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Gene Therapy of Experimental Autoimmune Thyroiditis by In Vivo Administration of Plasmid DNA Coding for Fas Ligand

Frédéric Batteux,*† Léa Tourneur,* Hélène Trebeden,* Jeannine Charreire,* and Gilles Chiocchia1*

Fas-Fas ligand (FasL) interaction is required for the maintenance of immune homeostasis and seems to be responsible for the privileged immune status of some tissues. Experimental autoimmune thyroiditis (EAT), which is characterized by autoreactive T and B cell responses and a marked lymphocytic infiltration of the thyroid, is a model of choice to study the therapeutic effects of FasL. Here, we provide evidence that direct injection of DNA expression vectors encoding FasL into the inflamed thyroid inhibited development of lymphocytic infiltration of the thyroid and induced death of infiltrating T cells. These results were paralleled by a total abrogation of anti-Tg cytotoxic T cell response in FasL-treated animals vs controls. In summary, our results show that FasL expression on thyrocytes may have a curative effect on ongoing EAT by inducing death of pathogenic autoreactive infiltrating T lymphocytes. The Journal of Immunology, 1999, 162: 603–608.

The Fas receptor (Fas, CD95, APO-1) and its natural ligand (Fas ligand (FasL),2 CD95L) are transmembrane proteins that belong to the TNF family of receptors and ligands (1). Upon engagement with FasL, most of the cells expressing Fas rapidly undergo apoptosis. Thus, the immune privilege of the anterior chamber of the eye (2) and of the testis (3) has been linked to the expression of FasL on certain cells of those tissues.

Fas-FasL interaction is required for the maintenance of immune homeostasis. A failure in that interaction may lead to autoimmune and lymphoproliferative disorders in mice (4–6) and humans as well (7–9). Therefore, it was expected that inducing ectopic FasL expression on the target cells of an autoimmune reaction or on a transplanted organ (10–12) would interfere with the deleterious immune process. Several groups have reported the generation of transgenic mice with FasL gene under the control of the insulin promoter to protect the animals from insulin-dependent diabetes mellitus (13, 14). The general lack of protection observed in those experiments may be explained by the high degree of susceptibility of pancreatic β cells to Fas-mediated death both in humans (15) and mice (13). In contrast, thyroid follicular cells (TFC) were especially attractive as sites of ectopic FasL expression, because they are known to be resistant to Fas-mediated apoptosis (16). Therefore, the therapeutic role of FasL was tested in mice with experimental autoimmune thyroiditis (EAT), which is a well established murine model of Hashimoto’s thyroiditis. The disease is characterized by autoreactive T and B cell responses and a marked lymphocytic infiltration of the thyroid. In the past, various preventive treatments of EAT have been reported. Those included Ag-specific approaches, such as i.v. injection of soluble thyroglobulin (Tg) before a subsequent immunization with Tg (17, 18), Tg-specific T cell lines (19), or clones (20). Nonspecific therapies included injection of anti-class II Abs (21), anti-CD4 (22), or IFN-γ mAb (23). More recently, we reported that IL-10 was able to prevent and cure EAT (24). To our knowledge, no attempt of gene therapy has ever been made for the treatment of EAT. We chose a nonviral transfer system that allies simplicity, absence of protein contaminations (thus minimizing host responses to the vector), and that may be applied to humans later on. Because TFC are transfectable with naked DNA (25), we have designed an original in vivo gene transfer technique allowing a rapid and lasting expression of plasmid DNA in the thyroid of mice. Our results demonstrate that FasL expression on TFC of mice with EAT dramatically reduced the lymphocytic infiltration of the thyroids, abrogated the anti-Tg cytotoxic T cell response, and induced a selective persistence of IgG1 anti-Tg Abs.

Materials and Methods

Mice

CBA/J female mice were purchased from Ifla Credo (L’Arbresle, France). Female mice between 7–10 wk of age were used in all experiments. Animals were maintained in standard environmental conditions with free access to food and water. After purchase, they were allowed to adapt to their environment for 1 wk before initiating the experiments.

