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Role and Specificity of T-Cell Subsets in Spontaneous Recovery from Friend Virus-Induced Leukemia in Mice

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Spontaneous recovery from Friend virus complex-induced leukemic splenomegaly in *H-2D*$^{b/b}$ mice correlated with the appearance of Friend virus complex-specific cytotoxic T lymphocytes (CTL) detectable directly in spleen cell populations. By testing CTL on target cells containing expression vectors encoding individual retroviral structural proteins, the main viral protein recognized was shown to be the Friend murine leukemia helper virus envelope glycoprotein. In vivo depletion of CD8-positive T cells drastically reduced the incidence of recovery, providing direct evidence for the role of CD8-positive CTL in the spontaneous recovery process. In vivo depletion of CD4-positive cells had little effect on the early stages of recovery but did cause a marked reduction in the final incidence of recovery at 60 to 90 days. Thus, CD8-positive cells were required for the initiation of the recovery process, whereas CD4-positive cells appeared to be required for maintenance of the recovered status.

Retroviruses are known to induce both neoplastic and nonneoplastic diseases in many species of animals and in humans (57). Protective immunity often can be generated by vaccination with killed virus or viral proteins prior to inoculation of infectious retrovirus (18, 23, 25, 31, 44); however, spontaneous recovery without previous vaccination is not commonly associated with most retroviral diseases. Nevertheless, in some mouse strains infected with certain retroviruses such as Friend virus and Moloney sarcoma virus, spontaneous recovery is regularly observed (11, 21, 34, 35). These models are useful for study of the immune mechanisms required for control of established retroviral infection and disease.

Friend virus complex (FV) consists of a defective spleen focus-forming virus (SFFV) and a replication-competent Friend murine leukemia helper virus (F-MuLV) (56). When inoculated into adult animals of susceptible genotypes, this complex causes rapid splenomegaly with polyclonal erythroid cell proliferation, leading ultimately to erythroleukemia (27). Severe immune suppression occurs in some mouse strains, depending on major histocompatibility complex (MHC) genotype (44, 45), but death is often due to complications of splenomegaly and a high (90%) hematocrit.

Our previous studies showed that some *H-2D*$^{b/b}$ mouse strains such as (C57BL/10 × A.BY)F_1 mice could spontaneously recover after infection with FV (11). MHC genes located in the *H-2D* and *H-2I-A* subregions as well as a non-*H-2* gene, *Rfv-3*, were shown to be critical for this spontaneous recovery process (5, 42). The MHC genes were associated with development of FV-specific cytotoxic T lymphocytes (CTL) and T-helper cells (3, 6, 40), and the *Rfv-3* gene was associated with generation of anti-FV neutralizing antibodies (7, 17). However, to date no definitive proof for the role of CTL or T-helper cells in spontaneous recovery has been obtained. Furthermore, the viral protein specificity of FV-specific CTL remains unclear. Previous data from our laboratory for studies using mutant leukemia cells as CTL targets suggested that the F-MuLV envelope protein was the main target recognized by CTL (14). However, data from other laboratories on secondary CTL generated in vitro indicated that many FV-specific CTL were directed against the *gag* polyprotein of F-MuLV (22, 30). Therefore, the experiments described in this report were designed to determine the viral protein specificity of CTL found in the spleens of spontaneously recovering *H-2D*$^{b/b}$ mice. We also studied the importance of CD8- and CD4-positive T lymphocytes in the recovery process in vivo.

MATERIALS AND METHODS

Mice. (C57BL/10 × A.BY)F_1 and [B10.A(2R) × A.BY]F_1 mice were used for CTL experiments and in vivo depletion of T-cell subsets. These mice have *Rfv-3*$^{r/s}$ and *H-2D*$^{b/b}$ genotypes and at least one *H-2I-A*^{b} allele, a combination of host genes that has been associated with a high incidence of spontaneous recovery from FV-induced leukemic splenomegaly (5). These two strains did not differ in any of the assays performed in these or previous studies; therefore, data from the two strains were pooled in the results presented in this report.

