with 0.2 ml of the MAIDS-inducing virus complex, LP-BM5.

experiments to determine their effect on the development of MAIDS. The donor B6.Fv-1^n cells were either fully differentiated anti-MAIDS tumor CTL raised by homologous secondary stimulation or spleen cells from B6.Fv-1^n mice that were primed with B6-1710 or B6-1153 tumor cells in vivo and transferred without further culture or stimulation. Two "read-out" responses were utilized to gauge the extent of immunodeficiency: the generation of primary allogeneic (anti-*H-2*^{d}) CTL and the production of MHC-restricted CTL to AKR/Gross MuLV-induced tumors following in vivo priming and in vitro secondary stimulation with B.GV and AKR.H-2^{b}SL1 tumor cells, respectively, as we have described elsewhere (14, 15). The *H-2K*^{b}-restricted anti-AKR/Gross MuLV CTL, specific for an octameric peptide located in the viral transmembrane envelope protein p15E, do not recognize MAIDS tumors (8, 37). Adoptive transfer of both effector cells raised against the immunodominant epitope of B6-1710 and those raised against B6-1153, largely directed against the cross-reactive determinant (8), resulted in a restoration of the read-out responses suppressed in mice receiving only LP-BM5. By using these criteria, substantial protection from MAIDS was observed in five of seven experiments. Somewhat more consistent protection was found with tumor-primed donor cells as opposed to secondary restimulated CTL. The level of restoration of the read-out CTL responses was often partial, as depicted in Table 1; for example, both the MHC-restricted anti-AKR/Gross MuLV CTL response to E♂G2 and AKR.H-2^{b}SL1 target cells after in vitro restimulation with AKR.H-2^{b}SL1, and especially the primary allogeneic response to P815, were significantly restored by the transfer of the B6-1710 tumor-primed B6.Fv-1^n cells (Table 1, compare regimens B and C). Frequently, it was easier to alleviate inhibition of the allogeneic response than that of the MHC-restricted, CTL response, in keeping with the reported greater relative resistance of allogeneic (versus MHC-restricted) CTL responses to LP-BM5-induced immunodeficiency (13, 31).

Effect on LP-BM5-induced MAIDS by adoptive transfer of CTL clones raised against MAIDS tumors. To confirm that the adoptive transfer of CTL per se could interfere with the course of LP-BM5-induced MAIDS, two interleukin-2 (Cetus Corporation, Emeryville, Calif.)-dependent clones, 0.3.1 and 10.2, were obtained from bulk cultures of B6-1710-primed and -restimulated CTL by limiting dilution. On the basis of their lysis of B6-1710, but not B6-1153 or other target cells (Table 2), the 0.3.1 and 10.2 clones appear to be specific for the immunodominant epitope preferentially expressed on B6-1710 (8). A third interleukin-2-dependent CTL clone, E4, originated from a primary proliferative culture of naive B6 spleen cells stimulated with irradiated B6-1710, that is, a superantigen culture as described by Hugin et al. (21). E4, though possessing less lytic activity than 0.3.1 or 10.2, also demonstrated a preferential specificity for B6-1710. By indirect immunofluorescence, all three CTL clones were only weakly positive at best for NK1.1 (data not shown) but strongly expressed H-2K^b, Thy 1.2, LFA-1, CD3, and α/β TcR (Table 3). Clone 0.3.1 was CD8$^+$, however, while both clones 10.2 and E4 were CD4$^-$ CD8$^-$. The 0.3.1 and E4 clones expressed TcR Vβ5 subunits, whereas clone 10.2 was Vβ8 positive. Monoclonal antibody blocking experiments showed that (i) clones 0.3.1 and 10.2 were *K*^{b} restricted, (ii) LFA-1 was involved in recognition by all three clones, and (iii) as predicted, only 0.3.1 showed a functional dependence on CD8 (Table 4). All three CTL clones were found to be MuLV negative on the basis of reverse transcriptase assays (data not shown).

