FBL-REACTIVE CD8⁺ CYTOTOXIC AND CD4⁺ HELPER T LYMPHOCYTES RECOGNIZE DISTINCT FRIEND MURINE LEUKEMIA VIRUS-ENCODED ANTIGENS

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Studies in animal tumor models have shown that localized and disseminated tumors can be eradicated by the adoptive transfer of tumor-specific syngeneic T cells (1–3). Analyses of the effector T cell subsets participating in tumor rejection have revealed disparate results in different tumor models. For example, in vivo generation of class I–restricted tumor-specific CTL has been shown to correlate with rejection of some murine tumors (4–8), while studies utilizing purified populations of class II–restricted tumor-specific Th, capable of inducing delayed-type hypersensitivity (DTH)[1] responses in vivo, have suggested that noncytolytic class II–restricted effectors are necessary and sufficient for tumor rejection (9–11). Moreover, in some tumor models both tumor-specific CTL and Th are required for tumor eradication (3). The disparities observed in the T cell responses required for tumor eradication in these different tumor models could reflect unique susceptibility by the tumor to a particular effector mechanism. However, most tumors are susceptible to lysis both by CD8⁺ CTL and macrophages activated by lymphokines released by CD4⁺ Th during DTH responses (12, 13). Therefore, an alternative explanation for these apparent differences in effector requirements for mediating tumor elimination in some models is that particular tumors may preferentially induce and/or activate either class I– or class II–restricted T cell responses.

Presentation of antigen for activation of MHC-restricted T cells requires that the antigen be processed to a peptide that can bind to the appropriate MHC molecule to form an immunogenic complex. The intracellular processing pathways that generate complexes of peptides and MHC antigens are separate for class I and class II molecules (14). Presentation with class II molecules appears to require that antigen enter via an exogenous chloroquine-sensitive pathway in which antigen is endocytosed and degraded in lysozomes (15, 16), whereas presentation with class I molecules appears to require antigen enter via an endogenous pathway in which proteins produced

[1] *Abbreviations used in this paper:* DTH, delayed-type hypersensitivity; env, envelope; F-MuLV, Friend murine leukemia virus; FRE, Fisher rat embryo fibroblast; vac-env, vaccinia (F-MuLV)-envelope recombinant virus.

in the cell, rather than endocytosed, are degraded outside lysozomes in the cytoplasm (17). Thus, antigens might preferentially interact with one class of MHC molecule and/or enter one pathway, resulting in predominant activation of class I- or class II–restricted T cells.

Previous studies in our laboratory (7, 8, 10, 11) have shown that immunization of C57BL/6 (B6) mice with the Friend retrovirus-induced syngeneic leukemia, FBL, induces both tumor-reactive CD8⁺ CTL and CD4⁺ Th, and each is independently capable of completely rejecting the tumor when transferred as a purified T cell subset or clone in adoptive therapy of mice bearing disseminated leukemia. Although the CD4⁺ Th population can proliferate and mediate its therapeutic DTH effect without the participation of CD8⁺ CTL (10, 11), maximal in vitro generation of FBL-reactive CD8⁺ CTL and the in vivo therapeutic activity of this subset are dependent on IL-2-producing CD4⁺ Th or exogenous IL-2 (7, 8, 18). Activation of these subsets, as assessed in vitro, has demonstrated that FBL-reactive CD4⁺ Th, but not CD8⁺ CTL, utilize a chloroquine-sensitive antigen-processing pathway (18). Thus, therapeutically effective FBL-reactive CTL and Th may recognize distinct FBL-derived peptides generated from separate metabolic pathways. Since the nature of the peptide may help determine which subset is activated, in this study, the antigenic determinants recognized by FBL-reactive CD8⁺ and CD4⁺ T cells were evaluated.

The major retroviral proteins expressed by FBL are products of the *envelope* (*env*) and *gag* genes of the helper Friend murine leukemia virus (F-MuLV) (19–21). Previous studies using purified viral protein have shown that FBL-reactive Th recognize the F-MuLV *env*-encoded glycoprotein gp70 (22). However, characterization of FBL-reactive CTL specificity has been more difficult, since CTL, unlike Th, require that antigen be endogenously produced and presented in association with class I MHC molecules rather than provided in soluble form for presentation by APC. Therefore, for this analysis, we have utilized rat fibroblast cells cotransfected with plasmids containing F-MuLV *gag* or *env* genes and the class I D^b gene, since immunogenetic studies have demonstrated restriction of FBL-specific CTL to the D^b allele (23, 24). The results demonstrate that in the Friend retrovirus-induced tumor model in which tumor rejection can be mediated by either CTL or Th, antigens derived from discrete retroviral proteins are responsible for activation of each T cell subset.

