Possible association of CEA expression with oxyphilic change but not with C-cell hyperplasia in Hashimoto’s thyroiditis

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Abstract. Reactive C-cell hyperplasia (CCH) has been observed in cases of autoimmune Hashimoto’s thyroiditis; however, its occurrence in Graves’ disease, the other major autoimmune disorder, has not yet been investigated. On the other hand, although Carcinoembryonic Antigen (CEA) serum levels have been reported elevated in patients with autoimmune thyroid disease (ATD), the source of CEA production at the cellular level is not elucidated. The aim of this study was to evaluate CCH and CEA immunohistochemical expression and comparatively analyze them in 136 ATD cases (107 Hashimoto’s and 29 Graves’ disease cases) and 20 cases of nodular hyperplasia (NH). Immunohistochemistry using monoclonal antibodies to chromogranin and CEA was performed. A scoring system for CCH and semiquantitative evaluation for CEA expression were applied. C-cell hyperplasia was absent in NH cases. In contrast, it was detected in 11% of ATD cases being more frequently observed in Hashimoto’s (12.1%) than Graves’ disease (6.8%) CCH associated to male sex and older age of Hashimoto’s patients. CEA was detected only in ATD cases (33.8%), in C-cells and in follicular cells as well, being more frequently detected in Graves’ (44.8%) than Hashimoto’s (30.8%) disease. An interesting finding was an emerging possible association of CEA expression with oxyphilic change but not with C-cell hyperplasia in Hashimoto’s thyroiditis. No significant correlation was established between CCH and CEA follicular cell expression in neither disease. In conclusion, C-cell hyperplasia and CEA expression may be encountered in the setting of Hashimoto’s thyroiditis and Graves’ disease.

Key words: C-cell hyperplasia, CEA, Oxyphilic change, Autoimmune thyroiditis

C-CELL hyperplasia (CCH) was first described in 1973 by Wolfe et al. in asymptomatic relatives of patients suffering from medullary thyroid carcinoma (MTC) [1]. Since then CCH has been mainly investigated as a putative precancerous lesion associated with the subsequent development of MTC [2-5]. However, apart from precursor of MTC, CCH has been described in physiologic conditions as a probable age related phenomenon in infants or elderly patients and in many different pathologic situations in association with hyperparathyroidism, hypercalcemia, hypergastrinemia and even adjacent to follicular cell tumors and non-Hodgkin lymphomas of the thyroid [6-12].

On the basis of previous observations, two types of CCH that differ by their physiological characteristics have been identified: neoplastic CCH and reactive (also called physiological) CCH [13]. Neoplastic CCH is thought to be caused by a germline mutation of the RET protooncogene in multiple endocrine neoplasia type 2 (MEN 2) syndrome. It progresses to MTC following a time line that depends on the RET mutation involved. CCH may actually be a misnomer for a neoplastic condition that some authors have proposed to call “in situ-MTC” [14]. On the other hand, the pathogenesis of reactive CCH, although poorly understood, has been attributed to overstimulation by thyroid stimulating hormone, hypercalcemia and other stimuli external to the C-cell [13]. In contrast to the neoplastic form, reactive CCH is not associated with MTC and its premalignant potential is not documented [13].

The occurrence of CCH in non neoplastic conditions of the thyroid has not been thoroughly investigated. Although CCH has been reported in cases of Hashimoto’s thyroiditis [12, 15-17], its occurrence in Graves’ disease, the other major organ-specific -
gan-stimulating thyroid disease, has not yet been studied. Moreover, although CEA serum levels have been reported elevated in patients with autoimmune thyroid disease [18, 19], the source of CEA production at the cellular level is not established. Hypothesizing that C-cells could be the source of CEA production, we investigated CEA immunohistochemical expression in relation to CCH in Hashimoto’s thyroiditis and Graves’ disease, comparatively and in each disease separately.

**Material and Methods**

Our material consisted of paraffin embedded tissue retrieved from the files of the 1st Department of Pathology, Medical School, University of Athens, Greece. A total of 156 thyroidectomy specimens examined in our Department between January 2006 and December 2008 was reviewed. Specimens included 136 cases of autoimmune thyroid disease (ATD) and 20 cases of nodular hyperplasia (NH). The weight of the thyroids ranged between 30 and 70 gr (a few too large or too small thyroids, examined in the same period, were not included in the study). Mean thyroid weight was 40gr for Hashimoto’s cases, 50 gr for Graves’ cases and 45gr for NH cases. Clinicopathological characteristics are presented in Table 1.

