

## **Potential targets for the hepatitis C virus-mediated autoimmune thyroiditis**

<sup>1</sup> Luís Jesuíno de Oliveira Andrade - <https://orcid.org/0000-0002-7714-0330>

<sup>2</sup> Gabriela Correia Matos de Oliveira - <https://orcid.org/0000-0002-8042-0261>

<sup>1</sup> Departamento de Saúde – Universidade Estadual de Santa Cruz - Ilhéus – Bahia, Brazil.

<sup>2</sup> Faculdade de Medicina – UniFTC – Salvador - Bahia, Brazil.

### **Author contributions**

**Conception and design:** Luis Jesuino de Oliveira Andrade

**Analysis and interpretation:** Luis Jesuino de Oliveira Andrade, Gabriela Correia Matos de Oliveira.

**Data collection:** Luis Jesuino de Oliveira Andrade, Gabriela Correia Matos de Oliveira.

**Writing the article:** Luis Jesuino de Oliveira Andrade, Gabriela Correia Matos de Oliveira.

**Critical revision of the article:** Luis Jesuino de Oliveira Andrade, Gabriela Correia Matos de Oliveira.

**Final approval of the article:** Luis Jesuino de Oliveira Andrade, Gabriela Correia Matos de Oliveira.

**Overall responsibility:** Luis Jesuino de Oliveira Andrade

### **Corresponding Author:**

Luís Jesuíno de Oliveira Andrade

UESC - Departamento de Saúde Campus Soane Nazaré de Andrade, Rod. Jorge Amado, Km 16 - Salobrinho, Ilhéus - BA, 45662-900e-mail: [luis\\_jesuino@yahoo.com.br](mailto:luis_jesuino@yahoo.com.br).

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## ABSTRACT

**Introduction:** The mechanisms by which hepatitis C virus (HCV) infection induces autoimmune thyroiditis (AIT) have been studied, and it was suggested that inflammatory cytokines during HCV infection would change the thyroperoxidase (TPO) signaling cascade and thyroglobulin (Tg) determining autoimmune thyroid disease.

**Objective:** To show the signaling pathway, of TPO and Tg, and their potential targets mediated HCV in individuals with hepatitis C.

**Methods:** The mapping of the signaling pathway was based on a review study approach and performed using the automatic annotation server of the Kyoto and Genome Encyclopedia (KEGG). PathVisio is free software for analysis and design of open source routes, and was used for the graphic representation of the signaling pathway.

**Results:** The *contigs* were extracted from the KEGG database and their mapped transcription represents the signaling pathway of the main biomolecules that triggers the AIT. The action of HCV or its treatment can trigger AIT that is characterized by the presence of autoantibodies against TPO and Tg. In AIT, autoreactive CD4 + T lymphocytes recruit B cells and CD8 + T cells in the thyroid. The progression of the disease leads to the death of thyroid cells and hypothyroidism.

**Conclusion:** HCV or its treatment activates several signaling pathways with thyroid cells damage resulting in AIT and secondary hypothyroidism to cellular apoptosis.

**Key words:** Autoimmune thyroiditis; hepatitis C virus; signaling pathway.

## INTRODUCTION

Signaling pathway, also called signal transduction cascades, is defined how the aggregation of molecules in a cell that regulates one or several functions of protection against damage to cell.

The thyroid hormone has a signaling pathway complex with multiple interactions being highly regulated due to tissue-specific thyroid cell expression, beyond cross-talk with other signaling pathways.<sup>1</sup> Thyroid peroxidase (TPO) is an enzyme and along with thyroglobulin (Tg) are involved in the thyroid hormone synthesis.

The autoimmune thyroiditis (AIT) is characterized by lymphocytic infiltrates with progressive destruction of thyroid follicles and production of antithyroglobulin and antithyroperoxidase antibodies (TPO-Ab).<sup>2</sup>

The hepatitis C virus (HCV) infection is associated to endocrine-metabolic disorders including AIT. Studies have shown that the therapy for HCV induces thyroid diseases, and female sex, positive TPO-Ab and elevated serum of thyroid-stimulating

hormone (TSH) concentration are important risk factors for AIT development.<sup>3</sup> It has been suggested that the elevation of interleukins in inflammation caused by HCV would alter the signaling cascade of TPO and Tg determining autoimmune thyroid disease.<sup>4</sup>

In this study, we show the signaling pathway of TPO and Tg, and their potential targets for the HCV-mediated AIT based on a review of the literature.

