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Immunology

Th1 CD4+ lymphocytes delete activated macrophages through the Fas/APO-1 antigen pathway

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ABSTRACT **The Fas/APO-1 cytotoxic pathway plays an important role in the regulation of peripheral immunity. Recent evidence indicates that this regulatory function operates through deletion of activated T and B lymphocytes by CD4$^+$ T cells expressing the Fas ligand. Because macrophages play a key role in peripheral immunity, we asked whether Fas was involved in T-cell–macrophage interactions. Two-color flow cytometry revealed that Fas receptor (FasR) was expressed on resting murine peritoneal macrophages. FasR expression was upregulated after activation of macrophages with cytokines or lipopolysaccharide, although only tumor necrosis factor-α rendered macrophages sensitive to anti-FasR antibody-mediated death. To determine the consequence of antigen presentation by macrophages to CD4$^+$ T cells, macrophages were pulsed with antigen and then incubated with either Th1 or Th2 cell lines or clones. Th1, but not Th2, T cells induced lysis of 60–80% of normal macrophages, whereas macrophages obtained from mice with mutations in the FasR were totally resistant to Th1-mediated cytotoxicity. Macrophage cytotoxicity depended upon specific antigen recognition by T cells and was major histocompatibility complex restricted. These findings indicate that, in addition to deletion of activated lymphocytes, Fas plays an important role in deletion of activated macrophages after antigen presentation to Th1 CD4$^+$ T cells. Failure to delete macrophages that constitutively present self-antigens may contribute to the expression of autoimmunity in mice deficient in FasR (*lpr*) or Fas ligand (*gld*).**

Recent studies suggest that perforin and Fas (APO-1/CD95 antigen)-based mechanisms may account for all cytotoxic T-lymphocyte effector function (1, 2). Whereas perforin-mediated killing by CD8$^+$ T cells may be most important in rapid cytolysis of virus-infected targets, Fas-mediated cytotoxicity is important in immunoregulation, a conclusion supported by the expression of lupus-like autoimmunity in Fas receptor (FasR)-deficient mice (for review, see ref. 3). The precise immunological contexts in which Fas normally prevents autoimmunity are not fully characterized, although most evidence suggests that the Fas ligand induces death of activated mature T and B cells (3). Significantly it is the Th1, rather than the Th2, subset of CD4$^+$ lymphocytes that kills activated B cells or whole lymphoid populations from spleen, lymph node, thymus, and bone marrow targets through the Fas pathway (4, 5). These findings emphasize the importance of deletion of activated lymphocytes by Th1 cells in the maintenance of peripheral tolerance.

Macrophages are "professional" antigen-presenting cells. Resting cells constitutively express the costimulatory molecule B7.2, consistent with the important role for macrophages during the early phase of an immune response (6). During an inflammatory response, T-cell costimulation is facilitated by the expression of B7.1 (7), but macrophages also express potentially injurious products such as proteolytic enzymes and free radicals (8). Because presentation of self-peptides (9, 10) in the presence of costimulatory molecules, as well as continued release of toxic products, would be deleterious to the host, mechanisms to eliminate activated macrophages are necessary. To explore the role of Fas in the deletion of murine macrophages, we examined FasR expression and the requirement for FasR during antigen presentation by macrophages to Th1 and Th2 T cell clones. The Fas-dependent, T-cell-mediated induction of apoptosis after antigen presentation suggests that, in addition to deletion of activated T and B cells, the Fas pathway is important for the elimination of activated macrophages.

MATERIALS AND METHODS

Mice. MRL/MpJ-*lpr/lpr* (MRL/*lpr*), MRL/Mp-+/+ (MRL/+), C3H (C3H/HeSnJ), C3H/*gld/gld* (C3H/*gld*), and BALB/c mice were originally obtained from The Jackson Laboratory and subsequently bred at the Hospital for Special Surgery. The mutant strain, CBA/*lpr*^{cg} (CBA/K1Jms/*lpr*^{cg}/*lpr*^{cg}), and the wild type, CBA/+ (CBA/K1Jms), were provided by Akio Matsuzawa (Institute of Medical Science, Tokyo) (11). MRL, C3H, and CBA are all H-2^k strains, whereas BALB/c is a H-2^d strain.

