CHRONIC FATIGUE SYNDROME CAUSED BY EPSTEIN BARR VIRUS INFECTION

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SUMMARY

Chronic fatigue syndrome or myalgic encephalomyelitis (ME/CFS) is a disease of currently unknown etiology, which appears suddenly in a previously active person and whose onset appears to be related to an acute infection in most cases. Until now, CFS patients have been studied without classification into pathogen subgroups. The Epstein Barr virus (EBV), like other pathogens, is able to generate a functional immunodeficiency acquired through the deficit of expression of class II molecules of the major histocompatibility complex in genetically predisposed individuals. However, some pathogens also succeed in reducing the class I molecules of MHC. This study aims to show how the viral cycle of EBV and its mechanism of immune evasion can generate CFS and what the metabolic and physiological consequences are.

INTRODUCTION

Chronic fatigue syndrome (CFS) is a currently unknown disease to which multiple triggers have been attributed, but none of these are present in all patients. Perhaps, the approach to take is that not only a determining factor or pathogen can produce this disease but several of them, and therefore it is necessary to analyze the etiopathogenesis of each and every one of the possible pathogens involved, as well as its way of evading the immune system, to understand how it can generate the metabolic and physiological changes produced in patients.

CFS usually begins with an infectious process in a generally active person who begins with fever, cough, odynophagia, myalgia, or flu-like symptoms. From this beginning, a permanent exhaustion is established that does not improve with rest, it worsens with both physical and mental activity that becomes persistent. When chronic symptoms are established, fatigue, fever (at the onset of the disease) or temperatures of 35ºC when the disease is more advanced, arthralgias, myalgias, cervical adenopathies, intestinal symptoms, increased respiratory allergies, hormonal alterations, etc., tend to predominate. It should be noted that all these symptoms are not present in all patients and that their heterogeneity may be due to the person's previous serological profile and the initial pathogen, which may generate different symptoms, such as arthritis, based on previous reactivations of past viruses.[1]

For this reason, this article aims to approach a subgroup of CFS whose origin is an infection with the Epstein Barr virus and demonstrate how its viral machinery can elude the immune system and establish the disease.

PRIMOINFECTION

Most acute and persistent viral infections begin at the periphery, often on the epithelial or endothelial cell surfaces. Infection of the cells at these sites usually induces a tissue-specific antiviral response that includes both an autonomic cellular response (intrinsic immunity) and a paracrine signaling of the infected cell to the surrounding uninfected cells by secreted cytokines (innate immunity). This local inflammatory response usually contains the infection. After several days, the adaptive immune response can be activated and the infection can be eliminated by the action of infection-specific antibodies and T cells (acquired immunity). Viral infections that are

beyond local control at the site of primary infection can spread to other tissues, where they can cause more serious problems due to robust virus replication or an exaggerated innate immune response. This latter reaction is sometimes called a cytokine storm because both pro-inflammatory and anti-inflammatory cytokines are elevated in serum, leading to vigorous systemic immune activity. Such a response in the brain is often devastating and can lead to meningitis, encephalitis, meningoencephalitis, or death.[2]

The Epstein-Barr virus (EBV) is the only human-adapted member of the Lymphocryptovirus genus, belonging to a lineage of Old World primates with gamma-1 herpesvirus that was transferred to a hominid ancestor about 12 million years ago, and is now responsible for nearly universal and lifelong human infections. Viral transmission usually occurs through saliva.[3]

This herpesvirus is present in more than 90% of the human population. However, it should be noted that its involvement in multiple diseases such as rheumatoid arthritis[4-5], multiple sclerosis[6], Hodking's lymphoma[7], Burkitt's lymphoma and other non-Hodking lymphomas has also been seen.

In childhood, primoinfection[8] tends to be asymptomatic and leads to persistence of the virus' life at intracellular level, but in adolescents and adults it can cause infectious mononucleosis, a generally self-limiting lymphoproliferative disease.

During the acute phase of infection, 1 in 104 circulating B cells are infected, as this virus predominantly affects these cells. In response, cytotoxic T cells proliferate and kill infected B cells. However, the virus has mechanisms in place to ensure that some EBV-infected B cells at rest do not present the antigen on their surface and thus avoid the immune response, so that they remain permanent in the B cell's DNA. These cells show different patterns of expression of EBV-coded genes:[9]

- Latency 0 and Latency I: are in memory B cells and are characterized by the lack of expression of any of the viral genes or EBNA-1 expression.

- Latency II: EBNA-1, LMP-1, LMP-2A, 2B is expressed in germ center infected centroblasts.

- Latency III: EBNA-1, -2, -3, -4, -5, -6, LMP1, LMP-2A, 2B is expressed in lymphoblasts.

These same programs of the latent viral cycle are also expressed in various malignant EBV tumors. Latency I is found in Burkitt's lymphoma, latency II in Hodgkin's disease, nasopharyngeal carcinoma, T / NK cell lymphoma, and latency III is characteristic of infectious mononucleosis and B-cell lymphomas found in transplant recipients and AIDS patients.[9]

VIRAL CYCLE

B cells are the main targets of EBV infection due to their expression of CD21, which is the main receptor of the virus. However, EBV can also infect T cells, endothelial cells, and epithelial cells through various processes including the transfer of the virus from infected B cells.
The virus persists through the latency systems in memory B-lymphocytes, both in IgD+ CD27+ and IgD-CD27+, but not in the naive B-cell, so it establishes itself in a latent state without expressing viral genes remaining hidden from immune system surveillance.9 However, the typical host immune response is sufficient to maintain control, not so in cases of lymphoma where the virus appears to have won the battle against the immune system. This is one of the simplest cases of how a virus infection is able to generate a tumor, and to understand the similarities between the cancer and a latent infection at the metabolic level that will be explained later.[9]

IMMUNE RESPONSE TO EBV

CD8 T-cells act specifically against lytic cycle proteins, but also against latency proteins such as EBNA -3A, -3B, -3C, LMP2-A, EBNA-1 and LMP-1. Studies of peripheral blood mononuclear cell tetramers (PBMCs) show that there is a massive expansion of more than 50% of specific T cells against lytic cycle proteins, more abundant than those of the latent cycle.[9]

Class II MHC tetramers have also been used to visualize how CD4+ T cells behave in acute EBV infection and healthy blood donors. These studies indicate that both lytic and latent proteins are directed by CD4+ T cells, while the relatively high frequencies of CD4+ T cells can be detected during infectious mononucleosis, the highest response being CD8+.[9]

Although T-cells are believed to be the main effector component of the immune response to EBV, NK cells also play a key role since a high number of NK cells are associated with lower viral loads in individuals with infectious mononucleosis. Studies in immunodeficient mice reconstituted with human cells indicate that NK cells are particularly important in the control of lytic EBV infection. NK cells also appear to play a role in the control of chronic viral infection. Thus, individuals with XMEN (a primary immunodeficiency associated with defects in NK cell function) exhibit high levels of EBV and are at increased risk for EBV lymphoproliferative disorders.[9]

In addition, males with X-linked lymphoproliferative disease (XLP) have defects in the protein associated with the lymphocyte activation molecule (SLAM), which is crucial for the cytotoxic function of NK cells and is unable to control EBV infections. While XLP and XMEN immunodeficiencies can affect T-cell response to EBV, other rare immunodeficiencies that are specific to NK cells are also associated with the development of malignant EBV neoplasms.[9]

EBV AND IMMUNE EVASION STRATEGIES

Like other herpesviruses, EBV uses a multitude of strategies to evade detection and elimination by the host immune system:[9]

1. The function of immune cells.
2. The routes of presentation of the antigen.
3. Apoptosis pathways.

1.The function of immune cells

EBV infects non-dividing B cells, activates them and encourages them to proliferate, thereby amplifying the viral genome load. Once activated, infected B cells acquire antigen-presenting cell properties. After infection, they rapidly present epitopes of structural proteins of incoming viral particles and transiently express lytic genes that are otherwise characteristic of the EBV production cycle.[10]

This prelatent phase of infection includes the expression of two genes encoding viral immunoevasins, BNLF2a and BCRF1, which inhibit the recognition of infected cells by EBV-specific effector T cells and natural killer cells (NK), respectively.[10]

The BCRF1 gene generates an IL-10 homologue (vIL-10), which can suppress the production of IFN-γ, IL-2 and IL-6 from CD4 + antiviral T-cells.[9]

BNLF2a prevents peptide loading on class I HLA molecules through interaction with the antigen processing associated transporter (TAP). So, it inhibits the recognition of infected cells by EBV-

specific effector T cells in the prelatent phase. During the latency phase, it does not prevent this recognition by the T cells. [9]