## **METHOD**

For search signaling pathways we use the Kyoto Encyclopedia for Genes and Genomes (KEGG) pathway as reference. The KEGG is a database where they found maps of biological interactions with cellular processes and pathways related to signal transduction, the understanding of functions of a cell from molecular-level information.<sup>5</sup>

PathVisio (version 3.2.4) is free software for biological pathways and design of open source routes, and was used for the graphic representation of the signaling pathway because it is a tool that enables displaying and editing of biological signaling pathways.<sup>6</sup>

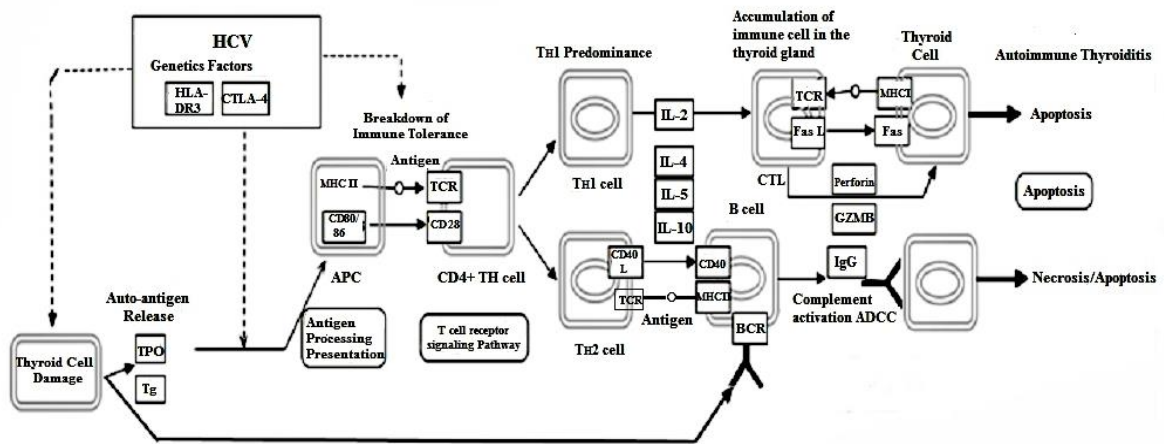
A concise map of the signaling pathway of TPO and Tg, and their potential targets for the hepatitis C virus-mediated autoimmune thyroiditis was developed based on a set of confidence criteria is also available at [www.genome.jp/kegg-bin/show\\_pathway?hsa05320](http://www.genome.jp/kegg-bin/show_pathway?hsa05320).

The connections were elaborated among all the molecules found in the literature evaluated. When the molecules recognized in the literature didn't have their interactions identified, the KEGG database was used to search for unidentified connections.

For the design of signaling pathway started with a schematic overview of all pathway maps where each pathway of HCV and of the AIT. Following an overview, started in a specific pathway and the drawing may then be extended step by step with additional linked pathways.

## **RESULT**

The HCV is an etiological factor of autoimmune thyroiditis because it may infect thyroid follicular cells, which presents CD8+ T-cells responses to HCV antigens expressed on a cell membrane, induced by a major histocompatibility complex (MHC) class II antigens DR expression, or non-specific secretion of interleukin production. Presumably, the HCV would alter immune regulatory signaling pathways allowing AIT to emerge (Figure 1).



**Figure 1.** Potential targets for the hepatitis C virus-mediated autoimmune thyroiditis - Signaling pathway. **Source:** Research result

## DISCUSSION

It has been hypothesized that individuals with genetic susceptibility for AIT exposed to HCV infection may develop thyroiditis. The HCV is both hepatic- and lymphotropic virus which triggers hepatic and extra hepatic manifestations including numerous autoimmune/lymphoproliferative and/or neoplastic disorders<sup>(7)</sup>. We show the signaling pathway of TPO and Tg, and their potential targets for the HCV-mediated AIT.

