Apoptosis of thyrocytes and effector cells during induction and resolution of granulomatous experimental autoimmune thyroiditis

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Abstract

Experimental autoimmune thyroiditis (EAT) with granulomatous histopathology (G-EAT) can be induced by cells from mouse thyroglobulin (MTg)-immunized donors activated in vitro with MTg and IL-12. G-EAT lesions reach maximum severity 18–21 days after cell transfer and, if some thyroid follicles remain, lesions almost completely resolve by day 35. CD8+ cells are required for G-EAT resolution. To begin to determine the mechanisms involved in G-EAT resolution, apoptosis in thyroids was analyzed by TUNEL staining. Apoptotic thyrocytes and inflammatory cells were present in the thyroids of both CD8+/c9059 and CD8-depleted recipient mice at day 19–21. By day 35, apoptotic cells were rare in thyroids of mice whose lesions had resolved; the few apoptotic inflammatory cells were generally in close proximity to thyroid follicles. Thyroids of CD8-depleted mice had ongoing inflammation at day 35 and most apoptotic cells were thyroid follicular cells. The expression of Fas and Fas ligand (FasL) mRNA in thyroids was also determined by RT-PCR in both CD8+ and CD8-depleted recipient mice. Fas was expressed in normal thyroids and its expression was relatively constant throughout the course of disease. FasL mRNA was not expressed in normal thyroids. FasL mRNA expression generally correlated with G-EAT severity, being maximal at day 21 and diminishing as lesions resolved. However, FasL mRNA expression in thyroids of CD8-depleted mice in which resolution was delayed was decreased compared to thyroids of CD8+ mice with comparable disease severity, suggesting that FasL expressed by CD8+ cells may play a role in G-EAT resolution.

Introduction

Experimental autoimmune thyroiditis (EAT) is characterized by infiltration of the thyroid gland by mononuclear cells and production of mouse thyroglobulin (MTg)-specific autoantibody (1). EAT can be induced by transfer of spleen cells from MTg-primed donor mice activated in vitro with MTg (1,2). Recipients of MTg-sensitized cells cultured with MTg and anti-IL-2R mAb or MTg, anti-IL-2R mAb and IL-12 develop a more severe, histologically distinct, granulomatous form of EAT characterized by follicular cell proliferation, large numbers of histiocytes, multinucleated giant cells and variable numbers of neutrophils in addition to T lymphocytes (3,4). Granulomatous EAT (G-EAT) lesions reach maximum severity 19–21 days after cell transfer and, if some thyroid follicles remain, these lesions almost completely resolve by day 35–60 (3,4). The primary effector cells for G-EAT are CD4+ T cells (3,4). CD8+ cells are not required for G-EAT development, but are required for the early resolution of G-EAT (5,6).

An important issue for understanding the healing or resolution of inflammation is to define the mechanisms that eliminate inflammatory cells when they are no longer needed. Apoptosis (programmed cell death) is one important mechanism for clearance of inflammatory cells during resolution of inflammation (7,8). Apoptosis has been shown to be involved in the resolution of some viral infections (9) and in resolution of G-EAT.
of lesions induced by *Leishmania major* (10). Apoptosis has also been suggested to play a role in the recovery phase of autoimmune diseases such as experimental allergic encephalomyelitis (EAE) (11–14). One mechanism of apoptosis is activation-induced cell death (AICD) which results after stimulation of T cells by antigen. Both the tumor necrosis factor (TNF) and Fas pathways are associated with AICD (8,15,16). Fas (CD95) is a member of the TNF receptor family of cell surface proteins, and Fas ligand (FasL) (CD95L) is a member of the TNF family of membrane and secreted proteins. Signaling through Fas leads to apoptotic cell death, with characteristic cytoplasmic and nuclear condensation and DNA fragmentation (15–17). Apoptosis due to Fas–FasL interactions can be induced in resolution of inflammation and also in mediating damage to target tissue in autoimmune diseases (11,12,14,18–24).