Cell Lines and Clones. A CD4$^+$, toxic shock syndrome toxin 1 (TSST-1)-reactive T-cell line (MRL-7), was isolated from the spleen of an 8-week-old MRL/+ mouse as described (12). A.E7, a CD4$^+$ Th1 T-cell clone (13), was provided by R. H. Schwartz (National Institutes of Health). Clone A.E7 is specific for the carboxyl-terminal fragment of pigeon cytochrome *c* (peptide 81–104) presented by I-E^k (14). Clone A.E7 was maintained as described (15) using moth DASp (provided by Marc Jenkins, University of Minnesota), a peptide consisting of moth residues 86–90 spliced to pigeon residues 94–104 (14). D10.G4.1, a CD4$^+$ Th2 T-cell clone (16), was obtained from the American Type Culture Collection. Clone D10.G4 is specific for conalbumin presented by I-A^k (17). Clone D10G.4 was maintained by stimulation with irradiated C3H (H-2^K) splenocytes and 1.25 μM conalbumin in Eagle's Hanks' amino acid medium/10% rat conditioned medium, as described (16).

Macrophage Isolation, Culture, and Activation. Resident peritoneal cells were obtained by peritoneal lavage with cold Hanks' balanced salt solution (HBSS). Elicited peritoneal exudate cells were obtained by i.p. injection of 2 ml of thioglycollate broth (Difco) followed by peritoneal lavage 3–4 days later. Macrophages were isolated from peritoneal cells by adherence to

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Abbreviations: PEM, peritoneal exudate macrophages; TNF-α, tumor necrosis factor α; IFN, interferon; FasR, Fas receptor; FasL, Fas ligand; IL, interleukin; MHC, major histocompatibility complex; LPS, lipopolysaccharide; TSST-1, toxic shock syndrome toxin 1; SFI, specific fluorescence index; MFI, mean fluorescence index; mAb, monoclonal antibody; RPM, resident peritoneal macrophages.

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plastic as described (18) and cultured at 37°C in RPMI 1640 medium containing supplements (10% fetal calf serum, 2 mM glutamine, streptomycin at 100 μg/ml, penicillin at 100 units/ml, fungizone at 10 μg/ml, and 50 μM 2-mercaptoethanol) in a humidified incubator with 5% CO_2/95% air. Macrophages isolated from peritoneal exudate cells are referred to as peritoneal exudate macrophages (PEM). In specific experiments, lipopolysaccharide (LPS) at 10 μg/ml derived from *Salmonella typhosa* (Difco), interferon (IFN) γ at 100 units/ml (Life Technologies, Gaithersburg, MD), tumor necrosis factor α (TNF-α) at 10 ng/ml (Genzyme), or interleukin (IL) 4 at 5 ng/ml (BioSource, Camarillo, CA) was added to the cultures. After 1–2 days, the adherent PEM were removed by gentle lavage with phosphate-buffered saline/2.5 mM EDTA and used for cytotoxicity assays and flow cytometry analysis.

Monoclonal Antibodies (mAbs) and Flow Cytometry Analysis. Antibodies reactive against murine cell-surface markers, Mac-1 (mAb M1/70.15.11.5.HL), F4/80; major histocompatibility complex (MHC) class II, anti-I-A^d (mAb BP107.2.2); B220 (mAb RA3-3A1/6.1); and Fc-γ receptor (mAb 2.4G2) were purchased from the American Type Culture Collection and grown as ascites in pristane-treated mice as described (19). The monospecific rabbit polyclonal anti-FasR antibody, IgG $(Fab')_2$ fragment, has been described (19). Secondary antibodies were purchased from Jackson ImmunoResearch. Single- and two-color flow cytometry were performed as described (19, 20).

Samples were analyzed on a FACScan instrument operating with the CELLQUEST software (Becton Dickinson). Lymphocyte and macrophage gates were defined based on anterior and right-angle scatter. To compensate for any potential differences in cell size, FasR expression is reported as the specific fluorescence index (SFI). SFI was determined by dividing mean fluorescence intensity of FasR-stained cells with mean background fluorescence obtained with normal rabbit immunoglobulin $F(ab')_2$ fragments as described (20). The mean fluorescence index (MFI) was used to compare changes in FasR expression in resting and stimulated cells—e.g., MFI = SFI of cells cultured with cytokines/SFI of *resident* peritoneal macrophages.