Cells. EL4 carcinogen-induced T-cell leukemia cells (8, 20), the 2C clone of Y57 FV-induced erythroleukemia cells (15), and Ψ2 (38), PA317 (39), and dunni (33) cells have all been previously described elsewhere. All cells were grown in RPMI 1640 medium supplemented with penicillin (200 U/ml), 10% fetal bovine serum, and 2×10^{-5} M 2-mercaptoethanol.

Viruses. The B-tropic form of the FV (FV-B) (36) was propagated by inoculation of BALB/c or (BALB/c × A)F_1 mice, and 20% spleen homogenates were made on days 10 to 14 as previously described (11). The FB-29 strain of F-MuLV (54) was propagated on dunni cells.

Immunofluorescence studies. The following monoclonal antibodies were used in indirect immunofluorescence studies to detect cell surface antigens on live cells (9) or cytoplasmic antigens on fixed cells (4): anti-P15^{gag} hybridomas R188, 257, and 34 (4), anti-F-MuLV *env* gp70 hybridoma 720 (51),

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and anti-hamster prion protein (PrP) hybridoma 3F4 (28). Undiluted tissue culture supernatants from hybridoma cultures were used in all immunoassays. For fluorescence-activated cell sorting (FACS) studies, the protocol for detection of cell surface antigens on live cells was followed (9) except that sodium azide was omitted and cells were incubated on ice throughout the procedure. Following the final incubation, cells were analyzed and sorted by using a FACStar cell sorter (Becton Dickinson).

In vivo T-cell depletion. For depleting T-cell subsets of mice in vivo, rat monoclonal antibodies specific for murine T-cell antigens were used. Antibody 2.43 reactive with an allelic determinant of murine CD8 was obtained from the American Type Culture Collection, Rockville, Md. (53). Anti-CD4 GK1.5 (16) was obtained from Robert Fritz, Emory University, Atlanta, Ga. Anti-CD4 191.1 (13, 46) and anti-CD8 169.4 (13, 46) were obtained from Michael Oldstone, Research Institute of Scripps Clinic, La Jolla, Calif. Monoclonal antibody I-5, reactive with an idiotype present on anti-(TG)-A-L antibodies, was used as a negative control and was kindly provided by Seth Pincus of the Rocky Mountain Laboratories. All monoclonal antibodies were of the immunoglobulin G2b isotype and were produced and used as ascites fluid or culture supernatant fluid. For depletion of CD4-positive cells, mice were given a total of approximately 300 μg of antibody GK1.5 or antibody 191.1 in four divided doses intraperitoneally on alternate days. For depletion of CD8-positive cells, mice were given a total of approximately 25 μg of antibody 2.43 in three divided doses intraperitoneally on alternate days. One day after the first antibody inoculation, mice were inoculated intravenously with 1,500 spleen focus-forming units (SFFU) of FV-B. Control uninfected mice were bled at 7 and 30 days after the last antibody inoculation to analyze the frequency of CD4- and CD8-positive cells. Mice inoculated with anti-CD4 had 90 to 93% reduction in circulating CD4-positive cells and normal CD8-positive cell numbers at both time points. Mice inoculated with anti-CD8 had 80 to 95% reduction in circulating CD8-positive cells and normal or marginally reduced CD4-positive cell levels.

Infection of EL4 cells with F-MuLV. EL4 cells were initially cocultivated with dunni cells chronically infected with F-MuLV strain FB-29. After cocultivation for 1 week, nonadherent cells were removed and analyzed for expression of F-MuLV envelope protein by membrane immunofluorescence and FACS analysis. Approximately 30% of the EL4 cells expressed F-MuLV envelope, and the 5% of the cells expressing the highest levels of gp70 were selected with a FACStar cell sorter. EL4 cells thus selected were cultured for several days, and two additional rounds of selection for cells expressing F-MuLV envelope protein were done on the cell sorter at weekly intervals. After the third round of cell sorting, single cell clones were generated by limiting dilution.

