T_H1 and T_H2 cytokine control of thyrocyte survival in thyroid autoimmunity

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Stassi *et al.*[1] have proposed that in thyroid autoimmunity thyrocyte survival depends on the differential effect of T_H1 and T_H2 cytokines. Their conclusion is based on the demonstration that the T_H2 cytokines IL-4 and IL-10, which are abundant in glands from patients affected by Graves' disease (GD) but not Hashimoto's thyroiditis (HT), are able to up-regulate expression of FLIP and of the anti-apoptotic gene that encodes Bcl-x_L on thyrocytes. *In situ*, both FLIP and Bcl-x_L are up-regulated in the thyroids of patients with GD but not HT. The up-regulation of these proteins would enable GD thyrocytes to become resistant to Fas-mediated apoptosis. The T_H1 cytokine IFN-γ, which is detected in HT thyroids, does not exert an effect on Bcl-x_L expression on HT thyrocytes. However, it can stimulate expression of caspase-8 and caspase-3 on these cells, which leads to apoptosis.

There are two points we wish to address: both relate to our difficulty in reconciling these results[1] with previous findings in the literature. First, in previous reports, no differential pattern of T_H1 and T_H2 cytokine expression has been shown in different thyroid autoimmune glands[2–5]. Although it has been suggested that a functionally, rather than morphologically, dominant subset of T cells is critical for the pathogenesis of either destructive or stimulating thyroid autoimmunity[6], clear documentation of a restricted T_H1- or T_H2-dependent profile has in general, remained elusive.

In particular, we have studied the expression of IL-10 in various thyroid conditions by reverse-transcribed polymerase chain reaction (RT-PCR), *in situ* hybridization and immunohistochemistry[5] and found the following. (i) By RT-PCR high amounts of IL-10 were seen in 3/6 GD samples and in 1/2 HT samples. In 3/6 GD samples the amount of IL-10 found was similar to that found in the controls. (ii) By *in situ* hybridization IL-10 expression was observed within the most intense infiltrates (not within the thyrocytes) not only of GD but also of HT glands. (iii) High amounts of IL-10 expression were associated with earlier stages of disease rather than with the severity of the lymphocytic infiltration. (iv) The highest amounts of IL-10 were present in 5/6 glands from autoimmune patients with the highest titers of circulating TPO antibodies, thus suggesting a role for this cytokine in B cell stimulation rather than in protecting against autoimmunity.

The second issue concerns the detection of "normal" amounts of Bcl-x_L expression on thyrocytes from thyroids affected by HT. Because it has been found that expression of another anti-apoptotic gene, which encodes Bcl-2, is decreased on thyrocytes taken from patients with this autoimmune-destructive condition[7,8] and Bcl-x_L is more easily induced than Bcl-2, at least on lymphocytes[9], we would have expected Stassi *et al.* to give an interpretation of their data in the light of these reports.

As autoimmune thyroid disease represents a broad spectrum of conditions with an extensive clinical overlap it is critical that one remains cautious when, from the results obtained in a small cohort of patients, hypotheses are formulated.

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Response

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Mirakian *et al.* question our recent results, which suggest that thyrocyte survival during thyroid autoimmunity depends on differential effects of T_H1 and T_H2 cytokines[1]. Thyrocyte destruction in autoimmune thyroiditis is a slow process that lasts several years. We hypothesized that in thyroid autoimmunity the balance between life and death in thyrocytes depends on the predominance over the time of T_H2 and T_H1 cytokines, whose action is not restricted to immune cells but involves direct modulation of key molecules responsible for survival or death of target cells[1].

As we discussed in our article, GD and HT are the two extremes of a wide clinical spectrum of thyroid autoimmunity that includes a series of mixed forms showing various degrees of tissue destruction. This heterogeneity may explain some conflicting results published in the literature.

However, before publishing our hypothesis[1], we analyzed cytokine expression in thyroid tissues from eight GD and eight HT patients. As described in our article, although the cytokine pattern is not "black and white", this analysis showed a clear prevalence of T_H2 cytokines in GD and T_H1 in HT[1] (and our unpublished data). These results were not published entirely because, considered in line with other published data[2,3], they were redundant.

Concerning the points specifically raised by Mirakian *et al*. in relation to their previous findings[4], we have the following comments. (i) In contrast to their data, IL-10 production has been clearly documented by others[5] in thyroids from patients with GD and not in thyroids from control patients. (ii) Mirakian *et al.* found IL-10 expression only in 1/2 HT-affected thyroids[4]. (iii) In contrast to what they claim, Mirakian *et al.* have shown that IL-10 was present only in the thyroid of the HT patient with the longest duration of disease (6 years)[4]. (iv) The protective role of IL-10 in autoimmune thyroiditis has been demonstrated *in vivo*[6].

Finally, we did not mention Bcl-2 in our article[1] because its overexpression in thyrocytes does not exert a clear protective effect during Fas-induced apoptosis in thyrocytes (our unpublished data).

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