**Method**

Haematoxylin-eosin sections were retrieved and the histological diagnosis reevaluated. Hashimoto’s thyroiditis cases were estimated according to the presence of oxyphilic cells and were classified as oxyphilic type in case of the presence of oxyphilic metaplastic cells leading to the formation of oxyphilic nodules of any size.

Four μm paraffin sections were prepared from 2 blocks of each case and immunohistochemistry was performed for the detection of chromogranin-positive C-cells as well as for the investigation of CEA expression in the tissue of the above cases. A two step immunoperoxidase method has been performed, using for the detection of the reaction the Chem Mate Real Envision Detection kit (Dako K5007). The primary antibodies used were the following: Monoclonal Chromogranin (Dako M867), dilution 1:10, incubation time 30 min, monoclonal CEA (Dako M707), dilution 1:50, incubation time 30 min. To enhance antigen retrieval microwaving (800W) for 10 min was applied. For the visualization of the reaction, DAB has been used as a chromogen counterstained with hematoxylin.

The evaluation of the immunohistochemistry has been performed according to the extent of the stain as following:

A. C-cell hyperplasia scoring system:
   - Number of chromogranin-positive C-cells in 5 LPF (X100).
     - Absent: <25 cells
     - Mild: 25-50 cells
     - Moderate: 50-70 cells
     - Severe and/or nodular: 70 cells or C-cell clusters of > 7 cells

B. CEA expression in follicular epithelium (semi-quantitative evaluation):
   - : < 5% of positive cells or very weak stain of any extent (negative expression)
   + : 5-25% strongly positive cells (mild positive expression)
   ++ : 25-50% strongly positive cells (moderate positive expression)
   +++ : > 50% strongly positive cells (strong positive expression)

**Statistical Analysis**

Statistical analysis was performed using Fisher’s Exact Test and Pearson’s Chi-Square. A p-value of less than 0.05 was considered as statistical significant.

**Results**

A. C-cell hyperplasia (CCH)

C-cell hyperplasia defined as >25 chromogranin positive cells / 5LPF (X100) was not observed in any of 20 nodular hyperplasia cases. In contrast, it was
detected in 11% of 136 autoimmune thyroid disease cases, being more frequently observed in Hashimoto’s (12.1%) than Graves’ disease (6.8%) (Table 2), (Fig. 1). However, this difference did not proved statistical significant (Pearson’s Chi-Square: 0.641). Regarding the degree of CCH, Graves’ disease positive cases demonstrated either moderate or severe CCH while in Hashimoto’s thyroiditis a substantial proportion of positive cases showed mild CCH (Table 2). However, the degree of CCH was not significant different between Hashimoto’s thyroiditis and Graves’ disease neither when cases with moderate and severe CCH were compared to cases with mild CCH (Fisher’s exact test: p=0.467) nor when cases with absent and mild CCH were compared to cases with moderate and severe CCH (Fisher’s exact test: p=0.679).

CCH demonstrated a slightly increased prevalence in males and older age patients.

### B. CEA immunohistochemical expression

CEA immunohistochemical expression was detected in the cytoplasm of C-cells while inflammatory or stromal cells were negative. In cases of autoimmune thyroid diseases but not in NH cases, CEA expression was also detected in follicular cells sometimes restricted in their apical border (Fig. 2). CEA expression in follicular cells was significantly associated with autoimmune thyroid disease compared to nodular hyperplasia (Pearson’s Chi-Square: p=0.022, value=9.594, df=3). Positive CEA expression in follicular cells was observed in 46 of 136 autoimmune thyroid disease cases (33.8%) being more frequently - though not significantly (Fisher’s exact test: p=0.186) - detected in Graves’ (44.8%) than Hashimoto’s disease (30.8%) (Table 3). Concerning the degree of CEA positivity, no significant difference between Hashimoto’s and Graves’ disease was observed with regard to mild or severe CEA expression while a two-fold higher percentage of moderate CEA positivity was noted in

#### Table 2  C-cell hyperplasia in nodular hyperplasia, Hashimoto’s and Graves’ disease

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Absent (%)</th>
<th>Mild (%)</th>
<th>Moderate (%)</th>
<th>Severe (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodular hyperplasia</td>
<td>20</td>
<td>20 (100)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Autoimmune thyroid disease</td>
<td>136</td>
<td>121 (88.9)</td>
<td>7 (5.1)</td>
<td>4 (2.9)</td>
<td>4 (2.9)</td>
</tr>
<tr>
<td>Hashimoto’s disease</td>
<td>107</td>
<td>94 (87.8)</td>
<td>7 (6.5)</td>
<td>3 (2.8)</td>
<td>3 (2.8)</td>
</tr>
<tr>
<td>Graves’ disease</td>
<td>29</td>
<td>27 (93)</td>
<td>0</td>
<td>1 (3.4)</td>
<td>1 (3.4)</td>
</tr>
</tbody>
</table>