Cytotoxicity and Cell Viability Assays. Peritoneal exudate cells were adhered to plastic dishes, cultured for 1–2 days with IFN-γ, and gently resuspended as described above. PEM (1×10^6) targets were labeled with 100 μCi of ^{51}Cr (1 Ci = 37 GBq; New England Nuclear) for 1 hr in 37°C, washed, and then used as targets in 4-hr cytotoxicity assays with effector cells as described (12). In some experiments, macrophages were preincubated with optimal concentrations of antigen (15, 16). Cell viability was also assessed by a fluorimetric assay using the dye Alamar blue (Alamar Bio-Sciences, Sacramento, CA), which gives identical results to the 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (ref. 21; unpublished results). Alamar blue was added to 10% final concentration for 4 hr at 37°C. Fluorescence was read on a Cytofluor 2350 plate reader (Millipore) with excitation at 530 and emission at 590 nm. The results were expressed as: 100 − [(FI of test sample/FI of control) × 100], where FI = fluorescence intensity.

Proliferation and Cytokine Assays. T-cell proliferation was assessed by the addition of 1 μCi of [^{3}H]thymidine (New England Nuclear) to cultures and harvesting DNA on a mini-Mash cell harvestor (BioWhittaker) 12 hr later. The concentrations of cytokines in T-cell culture supernates were quantified by sandwich ELISAs as described (22). The following mAbs were obtained from PharMingen: rat anti-mouse IL-4 (mAb BVD4-1D11) and biotinylated rat anti-mouse IL-4 (mAb BVD6-24G2) to quantify IL-4; rat anti-mouse IFN-γ (mAb R4-6A2) and biotinylated rat anti-mouse IFN-γ (mAb XMG1.2) to quantify IFN-γ. Recombinant mouse IL-4 and IFN-γ were used to construct standard curves.

Northern Blot Analysis. mRNA expression of Fas ligand (FasL) and the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase, were quantified by laser densitometry analysis of Northern blots as described (12).

RESULTS

FasR Is Expressed on Resident Peritoneal Macrophages (RPM). To determine whether the FasR was expressed on RPM, freshly isolated peritoneal cells were collected from unmanipulated 6- to 8-week-old MRL/+, MRL/*lpr* (FasR deficient), and BALB/c mice and analyzed for FasR expression by flow cytometry. As shown in a representative two-color flow cytometry experiment (Fig. 1), staining of MRL/+ (*A* and *C*), but not of MRL/*lpr* (*B* and *D*), peritoneal macrophages with anti-FasR induced a shift in the intensity of immunofluorescent staining of the entire F4/80+ population, indicating the presence of FasR on all resident macrophages. Similar results were obtained with BALB/c resident peritoneal cells and when phycoerythrin-conjugated mAb Jo2 anti-FasR was used for immunostaining (data not shown).

FasR Expression Is Upregulated After Macrophage Activation. IFN-γ has been reported to increase FasR expression on a murine macrophage line (23), human glioma cell lines (24), and on normal human macrophages (25). Because IFN-γ and TNF-α are potent stimulators of murine macrophages, we examined the effect of these cytokines at optimal concentrations (24, 25) on FasR expression. Results of three independent experiments are summarized (Fig. 2). IFN-γ or TNF-α alone or in combination increased expression of FasR 1.7- to 2-fold when compared to cells cultured in medium alone. FasR expression on cells cultured with both IFN-γ and TNF-α was not statistically significantly higher than cells cultured in the presence of either cytokine alone ($P > 0.4$, Mann–Whitney rank-sum test). To determine whether IFN-γ and TNF-α were specific in the ability to upregulate macrophage FasR expression, PEM were incubated with LPS and IL-4, which also activate macrophages (26). Fig. 2 shows that LPS and IL-4 induced FasR expression equivalent to either IFN-γ or TNF-α. FasR expression on peritoneal exudate macrophages before adherence or culture was slightly increased as compared to resident macrophages with an MFI of 1.32 ± 0.23.