Expression vectors

The plasmid expression vectors used in this study were: pcDNA3-β-gal encoding the Escherichia coli β-galactosidase (β-gal) gene under the control of the CMV immediate early promoter (a gift of G. Richter, Max Delbrueck Center, Berlin, Germany); pcDNA3.1-Fasl, and Tg promoter (pTg)-FasL encoding the rat Fasl under the control of either the CMV immediate early promoter or the bovine pTg (a gift of D. Christophe, Université Libre de Bruxelles, Bruxelles, Belgium). The cDNA of rat FasL contained in a pBluescript (Stratagene, Cambridge, U.K.) (1) was used as template to amplify a fragment of FasL by PCR.

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2 Abbreviations used in this paper: FasL, Fas ligand; Tg, thyroglobulin; pTg, thyroglobulin promoter; β-gal, β-galactosidase; EAT, experimental autoimmune thyroiditis; TFC, thyroid follicular cell; PLL, poly(L-lysine).
Plasmid formulation

In this study, gene transfer was performed with plasmids complexed with poly(ethylene-lysine) (PLL) (Sigma, St Quentin Fallavier, France) and Lipofectamine (Life Technologies, Eragny, France). Plasmids grown following standard procedures and purified by Qiagen Maxi column (Qiagen, Courtaulds, France) were stored at the concentration of 1 mg/ml in sterile water. PLL-DNA-liposome complexes were formed by mixing first 1 μg plasmid with 380 ng PLL in 10 μl Opti-MEM (Life Technologies). After 15 min incubation at room temperature to allow DNA-PLL condensation, the mixture was added to a 10-μl solution of 10 μg lipofectamine in Opti-MEM. After 30 min incubation, the preparation was delivered to the animals.

RT-PCR

For RT-PCR, whole thyroid extracts were homogenized and RNA was prepared by guanidine isothiocyanate-acid phenol extraction. Total RNA was treated with DNase I (Boehringer Mannheim, Mannheim, Germany), then 0.5 μg of RNA was used in a first-strand cDNA synthesis using oligo-dT primer, and PCR was performed from 1:20 of the cDNA reaction. The PCR reaction generated a 545-base pair fragment of rat FasL (sense primer 5'–AAGGACACATAGAGCTTGG-3' and anti-sense 5'–AATT GGTCAGCAAACGTTAG-3'), a 542-base pair fragment of mouse FasL (sense primer 5'–AGGGACACAAACACATACTG-3' and anti-sense 5'–GGTCAGCACTGTGTAAG-3'), or a 348-base pair fragment of mouse β actine (sense primer 5'–TGGAATCCTGTGGCATCCAT-3' and anti-sense 5'–TAAAAACGAGCTGAACTAAGTCCG-3'). The RT-PCR products were subjected to electrophoresis on a 2% agarose gel and stained with ethidium bromide.

Immunization of animals

Porcine Tg (Sigma) was emulsified in CFA for immunization on day 0 and on IFA for challenge on day 14. CFA suspension, which contained 1 mg/ml of Mycobacterium tuberculosis, strain H37Ra (Difco Lab, Detroit, MI) was injected i.d. with 100 μg of porcine Tg. Animals were killed at various times postimmunization as specified in the text.

Surgical procedure

Mice were anesthetized i.p. with 125 mg/kg avertine (Sigma). Using sterile procedures, a lateral neck dissection was performed to visualize the thyroid gland and dissect it free of surrounding fascia and muscle while maintaining the capsule intact and blood supply constant. The connective shaft surrounding the thyroid gland was gently dissected to form a gutter, where the plasmid preparation was applied using a 100-μl syringe. This procedure allowed for direct contact between the plasmid mixture and the thyroid gland without any effraction. After 1–2 min of contact, the wound was closed in layers using conventional surgical procedures.

Histopathological studies of thyroid specimens

The histological grade of EAT was assessed by three persons by blind evaluation of thyroid specimens. Infiltration indexes were evaluated on 5-μm thick sections stained with Masson Goldner’s trichrome solution. EAT was graded as a function of the mononuclear cell infiltration of the thyroid: grade 1, interstitial accumulation of inflammatory cells distributed around one or two follicles; grade 2, one or more foci of inflammatory cells reaching at least the size of one follicle; grade 3, 10–40% of the thyroid replaced by inflammatory cells; and grade 4, >40% of the thyroid replaced by inflammatory cells.