However, these two viral proteins are insufficient to overcome T-cell recognition. Within 7 to 10 days, EBV establishes a latent infection in infected B cells and expresses only a few or no viral genes, reducing its risk of being killed by the immunocompetent host.[10]

Thus, early infection may be the Achilles' heel of EBV, a window in which the infected cell expresses and presents many viral antigens to immune cells, but is insufficiently protected from the host immune response. But thanks to EBV miRNAs, they overcome this vulnerability by protecting newly infected B-lymphocytes from immune eradication by CD4+ T cells, supporting the lifelong success of EBV.[10]

The EBV expresses at least 44 miRNAs, most of them with unknown function, and two non-coding RNAs (EBERs). EBV-coded miRNAs have been found to control the expression of several cellular genes with antiapoptotic functions, but have also been shown to decrease the regulation of MICB, CXCL11, and NLRP3. Therefore, they interfere with innate immune responses and inflammation. Interestingly, MICB, a gene that encodes a ligand for the NKG2D receptor activator expressed in T and NK cells, is also a target of the miRNAs of the herpesvirus associated with Kaposi's sarcoma and human cytomegalovirus. These studies imply that certain herpesvirus-coded miRNAs target pathways involved in innate immune recognition.[10]

EBV miRNAs act by suppressing, in infected B-lymphocytes, the release of pro-inflammatory cytokines such as IL-12, resulting in the suppression of differentiation of CD4 naive + T-cells to Th1 cells. Th1 cells are important antiviral effectors, activating macrophages and NK lymphocytes to kill intracellular pathogens.[10]

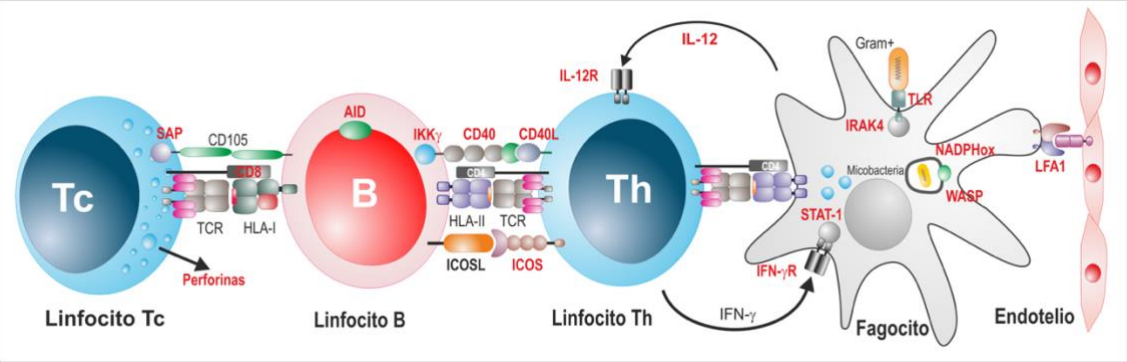

Figure 1: Mutations in proteins that are highlighted in red impair the function of certain leukocytes.[12]

2. The routes of presentation of the antigen.

Several EBV miRNAs modulate the immune recognition of newly infected B cells (preferably EBV target cells). Viral miRNAs in infected B cells control the gene expression of HLA class II and three lysosomal enzymes important for proteolysis and epitope presentation to CD4+ T cells. This allows them to interfere with peptide processing and class II HLA antigenic presentation. As a result of the decrease in HLA II antigenic presentation, the activation of EBV-specific CD4+ effector T cells and the death of infected B cells is reduced. These findings identify a hitherto unknown viral strategy of immune evasion. By rapidly expressing multiple miRNAs, EBV counteracts recognition by CD4+ T cells and establishes a program to reduce the immunogenicity of newly infected B cells, allowing the virus to express viral proteins necessary for the establishment of lifelong infection.[10]

The genes encoding the lysosomal enzymes actively involved in the processing of MHC class II peptides were inhibited by EBV miRNAs. They found that EBV miR-BART1, miR-BART2 and miR-BHRF1-2 could directly regulate the expression of the IFI30, LGMN and CTSB genes through their 3′-UTRs. It is important to note that the deterioration of these three genes resulted in a reduction in the presentation of exogenously charged protein antigens. These results show that EBV miRNAs interfere with the processes involved in MHC class II antigenic presentation at multiple levels, including lysosomal protein degradation, HLA class II expression, and co-stimulant molecule expression.[10]

Latent membrane protein 1 (LMP1) plays a central role in the transformation, survival and proliferation of EBV-infected B cells. LMP1 activates the CD40 pathway (receptor involved in the activation of B cells), inducing important immune co-receptors. But several viral BART miRNAs were found to control LMP1 expression. The results showed that viral miRNAs limit the expression of the LMP1 gene and thus indirectly inhibit the surface expression of some immune co-receptors and adhesion molecules.[10]

Also, to avoid the detection of EBV-specific CD4 T cells mentioned above, it was found that the EBV latent membrane protein 2A (LMP2A) plays a critical role in the negative regulation of the expression of class II MHC molecules in infected B cells. Functionally, LMP2A mimics constitutively activated BCR signaling; however, the LMP2A-activated PI3K pathway mediates suppression of HCM class II and CD74 in EBV-infected B cells. Previous studies have revealed that CIITA is a major regulator of the expression of MHC class II and CD74 molecules. They demonstrated that LMP2A mediated the reduction of CIITA levels by decreasing the expression of PU.1 and E47. Other viruses also evade the responses of antiviral CD4 T-cells through interference with the presentation of MHC class II antigens. For example, human cytomegalovirus, human parainfluenza virus type 3, and varicella zoster virus suppress IFN-induced expression of class II HCM-γ through inhibition of JAK-STAT activation and transcription pathway activator, resulting in reduced expression of IITC.[11]

In addition, there is a type of immunodeficiency, called class II HCM deficiency in antigen-presenting cells, that is associated with a severe decrease in CD4+ T cells. This absence of co-operating T-lymphocytes results in a deficiency of the humoral response (the defect of antigen presentation to the low number of CD4+ lymphocytes causes a defect in the collaboration between T- and B-lymphocytes), and cellular (by the intrinsic defect in the number of CD4+ T-lymphocytes). Patients suffer from recurrent infections, particularly of the digestive tract. The genetic defect of this severe immunodeficiency is found in several proteins that regulate the transcription of class II HLA genes.[12] The difference between this immunodeficiency and what occurs in patients with latent EBV infection is that the former is of genetic origin affecting the antigenic HLA II presentation of all antigen-presenting cells and the latter is acquired by pathogen infection only in infected cells.

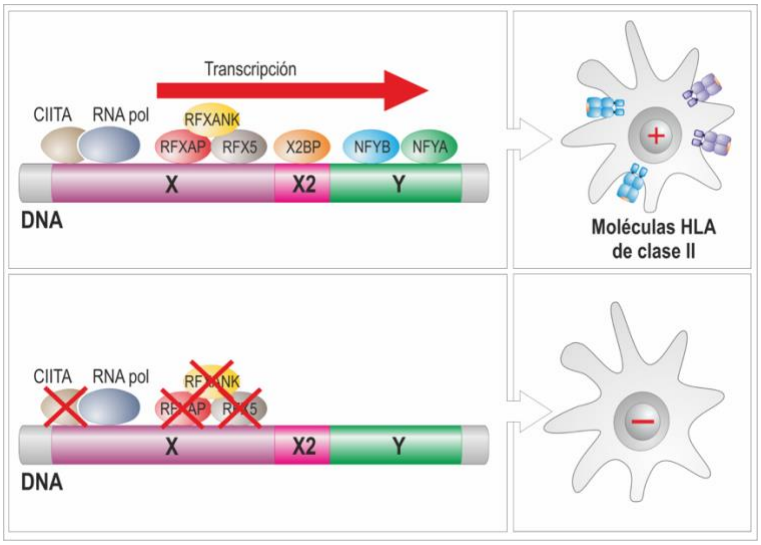

Figure 2: Regulation of class II HLA genes in normal individuals (above) and in patients with class II HLA deficiency (below). In patients, RFXANK, RFX5 or RFXAP mutations in CIITA (class II transactivator) prevent the expression of HLA-DR, -DQ, -DP and -DM, in addition to li(CD74)[12]