Anti-FasR Antibody Induces Apoptosis of TNF-α-Primed Peritoneal Macrophages. Despite the presence of FasR on

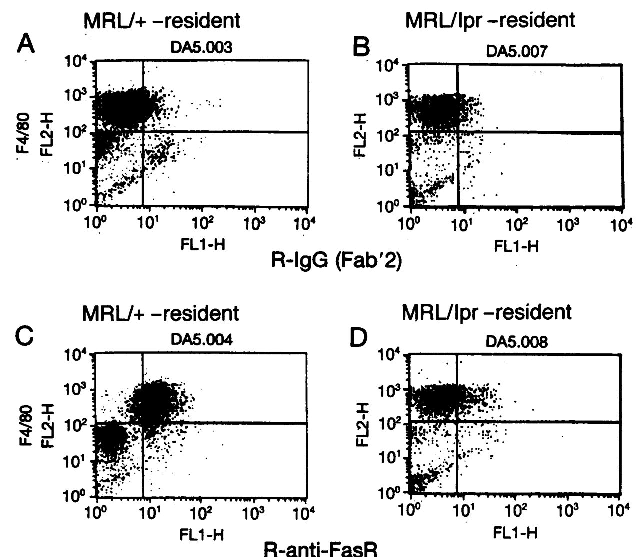

FIG. 1. RPM express FasR. Peritoneal cells obtained from 6- to 8-week-old MRL/+ (*A* and *C*) and MRL/*lpr* (*B* and *D*) mice by lavage were analyzed for FasR expression by two-color flow cytometry. Cells were stained with the macrophage-specific marker anti-F4/80+, and either normal rabbit $F(ab')_2$ IgG (R-IgG) in *A* and *B* or rabbit $F(ab')_2$ anti-FasR (R-anti-FasR) in *C* and *D*. Only cells falling in the large cell (macrophage) gate were analyzed.

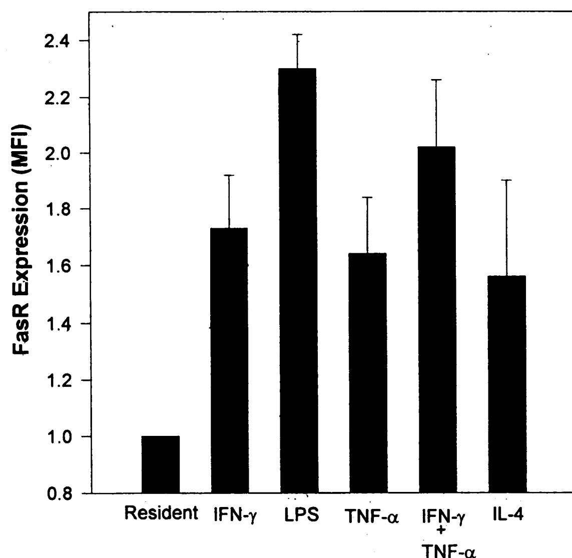

FIG. 2. IFN-γ and TNF-α upregulate macrophage FasR expression. PEM from MRL/+ mice were incubated with IFN-γ (100 units/ml), TNF-α (10 ng/ml), IFN-γ and TNF-α, LPS (10 μg/ml), or IL-4 (5 ng/ml). After 1–2 days, the adherent cells were removed and analyzed for FasR expression by two-color flow cytometry as in Fig. 1. The mean ± SD for three experiments using pooled cells from three to five mice is expressed as the MFI as described in text. A MFI of 1 represents the level of FasR on unmanipulated RPM.

normal lymphocytes and transformed cell lines (19, 27), ligation of the receptor does not always signal apoptosis (27, 28). To determine whether the agonist anti-FasR mAb, Jo-2, could kill peritoneal macrophages, PEM from CBA/+ mice were cultured for 24 hr with or without cytokines. mAb Jo2 or normal hamster IgG was then added to the cultures for 24 hr, and cell viability (>85% F4/80+) was assessed by Alamar blue incorporation 4 hr later. Fig. 3 shows that mAb Jo2 induced little cell death of PEM cultured in medium alone or with IFN-γ, LPS, or IL-4. In contrast, preexposure of cells to TNF-α alone or to the combination of IFN-γ and TNF-α significantly augmented mAb Jo-2-mediated killing ($P < 0.04$ and $P < 0.0001$, respectively, Student's *t* test). Similar results were seen in PEM from C3H and MRL/+ mice. To ensure that macrophages, rather than a contaminating cell type, were killed by mAb Jo2, the above experiment was repeated with F4/80+ macrophages (>96% F4/80+) purified by sorting on a FACS-Vantage cytometer. Incubation of these cells with IFN-γ and TNF-α followed by mAb Jo-2 also resulted in 30% cell death (data not shown).

