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Research Article

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Epstein - Barr virus Reactivation Associated with an Increased Thymidine Kinase and Normalized by an Immuno Modulatory Nano-Therapy: Three Case Reports

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Abstract

Epstein-Barr Virus (EBV), a common human herpes virus known to infect most of the world population, has been mentioned in the context of many diverse human pathologies while its participation during its latency phase is more and more often demonstrated in a growing number of chronic malignancies.The biological diagnosis of the virus activity is carried out using serological parameters on the one hand, and the measurement of the viral load on the other hand. Thymidine kinase (TK) is a key enzyme in the regulation of the intranuclear thymidine pool during cell cycle progression. The rise in its plasma level therefore systematically reveals an uncontrolled cellular proliferation evoking, of course, at first a neoplastic process. Nevertheless, EBV being a DNA virus, its reactivation or even a persistent primary infection are also likely to cause an increase in the blood level of TK.Using three examples, we will show that the neutralization of EBV by an immunomodulatory nano-therapy called Bio Immune (G)ene Medicine (BI(G)MED), is accompanied by a normalization of plasma levels of TK, thus underlining the close link between the virus and this marker of cell proliferation.

Keywords: EBV Reactivation, Thymidine Kinase, MicroRNAs, Sublingual Modulatory Immunotherapy, Ultra-low Doses

Abbreviations

EBV: Epstein-Barr Virus
TK: Thymidine kinase
BI(G)MED: Bio Immune (G)ene Medicine
PCR: Polymerase Chain Reaction
HP: Helicobacter pylori
HBV: Hepatitis B Virus
GPP: Good Pharmaceutical Practice

Introduction

Epstein-Barr virus (EBV) or human herpesvirus 4 is ubiquitous, and about 90% of adults throughout the world have antibodies against it [1]. The majority of the EBV-infected individuals tolerate the infection well, without any further symptoms after primary infection [2]. In developing countries and in socio-economically disadvantaged population in industrialized countries, a primary EBV infection usually occurs during infancy and early childhood. In more affluent population, in industrialized countries, EBV infection is also more common during early childhood, but about one-third of EBV cases occur during adolescence and early adulthood [3]. It is therefore understood that most patients who have been infected with the virus will not develop any clinically detectable diseases, and many of these "healthy carriers" will remain so throughout their lives.

However, in a significant number of them various clinical disorders will appear over time that can lead to sometimes formidable chronic diseases, whether autoimmune diseases or malignant tumors and hemopathies. The mechanisms of EBV latency have been carefully examined both because they represent the virus strategy to elude the response of the immune system of the host, and because they are correlated with those oncologic conditions associated to the viral persistence, particularly lymphomas and lymphoproliferative disorders [4]. But there are also much more benign cases, where the clinical picture will be dominated by symptoms of functional malaise at the origin, in such patients, of a medical wandering, which in the long run will prove to be penalizing for their daily life. This is particularly the case of all these pictures of chronic fatigue syndrome accompanied by the most diverse clinical manifestations, where the psychic argues the somatic, making it more difficult to make a proper etiological diagnosis.

In all these cases, serological methods, i.e. immunofluorescence, ELISA, or Western blot, are the methods of choice to come to an unequivocal diagnostic conclusion, while the detection and quantification of viral DNA through PCR plays a minor role. On the other hand, in a minority of the human population, prolonged EBV primary infection or EBV reactivation from latency may

be a serious and life-threatening complication which needs to be diagnosed as soon as possible, as seen above when EBV is associated or causally linked to autoimmune or malignant disease. The direct and quantitative detection of viral DNA are of importance for the diagnosis of such serious EBV diseases [2].

Many research works have been conducted to identify a causal link between EBV and some chronic diseases, and have often concluded that the latter is absent. In this context of etiological research, it is essential to avoid a fundamental trap, that of the monofactorial causality; it is clear today that most chronic diseases are multifactorial at the etiological level, and that it is the set of factors involved that will be responsible for triggering the pathological phenomena observed.

The reality of life is thus combined in terms of interconnectivity and modern research must comply with the requirements and rules of interactive pathophysiology. In this perspective,the BI(G)MED is close to the concept developed in the United States under the name of "systems biology".“Systems biology” is a recent and evolving interdisciplinary field that focuses on the systematic study of complex interactions in biological systems.Systems biology employs a holistic approach to study all components and interactions in the network of DNA (genes), RNA, proteins and biochemical reactions within a cell or an organism (fig.1). Chronic complex diseases are multifarious in origin with a variety of biologically culpable components and environmental factors being implicated. Complex diseases are clinically progressive through multiple interactions between the involved components and environmental parameters [5].

