Th17 Cells Undergo Fas-Mediated Activation-Induced Cell Death Independent of IFN-γ

Yingyu Zhang, Guangwu Xu, Liying Zhang, Arthur I. Roberts and Yufang Shi

*J Immunol* 2008; 181:190-196; doi: 10.4049/jimmunol.181.1.190

http://www.jimmunol.org/content/181/1/190

References

This article cites 34 articles, 14 of which you can access for free at:
http://www.jimmunol.org/content/181/1/190.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Th17 Cells Undergo Fas-Mediated Activation-Induced Cell Death Independent of IFN-γ

Yingyu Zhang, Guangwu Xu, Lifying Zhang, Arthur I. Roberts, and Yufang Shi

IL-17-secreting CD4+ T cells (Th17 cells) play a critical role in immune responses to certain infections and in the development of many autoimmune disorders. The mechanisms controlling homeostasis in this cell population are largely unknown. In this study, we show that murine Th17 cells undergo rapid apoptosis in vitro upon restimulation through the TCR. This activation-induced cell death (AICD), a common mechanism for elimination of activated T cells, required the Fas and FasL interaction: Fas was stably expressed, while FasL was up-regulated upon TCR reactivation of Th17 cells; Ab ligation of Fas induced Th17 cell death; and AICD was completely absent in Th17 cells differentiated from gld/gld CD4+ T cells. Thus, the Fas/FasL pathway is essential in regulating the AICD of Th17 cells. Interestingly, IFN-γ, a cytokine previously found to be important for the AICD of T cells, did not affect Th17 cell apoptosis. Furthermore, Th17 cells derived from mice deficient in IFN-γ receptor 1 (IFN-γRI−/−) underwent AICD similar to wild-type cells. Thus, AICD of Th17 cells occurs via the Fas pathway, but is independent of IFN-γ. The Journal of Immunology, 2008, 181: 190–196.

For almost two decades, the Th1/Th2 paradigm has been the classic framework for understanding the biology of CD4+ effector T cells and the interplay between the innate and adaptive immune systems (1, 2). However, some diseases previously considered to be Th1-mediated, such as experimental autoimmune encephalomyelitis are exacerbated by blockade of the Th1 cytokine IFN-γ (3, 4). IL-17 (now, more precisely defined as IL-17A, one of several homologous proteins of the IL-17 family) has been found to be involved in various inflammatory diseases and is considered a critical proinflammatory cytokine (5). IL-17 was first found to be highly expressed in CD4+CD45RO+ human T cells (6, 7). It remained a mystery how naive CD4+ T cells develop into IL-17-secreting cells and become IL-17-secreting memory cells until recent studies showed that the differentiation of murine IL-17-secreting CD4+ T cells (Th17 cells) requires TGF-β (8, 9) in combination with IL-6 or IL-21 (10). Other factors affecting the differentiation of Th17 have since been identified (11–13). In addition, the transcription factors, RORγt and RORαs were found to be critical for Th17 cell differentiation (14, 15). IL-17F, which shares sequence and functional similarities with IL-17A, was also found important for autoimmune and is also secreted by Th17 cells (16). The discovery of IL-17-producing T cells has provided new insights into immune regulation, immune pathogenesis, and host defense. These cells have been shown to play pivotal roles in the development of many autoimmune diseases in mice. They are also important for the recruitment of neutrophils (17) and in host defense against some fungal infections (18). Therefore, examination of the regulation of death of these cells could provide critical information about immune regulation in general.

Following an immune response, the elimination of activated T cells is important for the maintenance of immune homeostasis. Along with Bim-mediated cell death (19), activation-induced cell death (AICD) is believed to be important for the elimination of activated T cells and has been extensively studied in several experimental systems (20, 21). Studies of AICD have revealed that TCR signaling, which, in naive T cells, leads to activation, can instead lead to the deletion through apoptosis of activated peripheral T cells or thymocytes. Examination of the mechanism of AICD has revealed the importance of Fas signaling in the apoptosis of reactivated T cells (22–24). In addition, the survival or death of effector T cells is also affected by many cytokines, such as IFN-γ (25, 26), IL-4 (27, 28), and IL-2 (29).

