Two mechanisms of damage to thyrocytes in Hashimoto’s thyroiditis

Dwa mechanizmy uszkodzenia tyreocytów w zapaleniu tarczycy typu Hashimoto

Iwona Beń-Skowronek, Roman Ciechanek, Leszek Szewczyk, Elżbieta Korobowicz

1Dept. Paediatric Endocrinology and Diabetology, Medical University of Lublin, Poland
2Division of Surgery of Specialist Voivodship Hospital in Lublin
3Dept. Pathomorphology, Medical University of Lublin

Corresponding author: Iwona Beń-Skowronek I., Medical University, Dept. Paediatric Endocrinology and Diabetology, ul. Chodzki 2, 20-093 Lublin, Poland, e-mail: skowroneki@interia.pl, tel. +48 81 7185 440, fax +48 81 7185 120

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ABSTRACT/STRESZCZENIE

Introduction. The development of the Hashimoto’s thyroiditis is the result of the damage to thyrocytes, apoptosis, and autoimmune cytotoxic action of lymphocytes. The aim of the study is to present ultrastructural changes in thyroid cells in the course of damage in Hashimoto’s thyroiditis. Patients and methods. The study involved 40 children: 20 children with Hashimoto’s thyroiditis and 20 children as a control group. Specimens for ultrastructural investigations were obtained during thyroidectomy. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under the EM 900 Zeiss Germany Electron Microscope. Results. In the control group, the thyroid follicular cells were cuboidal or cylindrical and were lying on a thin basement membrane. Varied degrees of apoptosis were observed in the Hashimoto’s thyroiditis patients. The basement membrane of the follicles was thickened by deposition of numerous collagen fibers. The thyrocytes had irregular cell nuclei and an increased number of mitochondria in the basal pole. Different stages of thyrocyte necrosis were visible at the sites of contact between lymphocytes or plasma cells and thyrocytes. The nuclei of dying thyroid cells were deformed, the cisterns of the endoplasmic reticulum were swollen, and the cell membrane disrupted. Conclusion: Ultrastructural examinations of thyroid sections sampled from patients with Hashimoto’s thyroiditis suggest the following stages of process thyrocyte damage: thickening of the basement membrane caused by collagen deposition, thyrocyte apoptosis, stimulation of lymphocytes and plasma cells to cytotoxic reactions, and necrosis of thyrocytes damaged by the cytotoxic reaction.

Wstęp. Rozwój zapalenia tarczycy typu Hashimoto jest skutkiem uszkodzenia tyreocytów, apoptozy i autoimmune cytotoksycznej reakcji cytotoxycznej limfocytów. Celem badań jest prezentacja zmian ultrastrukturalnych w komórkach tarczycy przebiegu zapalenia tarczycy typu Hashimoto. Pacjenci i metody. Badania obejmowały 40 dzieci: 20 pacjentów z zapaleniem tarczycy typu Hashimoto i 20 dzieci stanowiących grupę kontrolną. Fragmenty tarczycy do badań ultrastrukturalnych były pobierane w czasie strumektomii. Skrawki ultracienkie były kontrastowane octanem uranyłu i cytrynicą ołowiu oraz badane w transmisyjnym mikroskopie elektronowym EM 900 Zeiss Germany.
Introduction

The development of the autoimmune thyroid disease – Hashimoto’s thyroiditis – is the result of the damage to thyrocytes, apoptosis, and the cytotoxic action of lymphocytes. The Nomenclature Committee on Cell Death (NCCD) proposes unified criteria for the definition of cell death and of its different morphologies, while formulating several caveats against the misuse of words and concepts that slow down progress in the area of cell death research [1]. Cell death can be classified according to its morphological appearance (which may be apoptotic, necrotic, autophagic, or associated with mitosis), enzymological criteria (with and without the involvement of nucleases or of distinct classes of proteases, such as caspases, calpains, cathepsins and transglutaminases), functional aspects (programmed or accidental, physiological or pathological) or immunological characteristics (immunogenic or non-immunogenic) [2].

Apoptosis or programmed cell death is an active process of self-destruction that requires the activation of a genetic program that may lead to changes in cell morphology [3]. Apoptosis is involved in the homeostasis of follicular cells of the thyroid gland as well as in the destructive mechanisms of autoimmune thyroiditis. The process of apoptosis is controlled by a diverse range of cell signals: extracellularly or intracellularly. Extracellular signals may include toxins, hormones, growth factors, nitric oxide or cytokines that must either cross the plasma membrane or transduce to effect a response [4,5].

Necrosis is the premature death of cells in living tissue. Necrosis is caused by factors external to the cell or tissue, such as infection, toxins, or trauma. This is in contrast to apoptosis, which is a naturally occurring cause of cellular death. Cells undergoing necrosis typically exhibit rapid swelling, lose membrane integrity, and shut down metabolism, and they release their contents into the environment. Cells that undergo rapid necrosis in vitro do not have sufficient time or energy to activate apoptotic machinery and will not express apoptotic markers [6].