Flow cytometry and assessment of apoptosis

In vitro cytotoxic responses to Tg-pulsed syngeneic macrophages

Cytotoxic responses were evaluated on 4-day in vitro porcine Tg-activated splenocytes. Then, 14 days after gene transfer (day 35 postimmunization), splenocyte cultures were prepared at 5 × 10^6/ml in medium. Cells were cultured in 100-mm petri dishes with 40 μg/ml porcine Tg and 1 nM IL-2 at 37°C for 4 days. At the end of the culture, cells were harvested, washed twice in HBSS, and referred to as effector cells. Peritoneal macrophages from thiglycylate-injected CBA/J mice were collected in HBSS-10% FCS, washed twice, and counted after staining with neutral red. Pelleted cells were then labeled with 100 μl of 37Cr per 10^6 cells. After 1 h incubation at 37°C under shaking, cells were washed twice in HBSS-10% FCS and 10^6 macrophages were distributed into each well of flat-bottom 96-well plates (Costar, No. 3799, Cambridge, MA). Then, 50 μg of porcine Tg were added under a volume of 100 μl for 4 h. Thereafter, the pulsed macrophages were washed with HBSS and 100 μl of effector cells at 2.5, 5, 10, and 20 × 10^5 cells/ml were added. After 6 h incubation, 100 μl of supernatant were collected and chromium release measured in a γ-scintillation counter (LKB, Bromma, Sweden). Spontaneous release was <23%. Spontaneous and maximal releases were defined by incubation of target cells with culture medium in the absence or the presence of Triton X-100 detergent (5% v/v solution in Tris buffer), respectively.

Levels and isotypes of Abs to pTg

Mice were bled by cardiac puncture at the time of death. Sera were stored at −20°C until use. Abs to porcine Tg were detected by ELISA as previously described (23). Briefly, flat-bottom microtiter plates (Costar, No. 3590) were coated overnight with 50 μl of 100 μg/ml porcine Tg at 4°C, then washed twice with PBS-Tween 20. Free protein binding sites were blocked by adding PBS-1% BSA for 2 h at 37°C. Serial 10-fold dilutions (1:10 to 1:10^3) of the sera from individual mice were incubated overnight at 4°C. After extensive washing of the plates, alkaline phosphatase-conjugated goat anti-mouse IgG (Miles-Yeda Laboratories, Rehovot, Israel), diluted 1:5000 in PBS-Tween, was added as second Ab and the colorimetric reaction revealed by substrate addition. The plates were read at 405 nm with a Titertek multiscan spectrophotometer (Dynatec MR 5000, Guyancourt, France). Isotypes of Abs to porcine Tg were determined in individual sera (serial-10-fold dilutions from 1:10^2 to 1:10^5) using alkaline phosphatase-conjugated goat anti-mouse IgG1, IgG2a, IgG2b, and IgG3 as secondary Abs (Southern, Birmingham, USA). For the IgG3 levels, we used a 1:10^5 to 1:10^6 dilutions. Arbitrary units of IgG, IgG1, IgG2a, IgG2b absorptions were defined as the amount of anti-Tg Ab providing the same OD as a standard serum diluted 1:10^6. One unit of IgG3 was reported to the OD provided by 1:10^3 standard serum. The standard serum was a pool of mouse sera with high amounts of anti-porcine Tg Abs.

Results

Expression of FasL in mouse thyroid after local in vivo gene delivery

FasL expression was measured by RT-PCR in normal mouse thyroid (Fig. 1A) and at various times following transfer of expression vectors. Whereas normal thyroid was negative for FasL expression, transfer of 1 μg of plasmid preparation as described in Materials and Methods induced FasL mRNA expression within 24 h and for at least 15 days (Fig. 1B). FasL expression was undetectable on day 30 after gene transfer (not shown). The same results were obtained following transfer of plasmid DNA encoding β-gal (not shown). Thus, as previously observed in rabbit by Sikes et al. (25), mouse thyroid cells were able to take up DNA and express the gene of interest.

