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Guest Editorial

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THE ARTICLE BY Giordano et al. in the current issue of *Thyroid* (1) highlights many interesting issues concerning the nature of apoptosis in the thyroid and the role it may play in autoimmune thyroid disease. The authors examined the expression of Fas antigen and ligand on thyroid cells from patients with Graves' and Hashimoto's diseases and compared this to normal thyroids. In addition, they evaluated levels of Bcl-2, an apoptosis regulatory protein, in these cells. They were also able to examine the expression of these molecules in lymphocytes infiltrating the thyroid in the autoimmune disorders. The results reinforce the hypothesis that regulation of apoptosis in thyroid cells may alter the expression of autoimmune thyroid disease, a concept currently advanced by several laboratories including my own (2–4). However, the authors' specific findings raise a number of questions concerning the nature of apoptosis regulation and the actual mechanisms involved in inducing cell death in autoimmune disease.

Giordano and colleagues found that there was differential expression of Fas pathway molecules in Graves' and Hashimoto's thyroid cells (see Fig. 1 for summary). While both types of thyroid cells reportedly expressed Fas ligand, there were marked increases in the expression of this protein in Hashimoto's thyrocytes as compared with Graves' or normal cells. The expression of Fas antigen was also upregulated in Hashimoto's thyrocytes. The authors also found that concentrations of Bcl-2 were elevated in Graves' cells compared to control thyroids, and suppressed in Hashimoto's cells. In contrast, they found that lymphocytes from Graves' disease patients have elevated levels of Bcl-2 and decreased expression of Fas ligand, and while Hashimoto's lymphocytes had decreased expression of Bcl-2. They interpret these findings to suggest that there is a general proapoptotic milieu for Hashimoto's thyroid cells and an antiapoptotic potential for Graves' cells. This was contrasted with a proapoptotic and noncytotoxic potential in the lymphocytes infiltrating Graves' disease thyroids and an antiapoptotic potential in the Hashimoto's gland lymphocytes. The authors believe that these results argue that the apoptotic potential of the thyroid cell is crucial to the manifestation of autoimmune disease, leading either to glandular destruction in thyroiditis or to growth in Graves' disease.

While I am in general agreement with the authors' hypothesis that apoptosis is regulated in thyroid cells, the actual role that the Fas pathway proteins play in thyroid cell apoptosis is not clear from this work. There is some support for the concept that Fas-mediated apoptosis is regulated through alterations in the levels of signaling and receptor proteins (5,6). However, the changes in the levels of expressed Fas antigen and ligand and observed in these cells may not be adequate to alter the induction of apoptosis (5). The authors' argument for regulation of the Fas pathway in this manner is further weakened by the absence of any functional data showing differences in signal transduction associated with these changes in Fas receptor and ligand. The most impressive difference the authors' demonstrate between Graves and Hashimoto's cells was in the expression of Fas ligand. Levels of Fas ligand protein on Hashimoto's thyrocytes were three logs higher than background straining, and a full log greater than the levels the authors document on lymphocytes from similar glands (Figure 4 in Giordano et al.'s article). This level of Fas expression in Hashimoto's thyroid cells is truly remarkable given that activated lymphocytes are one of the cells with the highest ob-

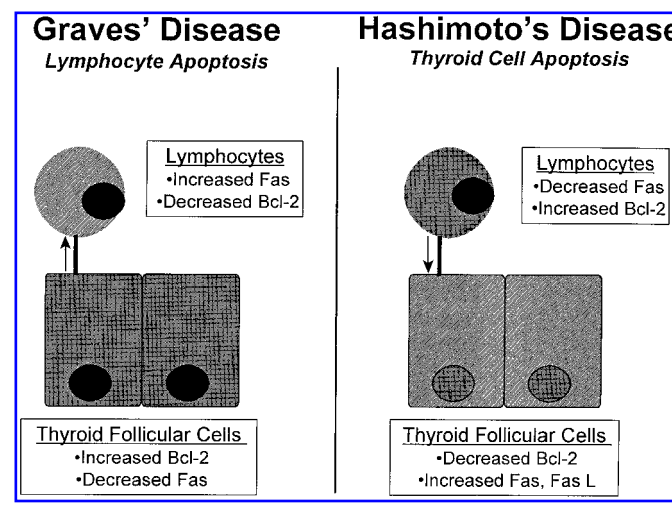

FIG. 1. Giordano et al's proposed differences in apoptotic molecules and potential for thyroid follicular cells and intrathyroidal lymphocytes in Graves' and Hashimoto's diseases.

