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## Autoimmune and non-autoimmune thyroid diseases have different patterns of cellular HLA class II expression

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### ABSTRACT

### INTRODUCTION

**Context:** Surface HLA-DR antigen is usually only expressed by antigen-presenting cells (APC). In autoimmune thyroid disease, follicle cells function as APC, thus expressing HLA-DR. However, non-autoimmune thyroid diseases may also express surface class II antigens.

**Objective:** To evaluate the presence and pattern of HLA class II expression in autoimmune and non-autoimmune thyroid disorders.

**Design:** Retrospective: histopathological and immunohistochemical analysis.

**Location:** Referral center, university hospital.

**Sample:** Ten histologically normal thyroids, 11 Graves' disease, 7 Hashimoto's thyroiditis, 10 atoxic multinodular goiter and 3 toxic adenomas were analyzed by immunohistochemistry, using a monoclonal antibody anti-HLA-DR.

**Main Measurements:** The presence of these antigens in thyroid follicular cells and their relation to inflammatory infiltrate was evaluated. The pattern of HLA-DR expression in thyroid follicular cells was analyzed: membrane, cytoplasmic or both.

**Results:** Although HLA-DR antigens were sparsely present in one of the 8 normal thyroids, in 6 of the 9 atoxic multinodular goiter and in 2 of the 3 toxic adenomas a net positivity could be seen in large areas. In all 5 Hashimoto's thyroiditis and in 7 of the 10 Graves' disease cases. This expression occurred in follicle cells either in contact with inflammatory cells or not. In non-autoimmune thyroid disease, HLA-DR positivity was essentially cytoplasmic, whereas in Graves' disease and Hashimoto thyroiditis it was mainly in cell membranes.

**Conclusions:** It is suggested that the HLA class II expression on the surface of follicle cells could be related to auto-antigen presentation to the immune system by these cells, leading to inflammation.

**Key-words:** Graves' disease. Autoimmune thyroiditis. HLA-DR antigens. Immunohistochemistry

Class II antigens of the human major histocompatibility complex (MHC), the human leukocyte antigens (HLA) DP, DQ and DR, are central elements in the presentation of exogenous antigens to T CD4+ lymphocytes and the regulation of immune response. Expression of these glycoproteins on the cell surface is restricted to B lymphocytes, macrophages, other antigen-presenting cells and the capillary endothelium.<sup>1</sup> Some authors described aberrant HLA class II expression antigens in follicular thyroid cells in Graves' disease (GD)<sup>2</sup> and Hashimoto thyroiditis (HT)<sup>3</sup>. Cell cultures from normal thyroids or GD cases generally express HLA-DR in follicles near areas infiltrated by lymphocytes, when stimulated by  $\gamma$ -interferon.<sup>2,4</sup> These cells probably present self-antigens to the immune system and perpetuate the autoimmune process.<sup>5</sup> HLA class II expression has also been observed in some normal thyroids, atoxic multinodular goiter (AMG), thyroid adenomas and carcinomas.<sup>6,7</sup> As there has been no report on the topographical pattern of HLA-DR expression in thyrocytes, the aim of the present study was to detail this aspect in normal, autoimmune and non-autoimmune thyroid tissue, using the immunoperoxidase technique.

## METHODS

Thyroid surgical specimens from 8 normal individuals, 10 with GD, 5 with HT, 9 with AMG and 3 with toxic adenoma (TA) were fixed in 10% formalin, embedded in paraffin, stained with hematoxylin and eosin and selected for immunohistochemical techniques. Specific immunostaining was performed to identify HLA-DR using a modified streptavidin-biotin-peroxidase technique.<sup>8</sup> Monoclonal primary anti-HLA-DR antibody (Dako), was diluted at 1:10 in 0.1% BSA. Detection of the streptavidin-biotin-peroxidase complex was achieved with diaminobenzidine and slight counterstaining with hematoxylin. At the end of the reaction, slides were mounted with Entellan (Merck). Immunostained sections were scored for the expression of HLA-DR using the following criteria: a) presence of HLA-DR positive follicular cells in contact with inflammatory infiltrate; b) presence of DR positive follicular cells in the absence of inflammatory infiltrate; c) presence of DR positive inflammatory infiltrate and d) presence of DR positive inflammatory infiltrate in the absence of DR positive follicular cells.

The pattern of HLA-DR expression in follicular cells was classified as: 1) restricted to the cell membrane; 2) restricted to the cytoplasm and 3) in both, cell membrane and cytoplasm.

