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ORIGINAL ARTICLE

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Expression of bcl-2, Bax and Fas in oxyphil cells of Hashimoto thyroiditis

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Abstract Immunoreactivity for bcl-2, Bax and Fas was analysed in 16 cases with Hashimoto thyroiditis. Bcl-2-expression was constantly seen in regular thyrocytes and in the mantle-zone of lymphofollicular infiltrates. However, thyrocytes in the vicinity of lymphoid infiltrates and, especially, mitochondria-rich oxyphil cells exhibited reduced staining or none at all for bcl-2. Bax was found to be weakly reactive or negative in normal thyrocytes and was not up-regulated in bcl-2-deficient epithelial cells. In contrast, expression of Fas was markedly increased both in typical thyrocytes and in oxyphil cells within areas of lymphocytic infiltration. In conclusion, focal lack of bcl-2 expression together with up-regulation of Fas is a constant feature of Hashimoto thyroiditis. The reaction pattern of oxyphil cells is identical to that of affected typical thyrocytes without proliferation of mitochondria. Loss of bcl-2 with up-regulation of Fas is therefore likely to precede oncocytic change. Whether these alterations are involved in the process of oncocytic transformation remains to be clarified, however.

Key words Thyroid · Hashimoto thyroiditis · Oxyphil cell · Immunohistochemistry · bcl-2 · Bax · Fas

Introduction

Oxyphil cell metaplasia of the thyroid is a characteristic feature of Hashimoto thyroiditis [15, 42, 43]. The significance of this cell type is still unclear, however. In recent studies we have shown that defects of cytochrome-c-oxidase (complex IV of the respiratory chain) and a 4977-bp deletion (common deletion) of mitochondrial DNA may occur in the cells, which are very rich in mitochondria [38]. Furthermore, glucose transporter type IV is highly expressed in these cells [39].

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The bcl-*2* gene was first described in follicular lymphomas in which the chromosomal translocation, t (14; 18) juxtaposes the bcl-*2* gene to the immunoglobulin gene locus, resulting in high bcl-2 protein expression and prevention of apoptosis [58, 59]. As well as being expressed in various tumors, including carcinomas of the thyroid [1, 4, 8, 11, 19, 30, 41, 46–49, 53–55], bcl-2 is usually expressed in nonneoplastic lymphocytes and in regular tissue of the thyroid [30, 48, 49], the breast [25], the endometrium [21, 61] and the adrenals [9].

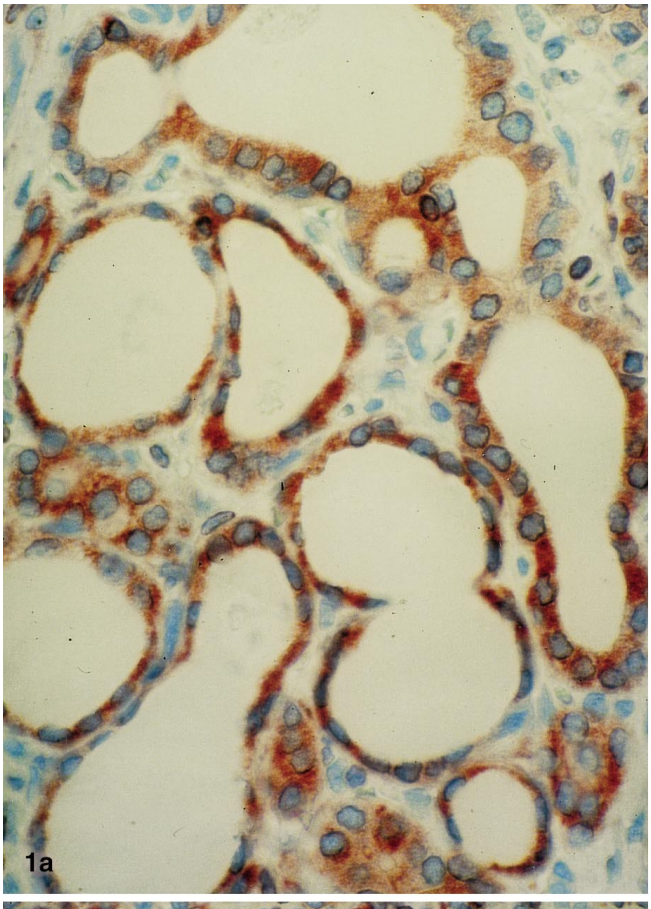
Bcl-2 expression in the thyroid has been found to be associated with differentiated follicular and papillary carcinomas, whereas anaplastic carcinomas, but also oncocytic tumors, mostly lack bcl-2 expression [30, 37, 48, 49].