Several studies have suggested that apoptosis of thyroid follicular cells is one mechanism for tissue destruction in patients with Hashimoto’s thyroiditis, subacute thyroiditis and Grave’s disease (25–35). Apoptosis in thyroid of Hashimoto’s thyroiditis patients was suggested to result from the interaction of Fas and FasL, both expressed by thyrocytes (31–33). However, results reported using a mouse model of EAT suggested that FasL expression by thyrocytes could inhibit tissue destruction by inducing apoptosis of infiltrating T cells (23,24). It has also been shown that CD8+ T cells can regulate immune responses by eliminating CD4+ T cells via Fas–FasL-induced apoptosis or other mechanisms (36–38) and activated Fas+ T cells can be deleted through interaction with FasL expressed on epithelial cells or dendritic cells (39,40). Thus, apoptosis mediated by the Fas and FasL pathway may play an important role in regulating inflammation in autoimmune diseases.

This study was undertaken to determine if apoptosis and/or Fas and FasL interactions might play a role in the induction and/or resolution of G-EAT. Our results suggest that apoptosis may be involved in destruction of thyrocytes as well as effector CD4+ T cells and that FasL, expressed by CD8+ cells may play a role in the resolution of thyroid autoimmune inflammatory responses.

Methods

**Mice**

CBA/J mice were obtained through Mr C. Reeder (National Institutes of Health, Bethesda, MD). Female mice, 6–8 weeks old, were used for all experiments.

**G-EAT induction**

G-EAT was induced as previously described (3). Briefly, mice were injected i.v. twice at 10 day intervals with 150 µg MTg and 15 µg lipopolysaccharide (*Escherichia coli* 011:B4; Sigma, St Louis, MO) in a volume of 0.5 ml. Seven days later, donor spleen cells were re-stimulated for 72 h with 25 µg/ml MTg in the presence of 5% final concentration of culture supernatant containing the anti-IL-2R mAb M7/20 and 5 ng/ml IL-12 (3,4). Cells were harvested, washed twice with balanced salt solution and 3.5×10^7 cells were transferred iv. into 600 rad-irradiated syngeneic recipients. Recipient thyroids were collected at different times after cell transfer for histologic evaluation of EAT. CD8+ T cells were depleted by i.p. injection of 250–300 µg anti-CD8 mAb (116-13.1) (ATCC HB-129) on days 1 and 12 after cell transfer, and at 10 day intervals thereafter (5,6). Effectiveness of splenic CD8+ T cell depletion (<2% of spleen cells) was confirmed by flow cytometry when thyroids were removed.

**Evaluation of EAT**

Thyroids were scored quantitatively for EAT severity (the extent of thyroid follicle destruction) using a scale of 1+ to 5+ as described previously (3,4). 1+ thyroiditis is defined as an infiltrate of at least 125 cells in one or several foci, 2+ is 10–20 foci of cellular infiltration involving up to 25% of the gland, 3+ indicates infiltration of 25 to 50% of the gland, 4+ indicates that >50% of the gland is destroyed and 5+ indicates almost complete destruction of the gland with few or no remaining follicles. Thyroid lesions were also evaluated qualitatively (4). The thyroid infiltrates in lymphocytic EAT consist of lymphocytes and some plasma cells with relatively few neutrophils. Thyroids with 1–2+ severity scores have primarily lymphocytic features. More severely destroyed thyroids (3–5+ severity scores) had granulomatous changes with proliferation and enlargement of thyroid follicular cells, and infiltration by histiocytes, multinucleated giant cells, lymphocytes and increased neutrophils. Very severe 4–5+ granulomatous lesions also had microabscess formation, necrosis and fibrosis (4).

**Immunohistochemistry and TUNEL staining**

Apoptosis was determined by the TUNEL technique using the ApopTag kit (Intergen, Purchase, NY) with paraffin-embedded formaldehyde-fixed thyroid sections according to the manufacturer’s instructions. To determine if CD4+ T cells were apoptotic, TUNEL staining was combined with immunohistochemistry (41). Frozen thyroid sections were fixed with 4% paraformaldehyde for 10 min, followed by digestion with proteinase K for 15 min. Slides were incubated with 1% PBS/BSA for 30 min, then incubated with the primary antibody [anti-CD4 (GK1.5) (ATCC TIB 207) or isotype control] for 30 min at room temperature. Slides were rinsed in PBS and incubated with a biotinylated goat anti-rat IgG (Caltag, Burlingame, CA) for 30 min. Slides were incubated with 0.3% H₂O₂/0.1% sodium azide in PBS for 45 min to quench endogenous peroxidase activity. The antibody–biotin conjugates were detected with an avidin–biotin–horseradish peroxidase complex (Vectastain Elite ABC; Vector, Burlingame, CA) according to the manufacturer’s instructions. Enzyme activity was detected with diaminobenzidine. Apoptotic cells were then determined by TUNEL staining. TUNEL-positive cells were revealed by VIP substrate (Vector) and slides were counterstained with hematoxylin.