The mechanisms of T cell death can vary, even in the case of AICD (30–32). Because Th17 cells were identified and recognized as a distinct T cell population, extensive studies have focused on their characteristics and differentiation processes, but little is known about the regulation of their survival and death. In this study, murine Th17 cells were differentiated from naive CD4+ T cells and their mechanism of AICD examined in vitro. We found that FasL-triggering of the Fas pathway plays a key role in the AICD of Th17 cells. Unlike in Th1 cells, however, AICD in Th17 cell was unaffected by IFN-γ. This finding provides new knowledge for understanding the pathogenesis of many diseases, especially autoimmunity and certain fungal infection.

Materials and Methods

Reagents, Abs, and mice

RPMI 1640 medium and α-MEM were from Sigma-Aldrich. FBS was from Invitrogen. Caspase inhibitor z-VAD-FMK was from R&D systems. Neutralizing Abs used were: anti-IFN-γ (R&D Systems), anti-FasL (MFL3), and anti-Fas (Jo2) (BD Pharmingen). Recombinant DR5 (soluble receptor for TRAIL) was a gift from Dr. Youhai Chen of the University of

1 This work was supported in part by grants from the New Jersey Commission on Science and Technology (2042-014-84), National Institutes of Health grants (AI43384, AI057596), and the National Space Biomedical Research Institute (MFL3), and RORαs (IIH00405), which is supported by the National Aeronautics and Space Administration (2042-014-84), National Institutes of Health grants (AI43384, AI057596), and the National Space Biomedical Research Institute (MFL3), and anti-Fas (Jo2) (BD Pharmingen). Recombinant DR5 (soluble receptor for TRAIL) was a gift from Dr. Youhai Chen of the University of

2 Y.Z. and G.X. contributed equally to this work.

3 Address correspondence and reprint requests to Dr. Yufang Shi, Robert Woods Johnson Medical School, University of Medicine and Dentistry of New Jersey, 661 Hoes Lane, Piscataway, NJ 08854. E-mail address: shiyu@umdnj.edu

Received for publication October 10, 2007. Accepted for publication April 30, 2008.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Copyright © 2008 by The American Association of Immunologists, Inc. 0022-1767/08/$2.00

www.jimmunol.org
Pennsylvania (Philadelphia, PA). Staining Abs were FITC-conjugated anti-Fas (Jo2), biotin-conjugated anti-Fasl (MFL3) (BD Pharmingen), and PE-conjugated streptavidin-avidin (eBioscience); FITC-conjugated anti-IFN-γ (clone XMGI12, E BioScience) and PE-conjugated anti-IL-17 (clone TCI11-18H10.1; E BioScience). Abs for Western blotting against caspase 3 (cat. no. 9662), caspase 8 (clone 1C12), and Bid (cat. no. 2003) were from Cell Signaling Technology. Anti-cleaved Bid was from Calbiochem.

Mice were obtained from The Jackson Laboratory (C57BL/6, B6.129S7-Ighr1mpj/H11032 (Ighrl/1−/−) on the C57BL/6 background), and B6.129S7-Ighr1mpj/H11032 (Ighrl1−/−) on the background) or from the National Cancer Institute (BALB/cAn mice). All mice were housed under specific pathogen-free conditions in an American Association for Accreditation of Laboratory Animal Care- accredited animal facility at Robert Wood Johnson Medical School, and all studies were conducted under approval of the Institutional Animal Care and Use Committee.