In contrast to bcl-2, which prevents cell death, Bax protein promotes cell death by forming homo- and heterodimers with bcl-2 [45].

In thyroid carcinomas Bax protein has been found to be almost completely lacking in well-differentiated carcinomas and to be coexpressed with bcl-2 in poorly differentiated carcinomas, but it is selectively present in anaplastic carcinomas [30].

In recent studies Apo-1/Fas, a member of the TNF receptor family involved in triggering apoptosis [44, 64], has been shown to be up-regulated in Hashimoto thyroiditis [10, 14, 32]. The reactivity of oxyphil cells has not, however, been analyzed specifically.

In the present study we investigated the reaction pattern of oxyphil cells for Fas, Bax, bcl-2 and were especially interested to find whether oxyphil cells in Hashimoto thyroiditis lack bcl-2 expression, as is the case in oncocytic neoplasms of the thyroid gland [37]. Furthermore, the reactivity of oxyphil cells was compared with the reaction profile of affected nonoxyphil thyrocytes to see whether alterations of these proteins might be involved in the pathogenesis of oxyphil metaplasia in Hashimoto thyroiditis.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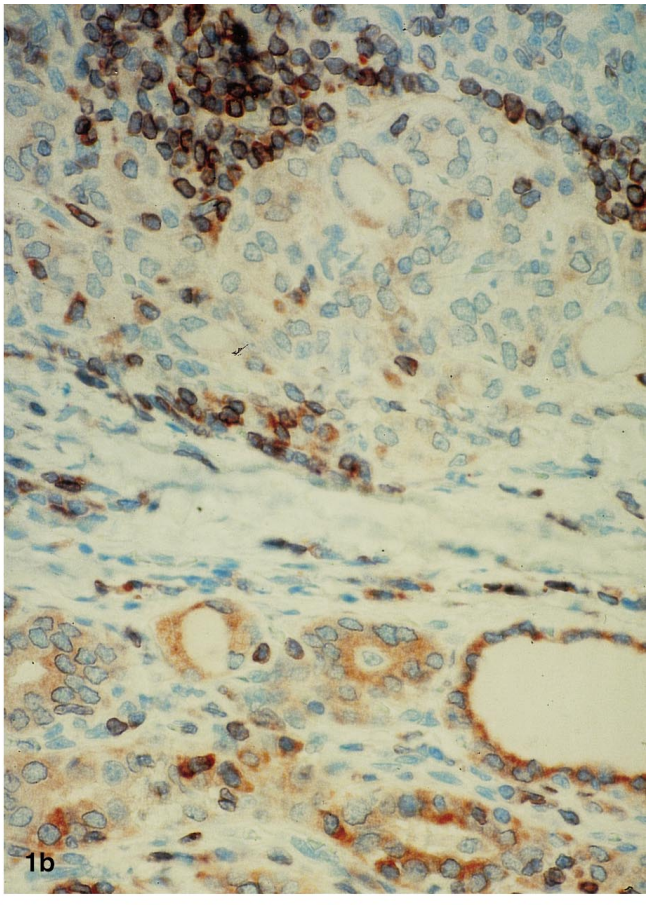
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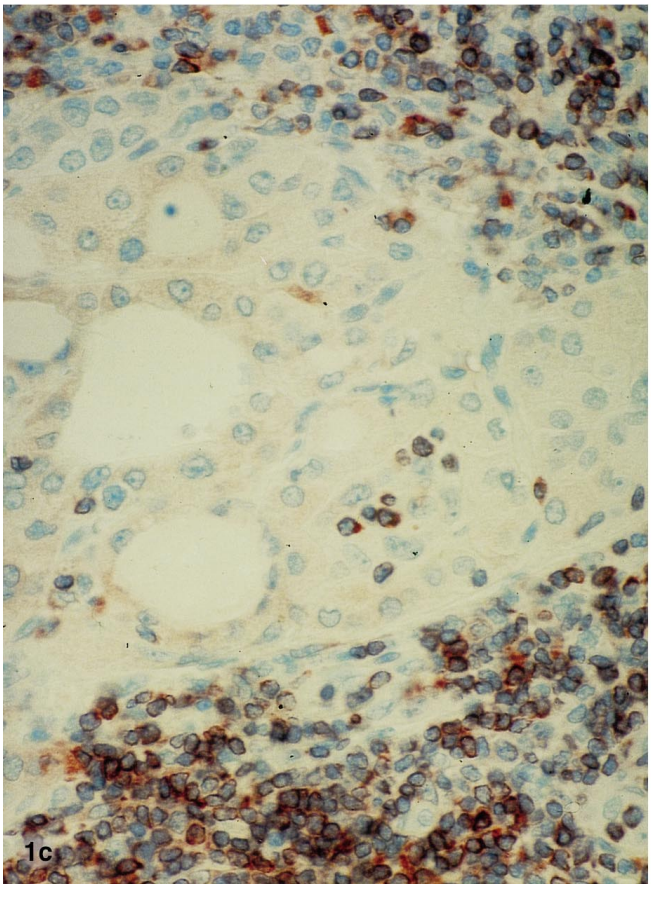
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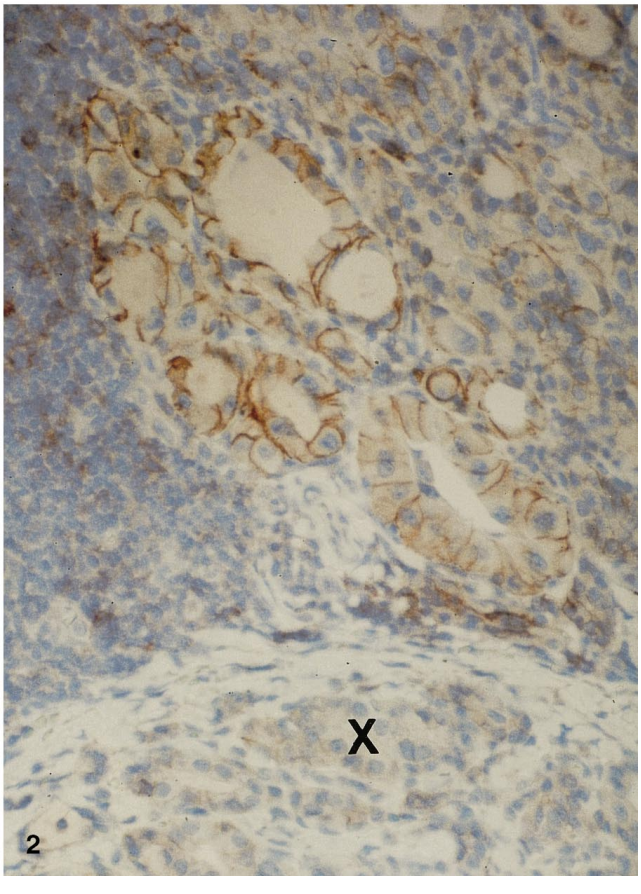
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Materials and methods