**RT-PCR amplification**

Thyroid lobes were removed from recipient mice, individually snap-frozen in liquid nitrogen and stored at −70°C until used. Blood was excluded as much as possible and thyroids were trimmed to remove non-thyroid tissue. Thyroid tissue was homogenized by grinding the frozen thyroid lobe between the frosted ends of two glass microscope slides in 1 ml Trizol
(BRL/Life Technologies, Gaithersburg, MD) and RNA was isolated according to the manufacturer's instructions. The RNA pellet was dissolved and total RNA was converted to cDNA as previously described (6,42,43). To determine the relative initial amounts of target cDNA, each cDNA sample was serially diluted 1/5, 1/25 and 1/125, and each dilution was amplified with cytokine-specific primers. HPRT was used as a housekeeping gene to verify that the same amount of RNA was amplified (6). Primer sequences used in this study were: Fas sense: ATC GCA GCT CTG AGG AGG CCG GTT CAT GAA AC; anti-sense: GGA GGT TCT AGA TTC GGT GCC GTT TCC; CD3 sense: GCA GCT GGC AAA GGT GGT GTC TTC; anti-sense: TAT GGC ACT TTG AGA AAC CTC CAT. CD8 sense: TGG CAC GAC AGA ACT GAA GTA CAT C. To compare relative levels of mRNA transcripts between different groups, samples were reverse transcribed and amplified at the same time using aliquots of reagent from the same master mix. PCR was performed as previously described (6,42,43). PCR products were separated by electrophoresis in 3% agarose gels, visualized by UV light following ethidium bromide staining and analyzed using a digital imager. Samples within the linear relationship between input cDNA and final PCR products (usually 1/25 cDNA dilution) were collected, and the densitometric units for each cytokine band was determined with cytokine-specific antibodies. PCR results were confirmed by quantitative real-time PCR as previously described (6,42,43).

Donor spleen cells were activated with MTg and anti-IL-2R (rows 4–7) or with MTg, anti-IL-2R and IL-12. Anti-CD8 mAb was given to donors (rows 3, 6 and 7) 5 and 2 days before culture; CD8 depletion in the spleen was determined to be complete by flow cytometry. Recipients (rows 2, 5 and 7) were given anti-CD8 mAb 1 and 12 days after cell transfer, and at 10 day intervals thereafter.

Number of mice with various degrees of severity of EAT at day 19 or day 35 or 60 after cell transfer (day 35 for mice in lines 1–3 and day 60 for mice in lines 4–7). 

### Expression of Fas and FasL mRNA in G-EAT thyroids

Apoptosis can contribute to tissue damage in autoimmune inflammatory lesions (8) and may also be a major mechanism for clearance of inflammatory cells during resolution of inflammation (7–14). Fas and FasL interactions can play an important role in induction of apoptosis (15,16). To begin to determine if Fas–FasL interactions might have a role in the induction or resolution of G-EAT, the kinetics of expression of Fas and FasL mRNA in the granulomatous thyroids were

### Table 1. Requirement for recipient CD8+ T cells for resolution of G-EAT

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aDonor spleen cells were activated with MTg and anti-IL-2R (rows 4–7) or with MTg, anti-IL-2R and IL-12. Anti-CD8 mAb was given to donors (rows 3, 6 and 7) 5 and 2 days before culture; CD8 depletion in the spleen was determined to be complete by flow cytometry. Recipients (rows 2, 5 and 7) were given anti-CD8 mAb 1 and 12 days after cell transfer, and at 10 day intervals thereafter.

bNumber of mice with various degrees of severity of EAT at day 19 or day 35 or 60 after cell transfer (day 35 for mice in lines 1–3 and day 60 for mice in lines 4–7). G = number of mice with granulomatous thyroid lesions.