These CTL clones were employed in adoptive transfer experiments in which between 1×10^6 and 2×10^6 donor CTL cells were infused into B6 mice one or two times during the 2- to 6-day period following injection of LP-BM5 MuLV. Subsequently the mice were immunized with B.GV tumor cells to prime them for AKR/Gross virus CTL production, and later the primed responder cells were cultured with appropriate stimulator cells for primary allogeneic, or secondary anti-AKR/

TABLE 2. Specificity of MAIDS CTL clones

Expt and CTL clone[a]	E:T ratio[b]	Target cell % specific lysis[c]		
		B6-1710	B6-1153	AKR.H-2^{b}SL1
Expt 1				
0.3.1	10:1	95	4	13
0.3.1	2:1	91	6	6
10.2	10:1	80	6	3
10.2	2:1	70	3	3
E4	10:1	28	12	3
E4	2:1	15	8	0
Expt 2				
0.3.1	10:1	96	2	3
0.3.1	2:1	99	4	−1
E4	10:1	36	11	4
E4	2:1	27	9	1

[a] Polyclonal secondary anti-B6-1710 CTL that showed a high specific lytic activity for only B6-1710 were cloned by limiting dilution with media containing 15 U of interleukin-2, 5×10^6 irradiated B6-1710 cells, and 10^6 irradiated B6 spleen cells (antigen and feeder cells) per ml. Alternatively, the E4 clone was obtained from a primary in vitro culture of naive B6 spleen cells stimulated with irradiated B6-1710 tumor cells at a responder to stimulator cell ratio of 2:1 (21). CTL clones were maintained by feeding with interleukin-2 media thrice weekly and restimulation with antigen and feeder cells every other week.

[b] E:T ratio, effector/target ratio.

[c] Other ^{51}Cr-release experiments have shown additional targets, including clone 18-5 (10, 11), E♂G2, P815, LB27.4 (a murine *H-2^{bxd}* B-cell hybrid), and YAC-1 (a natural killer cell target) to be insusceptible to lysis by these CTL clones. The level of spontaneous release of targets in both experiments was 20% or lower. Percent specific lysis was determined as described in Table 1, footnote *c*.

Gross MuLV, CTL generation to measure the degree of immunodeficiency. Transfer of the 0.3.1 CTL clone was effective in substantially restoring the read-out antiviral CTL response and completely restored allogeneic CTL production, relative to responses by mice receiving only LP-BM5 (Table 5, experiment 1). In repeat experiments, adoptive transfer of clone 0.3.1 was also able to protect from immunodeficiency, as evidenced by restoration of either the allogeneic and/or the MHC-restricted antiviral CTL responses in five of seven experiments. Although the protection from LP-BM5-induced immunodeficiency was frequently partial, especially for the antiviral CTL response, in one experiment the anti-AKR/Gross MuLV (as well as the anti-allogeneic) CTL response was fully restored. The E4 CTL clone was also shown to restore the read-out CTL responses in two of three experiments, including one in which both the anti-AKR/Gross MuLV and anti-*H-2^d* allogeneic CTL responses were essentially completely restored (Table 5, experiment 2). In the same experiment, a parallel transfer of slightly larger numbers of a control CTL clone (G8; see the description below) provided no protection from LP-BM5 immunodeficiency. Thus, despite its derivation from a superantigen-stimulated proliferation assay and CD4$^-$ CD8$^-$

TABLE 4. CTL recognition by MAIDS CTL clones[a]

Clone	Restriction element	Inhibition of lysis by pretreatment with antibody against:		
		CD4	CD8	LFA-1
0.3.1	K^b	−	+	+
10.2	K^b	−	−	+
E4	ND[b]	−	−	+

[a] As determined by ^{51}Cr-release antibody blocking assays in which for restriction element determination the lysis of antibody treated targets was compared with that of targets pretreated with media alone to arrive at a percentage of lysis blocked. Alternatively CTL clones were pretreated by antibodies to CD4, CD8, or LFA-1 and tested against untreated ^{51}Cr-labeled targets. The level of blocking was defined as ≤29% inhibition (−) or ≥80% inhibition (+).