Construction of plasmids expressing FV proteins. A 1.7-kb *Bam*HI-to-*Sac*I fragment consisting of the 3′ two-thirds of the F-MuLV envelope gene and most of the U-3 region of the long terminal repeat was obtained from the infectious molecular clone of strain F-MuLV-57 (47, 48). This fragment was cloned into pUC19, and then a 0.83-kb *Bam*HI fragment from the 5′ portion of the F-MuLV-57 envelope gene was inserted into this plasmid to reconstruct the entire envelope protein open reading frame. Subsequently, this plasmid was digested with *Sal*I and *Eco*RI to excise the 2.5-kb F-MuLV insert, and this fragment was cloned into the *Xho*I and *Eco*RI sites of plasmid pSFF (1) to create p12-1 (Fig. 1). After

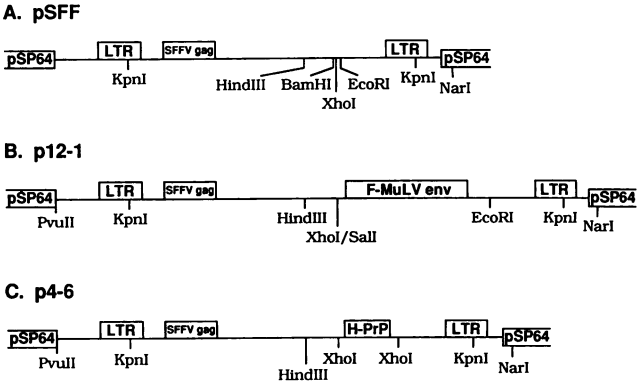

FIG. 1. Retroviral expression vectors used in these experiments. In pSFF, cloning sites for expressing other genes are at *Bam*HI, *Xho*I, and *Eco*RI sites. p12-1 is pSFF containing the F-MuLV *env* gene; p4-6 is pSFF containing the hamster PrP (H-PrP) open reading frame. All of these plasmids express the SFFV *gag* polyprotein, p42, which is a deleted form of the F-MuLV *gag* polyprotein, pr65^{gag} (12). LTR, long terminal repeat.

transfection into mixed packaging cell cultures consisting of Ψ2 and PA317 cells, this plasmid induced expression of both F-MuLV envelope protein detected by monoclonal antibody 720 and SFFV p42^{gag} polyprotein detected by monoclonal antibodies R188 and 34. As a control, plasmid p4-6 was constructed by cloning a 1-kb *Xho*I fragment of hamster PrP cDNA (50) into the *Xho*I site of pSFF. After transfection into a mixed culture of Ψ2 and PA317 cells, this plasmid induced expression of SFFV *gag* polyprotein and of hamster PrP detected by membrane immunofluorescence using monoclonal antibody 3F4.

Expression of Friend envelope protein and hamster PrP in EL4 cells. After expression of Friend envelope protein and hamster PrP in mixed packaging cell cultures as described above, cultures were incubated for 7 to 14 days to allow spread of the retroviral vector throughout the culture. When sufficient spread was achieved as demonstrated by analyses using immunofluorescence with antibody 720 or 3F4, infection was transferred to EL4 cells by cocultivating EL4 cells on the packaging cell lines for several days. Subsequently, nonadherent EL4 cells were removed, analyzed by immunofluorescence, and sorted for expression of Friend envelope protein or hamster PrP in a FACStar cell sorter. After two rounds of sorting, each cell line was cloned by limiting dilution, and clones expressing high levels of either Friend envelope protein (EL4-F-ENV) or hamster PrP (EL4-H-PrP) were selected and used for subsequent experiments.