**Th17 differentiation and induction of cell death in activated Th17 cells**

CD4+ T cells were isolated from mouse spleenocytes by immunomagnetic cell sorting using negative selection (Miltenyi Biotec). To generate Th17 cells, purified CD4+ T cells were stimulated with plastic-bound anti-CD3 and anti-CD28 in 25 cm2 tissue culture flasks and cultured in complete medium (RPMI 1640 medium supplemented with 10% FBS, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Invitrogen) supplemented with anti-IFN-γ (10 μg/ml), anti-IL-4 (10 μg/ml), IL-6 (20 ng/ml), recombinant human TGF-β1 (5 ng/ml), and recombinant mouse TNF-α (2 μg/ml) (all from R&D Systems) for 3 days. Cells were then rested for 2 days in culture with anti-IFN-γ, anti-IL-4, IL-6, and recombinant human TGF-β1. After separation by density gradient centrifugation on Ficoll (Amersham Biosciences), the resultant viable Th17 cells were restimulated with PMA (50 ng/ml) and ionomycin (1 μM) for 6 h for determination of IL-17 production, or were seeded into anti-CD3-coated 24-well plates (2.5 × 10^5 cells per well) to induce AICD.

**Flow cytometric analysis of intracellular cytokines**

To identify individual cytokine-producing cells, cells were activated with PMA (50 nM) and ionomycin (1 μM) in the presence of brefeldin A for 6 h and then stained by direct immunofluorescence for intracellular cytokines. In brief, cells were harvested, washed twice with PBS, and resuspended in Ficoll/Perm buffer (BD Biosciences) for 15 min. After washing twice, cells were stained with FITC-conjugated anti-IFN-γ (clone XMGI12; E BioScience) and PE-conjugated anti-IL-17 (clone TCI11-18H10.1; E BioScience). Other cells were stained for surface markers following blocking with anti-CD16 (clone 93; eBioscience). Cells were then analyzed using a FACScan flow cytometer (BD Immunocytochemistry), with CellQuest software for data acquisition and analysis.

**Determination of apoptosis by DNA content analysis**

To determine the extent of apoptosis, the percentage of cells with hypodiploid DNA content was determined. In brief, cells were harvested and washed once with PBS and resuspended in DNA staining buffer consisting of 50 μg/ml propidium iodide, 0.25% saponin, and 40 μg/ml RNase A (Roche). After a 30-min incubation at room temperature, the cells were analyzed by flow cytometry.

**Real-time PCR**

Total RNA was isolated from cell pellets using an RNAeasy Mini kit (Qiagen). Genomic DNA was removed from total RNA before cDNA synthesis using the RNase-Free DNase Set for DNase digestion during RNA purification. First-strand cDNA synthesis was performed for each RNA sample using MMLV reverse transcriptase (Invitrogen). Oligo(dT17) was used to prime cDNA synthesis. mRNA expression was determined by real-time PCR using the RNase-Free DNase Set for DNase digestion during RNA synthesis (Qiagen). Genomic DNA was removed from total RNA before cDNA synthesis. Relative expression levels were calculated by the 2−ΔΔCT method (Amersham Biosciences).

**Western blotting analysis of protein expression**

To analyze protein expression, cells were washed 3 times with PBS and the pellet resuspended in 100 μl lysis buffer. Whole cell protein was extracted and immunoblotting was conducted according to the manufacturer’s protocol (Cell Signaling Technology). Briefly, 30 μg of protein extract was added to 3× sample buffer (125 mM Tris-HCl, pH 6.8, 1% SDS, 2% 2-ME, and 0.01% bromophenol blue), boiled for 5 min, and loaded onto a 10% SDS-polyacrylamide gel. After separation, proteins were transferred to a nitrocellulose membrane by electroblotting (Bio-Rad). The membrane was blocked with 5% nonfat milk in TBS with 0.1% (w/v) Tween 20 for 1 h at room temperature, probed for 2 h with the specified primary Abs against mouse caspase 8, caspase 3, Bid, or BID, and incubated with a HRP-linked secondary Ab for 1 h before the membrane was washed with 1× TBS with Tween 20 for 1 h. Protein bands were visualized by chemiluminescence (Amersham Biosciences). The membrane was then stripped according to the manufacturer’s protocol (Amersham Biosciences) and reprobed with Ab for a control protein, usually β-actin (Sigma-Aldrich).