When a cell is infected by intracellular pathogens (viruses, protozoa or bacteria), the mechanisms for processing and presenting antigens on the surface of the infected cell begin. In this process, the peptides that are derived from the antigens are presented by the class I MHC molecules forming foreign peptide/MHC I complexes. Tc (cytotoxic) lymphocytes, through specific recognition by the TCR of these foreign peptide/MHC I complexes, are able to distinguish those cells that are infected from the rest of healthy neighboring cells.12 In the case of EBV, their miRNAs interfere with the recognition and destruction of EBV-infected cells by CD8+ T cells. They identified several mechanisms for this inhibition. First, miRNAs directly target TAP2, negatively regulate the entire TAP complex, and reduce HLA class I allotypes that preferentially have TAP-dependent epitopes. Second, they repress EBNA1, a protein expressed in most forms of EBV latency and a target of EBV-specific CD8+ T-cells. Third, miRNAs decrease the release of IL-12 by infected B cells, as IL12B is directly suppressed by these miRNAs in infected cells. This repression of IL12B not only can reduce the differentiation of CD4+ T cells, it can also regulate the functions of effector T cells, decreasing the activity of CD8+ T cells specific to EBV. Therefore, miRNA-mediated reduction of IL-12 may lead to decreased recognition at different stages of infection.[10,13]

This leads us to the fact that B-lymphocytes do not have class I HLA antigens and therefore avoid the immune response of CD8 T-lymphocytes, as they cannot detect intracellular infection. This prevents the Tc lymphocytes from initiating the release of cytolysins, such as perforins, to lyse infected cells.[13]

3. Apoptosis pathways.

EBV also develops several tactics to prevent apoptosis of the infected cell in order to increase viral persistence. A functional bcl-2 counterpart encoded by BHRF1 can inhibit apoptosis induced by a range of stimuli, at least in part by binding to the pro-apoptotic protein Bim. EBV-infected B-cell lymphomas have been shown to be resistant to induction of apoptosis via cell death receptor mediated pathways, the Fas/Fas ligand and TRAIL/RD. This process depends on signaling by latent membrane protein 1, LMP1, which plays a central role in the transformation, survival and proliferation of EBV-infected B cells. LMP1 signaling in human B-cell lymphoma cell lines induces the expression of c-FLIP cell protein that interferes with the formation of the death inducing signaling complex (DISC), required to initiate activation of dandruff-8 after

binding with death receptors. Induction of cFLIP by LMP1 is NF-KB dependent and provides a mechanism for EBV to prevent host cell apoptosis.[9]

Other considerations.

Although most of the cells that are infected are B-lymphocytes, epithelial cells may also initially harbor EBV including NKV. It has been shown in vitro that EBV has the ability to infect NK by the HLA-II molecule without the presence of CD21 on its surface.[14] This predisposes it to a much more severe clinical picture, as it blocks innate immunity to viral destruction and the ability to detect flaws in antigenic presentation (HLA-I lowering) in B cells, since the absence of NK cannot be detected and infected B cells can perpetuate their immortality.

There is evidence that loss of immune control by NKNs predisposes to EBV-associated diseases. This has been seen in primary immunodeficiencies that are more prone to EBV-associated malignancies. The differentiation of NK is interrupted by mutations in GATA2 and MCM4 complex. Patients with GATA2 mutations are diagnosed with chronic EBV active infections (EBVCA) and virus-positive smooth muscle tumors. Therefore, NKD alteration is associated with uncontrolled EBV infection.[15]

We can transfer this to what happens in some patients with EBV CFS. In those patients where the primary infection will also affect the NK cells or who have a genetic alteration in GATA 2 or MCM4, they will generate an infection of greater severity than those who only have latent infection in the B cells. This involvement could be reflected in the analyses by the decrease in NK cells and perforin levels together with high viral loads of EBV by serum PCR. EBV in NK cells can behave differently by initiating the lytic cycle and not by generating latency. An in vitro study16, which aimed to study the behaviour of EBV in KNs, showed both latent and lytic infections in the early stage of EBV infection in two lines of KN cells. However, EBER cells positive for latent EBV behaved strangely and became apoptosis after 72 hours of exposure to the virus, which explains the difficulties in generating NK clones. Therefore, the virus would make a complete replication in the NK cells and be expelled. This is the reason why, in the face of this subgroup of Epstein Barr virus infection that affects NKD, a decrease in the number of NKDs is generated, and consequently also in the levels of perforins. In addition, viral loads could be visible by PCR (this is only detected in more severe patients), behaving similarly to CAEVB (chronic active infection with Epstein Barr virus) of genetic origin. Thus, the number of NKs and the quantification of their activity by means of the levels of perforin can give us an indication of a higher severity in CFS by EBV.

BCRF1

It is important to remember that cooperating T-cells can be divided into two types, Th1 and Th2, according to the cytokines they produce and their effecting function. The differentiation to Th1 cells, which generate IL-2, INF-g and lymphotoxin, is stimulated by IL-12 and INF-g, while the differentiation to Th2 cells, which originate IL-4, IL-5, IL-10 and IL-13, depends on IL-4.

As noted above, EBV-infected B-lymphocytes generate an IL-10 homologue (VIL-10), encoded by the EBV BCRF1 gene during the prelatent phase (lytic phase of viral replication).9 Although recent evidence indicates that it is also expressed during the latent phase.[17]

In several studies, IL-10, produced mainly by activated macrophages, has been shown to be an inhibitor of activated macrophages and therefore intervenes in the homeostasis control of innate and cellular immunity reactions as a negative feedback regulator. In an acute infection, macrophages respond to microorganisms by releasing cytokines and expressing co-stimulators that enhance T-cell activation and cellular immunity. But IL-10 acts on activated macrophages to terminate these responses and restore the body's resting state once microbial infection has been eradicated.18 Like mast cells, Th2 lymphocytes can also secrete IL-10 to inhibit the production

of cytokines (IL-2 and IFN-g) by Th1 cells. Thus, vIL-10 (IL-10 homologous) may act on multiple cell types and inhibit cytokine synthesis in T-cells and NK.19 cells.
In addition, remember that EBV miRNAs act by suppressing the release of IL-12 in infected B cells, causing an inhibition of differentiation of CD4 naive + T cells to Th1 cells (this leads to an increase of Th2).[10,13]

IL-10 modulates the expression of cytokines, soluble mediators and cell surface molecules: IL-1a, IL-1b, IL-6, IL-10, IL-12, IL-18, GM-CSF, G-CSF, M-CSF, TNF, LIF and PAF by activated monocytes/macrophages, as well as the production of chemokines (CC and CXC) such as IL-8, IP-10, MIP-2, KC (Gro-a) by activated monocytes. These chemokines are involved in the recruitment of monocytes, dendritic cells, neutrophils and T-cells. In this way, IL-10 prevents the expression of many inducible chemokines involved in inflammation, increasing the production of the antagonist receptor for IL-1 (IL-1RA) and the soluble receptors of TNFR p55 and p75; it also inhibits the expression of IL-1RI and IL-1RII in activated monocytes, indicating that IL-10 not only deactivates monocytes, but induces the creation of anti-inflammatory molecules. In addition, IL-10 also prevents the generation of E2 prostaglandins (PGE2), thus reducing the expression of cyclooxygenase 2 (COX-2) and MHC class II antigens, CD54 (ICAM-1), CD80 (B7-1) and CD86 (B7-2) in monocytes, even after IL-4 or IFNg have induced their creation; it also prevents the production of IL-12 and the expression of co-stimulating molecules for various types of dendritic cells. 1[8]

The dendritic cell presents the antigens through HLA-II (this constitutes the signal 1) but also expresses co-stimulatory signals that do so through CD80 ligands, CD86 that interacts with CD28 so that the T lymphocyte is cloned exponentially. Co-stimulation (i.e., signal 2) is not a priority in dendritic cells, but if absent, the T cell refuses to respond correctly and will often self-destruct through programmed cell death (apoptosis).[20] Virgin T cells require both signals 1 and 2 of a CPA (antigen-presenting cell) to become correctly active.

LMP2A directly decreases the HLA-II antigenic presentation of infected cells and vIL-10 (IL-10 counterpart) decreases HLA-II of nearby antigen-presenting cells. All this leads to a decrease in activated CD4 lymphocytes.

On the other hand, the only way left to eliminate Epstein Barr virus-infected B-lymphocytes, having inhibited the antigenic HLA-I presentation in B-lymphocytes, would be through NKs alone. And remember that CD8 T-cells played an important role in containing the virus during primary infection.

Recently, direct effects of vIL-10 were detected in ex vivo isolated NK / NKT cells as well as in CD4+ T cells. NK cells lysate EBV-infected B cells preferably when they enter the productive lytic cycle. Although they showed that NK cells also lyse newly infected B cells, vIL-10 interfered with this effector function. The presence of CD4+ T-cells also supported NK-mediated lysis, especially when vIL-10 was not expressed. This phenomenon is probably attributable to two different observations: (i) infections with BCRF1 deficient viruses led to higher levels of Th1 cytokines, suggesting that increased secretion of Th1 cytokines by CD4+ T cells stimulated the activity of NK cells, and (ii) experiments indicated a direct inhibitory effect of VIL-10, as well as IL-10, on the activity of NK and T-CD4+ cells. [21]

That is, vIL-10 released by infected B cells can act on multiple cell types and inhibit cytokine synthesis in T cells (inhibits production of IL-2 and IFN-g by Th1 cells) and NK cells. This allows the antiviral functions of effector CD4+ T cells to be suppressed and the NK cell-mediated death of infected B cells to be reduced. It is also a potent inhibitor of antigenic presentation, reducing the expression of MHC II and the accessory co-stimulation molecules CD80 and CD86 in dendritic cells.