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**Results**

**CD8+ cells are required for resolution of G-EAT**

G-EAT was induced by spleen cells from MTg/LPS primed donor mice activated in vitro with MTg, anti-IL-2R mAb and IL-12 (Table 1). G-EAT lesions reached maximum severity 19–21 days after cell transfer (3,4) (Table 1). When recipient mice developed 3–4+ G-EAT with some follicles remaining at day 19–21, lesions almost completely resolved (0–1+) in most mice by day 35 (Table 1, rows 1 and 3). Treatment of recipient mice with anti-CD8 mAb, which eliminates both donor and recipient CD8+ cells, delayed G-EAT resolution so that at day 35 or 60, thyroid lesions were similar in severity to those observed at day 19 (Table 1, rows 2, 5 and 7). Depletion of only donor CD8+ cells had no effect on resolution (Table 1, rows 3 and 6), suggesting that the CD8+ cells that promote resolution of G-EAT can be primarily of recipient origin, and do not need to be primed by MTg and LPS. Depletion of donor CD8+ cells or both donor and recipient CD8+ cells had little effect on the severity of lesions at day 19, and thyroid lesions in both control and CD8-depleted mice developed with the same kinetics through day 19–21 (Table 1 and data not shown). This suggests that neither donor nor recipient CD8+ cells are required for induction of G-EAT in this model, but recipient CD8+ T cells are required for the early resolution of G-EAT. Figure 1 shows typical thyroid histopathology for untreated recipients of sensitized, activated lymphocytes from untreated or anti-CD8-treated donors and from anti-CD8-treated recipient mice 19 and 35 days after cell transfer. Many thyroid follicles were destroyed by infiltrating inflammatory cells, including neutrophils, and mononuclear cells in all three groups at day 19 (Fig. 1A, C and E). These changes persisted in anti-CD8 treated recipients at day 35 (Fig. 1F), while thyroids of untreated recipients had only minimal infiltration at day 35 (Fig. 1B). Thyroids of recipients of cells from anti-CD8-treated donors were comparable to those of untreated recipients at both day 19 and day 35 (Fig. 1C and D).
CD8⁺ T cells are required for resolution of G-EAT. Shown are H & E-stained thyroid sections from recipients of spleen cells from MTg- and LPS-immunized CBA/J donors that were cultured for 72 h with MTg and anti-IL-2R mAb in the presence of IL-12. Recipients were either untreated (A–D) or given anti-CD8 mAb (E and F) on day 1 and 12 after cell transfer, and at 10 day intervals thereafter. In (C) and (D), donors were CD8-depleted and recipients were untreated. Thyroids were removed 19 (A, C and E) or 35 (B, D and F) days after cell transfer. Magnification ×100. At day 19, thyroids from all three groups had similar G-EAT severity scores (4+); by day 35, lesions in untreated recipients of CD8-depleted or untreated donor cells (B and D) had resolved (1+), while thyroids of anti-CD8-treated recipients (F) had ongoing inflammation (4+).

analyzed by RT-PCR (Fig. 2). FasL mRNA was not detected in thyroids of normal mice or of mice that received effector cells but had not yet developed EAT 3 days after cell transfer. FasL mRNA was increased in thyroids of recipient mice with G-EAT. FasL mRNA expression was maximal at day 21 and diminished through day 35 as lesions resolved (Fig. 2).
general, levels of expression of FasL mRNA correlated with the EAT severity score, which is indicative of the number of infiltrating cells. This suggests that some infiltrating cells may express FasL, while thyrocytes may be FasL negative and/or that FasL may be induced on thyrocytes during inflammation (28–31,34,35). In contrast to FasL expression, Fas mRNA was detected in both normal and G-EAT thyroids. Fas mRNA expression was relatively constant throughout the course of G-EAT, although it was usually greatest at the time of peak disease severity, 21 days after cell transfer (Fig. 2). These results suggest thyrocytes constitutively express Fas mRNA, as demonstrated by others (28–31).