CTL assays. Splenocytes were obtained by dissociating spleens with forceps in RPMI 1640. Cell clumps were broken up by gentle pipetting, and cells were washed once by centrifugation and resuspension in medium with 10% fetal calf serum (FCS). CTL were assayed by mixing 10^4 ^{51}Cr-labeled 2C erythroleukemia cells plus 2×10^6 splenocytes taken directly from mice infected with 1,500 SFFU of FV-B intravenously or intraperitoneally 7 to 40 days previously (14). Cells were incubated in a total volume of 200 μl of RPMI 1640 with 10% FCS in quadruplicate wells of 96-well flat-bottom trays for 12 to 14 h at 37°C. Supernatant fluid was then sampled, and ^{51}Cr was counted to determine percent target cell lysis calculated as described previously (6); 100% ^{51}Cr release was determined by lysing cells in 1% Nonidet P-40. CTL inhibition assays were done in a similar fashion

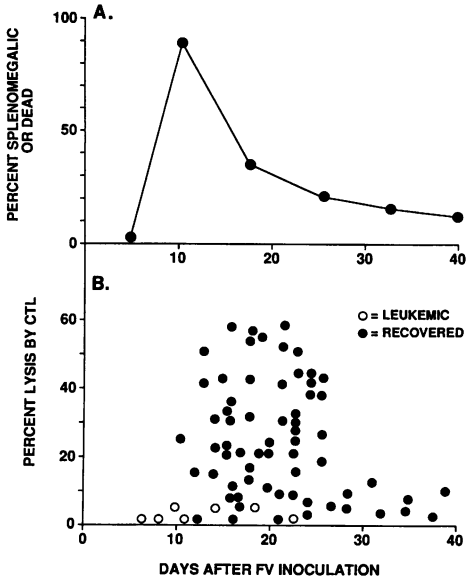

FIG. 2. (A) Spontaneous recovery from FV-induced leukemic splenomegaly in *H-2D*$^{b/b}$ mice after intravenous inoculation of 1,500 SFFU of FV-B. Splenomegaly was assessed by spleen palpation under anesthesia. Data were pooled from several experiments involving a total of 63 mice. (B) Detection of primary FV-specific CTL in *H-2D*$^{b/b}$ mice at various times after FV-B inoculation as described for panel A. CTL were detected by incubating ^{51}Cr-labeled 2C erythroleukemia target cells with a 200-fold excess of mouse splenocytes for 12 h at 37°C. Most assays were also done at ratios of 100:1 and 50:1, and this showed a progressive decrease in lysis obtained. Effector/target cell ratios higher than 200:1 often gave lower specific lysis because of higher background release. Open circles represent individual mice with leukemic splenomegaly; closed circles represent individual mice recovered from splenomegaly. No CTL were detected in mice with splenomegaly. Previous experiments have shown that FV leukemia spleen cells can competitively inhibit FV-specific CTL in vitro (6, 14). In approximately 80% of recovered mice, CTL were detected in a direct assay between 10 and 26 days after virus inoculation. After 28 days, CTL were not usually detected even in recovered mice.

except that unlabeled inhibitor cells were added to wells in various numbers prior to addition of ^{51}Cr-labeled target cells (14). In vitro CTL blocking experiments were done by preincubating effector spleen cells with monoclonal antibodies reactive with CD8 or CD4 for 15 min on ice prior to addition of labeled target cells.

Recovery experiments. Recovery from FV-induced leukemic splenomegaly was followed by spleen palpation under anesthesia as previously described (11).

RESULTS

Detection of primary FV-specific CTL correlated with the time of recovery from FV-induced leukemic splenomegaly. *H-2D*$^{b/b}$ mice of strains (C57BL/10 × A.BY)F_1 and [B10.A (2R) × A.BY]F_1 were inoculated intravenously with 1,500 SFFU of FV-B and examined under anesthesia for FV-induced splenomegaly every 5 to 7 days thereafter. Most of these mice developed palpable splenomegaly (>0.5-g spleen) 7 to 10 days after inoculation with FV, but 80 to 90% showed spontaneous recovery from splenomegaly during the next 1 to 3 weeks (Fig. 2A). In a separate experiment, groups of mice were sacrificed at various days after inoculation with FV, and their spleen cells were used directly in CTL assays