Studies of systemic lupus erythematosus (SLE) from Epstein Barr virus infection showed a decrease in IL-12, IFN gamma and as a result also a decrease in IL17 and IL6 by the EBNA1 protein present in the latent cell. Both gamma IFN and IL12 are important factors for the differentiation of Th1 and crucial for the correct activity of CD8 and NK. IL12 stimulates the production of gamma IFNs in both CD8 and NK as well as activating macrophages. Therefore, it has been demonstrated that in SLE there is a dysfunction of the Th1 response and therefore of the activity of CD8 and NK T-lymphocytes against latent infection with Epstein Barr.[22]

Even so, the immune system has a series of mechanisms to detect these failures and prevent the virus from avoiding them. This balance is what plays a role between the generation of a cancer or the perpetuity of a latent infection with the metabolic consequences that this entails. NK+ cells are able to detect a decrease in HLA-I in B cells and are activated by increasing levels of perforin (these increases may be visible in patients with chronic fatigue syndrome) if they have not infected NK cells, otherwise a decrease in perforin levels and a decrease in the number of NK cells would be visualized, as explained above.

It is known that EBV influences the regulation of T-bet/GATA 3 (Th1/Th2) in T[23] cells by positively regulating the expression of GATA 3 in vitro. On the other hand, the mir-BART20-5p[24] gene that uses the EBV to perpetuate latency, since it inhibits the generation of the lithic cycle, it also inhibits the T-bet translation, thus blocking differentiation towards Th1. Therefore, from an Epstein Barr virus infection, an increase in the Th2 response can be generated with a decrease in Th1 (miRNAs also decrease the differentiation of CD4 T-cells naives to Th1). In patients with chronic fatigue syndrome, this increased activity of Th2.[25,26] This can be measured directly from cytokines or indirectly measured, such as the elevation of eosinophilic cationic protein27 that may be part of the markers of this disease. All this altered Th2 response is the one that generates the increase in allergies at both respiratory and intestinal levels that are present in these patients and that is common for them to have developed as a result of the initial process. This is a response not mediated by IgE, but by a T-lymphocyte-mediated type IV hypersensitivity that would be related to diarrhoea, multiple emerging food intolerances, the breakdown of the intestinal barrier and bacterial translocation in these types of patients, which can be assessed by elevation of sCD14.

This elevation of sCD14 would also be recommended as one of the markers to check the affectation of the intestinal barrier in those subtypes that present diarrhoeal symptoms. There has also been an increase in the levels of 5-HIAA[28] (serotonin metabolic product) and histamine[29], which again show excessive Th2 activity.

EBV CAN INFECT THE CNS

Some human-adapted viruses have access to the Central Nervous System (CNS) as a result of decreased host defenses that fail to limit peripheral infections (e.g., Epstein-Barr virus, human cytomegalovirus, and JC virus or John Cunningham virus). Surprisingly, many alpha herpesviruses efficiently enter the peripheral nervous system (PNS) of their hosts and establish a quiescent infection with little or no CNS pathogenesis. Its initial peripheral infection stimulates a well-controlled intrinsic and innate immune response, as well as a long-lasting adaptive immune response. The herpesvirus alpha genomes remain at rest in PNS neurons for the life of their hosts, reactivating only occasionally to produce virions that can reinfect peripheral tissues and spread to other hosts. Perhaps the cyto-resistant pro-survival nature of mature neurons facilitates the establishment of such persistent and reactivable infections.[30]

PNS is relatively more accessible to peripheral infections because the nerves are in direct contact with tissues of all kinds, but the CNS itself has several layers of protection. The spread of infection from the blood to the cerebrospinal fluid and central nervous system cells is limited by the blood-brain barrier (BBB). BBB is composed mainly of endothelial cells, pericytes, astrocytes and the basal membrane. The experts project finger-like processes to surround the capillary wall and

coordinate the neurovascular functions of the BBB. Star-shaped astrocytes (i.e., astroglia) are the most important type of glial cell in the CNS. The projections of the final feet of the astrocyte reach the capillary, regulating the BBB homeostasis and blood flow. The microvascular endothelial cells of the human brain (HBMEC), which line the vasculature of the CNS, are connected by narrow joints not found in the capillaries of other tissues. These bonds restrict the release of bacteria, virus particles and large protein molecules from the blood vessel lumen, allowing the transport of metabolites, small hydrophobic proteins and dissolved gases into and out of the CNS. The basement membrane, a thick extracellular matrix, also surrounds these capillaries, further limiting the movement of pathogens. Perivascular macrophages (i.e. migroglia), which reside between endothelial and glial cells, also provide immune surveillance in CNS tissue. Viral infections that leave the periphery and find their way into the PNS or CNS either by direct infection of the nerve endings in the tissues, or by infecting the cells of the circulatory system that eventually carry the infection through the BBB to the CNS.[30]

There are several ways in which viruses can access the central nervous system, but we will focus on infection of the microvascular endothelium of the brain with BBB.
In some cases, virus particles in the circulatory system can reach and infect HBMEC, one of the main components of BBB. RNA viruses such as West Nile virus (WNV), hepatitis C virus (HCV), HTLV-1 and DNA viruses such as JCV, Epstein-Barr virus (EBV), human cytomegalovirus (HCMV) and mouse adenovirus 1 (AVM-1) can infect HBMEC. Infection of these cells often leads to alteration of the integrity of BBB, which is accompanied by uncontrolled migration of immune cells in the brain parenchyma. Inflammation in brain tissue induced by the activity of these cells is often the cause of observed neurological disorders.[30]

WNV can access the CNS by infecting sensory nerve endings, olfactory neurons or through the bloodstream, but not through neuromuscular junctions (NMJ). A distinctive feature of WNV neuropathogenesis is the alteration of BBB that causes uncontrolled entry of immune cells into the brain. The infection not only stimulates the loss of proteins from narrow-binding epithelial and endothelial cells, but also induces the production of matrix metalloproteinases that degrade the basement membrane. As a result, leukocytes pass through capillaries into surrounding tissue and release cytokines by recognizing double-stranded RNA of WNV (dsRNA) through signaling of the toll 3 receptor (TLR3) and alpha tumor necrosis factor (TNF-alpha). HIV, HTLV-1, and MAV-1 infections can also alter BBB by affecting the proteins in tight joints.[30]

Another HCV flavivirus, which mainly infects hepatocytes, has also been associated with CNS abnormalities such as cognitive dysfunction, fatigue and depression. HBMECs are the only type of cell in the brain that expresses the four receptors required for HCV entry (vector receptor BI, CD81, claudin-1, and ocludin). In vitro HCV infection suggests that they may be the reservoir of HCV in the CNS. HCV infection in the CNS is also associated with increased expression of proinflammatory cytokines (IL-1, TNF-alpha, IL-12, and IL18), choline, creatine, and inositol, all of which trigger the activation of microglia and astrocytes, leading to pronounced neurological disorders.[30]

Prevalent human DNA viruses, which establish persistent and well-controlled life-long infections in other tissues, can also damage the CNS by infecting HBMECs under immunosuppressive conditions. These pathogens include CMV (cytomegalovirus) beta herpesvirus, EBV gamma herpesvirus, and polio, John Cunningham virus, or JC virus (JCV). CMV establishes lifetime latency, predominantly in myeloid lineage cells. If primary infection occurs during pregnancy, transmission to the fetus, which is not yet immune, can result in a variety of deadly CNS abnormalities, such as mental retardation and hearing loss. In addition to HBMEC, astrocytes, pericytes, neurons, microglial cells, and most importantly, all neural stem cells can be infected with CMV. Astrocytes and microglial cells naturally produce large amounts of inflammatory cytokines in response to CMV infection and this response can promote fetal neurodevelopmental diseases.[30]