Materials and Methods

Mice. Female C57BL/6 (B6) H-2^b mice and CBA/J H-2^k mice were obtained from The Jackson Laboratories, Bar Harbor, ME.

Cell Lines and Viruses. FBL is a Friend virus-induced erythroleukemia of B6 origin that expresses F-MuLV *env*- and *gag*-encoded determinants (19–21). The transfectant fibroblast cell lines expressing the class I–restricting antigen D^b and/or Friend retrovirus proteins were established as previously described (24). Briefly, plasmids containing the D^b gene and the selectable marker pSV2neo were transfected into Fisher rat embryo fibroblasts (FRE) cells, cultured with 400 µg/ml G418 (gentimycin sulfate; Gibco Laboratories, Grand Island, NY), and stable G418-resistant transfectants were isolated (cell line B2). Plasmids consisting of the F-MuLV provirus (pF-MuLV₅₇), or F-MuLV *gag* or F-MuLV *env* genes were constructed as previously described (24), and cotransfected into FRE cells along with D^b in pSV2neo. Stable G418-resistant cell lines were isolated that express D^b plus F-MuLV, F-MuLV gag, or F-MuLV env, and termed FB2, Ps6, and N34, respectively.

Recombinant vaccinia virus containing the F-MuLV *env* (vac-*env*) gene was constructed

and characterized previously, and kindly provided by Dr. Bernard Moss (NIAID, Bethesda, MD) (25).

Immunofluorescence of Rat Fibroblast Transfectant Cell Lines. The rat fibroblast (FRE) cell lines transfected with the viral expression vectors described above were briefly trypsinized, washed, and resuspended in HBSS with 0.1% NaN_3. 10^6 cells were incubated on ice for 30 min with either F-MuLV gp70^{env}-specific (hybridoma 350) or p15^{gag}-specific (hybridoma 256) mAb (kind gifts of Dr. Bruce Chesebro, Rocky Mountain Laboratory, Hamilton, MN) (26). The cells were then centrifuged, washed twice with HBSS, and resuspended in 100 μl of a 1:25 dilution of FITC-conjugated goat anti-mouse Ig serum (Tago Inc., Burlington, CA). After 30 min at 0°C, cells were centrifuged, washed, and resuspended in HBSS with 5% BSA and examined for membrane fluorescence.

Lymphocyte Cultures. Spleen cells from B6 mice inoculated intraperitoneally with three doses of 2 × 10^7 irradiated FBL tumor cells (B6$_{\alpha FBL}$) at 2-wk intervals, with recombinant vaccinia F-MuLV envelope (B6$_{\alpha vac-env}$) virus or with recombinant vaccinia influenza hemagglutinin (B6$_{\alpha vac-flu}$) virus (10^7 plaque-forming units, via tail scratch) were obtained at least 6 wk after in vivo sensitization for in vitro assays. Cytolytic reactivity was assessed after in vitro sensitization of these responder cells in MLC. 6 × 10^6 responder spleen cells were cultured for 5 d in 24-well tissue culture plates (Costar, Cambridge, MA) in supplemented RPMI 1640 medium containing 10% FCS, and stimulated with either 1.5 × 10^6 autologous uninfected splenocytes, 1.5 × 10^6 splenocytes infected with recombinant vaccinia F-MuLV envelope virus (multiplicity of infection, 10:1) or 1.5 × 10^6 irradiated allogeneic stimulator cells, or 0.3 × 10^6 irradiated FBL.