Sixteen resection specimens from patients with a diagnosis of Hashimoto thyroiditis were obtained from the files of the Department of Pathology of the University of Munich for immunohistochemical analysis. The male-to-female ratio was 1:3, and the median age of the patients, 51 years (mean age 54 years, range 27–77 years).

Surgical resection had been performed mainly because of goitrous enlargement partly caused by the additional presence of endemic goitrous nodules and because of unclear cytological results of fine-needle biopsies.

The diagnosis of Hashimoto thyroiditis was made mainly with reference to morphological criteria. These encluded a bilateral involvement, the presence of a predominantly diffuse lymphocytic infiltrate, often with formation of germinal centers, atrophy and destruction of follicular epithelium associated with oxyphilic cell metaplasia.

Immunohistochemical analysis was performed on formalin-fixed paraffin-embedded 5-µm sections, deparaffinized and rehydrated through graded alcohols. Immunocytochemical reactivity was tested for bcl-2 (monoclonal, mouse, 1:10, Dako), Bax (polyclonal, rabbit, 1:20, Calbiochem) and Fas (monoclonal, mouse, 1:40, Dako).

The slides were subjected to pretreatment by microwaving in citrate cytobuffer at pH 6 in a 750-W oven for 2×10 min. Before further staining slides were also pretreated with avidin/biotin blocking reagent (Dako) for 15 min each at room temperature.

The specific antibodies were applied for 30 min at room temperature. Detection was performed with streptavidin-conjugated peroxidase (Dako) and for Bax protein with ABC Elitekit (Vector), using aminoethylcarbazole (AEC) as chromogen.

For negative controls the primary antibody was omitted and the primary antibody was substituted by nonimmune immunoglobulin of the same class as the primary antibody.