**Results**

**Th17 cells undergo rapid apoptosis upon TCR crosslinking**

Th17 cells are a newly identified subset of effector T cells shown to play a pivotal role in the development of various autoimmune diseases and fungal infections. Their homeostasis may involve TCR-mediated induction of apoptosis, as modeled in vitro by AICD. To investigate Th17 cell homeostasis, we differentiated these cells from CD4+ T cells isolated from both C57BL/6 and BALB/c mice according to a previously published protocol (9, 14), with modifications. The identity of these cells was verified by their abundant expression of IL-17 and low expression of IFN-γ in comparison to Th1 cells, as determined by intracellular cytokine staining and mRNA levels of these cytokines (Fig. 1).

To determine whether Th17 cells are sensitive to AICD after reactivation through the TCR, differentiated Th17 cells were stimulated with plastic-bound anti-CD3 for up to 24 h, and apoptotic...
cells identified by hypodiploid DNA content, revealed by staining with propidium iodide after permeabilization. Cell viability was also tested by exclusion of propidium iodide in non-permeabilized cells (data not shown). Similar results were obtained with both methods; thus, only DNA content analysis is shown. We found that in the absence of IL-2 or IL-4, the differentiated Th17 cells undergo significant spontaneous cell death. Reactivation did not increase cell death under this condition (Fig. 2A). Addition of IL-2 or IL-4 strongly inhibited spontaneous cell death while cells underg0 robust apoptosis after reactivation (Fig. 2A). These experiments were conducted with cells from both BALB/c and C57BL/6 mice; only results from C57BL/6 mice are shown in Fig. 2. We also found that Th17 cells underwent robust apoptosis within 24 h of restimulation (Fig. 2B). The extent of AICD in Th17 cells (13.7 ± 1.5% after 12 h and 24.0 ± 1.0% after 24 h; adjusted for background death of cells treated only with IL-2), while extensive, was not as great as that of Th1 cells (34.9 ± 2.9% and 47.9 ± 1.0%), respectively (Fig. 2C).

**AICD in Th17 cells requires the Fas/FasL pathway**

To investigate the mechanism by which Th17 cells undergo AICD, cells from C57BL/6 mice were restimulated in the presence of specific inhibitors of apoptosis: blocking Ab against FasL (MFL3), soluble death receptor-5 (DR5, a TRAIL receptor), or z-VAD-FMK (a pan-caspase inhibitor). Both z-VAD-FMK and anti-FasL effectively inhibited AICD of Th17 cells as detected by DNA content analysis. There were 35.3 ± 3% of anti-CD3 treated cells possessing hypodiploid DNA content, while the inclusion of z-VAD-FMK (14.0 ± 0.7%) or MFL3 (12.0 ± 1.6%) completely inhibited AICD in Th17 cells (13.9 ± 2% in untreated control cells) (ANOVA; p < 0.001), whereas DR5 had no effect (Fig. 2B). These results suggest that AICD in Th17 cells is initiated by FasL.

