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Research Article

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Epstein – Barr Virus Associated with Hashimoto's Thyroiditis and Treated with a Novel Nanobiological Therapy Based on Micro RNAs: A Case Report

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Abstract

Hashimoto's thyroiditis, on the one hand, represents one of the most common autoimmune diseases in general, and on the other hand displays a proven genetic and environmental determinism. Its prevalence has been rising up in the last decades everywhere and the disease shows multifactorial etiologies. For some time now, different viruses have been regularly mentioned among the environmental factors involved in the onset of the disease, but it is only recently that Epstein-Barr Virus, a common human herpes virus known to infect most of the world population, has been mentioned in this context of human pathology, while its participation during its latency phase is more and more often demonstrated in a growing number of autoimmune diseases. MicroRNAs (miRNAs) are small noncoding endogenously produced RNAs that play key roles in controlling the expression of many cellular genes at a post-transcriptionally level. After cytoplasmic recruitment and incorporation into a ribonucleoprotein complex, they can target specific mRNAs and interfere in their translation into proteins. Consequently, miRNAs can regulate many cellular pathways and processes, and their dysregulation may largely contribute to several kinds of diseases, particularly those related to autoimmune mechanisms. We will show with the help of a clinical case that such a pathological association can be regulated thanks to a new method of immunotherapy in ultra-low doses.

Keywords (MeSH): EBV reactivation, Hashimoto thyroiditis, Immunomodulation, MicroRNAs, Sublingual immunotherapy, Ultra-low doses

Abbreviations

Anti-Tg: anti-Thyreoglobuline
AITD: AutoImmune Thyroid Diseases
BI(G)MED: Bio Immun (G)en Medicine
CTLA-4: Cytotoxic T Lymphocyte Associated Antigen-4
EA: Early Antigen
EBNA: EBV Nuclear Antigen
EBV: Epstein - Barr virus
GWAS: Genome-Wide Association Study
HCV: Hepatitis C Virus
HLA: Human Leucocyte Antigen
HT: Hashimoto's Thyroiditis
LMP1: Latent Membrane Protein-1
PTPN22: protein tyrosine phosphatase -22
TSH: Thyroide Stimulating Hormone
VCA: Viral Capsid Antigen

Introduction

Hashimoto's thyroiditis, also known as chronic lymphocytic thyroiditis, is the most common cause of hypothyroidism in iodine-sufficient areas of the world. It is an autoimmune disorder in which antibodies directed against the thyroid gland lead to chronic inflammation. It is not known why some people make antibodies, although this condition tends to run in families. Over time, however, this results in impaired ability of the thyroid gland to produce thyroid hormones, leading to gradual decline in function and eventually after a few years of evolution to hypothyroidism. Hashimoto's thyroiditis is seen in up to 10 percent of the population and its prevalence increases with age, and this incidence makes autoimmunity against thyroid one of the most frequent autoimmune disease in humans, that represents the prototype of autoimmune organ specific disease [1].

The disease occurs most commonly in middle aged women, but can be seen at any age, and can also affect men, and children. The exact factors that can lead to the onset of Hashimoto's thyroiditis have not yet been sufficiently clarified. In addition to a family (genetic) pre-stress, there are also stress, ongoing viral diseases (such as mononucleosis, herpes zoster), dysfunction of the adrenal cortex, microchimerism and environmental effects.

Hashimoto's thyroiditis is predominantly the clinical expression of cell-mediated immunity leading to destruction of thyroid cells, which in its severest form causes thyroid failure [2]. Some authors suggest that Hashimoto's thyroiditis, primary myxedema, and Graves' disease are different expressions of a basically similar

autoimmune process, and that the clinical appearance reflects the spectrum of the immune response related to each particular patient [3]. This response may include cytotoxic antibodies, stimulatory antibodies, blocking antibodies, or cell mediated immunity.

The interaction between genetic susceptibility and environmental factors appears to be of fundamental importance to initiate the process of thyroid autoimmunity [4]. In recent years, the genetics of autoimmune thyroid diseases, like many other human pathologies, have benefited greatly from the contributions of GWAS [5]. Currently, three gene regions are clearly associated with Hashimoto disease and other AI-related thyroiditis such as Morbus Graves; it is about the gene region HLA, which is so far recognized the most important susceptibility factor, the gene CTLA-4, and the gene PTPN22 [6,7].