EBV establishes a lifetime latency in memory B cells. EBV infection in young adults causes infectious mononucleosis. Horwitz and his team showed that EBV can infect human BMEC, where it becomes dormant. Reactivation of the virus in these cells increases the expression of inflammatory cytokines and chemokines that affect the integrity of local BBB, which could lead to progression of inflammatory neurological disease, multiple sclerosis (MS). In transgenic TCR mouse models, MS is only activated when the blood-brain barrier (BBB) is compromised. The presence of specific autoactive myelin lymphocytes is not sufficient to cause MS as such cells have been isolated from healthy individuals. In patients with MS, lesion and plaque formations are associated with a BBB disorder. Therefore, increased permeability to BBB, together with activation of self-reactive T-cells, is a prerequisite for the development of MS. However, it is not clear how changes in BBB are initiated prior to the initial entry of immune cells into the brain.[30]

It was hypothesized that EBV infection of a subset of brain endothelial cells would increase the potential for inflammatory breakdown of the blood-brain barrier (BBB), particularly after reactivation of the latent virus. Previous work has established that EBV can infect macrovascular endothelial cells in both human tissue and culture with human umbilical vein endothelial cells (HUVECs). However, BBB is composed of microvascular endothelial cells that differ significantly from macroscopic endothelial cells, such as HUVECs, in a number of aesthetic features, particularly in terms of susceptibility to viral Herpesviridae infection.[30]

Microvascular endothelial cells of the human brain (HBMEC) isolated from three different donors were successfully infected with EBV under standard laboratory culture conditions. The viral genome was detected by standard PCR in infected HBMECs and was absent from simulated infected HBMECs, indicating that donor endothelial cells were EBV negative.[30]

Latency and immediate expression of early genes were detected in separate experiments with cells derived from two different donors, demonstrating viral replication and splicing of EBV genes. The expression of BZLF-1 and EBNA-1, both involved in the transcription transaction of other viral genes, were detected post-infection in HBMEC from one donor; while LMP2B and EBNA-1 were found to be expressed post-infection in HBMEC from a second donor.[30]

BZLF-1 is an immediate initial gene responsible for the transition from latency to reactivation of the lytic cycle.
EBNA-1 is a latency gene responsible for maintaining the viral genome during host cell replication.
LMP-2B is a latency gene that promotes the motility and spread of epithelial cells, while its function in EBV-infected B cells is poorly understood.

Although these patterns are different from those seen in infected B cells, they are similar to those found in EBV-infected epithelial cells. In fact, primary in vitro epithelial cells have been shown to express EBNA-1, LMPs and BZLF-1 on the 5th day post infection with similar levels of variability at the single cell level and with primary cultures from different donors. This was the first report to demonstrate the success of EBV infection and gene expression in BHMEC.[30]

It was then investigated whether EBV infection of HBMECs could lead to activation and increased production of proinflammatory molecules. Non-infected HBMEC expressed baseline levels of CCL-2 (MCP-1) and IL-8 consistent with previous reports. An increase in CCL-5 production was observed at 24 and 48 h after infection in cultured supernatants. In addition, the surface expression of the adhesion molecule, ICAM-1, increased significantly in 48 h post-infection, while the VCAM-1 expression was very low in EBV-infected or harmless HBMECs. It is important to note that both ICAM-1 and CCL-5 are involved in the firm adhesion of white blood cells to the endothelium. To determine whether the observed EBV-mediated upregulation of ICAM-1 and CCL-5 was sufficient to increase the adhesion of peripheral blood mononuclear cells (PBMCs), they performed PBMC adhesion trials. While only a few PBMCs adhered to native HBMECs, significantly more PBMCs adhered to EBV-infected HBMECs. As a positive

control, HBMECs treated with TNF-α for 24 hours showed significant levels of PBMC adhesion.[30]

The supernatant used for infection was derived from a B cell line (B95.8) transformed by EBV. To rule out the possibility that cytokines produced by B95.8 cells are contributing to the changes observed in HBMECs, supernatant B95.8 was tested for the presence of pro-inflammatory mediators using a human kit with cross reactivity with non-human primates. Supernatant B95.8 was positive for IL-10 but negative for IL-6, IL-1, TNF-α and CCL-5. In summary, EBV infection regulates inflammatory molecules in HBMEC.[30]

Interestingly, both CCL-5 and ICAM-1 have been associated with MS. The polymorphisms in the CCL-5 and its receptor, the CCR5, modify the course and outcome of MS. The low-production CCL-5 allele is associated with a reduced risk of axonal loss; while the high-production CCL-5 allele is associated with more severe clinical disease. The expression of CCL5 is regulated upwards in BBB cells before clinical signs appear. MS-derived HBMECs express higher levels of ICAM-1 and circulating leukocytes from patients with MS express higher levels of LFA-1, the ICAM-1 ligand. [30]

Therefore, the ability of EBV to better up-regulate CCL-5 and ICAM-1 in HBMEC in a manner similar to what is observed in the BBB of MS patients, could in part describe the mechanism of MS pathogenesis.[30]

Here it was demonstrated that EBV infection of EBV in HBMEC leads to activation of endothelial cells and adhesion to PBMCs. They proposed that reactivation of latent EBV infection in brain endothelial cells could regulate cytokines, chemokines and adhesion molecules that would induce a local rupture in BBB and attract autoreactive lymphocytes to the brain. In an individual with a higher level of self-reactive peripheral T-cells, this may lead to an initial localised entry of immune cells and the development of CNS lesions. Thus, EBV would only need to infect a small population of HBMEC and the required reactivation within a minority of these cells. This proposed model would explain the inconsistent detection of EBV infection in the brains of MS.[30]

This mechanism also explains important features of MS, including: the lack of detectable virus in MS plaques; the infiltration of specific macrophages and lymphocytes, both viral and myelin; the presence of oligoclonal antibodies in the cerebrospinal fluid; and the success of antiviral treatments such as interferon-β in preventing MS relapse.[30]

But this should not only occur in MS, but also in other EBV-associated diseases where there are no self-reactive myelin T-lymphocytes present. EBV infection of HBMEC leads to the breakdown of adhesion molecules or narrow BBB bonds, leading to the passage of leukocytes (including EBV-infected B cells) through the capillaries into the surrounding tissue. B cells with latent EBV infection are able to release EBERs (two non-coding RNAs). Where the release of EBER1 induces the activation of TLR3 signaling resulting in an increase in proinflammatory cytokines, generating inflammation in the tissue.[31]

GENETIC PREDISPOSITION AND AUTOIMMUNITY

Tolerance to one's own is a process that is acquired during the development of T and B lymphocytes in the primary lymphoid organs (central tolerance) and in the periphery (peripheral tolerance), by elimination or inactivation of potentially self-reactive cell clones.[12]

Due to the dependence of B and Tc (cytotoxic) lymphocytes on Th (helper), most autoimmune responses are considered to begin with the activation of self-reactive Th-lymphocytes. The specific cause(s) of activating self-reactive T-lymphocytes and triggering autoimmunity are not yet known, but all known data suggest that they are triggered by the action of an environmental factor acting on a genetically predisposed individual. It has been suggested that infections may

be the most important environmental trigger. Infections do not cause a break in central tolerance, but a number of mechanisms have been suggested by which peripheral tolerance can be broken:[12]

-The structural similarity between microbial and autoantigenic antigens (molecular mimicry) can lead to cross reactions that damage the antigens themselves. In this case, the antibodies or T lymphocytes recognize, in addition to the antigens of the infectious agent, other antigens of the organism of similar structure.

-B-lymphocytes capable of recognizing their own antigen interact with it when it is associated with the "carrier" bacteria, and can then be helped by the Th lymphocytes.

-It has also been suggested that polyclonal activation by microbial superantigens of large numbers of T or B lymphocytes (some of them self-reactive) may be a mechanism of loss of tolerance.

-They can also release self-antigens seized from immunologically privileged sites through trauma or infection. For example, damage to the blood-brain barrier can bring the autoantigens of the central nervous system into contact with lymphocytes, triggering autoimmunity.

-They can induce the non-specific activation of self-reactive T-lymphocytes by cytokines (spectator effect) or the expression of MHC class II molecules and/or co-stimulatory signals in the antigen-presenting cell, which will now specifically activate self-reactive T-lymphocytes that it was previously unable to activate.

In the development of many, if not all, autoimmune diseases, there is not only an environmental component (such as infections), but also an important genetic component. However, these diseases are not transmitted as classical monogenic diseases. Most autoimmune diseases are polygenic, i.e. there are numerous genes (actually certain polymorphisms of these genes) of susceptibility that act together to produce a given disease. These genes often have complex interactions, with low and incomplete penetration and non-Mendelian inheritance patterns. In addition, some polymorphisms may play a protective role against the disease. These allelic variants are normal in the population and by themselves do not determine whether or not an individual will develop the disease (they only increase or decrease the risk of developing them); only when they act together and there is an environmental factor (probably infectious) will the disease develop. Therefore, there is a great genetic heterogeneity among patients who develop an autoimmune disease, which manifests itself as a great phenotypic variability among different patients suffering from the same disease.[12]

The genes that are most strongly associated with most autoimmune diseases are those of certain HLA molecules, mainly class II, although there are also class I associations. This idea is not at all far-fetched, since not all HLA molecules will have the same ability to deliver autoantigen-derived peptides to T-lymphocytes, nor will they be equally effective in developing an autoimmune response. In addition to HLA molecules, other genetic factors, some not directly related to the immune system, such as sex (they are more common in females than in males), are important in the development of autoimmune diseases.[12]

Genetic predisposition to develop EBV-associated diseases.

