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Immunology

Participation of target Fas protein in apoptosis pathway induced by CD4+ Th1 and CD8+ cytotoxic T cells

(lpr/cell death mechanism)

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ABSTRACT **The results presented here provide evidence that the presence of Fas protein in target cells is essential to permit cytotoxicity (resulting in apoptosis) mediated by cloned CD4+ Th1 cells. Using mitogen-activated B cells as targets, antigen-dependent lysis by CD4+ Th1 effectors was observed with MRL/MpJ+ but not with MRL/MpJ-lpr targets. The congenic MRL/MpJ-lpr strain is defective in Fas expression. Target cells from various lymphoid tissues of C3H.MRL-lpr mice were also resistant to the lectin-dependent cytotoxicity of Th1 effectors, whereas C3H/HeJ targets were sensitive. Moreover, a rapid DNA fragmentation prior to ^{51}Cr release was induced only in C3H/HeJ targets. Thus, cytotoxicity induced by Th1 effectors correlates with target Fas expression. In contrast to Th1 effectors, CD8+ cytotoxic T lymphocytes (CTLs) killed C3H.MRL-lpr targets. When cytotoxicity was assayed in the presence of EGTA and $MgCl_2$, which chelates extracellular Ca^{2+} [$(Ca^{2+})_{ext}$], only C3H.MRL-lpr targets became resistant to CD8+ CTLs. This $(Ca^{2+})_{ext}$-independent cytotoxicity of both Th1 and CD8+ effectors could be inhibited with unlabeled C3H/HeJ thymocytes or with a transfectoma carrying a murine Fas–human μ gene construct. In comparison, C3H.MRL-lpr thymocytes and the nontransfected parental cell line were poor inhibitors. Our study demonstrates that CD4+ Th1 cells and CD8+ CTLs differ in their $(Ca^{2+})_{ext}$-dependent cytotoxicity but share a $(Ca^{2+})_{ext}$-independent cytotoxicity that requires participation of Fas molecules for cytotoxic signal transduction leading to target apoptosis.**

Both CD4+ and CD8+ cytotoxic T lymphocytes (CTLs), upon activation and formation of conjugates with target cells, deliver a cytotoxic signal that induces apoptosis in target cells (1–7). The identity of the molecules that send the cytotoxic signal and the identity of the receptors for the cytotoxic signal have been enigmas for many years. It has been demonstrated that perforin and granzyme A from CD8+ CTLs and perforin and fragmentins from natural killer (NK) cells can induce apoptosis in target cells (8, 9) by using a mechanism(s) that is strictly dependent on extracellular Ca^{2+} [$(Ca^{2+})_{ext}$] (8–11). However, CD8+ CTLs also mediate a $(Ca^{2+})_{ext}$-independent cytotoxicity (12–15). This cytotoxic activity kills B6 thymocytes that express normal Fas protein but not B6.MRL-lpr thymocytes that are defective in Fas expression (16).

Upon activation, murine CD4+ Th1 clones acquire cytotoxic activity that requires *de novo* protein synthesis (17–19). The cytotoxicity has a half-life of 1–3 hr and requires direct cell contact with target cells (6, 19). The cytotoxic molecule is neither tumor necrosis factor nor perforin (17, 18, 20). Because cytotoxic Th1 cells induced apoptosis in targets (2, 6), we wanted to determine whether target Fas protein participated in the cytotoxic process. We compared lpr targets with their normal counterparts with regard to their susceptibility to CD4+ Th1 and CD8+ CTL clones. Our results demonstrated that Th1 clones killed lymphoid cells of normal mice but not the lymphoid cells of lpr mice. In contrast, targets from both strains of mice were sensitive to CD8+ CTLs in conventional CTL assays. However, under $(Ca^{2+})_{ext}$-independent conditions, only target cells from normal mice were sensitive. This Fas-dependent cytotoxicity could be inhibited specifically by cold targets that naturally expressed Fas or that expressed Fas as a result of transfection with an appropriate construct. Our study demonstrated that CD4+ Th1 and CD8+ CTLs differ in the expression of $(Ca^{2+})_{ext}$-dependent cytotoxicity but share a common $(Ca^{2+})_{ext}$-independent cytotoxicity, which critically involves target Fas protein during transduction of the apoptotic signal.

MATERIALS AND METHODS

Mice. Inbred mouse strains of BALB/cByJ, C3H/HeJ, C3H/HeJ-gld, C3H.MRL-lpr (lpr of MRL/MpJ-lpr on C3H/HeJ background), MRL/MpJ+, and MRL/MpJ-lpr at 4–7 weeks of age were purchased from The Jackson Laboratory. They were maintained with laboratory chow and acidified water for 1–2 weeks before use.