Proliferative responses of FBL- and vaccinia (F-MuLV)-envelope recombinant virus (*vac-env*)-primed T cell subsets were evaluated as previously described (27). Briefly, immune spleen cells were RBC lysed and either αlyt-2.2 (hybridoma HO-2.2 from American Type Culture Collection, Rockville, MD) plus C treated (denoted CD4$^+$ T cells) or αL3T4 (hybridoma GK-1.5, a kind gift of Dr. Frank Fitch, University of Chicago) plus αrat κ (hybridoma Mar 18.5, a kind gift Dr. Lewis Lanier of Becton Dickinson & Co., Mountain View, CA) plus C treated (denoted CD8$^+$ T cells), and then reconstituted to viable cell number. The effectiveness and specificity of antibody plus C treatment in depleting the appropriate T cell subpopulation was assessed by binding of fluoresceinated antibodies to the CD4 and CD8 molecules as previously described (28). 8 × 10^5 responder T cells were incubated with either 8 × 10^4 irradiated (FBL)$_x$ (10,000 rad) tumor cells or 4 × 10^5 irradiated (B6)$_x$ (2,500 rad) spleen cells for 6 d. [^{3}H]Thymidine, 1.0 μCi/well, was added for the final 24 h of incubation.

T Cell Clones. FBL-specific CD8$^+$/CD4$^-$ (2.H8) and CD4$^+$/CD8$^-$ (B10 and C8, kind gifts of Dr. Michael Bookman, Medical Branch, NCI, NIH) T cell clones were established from FBL-specific T cell lines generated from draining lymph nodes of B6 mice immunized with irradiated (FBL)$_x$ in CFA as previously described (8, 22, 29, 30). Proliferative assays were performed in 96-well plates with 2 × 10^4 T cell clones/well, 5 × 10^5 irradiated syngeneic (B6)$_x$/well, and with or without 2 × 10^4 irradiated FBL stimulator cells (10,000 rad). Proliferation was assessed after 3 d by the incorporation of [^{3}H]thymidine (1.0 μCi/well) added during the final 18 h of culture. Cytolytic reactivity was determined after culture of 5 × 10^5 T cell clones/well (24-well tissue culture plate, Costar, Cambridge, MA) with 5 × 10^6 irradiated (B6)$_x$ and 2 × 10^5 irradiated (FBL)$_x$ for 2 d.

Cytotoxicity Assay. Cytolytic activity was determined, as previously described (7, 8), by a 4–6-h incubation of effector cells with 5 × 10^3 ^{51}Cr-labeled FBL tumor targets, transfected fibroblast targets, or EL4 tumor cells (an antigenically distinct noncrossreacting tumor of B6 origin) infected with either vaccinia or influenza (multiplicity of infection of 10:1). The data shown are the means of triplicate determinations with spontaneous release values <25%.

Results

Expression of Cloned F-MuLV gag and env Gene Products in Transfected Rat Fibroblast (FRE) Cells. Before assessing the cytolytic reactivity of FBL-specific T cells to the transfected FRE lines, expression of the viral antigens was defined by immunofluorescence (data not shown). FRE cells transfected with the F-MuLV provirus (FB2) reacted

TABLE I

Recognition of F-MuLV GAG-encoded Determinants by FBL-reactive Tc

Target cells[ǂ]	Components	Percent specific lysis of target cells from effector populations[*]		
		FBL specific ($[B6_{aFBL}][FBL]_x$)	Allospecific ($[CBA][B6]_x$)	Unprimed ($[B6][FBL]_x$)
FBL	F env + F gag/K^b/D^b	80	78	3
B2	D^b only	2	49	5
FB2	F env + F gag D^b	34	25	1
Ps6	F gag/D^b	29	24	2
N34	F env/D^b	6	25	1

[*] Spleen cells from B6 (H-2^b) mice previously primed in vivo with irradiated FBL ($B6_{aFBL}$) or unprimed B6 or CBA/J (H-2^k) spleen cells were cultured for 5 d with irradiated (FBL)$_x$ (10,000 rad) tumor cells or irradiated (B6)$_x$ (3,000 rad) spleen cells as indicated. Effector cells were harvested after 5 d and tested in a 4-h Cr-release assay at an 100:1 E/T ratio with labeled tumor or transfected fibroblast lines.

[ǂ] Target cell lines used for analysis consisted of the Friend virus-induced tumor, FBL, or rat fibroblasts transfected with the class I-restricting element D^b alone (B2), D^b and F-MuLV (FB2), D^b and the gag region of F-MuLV (Ps6), or D^b and the env region of F-MuLV (N34).

with both F-MuLV anti-gp70^{env}-specific mAb as well as F-MuLV p15^{gag}-specific mAb. The gag-expressing FRE line (Ps6) bound anti-p15^{gag} but failed to react with anti-gp70^{env}, while the env-expressing rat fibroblast line (N34) bound anti-gp70^{env} but did not react with anti-p15^{gag}. FRE cells transfected with the class I-restricting molecule D^b alone (B2), however, were negative for staining with both anti-gp70^{env} and anti-p15^{gag}.