TABLE 1. Inhibition of FV-specific CTL by anti-CD8 monoclonal antibodies[a]

Antibody	% Specific lysis	
	Expt 1	Expt 2
Anti-CD8 (169.4)	18	15
Anti-CD4 (GK1.5)	55	55
None	58	52

[a] A total of 2 × 10^6 spleen cells from (C57BL/10 × A.BY)F_1 mice inoculated intravenously 19 days previously with 1,000 SFFU of FV-B were incubated in 96-well plates for 15 min on ice with 2 μl of concentrated hybridoma culture supernatant containing 200 ng of antibody in 100 μl of RPMI 1640 plus 10% FCS. Next, 10^4 ^{51}Cr-labeled 2C FV erythroleukemia cells were added in 100 μl of RPMI 1640 plus 10% FCS, and trays were incubated for 14 h at 34°C. Supernatant fluid was then sampled and assayed for percent ^{51}Cr release as usual. Each experiment represents the mean of triplicate wells, using spleen cells from one mouse.

by mixing with ^{51}Cr-labeled cells of the Friend erythroleukemia cell line 2C. Significant CTL activity was detected as early as 10 days after challenge and was detectable between 10 and 26 days after infection in the majority of mice that had recovered from splenomegaly (Fig. 2B). This CTL activity was due to T cells because lysis could be eliminated when spleen cell populations were treated with anti-Thy 1.2 antibodies and complement prior to assaying for CTL activity (6). This lysis was due to CD8-positive rather than CD4-positive CTL, since it could be blocked by preincubation of effector cells with anti-CD8 antibodies (Table 1).

These experiments did not determine which FV proteins were recognized by the CTL because 2C cells expressed all structural proteins of both the helper F-MuLV and the defective SFFV (Table 2). To analyze the viral protein specificity of the CTL, we used EL4 cells infected with the entire F-MuLV helper virus strain FB-29 or infected with pSFF expression vectors encoding the SFFV *gag* polyprotein and F-MuLV envelope protein or an unrelated protein, hamster PrP, as a negative control (Fig. 1). Target cells were analyzed for expression of retroviral proteins by using monoclonal antibodies, and all cell lines were also tested as targets for primary CTL derived from spleens of FV-inoculated *H-2D*$^{b/b}$ mice (Table 2). In these experiments, high susceptibility to CTL-mediated lysis was observed in EL4-

TABLE 2. Correlation of F-MuLV and SFFV protein expression with susceptibility of target cells by lysis by anti-FV CTL from spontaneously recovering mice

Cell line[a]	Antigen expressed[b]				% Specific lysis[c]	
	SFFV Gag	F-MuLV Gag	F-MuLV Env	Hamster PrP	Mean	Range
2C	+	+	+	−	37	12–58
EL4-FB29	−	+	+	−	32	17–58
EL4-F-ENV	+	−	+	−	27	7–55
EL4-H-PrP	+	−	−	+	12	5–21
EL4	−	−	−	−	3	0–8

[a] CTL target cells were derived as described in Materials and Methods.
[b] Determined by indirect immunofluorescence using the following monoclonal antibodies: 720, F-MuLV Env; 3F4, hamster PrP; R188 and 34, SFFV Gag; 257, F-MuLV (strain FB29) Gag. Antibodies 720 and 3F4 were used on live unfixed cells, and antibodies R188, 34, and 257 were used on acetone-fixed cells.
[c] CTL data represent assays using spleen cells from four to eight individual *H-2D*$^{b/b}$ mice {[C57BL/10 × A.BY]F_1 or [B10.A(2R) × A.BY]F_1} inoculated with FV-B 14 to 22 days earlier.