FasL expression by TFC abrogates lymphocytic infiltration of the thyroid

To determine whether FasL expression could alter the course of ongoing EAT, CBA/J mice were immunized with Tg using standard procedures. Because the first signs of EAT appear around 2 wk postpriming (27), mice were treated on day 21 with different plasmid DNA preparations. The animals were then killed 1 or 2 wk after DNA injection (day 28 or day 35 postimmunization), at the time when the inflammation usually reaches its peak in untreated immunized animals. Table I summarizes the data obtained in three
consecutive experiments. FasL expression resulted in an highly and significant decrease in the infiltration of the thyroid glands on day 28 and on day 35 as well. The mean infiltration index per mouse was 1.56 ± 0.24 in PBS-treated group, 0.40 ± 0.14 (p < 0.001) and 0.70 ± 0.30 (p < 0.05) after injection of pCMV-FasL and pTg-FasL, respectively. In controls (empty vectors and β-gal-encoding vectors), the disease was similar to that in immunized mice after saline treatment, implying that the vectors did not affect the course of the disease (Table I). All of the nonimmunized animals infused with the various constructions (five mice per group) showed normal thyroids (data not shown). Thus, FasL expression on thyroid cells under autoimmune attack inhibits lymphoid infiltration of the gland.

**FasL gene transfer induced apoptosis of infiltrating lymphocytes**

To understand the mechanism of the lymphocytic thyroid infiltrate abrogation, we investigated whether the lymphoid cells had undergone apoptosis in the thyroid of animals treated by FasL gene constructs.

Four thyroids per group were pooled and treated as described in Materials and Methods before flow cytometry analysis. In agreement with the lower index of lymphocytic infiltration mentioned above (Table I), a strong reduction in the absolute number of infiltrating cells was observed in the FasL gene transferred groups compared with PBS-treated group. The number of intrathyroidal lymphocytes was reduced by 47% and 37% in pCMV-FasL- and pTg-FasL-treated groups, respectively, compared with PBS-treated group. Furthermore, the percentage of apoptotic lymphocytes was higher in FasL- than in PBS-treated groups (2.28-fold and 1.42-fold increases in cells labeled with annexin V-FITC following pCMV-FasL and pTg-FasL treatments, respectively). To investigate whether the proapoptotic effect of FasL expressed on TFC was selectively exerted on some cell subsets, CD4-, CD8-, B220-, and MAC-1-positive cells were enumerated in suspensions of infiltrating cells. Both pCMV-FasL- and pTg-FasL-treated groups showed a similar reduction in intrathyroidal CD4- and CD8-positive cells (Table II). However, B220-positive cells were in higher percentages in both groups compared with PBS-treated group. There was no alteration in the macrophage-gated population based on forward and side scatters and on MAC-1 expression (Table II). These results strongly suggest that expression of FasL by thyroid with autoimmune inflammation induces intrathyroidal T lymphocyte apoptosis.

**FasL gene transfer inhibited Tg-specific cytotoxic T cell response**

Because Tg-specific cytotoxic T cells are the main effectors in EAT, FasL gene transfer was tested for its ability to modulate this response. Splenic cytotoxic T cell response toward Tg-pulsed syngenic macrophages was measured on day 35 postimmunization (2 wk after treatment). Whereas cytotoxic T cells were always detected in splenocytes from PBS- and empty vector-treated animals, they were undetectable in both FasL-treated groups (Fig. 2). Thus, transgenic FasL expression at the site of inflammation induces systemic elimination of Tg-reactive cytotoxic T cells.

**Effects of FasL gene transfer on anti-Tg B cell response**

The impact of FasL expression on Ab responses was studied in the sera of animals with ongoing EAT in experiment 3 (Table I). Transfection with the pTg-FasL vector induced a 2.17-fold increase in anti-Tg IgG1 level (p < 0.01 vs PBS-treated group) without affecting any other subclass responses (Fig. 3). The same