---

**FIGURE 2.** AICD in Th17 cells was blocked by a pan-caspase inhibitor and anti-FasL. **A,** Extent of AICD in Th17 cells is revealed in differentiated Th17 cells from either BALB/c or C57BL/6 mice after restimulation with plastic-bound anti-CD3 with or without IL-2 or IL-4 supplementation for 12 h. Cells were permeabilized and stained with propidium iodide for determination of apoptosis by flow cytometric DNA content analysis. The percentage of apoptotic cells as represented by the hypodiploid peak is given. Differences of apoptosis before and after anti-CD3 reactivation were analyzed by ANOVA (***, p < 0.001). Because IL-2 supplementation prevented cell death from cytokine deprivation, IL-2 was added to all cultures in all subsequent experiments. **B,** Differentiated Th17 cells from C57BL/6 mice were restimulated with plastic-bound anti-CD3 with or without DR5, z-VAD-FMK, or MFL3 for 24 h, and apoptosis tested as above. Differences among groups were tested by ANOVA (p < 0.01). **C,** AICD in Th1 and Th17 cells was compared after 12- or 24-h restimulation with anti-CD3, and the extent of apoptosis was determined. Percentages of apoptotic cells indicated were determined after subtracting background cell death occurring in presence of IL-2 alone. Differences between Th1 and Th17 cells were analyzed by ANOVA (**, p < 0.01). **D,** Expression of Fas and FasL was determined in Th17 cells after restimulation with anti-CD3 for 12 h, and surface expression of Fas and FasL analyzed by direct immunofluorescence and flow cytometry. Plot depicts Th17 cells without restimulation (gray line), after restimulation (heavy line), and after restimulation stained with isotype control (light line). **E,** The induction of apoptosis by Ab ligation of Fas was tested in Th17 cells cultured with anti-Fas (JO2) for 24 h. All results are representative of two separate experiments.
(CD95L), but not TRAIL, and proceeds through a caspase-dependent pathway.

To further confirm the role of Fas and FasL in AICD of Th17 cells, the expression of Fas and FasL on the cell surface was examined by flow cytometric analysis before and after anti-CD3 restimulation. We found that differentiated Th17 cells constitutively express Fas at high levels, whereas FasL expression was significantly up-regulated following restimulation (Fig. 2D). Next, to determine whether Th17 cell apoptosis could be induced by direct Ab ligation of Fas, we added anti-Fas (JO2) to Th17 cells and found significant apoptosis within 24 h (Fig. 2E). Thus, Th17 cells express Fas and FasL, and undergo apoptosis in response to Fas signaling.

Previous studies of AICD in activated T cells have found that apoptosis can be induced by FasL in either the membrane-associated or soluble forms. We differentiated between these two alternatives by using the Transwell system. In brief, Th17 cells were seeded into both the upper and lower chambers separated by a semipermeable membrane (0.4 μm pores) in a 6-well plate with only the lower chambers coated with anti-CD3 or control IgG. We found no increase in apoptosis in unstimulated cells from the upper chamber, whereas apoptosis did occur in the lower chamber, as expected (Fig. 3A). This suggests that AICD in Th17 cells depends mainly on cell surface-associated FasL. These cells are sensitive to soluble FasL, however, because addition of exogenous FasL directly did induce Th17 cell death (Fig. 3B).

To further verify the involvement of Fas/FasL in the deletion of activated Th17 cells, we generated Th17 cells from splenocytes of gld/gld mice, which have a functional mutation in the Fasl gene. Anti-CD3-restimulation failed to induce AICD in gld/gld Th17 cells (Fig. 4, A and B). Thus, FasL deficiency renders Th17 cells resistant to AICD, providing further evidence that AICD in Th17 cells is mediated by Fas-FasL interaction. Furthermore, stimulated gld/gld CD4+ T cells expressed significantly higher levels of both IL-17A and IL-17F compared with that of wild-type cells (Fig. 4C).