Reagents. 4β-Phorbol 12β-myristate 13α-acetate (PMA), A23187, EGTA, and ConA were obtained from Sigma. $Na_2{}^{51}CrO_4$ was purchased from New England Nuclear. [^{3}H]Thymidine was purchased from ICN.

T-Cell Clones. The derivation and maintenance of the CD4+ Th1 clones C7 (keyhole limpet hemocyanin specific, I-E^d restricted), E10 [poly(Glu, Lys, Tyr) specific, I-E^d restricted], A.E7 [pigeon cytochrome *c* (Cyt *c*) specific, I-E^k restricted], and CD8+ CTL clone OE4 [H-2^d specific; kindly provided by W. R. Clark (University of California at Los Angeles)] have been described (17, 21–23). The lprvb2 is an Ia^k-reactive T-cell line derived from a MRL/MpJ-lpr mouse. This cell line has been maintained *in vitro* continuously for 2 years. It contains Th1-type cells. Resting cells were purified over a Ficoll/Hypaque gradient and washed before use.

Target Cells. Single cell suspensions were prepared from spleens, lymph nodes, thymi, and bone marrow. Erythrocytes were lysed by 0.85% ammonium chloride. Targets (10^7) were labeled for 2 hr with $Na_2{}^{51}CrO_4$ as described (17).

Cells (7.5×10^6 per 2 ml) from each of the lymphoid organs were also cultured in 24-well Costar plates in the presence of Con A (3 μg/ml) and recombinant interleukin 1 (rIL-1) [2.5×10^3 units/ml, provided by C. Martin (Brigham and Women's Hospital, Harvard Medical School)]. Proliferating cells were labeled with [^{3}H]thymidine (50 μCi/ml; 1 Ci = 37 GBq).

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Abbreviations: $(Ca^{2+})_{ext}$, extracellular calcium ion; CTL, cytotoxic T lymphocyte; Cyt *c*, pigeon cytochrome *c*; LPS, lipopolysaccharide; MHC, major histocompatibility complex; PMA, 4β-phorbol 12β-myristate 13α-acetate; rIL-1, recombinant interleukin 1.

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Aliquots were used as targets for the DNA fragmentation assay. The remaining cells were labeled with $Na_2{}^{51}CrO_4$ and used for the ^{51}Cr-release assay.

Splenic cells were treated with Thy-1 monoclonal antibody plus rabbit complement as described (24). Viable cells were activated with bacterial lipopolysaccharide (LPS; 100 μg/ml; Difco) for 3 days. Antigen-pulsed B cells were prepared by the addition of 0.2 mg of Cyt *c* per ml 12 hr before harvest. The activated B cells were purified over a Ficoll/Hypaque gradient. Purified cells were labeled with $Na_2{}^{51}CrO_4$ and used as targets.

^{51}Cr-Release Assay. The ^{51}Cr-release assay was carried out as described (17). For $(Ca^{2+})_{ext}$-independent cytotoxicity, T cells were activated with PMA (20 ng/ml) and A23187 (0.5 μM) for 1 hr, washed once, and added to target cells; the assays were carried out in the presence of EGTA (6 mM) and $MgCl_2$ (3 mM). All experiments were carried out in duplicate with <5% variation.

DNA Fragmentation Assay. DNA fragmentation assays were carried out as described (2).

Cold Target Inhibition. Clones were activated with PMA plus A23187 for 1 hr and washed. They were added to wells containing ^{51}Cr-labeled P815 (for C7 effectors) or BW5147 (for OE4 effectors) and various numbers of cold target cells in the presence of EGTA and $MgCl_2$. The specific ^{51}Cr release was determined 4 hr after culture. The cold targets tested were P815, BW5147, thymocytes from C3H/HeJ or C3H.MRL-lpr that were activated with Con A plus rIL-1 for 3 days, J558L myeloma cells, and clone 1.4.7. Clone 1.4.7 was a transfectant containing a murine Fas–human μ construct (see below).