**FasL mRNA is decreased in thyroids of mice depleted of CD8+ cells**

Expression of FasL mRNA in thyroids of untreated and anti-CD8-treated recipients was compared using RT-PCR. Compared to thyroids with 4–5+ G-EAT (day 21 untreated and anti-CD8 treated mice), lower levels of mRNA for CD3 and FasL were detected in thyroids of untreated mice with resolving (1+) lesions at day 35 (Fig. 3b). Thyroids of anti-CD8-treated mice still had 4–5+ G-EAT 35 days after cell transfer. CD8 mRNA was markedly decreased compared to thyroids of untreated mice at both day 21 and 35 after cell transfer, indicating that anti-CD8 treatment greatly reduced the numbers of CD8+ cells in the thyroid infiltrates as demonstrated previously using flow cytometry for analysis of thyroid-infiltrating cells (44). Thyroids of both untreated and anti-CD8 treated recipient mice with 4–5+ G-EAT had similar levels of both Fas and CD3 mRNA. CD3 message in thyroids of untreated and anti-CD8-treated mice is comparable despite the marked decrease in CD8+ T cells in the latter, because CD8-depleted thyroids have increased numbers of CD4+ T cells compared to thyroids of untreated mice (44 and Y. Wei, unpublished results). Interestingly, FasL mRNA expression was decreased in thyroids of anti-CD8 treated mice with 4–5+ G-EAT at day 35, suggesting that CD8 depletion decreased the number of FasL-expressing cells (Fig. 3b). In most experiments, FasL mRNA was also reduced in thyroids of anti-CD8-treated mice compared to untreated mice at day 21, but was comparable to that of untreated mice at day 10 (Fig. 3a). These results suggest that CD8+ cells may contribute, in part, to FasL mRNA expression in thyroid infiltrates, particularly from day 21 through resolution, and FasL mRNA expression is decreased in thyroids of CD8-depleted mice with delayed resolution of inflammatory lesions.

**Apoptosis of thyrocytes and CD4+ T cells in G-EAT thyroids**

To determine if apoptosis could play a role in development or resolution of G-EAT, in situ apoptosis in thyroids was determined using the TUNEL technique. Numerous apoptotic cells were detected in granulomatous thyroids 21 days after cell transfer (Fig. 4b–f). Apoptotic thyroid epithelial cells were usually enlarged and cuboidal, in contrast to normal thyroid follicular cells, which were small and flat in paraffin sections (Fig. 4f). Both thyroid follicular cells and infiltrating cells were TUNEL-positive, indicating that both target and infiltrating cells can be apoptotic (Fig. 4b, f). Apoptotic cells, including both thyrocytes and some inflammatory cells, were also present in thyroids of CD8-depleted mice at day 21 (Fig. 4c). Apoptotic cells were evident in most thyroid sections from both control and CD8-depleted mice 14–21 days after cell transfer (data not shown). Normal thyroids had no detectable apoptotic cells under the same experimental conditions (Fig. 4d). Combined immunohistochemistry and TUNEL analysis indicated that some CD4+ T cells in day 21 G-EAT thyroids were apoptotic (Fig. 4e). These results suggest that apoptosis occurs in both target (thyrocytes) and effector CD4+ T cells in G-EAT.

At 35 days after cell transfer, thyroid lesions of untreated recipients had mostly resolved (1+ severity). Thyrocytes were no longer apoptotic and only a few apoptotic inflammatory cells were detected (Fig. 5a). The scattered apoptotic inflammatory cells were generally located in close proximity to thyroid follicles (Fig. 5b). Thyroids of CD8-depleted mice had ongoing inflammation (4+) at day 35 and there were numerous...
Apoptosis of target and effector cells in G-EAT

Fig. 3. Expression of Fas, FasL, CD8 and CD3 mRNA in thyroids of untreated and anti-CD8-treated recipients 10, 20 or 35 days after cell transfer. Results are expressed as the ratio of Fas, FasL, CD8 or CD3 to HPRT densitometric units for 1/25 cDNA dilutions ± SEM for individual thyroids from four or five mice per group as described in the legend to Fig. 2.

apoptotic cells. These included both thyrocytes and inflammatory cells (Fig. 5c and d), although there were usually fewer apoptotic inflammatory cells compared to day 21 thyroids (Fig. 4b and c, and data not shown).

Discussion

In this study, apoptotic thyroid follicular cells and infiltrating inflammatory cells were demonstrated in thyroids of mice with G-EAT using TUNEL staining. Combined immunohistochemistry and TUNEL staining indicated that some infiltrating CD4+ T cells and numerous thyrocytes were apoptotic when G-EAT severity was maximal. These results suggest that apoptosis could be involved both in inducing damage to thyrocytes and in eliminating CD4+ effector T cells or other inflammatory cells during the course of G-EAT. Since thyroid lesions in G-EAT almost completely resolved between 21 and 35 days after cell transfer (Table 1 and Fig. 1), elimination of inflammatory cells by apoptosis may be one mechanism for resolution of these lesions.