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served levels of Fas ligand expression (7), and would suggest a unique activation of the Fas ligand transcription in these cells. However, these data are controversial because the expression of Fas ligand has not been consistently observed in thyroid cells (2–4,8,9) and this level of expression has not been previously observed in other cells (5).

The high level of Fas antigen expression on Hashimoto's thyrocytes also observed in this report may have functional significance as it could overwhelm inhibitory molecules that compete for caspace binding with Fas such as FAP, which has been identified in the thyroid (8,9). Potential support for this concept comes from a 1999 report from my laboratory that showed papillary thyroid cancer cells express high levels of Fas antigen and this expression appeared to relate to increase susceptibility to Fas pathway activation (10). However, a majority of publications do not support alterations in the levels of Fas antigen on thyroid cells in autoimmune diseases (11,12). In particular, a recent article in *Nature Medicine* by Stassi and coworkers (13), also from the University of Palermo and occasional collaborators of Giordano, did not show significant differences in the expression of Fas antigen or ligand in Graves and Hashimoto's thyrocytes. That report and an earlier publication from my laboratory (14) both indicate that the regulation of the Fas pathway in thyroid cells involves inflammatory cytokines and that alterations in signal transduction occur without changes in the concentrations of pathway components. Thus, while regulation of Fas mediated apoptosis may be important in the expression of autoimmune thyroid disease, the mechanisms regulating this and the relative role this pathway plays in mediating disease require further clarification.

The authors' also hypothesize that Bcl-2 is important in regulating apoptosis in thyroid cells, and this concept is better supported by publications from other groups (9). In particular, work suggesting that Bcl-2 regulation is important in the apoptotic potential of cells has come primarily from cancer cell lines where increased levels of Bcl-2 or decreased levels of proapoptotic proteins, such as Bclx, has been associated with increased survival potential and resistance to apoptosis (15,16). The Bcl-2 family of proteins is important in regulating apoptosis triggered by several mechanisms including oxidative stress, radiation damage, or abnormal cell division (15,17). However, the role that Bcl-2 might play in preventing apoptosis associated with autoimmune attack is not clear. This is especially true for signaling occurring through membrane receptors, such as Fas, where Bcl-2 has consistently been unable to block apoptosis (18,19). In addition, in pathways where Bcl-2 does block apoptosis it is the ratio of proapoptotic to antiapoptotic proteins that determines the overall potential for apoptosis (15). Unfortunately, the current article fails to provide levels of proapoptotic proteins for comparison and presents no functional data supporting a role for Bcl-2 in this particular setting. Thus, while Bcl-2 may alter the apoptotic potential of thyroid cells, it would not be expected to prevent apoptosis mediated by attacking lymphocytes through either the tumor necrosis factor, Fas, or TRAIL receptors that are present on thyroid cells (20).

The reasons behind the changes in apoptotic proteins observed by Giordano et al. are also of interest. As suggested above, one might presume that these changes are secondary to inflammatory events in the thyroid. This would certainly make sense when examining the alterations in the thyroid cells, but does not fully explain why the lymphocyte populations would be so different. In that regard, it could be that reported differences in the expression of cytokines or costimulatory molecules by thyroid cells in Graves' versus Hashimoto's diseases would yield different lymphocytes populations (21,22). It is also possible that cytokines elaborated as a result of the "thyrotoxic state" could alter either thyroid follicular cells or lymphocytes (23). What seems less likely is a genetic difference in the regulation of these pathways in patients with Graves' or Hashimoto's diseases. It is still possible that genetic factors could be involved, but this would probably also require environmental inputs to mediate the specific and contrary status of the apoptotic machinery on these two cell types.

In conclusion, while regulation of apoptosis is likely to be important in the expression of autoimmune disease, the specific mechanisms of regulation in the thyroid remain unknown. Activation of apoptosis can occur through a number of pathways that are similar in function but entirely independent in signal transduction. It is also likely that regulation of apoptosis or the apoptotic potential of thyroid cells may change over the course of autoimmune disease, allowing for the initiation or perpetuation of thyroid damage. Because it is impossible to examine the human thyroid during the evolution of the autoimmune process, it may be important to evaluate mouse models of Graves' and Hashimoto's disease to better clarify the role of proapoptotic and antiapoptotic forces that play a role in mediating the destruction or growth of human thyroid cells. This remains an extremely important area for clarification because it may allow for the manipulation of apoptotic pathways, perhaps leading to therapies that prevent thyroid dysfunction in patients with antithyroid autoimmunity (24).

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