Construction of Fas Transfectomas. A cDNA spanning the entire coding region of the murine Fas gene was obtained by reverse transcription PCR using oligonucleotides corresponding to the sense nucleotides 5′-TGGAATTCCGCTGTTTTC-CCTTGCTGCA-3′ (underlined sequence corresponds to positions 26–46 of the 5′ untranslated sequence) and nucleotides 5′-TGGTCGACCAGGAGTTGCCAATGTCAAT-3′ (underlined sequence is antisense to positions 1131–1110 of the 3′ untranslated sequence) of the Fas sequence (25). A *Sac* I restriction site was added to the 5′ untranslated region and a *Sal* I site was added to the 3′ end of the coding region by a second round of PCR using 5′-GCTTGAGCTCCGCTGTTT-TCCCTTGCTGCA-3′ and 5′-ACTTGTCGACCTCACC-CTCCAGACATTGTCCTTC-3′ as primers (underlined is original sequence). A consensus 3′ splice site (boldface) was added to the 3′-end oligonucleotide to allow proper splicing next to the CH2 domain of the human μ-chain gene. The 1-kb PCR product confirmed by sequencing was cloned into a human μ-chain-expressing vector [pCD4-Hu; a gift of C. Ianelli (Tufts University)]. The construct was transfected into J558L by spheroplast fusion. Clone 1.4.7 was selected for use because recombinant molecules could be detected with an anti-IgM reagent in cell lysate but not in culture supernatant (<1 ng/ml). Western blot analysis of SDS/PAGE under nonreducing conditions detected a product of ≈100 kDa, which is the expected value of the Fas–μ chimeric protein.

RESULTS

Lymphoid Target Cells from C3H.MRL-lpr Were Resistant to Cytotoxic CD4+ Th1 Clones. Cells were obtained from bone marrow, lymph node, spleen, and thymus of C3H/HeJ-gld, C3H.MRL-lpr, or their normal counterpart C3H/HeJ mice. These cells were labeled with ^{51}Cr and used as targets for cytotoxicity mediated by Con A-activated CD4+ Th1 clones. Con A was chosen because it bypasses the requirements of antigenic peptides and major histocompatibility complex (MHC) molecules on target cells and permits comparison between targets for sensitivity to the cytotoxic Th1 effectors. A representative experiment using clone C7 effectors is shown in Fig. 1. All targets from C3H/HeJ mice were sensitive. In contrast, targets from C3H.MRL-lpr spleen and lymph node were resistant (Fig. 1*a*). A weak lysis was consistently observed with C3H.MRL-lpr thymocytes, whereas the weak lysis of bone marrow targets was not always observed (Fig. 1*a*). All targets from C3H/HeJ-gld mice were sensitive, indicating that the resistance of C3H.MRL-lpr targets was a unique defect not specifically associated with lymphoproliferative disease (Fig. 1*b*).

Sensitivity of Mitogen-Activated Targets. After activation with Con A and rIL-1, DNA of target populations was labeled and target susceptibility was tested by the DNA fragmentation assay. An early target DNA fragmentation has been shown to coincide with an electrophoretic ladder pattern of DNA extract and is considered a hallmark for apoptosis (2). As shown in Fig. 2, DNA from all four of the C3H/HeJ targets was strongly fragmented within 2.5 hr after effector/target interaction, whereas DNA from C3H.MRL-lpr targets was not (Fig. 2*a*). Similar results were observed for the 5-hr ^{51}Cr-release assay (Fig. 2*b*). When compared with targets freshly obtained from mice (Fig. 1*a*), mitogen-activated targets were more sensitive. Interestingly, mitogen-activated