Results

Routine hematoxylin and eosin staining disclosed the typical features of Hashimoto thyroiditis with a prominent diffuse and lymphofollicular infiltrate irregularly distributed in the thyroid tissue and multiple foci of follicular epithelium destruction. To a varying extent pronounced oxyphil change of thyrocytes was seen (Fig. 1C).

Bcl-2

Bcl-2 staining showed a cytoplasmic type of reaction in normal thyrocytes distant from lymphocytic infiltrates (Fig. 1A), which was identical to the staining patterns in thyroids not affected by inflammatory disease. The reaction was equally developed in all follicles, and a high staining intensity predominated. Typically the lymphocytic infiltrates also were stained (Fig. 1B, C), the germinal centers being spared. In contrast, expression of bcl-2 was reduced or lacking in small aggregates of nonoxyphilic thyrocytes closely associated with lymphocytic infiltrates (Fig. 1B). Oxyphil cells in particular were predominantly negative for bcl-2 (Fig. 1C). Only occasionally was a faint cytoplasmic reaction seen (not shown).

Bcl-2 staining was lacking both in single oxyphil cells and in oxyphilic cell clusters with and without inflammatory infiltrate in its vicinity.

Bax

The antibody to Bax was found to be weakly reactive or negative in most regular thyrocytes and oxyphil cells, whereas immunoreactivity was seen especially in the germinal center cells of lymphofollicular infiltrates (not shown).

Fas

Thyrocytes from normal thyroid tissue showed only a weak reaction or none at all in the cytoplasm. A membranous staining pattern was not seen. In contrast, thyroid follicles near or within lymphocytic infiltrates exhibited intensive staining (Fig. 2). The positive staining reaction was seen both in nonoxyphil follicular cells and in typical oxyphil cells. Especially in oxyphil cells the staining pattern was mainly membranous. A clear positive reaction was also observed in the infiltrating lymphocytes, particularly at the center of lymphoid follicles, whereas the reaction was predominantly lacking in the adjacent mantle zone.

Discussion

Bcl-2 belongs to a group of genes (bcl-2 gene family) that modulates cell death [3]. The expression of the gene family seems to be regulated differentially among cell types and stage of differentiation. The biological effect on a cell is therefore dependent on its level, selective expression and dimerization status [28]. Bcl-2 is expressed in several normal and various malignant human tissues [17, 27, 28]. Interestingly, bcl-2 is expressed in most developing epithelial tissues and appears to be restricted to cells at specific stages of differentiation [27, 28].

In adult epithelia bcl-2 is found in duct cells of exocrine cells, including pancreas, salivary and sweat glands [28], and in hormone-responsive cells, such as those in the endometrium, mammary glands, prostate, adrenals [9, 21, 25, 61] and thyroid [30, 48, 49].

In thyroid carcinomas bcl-2 expression depends on the degree of differentiation, being absent in anaplastic

◀ **Fig. 1A–C** Bcl-2-immunohistochemistry. **A** Normal thyroid follicles with cytoplasmic reactivity. **B** Nonoxyphilic thyroid follicles in the vicinity of lymphocytic infiltrates are devoid of immunohistochemically detectable bcl-2 in the cytoplasm. **C** In oxyphil thyrocytes the typical cytoplasmic reaction is also lacking. **B, C** Lymphocytes stain positive. ×540

Fig. 2 Immunohistochemical detection of Fas. In areas with inflammatory infiltrates intensive expression of Fas is seen with a predominantly membranous staining pattern in oxyphil cells. Adjacent regular thyroid follicles (*X*) show only weak expression or none at all. ×350

tumors [30, 48, 49]. This finding is in agreement with the idea that bcl-2 expression allows epithelia to differentiate and morphogenesis to proceed [28].

Interestingly, anaplastic thyroid carcinomas express the Bax protein exclusively, which forms homo- and heterodimers with bcl-2 in vivo [30]. Thereby, bcl-2 is inactivated and programmed cell death may proceed [45].

We analyzed the expression of bcl-2, Bax and Fas in Hashimoto thyroiditis with special reference to the reaction pattern of the mitochondria-rich oxyphil cells. It was found that bcl-2 is down-regulated in Hashimoto cells, particularly at inflammatory sites, down-regulation being most evident in oxyphil cells. Conversely, Fas was found to be up-regulated in thyrocytes near the inflammatory reaction sites, with oxyphil cells showing an intensive, often membranous staining reaction.