In virtually all vertebrate species, MHC molecules are highly polymorphic. This polymorphism reflects an immune system strategy to prevent the evasion of immune system pathogens. Having different MHC molecules, individuals deal with microbes in a different way, with individuals in a given population being more susceptible and more resistant to a given disease. Repeated exposure to certain pathogens over the course of evolution may select those individuals who express MHC alleles most suitable for responding to infection. For example, the HLA-B53 allele is associated with resistance to a lethal form of malaria. This allele is very common in individuals in Africa where malaria is abundant, but is not common in places where malaria is not endemic.[12]

Paradoxically, EBV has adapted to the use of MHC class II molecules as input co-receptors through their interaction with gp42. This interaction is essential for EBV infection. Although the binding of gp42 to HLA involves only the β chain outside the peptide slot of the heterodimer αβ, the binding of gp42 also interferes with the interaction of HLA-DR with the T-cell receptor, inhibits the generation of cytotoxic T-cells, and prevents the presentation of the antigen. Therefore, gp42 may have developed multiple functions that inhibit the cellular immune response to the virus.[32]

Primary infection with EBV was demonstrated many years ago as the leading cause of infectious mononucleosis (IBD). Symptoms of IM are caused by the immune response to the infection. Although EBV mainly infects B cells and can cause their proliferation, the excessive number of lymphocytes observed, which are responsible for mononucleosis, are mostly T cells. The specificity of these T cells is largely directed at the EBV proteins produced in infected B cells. Cytokines produced during the chaotic immune response that occurs in IM produce characteristic fever, malaise and other inflammatory symptoms. The disease decreases as the immune response adjusts, until it becomes more like that of an asymptomatically infected person. EBV persists throughout the host's life because it expresses a series of proteins that inhibit HLA-II and HLA-I antigenic presentation to prevent recognition by the immune system. However, about 30% of adults develop IM after EBV infection, while most seroconvert without noticeable symptoms. This leads us to think that there is a genetic predisposition to developing IB and other diseases associated with this virus.[33]

The data presented by McAulay et al. clearly show a tendency to link certain HLA alleles to IM and indicate that genetic variation in T cell responses influences the outcome of primary EBV infection and the level of viral persistence. Since class I HLA determines the effectiveness of the presentation of viral peptides to T cells, it is easy to predict how this genetic variation might affect the immune response against EBV infection. A suboptimal response of T cells to the virus during IM could result in a higher level of viral persistence in B cells, thus increasing the chance of EBV infection of these cells and the subsequent survival of abnormal B cells that have malignant potential. The fact that the same class I HLA alleles (class I HLA markers D6S510 and D6S265) that influence the frequency of IM have also been associated with EBV-associated Hodgkin's lymphoma (LH) suggests a genetic basis for the increased risk of EBV-positive LHV in those individuals who have had IM.[34]

One of the autoimmune diseases associated with EBV is multiple sclerosis (MS), a demyelinating and inflammatory disease of the central nervous system that often leads to neurodegeneration and long-term disability despite current treatment strategies. It is clear that the main component of the genetic risk is associated with the HLA-DR locus but especially with the DR15/DQ6 haplotype [35,36] (DR2/DQ1 subtypes). Several lines of evidence link specific immunity to EBV to the risk of developing multiple sclerosis. Both serologic and CD4 T-cell responses directed against EBNA-1 have been associated with multiple sclerosis. It is believed that self-reactive T-lymphocytes may arise as a result of cross-reactivity with specific immune responses against EBNA-1. Several groups have demonstrated increased seroprevalence of EBV in patients with MS compared to controls and late (adult) EBV infection, particularly if manifested as infectious mononucleosis, has also been shown to increase a person's risk of MS.[3]

AntiEBNA-1 IgG antibody titers are another risk factor for multiple sclerosis (MS), regardless of the DR15 allele. Carriers of the DR15 allele with elevated titers of antiEBNA-1 antibodies may have a significantly increased risk of developing multiple sclerosis.[37]

Other observations include that the oligoclonal bands of cerebrospinal fluid that are a hallmark of MS can be specifically targeted at EBNA-1 and another group has further identified the presence of EBV-infected B cells within white matter MS lesions at all stages of the disease, although this finding has not been replicated in other studies.[3]

A recent study has also explored the use of Epstein-Barr virus-specific adoptive immunotherapy for progressive multiple sclerosis, with promising preliminary results. These effects can be explained by the death of EBV-infected B cells in the CNS by adoptively transferred CD8+ T cells.[38]

In summary, patients with genes from MHC class I and II molecules that may develop EBV-related diseases will have difficulty fighting EBV infection. As most of these diseases have numerous polymorphisms of these susceptibility genes, there is a great genetic heterogeneity among patients who develop one of these diseases, which is manifested as a great phenotypic variability among different patients suffering from the same disease.

WARBURG EFFECT

The predominant symptom in chronic fatigue is extreme prolonged or intermittent fatigue in these patients. This extreme tiredness is accompanied by the Warburg effect, which refers to the fact that virus-infected cells produce energy, mainly in the cytosol, by a process of anaerobic glycolysis even under suitable oxygen conditions.

The expression of an EBV oncogene (expressed during many forms of latency) LMP-1, would induce the expression of HK2 (hexokinase 2). Hexokinase 2 is an enzyme that limits the speed of glycolysis, which, thanks to its activation, would lead to an increase in glycolysis and therefore lactate production. In addition, it is able to increase cell survival by inhibiting apoptosis by binding to the external mitochondrial membrane, interacting with the voltage-dependent ion channel (VDAC) to block the release of cytochrome C and thus caspase 9-dependent apoptosis. [39]

However, the use of glycolysis as the primary metabolic pathway for glucose, requires an increase in extracellular glucose uptake (increased surface expression of GLUT1) [40,41] to match the higher metabolic rate. To boost glucose uptake, recent studies on the behaviour of cancer cells have shown the emergence of a mechanism by which these cells can increase the consumption of ATP, thus leading to a decrease in ATP reserves as a result. During this process glucose is incorporated into biosynthesis pathways (lipid biosynthesis and protein glycosylation pathways) and is not intended for the production of ATP. In both cancer and latently infected cells, there is a metabolism that is not adapted to support ATP production, but to be consumed. It should be noted that one of the symptoms that has traditionally been associated with a tumour process is the constitutional syndrome, so the feeling of lack of energy is a common symptom in both pathologies.
To alter glucose metabolism the cell needs the 3-kinase phosphoinositol (PI3K) signaling pathway, which is activated in many cancers and which has been shown to also activate PI3K[42,43] in cells with latent EBV infection, both LMP1 and LMP2A. Its functions include the regulation of cell growth and survival. AKT serine / threonine kinase, an important PI3K effector, promotes glucose uptake and increases the activity of glycolytic enzymes.

The most important mechanism caused by AKT signaling is the increase in the Warburg effect. The activation of Akt promotes protein glycosylation in the endoplasmic reticulum, which increases the intake of ATP and disinhibits a speed-limiting enzyme in glycolysis, which should be inhibited by a high proportion of the ATP/AMP ratio and thus is not abnormally increasing the glycolysis process. This exaggerated increase in glycolysis causes systems to become saturated so that the cell uses both the aerobic and anaerobic pathways to achieve ATP.

Under normal aerobic conditions, the pyruvate generated by glycolysis is transported to the mitochondria, where it is converted to acetyl-CoA by the pyruvate dehydrogenase complex (PDH). Under anaerobic conditions, when mitochondrial respiration is inhibited, pyruvate accumulates in the cytosol, leading to increased cellular production and excretion of lactate.

The activity of PDH is controlled by PDKs that inhibit the activity of the PDH enzyme by phosphorylation and the phosphatases that catalyze dephosphorylation. Significant increases in PDK1, PDK2, and PDK444 inhibitor kinases have been seen in CFS, while PDK3 remained unchanged. These enzymes are found, among other places, in skeletal muscle, heart and brain. Activations of the PDKs are directly regulated through increased levels of ATP, NADH and acetyl-coA. Instead it is inhibited by ADP, NAD+, CoA-SH and pyruvate.