#### Table 3  CEA expression in follicular thyroid cells in our cases

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>CEA positivity in follicular cells (%)</th>
<th>(+) (%)</th>
<th>(+++) (%)</th>
<th>(++) (%)</th>
<th>- (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodular hyperplasia</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20 (100)</td>
</tr>
<tr>
<td>Autoimmune thyroid disease</td>
<td>136</td>
<td>46 (33.8)</td>
<td>20 (14.7)</td>
<td>20 (14.7)</td>
<td>6 (4.4)</td>
<td>90 (66.2)</td>
</tr>
<tr>
<td>Hashimoto’s disease</td>
<td>107</td>
<td>33 (30.8)</td>
<td>15 (14)</td>
<td>13 (12)</td>
<td>5 (4.6)</td>
<td>74 (69)</td>
</tr>
<tr>
<td>Graves’ disease</td>
<td>29</td>
<td>13 (44.8)</td>
<td>5 (17.2)</td>
<td>7 (24)</td>
<td>1 (3.4)</td>
<td>16 (55)</td>
</tr>
</tbody>
</table>
Graves’ cases compared to Hashimoto’s ones (Table 3). However, the degree of CCH was not significant different between Hashimoto’s thyroiditis and Graves’ disease (Pearson’s Chi-Square: $p=0.370$, value=3.143, $df=3$). No correlation of CEA expression with the age and sex of the patients emerged.

C. Association of CEA expression and CCH with oxyphilic change in Hashimoto’s disease

An increased CEA expression was observed in Hashimoto’s cases with oxyphilic change of the follicular epithelium. Oxyphilic cells themselves demonstrated CEA staining which, of note, was observed in a biotin free environment. More specifically, 48 out of 107 Hashimoto’s cases (44.85%) demonstrated oxyphilic change. In approximately half of them (23/48 ~ 48%) CEA expression and/or CCH was recorded as following: 17 cases (35.4%) showed positive CEA expression in the oxyphilic cells (mild: 47%, moderate: 47%, strong: 5.8%) without coexistent CCH, 4 cases (8.3%) showed positive CEA expression in the oxyphilic cells and presence of CCH and 2 cases (4%) showed only CCH moderate and intense respectively. These findings suggest a possible association between oxyphilic change and CEA expression rather than CCH.

D. Comparison between CCH and CEA expression

We isolated the cases of Hashimoto’s and Graves’ disease showing more than moderate CCH and/or more than moderate CEA expression: there were 20 such Hashimoto’s cases and 9 such Graves’ cases. Among the 20 Hashimoto’s cases, 6 (30%) demonstrated simultaneous strong CEA expression and CCH (moderate or severe). Among the 9 Graves’ cases, one (11.1%) showed simultaneous strong CEA expression and moderate or severe CCH. Although the number of cases in each subgroup is quite limited for statistical analysis, these findings suggest that there is no strong correlation between CCH and CEA expression in neither disease.

Discussion

C-cell hyperplasia can be defined as an increased number of C-cells in the thyroid gland [20]. This definition suffers from the difficulty to precisely assess the total number of C-cells in a normal thyroid gland and unanimity is far from being reached in the delineation between what can be considered to be a normal C-cell population and what represents C-cell hyperplasia [6-8, 21-24]. Relatively few studies using necropsy specimens have documented the normal C-cell population in the thyroid gland [23]. Such studies were confronted with many difficulties: C-cells represent a very low proportion of the thyroid cells, with a heterogeneous distribution in the gland (highest concentrations being found in the junction zone between the upper and middle thirds of each lobe), and their density varies greatly according to age, and even between two individuals of the same age [17]. Furthermore, methodology varied from one study to another and, because the choice of the thyroid site examined and the extent in sampling have a direct bearing on the C-cell density, this prevents any relevant comparison between the two variables. In the present study, we used a grade-scale in order to evaluate the possible implications of gradual C-cell increase.