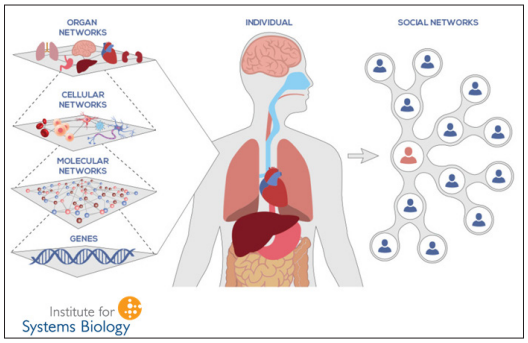

Figure 1: What is Systems Biology?

A network of networks. *https://www.systemsbiology.org/about/what-is-systems-biology*

The privileged place of a virus such as EBV is obvious in such an interconnected system as shown on next figure (fig.2), where the microbiome is positioned as a constituent element of this vast interactive network represented by the whole organism [6].

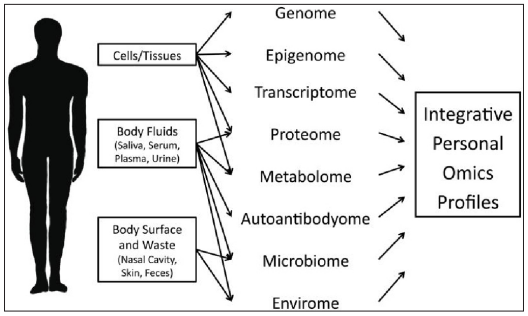

Figure 2: Integrative Personal Omics Profile (iPOP) Analysis.

Various types of systems data can be generated and integrated with the iPOP analysis. Note that this approach is highly modular and can be tailored to meet specific needs of different studies [6].

Studies in this sense have already been carried out within the limited scope of work on the origin recognition complex and demonstrating how EBV uses its own genes to initiate its replicative machinery [7]. Moreover, at the clinical level, the causal role played by EBV has been demonstrated, in connection with other viral factors and immune disorders involved in the etiopathogenesis of systemic lupus erythematosus [8].

Thymidine kinase (TK) is an enzyme, a phosphotransferase (a kinase): 2'-deoxythymidine kinase, ATP-thymidine 5'-phosphotransferase,whose key function is the synthesis of DNA and therefore plays an important role in cell division, as it has part of the unique reaction chain to introduce thymidine into the DNA.Certain viruses also have genetic information for expression of viral thymidine kinases. For example, it was demonstrated that upregulation of serum TK1 levels is an early event in cancer development [9]. In proliferating normal and tumor cells, the level of TK1 starts to increases at late G_1 phase, and reaches a maximum in late S-phase/early G_2 phase of the cell cycle, but in quiescent cells, TK1 is almost completely absent; this singles out TK1 as a useful indicator of cellular proliferation, and hence for malignancy in a lot of cases. Serum TK1 protein (STK1p) level, determined by non-invasive serological methods, was found to be an emerging potential cell proliferation biomarker for the prognosis of cancer patients, for monitoring tumor treatments, relapse, follow-up and survival, and particularly for the early detection of cancer development risk [10]. In another study conducted in China, follow-up data (3–6 years) of individuals with elevated TK showed a 3- to 5-fold higher risk of developing malignancies, compared to individuals with normal TK values in the serum [11].

But there are also patients with high levels of TK without confirmed underlying malignancy, but very often presenting different states of hyperplasia, polyps, Helicobacter positivity, obesity, fatty liver and HBV infections, but also with various types of pre-cancerous states of more or less severe intensity. All of these situations share the same risk of promoting the further development of neoplasia within six years [12].

It emerges that the measurement of the TK can in such a context have a real predictive value. And they are precisely the patients recruited in such conditions, which are of the greatest clinical interest because they may be able to benefit from a preventive therapeutic approach.This is especially true since the increase of the plasma level of TK expresses either a state of reactivation of EBV or a chronic mononucleosis (that means a persistent primary infection) given that it is a DNA virus [13].

The concentrations of TK-serum levels were measured using a commercial Kit based on an enhanced chemiluminescent dot blot assay as described by the manufacturer (Diasorin – France), the EBV DNA determination was performed by a real time PCR assay, and EBV serologies performed using an immunofluorescence technique.

The preventive treatment will be implemented in the form of a regulatory nanotherapy affecting the immuno-genetic level, which will in particular use ultra-low doses of micro RNAs able to exert a regulatory effect at the post-transcriptional level [14]. This kind

of treatment has the advantage of allowing regulation of both the epigenome of the cell and of the virus.

Observation of three very different cases in their clinical presentation will allow us to highlight the relationship between an "unexplained" increase of TK and an uncontrolled activity of EBV.