Breakdown of immune tolerance due to chronic HCV infection occur due to a lack or deficiency of subpopulation of regulatory T-cells with suppressive function, and the cytotoxic T-cells antigen-4 (CTLA-4) plays a crucial role taking to maintain self-tolerance leading to initiation of a primary autoimmune response.<sup>8</sup>

The HCV infection can cause thyroid cell damage in genetically susceptible individuals by an immune response, with a nonspecific production of TNF- $\alpha$  secondary, thereby increasing the MHC class expression on a cell membrane, making these cells seems like antigen presenting cells (APC) increasing antibodies production against TPO and Tg.<sup>9</sup> Thus, in the initial stage, agglutination of MHC class II positive APC in the thyroid, that present thyroid-specific autoantigens to T-cells with clonal expansion and full development of autoreactive T and B lymphocytes.<sup>10</sup>

The antigens produced by the HCV through an interaction of membrane T-cells receptors (TCR) are recognized by the T-cells that are activated resulting in greater

expression of the CTLA-4 molecule that shares homology with CD28, in this way it acts as a competitive inhibitor to CD28 besides acting as negative regulator of T-cell activation. Another important aspect is that an increase of CTLA-4 that can inhibit the early T-cell activation by blocking the interaction of CD80/CD86 with the co-stimulatory receptor CD28, and the increased expression of co-stimulatory molecules CD80/86 arising from transport of MHC molecules to the cell surface has been established during an immune response.<sup>11,12</sup>

The T-cells CD4<sup>+</sup> execute important functions in the pathogenesis of AIT. After the identification of autoantigens by the lymphocytes, an exaggerated increase will occur in the production of autoreactive CD4<sup>+</sup> T-cells because they are excessively stimulated, as well as immunoglobulin G (IgG) autoantibodies, and CD8<sup>+</sup> cytotoxic T-cells.<sup>13</sup>

Recently showed that HCV envelope protein E2 induces strong inflammatory responses in thyroid cells, producing interleukin (IL)-8, IL-6, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). Besides that, the E2 protein induces production of other proteins as HSP60, HSP70p12A, and HSP10, in the primary thyrocytes.<sup>14</sup>

The Th1 cells stimulate autoimmune inflammatory reactions stimulating APC, and secrete IL-2, IFN- $\gamma$ , and TNF- $\alpha$ , whereas Th2-cells enhance the production of autoantibodies and secrete IL-4, IL-5, IL-6, IL-10. The TCR affinity will determine whether the immune response will be by Th1 or Th2 cells. Thus, the preservation of thyroid cells by the induction of anti-apoptotic and pro-apoptotic proteins is regulated by Th1-cells or Th2-cells. The initial process of apoptosis of thyroid cells in AIT is TH1 cells-mediated by mechanisms involvement cytokine-regulated apoptotic pathways, and involvement of death receptors cell.<sup>15</sup>

The B-cells can internalize an antigen linking to the B-cell receptors (BCR) incorporating it into the MHC II molecule. Low antigen concentrations make the B cells to function as superior APC, and capture antigen with antigen-specific BCR, concentrating the immune response in a specific antigen, while macrophages and dendritic cells perform pinocytosis of antigen.<sup>16</sup>

The AIT has as a histological feature accumulation of immune cells in the thyroid gland, leading to disease progression, since central tolerance causes self-reactive T cells to accumulate in the thyroid by altering the site of immunoregulation with secretion of lymphokines or cytolysis.<sup>17</sup> The thyroid disorders due HCV infection increases the CXCL10 expression of thyroid cells, with a grouping of Th1 lymphocytes

into the gland, unleashing the onset of AIT in individuals with risk factors as well as in predisposed individuals.<sup>18</sup>

The Fas (CD95, TNF receptor superfamily member 6) is membrane receptor that belongs to the TNF- $\alpha$  family of death receptors that intermeddles T-cells responses, and T-cell-mediated cytotoxicity happens due to mechanisms Fas-dependent cytotoxicity and perforin/GZMB. The Fas/Fas ligand interaction among thyrocytes favors for the pathogenesis of autoimmune thyroiditis with apoptotic cell death.<sup>19</sup>

The complement activation of antibody-dependent-cell-cytotoxicity carry antibodies to target cells including those against TPO and Tg mediated by IgG, but other unknown antibody-antigen complexes may also contribute to thyroid cell destruction.<sup>20</sup>

## **CONCLUSION**

Thus, the data indicate that HCV infection acts in the breakdown of immune tolerance unleashing thyroid cell damage through of an auto-antigen released against TPO and Tg, with activation of several signaling pathways resulting in AIT and secondary hypothyroidism to cellular apoptosis.

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