A number of studies suggest a significant association of Hashimoto disease with Caucasians with the following alleles; HLA-DR03, -DR04 and / or -DR05, HLA-DQ02 and -03 could be validated. Conversely HLA-DR07 plays a protective role. Secondary compounds with HLA alleles of class I are additionally described [5].

There have been numerous environmental triggers identified as risk factors for the development of AITD; these include smoking, stress, iodine intake, medications, bacterial and viral infections, irradiation, pollutants, and pregnancy [8]. For our part, we are particularly interested in the impact of viral factors on the development of autoimmunity mechanisms.Components of several viruses such as hepatitis C virus, human parvovirus B19, coxsackie virus and herpes virus are detected in the thyroid of Hashimoto's thyroiditis patients [9,10]. Hashimoto thyroiditis has been shown to demonstrate the presence of parvovirus B19 capsid proteins and nucleic acidbelonging to the core of this virus in thyroid tissue samples [11,12]. Chronic HCV infection has also been shown to be associated with increased incidence of clinical and subclinical autoimmune thyroiditis [13,14]. HCV infection is at the moment the only infectious agent that is clearly associated with an increased risk for autoimmune thyroiditis [15]. More recently, various members of the Herpesviridae family have been involved in the pathogenesis of Hashimoto's disease [16]. We are especially interested in the potential role of EBV, which we personally consider to be a virus involved in a very large number of chronic diseases, in particular of autoimmune origin. There is indeed a large number of studies incriminating EBV as being more or less responsible for the initiation and progression of various autoimmune diseases such as rheumatoid arthritis, SLE or multiple sclerosis.

EBV causes a persistent infection with a tight latency program in memory B-cells and epithelial cells, which enables evasion of the immune defense. Numerous immune escape mechanisms and immune-modulating proteins have been described for EBV. These immune modulating functions make EBV a good candidate for initiation of autoimmune diseases and exacerbation of disease progression [17]. EBV, also known as human herpes virus 4, is a gamma-herpes virus that infects most of the world's population.

Initial infection with EBV is often asymptomatic but can also manifest as infectious mononucleosis. Following acute lytic replication in epithelial cells, EBV infects B cells where a distinct set of latency-associated genes and transcripts are expressed. EBV causes a persistent infection with a tight latency program in memory B-cells, which enables evasion of the immune defense. A number of immune escapemechanisms and immune-modulating proteins have been described for EBV [18]. **Table I** shows the main characteristics of EBV infection and the disorders caused by this virus [19].

Table I: main characteristics of EBV infection and the disorders caused by this virus

Who	How	When	Consequences
EBV infects most of the population; it can be detected in almost every adult	The most common route of transmission is with the saliva ("kissing disease")	The infection occurs mainly during childhood and can be followed by infectious mononucleosis, the incidence of which increases linearly with age at infection	EBV is the unique or the most common causative agent of several malignancies. It may contribute to the pathogenesis of several autoimmune disorders.

These immunemodulating functions make EBV a good candidate for initiation of autoimmune diseases and exacerbation of disease progression. Among these diseases of autoimmune origin feature rheumatoid arthritis, SLE, Sjögren's syndrome or multiple sclerosis [20]. Enhanced lytic replication may result in new infection- and transformation-events and thus is a risk factor both for malignant transformation and the development of autoimmune diseases. An increased viral load or a changed presentation of a subset of lytic or latent EBV proteins that cross-react with cellular antigens may trigger pathogenic processes through molecular mimicry that result in the different diseases listed above , but research published in recent years has revealed links between EBV and other autoimmune diseases, including thyroiditis and in particular Graves' disease.The initiation of EBV-linked autoimmune thyroiditis could start with changes in the follicular epithelium caused by transforming latency type III EBV infection characterized by LMP1 expression. These epithelial changes may also involve the production of inflammatory mediators leading to the recruitment of lymphocytes [21,22]. But much older work had already shown an increase in anti-VCA antibodies in patients with Hashimoto thyroiditis [23].