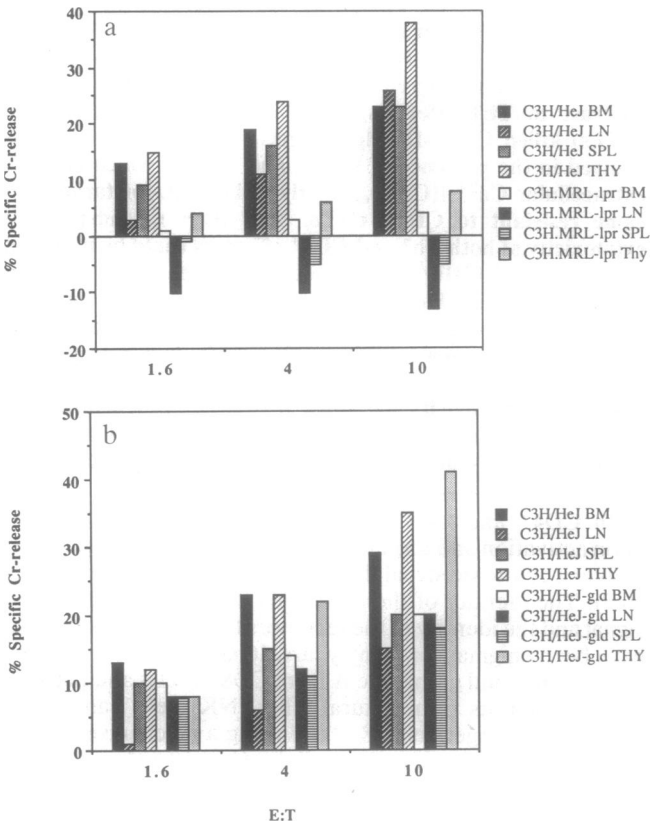

FIG. 1. Targets from lymphoid tissues of C3H.MRL-lpr are resistant to CD4+ Th1 clones. Bone marrow (BM), lymph node (LN), spleen (SPL), and thymus (THY) were obtained from C3H/HeJ, C3H.MRL-lpr, and C3H/HeJ-gld mice. Single cell suspensions were prepared, labeled with $Na_2{}^{51}CrO_4$, and used as targets. E:T, effector/target cell ratio. Clone C7 cells were used as effector cells. Cytotoxicity was carried out as described. The percentage specific ^{51}Cr release was determined after incubation for 5 hr. Nonspecific ^{51}Cr release from targets that were cultured in the absence of Con A or in the absence of C7 was not different from background ^{51}Cr release, which ranged from 9% to 38% among various targets. (*a*) Comparison of target sensitivity between C3H/HeJ and C3H.MRL-lpr targets. (*b*) Comparison of target sensitivity between C3H/HeJ and C3H/HeJ-gld targets. Negative values of percentage specific ^{51}Cr release from lymph node and spleen targets of C3H.MRL-lpr were consistently observed in four other experiments.

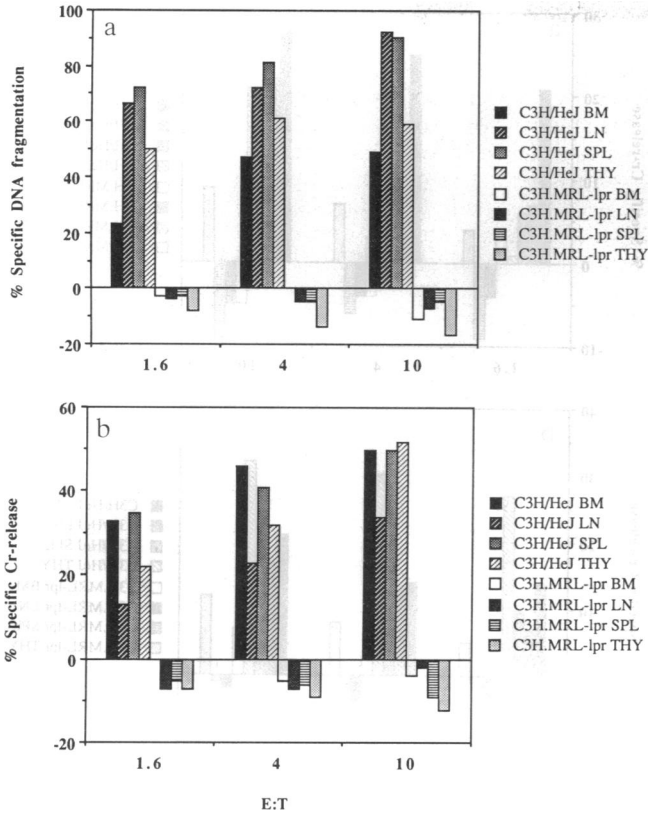

FIG. 2. Target sensitivity changes after mitogen activation as determined by DNA fragmentation assay (*a*) and ^{51}Cr-release assay (*b*). Target cells were cultured in the presence of Con A and rIL-1 for 60 hr and labeled with [^{3}H]thymidine for 12 hr as described. Cells were purified over a Ficoll/Hypaque gradient and used. Background release was 16–40% among various targets. Aliquots were labeled with $Na_2{}^{51}CrO_4$ for ^{51}Cr-release assays. Background ^{51}Cr release was 14–34% among various targets. The effectors were clone C7 cells. Abbreviations are as in Fig. 1.