Materials and Methods

Case 1

This is a 62-year-old woman at the time of her first consultation, where she complains of various disorders mainly focused on the digestive tract and evoking a possible cholelithiasis; the main demand is in fact the practice of a more or less extended biological assessment, whose report reveals nothing particular including among others a normal rate of TK and a quite trivial EBV serology.

Several similar reports show no significant anomaly until 2016 when, in a context of significant family stress, externalizing with violent tinnitus, appear concomitantly an EBV reactivation associated for the first time with a rise in the rate of TK. At the same time, we can observe an increased value of haptoglobin indicating a chronic inflammation somewhere, which finally proved to be connected to large gallstones, which the patient however refused to operate.

Table 1: EBV serology by immunofluorescence before treatment (patient 1)

IgM anti-VCA	∅
IgG anti-VCA	1/320
IgG anti-EBNA	1/160
IgG anti-EA	1/40
Positive level at 1/40	

Table II: TK value before treatment (patient 1)

TK	10,5 U/l	normal range: from 2,0 to 7,5

A real time PCR assay shows a weakly positive quantification of EBV-DNA. 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 Immune (G)ene Medicine, a very innovative sublingual immunotherapy. 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 and meaning that activating effects at low concentration (eg $1x10^{-5}$Mol) become more and more inhibitory as dilution increases (eg $1x10^{-10}$) [15]. 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 [16]. 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 [17].

Case 2

This is a 62-year-old woman at the time of her first consultation for chronic gastralgia linked to an infection with Helicobacter pylori (HP) evolving in the context of a chronic fatigue syndrome. A biological assessment is performed shortly after treatment with antibiotics to eradicate HP and shows a persistent primary infection related to EBV so as a weakly increased value of TK; there was also a huge increase of anti-HBs antibodies following vaccination.

Table III: EBV serology by immunofluorescence at first consultation (patient 2)

IgM anti-VCA	Ø
IgG anti-VCA	1/80
IgG anti-EBNA	1/20
IgG anti-EA	Ø
positive level at 1/40	

Table IV: TK value at first consultation (patient 2)

TK	9,8 U/l	normal range: from 2,0 to 7,5

Table V: Anti-HB antibodies value at first consultation (patient 2)

Anti-HBs Ab	16049	threshold of protection: 10 U/l

CASE 3

This is a 66-year-old man at the moment of the first consultation, in which a colon carcinoma was discovered a year ago following a large state of fatigue and the presence of occult blood in the stool. Immediate treatment consisted of 25 radiotherapy sessions combined with neo-adjuvant chemotherapy with 5-fluorouracil. The surgical excision of the tumor is planned one week after this first consultation. A biological assessment carried out a few months after the surgical removal of the tumor reveals a minimal increase in TK causing suspicion either local recurrence of the neoplastic process or the existence of distant metastases.But the report highlights at the same time a reactivation of EBV. Moreover, this report obviously showed a completely impaired cell-mediated immunity and an active chronic inflammatory syndrome at the level of humoral immunity.At this moment the patient is exhausted, adynamic, without spring, quite the opposite of the hyperactive man he was usually.

Table VI: EBV serology by immunofluorescence before treatment (patient 3)

IgM anti-VCA	∅
IgG anti-VCA	1/640
IgG anti-EBNA	1/80
IgG anti-EA	∅
positive level at 1/40	

Table VII: TK value before treatment (patient 3)

TK	8,6 U/l	normal range: from 2,0 to 7,5

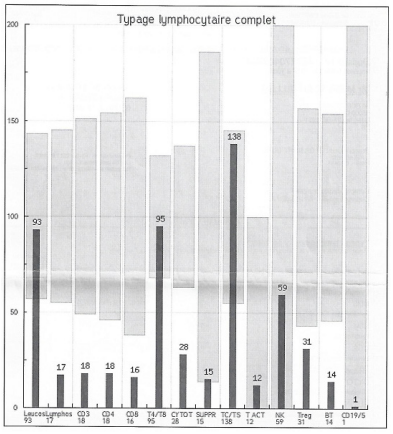

Figure 3: Lymphocyte typing before treatment (patient 3)

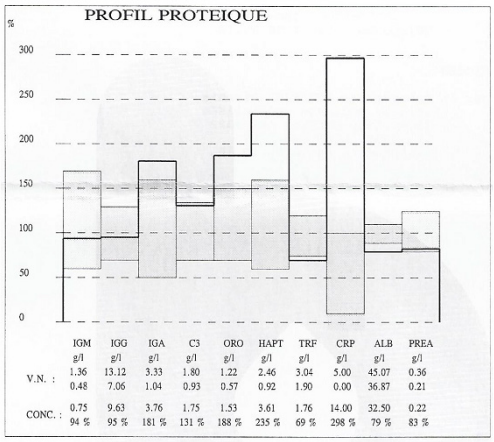

Figure 4: Protein profile before treatment (patient 3)

Results and Discussion

Case 1

The recommended treatment, administered in capsules containing xylitol-based globules, and GPP-certified (Good Pharmaceutical Practice), lasted six months after which the patient felt an undeniable clinical better,but the most interesting was the evolution of her biological parameters with a normalization of the EBV serology and thymidine kinase value as well, and a negativation of the viral load related to the EBV. Only haptoglobin remained increased in relation to the biliary lithiasis still present.