Recognition of F-MuLV Antigenic Determinants by FBL-reactive CTL. To assess the specificity of FBL-reactive CTL, responder FBL-primed B6 (H-2^b) spleen cells, or as controls, unprimed B6 or CBA/J (H-2^k) spleen cells, were stimulated for 5 d with either irradiated FBL or B6 splenocytes in vitro, and lytic activity assessed in a 6-h ^{51}Cr-release assay against either FBL tumor cells or FRE lines transfected with D^b plus the full-length F-MuLV genome, D^b plus the F-MuLV gag gene, D^b plus the F-MuLV env gene, or D^b alone (Table I). All targets were lysed by the CBA/J alloreactive CTL specific for H-2^b, insuring that the class I-restricting element D^b was adequately expressed on all targets. Target cells expressing F-MuLV gag plus the class I-restricting element D^b (FBL, FB2, Ps6) were susceptible to lysis by FBL-specific CTL, but only background lysis by FBL-specific CTL was observed with the FRE lines transfected with either D^b alone (B2) or F-MuLV env plus D^b (N34). Unprimed B6 splenocytes did not lyse any of the targets tested, confirming that in vivo priming is required for in vitro generation of FBL-reactive cytotoxicity (31). Thus, F-MuLV gag gene-encoded antigens were recognized by FBL-reactive CTL, but CTL specific for env products could not be detected in significant numbers in populations of FBL-reactive CTL effectors.

F-MuLV Env Transfectant Fibroblast Cells Express an Immunologically Functional Env Product. The failure to detect in the above analysis F-MuLV env-specific CTL could potentially have resulted from the inability of the env-expressing transfectant line (N34) to produce immunologically active env proteins rather than a lack of env-specific CTL. Since previous studies (22) have suggested that FBL-specific CD4⁺

F-MuLV Envelope Transfectant Fibroblast Cell Line Expresses Immunologically Functional Gene Products

		Δcpm*		
		CD4⁺/CD8⁻		CD8⁺/CD4⁻
Stimulator[‡]	Components	B10	C8	2.H8
FBL	F *env* + F *gag*	47,631	37,271	29,803
B2	None	− 387	210	− 1,585
Ps6	F *gag*	786	567	36,829
N34	F *env*	49,173	48,924	− 417

* 2 × 10⁴ FBL-reactive CD4⁺ (B10 and C8) or CD8⁺ (2.H8) T cell clones were stimulated with or without 2 × 10⁴ irradiated (FBL)ₓ, (B2)ₓ, (Ps6)ₓ or (N34)ₓ (10,000 rad), and 5 × 10⁵ irradiated (B6)ₓ (3,000 rad). IL-2 (10 U/ml) was added to cultures containing 2.H8 responder cells. Proliferation was assessed after 3 d by the incorporation of [³H]thymidine (1.0 μCi/well) added during the final 18 h of culture. Data presented represents the difference between the mean values of triplicate determinants of cultures stimulated with antigen or with (B6)ₓ cells.
‡ The stimulator cell lines used for analysis are described in Table I.

T cells recognize gp70, the ability of FBL-specific CD4⁺ T cell clones to proliferate in response to FBL tumor cells and to the transfected fibroblast lines was examined as an indicator for immunogenic env expression (Table II). The FBL-specific CD4⁺/CD8⁻ T cell clones C8 and B10 proliferated to FBL and the fibroblast line expressing gp70ᵉⁿᵛ (N34), but not to the line expressing Dᵇ alone (B2) or the *gag*-transfected line (Ps6). The response of a CD8⁺/CD4⁻ T cell clone, denoted 2.H8, was also examined, and, consistent with the specificity of the FBL-reactive nonclonal CD8⁺ population to F-MuLV gag, this clone proliferated in response to gag-expressing FBL and Ps6, but not B2 or N34. Thus, the inability to detect F-MuLV-env-specific CTL was not due to expression of a defective F-MuLV *env* gene product by the env-transfected fibroblast cell line. Moreover, these results, in conjunction with previous reports analyzing the antigen specificity of FBL-reactive Th clones (22, 29), suggest that priming B6 mice with FBL induces predominantly or exclusively Th responses specific for products of the env gene.