A Thl, Superantigen-Reactive T-Cell Line Is Cytotoxic for Wild-Type, but not *lpr*, Macrophages. Previous studies have shown that certain $CD4^+$ T-cell lines can kill antigen-pulsed macrophages and that the macrophages require preexposure to IFN-γ to become sensitive to lysis (29, 30). To examine whether macrophage cytotoxicity was mediated through the FasR, IFN-γ-treated PEM harvested from CBA/+ ($FasR^+$) and CBA/lpr^{cg} ($FasR^-$) mice (31) were used as targets in a standard ^{51}Cr cytotoxicity assay with the TSST-1-reactive T-cell line, MRL-7, as effectors. MRL-7 is >99% $CD4^+$ and belongs to the Thl subset because the cells produced high levels of IFN-γ (>1.2 μg/ml) but not IL-4 (<0.1 μg/ml) after stimulation with TSST-1.

Fig. 4 shows that $FasR^+$ target cells were lysed by MRL-7 T cells in a dose-dependent manner, and lysis absolutely depended upon the presence of the superantigenTSST-1. In contrast, $FasR^-$ macrophages were not lysed either with or without TSST-1, showing that macrophage lysis occurred exclusively by a Fas-mediated pathway. Similar results were obtained when the incubation time was prolonged up to 16 hr

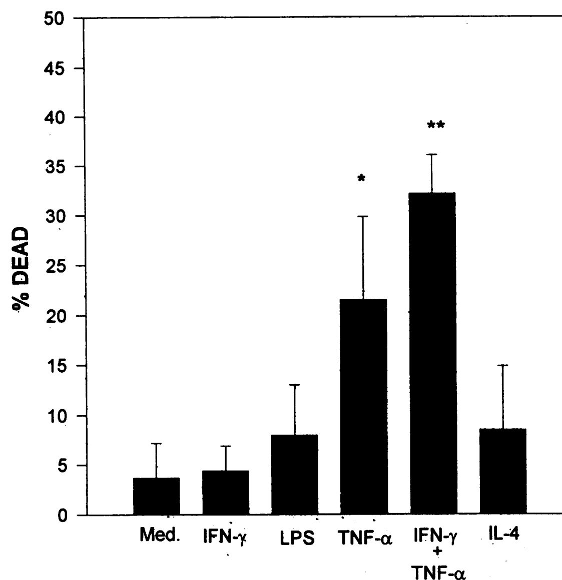

FIG. 3. Anti-FasR antibody induces macrophage cell death. PEM obtained from MRL/+ mice were cultured for 24 hr in either medium alone (Med.), LPS, IFN-γ, TNF-α, or IL-4 as in Fig. 3. Cells (4 × 10^4 cells/ml) were incubated with either anti-FasR mAb or normal hamster IgG (1.5 μg/ml) for 24 hr. Viability was measured with Alamar blue (21), and results were expressed as percentage of live cells compared with a population of cells exposed to normal hamster IgG at 1.5 μg/ml. Results indicate mean ± SD of three independent experiments done on pooled cells from three to five mice. Similar results were obtained with both BALB/c and C3H macrophage targets. *, $P < 0.04$; **, $P < 0.0001$ Student's *t* test.

and when MRL/+ ($FasR^+$) and MRL/*lpr* ($FasR^-$) macrophages were used as targets (data not shown). When cytotoxicity experiments were repeated with TNF-α-treated macrophages, significant (40% at effector-to-target ratio of 40:1)