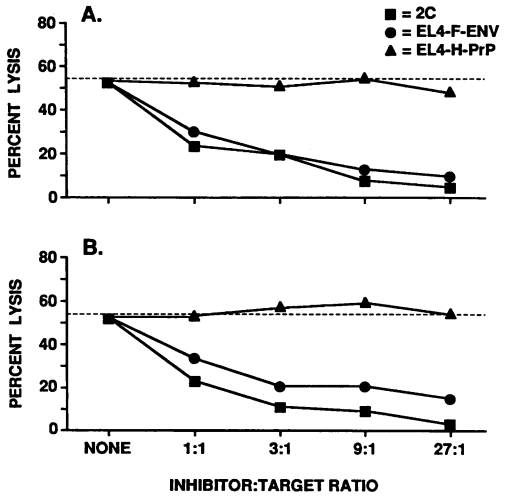

FIG. 3. Competitive inhibition CTL assays using 10^4 ^{51}Cr-labeled 2C cell targets plus 2×10^6 splenocytes from FV-B-inoculated syngeneic mice. Inhibitors were various numbers of unlabeled 2C cells (■), EL4 cells expressing SFFV Gag and F-MuLV Env (EL4-F-ENV) (●), or EL4 cells expressing SFFV Gag and hamster PrP (EL4-H-PrP) (▲). Normal EL4 cells gave no inhibition, similar to EL4-H-PrP cells. Panels A and B represent two separate experiments.

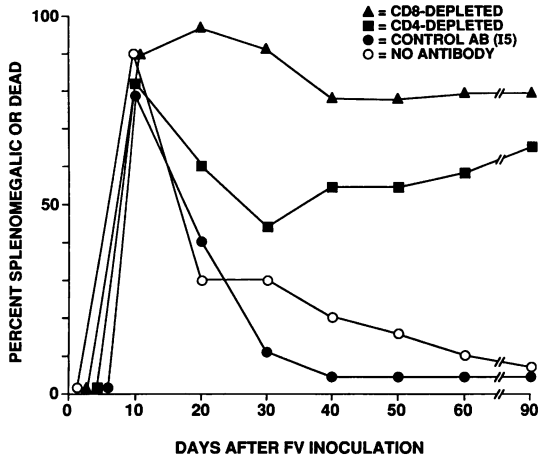

FIG. 4. Effects of T-cell subset depletion on spontaneous recovery from FV leukemia. CD4 and CD8 depletion was done by using rat monoclonal antibodies as described in Materials and Methods. *H-2D*$^{b/b}$ mice were inoculated intravenously with 1,500 SFFU of FV-B, and disease was monitored by spleen palpation under anesthesia. Symbols: ○, no antibody (30 mice); ●, control antibody, I-5 (21 mice); ▲, CD8 depleted with antibody 2.43 (56 mice); ■, CD4 depleted with antibody 191.1 (30 mice) or antibody GK1.5 (16 mice). No differences were observed between groups of mice treated with either 191.1 or GK1.5; therefore, data were pooled. CD8- and CD4-depleted groups were statistically different from each other on days 20 and 30 only ($P < 0.001$). Both of these groups differed significantly from both control groups on days 60 and 90 ($P < 0.001$).

FB29 cells, which expressed both F-MuLV *gag* and envelope proteins. The results showed that the helper virus, F-MuLV, was sufficient to encode the major CTL target antigen(s) and that the defective virus, SFFV, was not required for recognition of target cells by CTL. In addition, high CTL activity was detected on EL4-F-ENV cells, which expressed the F-MuLV envelope protein and the SFFV *gag* protein, p42, whereas only a low level of lysis was seen in EL4-H-PrP cells, which expressed the SFFV *gag* protein and hamster PrP. Thus, it appeared that the majority of CTL activity was directed at the F-MuLV envelope protein; however, these results could not rule out the possibility that a low level of CTL activity was also directed at the SFFV or F-MuLV *gag* protein.