Cytolytic Reactivity after In Vivo Priming with Recombinant Vaccinia F-MuLV Env Virus. Our inability to detect F-MuLV env-specific CTL seemed surprising, since env-specific CTL have been demonstrated to other retrovirus-induced tumors (24, 32), and the F-MuLV *env* gene products are expressed in an immunogenic fashion by the transfected FRE cell lines (Table II). The lack of reactivity in our system could potentially result from the FBL tumor cell inappropriately processing the F-MuLV *env* gene product via the endogenous pathway for presentation with class I molecules. Therefore, an alternative mechanism for expressing the *env* gene in a fashion known to induce CTL was explored. A recombinant vaccinia F-MuLV virus was utilized, because previous studies have demonstrated that viral genes incorporated into vaccinia virus can serve as targets for antigen-specific CTL. Moreover, in vivo immunization with recombinant vaccinia viruses has been shown to induce CTL specific for products of the inserted gene (33).

B6 mice were primed in vivo either with FBL or vac-*env* virus, and the specificity

TABLE III

Priming B6 Mice with Recombinant Vaccinia F-MuLV ENV Fails to Induce ENV-specific Tc

MLC[‡]		Percent specific lysis of targets*					
Responder	Stimulator	FBL (F $env/gag/K^bD^b$)	B2 (D^b)	N34 (F env/D^b)	Ps6 (F gag/D^b)	EL-4 (K^bD^b)	EL-4/vac (vac + K^bD^b)
B6αFBL	(FBL)ₓ	78	3	2	25	1	2
	vac-env	5	6	1	0	3	2
B6αvac-env	(FBL)ₓ	5	4	0	0	6	7
	vac-env	4	3	1	0	4	59
B6	(FBL)ₓ	8	7	0	0	2	3
	vac	3	2	0	0	2	7
BALB/c	(B6)ₓ	52	44	29	25	60	66

* Effector cells were harvested after 5 d and tested in a 4-6-h Cr-release assay at an 80:1 E/T ratio with labeled FBL, EL-4, vac-infected EL-4 (10:1), or transfected fibroblast lines as detailed in Table 1.

‡ Spleen cells from B6 mice were immunized with irradiated FBL (B6αFBL), live vaccinia F-MuLV *env* recombinant (B6αvac-env); or left unprimed (B6). Responder splenocytes were cultured with irradiated stimulator (FBL)ₓ, (B6)ₓ, or vac-*env*-infected (multiplicity of 5:1) syngeneic B6 spleen cells, as indicated.

of the cytolytic T cell response was assessed after in vitro stimulation with either FBL or vac-*env*-infected syngeneic B6 splenocytes. The recombinant vaccinia *env* virus used for these studies has been shown to induce expression of both the gp85 env precursor protein and the processed gp70 and p15E expressed by FBL (25). As previously observed, CTL generated by immunization to FBL recognized FBL and the D^b- plus gag-expressing transfectant, but not the transfectant expressing env plus D^b or D^b alone (Table III). The effector T cells from mice primed with vac-*env* did not contain detectable CTL that lysed either the env-expressing transfected line or the FBL tumor. This priming did induce CTL, since stimulation of vac-*env*-primed splenocytes with vaccinia-infected stimulator cells induced effector cells that lysed vaccinia-infected target cells (Table III). Thus, priming B6 mice with env antigens in the context of a recombinant vaccinia virus still failed to induce env-specific CTL.