The data on expression of bcl-2 and Fas have been corroborated by two recent studies [14, 32]. In these studies the reactivity of mitochondria-rich oxyphil cells was not specifically analyzed, however. Furthermore, our results indicate that up-regulation of Bax is not apparently involved in the pathogenesis of Hashimoto thyroiditis. This is consistent with the finding that apoptosis in Hashimoto thyroiditis is induced by Fas-ligand up-regulation [10, 14, 32]. Since Fas belongs to the TNF receptor family, it is interesting to note that TNF receptor II has recently been localized in the inner mitochondrial membrane of preadipocytes [24].

Bcl-2 has been localized to the outer membrane of mitochondria, in a patchy distribution probably related to contact sites between the outer and inner mitochondrial membrane [22, 34, 52]. A variety of mitochondrial events such as the sensitivity to oligomycin inhibition of complex V, to cyanide and the release of sequestrated calcium in the matrix appear to be modulated by bcl-2 [12]. Disruption of the transmembrane potential (electrochemical proton gradient) of the inner mitochondrial membrane is an early event in apoptosis, which can be prevented by bcl-2 overexpression [23]. Bcl-2 blocks apoptosis in cells without mitochondrial DNA and therefore with a nonfunctional respiratory chain [18, 31].

What precisely causes dysregulation of bcl-*2* and Fas genes in Hashimoto thyroiditis is not yet known. Down-regulation of bcl-*2* has been shown to be the first step in Fas-mediated apoptosis in the male reproductive tract [57], and thyroid-stimulating hormone (TSH) inhibits Fas-mediated apoptosis [20]. The altered immunohistochemical profile of bcl-2 and Fas, especially in the vicinity of inflammatory infiltrates, is most consistent with the involvement of cytokine effects. In fact, TNF alpha has been shown to decrease bcl-2 expression and up-regulate Fas in human cell lines [5, 29]. In addition, interleukin-1 beta has been shown to up-regulate Fas and induce apoptosis in Hashimoto thyroiditis [10]. Recently a deletion of C-terminal signal anchor sequence has been found to interfere with targeting of bcl-2 to the outer mitochondrial membrane and with bcl-2-mediated suppression of apoptosis [23]. Furthermore, it should be mentioned that bcl-2 is down-regulated by the suppressor gene *p53* [13, 33].

We recently reported on reduced expression of bcl-2 in oncocytic adenomas and carcinomas of the thyroid [37]. In these tumors no up-regulation of Fas could be found, however (unpublished results). Since the lack of bcl-2 in anaplastic thyroid carcinomas [30, 48, 49] is associated with up-regulation of Bax – which was not the case in the bcl-2-negative oxyphil cells in Hashimoto thyroiditis – it may be assumed that pathogenetic mechanisms are different in neoplastic and metaplastic oxyphilic transformation.

The pathogenesis of oncocytic transformation, and thus of mitochondrial proliferation, is still unresolved. Generally the rate of ATP consumption, hypoxia and nuclear-regulatory factors are assumed to be of importance for the regulation of mitochondrial proliferation. The role of cytokines is still unclear. Our results have shown that the immunoreactive profile of oxyphil cells for bcl-2, Bax, and Fas does not differ from affected follicular cells without mitochondrial proliferation. Dysregulation of bcl-2 and Fas is therefore likely to precede oncocytic metaplasia and could be involved in its pathogenesis. Mitochondrial DNA, at least, is not a necessary prerequisite, since mitochondrial proliferation is also seen in case of depletion of mitochondrial DNA occurring under various conditions [2, 6, 26, 35, 36, 60].

Various studies indicate that mitochondria are possible primary sites for the action of thyroid hormones, and especially of T3 [7, 16, 50, 51, 56, 62, 63], and it has been shown that T3 induces biogenesis of mitochondria [50, 51]. Furthermore, a 43-kDa protein related to c-ErbA alpha 1 has been located in the mitochondrial matrix and could act as a mitochondrial T3-dependent transcription factor in a similar way to its function in the nucleus [63]. It would be interesting to know whether there is a change in the receptor status for TSH and thyroid hormones in oxyphilic cells.

In summary, we have shown that focal loss of bcl-2 and up-regulation of Fas is a characteristic feature of Hashimoto thyroiditis occurring prior to oncocytic metaplasia. It remains to be seen, however, whether the altered cells without a typical oxyphil appearance are already harboring some type of nuclear and/or mitochondrial genetic alteration responsible for mitochondrial proliferation. These factors are still unclear.

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