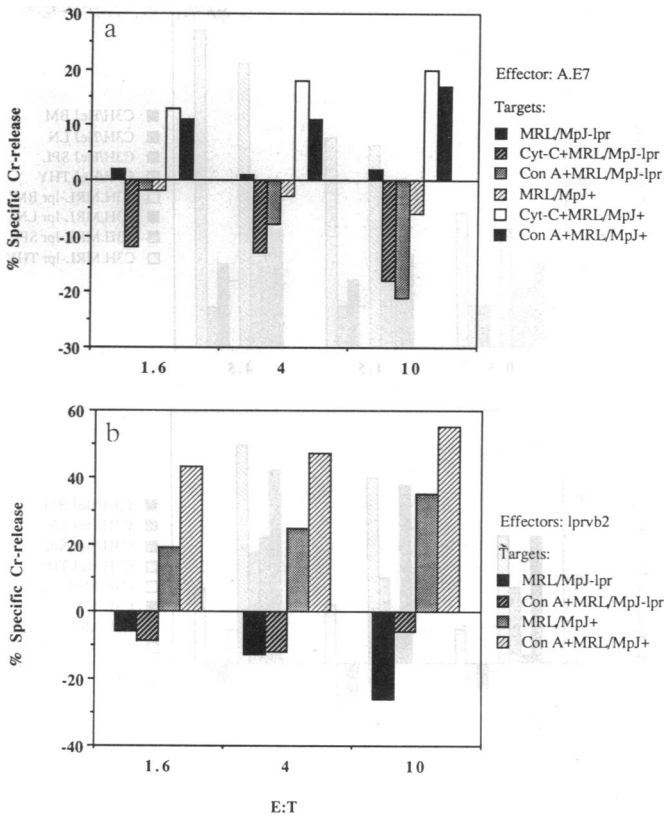

FIG. 3. Antigen-specific and MHC-restricted lysis by specific CD4$^+$ Th1 cells could be demonstrated with the LPS-activated B cells from MRL/MpJ+ mice but not with the LPS-activated B cells from MRL/MpJ-lpr mice. The CD4$^+$ Th1 effectors were A.E7 (*a*) and lprvb2 (*b*). Lectin-dependent cytotoxicity assays were also included. Background ^{51}Cr release was 34–54% among various targets.

thymocytes of C3H.MRL-lpr were completely resistant. This suggests that the sensitive cells responsible for the weak killing of freshly isolated C3H.MRL-lpr thymocytes either differentiated into resistant targets or were not selected by mitogen activation.

Sensitivity of Antigen-Presenting B Cells. LPS-activated, antigen-pulsed or unpulsed B cells were prepared from MRL/MpJ+ and MRL/MpJ-lpr mice as described. The sensitivity of these targets to the Cyt *c*-specific, I-E^k-restricted Th1 clone A.E7 (Fig. 3*a*) and to the Ia^k-restricted autoreactive Th1 cell line lprvb2 (Fig. 3*b*) was determined. In both cases, antigen-specific and MHC-restricted lysis was observed only with LPS-activated MRL/MpJ+ targets. The LPS-activated MRL/MpJ-lpr targets were resistant. Both targets induced IL-2 production by the appropriate T cells (data not shown). In addition, similar results were observed using Con A as an activation agent.

Lymphoid Targets from C3H.MRL-lpr Are Not Resistant to CD8$^+$ CTL Effectors. Target sensitivity to CD4$^+$ Th1 and CD8$^+$ CTL effectors was studied. Con A was used so that target sensitivity to Th1 and CD8$^+$ CTL effectors could be compared. The results shown in Fig. 4 demonstrated that targets from C3H.MRL-lpr mice were sensitive to CD8$^+$ CTL clone OE4 under the conventional CTL assay condition (Fig. 4*a*). In contrast, these targets were resistant to CD4$^+$ Th1 clone E10 (Fig. 4*b*). Again, C3H.MRL-lpr thymocytes were lysed weakly. As controls, both effectors killed C3H/HeJ targets. These results indicate that distinct cytotoxic mechanisms were used by CD4$^+$ Th1 and CD8$^+$ CTL clones to kill these targets.

C3H.MRL-lpr Targets Are Resistant to $(Ca^{2+})_{ext}$-Independent Cytotoxicity. The possibility that CD8$^+$ CTL clone OE4 kills C3H.MRL-lpr targets by a $(Ca^{2+})_{ext}$-dependent cytotoxic mechanism was examined. We have previously shown that after activation with PMA plus A23187, CD4$^+$ Th1 clones can exhibit cytotoxic activity in the presence of EGTA and $MgCl_2$—i.e., via $(Ca^{2+})_{ext}$-independent cytotoxicity (6). This cytotoxicity is not inhibited by cycloheximide; presumably, the cytotoxic machinery has already been induced (6). Therefore, both Th1 clone C7 and the CD8$^+$ CTL clone OE4 were activated with PMA plus A23187 for 1 hr and tested for $(Ca^{2+})_{ext}$-independent cytotoxicity. Under this experimental condition, targets from C3H/HeJ, but not C3H.MRL-lpr, were sensitive to clone C7 (Fig. 5*a*). In contrast to their susceptibility to clone OE4 under conventional CTL assay conditions, targets from C3H.MRL-lpr mice were resistant when assays were carried out in the presence of EGTA and $MgCl_2$ (Fig. 5*b*). This was not due to nonspecific inhibition by EGTA and $MgCl_2$ because killing of C3H/HeJ targets by both effectors was still observed. The data suggest that the $(Ca^{2+})_{ext}$-dependent cytotoxicity of CD8$^+$ CTL was responsible for the killing of C3H.MRL-lpr targets and that CD4$^+$ Th1 effectors lacked this cytotoxic mechanism. Moreover, both Th1 and CD8$^+$ CTL effectors use a cytotoxic mechanism that does not act on C3H.MRL-lpr targets lacking functional Fas protein.