## RESULTS

HLA-DR expression in one of the 8 normal thyroid tissue cases, 6 of the 9 AMG and 2 of the

3 TA was restricted to small scattered groups of follicular cells. However, in 7 of the 10 GD cases and especially in all 5 HT cases, expression was more diffuse and extensive, generally observed in close relation to a lymphoplasmacytic infiltrate. In addition, in 6 GD and 4 HT cases, positive follicles were also found outside these areas.

The pattern of HLA-DR expression in follicular cells in AMG (5 of 6) and in TA (2 of 2) could only be verified restricted to the cytoplasm. In one normal thyroid that presented a small group of DR positive follicular cells, the expression was in the membrane. In GD, HLA-DR was always expressed in the membrane (7 of 7) and in 2 cases it was also observed in the cytoplasm. In 5 of the 5 HT cases, the positive membrane staining was more intense and was accompanied by cytoplasmic expression in 4 of the 5. (Figure 1, Table 1)

## DISCUSSION

This study has shown that various benign thyroid diseases may express HLA class II, in agreement with other authors.<sup>6</sup> However, the pattern of expression was different when autoimmune and non-autoimmune thyroid diseases were compared. Only in autoimmune processes was there significant, extensive membrane expression of HLA-DR whereas in non-autoimmune processes it was generally limited to the cytoplasm.

The genes of the human MHC situated in the HLA region are related to the immune response

**Table 1 - Patterns of HLA-DR expression in thyrocytes of normal tissue and of tissue from patients with Graves' disease, Hashimoto's thyroiditis, atoxic multinodular goiter and toxic adenoma**

Diagnosis	Number of cases	HLA-DR expression	HLA-DR expression type		
			Membrane	Cytoplasmic	Both
Normal	8	1	1	0	0
Graves' disease	10	7	5	0	2
Hashimoto's thyroiditis	5	5	1	0	4
Multinodular goiter	9	6	1	5	0
Toxic adenoma	3	2	0	2	0
<b>Total</b>	<b>35</b>	<b>21</b>	<b>8</b>	<b>7</b>	<b>6</b>

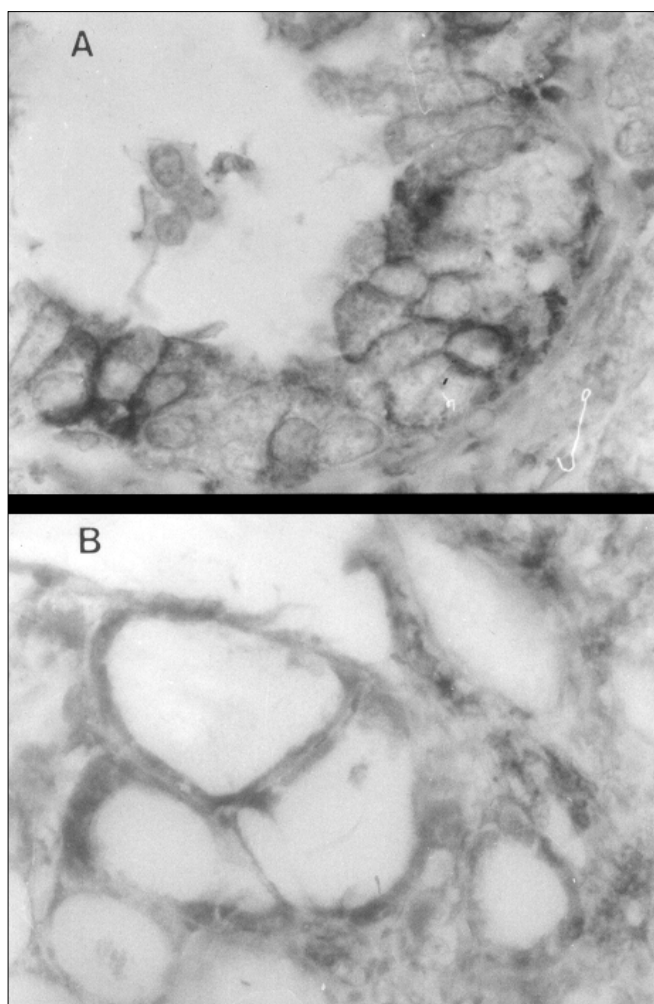
to protein antigens. Thus, CD4+ or CD8+ T lymphocytes only recognize peptides processed by antigen-presenting cells, the macrophages, B lymphocytes, capillary endothelium and dendritic cells, when associated with products of the MHC genes expressed on the cell surface.<sup>9</sup> In the 1980's the inadequate expression of HLA class II in follicular cells of GD and HT cases was demonstrated, most frequently in areas close to the lymphoplasmacytic infiltrate. This suggests that these thyrocytes could play a role in the development and perpetuation of the autoimmune disturbance.<sup>6,7,10</sup> In agreement with these data, in our study a significant number of positive cases (12 out of 21) exhibited areas of follicular cells expressing HLA-DR in the presence of inflammatory cells. In 19 of these 21 cases there was also HLA-DR expression in areas distant from the inflammatory infiltrate.