The initial sudden increase of pyruvate by glycolysis produces an inactivation of the PDK and therefore leaves the PDH to perform its function and begin the Krebs cycle in the mitochondrial chain to generate ATP. However, the speed of the Krebs cycle is limited, so the rest of the pyruvate is consumed anaerobically, increasing lactate levels. So far, the cell is using both pathways for ATP production. The lactate generated can be used by the muscle cells for fueling or by the liver through the Cori cycle to generate glucose again.

However, the increase of lactate at the cytosolic level of infected cells would activate the HIF1A protein, whose active function is to inhibit ATP synthetase, i.e. it interrupts the production of ATP by the mitochondrial oxidative pathway. Although the electron transport chain is inhibited by the HIF1 protein, the cell can still generate Krebs cycle intermediates because the other enzymes are not inhibited. As a result, an increase in citrate in the mitochondria is produced with expulsion to the cytosol to generate acetyl coA and for it to be integrated into the fatty acid biosynthesis pathways. At the same time, the pyruvate accumulated in the cytosol is consumed as lactate for the generation of ATP, since it is the fastest way to obtain energy and the only one in this case because the conveyor chain is inhibited. When an accumulation of acetyl coA (by the synthesis of fatty acids) in the cytosol, the PDH would be inhibited via PDK and the pyruvate would begin to accumulate. Again, the elevated pyruvate would activate this pathway and begin the process. An increase in KDPs has been reported in CFS patients, so this may be the explanation.

In order to follow a continuous glycolysis process, NAD needs to be replenished constantly, this can be replaced by the aerobic or anaerobic route, in this case it would be the anaerobic route, because we have already said that aerobic has been inhibited, therefore, NAD is achieved by converting pyruvate to lactate by lactate dehydrogenase since NADH is consumed during glycolysis.

When the transport of mitochondrial electrons decreases for any reason there are fewer oxygen molecules converted to water (H2O) by the cytochrome c oxidase enzyme. If the capillary delivery of oxygen to the cell does not change, the concentration of dissolved oxygen rises in the cell. This activates dozens of enzymes that are kinetically regulated by the availability of dissolved oxygen and can act as oxygen sensors. Some of these enzymes are NADPH oxidase (Nox4 gene)[45] which increase levels of hydrogen peroxide (H2O2) to neutralize excess oxygen (O2). The reaction would be:

$NAD(P)H + 2O_2 \rightarrow NAD(P)^+ + 2O_2^- + H+$

It should also be noted that the enzyme NADPH oxidase, which catalyzes the oxidation reaction of NADPH to NADP, uses calcium, FAD and the heme group as co-factors, and Naviaux studies of metabolites in CFS have shown a reduction in FAD.

This O2- is used by SOD (superoxide dismutase) to form H202 that would go into the peroxisome and then be neutralized by catalase to H2O. But catalase has its limited speed so that the rest of H2O2 has to be used in another way, so that glutathione, which is normally in its reduced form (GSH) is oxidized to GSSG by the enzyme glutathione peroxidase.

2 Glutathione (GSH) + H2O2 ⇌ Glutathione disulfide (GSSG) + 2 H2O.

The route of the pentose phosphate is regulated by the concentration of NADP in cytosol. The sudden increase of NADP by NADPH oxidase in response to excess oxygen generates the beginning of the route of the pentose phosphate. When NADPH accumulates along with the production of elevated levels of acetyl guava as a result of increased glucose uptake, both provide a stimulus for fatty acid synthesis.

ROLE OF PEROXISOMES

Peroxisomes play a key role in both the production and neutralization of reactive oxygen species (ROS) along with lipid metabolism. Peroxisomes generate significant amounts of hydrogen peroxide through the action of several peroxisomal oxidases (e.g., their oxidase acyl-CoA reductase). However, peroxisomes also contain multiple antioxidant enzymes such as catalase, SOD, glutathione peroxidase, epoxide hydrolase, peroxyrredoxin I... which contribute to the regulation of intracellular ROS levels and thus to oxidative stress. [46]

Latent HVSK infection (HHV8) has been shown to increase peroxisome proliferation. They found that lipid metabolism (especially very long-chain fatty acids) in the peroxisome was necessary for survival from latent herpesvirus infection.[47]

This proliferation of peroxisomes is induced by PPAR γ (gamma receptor activated by peroxisome proliferating factor). PPAR is activated in both cancer cells and latent herpesvirus infections where it promotes increased oxidation of fatty acids in peroxisomes.[48,49] In a study of prostate cancer, it was shown that peroxysomal over-activation with increased peroxisomal b-oxidation of branched-chain fatty acids occurred in cancer cells[50].

All this indicates that peroxisomes play a role in the survival of cancer cells or latent infections. The peroxisomal beta-oxidation produced in these cells would serve to consume some of the long chain fatty acids harmful to them and thus provide a more acetyl CoA product to be used again by the cell in the synthesis of fatty acids. During this process, energy is not produced in the form of ATP, but is dissipated in the form of heat.

In the peroxisome during beta-oxidation of fatty acids FADH2 is formed which reduces the oxygen to H_20 to reuse the FAD in order to follow the beta-oxidation. This H_20_2 being harmful is reduced in a second step through the catalases of the peroxisomes where H20 and ½ O_2 would be formed.

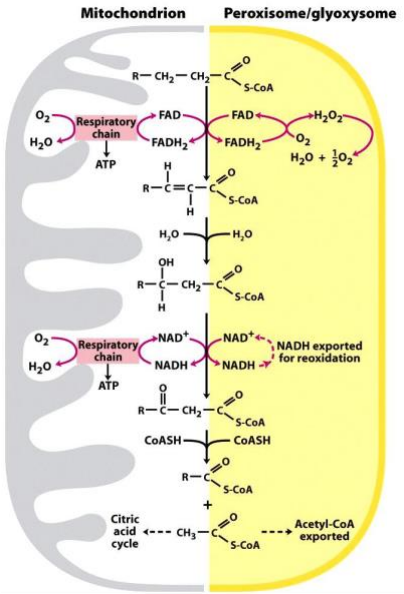

Figure 3: Comparison of β-oxidation in mitochondria and peroxisomes/glioxisomes.[51]

In addition, recent studies have shown reduced levels of CoQ10, decreased mitochondrial membrane potential (hence no production of mitochondrial ATP), increased levels of mitochondrial superoxide, and increased levels of lipid peroxidation in mononuclear cells in the blood of patients with fibromyalgia.[52]

FORMATION OF FREE RADICALS

The hydroxyl radical -OH has a very short half-life (1 nanosecond) which allows it to act only at the place of its formation or in its proximity. It can react on the structure of DNA. Biological damage would be the chain reaction known as lipid peroxidation or lipoperoxidation. When this radical is generated near biological membranes, it can attack the fatty acids of the phospholipids, which are preferably polyunsaturated, such as arachidonic acid. It is a chain reaction in which an OH can cause hundreds of fatty acid molecules to become lipid hydroperoxides, the accumulation of which disrupts the membrane function and can even destroy it.[53]

The formation of the hydroxyl radical depends on a reaction catalyzed by ions of transition metals (iron and copper), the iron ions being the promoters of free radicals. This is called a Fenton reaction where the ferrous ion reacts with hydrogen peroxide to form the highly reactive hydroxyl radical that interacts rapidly with DNA, proteins and lipids.[53]

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$ (Fenton reaction)

The cell receives iron from the blood as an iron ion bound to transferrin. The complex formed by transferin and iron is captured by specific receptors on the cell surface and internalized by endocytosis.