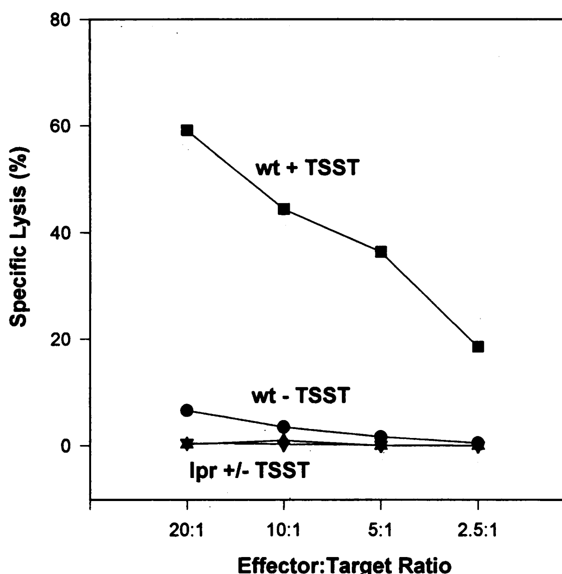

FIG. 4. A Thl, superantigen-reactive, $CD4^+$ T-cell line induces Fas-mediated macrophage cytotoxicity. PEM were isolated from wild-type (wt) CBA/+ and FasR-deficient CBA/lpr^{cg} (*lpr*) mice and cultured for 2 days with IFN-γ at 100 units/ml. The PEM targets were labeled with ^{51}Cr and exposed to the Thl, TSST-1-responsive, $CD4^+$ T-cell line MRL-7 from a MRL/+ mouse. After incubation of MRL-7 with macrophages at various effector-to-target ratios with (+) or without (−) TSST-1 at 10 ng/ml, specific ^{51}Cr release (% lysis) was calculated. A representative experiment of three performed is shown.

lysis of macrophages was seen, but lysis was significantly lower than IFN-γ-primed cells (data not shown).

Thl, But Not Th2, T-Cell Clones Mediate Macrophage Cytotoxicity Through FasR in an Antigen-Specific MHC-Restricted Interaction. To examine the requirements for Fas-mediated cytotoxicity by $CD4^+$ T-cell clones, IFN-γ-treated PEM from CBA/+ mice were pulsed with antigen and labeled with ^{51}Cr. The labeled cells were then incubated with the MHC class II H-2^k-restricted $CD4^+$ T-cell clones, A.E7 (a Th1 clone) or D10.G4 (a Th2 cell clone), and tested for cytotoxicity. As shown in Fig. 5*A*, clone A.E7 lysed CBA/+ (H-2^k) macrophages prepulsed with cytochrome *c* peptide in a dose-related fashion, whereas clone D10.G4 had virtually no effect on CBA/+ macrophages prepulsed with conalbumin. The failure of clone D10.G4 to kill cannot be explained by lack of activation of the T cells because these cells proliferated (stimulation index of 5 as assessed by [^{3}H]thymidine incorporation) under these conditions.

To examine the requirements for FasR and for H-2-restricted T-cell recognition, clone A.E7 effectors were incubated with different IFN-γ-treated PEM targets prepulsed with cytochrome *c* peptide. Fig. 5*B* shows that H-2^k, $FasR^+$ (CBA/+), but not H-2^k, $FasR^-$ (CBA/lpr^{cg}), macrophages were lysed in the presence of A.E7 effector cells. C3H/*gld* PEM (H-2^k, $FasR^+$, $FasL^-$) were also lysed efficiently by A.E7 effector cells (data not shown). When MHC-mismatched (H-2^d) BALB/c macrophages were prepulsed with antigen and used as targets, virtually no lysis was seen (Fig. 5*B*). Together, these findings confirm the requirement for a functional FasR on macrophages for induction of cytotoxicity, that failure to lyse *lpr* macrophages was not a consequence of autoimmunity, and that specific T-cell recognition of antigen in the context of MHC was necessary. As expected (29, 30), PEM cultured without IFN-γ or TNF-α and pulsed with antigen were not lysed by A.E7 effector cells (data not shown).

To determine the effect of antigen processing on FasR-mediated cytotoxicity, IFN-γ-treated PEM from CBA/+ mice were prepulsed with equimolar concentrations of the cytochrome *c* protein, the cytochrome *c* peptide (DASp), or without specific antigen. No killing of macrophages was seen without cytochrome *c*, and somewhat higher cytotoxicity was seen when cells were pulsed with the peptide compared to the whole protein (Fig. 5*C*). These findings show that macrophage cytotoxicity absolutely depended upon the presence of antigen and that antigen processing did not enhance susceptibility of the macrophages to cell death.