### Table 1. Beneficial effect of FasL gene transfer on EAT

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Treatment</th>
<th>n</th>
<th>Day 28</th>
<th>Day 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PBS</td>
<td>3</td>
<td>1.33 ± 0.17</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>pCMV-β-gal</td>
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<td>1.65 ± 0.17</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>pCMV-FasL</td>
<td>4</td>
<td>0.25 ± 0.14</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>pTg-FasL</td>
<td>5</td>
<td>0.25 ± 0.16</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>PBS</td>
<td>(6, 3)</td>
<td>1.67 ± 0.36</td>
<td>2.17 ± 0.59</td>
</tr>
<tr>
<td></td>
<td>pCMV-β-gal</td>
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<td>1.60 ± 0.24</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>pCMV-FasL</td>
<td>(6, 4)</td>
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<td>1.38 ± 0.24</td>
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<tr>
<td></td>
<td>pTg-FasL</td>
<td>(6, 4)</td>
<td>1.08 ± 0.49</td>
<td>1.00 ± 0.67</td>
</tr>
<tr>
<td>3</td>
<td>PBS</td>
<td>7</td>
<td>1.89 ± 0.22</td>
<td>ND</td>
</tr>
<tr>
<td></td>
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<td>5</td>
<td>1.70 ± 0.37</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>pCMV-FasL</td>
<td>6</td>
<td>2.04 ± 0.36</td>
<td>ND</td>
</tr>
<tr>
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<td>pTg-FasL</td>
<td>7</td>
<td>1.65 ± 0.20</td>
<td>ND</td>
</tr>
<tr>
<td>1 + 2 + 3</td>
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<td>1.56 ± 0.24</td>
<td>1.95 ± 0.20</td>
</tr>
<tr>
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<td>pCMV-β-gal</td>
<td>5</td>
<td>1.70 ± 0.37</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>pCMV-FasL</td>
<td>(10, 6)</td>
<td>1.63 ± 0.14</td>
<td>2.04 ± 0.36</td>
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<tr>
<td></td>
<td>pTg-FasL</td>
<td>(10, 11)</td>
<td>0.40 ± 0.14†</td>
<td>1.28 ± 0.21†</td>
</tr>
<tr>
<td></td>
<td>pTg-FasL</td>
<td>5</td>
<td>1.65 ± 0.20</td>
<td>ND</td>
</tr>
</tbody>
</table>

*EAT was graded as described in Materials and Methods.

†, p < 0.05; †, p < 0.02; †, p < 0.001. All p values were defined in comparison to PBS-treated group (Student’s t test).
alterations, although less marked, were observed on day 28 (data not shown). None of the treatment induced any significant change in the level of anti-Tg IgG3 (Fig. 3). The decrease in total anti-Tg IgG observed in serum from animals treated with pCMV-FasL essentially resulted from a drop in anti-Tg IgG2a concentration \( (p < 0.05 \text{ vs PBS-treated group}) \) (Fig. 3). The anti-Tg IgG2b and IgG3 responses were also diminished, although not significantly, and IgG1 titers were not affected. The same results were obtained in experiments 1 and 2 depicted in Table I (data not shown). Thus, FasL expression on thyroid cells of mice with EAT induced a marked and isotype-specific modulation of the anti-Tg Ab response.

Discussion

Over the past few years, numerous reports have focused on the role of FasL in deleting Fas-positive activated lymphoid cells, suggesting that this phenomenon was involved in the maintenance of natural tolerance and the prevention of autoimmune diseases (28). To test whether FasL expression on thyrocytes could inhibit EAT in mice, plasmids carrying the FasL gene were transferred into thyroids of animals with ongoing EAT. We took the opportunity of the ability of TFC to take up and express genes from expression plasmids (25) to transfer the FasL gene in thyroid tissue. However,
the method of naked DNA transfer used so far has allowed expression of the transgene during only 3 to 5 days (Ref. 25 and data not shown) and requires up to 100 μg of plasmid DNA, which is inappropriate for gene transfer into small organs such as the mouse thyroid. Therefore, we designed a new procedure of gene transfer based on the condensation of plasmid DNA with a mixture of liposomes and PLL (29, 30) that allowed the use of smaller amounts of plasmids and prolonged transgene expression in the target tissue. Two promoters were used in this study: the immediate early CMV promoter that allows strong expression of the transgene, and a tissue-specific promoter (pTg) that induces FasL gene expression on TFC only. FasL could be detected on the thyroid tissue for more than 15 days with both promoters, pCMV and pTg.

In thyroids of animals with EAT, the expression of FasL resulted in a dramatic decrease in lymphocytic infiltration. Because the apoptosis rate evaluated by flow cytometry was higher in FasL-positive thyroids than in controls, the decrease in lymphocytic infiltration could be reported to the apoptotic effect of FasL interaction with activated Fas-positive lymphoid cells. The proapoptotic consequences of FasL expression were observed in both CD4 and CD8 lymphocyte subsets.