Cold Target Inhibition of Fas-Dependent Cytotoxicity. To provide molecular evidence that Fas molecules were directly involved in signal transduction for target apoptosis, we

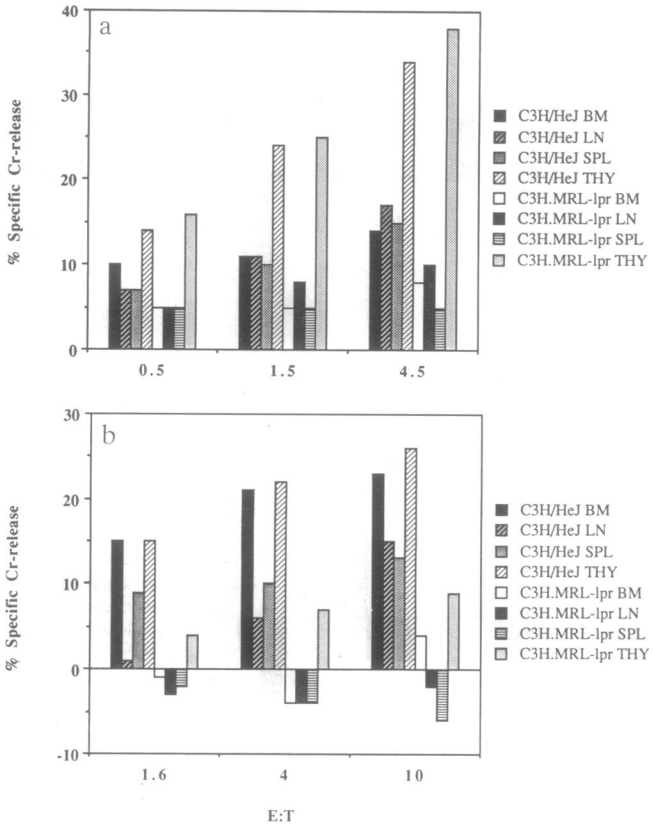

FIG. 4. Targets from C3H.MRL-lpr were resistant to CD4+ Th1 clone E10 but were sensitive to CD8+ CTL clone OE4. Targets were prepared as described in Fig. 1 and tested for sensitivity to OE4 (*a*) and to E10 (*b*). Abbreviations are in Fig. 1.