**AICD of Th17 cells is not affected by IFN-γ signaling**

IFN-γ has been shown to be critical for AICD of activated T cells (25), especially Th1 cells (26). Because the differentiation of Th17 cells does not require IFN-γ, yet these cells are still susceptible to AICD, we wondered whether IFN-γ has a role in controlling AICD in Th17 cells. To test this, we first removed or supplemented IFN-γ during the induction of AICD. We found that neither Ab blockade of IFN-γ nor exogenously added IFN-γ had any effect on Th17 AICD (Fig. 5A). In addition, we differentiated Th17 cells from mice deficient in the IFN-γ receptor 1 (IFN-γR1−/−). Differentiation quality was also checked by intracellular staining of IL-17A (data not shown) and we found that Th17 cells derived from these mice underwent AICD after anti-CD3-restimulation to the same extent as wild-type Th17 cells (Fig. 5B). Thus, the absence of the receptor for IFN-γ did not affect AICD of Th17 cells, providing evidence that IFN-γ is not involved. Furthermore, IFN-γR1−/− Th17 cells continuously expressed Fas, demonstrated up-regulation of FasL upon restimulation, and were also sensitive to crosslinked anti-Fas-induced apoptosis (Fig. 5, C and D).

Because IFN-γ has been shown to be required for the up-regulation of caspase-8 in restimulated T cells, we next examined caspase-8 expression in Th17 cells using Western blotting analysis. We found that caspase-8 levels in IFN-γR1−/− Th17 cells were as high as that in wild-type controls (Fig. 6). Furthermore, caspase-3 cleavage was also found to be similar in both cell types (Fig. 6). Thus, the expression and activation of caspases in Th17 cells seems to be unaffected by the absence of IFN-γ signaling. Next, because Fas signaling in restimulated T cells may also induce the mitochondrial pathway of apoptosis, we examined the expression of the pro-apoptotic Bcl-2 family protein BID and its truncated form tBID. We found that Th17 cells from both IFN-γR1−/− and wild-type mice had similar levels of both BID and tBID up-regulation after reactivation. Taken together, these results demonstrate that re-stimulated Th17 cells undergo AICD through a Fas/FasL-mediated pathway that is unaffected by IFN-γ.
Discussion
Proper elimination of activated T cells that have completed their effector function is important for immune homeostasis. Induction of apoptosis following re-stimulation through the TCR, as modeled in vitro by AICD, is believed to be important for removal of activated T cells under certain circumstances, and elucidation of its mechanisms has provided important clues for understanding the nature of various immune responses and autoimmune diseases. Although AICD in activated T cells is believed to be mediated through Fas, other mechanisms of AICD have been reported in different subsets of effector T cells (32). Because Th17 cells are believed to be critical in the pathogenesis of autoimmune diseases and some infections, elucidation of the mechanisms through which these cells undergo AICD is important in advancing our understanding of the regulation of this important helper T cell population. In the present study, we showed that Th17 cells undergo apoptosis after reactivation through their TCR. It has been shown that AICD in other T cell subsets requires IL-2 and IL-4 (28, 33), and we found that without IL-2 or IL-4 supplementation, differences in cell death with or without reactivation are minimal. Therefore, IL-2 is included in all our experiments. We found that Th17 cells constitutively express Fas and up-regulate FasL expression upon TCR activation. We presented evidence that the induction of AICD in Th17 cells is mediated mainly by the Fas-FasL pathway, as it was completely blocked by Ab neutralization of FasL, whereas soluble DR5 (to block TRAIL binding) had no effect. We also found that Th17 cells were susceptible to agonistic anti-Fas-induced apoptosis without reactivation. Furthermore, Th17 cells generated from gld/gld mice were completely resistant to AICD. Moreover, after PMA and ionomycin stimulation of CD4+ T cells in gld/gld mice, a higher level of IL-17A and IL-17F mRNA levels were shown compared with those in the control mice. These data suggest that the Fas-FasL pathway is involved in the deletion of Th17 cells, especially in the process of AICD.

We also showed that when separated by a permeable membrane, resting Th17 cells were not killed by reactivated Th17 cells despite increased FasL expression on the cell surface of reactivated Th17 cells, indicating that the FasL that mediates AICD in Th17 cells are mostly membrane-associated. Thus, we conclude that membrane associated FasL is the prime mediator of AICD in Th17 cells.