Morphological examination by electron microscopy is considered a gold standard technique to demonstrate the classical features of apoptosis and necrosis [7].

The aim of the study is to present ultrastructural changes in thyroid cells in the course of damage in Hashimoto’s thyroiditis.

Patients and methods

Patients

A group of children and adolescents was chosen for the study to exclude the impact of aging processes and other diseases connected with age: circulatory disorders, arterial sclerosis, and drug use.

The study involved 40 children: 20 children with Hashimoto’s thyroiditis and 20 children as a control group. The children were treated at the Department of Pediatric Endocrinology and Diabetology in Lublin and at the Pediatric Department in Rzeszow in the years 1994–2007 and operated on at the Surgery Department of the V oivodship Hospital in Lublin and of the Voivodship Hospital in Rzeszów.

Control group

The control group consisted of 20 children aged 6-19. The specimens were taken during a surgical resection of thyroglossal cysts and during surgery of parathyroid glands. These were fragments of routinely sampled tissue specimens for standard pathologic investigations. All the children were euthyroid (Tab. 1).

The patient qualification procedure

All parents and patients signed an informed consent before these investigations.
All the patients received physical examination to assess the goiter and clinical signs and symptoms of thyroid disorders. The TSH (Thyroid-stimulating hormone), fT4 (free thyroxin) and TT3 (total triiodothyronine) hormones were assayed by MEIA (Abbott Kit, Langford, Ireland). The levels of TSH receptor antibodies were measured by RIA (TRAb assay BRAHMS Diagnostica GmbH, Berlin, Germany). The thyroperoxidase (TPO normal ranges 0-34 IU/l)) and thyroglobulin (TG normal ranges 0-115 IU/l)) antibodies were assayed by LIA (Lumitest BRAHMS Diagnostica GmbH, Berlin, Germany).

Hashimoto’s thyroiditis was recognized in patients with parenchymal or nodular goiter in the phase of euthyreosis or hypothyreosis, rarely in hyperthyreosis (Hashitoxicosis). In ultrasonography, a non-homogenous structure of the thyroid was observed. The levels of TPOAb and TGAb were increased, but the levels of the TRAb were in normal ranges. Mononuclear lymphatic infiltrations in the thyroid parenchyma were detected during a histopathological examination and Hashimoto’s thyroiditis was diagnosed. Before surgery, these patients were usually treated with l-thyroxin 25-100 µg/day; they were operated on for a large-sized goiter which exerted pressure on other neck structures (Tab. I).

**Results**

In the control group, the thyroid follicular cells were cuboidal or cylindrical and they were lying on a thin basement membrane adjacent to the fenestrated membrane of capillaries. The basement membrane was made of loosely arranged connective tissue fibers and protein substances. The basal pole of the thyrocytes contained mitochondria, cisterns of rough and smooth endoplasmic reticulum, and a round, one-celled nucleus filled with a big amount of euchromatin, and a small amount of heterochromatin with a prominent nucleolus. Numerous mitochondria, cisterns of rough and smooth endoplasmic reticulum, Golgi apparatus, secretory vesicles, and colloidal vesicles were visible in the apical pole as well; numerous microvilli were found on the luminal surface. Junctions of the zonula occludens type were present between the thyrocytes (Fig. 1).

Varied degrees of destructive changes were observed in the all Hashimoto’s thyroiditis patients. The basement membrane of the follicles was thickened at the site of normal thyrocytes, which was caused by deposition of numerous collagen fibres. The thyrocytes present at those sites had irregular cell nuclei and an increased number of mitochondria in the basal pole (Fig. 2).
Deep recesses in the nuclear membrane (karyorrhexis) were visible in other thyroid follicles surrounded by the thickened basement membrane. A part of the thyrocytes exhibited typical features of apoptosis: chromatin condensation, karyorrhexis, mitochondrial swelling, and condensation of other elements of the cytoplasm. Retraction of microvilli, reduction in the cellular volume (pyknosis), chromatin condensation, nuclear fragmentation, classically little or no ultrastructural modifications of cytoplasmic organelles, plasma membrane blebbing (although its integrity was maintained until the final stages of the process) were observed in thyrocytes surrounded by the thickened basement membrane. Some follicles displayed a thinned thyrocyte layer, which formed the epithelium (Fig. 3).

Fibrotic processes in the basement membrane lead not only to the typical apoptotic process. The follicles in contact with lymphoid cells infiltrating the thyroid glands exhibited damage to the thyrocyte cell membrane.