Priming with Vac-Env Induces CD4⁺ Class II–restricted T Cell Responses. The inability to detect F-MuLV env-specific CTL after priming with vac-*env* could reflect inefficient expression in vivo of the inserted gene encoding F-MuLV *env*, resulting in failure to induce env-specific T cell responses. Therefore, to determine if the *env*-encoded gene product was expressed in a potentially immunogenic form during vac-*env* priming, the proliferative response of vac-*env* and FBL-primed CD4⁺ and CD8⁺ T cell subsets to the F-MuLV env-expressing tumor cell FBL was assessed (Fig. 1). Purified class II–restricted CD4⁺ T cells, from both FBL and vac-*env*-primed B6 mice proliferated in response to FBL, demonstrating that both immunogens primed FBL-specific class II–restricted T cells. By contrast, purified CD8⁺ splenocytes from FBL-primed but not vac-*env*-primed mice proliferated in response to FBL, consistent with the observed inability of class I–restricted T cells with cytolytic function to recognize F-MuLV env antigens. Moreover, the purified CD8⁺ T cell population from vac-*env*-primed mice exhibited a class I–restricted proliferative alloresponse to B6ᵇᵐ¹ class I mutant stimulator cells, demonstrating that immunization with vac-*env* did not interfere with expression of CD8⁺ proliferative responses. These results demonstrate that *env*-encoded gene products are expressed during in vivo immunization with FBL or vac-*env* recombinant virus, but env antigens effectively prime only FBL-specific CD4⁺ T cells and not CD8⁺ T cells.
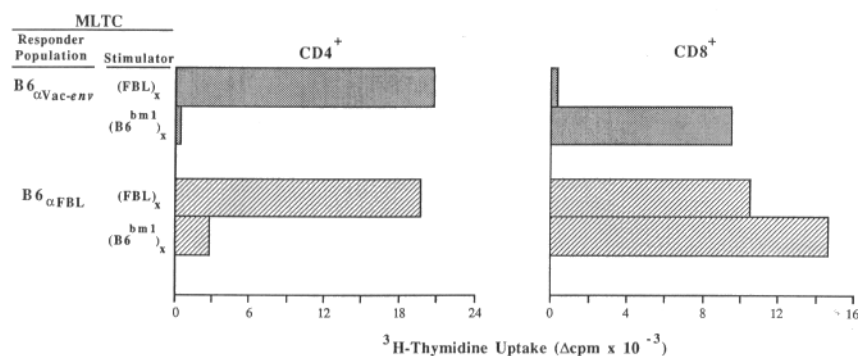
FIGURE 1.   Ability of CD4$^+$ or CD8$^+$ T cells primed in vivo with vac-*env* or FBL tumor cells to proliferate in vitro in response to (FBL)$_x$. Spleen cells from unprimed B6 mice (B6) or mice primed with irradiated FBL (B6$_{\alpha FBL}$) or live vaccinia F-MuLV *env* recombinant (B6$_{\alpha Vac\text{-}env}$) virus were purified into CD8$^+$ or CD4$^+$ T cell subsets as described in Materials and Methods. 8 × 10^5 responder cells/well were cultured with 8 × 10^4 (FBL)$_x$ tumor cells or 4 × 10^5 (B6)$_x$ spleen cells for 5 d, and 1.0 μCi/well [^{3}H]thymidine was added during the final 24 h of culture. Data presented were calculated as described in Table 1.

Discussion

The data presented in this study show that FBL-reactive T cell subsets predominantly recognize tumor antigens derived from discrete retroviral proteins, with FBL-reactive CD8$^+$ CTL specific for F-MuLV *gag*-encoded determinants and FBL-reactive CD4$^+$ Th specific for F-MuLV *env*-encoded determinants. This failure of immunization with FBL to induce F-MuLV env-specific CD8$^+$ T cells might result from: (*a*) FBL processing env antigens poorly via the endogenous pathway and failing to present env in the context of class I molecules; an hypothesis supported by recent data demonstrating that different APCs, such as B cells, macrophages, and fibroblasts, process antigens differently and may present different epitopes (34); (*b*) env antigens being unable to interact with H-2^b class I molecules; or (*c*) the B6 mouse strain being a genetic nonresponder to the Friend env protein presented with H-2^b class I molecules.

To address the issue of the adequacy of presentation of env antigens by FBL, an infectious recombinant vaccinia F-MuLV *env* virus was utilized as an alternative mechanism for endogenously expressing and presenting the F-MuLV *env* gene. Effector T cells from mice primed with vac-*env*, as observed with FBL-reactive CTL, failed to lyse either FBL tumor cells or the env-expressing transfectant cell line, despite the fact that priming with vac-*env* induced immunogenic expression of env antigens. Therefore, the failure to generate env-specific CTL cannot be due to a unique tumor-associated env-processing defect in the transformed FBL tumor, since this defect would also need to be present in cells infected with the recombinant vaccinia env virus and/or the transfected fibroblast line.