[b] ND, not determinable.

TABLE 3. Characteristics of MAIDS CTL clones

Cell surface antigen[a]	Results for clone					
	0.3.1 (expt 1)		10.2 (expt 1)		E4 (expt 2)	
	% of cells +	ΔTMFI[b]	% of cells +	ΔTMFI	% of cells +	ΔTMFI
DNP (control)	5		5		4	
Thy1.2	99	1,292	100	1,327	100	1,372
CD8	100	405	5	0	8	8
K^b	100	530	100	396	99	781
Mac-1 (control)	5		5		5	
LFA-1	100	1,372	100	1,415	99	2,282
CD4	2	−1	4	0	3	−9
Vβ11	1	−1	6	0	4	2
Vβ6	2	0	4	0	3	2
P3 (control)	4		6		4	
Vβ5.1+5.2	100	124	5	0	98	307
Vβ13	6	1	7	1	4	−1
HuT cells (control)	4		5		3	
Vβ8	4	0	99	164	3	2
TNP (control)	4		5		15[c]	
CD3	99	144	98	114	100	455
α/β TcR	100	74	97	61	94	89
γ/δ TcR	16	4	6	1	21	3

[a] As determined by flow cytometric analysis on a Becton Dickinson FACScan.

[b] ΔTMFI, Δ total mean fluorescence intensity, was calculated by subtracting the indicated isotype control TMFI from each appropriate experimental TMFI.

[c] Negative control cells were stained with media followed by secondary fluorescein isothiocyanate-conjugated antibody.

TABLE 5. Effect of adoptive transfer of CTL clone 0.3.1 or E4 on the LP-BM5 MuLV-induced immunodeficiency of B6 mice

Expt and in vivo sequence and inoculation regimen[a]	Stimulation source	E:T ratio[b]	Target cell % specific lysis[c]		
			Anti-AKR/Gross MuLV		Antiallogeneic P815 ($H\text{-}2^d$)
			E6G2	AKR.H-2^bSL1	
Expt 1					
Sequence A: day 10, 10^6 B.GV	None	100:1	3	7	4
	AKR.H-2^bSL1	100:1	61	73	
	AKR.H-2^bSL1	20:1	46	64	
	P815	100:1			57
	P815	20:1			31
Sequence B: day 0, LP-BM5 virus; day 10, 10^6 B.GV	None	100:1	0	4	2
	AKR.H-2^bSL1	100:1	27	42	
	AKR.H-2^bSL1	20:1	11	23	
	P815	100:1			18
	P815	20:1			8
Sequence C: day 0, LP-BM5 virus; days 3 and 6, 2×10^6 clone 0.3.1; day 10, 10^6 B.GV	None	100:1	3	12	8
	AKR.H-2^bSL1	100:1	42	70	
	AKR.H-2^bSL1	20:1	20	45	
	P815	100:1			77
	P815	20:1			42
Expt 2					
Sequence A: day 14, 10^6 B.GV	None	100:1	0	6	4
	AKR.H-2^bSL1	100:1	64	69	
	AKR.H-2^bSL1	20:1	43	38	
	LB27.4	100:1			75
	LB27.4	20:1			62
Sequence B: day 0, LP-BM5 virus; day 14, 10^6 B.GV	None	100:1	0	9	17
	AKR.H-2^bSL1	100:1	11	22	
	AKR.H-2^bSL1	20:1	7	23	
	LB27.4	100:1			16
	LB27.4	20:1			7
Sequence C: day 0, LP-BM5 virus; day 3, 1.2×10^6 clone E4 (IV); day 14, 10^6 B.GV	None	100:1	4	4	2
	AKR.H-2^bSL1	100:1	86	57	
	AKR.H-2^bSL1	20:1	42	40	
	LB27.4	100:1			74
	LB27.4	20:1			54
Sequence D: day 0, LP-BM5 virus; day 3, 1.5×10^6 clone G8 (IV); day 14, 10^6 B.GV	None	100:1	4	9	2
	AKR.H-2^bSL1	100:1	4	4	
	AKR.H-2^bSL1	20:1	3	3	
	LB27.4	100:1			26
	LB27.4	20:1			10