A recent study of the relationship between cell surface glycosylation and susceptibility to cell death showed that Th1 and Th17 cells share a common glycan motif, which may contribute to a shared pathway of AICD (34).

Various cytokines and other factors have been demonstrated to modulate AICD. Among them, IFN-γ has been shown by a series of studies to be essential for the AICD of T cells, including Th1 cells (25, 26). In the present study, however, we found that IFN-γ R1-deficient Th17 cells underwent apoptosis as well as their wild-type counterparts, and the addition of anti-IFN-γ or recombinant
mouse IFN-γ had no effect on the reactivation-induced apoptosis of Th17 cells. These data suggest that the AICD of Th17 cell is independent of IFN-γ.

Some studies have shown that IFN-γ promotes T cell death by increasing the expression of FasL (22), whereas others have demonstrated that STAT-1-deficient T cells have normal FasL expression (25). Consistent with the latter, we found that normal levels of FasL are induced in IFN-γR1−/− Th17 upon reactivation, and the death of these cells can also be largely abolished by Ab neutralization of FasL. It has been previously reported that IFN-γ-deficient T cells are resistant to anti-Fas-induced apoptosis and that signaling events downstream of Fas, including caspase up-regulation and activation, are also defective in these cells. However, unlike a report that found that T cells from IFN-γ−/− or STAT-1−/− mice are resistant to anti-Fas-induced apoptosis (25), we showed that Th17 cells from IFN-γR1−/− mice are sensitive to anti-Fas-induced apoptosis. We also showed that the levels of expression of caspase 8 and caspase 3, including cleaved caspase 3, were comparable in IFN-γR1−/− Th17 cells and their wild-type counterparts. Similar increases in Bid expression after TCR ligation were observed in both IFN-γR1−/− and wild-type Th17 cells. Therefore, our data indicate that the AICD of Th17 cells, unlike that of other T cell subsets, is unaffected by IFN-γ signaling. The IFN-γ-independent AICD of Th17 cell may be derived from the education they receive from factors or cytokines during their differentiation, which may sensitize Th17 cells to Fas-induced death. Our finding that AICD in Th17 cells is mediated mainly by the Fas/FasL interaction and is independent of IFN-γ reveals a novel aspect of Th17 cell homeostasis. Further research into the mechanisms regulating activation or Ag-induced apoptosis of Th17 cells may eventually lead to improved modalities for clinical modulation of the immune response for the treatment of autoimmune diseases.

**FIGURE 5.** IFN-γ is not required for AICD in Th17 cells. A, Differentiated Th17 or Th1 cells were restimulated with plastic-bound anti-CD3 for 24 h in the presence of anti-IFN-γ (10 μg/ml) or exogenous recombinant IFN-γ (10 ng/ml), and apoptosis determined. B, Live Th17 blasts from IFN-γR1−/− or wild-type mice were purified on Ficoll, restimulated with plastic-bound anti-CD3 for 24 h, and apoptosis determined. C, Th17 cells generated from IFN-γR1−/− mice were examined before and after restimulation for 12 h for the expression of Fas and FasL by cell surface immunofluorescence staining and flow cytometry. Plot depicts Th17 reactivity determined after 24 h. Representative of two separate experiments.

**FIGURE 6.** Expression and activation of apoptosis mediators in Th17 cells. IFN-γR1−/− and wild-type Th17 cells were cultured with (+) or without (−) anti-CD3 restimulation in the presence of IL-2 for 12 h, as indicated. Cells were harvested, total protein was isolated, and apoptosis mediators were analyzed by Western blotting. The presence of caspase 3 (both full-length procaspase-3 [35 kD] and the active cleaved large fragment [17–19 kD]), procaspase-8, Bid (22 kD), and cleaved Bid (tBid, 15 kD) were determined. β-actin served as a loading control. Representative of two separate experiments.
Disclosures
The authors have no financial conflict of interest.

References