An alternative explanation for the failure of FBL to induce env-specific CTL is that env-encoded gene products are unable to bind to the H-2^b class I molecules. This is not true for all retroviral *env* products, since env-specific CTL can be detected in B6 mice in response to Gross MuLV (32). Moreover, one of us (F. Lilly) has previously shown that BALB.B mice, which are *H-2* congenic to B6, can generate

F-MuLV env-specific CTL (24). Thus, the failure to detect F-MuLV env-specific CTL is unlikely, due to the inability of retroviral *env* products to react with B6 class I molecules. These data suggest that non-MHC genes can regulate the responses of CD8⁺ T cells to retrovirally encoded products, as has been shown for regulation of T cell responses to other proteins (35, 36), and that such background genes are responsible for the nonresponder status of B6 mice in regard to class I–restricted response to F-MuLV env. This cannot represent global tolerance to env proteins, since B6 mice generate an env-specific CD4⁺ T cell response. While the mechanism(s) by which relatively large complex proteins, such as F-MuLV *gag* and *env*-encoded gene products, uniquely activate separate T cell subsets remain to be defined, these data imply that, even in the setting in which human tumors are found to express unique tumor antigens capable of inducing a T cell response, host factors such as MHC and non-MHC genes may significantly influence an individual's ability to generate tumor-specific CD4⁺ or CD8⁺ T cell responses, and may determine which effector population is most efficient at eradicating the tumor.

In addition to the role of T cells in tumor rejection, the host T cell response to viruses is critical for protection from viral diseases. For example, the generation of viral-specific CTL has been shown to correlate with protection from a wide variety of murine and human viruses, and may be important in protection from human retrovirus-related diseases (37–40). The results of this current study, which has shown that the major retroviral proteins may induce selectively only CD8⁺ CTL or CD4⁺ Th immune responses in the host, suggests that retroviral subunit vaccines may uniquely or predominantly activate only one T cell subset in some individuals, and thus, may not provide the population with adequate protection against viral infection. Preliminary studies evaluating the responses of chimpanzees vaccinated with a recombinant vaccinia virus containing the HIV *env* gene may illustrate this concept, since class II–restricted T cells and neutralizing antibody responses but no class I–restricted CTL were detected, and the chimps were not protected from challenge with HIV (40). Consistent with our findings that class I–restricted FBL-specific CTL can recognize *gag*-encoded determinants, recent reports have demonstrated HIV-specific CTL that recognize gag proteins (41), and such responses may be particularly important to individuals not able to generate class I–restricted env-specific T cell responses. Further studies, using similar approaches to analyze the immunogenetics of responses to distinct retroviral and/or tumor antigens, may provide important insights into the requirements for eliciting protective host responses to infectious retroviruses and transformed cells.

Summary

Immunization of C57BL/6 (B6) mice with FBL, a Friend murine leukemia virus (F-MuLV), induces both tumor-specific cytolytic CD8⁺ (CTL) and lymphokine-producing CD4⁺ Th that are effective in adoptive therapy of B6 mice bearing disseminated FBL leukemia. The current study evaluated the F-MuLV antigenic determinants expressed on FBL that are recognized by FBL-reactive CD8⁺ and CD4⁺ T cells. To identify the specificity of the FBL-reactive CD8⁺ CTL, Fisher rat embryo fibroblast (FRE) cells transfected with plasmids encoding F-MuLV *gag* or *envelope* (*env*) gene products plus the class I–restricting element D^b were utilized. FBL-reactive CTL recognized FRE target cells transfected with the F-MuLV *gag*-encoded

gene products, but failed to recognize targets expressing F-MuLV env. Attempts to generate env-specific CD8⁺ CTL by immunization with a recombinant vaccinia virus containing an inserted F-MuLV *env* gene were unsuccessful, despite the generation of a cytolytic response to vaccinia epitopes, implying that B6 mice fail to generate CD8⁺ CTL to env determinants. By contrast, CD4⁺ Th clones recognized FRE target cells transfected with *env* and not *gag* genes, and immunization with the recombinant vaccinia virus induced an env-specific CD4⁺ T cell response. These data show that in a Friend retrovirus-induced tumor model in which tumor rejection can be mediated by either CTL or Th, antigens derived from discrete retroviral proteins are predominantly responsible for activation of each T cell subset.

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