Subsequent reports have shown that follicular cells in AMG, TA and carcinomas also express HLA-DR, especially in relation to foci of inflammatory infiltrate. Studies using primary cultures of normal thyroid cells, GD and other thyroid diseases, have demonstrated that thyrocytes in autoimmune diseases could function as antigen-presenting cells, which apparently does not occur in non-autoimmune processes.<sup>6,11</sup> In other investigations using cultures of thyroid murine cells or FRTL-5, it was observed that these cells can express the class II MHC molecules, but were unable to present antigens.<sup>12,13</sup> These conflicting results may reflect contamination of the thyroid cell cultures by dendritic cells, which are abundant in this tissue<sup>14</sup> and are powerful antigen-presenting cells.

Our findings indicate a fundamental difference in the pattern of HLA class II expression in the follicular cells. Thus, in non-autoimmune thyroid diseases, such as AMG and TA, this expression was almost exclusively observed in cell cytoplasm, and in GD and HT, HLA-DR was expressed on the cell membrane in all cases irrespective of the presence of cytoplasmic expression. The pattern of HLA-DR expression on the cell membrane in autoimmune thyroid diseases confirms that these thyrocytes play a role in

antigenic presentation. On the other hand, in cells of non-autoimmune thyroid diseases such expression may reflect a general activation of the mechanisms of cell proliferation and such activation may not result in hormone overproduction, since it was also observed in AMG.<sup>5,6,12,13,15</sup>

Our results provide evidence that, independent of the thyroid function, class II MHC products are expressed on the surface of thyrocytes in GD and HT, and that there are clear-cut differences in the patterns of HLA-DR expression in autoimmune and non-autoimmune thyroid diseases.



**Figure 1** - A: In Graves' disease, HLA-DR antigen is expressed on the cell membrane (anti-HLA-DR, Streptavidin-biotin peroxidase reaction, 1000x). B: In contrast, in a case of toxic adenoma cytoplasmic pattern predominates (anti-HLA-DR, StreptABP reaction, 564x).

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## RESUMO

**Contexto:** Normalmente, apenas células apresentadoras de antígenos expressam o HLA-DR em sua superfície. Na doença tireoidiana auto-imune, as células foliculares adquirem o papel de apresentar antígenos e assim expressam o HLA-DR. Entretanto, doenças tireoidianas não auto-ímmunes também podem expressar antígenos de classe II. **Objetivo:** Avaliar a presença e o padrão de expressão do HLA de classe II em tiróides de pacientes com doenças tireoidianas auto-ímmunes e não auto-ímmunes. **Tipo de estudo:** Retrospectivo histopatológico e imunohistoquímico. **Local:** Centro de referência, hospital universitário. **Amostra:** 10 tiróides histologicamente normais, 11 com doença de Graves, 7 com tireoidite de Hashimoto, 10 com bócio multinodular atóxico e 3 com adenoma tóxico foram analisadas através de imunohistoquímica usando um anticorpo monoclonal anti-HLA-DR. **Variáveis estudadas:** Avaliamos a presença desses antígenos na célula folicular tireoidiana, sua relação com o infiltrado inflamatório e o padrão de expressão do HLA-DR nas células foliculares, se presente na membrana, no citoplasma ou em ambos. **Resultados:** A expressão do HLA-DR ocorreu focalmente em uma das 8 tiróides normais, 6 dos 9 casos de bócio multinodular atóxico e em 2 dos 3 adenomas tóxicos. Entretanto, foi nítida sua positividade em áreas extensas de células foliculares tireoidianas nos 5 casos de tireoidite de Hashimoto e, em menor grau, em 7 dos 10 casos de doença de Graves, quer em associação ao infiltrado inflamatório, longe dele ou apenas nas células linfóides. Nas doenças tireoidianas não auto-ímmunes, o padrão de expressão do HLA-DR foi essencialmente citoplasmático, enquanto que na doença de Graves e tireoidite de Hashimoto esta foi verificada na membrana celular, muitas vezes associada à citoplasmática. **Conclusões:** Estes fatos sugerem que a expressão do HLA de classe II na superfície das células foliculares estaria relacionada à apresentação dos auto-antígenos ao sistema imunológico por estas células, levando ao processo inflamatório.