Anti-Tg proliferative response of lymph node T cells was only slightly diminished in pCMV-FasL-treated group compared with control on day 7 after gene transfer (data not shown). In contrast, the decrease in T cell infiltration was paralleled by a total abrogation of the Tg-specific cytotoxic T cell response. The same differential effects on the proliferative and cytotoxic responses have been observed using IL-10 as a curative treatment of EAT (24). Several possibilities such as the systemic release of the pDNA/liposome complexes or the migration of transfected cells in the periphery could explain why the administration of pDNA encoding FasL to the thyroid would lead to reduced Tg-specific CTL reactivity in the spleen. The possibility that few transfected cells have emigrated to the periphery cannot be formally ruled out. Nevertheless at least two of our results argue against this possibility. First, we used the rat FasL gene, and this allowed us to follow specifically the cells expressing the gene in various organs, namely draining lymph nodes, spleen, and kidney. Never have we been able to detect rat FasL expression by RT-PCR (data not shown). Second, the use of pTg-FasL induced the same effect that pCMV-FasL, whereas the expression of FasL in the case of pTg-FasL is driven by a promoter that allow expression of the transgene only in thyroids. Thus, the abrogation of the Tg-specific cytotoxic T cell response is certainly due to the local deletion of autoreactive CD8 T cells in both groups treated with FasL gene.

In contrast to T lymphocytes, B cells were found in higher percentages in FasL-treated thyroids than in PBS-treated thyroids. Because FasL cytotoxicity is a negative regulatory control for both T and B lineages (31), the opposite effects of FasL on those cell populations in our model seem paradoxical. However, various lines of evidence indicate that mature B cells are resistant to Fas-dependent mediated apoptosis when activated through their Ag receptor (32, 33). Thus, intrathyroidal autoreactive B cells, which are essentially activated cells, could be protected from Fas-mediated death.

B cell infiltration in pTg-FasL-treated group was correlated with the anti-Tg Ab response, which was more pronounced than in controls. The increase in anti-Tg IgG titers was mainly related to a higher production of IgG1 in the pTg-FasL-treated group. In the pCMV-FasL-treated group, all the anti-Tg IgG subclasses decreased except for IgG1. Those data could be explained by the preferential survival of Th2 cells in the FasL-treated animals. Indeed, it has been reported that activation-induced cell death in CD4 Th cells could be responsible for imbalances in Th1 and Th2 subsets because Th1, but not Th2, undergo rapid Fas/FasL-mediated apoptosis (34–36). During the Th2 response, B cells are protected from Fas/FasL-induced apoptosis, and IgG1 production is elicited (37). Whether this mechanism could be involved in our model is unclear because no production of IL-4 and no difference in IFN-γ production by lymph node cells could be observed to support an imbalance in Th1/Th2 T cell populations (data not shown). Another nonexclusive possibility to explain our results can be linked to the ubiquitous character of the viral promoter that may have led to the expression of the transgene not only on TFC, but also on other cells such as the infiltrating lymphocytes. Under those conditions, it is highly probable that a cis-mediated cell death of infiltrating lymphocytes may have been associated to the trans-mediated cell death triggered by FasL expression on thyrocytes. This result could be reported to the higher efficiency of the pCMV-FasL treatment on day 7 after gene transfer (Table I).

In humans, recent data indicate that thyrocytes express FasL in Hashimoto’s thyroiditis (38), and the disease seems related to the simultaneous expression of Fas and FasL by TFC. Whether FasL is also expressed on normal human thyrocytes is still a matter of debate (39, 40). It has been proposed that FasL expression on those cells prevents autoimmunity, which is in contradiction with a possible role of FasL in Hashimoto’s disease (41). Discussing the role of FasL in Hashimoto’s thyroiditis is rendered still more difficult by new insights in the control of intrathyroidal apoptosis: first, it has been reported that the thyroid-stimulating hormone inhibits Fas A-mediated apoptosis of human thyrocytes in vitro (42); second was the report on the constitutive expression of an inhibitor of the Fas pathway apoptosis by normal thyrocytes (16).

In conclusion, this report is in agreement with the hypothesis of Dayan et al. (41) supporting the beneficial effect of FasL expression on thyrocytes to avoid autoimmune thyroiditis. In addition, our results show that FasL expression restricted to TFC may have a curative effect on ongoing EAT in the mouse by inducing death of infiltrating lymphocytes and particularly of pathogenic autoreactive T cells.

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