FasL Expression by Northern Blot Analysis. As reported (12), the superantigen-reactive line MRL7 expressed high levels of FasL mRNA after activation with superantigen or phorbol 12-myristate 13-acetate and ionomycin. Similarly, FasL mRNA was highly expressed in the Th1 cell line A.E7 but was not detected in the Th2 line D10G.4 after activation by either antigen or phorbol 12-myristate 13-acetate and ionomycin (data not shown).

DISCUSSION

In this study, we report that murine RPM express FasR, that FasR expression is upregulated after macrophage activation, and that macrophages exposed to IFN-γ or TNF-α are susceptible to Fas-mediated cytotoxicity by Th1, $CD4^+$ T cells. These findings suggest that, in addition to T–T (32–34) and T–B (35, 36) lymphocyte cytotoxicity, the Fas ligand mediates T-macrophage cytotoxicity as a third potential mechanism for regulation of peripheral tolerance.

FasR was constitutively expressed on RPM obtained from either autoimmune (MRL/+) or normal (BALB/c) mice, but RPM were not susceptible to anti-FasR-mediated death. This observation resembles that seen with normal resting T cells (28, 37) and is most likely explained by the presence of functional inhibitor(s) of Fas-mediated apoptosis located on the C terminus of the receptor (38, 39). Expression of FasR and macrophage susceptibility to anti-FasR antibody-mediated apoptosis has also been described in human peripheral blood macrophages (25).

As seen in a number of other cell types (23, 24), macrophage activation induced by either IFN-γ or TNF-α enhanced macrophage FasR expression, although IFN-γ alone did not facilitate anti-FasR antibody-mediated cytotoxicity. The ability of TNF-α to prime macrophages for anti-Fas-mediated cell death is important in view of the central role of this cytokine in macrophage activation (40–42) and suggests that, in addition to its proinflammatory properties, TNF-α also helps limit the survival of macrophages exposed to FasL (see below).

Macrophages phagocytose, process, and present peptide antigens to T cells. T cells that recognize the specific antigen become activated, proliferate, and perform their effector functions. On the basis of their functions and cytokine profiles, two subsets of $CD4^+$ T cells are recognized. Th1 cells secrete IL-2 and IFN-γ and mediate cell-mediated cytotoxicity, whereas Th2 cells secrete IL-4, IL-6, and IL-10, which facilitate humoral immunity (43). Ju *et al.* (29) reported that certain $CD4^+$ T-cell clones killed macrophages after antigen presentation. In the present study, we report that $CD4^+$ T cells of the