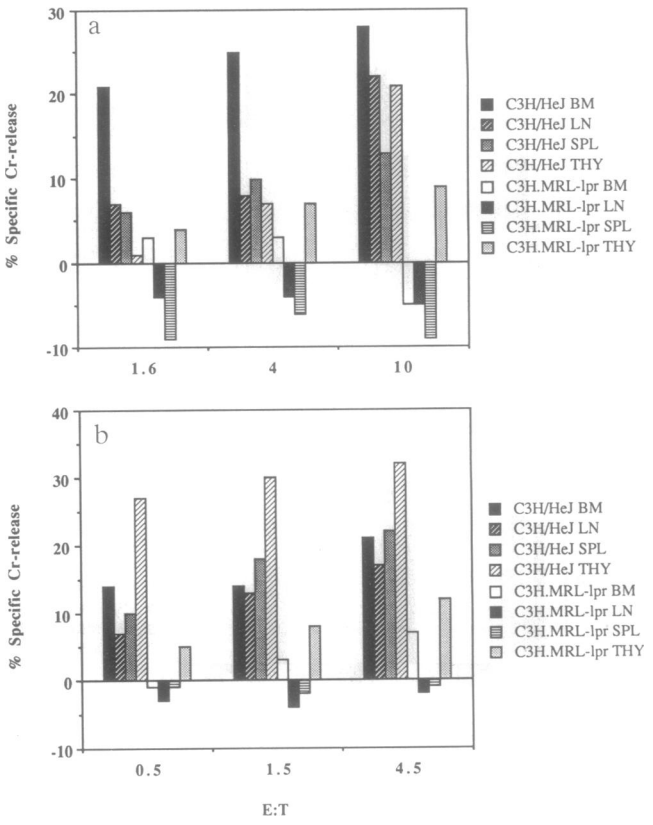

FIG. 5. Targets from C3H.MRL-lpr mice were resistant to $(Ca^{2+})_{ext}$-independent cytotoxicity of both Th1 and CD8+ CTL effectors. Clone C7 (*a*) and clone OE4 (*b*) were activated with PMA and A23187 for 1 hr, washed, and used as effectors. Cytotoxicity assays were conducted in the presence of EGTA and $MgCl_2$. Background ^{51}Cr release was 12–38% among various targets. Abbreviations are as in Fig. 1.

carried out cold target inhibition experiments. Tumor targets P815 and BW5147 were used as labeled targets. The experimental conditions of cold target inhibition have been described. The results are shown in Fig. 6. Little or no inhibition of lysis was observed with C3H.MRL-lpr thymocytes, whereas C3H/HeJ thymocytes inhibited >60% of target lysis and the inhibition was observed in a dose-dependent fashion. Both P815 and BW5147 cold targets strongly inhibited the lysis of P815 by C7 and the lysis of BW5147 by OE4. The LFA-1/ICAM-1 molecules of cold targets were not responsible for the inhibition because nearly identical levels of LFA-1/ICAM-1 expression were observed on C3H/HeJ and C3H.MRL-lpr thymocytes. Moreover, the tumor cold targets expressed fewer adhesion molecules than thymocytes and yet were stronger inhibitors (data not shown).

In another approach, we transfected J558L cell line with a hybrid construct containing the normal murine Fas gene and part of the human μ-chain gene. When tested, clone 1.4.7 transfectant was a stronger cold target inhibitor than the parental J558L cells. The inhibitory activity of transfectant 1.4.7 was almost comparable to that of autologous cold targets (Fig. 6).

DISCUSSION

The present study demonstrated that target cells from C3H.MRL-lpr mice that fail to express functional Fas molecules were resistant to the cytotoxic signal of CD4+ Th1 effector cells, whereas target cells from normal control C3H/HeJ mice were sensitive. In contrast, CD8+ CTLs killed both C3H.MRL-lpr and C3H/HeJ targets, indicating that the cytotoxic mechanism(s) of CD8+ CTLs was different from that of CD4+ Th1 effectors. However, when both effectors were activated with PMA plus A23187 and the assays were carried out in the presence of EGTA and $MgCl_2$, both effectors failed to kill targets from C3H.MRL-lpr mice, although C3H/HeJ targets were still lysed. A strong correlation between Fas expression and its recognition by effector T cells was demonstrated by the cold target competition experiments in which lysis was inhibited by C3H/HeJ thymocytes and Fas transfectants. The data demonstrated that target Fas molecules are critically involved in the cytotoxicity of CD4+ Th1 and CD8+ CTL effectors.

The rapid DNA fragmentation of targets expressing normal Fas protein and the lack of such exhibition in lpr targets when they were under the attack of Th1 effectors are consistent with the model that Fas is involved in target apoptosis. Moreover, Fas expression is high in thymocytes and low in spleens and lymph nodes freshly prepared from normal mice (25–27). The levels of Fas expression among these targets coincide with their sensitivity to the CD4+ Th1 effectors. In addition, thymocytes that had been activated with Con A and rIL-1 and splenic B cells that had been activated with LPS became more susceptible. It has been shown that Fas expression increased in activated T and B cells (26).

We confirmed and extended the study by Rouvier *et al.* (16), who demonstrated that B6.MRL-lpr thymocytes were resistant to the $(Ca^{2+})_{ext}$-independent cytotoxicity of *in vivo* generated CTLs in peritoneal exudate. Our studies demonstrate that CD4+ Th1 and CD8+ CTL clones share a common cytotoxic activity, the expression of which is $(Ca^{2+})_{ext}$ independent and Fas mediated. The cold target inhibition studies suggest strongly that Fas was a receptor for the cytotoxic signal (i.e., Fas ligand) and that Fas ligand interaction with this receptor transduced the death signal and induced target apoptosis. In this

