Thyrocytes — not innocent bystanders in autoimmune disease

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It is known that thyrocyte apoptosis is significantly more pronounced in Hashimoto's thyroiditis (HT) than in Graves' Disease (GD)[1]. In their recent contribution to *Nature Immunology*[2], Stassi and coworkers confirmed higher thyrocyte apoptosis in HT than in GD thyroids. However, they also found that Fas and Fas ligand (FasL) expression were only moderately reduced in seven GD-affected *versus* five HT-affected thyroids, suggesting that other mechanisms may affect apoptosis. They propose that differences in the production of cytokines by infiltrating lymphocytes can explain the differences in thyroid follicular cell (TFC) apoptosis that are observed in HT and GD.

In contrast to the findings of Stassi *et al.*[2], we found that Fas expression was invariably higher in HT and lower in GD thyrocytes.[3] Immunohistochemical analysis showed limited areas of Fas^+ TFCs in GD, which were most often observed near to mononuclear infiltrates, the majority of follicles being Fas^-. TFC apoptosis was accordingly minimal. These findings suggest that the homophilic interaction between Fas and its ligand, expressed by thyrocytes, is far more likely to occur in HT than in GD glands.

After TFC depletion, we found significant differences in the expression of Fas by infiltrating lymphocytes in HT and GD glands. Fas expression in intrathyroidal lymphocytes was higher in GD, as shown by quantitative mRNA, immunoblotting and cytofluorimetric analyses. We also observed marked differences in the proportion of apoptotic lymphocytes, which were far higher in GD than in HT. Comparative immunohistochemical analysis of GD and HT specimens by double-staining with CD3 mAbs and TUNEL, or cytokeratin mAb and TUNEL, showed that apoptosis was mainly present in infiltrating lymphocytes in GD and in TFCs in HT.

Stassi *et al.*[2] show that infiltrating lymphocytes are more prone to apoptosis in GD than in HT. We propose that FasL expression by GD thyrocytes may promote lymphocyte apoptosis. This hypothesis is consistent with the previously proposed concept[4] that T_H1 cells are expected to be more sensitive to Fas-mediated apoptosis than T_H2 cells. Thus, in GD thyrocytes may preferentially stimulate apoptosis in T_H1 lymphocytes, whereas the surviving T_H2 cells may promote TFC resistance to apoptosis *via* the up-regulation of cFLIP and Bcl-x_L. In contrast, we did not observe significant apoptosis in lymphocytes infiltrating HT glands, which suggests that the HT microenvironment does not promote lymphocyte apoptosis. We also found that the expression of the anti-apoptotic molecule Bcl-2 is increased in HT lymphocytes and reduced in thyrocytes.

In conclusion, it is conceivable that T cell–derived cytokines play a crucial role in regulating TFC apoptosis in GD and HT, as shown by the elegant experiments by Stassi and coworkers. However, our findings suggest that thyrocytes are likely to play a crucial role in regulating intrathyroidal T cell survival and selection, which supports the concept that the interaction between tissue-specific epithelial cells and infiltrating lymphocytes plays a major role in autoimmune disease.

1. Davies, T. F. *Thyroid* **10**, 525–526 (2000).
2. Stassi, G. *et al. Nature Immunol.* **1**, 483–488 (2000).
3. Giordano, C. *et al. Thyroid* **11** (in the press, 2001).
4. Pinkoski, M. J. & Green, D. R. *Nature Immunol.* **1**, 461–462 (2000)

Response

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Giordano *et al.* propose that thyrocytes play a crucial role in the regulation of the autoimmune response during GD. According to them, Fas is weakly expressed in GD thyrocytes, whereas FasL is responsible for a specific deletion of infiltrating T_H1 cells and maintains a T_H2 phenotype in the lymphocytic infiltrate.

Data published so far reports unambiguous Fas expression in GD thyrocytes[1–5], although there is one exception to this. In this one report the authors may have used an insensitive detection technique because they found "weak staining" for Fas in HT thyrocytes[6], which was definitely lower than the "large amounts of Fas" found by Giordano *et al.*[7]. In addition, where the largest number of cases were analyzed, Fas expression was higher in GD, compared to HT, thyrocytes[2].

Nevertheless, we agree that there is higher Fas expression in HT thyrocytes compared with GD thyrocytes located far from lymphocytic infiltration areas. This is likely to be the result of the higher interferon γ production observed in HT because this cytokine is the strongest Fas, inducer in thyrocytes[8] (and our unpublished results). This is in line with our hypothesis that T_H cytokines control autoimmune thyrocyte destruction.

Although immunohistochemistry allows easy *in vivo* evaluation of Fas expression, it is not a sensitive tool and may well underestimate the number of positive cells, which is likely to vary depending on the techniques used in different laboratories[9]. On the other hand, cultured thyrocytes derived from normal or nonautoimmune thyroids express high amounts of Fas, which cannot trigger apoptosis without appropriate cytokine priming[1,8]. In addition, T_H2 cytokines are able to prevent Fas-induced apoptosis by inducing anti-apoptotic proteins and without interfering with Fas expression[1]. Thus, we believe that the intensity of Fas expression in thyrocytes is not strictly related to its function.

Giordano *et al.* propose that GD thyrocytes selectively kill infiltrating T_H1 cells through Fas-induced apoptosis, which promotes the prevalence of T_H2 cytokines. In contrast, because of increased Bcl-2 expression[10], infiltrating T_H1 cells would be spared in HT. They also claim to have compared Fas expression in GD and HT lymphocytes with various techniques to show that Fas is more active in GD lymphocytes. The selective killing of T_H1 cells in GD is an interesting hypothesis that needs to be supported by specific data. Although the extensive evaluation of Fas expression in GD and HT lymphocytes is appreciated, functional analyses are required to confirm these hypotheses, particularly when they are discrepant from literature data[11].

1. Stassi, G. *et al. Nature Immunol.* **1**, 483–488 (2000).
2. Tanimoto, C., Hirakawa, S., Kawasaki, H., Hayakawa, N. & Ota, Z. *Endocrinol. J.* **42,** 193–201 (1995).
3. Kawakami, A. *et al. Clin. Exp. Immunol.* **110**, 434–439 (1997).
4. Stokes, T. A. *et al. Science* **279**, 2015 (1998).
5. Kawakami, A. *et al. Lab. Invest.* **80**, 471–484 (2000).
6. Hammond, L. J. *et al. J. Pathol.* **182**, 138–144 (1997).
7. Giordano, C. *et al. Science* **275**, 960–963 (1997).
8. Bretz, J. D., Arscott, P. L., Myc, A. & Baker, J. R., Jr *J. Biol. Chem.* **274**, 25433–25438 (1999).
9. Borgerson, K. L., Bretz, J. D. & Baker, J. R. Jr *Autoimmunity* **30**, 251–264 (1999).
10. Mitsiades, N. *et al. J. Clin. Endocrinol. Metab.* **83**, 2199–2203 (1998).
11. Stassi, G. *et al. J. Immunol.* **162**, 263–267 (1999).