MicroRNAs (miRNAs) are ~19-24 nt non-coding RNAs that post-transcriptionally and negatively regulate gene expression by binding to target messenger RNAs (mRNAs) [24]. As miRNAs require only limited complementarity for mRNA binding, they are able to modulate the expression of multiple genes. Conversely, different miRNAs can control a single mRNA, building a particularly complex miRNA regulatory network [25]. They are implicated in almost every biological process, including pathways involved in immune homeostasis, such as immune cell development, central and peripheral tolerance, and T helper cell differentiation. Alterations in miRNA expression and function can lead to major dysfunction of the immune system and mediate susceptibility to autoimmune disease [26]. Recent work has identified DNA fragments, generated following cell death, and micro RNAs as potential factors in HT pathogenesis [27]. At the same time, recent studies have revealed that some miRNAs are also involved in the development of AITD, and most of them have been found to be intensively involved in

modulating the differentiation or activation of immune cells and immune response.

For example,there are several studies providing evidence for the abnormal expression of miR-155-5p and miR-146a-5p in AITD patient [27,28]. miR-142-5p was also highly expressed in HT patients and was positively correlated with TgAb (Thyreoglobulin Antibodies) [29].

Here we present the case of a patient with a Hashimoto thyroiditis, whose evolution is closely related to that of a reactivated EBV.

Material and Methods

The patient is a 60-year-old woman at the time of her first consultation. At that time, a Hashimoto thyroiditis has been known for three years. She complains of persistent nervousness and chilliness as well as a lazy bowel. At the biological level, there is a hypercholesterolemia and a slight elevation of ultrasensitive TSH. There is no substitution treatment in progress. Anti-Tg IgG are moderately increased (152 IU / ml - N <115) and will evolve in a variable way as time goes by. Sonography reveals two micro-nodules of the right lobe and large hypoechoic areas, which will remain very stable. At this time, the EBV serology by immunofluorescence is perfectly trivial **(Table II)**.

Table II: EBV serology by immunofluorescence at first consultation

IgM anti-VCA	Ø
IgG anti-VCA	1/160
IgG anti-EBNA	1/80
IgG anti-EA	1/10
Positive level at 1/40	

Two years later, after a period of family stress, the anti-Tg antibodies rise up to 275 IU / ml and at the same time appears an EBV reactivation with anti-VCA IgG at 1/1280.

In order to neutralize the reactivated EBV, we use a nanobiotechnology method using ultra low doses of microRNAs associated with immunoregulatory molecules and called Bio Immun (G)en Medicine (BI(G)MED), a very innovative sublingual immunotherapy.The BI(G)MED-nanovectors are so-called xylitol globules produced and analysed by Remedy Bank (Hoboken, Belgium).The effectiveness of these very low molecular concentrations is based on the principle of Hormesis, which makes it possible to understand the law of reverse action according to the dilution meaning that activating effects at low concentration (eg 1x10-5Mol) become more and more inhibitory as dilution increases (eg 1x10-10). A response in cells or organisms induced by a low, subtoxic dose of a compound that can induce changes in the environment is called hormesis [30].

A better understanding of hormesis mechanisms at the cellular and molecular levels is leading to novel approaches for the prevention and treatment of many different diseases [31]. For this purpose, we use xylitol-based nanovectors for rapid absorption at the level of oropharyngeal mucosa and equally fast propagation of molecular signals through the lymphatic network.

Results and Discussion

After six months of BI(G)MED treatment we observed fully negative anti-Tg IgG and a normalized EBV-serology**(Table III)**.

Table III: EBV serology after 6 months of treatment

IgM anti-VCA	1/10
IgG anti-VCA	1/160
IgG anti-EBNA	1/160
IgG anti-EA	Ø

For the whole duration of this treatment the thyroid hormones did not move, showing that the problem was immunological and microbiological but not endocrine.

This apparently simple clinical case nevertheless provides very interesting information in several fields. It clearly highlights that the evolutionary course of a Hashimoto thyroiditis can be influenced by the reactivation of a latent and recurrent virus such as EBV; that this viral reactivation can be triggered by emotional stress, and that the neutralization of the Epstein-Barr virus results in the cessation of the autoimmune process attested by the disappearance of autoantibodies in the peripheral blood.

This means in clear that any experienced clinician should systematically check the biological status of EBV when discovering an autoimmune disease such as Hashimoto's thyroiditis.

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