[a] Groups were composed of three B6 mice each. All injections were given intraperitoneally unless otherwise noted. Mice in experiment 1 received 0.15 ml and in experiment 2, 0.25 ml of the LP-BM5 virus.
[b] E:T ratio, effector/target ratio.
[c] Percent specific lysis was determined as described in Table 1, footnote *c*.

phenotype, E4 was similar to clone 0.3.1 in terms of its ability to interfere with LP-BM5-induced MAIDS.

In contrast, no consistent evidence for interference with LP-BM5-induced immunodeficiency was observed for the 10.2 CTL clone (three of three experiments), including in an experiment in which 0.3.1 demonstrated substantial restoration of read-out responses. With regard to its inability to interfere with LP-BM5-induced immunodeficiency, clone 10.2 was thus similar to several control CTL clones tested, including CTLL-2 (9), the interleukin-2 indicator line which in our hands is nonlytic, and G8. The anti-AKR/Gross MuLV G8 clone is highly active, K^b restricted (2, 10), and specific for an octameric peptide of p15E not encoded by the defective virus and altered so as not to be recognized in the B-ecotropic helper virus of MuLV (24), and it recognizes the MAIDS tumors very poorly, if at all. G8 failed to interfere with LP-BM5-induced immunodeficiency in three of three experiments, including head-to-head comparisons with protective clones (e.g., see Table 5, experiment 2).

Collectively, these results thus demonstrate that polyclonal anti-MAIDS tumor CTL and some CTL clones directed against the B6-1710 B-cell lymphoma can interfere with LP-BM5 MuLV pathogenesis in MAIDS-sensitive B6 mice. The basis for the inability of the 10.2 CTL clone to show such protection is unclear: the protective E4 CTL clone is always less lytically active than clone 10.2 in ^{51}Cr-release assays (e.g., see Table 2). Clone 10.2 appears to have a target cell specificity and class I K^b restriction element requirement indistinguishable from that of the protective clone 0.3.1. The three CTL clones produce gamma interferon at roughly equivalent levels

(data not shown). The $CD4^-$ $CD8^-$ cell surface phenotype of clone 10.2 appears unrelated to its inability to protect, because clone E4 is also $CD4^-$ $CD8^-$. Although it is possible that an adhesion molecule or some other cell surface molecule may be missing, the other phenotypic characteristics of clone 10.2 are indistinguishable from those of the two protective CTL clones, except for the β-chain subunit of the α/β TcR. Clones 0.3.1 and E4 are $V\beta5^+$, whereas clone 10.2, like most $CD4^-$ $CD8^-$ T cells immortalized by radiation leukemia virus (34), is $V\beta8^+$. The common usage of Vβ5 by the two protective CTL clones is also intriguing in that Vβ5 (along with Vβ11) TcR-bearing T cells have been reported to expand in response to a viral superantigen on the B6-1710 tumor (21). Whether interference with LP-BM5-induced MAIDS correlates with the utilization of Vβ5 type TcR and/or recognition of superantigen determinants remains to be determined in further experiments. Regardless of the precise mechanism by which protective clones recognize MAIDS tumors in vitro and nontransformed cells in LP-BM5-infected mice in vivo to restore immune reactivity, these experiments clearly show the potential relevance of CTL in the disease process and offer promise that immunization strategies to raise such CTL in MAIDS-susceptible mice may also provide protection from LP-BM5-induced immunodeficiency.

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