Autoimmune disease is characterized by the destruction of target tissue by infiltrating inflammatory cells. Several studies have suggested that apoptosis is involved in the pathogenesis of autoimmune thyroid diseases. Apoptotic cells have been demonstrated in thyroids of patients with Hashimoto’s thyroiditis, subacute thyroiditis and Grave’s disease (25–35). Most apoptotic cells in Hashimoto’s thyroiditis were in areas of disrupted follicles and infiltrating lymphoid cells (30–32), and in granulomas and areas of regenerating follicles in subacute thyroiditis (33). The mechanism of thyroid damage by apoptosis is not completely understood. One group showed that co-expression of Fas and FasL by thyrocytes may lead to apoptosis and tissue damage. Both normal and inflamed thyroids expressed FasL, while Fas was up-regulated during inflammation (26,27). However, other studies have shown that normal human thyroids express Fas, but not FasL both at the mRNA and protein level, although both Fas and FasL can be expressed by thyrocytes in inflamed thyroids (28,30,32,33).

Similarly, normal mouse thyroids were shown in this study (Fig. 2) and by others (24) to be FasL–, and our results showed that normal thyroids expressed Fas mRNA (Fig. 2). FasL mRNA was expressed only in G-EAT thyroids and the level of expression generally correlated with disease severity (Fig. 2). While these results could suggest that FasL is expressed primarily by thyroid infiltrating cells, assessment of FasL mRNA expression by RT-PCR does not allow us to draw any conclusions about the cellular source of the FasL mRNA. Recent results using in situ hybridization and immunohistochemical staining for FasL indicate that both thyrocytes and infiltrating cells in G-EAT thyroids express FasL, and FasL expression is decreased in thyroids of anti-CD8-treated mice (Wei et al., manuscript in preparation). These results suggest
Fig. 4. Detection of apoptotic cells in thyroids of untreated and anti-CD8-treated recipients 21 days after cell transfer. G-EAT was induced as described in Methods. Apoptotic cells (brown) were determined using the TUNEL technique. Sections were counterstained with hematoxylin. (A) Thyroid from an untreated recipient with 4+ G-EAT in which the terminal deoxynucleotide transferase was omitted shows no apoptotic cells. (B) A 4+ G-EAT thyroid stained using terminal deoxynucleotide transferase shows numerous apoptotic cells, including both thyroid follicular cells and infiltrating cells. (C) Thyroid from an anti-CD8-treated recipient at day 21 (4+ G-EAT) demonstrates similar staining of apoptotic thyroid follicular cells and some infiltrating cells. (D) Normal CBA/J thyroids are negative for apoptosis. (E) Co-localization of CD4+ T cells and apoptotic cells in a thyroid with 4+ G-EAT 21 days after cell transfer. Thyroid frozen sections were fixed with 4% paraformaldehyde and CD4+ T cells were labeled using anti-CD4 (GK 1.5) followed by anti-rat IgG. Positive cells were revealed by DAB (brown). Apoptotic cells were detected by TUNEL and VIP (purple) was used as substrate. The slide was counterstained with hematoxylin. (F) High-power view demonstrating enlarged TUNEL-positive thyroid follicular cells (upper left) and a non-apoptotic thyroid follicle in which the follicular cells are not enlarged (lower right). Magnification A–D, ×400; E and F, ×1000.
that FasL may be induced on thyrocytes during inflammation, as has been reported for human autoimmune thyroid diseases (26–30,32–34). We are currently examining the possibility that FasL expression on thyrocytes is induced by the infiltrating CD8+ cells, since activated T cells can induce FasL expression on some non-lymphoid tissues (39).