The presence of CCH in association with chronic lymphocytic thyroiditis has been investigated in only a few studies [12, 15-17]. Libbey et al. [15] in 1989 and Biddinger et al. [16] in 1991 reported on the cases of a 63 year-old woman and a 58 year old man respectively, both suffering from Hashimoto’s disease accompanied by hypercalcitoninemia. Immunohistochemical examination of their thyroids showed marked CCH. Nine other cases of chronic lymphocytic thyroiditis were then retrospectively analysed among which two showed several LPFs containing from 25 to 90 C-cells; none of these patients had a history of MEN or MTC familial disease [16]. In a study of 112 cases, chronic lymphocytic thyroiditis was associated in 20% of the cases with CCH, defined as a C-cell density > 40 cells/cm$^2$ and the presence of at least three LPFs containing more than 50 C-cells [17]. In the same study, Guyetant et al. [17] showed that CCH associated with chronic lymphocytic thyroiditis can occasionally be accompanied by an increase in calcitonin levels, which may be important enough to suspect the presence of a medullary thyroid carcinoma. The undetectable calcitonin levels in the postsurgery tests confirmed the thyroid origin of this hypersecretion. Clinicians must be aware of this possibility, which could lead to thyroidectomy in the absence of any MTC and most probably in the absence of any increased risk of MTC.

The present study is one of the few ones investigating the occurrence of CCH and of CEA immuno-
hypothesis of CCH in the setting of autoimmune thyroiditis remains to be established. It has been shown that neuroendocrine cells can rapidly proliferate in response to or in association with a variety of reactive, regenerative and chronic inflammatory stimuli; this has been well documented in the bronchopulmonary tract and in the small intestine [24, 25]. The pathogenesis of CCH associated with chronic lymphocytic thyroiditis may involve an immunopathological mechanism or an effect of the inflammatory mediators and cytokines secreted by the infiltrating leukocytes in the thyroid parenchyma. The role of a C-cell growth factor, such as the gastrin-related peptide whose gene is overexpressed in hyperplastic C-cells adjacent to follicular cell tumors has also been suggested [26].

CEA has been mainly studied in the serum but not the tissue of patients with autoimmune thyroid diseases [18, 19]. Increased levels of serum CEA have been observed in hyperthyroid patients with Graves’ disease possibly as a result of stimulation by cytokines and especially interferon-γ and IL-6 known to be upregulated in Graves’ disease [19, 27]. On the other hand, and in contrast to the previous studies, Amino et al. recorded a significantly high frequency of elevated serum CEA in hypothyroid patients with Hashimoto’s disease [18]. The authors suggest that CEA elevation in hypothyroidism may be caused by decreased degradation of CEA [18].

In our study, CEA expression was investigated at the tissue level in patients with Graves’ disease and thyroiditis Hashimoto’s. In both conditions, CEA expression was detected in C-cells as well as in follicular cells restricted in some cases in their apical border. No relation between C-cell hyperplasia and follicular CEA expression was observed rendering less possible the existence of either a common pathogenetic mechanism explaining both expressions or a paracrine interaction between follicular and C-cells. Taking into account the putative stimulatory role of cytokines in the pathogenesis of both C-cell hyperplasia and CEA expression, one could speculate that different groups of cytokines might be activated to generate either C-cell hyperplasia or CEA expression. This concept is further enhanced by the observed in our study tighter correlation between: a) CEA immunohistochemical expression and Graves’ than Hashimoto’s disease and b) C-cell hyperplasia and Hashimoto’s than Graves’ disease. This differential predominance of C-cell hyperplasia and CEA expression in Hashimoto’s thyroiditis and Graves’ disease respectively could reflect the different cytokine profile of the two diseases known to be “organ stimulatory” in Graves’ with a shift to a more Th2-driven cytokine pattern, and “organ destructive” in Hashimoto’s with increased levels of Th1 related cytokines indicating predominantly cell-mediated cytotoxic processes [28, 29].

Another interesting finding was the increased CEA expression in Hashimoto’s disease associated with the synchronous presence of oxyphilic nodules. Although the pathogenesis of oxyphilic change is not clear, it might also be related to cytokine release. Caturgeli and Kimura developed a mouse model of Hashimoto thyroiditis based on the chronic production of interferon-γ by the thyroid follicular cell via transgenesis [30-32]. Interestingly, the thyrocytes of interferon-γ transgenic mice demonstrated increased levels of immunoproteasome subunits like LMP2 and assumed a morphology resembling that of the human oncocyte suggesting an important role of interferon-γ and immunoproteasome in oncocyte pathogenesis [30-32]. Taking into account the reported in many previous studies [27, 33, 34] in vitro upregulation of CEA by interferon-γ, our finding of increased CEA in cases with more abundant oxyphilic cells could be attributed to the involvement of interferon-γ in both oxyphilic change and CEA upregulation as well.
References