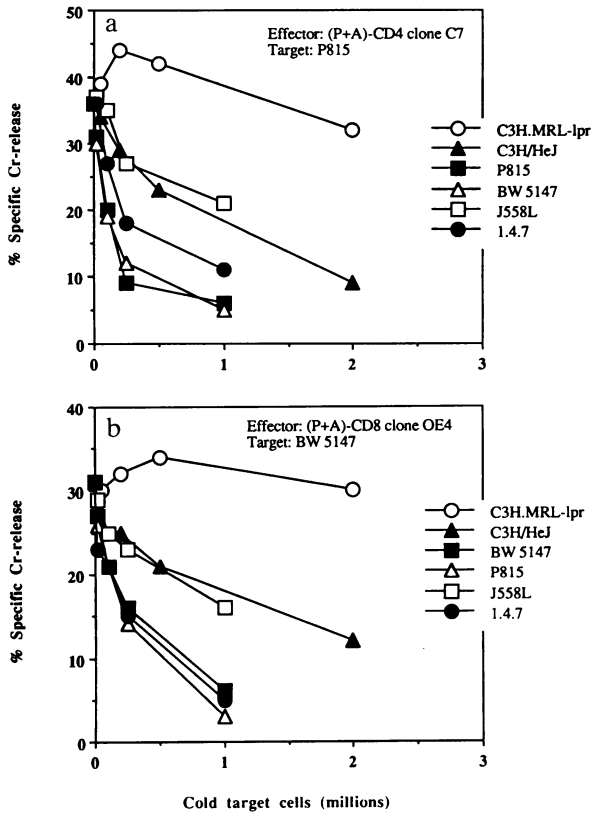

FIG. 6. Cold target inhibition of the $(Ca^{2+})_{ext}$-independent cytotoxicity of Th1 clone C7 (*a*) and of CD8+ CTL clone OE4 (*b*). See text for details. (P+A), PMA and A23187.

regard, it has been suggested that the autoimmune gld strain has a defect in Fas ligand expression (27). We are deriving CD4+ Th1 clones from C3H/HeJ-gld mice to determine whether they express the Fas ligand-mediated cytotoxicity.

An interesting observation is the negative values of the specific ^{51}Cr release when lpr targets were exposed to CD4+ Th1 effectors. This "protective" activity was consistently observed with lymph node, spleen, and mitogen-activated thymocyte targets. In the antigen-specific system, only LPS-activated B cells bearing the appropriate antigens were protected, indicating that the decrease in spontaneous ^{51}Cr release was not due to a nonspecific effect of additional T cells in the culture (Fig. 3). The data suggest that when Fas-dependent and perforin-dependent cytotoxic activities were absent, a hidden antideath activity of CD4+ Th1 cells was detected. These observations suggest that resistance to Fas-dependent cytotoxicity may have a physiological consequence on activated B cells during T-/B-cell interaction.

The comparison between CD4+ Th1 and CD8+ CTL effectors on C3H/HeJ and C3H.MRL-lpr targets reveals that CD8+ CTLs, but not Th1 effectors, express a $(Ca^{2+})_{ext}$-dependent cytotoxicity capable of killing C3H.MRL-lpr targets. The $(Ca^{2+})_{ext}$-dependent cytotoxicity mediated by perforin and serine proteases from CD8+ CTLs may be responsible for the killing (8–11). This possibility is further supported by the observations that murine CD4+ Th1 clones failed to express perforin and secreted few trypsin-like serine proteases under the condition of $(Ca^{2+})_{ext}$-dependent activation (17, 18, 20). Although CD4+ Th1 clones express a $(Ca^{2+})_{ext}$-dependent cytotoxicity (17, 18), they fail to kill C3H.MRL-lpr targets. This is consistent with the idea that $(Ca^{2+})_{ext}$ is required for activation and synthesis of Fas ligand by Th1 cells. Moreover, after activation with PMA plus A23187 and presumably Fas ligand has been produced, both effectors express a $(Ca^{2+})_{ext}$-independent cytotoxicity that depends on target Fas expression. These results suggest that in contrast to CD8+ CTLs, Fas-dependent killing is the major cytotoxic activity of CD4+ Th1 effectors.

Finally, our study has biological implications. A defect in Fas expression has serious consequences. The defect in the Fas gene leads to uncontrolled lymphoproliferation and severe autoimmune diseases. Fas has been implicated in the negative selection of autoreactive T cells. Our demonstration that lpr thymocytes are not totally defective in expressing a Fas-dependent function is consistent with this idea and suggests that young lpr mice have some capacity of negative selection. This could explain the different levels of negative selection in various systems examined in lpr mice (28–30). The observation that lpr B cells are resistant to cytotoxic Th1 effectors during antigen-specific, Ia-restricted interaction is consistent with the hyperactivity of lpr B cells and the excessive production of IgG autoantibodies (31). Thus, the Fas defect induces abnormal expression of immunity in both the T-cell compartment and the B-cell compartment in lpr mice.

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