Table VIII: EBV serology by immunofluorescence after 6 months of treatment (patient 1)

IgM anti-VCA	∅
IgG anti-VCA	1/160
IgG anti-EBNA	1/40
IgG anti-EA	1/10
positive level at 1/40	

Table IX: TK value after 6 months of treatment (patient 1)

TK	4,4 U/l	normal range: from 2,0 to 7,5

Case 2

In the same way as in the previous case, the patient's treatment consisted in taking two regulatory nanotherapeutic remedies for a period of six months.At the end of this immunoregulatory therapy the patient no longer expressed any clinical complaint, and her biological assessment showed a standardized EBV serology and a rate of TK returned to the norms.

Table X: EBV serology by immunofluorescence after 6 months of treatment (patient 2)

IgM anti-VCA	∅
IgG anti-VCA	1/80
IgG anti-EBNA	1/40
IgG anti-EA	∅
positive level at 1/40	

Table XI: TK value after 6 months of treatment (patient 2)

TK	6,7 U/l	normal range: from 2,0 to 7,5

Case 3

The treatment set up by myself consisted, on the one hand, in an onco-regulation with the help of compounds (so-called "onco-complex") and, on the other hand, a treatment aimed to neutralizing EBV.Six months later, and without taking any other medication, the patient returned to good health and refused the neoadjuvant chemotherapy that he was offered.The biological control balance shows several parameters whose evolution is very satisfactory and highlights once again the relationship between EBV and TK, both of which have been completely normalized.There is also a clear improvement in cellular and especially humoral immunity.

Table XII: EBV serology by immunofluorescence after 6 months of treatment (patient 3)

IgM anti-VCA	∅
IgG anti-VCA	1/320
IgG anti-EBNA	1/80
IgG anti-EA	∅
positive level at 1/40	

Table XIII: TK value after 6 months of treatment (patient 3)

TK	1,1 U/l	normal range: from 2,0 to 7,5

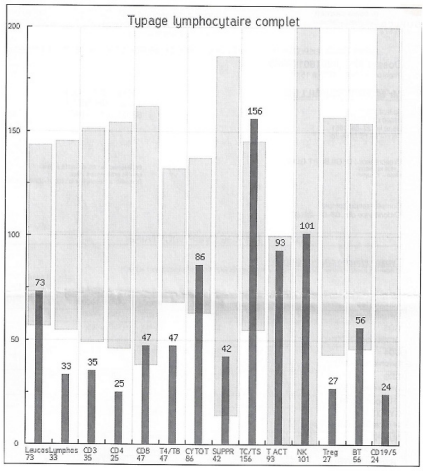

Figure 5: Lymphocyte typing after 6 months of treatment (patient 3)

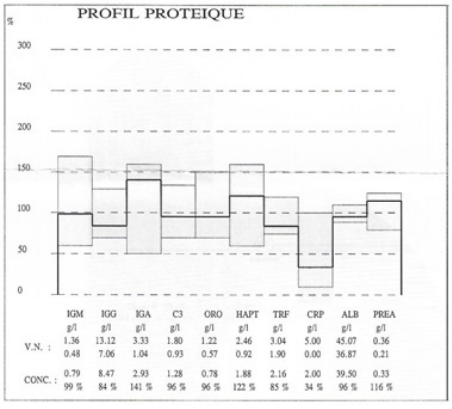

Figure 6: Protein profile after 6 months of treatment (patient 3)

Conclusion

EBV is an ubiquitous virus in human pathology, which in my opinion is involved in a majority of chronic diseases of all types, where it is not necessarily the etiological factor but rather a contributory element by the deleterious effect that it exerts on the immune system especially adaptive at the level of B-lymphocytes.

In my practice, I look for it systematically, first in the context of serological tests and otherwise through the measurement of the viral load.In the latter case in particular but very often elsewhere also, the TK level appears high outside any pathological cellular proliferation. This close correlation is underlined by the concomitant normalization of the two parameters in the context of an immunoregulatory nano-therapy called BI (G)MED.

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