The thyroid gland of patients with Hashimoto’s thyroiditis contained numerous cells with the T-cell ultrastructure – a pyknotic nucleus and a small amount of cytoplasm, as well as plasma cells with a characteristic nucleus and numerous concentrically arranged cisterns of the rough cytoplasmic reticulum (Fig. 4). Various stages of thyrocyte necrosis were visible at the sites of contact between lymphocytes or plasma cells and thyrocytes. The nuclei of dying thyroid cells were deformed, the cisterns of the endoplasmic reticulum were swollen, and the cell membrane disrupted (Fig. 4, 5). Debris of thyrocyte cytoplasm or nuclei was visible in many sites.
Discussion

The term ‘apoptosis’ should be applied exclusively to cell death events that manifest several among these morphological features [8]. Several proteins are involved in apoptosis, but two main methods of regulation have been identified: targeting mitochondria functionality, or directly transducing the signal via adaptor proteins to the apoptotic mechanisms. Another extrinsic pathway for initiation identified in several toxin studies is an increase in calcium concentration within a cell caused by drug activity, which also can cause apoptosis via calcium binding protease calpain [9,10].

Apoptotic proteins that target mitochondria affect them in different ways. They may cause mitochondrial swelling through the formation of membrane pores, or they may increase the permeability of the mitochondrial membrane and cause apoptotic effectors to leak out [11]. There is also a growing body of evidence indicating that nitric oxide is able to induce apoptosis by helping to dissipate the membrane potential of mitochondria and therefore make it more permeable [5].

http://en.wikipedia.org/wiki/Apoptosis - cite_note-NO-12

A research done in 1999 exhibits how NO can both initiate and inhibit apoptosis due to the cellular variables [5]. Mitochondrial proteins known as SMACs (small mitochondria-derived activator of caspases) are released into the cytosol following an increase in permeability. SMAC binds to inhibitor of apoptosis proteins and deactivates them, preventing these proteins from arresting the apoptotic process and therefore allowing apoptosis to proceed. Inhibitor of apoptosis proteins also normally suppresses the activity of a group of cysteine proteases called caspases, which carry out the degradation of the cell, therefore the actual degradation enzymes can be seen to be indirectly regulated by mitochondrial permeability [12]. Cytochrome c is also released from mitochondria due to formation of a channel, the mitochondrial apoptosis-induced channel, in the outer mitochondrial membrane [13]. This process is regulated by various proteins, such as those encoded...
Two theories of the direct initiation of apoptotic mechanisms in mammals have been suggested: the TNF-induced (tumour necrosis factor) model and the Fas-Fas ligand-mediated model, both involving receptors of the TNF receptor (TNFR) family [15]. This way of induction of apoptosis may result from the thickening of the basement membrane. Impaired exchange of thyrocyte metabolites (hormone secretion, excretion of waste products) leads to condensation of the contents of the rough and smooth endoplasmic reticulum. Finally, the metabolic disorders within the cell lead to fragmentation of the nucleus and eventual death of the thyrocyte.

A characteristic morphology was observed in the thyrocytes undergoing apoptosis: cell shrinkage due to the breakdown of the proteinaceous cytoskeleton by caspas. Because of the damage to the transcellular and thyrocyte-capillary vessel, the cytoplasm appears dense, and the organelles appear tightly packed. Chromatin undergoes condensation into compact patches against the nuclear envelope in a process known as pyknosis, a hallmark of apoptosis. The nuclear envelope becomes discontinuous and the DNA contained in it is fragmented in a process called karyorrhexis. The nucleus breaks into several discrete chromatin bodies or nucleosomal units due to the degradation of DNA [16]. The cell membrane shows irregular buds known as blebs. The cell breaks apart into several parts called apoptotic bodies, which are then phagocytosed by thyrocytes and lymphatic cells. During karyorrhexis, endonuclease activation leaves short DNA fragments, regularly spaced in size.

As long as the process involves a small number of cells, the apoptotic thyrocytes are phagocytized by neighboring cells. In the case of more pronounced apoptosis accompanied by damage to the thyroid follicle cell membrane, intracellular antigens that can stimulate T and B cells to migrate and settle in a thus damaged thyroid are secreted into the bloodstream and tissue fluid. In the thyroid lymphatic follicles, B cells proliferate and mature, differentiating into plasma cells which produce antibodies against thyroglobulin, thyroid peroxidase, and others antigens. Cytotoxic T cells proliferating in the lymphatic follicles can damage other thyrocytes through antibody-dependent cytotoxicity.
Necrotic cell death or necrosis is morphologically characterized by a gain in cell volume (oncosis), swelling of organelles, plasma membrane rupture, and subsequent loss of intracellular contents. For a long time, necrosis has been considered merely as an accidental uncontrolled form of cell death, but evidence is accumulating that the execution of necrotic cell death may be finely regulated by a set of signal transduction pathways and catalytic mechanisms [17]. In the second reaction stage, thyrocytes undergo necrosis at the sites of the greatest damage to the thyroid, and the thyrocyte debris present in the gland enhances the already ongoing autoimmune response.

**Conclusion**

Ultrastructural examinations of thyroid sections sampled from patients with Hashimoto’s thyroiditis suggest the following stages of the process of thyrocyte damage:

- thickening of the basement membrane caused by collagen deposition,
- thyrocyte apoptosis,
- stimulation of lymphocytes and plasma cells to cytotoxic reactions,
- necrosis of thyrocytes damaged by the cytotoxic reaction.

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**REFERENCES/PIŚMIENNICTWO**