Although CD4+ T cells are the primary effector cells for G-EAT (3,4), CD8+ T cells outnumber CD4+ T cells in G-EAT thyroids (44). Although others have suggested that CD8+ T cells can contribute to thyroid damage in EAT (45), depletion of donor or both donor and recipient CD8+ cells has little effect on the kinetics of disease induction or on the severity of the lesions that develop through day 21 in our adoptive transfer G-EAT model (5) (Table 1). Our studies indicate that CD8+ T cells have a regulatory role in G-EAT since depletion of CD8+ T cells in recipient mice delays the resolution of granulomatous thyroid lesions (5,6) (Table 1). The results presented here extend these observations by demonstrating that the CD8+ cells that promote resolution of G-EAT can be derived from non-immunized recipient mice, since depletion of only donor CD8+ cells had no effect on resolution (Table 1). We have previously reported that the CD8+ T cells in G-EAT thyroids contribute minimally, if at all, to cytokine expression (6), although they express IL-2R and have a memory phenotype (44), and may express FasL (Fig. 3). This suggests the thyroid-infiltrating CD8+ cells become activated before or after they migrate to the thyroid, possibly by thyroid antigens released when the gland is damaged (46) or by the CD4+ effector T cells (38,47). It is also possible that the recipient CD8+ cells that promote G-EAT resolution could be CD8+ dendritic cells and not CD8+ T cells, since CD8+ dendritic cells can express FasL and induce apoptosis of CD4+ T cells (40). Dendritic cells could also activate CD8+ T cells by presenting thyroid antigens or peptides derived from apoptotic or necrotic CD4+ T cells via cross-priming (48,49).

Studies are currently in progress to address these important issues.

Although a regulatory role for CD8+ T cells in autoimmune
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Diseases have been demonstrated (49–52), the mechanism of CD8+ T cell-dependent regulation is still poorly understood. One mechanism by which CD8+ T cells can kill target cells or regulate immune responses is through Fas–FasL interactions (15,16). In an animal model of acute graft-versus-host disease, Fas and FasL up-regulation was critically dependent on antigen-specific CD8+ T cell activation (37). In addition, activated CD8+ T cells can induce Fas-mediated apoptosis of antigen-activated CD4+ T cells (36) and CD8+ dendritic cells can induce apoptosis of CD4+ T cells (40). Activated CD8+ T cells can also delete CD4+ T cells via recognition of their TCR determinants (38) or by as yet undefined mechanisms (53). In this study, depletion of recipient CD8+ cells resulted in decreased expression of FasL mRNA in thyroids (Fig. 3), suggesting that CD8+ cells in thyroid infiltrates may express FasL. FasL-expressing CD8+ cells could promote G-EAT resolution by inducing apoptosis of Fas-expressing inflammatory cells. Fas–FasL interactions have been shown to be involved in resolution of lesions induced by L. major (10), in resolution of viral infections (9) and in recovery from EAE (11–14,54). Although normal thyroids do not express FasL (24) (Fig. 2), FasL is expressed on thyroglobulin during inflammation (28,30,32,33 and Wei et al., in preparation). Moreover, thyroglobulin that over-express FasL can induce apoptosis of infiltrating inflammatory cells (27) and prevent thyroid inflammation in EAT (23,24). We are currently testing the hypothesis that CD8+ cells promote G-EAT resolution by inducing FasL expression on thyroglobulin, resulting in apoptosis of CD4+ effector cells and/or other inflammatory cells. Such a role for CD8+ cells would be consistent with the observation that activated T cells can induce FasL expression on epithelial cells that normally do not express this molecule (39).

In summary, these results suggest that apoptosis may play an important role both in inducing damage to the thyroid and in mediating resolution of infection in G-EAT. Although the evidence provided here suggests apoptosis could be mediated through the Fas–FasL pathway, apoptosis can also result when cytokine production declines, from perforin-dependent lysis of target cells, or following interaction of TNF and TNF receptors (8,15,16). One or more of these mechanisms could be involved in damaging thyroglobulin or eliminating effector cells and further studies are required to determine which pathways are most important in this model.

The G-EAT model used for these studies is one in which the host’s own immune system leads to resolution or healing of the autoimmune inflammation. Our results suggest that apoptosis is involved both in the destruction of thyroid tissue and in the resolution of granulomatous autoimmune inflammatory responses in the thyroid. This model will be useful to further elucidate the mechanisms of apoptosis of activated T cells and the role of CD8+ cells in the resolution of autoimmune inflammatory responses. Granulomatous lesions are a major pathologic feature of several human autoimmune diseases such as Wegener’s granulomatosi, allergic granulomatosi, giant cell arteritis and sarcoidosis. Information gained from these studies is not only relevant for autoimmune thyroiditis, but may also provide important information for understanding the mechanisms involved in healing or resolution of other granulomatous autoimmune diseases.

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Abbreviations

AICD: activation-induced cell death
EAE: experimental allergic encephalomyelitis
FasL: Fas ligand
G-EAT: granulomatous experimental autoimmune thyroiditis
MTg: mouse thyroglobulin
TNF: tumor necrosis factor

References