Competitive inhibition studies. To determine whether the antigens seen on 2C erythroleukemia target cells were identical to those detected on the various EL4 target cell lines, competitive inhibition CTL assays were carried out. As shown in Fig. 3, EL4 cells expressing SFFV *gag* polyprotein and Friend envelope protein were as effective as 2C cells in eliminating the lysis of ^{51}Cr-labeled 2C target cells. In contrast, EL4 cells expressing the SFFV *gag* protein and hamster PrP showed no significant inhibition of the lysis of 2C target cells in these experiments. Thus, Friend envelope protein appeared to be a major target antigen recognized by primary CTL in mice spontaneously recovering from FV-induced splenomegaly.

Depletion of T-cell subsets influences recovery from FV leukemia. Because the recovery of *H-2D*$^{b/b}$ mice correlated with the appearance of CTL capable of lysing target cells which expressed the envelope protein of Friend virus, we next sought to determine whether depleting CD8 or CD4 T-cell subsets in vivo could prevent recovery. Intraperitoneal inoculation with monoclonal antibodies reactive with CD4- or CD8-positive T cells has previously been shown to result in reduction in the levels of these cells, resulting in depression of several types of immune responses (2, 13, 46, 49, 52).

In our experiments, treatment of high-recovery *H-2D*$^{b/b}$ mice with anti-CD8 antibody had a profound effect on recovery from FV leukemia. Nearly 90% of mice had splenomegaly by day 10, and 79% were still splenomegalic at 90 days (Fig. 4). In contrast, less than 10% of the mice treated with no antibody or those treated with the control antibody, I-5, still had splenomegaly 90 days after challenge with FV. Treatment of mice with anti-CD4 antibody also significantly affected recovery from FV leukemia. At 20 and 30 days, the mice treated with anti-CD4 antibodies appeared to be recovering, and this group was significantly different from the group treated with anti-CD8 ($P < 0.001$). However, subsequently more CD4-depleted mice developed splenomegaly, and at 90 days, 64% were splenomegalic or dead (Fig. 4). At this time, CD4- and CD8-depleted groups were both significantly different from control groups ($P < 0.001$). These results indicated that both CD4- and CD8-positive T cells were important for spontaneous recovery from FV leukemia. However, the kinetic differences in the responses suggested that the mechanisms might be quite different.

DISCUSSION

The results presented above indicated that both CD8- and CD4-positive T lymphocytes were essential for the spontaneous recovery from FV-induced leukemia in *H-2D*$^{b/b}$ mice. The effect of CD8 depletion was dramatic, as nearly 80% of the mice remained leukemic throughout the experiment. This result was in good agreement with our present and previous findings that spontaneous recovery from FV leukemia was usually followed by the appearance of FV-specific CTL directly detectable among the spleen cells of recovering mice (Fig. 2) (5, 6). Thus, it seems likely that FV-specific CD8-positive CTL are an essential component of the spon-

taneous recovery process in this model. The situation in CD4-depleted mice was somewhat different. At 20 to 30 days after FV inoculation, some CD4-depleted mice began to recover; however, they showed a progressive increase in splenomegaly after day 30, in contrast to control mice, which continued to recover during this time period. Thus, CD4-depleted mice appeared to be able to restrict the incidence of leukemic splenomegaly for up to 30 days after FV inoculation but were unable to mediate continued restriction of the leukemic process. The mechanisms involving CD4-positive T lymphocytes in the spontaneous recovery process are unclear at this time. The kinetics were surprising because in a different system, CD4-positive cells appeared to be more important in the earlier phases of the antiviral immune response (26). Nevertheless, it seems likely that CD4-depleted mice might be able to control FV leukemia initially through the development of FV-specific CD8-positive CTL as has been seen in several other virus systems (43, 46). The subsequent disease progression observed in CD4-depleted mice might be due to lack of CD4-positive T-helper cells required for amplification and maintenance of both the CTL and humoral antibody responses (29) or possibly to lack of CD4-positive virus-specific CTL (32, 37, 55), which could be required at some later stages of the recovery process.