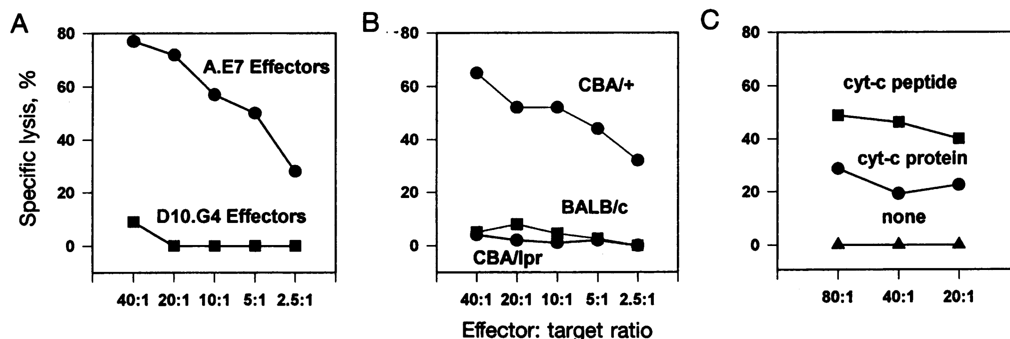

FIG. 5. Requirements for Fas-mediated macrophage cytotoxicity by T-cell clones. PEM were cultured with IFN-γ, pulsed with antigen, and labeled with ^{51}Cr. The PEM targets were then incubated with T-cell effector clones, and cytotoxicity was measured by specific ^{51}Cr release as for Fig. 4. (*A*) Thl, but not Th2, T-cell clones induce macrophage cytotoxicity. The cytochrome *c*-specific Thl, $CD4^+$ T-cell clone A.E7 and the conalbumin-specific Th2, $CD4^+$ T-cell clone D10.G4—both H-2^k restricted—were used as effector cells, and CBA/+ (H-2^k) PEM were used as targets. The macrophages were preincubated with either cytochrome *c* peptide (2.4 μM) or conalbumin (1.25 μM) before exposure to A.E7 or D10.G4 cells, respectively. (*B*) Macrophage cytotoxicity requires MHC-restricted T-cell recognition. A.E7 cells were used as effectors and either FasR+, H-2^k (CBA/+); FasR-, H-2^k (CBA/lpr^{cg}); or FasR+, H-2^d (BALB/c) PEM targets pulsed with cytochrome *c* peptide as targets. (*C*) Macrophage cytotoxicity does not require antigen processing. CBA/+ PEM were prepared as described for Fig. 4 and incubated with either cytochrome *c* peptide [cyt-c peptide (DASp); 2.4 μM], whole pigeon cytochrome *c* (cyt-c protein; 2.4 μM), or no antigen (none). A.E7 was used as the effector cell.

Th1 subset induced macrophage death through the Fas pathway because macrophages obtained from MRL/*lpr* and CBA/*lpr*^{cg} mice totally resisted T-cell-mediated lysis.

Using the cytochrome *c*-specific, CD4$^+$ Th1 T-cell clone A.E7, we showed that Fas-mediated macrophage cytotoxicity had the same recognition requirements as T-cell proliferation—namely, antigen-specific recognition and MHC restriction. In contrast to only ≈20–30% killing of activated macrophages by the anti-FasR agonist antibody, the superantigen-reactive or antigen-specific Th1 CD4$^+$ cells killed ≈50–80% of normal FasR$^+$ macrophages. This finding suggests either that the authentic FasL on T cells is more efficient at inducing cell death or that other T-cell-derived factors enhance Fas-mediated lysis of macrophages. These factors may be cytokines, adhesion molecules (e.g., ICAM-1 and lymphocyte function-associated protein 1), or costimulatory (B7 and CD28/CTLA-4) molecules facilitating cognate interactions. Alternatively, the perforin pathway may be recruited and augment Fas-mediated killing.

FasL is rapidly upregulated after T-cell activation through the T-cell antigen receptor, mitogens, or by phorbol 12-myristate 13-acetate and ionomycin (44). Functional studies suggest that the ligand is expressed by activated Th1, but only rarely on Th2, cell lines (4, 5, 44)—consistent with our findings. Differential upregulation of FasL on this functional subset, therefore accounts for the selectivity of macrophage cytotoxicity by Th1, but not Th2, CD4$^+$ T cells in this study. Because Fas-dependent cytotoxicity of B cells has also been seen after T-cell activation (5, 35, 36), these findings suggest that the deletion of different types of antigen-presenting cells is a normal physiological accompaniment of antigen presentation to Th1 CD4$^+$ T cells.

What are the implications of Fas-mediated macrophage cytotoxicity *in vivo*? Most peptides presented by MHC class II molecules are self-antigens (10). Because Fas-mediated macrophage cytotoxicity requires both T-cell recognition and macrophage priming, normal resting macrophages would not be deleted by this mechanism. In contrast, in inflammation, the risk of presentation of self-peptides to autoreactive T cells is heightened due to the enhanced expression of costimulatory molecules. In normal mice, the production of IFN-γ and TNF-α would render the macrophages susceptible to Fas-mediated T-cell lysis and would limit the opportunity for an autoimmune response. Mice with mutations in FasR (*lpr*) or FasL (*gld*) are unable to delete macrophages through the Fas pathway, so that T-cell recognition of self-peptides would be considerably facilitated. This observation may, in part, explain how Fas mutant mice develop a systemic autoimmune disease similar to systemic lupus erythematosus (45). Consistent with the defect in T-cell-mediated cytotoxicity of *lpr* macrophages observed in the present study, MRL/*lpr* mice have significantly more peritoneal macrophages (46) and produce more proinflammatory cytokines, IFN-γ and TNF-α, than control mice (47). Further studies will be required to evaluate the specific role of FasL-mediated macrophage cytotoxicity in human diseases.

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