We also analyzed the viral protein specificity of CTL detected in spontaneously recovering mice (Table 2 and Fig. 3). The results indicated that the major viral protein recognized by CTL was the F-MuLV envelope protein. These results were in agreement with those of previous experiments using mutant FV-induced erythroleukemia target cell lines expressing various retroviral proteins (14). Although we also found some evidence for weak CTL activity possibly directed against the SFFV *gag* protein, which is a partially deleted version of the F-MuLV *gag* polyprotein (12), competitive inhibition CTL experiments showed that cells expressing SFFV *gag* plus hamster PrP were unable to compete significantly with the lysis of 2C target cells (Fig. 3). Moreover, since EL4-F-ENV cells expressing SFFV *gag* protein and F-MuLV envelope protein could completely inhibit CTL lysis of 2C erythroleukemia target cells, there was no requirement for expression of F-MuLV *gag* protein for maximum inhibition of CTL in this system. These results differ significantly from recently published data showing that in some situations, FV-specific CTL primarily recognize F-MuLV *gag*-encoded proteins (22, 30). Several factors might account for the differences in these results. First, we analyzed primary CTL taken directly from the spleens of animals without further restimulation by in vitro culturing, whereas other laboratories used in vitro stimulation to augment CTL activity to detectable levels. Second, we used live FV to immunize mice by an infectious process and detected CTL after spontaneous recovery; in contrast, other groups used killed virus or formalin-fixed FV leukemic spleen cells for immunization in vivo and in vitro (22) or studied tumor immunity by using the FBL-3 lymphoid leukemia cell line from C57BL/6 mice (30). The FBL-3 tumor was originally induced by the F-MuLV helper virus but does not express the SFFV component of FV (8, 19). Lastly, it is possible that the different mouse strains used in these experiments may have some influence on the CTL specificity detected.

Spontaneous recovery appears to involve genes of both the *H-2I-A* subregion and the *H-2D* subregion, with one *H-2I-A*^{b} allele and two *H-2D*^{b} alleles being required for maximum recovery incidence (5, 42). Previous data from our laboratory have demonstrated that FV-specific CTL are restricted in their recognition of target cells by the *H-2D* region genotype (5). Thus, these previous genetic results together with the present findings on CD8 depletion support the conclusion that *H-2D*-restricted FV-specific CTL are an essential effector mechanism in the spontaneous recovery process. In contrast to these results on spontaneous recovery, there appears to be no *H-2D*^{b} requirement for induction of protective immunity generated by vaccination or immunization prior to FV infection (18, 44). The *H-2I-A*^{b} allele appears to be essential for development of a good CD4-positive T-cell response to the F-MuLV envelope protein (25, 40). However, when killed viral particles are used in potent immunological adjuvants, the *H-2I-A* genotype appears to have little influence on development of protective immunity, suggesting that FV envelope-specific CD4-positive T-helper cells can be generated if appropriate adjuvants or immunological carrier proteins are used (24, 25). Similar results have been found with use of vaccinia virus recombinants expressing the F-MuLV *gag* polyprotein (41).

The data in this report demonstrate the importance of F-MuLV envelope-specific CTL in the process of spontaneous recovery from FV-induced leukemia. The recovery process appears to be quite complex, involving both CD4- and CD8-positive T cells as well as humoral antibodies influenced by the *Rfv-3* gene (5, 7, 10). These data provide concrete evidence that even highly virulent retroviral infections can be successfully controlled by the immune system of genetically resistant host animals. An understanding of the mechanisms involved in the spontaneous recovery process should provide additional clues to methods for immunization or therapy to prevent or reverse retroviral disease in individuals lacking one or more of these immune mechanisms (5). The data presented above support the concept that early generation of virus-specific CTL is necessary, but not sufficient, for the spontaneous recovery process and that maintenance of the recovery state requires CD4-positive cells and some other effector mechanisms, including humoral antibodies.

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