The acidic medium of the endosome releases the ferric ion of transferrin which can be incorporated into some proteins or stored as ferritin. While the transferrin-receptor complex is recycled to the cell surface. The ferric ion, in addition to being released from transferrin, can also be mobilized from ferritin, forming the pool of iron not bound to the proteins necessary for oxidative damage and which can be removed from circulation by ferric ion chelators, such as desferroxamine. The ferric ion reacts with superoxide.[53]

$Fe^{3+} + O_2^{\cdot -} \rightarrow Fe^{2+} + O_2$

These two reactions together are known as the Haber-Weiss reaction. The net result of both of them is thus represented:

$O_2^{\cdot -} + H_2O_2 \rightarrow \cdot OH + OH^- + O_2$

All reactions that generate $O_2^{\cdot -}$ due to the reaction of SOD also form H_20_2. Having continuous flow of $O_2^{\cdot -}$ it can react with hydrogen peroxide to generate ·OH is why the action of antioxidants is so important.[53]

ANTIOXIDANT MECHANISMS: ENZYMES AND VITAMINS

The primary antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT) present in peroxisomes and glutathione peroxidase (GSH-Px). SOD catalyzes the enzymatic conversion of O2- into H202. CAT removes H202 (hydrogen peroxide). GSH-Px complements the activity of catalase in the metabolism of H202, being predominantly located in the cytoplasm. This enzyme has two forms, one that requires selenium for its activity and uses hydrogen peroxide as its substrate, and the other that does not require selenium and catalyzes the degradation of organic peroxides, especially lipoperoxides. The reduction of these peroxides is coupled with the

oxidation of reduced glutathione (GSH), generating oxidized glutathione (GSSG). The mechanism of regeneration of GSH from GSSG is carried out by the action of the enzyme glutathione reductase required for its activity by the NADPH coenzyme. NADPH is supplied by glucose metabolism through the pentose cycle.[53]

Reduced glutathione is also important for maintaining a pool of reduced ascorbic acid (vitamin C) used to suppress free radicals. Peroxides can also be eliminated, although to a lesser degree, by the action of the enzyme glutathione S-transferase, since glutathione conjugated compounds are metabolically inactive and are excreted. When there is a deficit of GSH-Px the activity of glutathione-S-transferase increases as a possible compensatory mechanism.[53]
Vitamin E is one of the most important antioxidants, especially the alpha-tocopherol form, present in cell membranes and in LDL (low-density lipoprotein). Its importance lies in the fact that it is capable of preventing the peroxidation of polyunsaturated fatty acids by the presence in its structure of a group -OH (alpha-tocopherol-OH) whose hydrogen is easily separable from the molecule.

During peroxidation, peroxyl and alkoxyl radicals are generated, which are preferably combined with alpha-tocopherol rather than with the adjacent fatty acid, ending the chain reaction. The alpha-tocopherol-O- (radical tocopherol) that is formed is very little reactive, being unable to attack the lateral chains of the adjacent fatty acids. It can migrate to the membrane surface and be converted back to alpha-tocopherol by a reaction with ascorbic acid. It is likely that reduced glutathione is also involved in the regeneration of alpha-tocopherol from its radical. On the other hand, vitamin C is a good eliminator of oxidants such as H_2O_2, $O_2^{\cdot-}$ and ·OH. It is a water-soluble vitamin that is found in cytosol and extracellular fluids where it is oxidized by various oxidants to dehydroascorbate that protects lipidic particles and membranes from potential oxidation. Dehydroascorbate is again reduced to ascorbate in a reaction involving reduced glutathione.[53] In addition, the reduced form of coenzyme Q10, ubiquinol, is very effective as a lipidic peroxyl radical remover and can also function as a regenerator of oxidized vitamin E.[54] Uric acid can also eliminate free radicals, being a stabilizer of ascorbate. Glucose and pyruvate can also eliminate ·OH radicals, and according to some studies, NADPH and carnitine can also reduce oxidative stress.[53] In this way, a decrease in vitamin C, vitamin E, Q10 and serum carnitine could be a marker of marked oxidative stress. In fact, in HIV, a decrease in carnitine levels[55] and a possible therapeutic pathway has been visualized associating retrovirals with carnitine, obtaining better CD4 counts.

ROLE OF CYSTEINE AND GLUTAMINE

Low levels of cysteine and glutamine have been found in patients with sepsis, major surgery, liver cancer, Crohn's disease, ulcerative colitis, and chronic fatigue syndrome. Both in sepsis, major surgery, HIV... you can see a high production of urea with negative nitrogen balance, low levels of cysteine and glutamine, with elevated levels of glutamate and loss of skeletal muscle mass. This is also true for high-performance athletes. Studies show abnormally low levels of glutamine, high levels of urea production, impaired immune function, and an increased incidence of opportunistic infections in high-performance athletes.[56]

While weight loss in starvation affects virtually all organs including the heart, spleen and liver, it has been shown that cachexia present in cancer and sepsis mainly affects skeletal muscle tissue to save heart, spleen and liver.

Due to the high consumption of glucose in the Warburg cells, blood glucose is reduced. This results in a stimulus for glucagon and the liver begins to perform glucogenolysis and gluconeogenesis from lactate, amino acids such as alanine and glutamine present in the muscles and from glycerol, resulting from the triglycerides of the adipose tissue.

The triglycerides in adipose tissue are degraded by lipase to fatty acids and glycerol. The fatty acids have to be transported by the albumin and the glycerol goes to the liver to perform glyconeogenesis. Glycerol is phosphorylized and oxidized to dihydroxyacetone phosphate and is isomerized to glyceraldehyde 3 phosphate, an intermediate in the gluconeogenic pathway. The fatty acid in the cytosol is activated by acyl-CoA synthetase + ATP and forms acyl-CoA and AMP. Small fatty acids can pass directly into the mitochondria, but long ones need carnitine. The acyl group is transferred to the carnitine formane acilcarnitine (catalyzed by carnitine acyltrasphase I) which is in the mitochondrial outer membrane. Acylcarnitine transfers the acyl to a coA in a reaction catalyzed by Carnitine Acyltransferase II and the translocasa returns the carnitine to the cytosolic by exchanging it for another acyl-Carnitine. Beta-oxidation occurs in the mitochondria and this ATP is used for gluconeogenesis.

Muscle can degrade proteins for fuel. The NH4 groups from their degradation are transformed into glutamate by aminotransferases. Glutamate transfers its amino group to pyruvate from glycolysis to form alanine and restore alpha-ketoglutarate through alanine aminotransferase. The formation of alanine serves to transport non-toxic amino groups in the blood to the liver, this is the so-called glucose-alanine cycle.

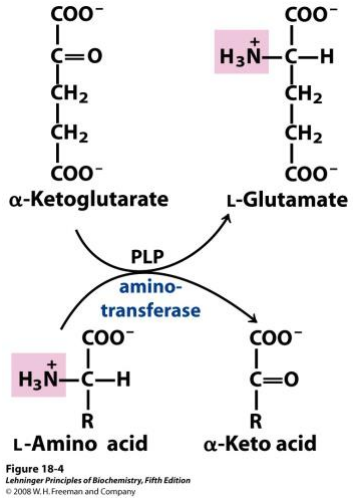

Figure 4: Enzymatically catalysed transamination. In the reactions of many aminotransferases alpha-ketoglutarate is the acceptor of the amino group.[51]

Another option for the transport of amino groups would be the formation of glutamine from glutamate by glutamine synthetase. Both glutamine (this glutamine part could be captured by infected or cancerous cells to replace the intermediates of their Krebs cycle) and alanine pass into the bloodstream until they are captured by the liver. Once alanine enters the liver, it passes its amino group to an alpha-ketoglutarate through alanine aminotransferase to form pyruvate and glutamate. This pyruvate in the liver is used to produce glucose (gluconeogenesis).

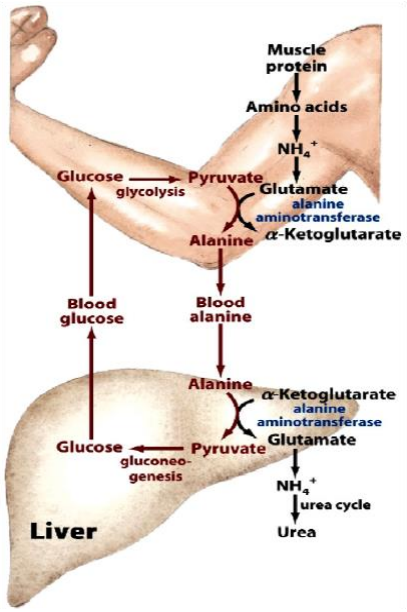

Figure 5: Glucose-alanine cycle. Alanine acts as a carrier of ammonia and the carbonate skeleton of pyruvate from skeletal muscle to the liver. Ammonia is excreted and pyruvate is used to produce glucose that returns to the muscle. [51]

In mitochondria, the enzyme glutamate dehydrogenase releases ammonia and alpha ketoglutarate (used in the citric acid/ gluconeogenesis cycle) from glutamate. Ammonia would pass into the urea cycle

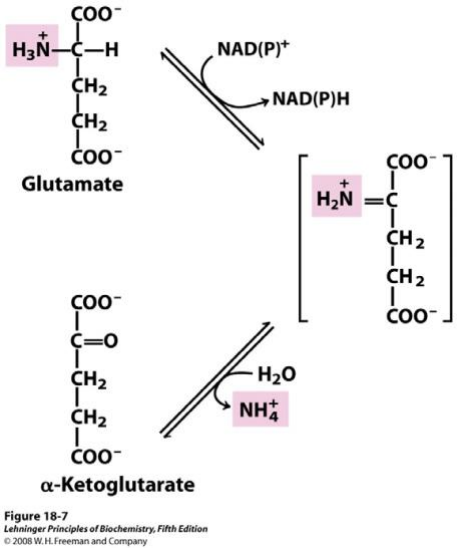

Figure 6: Reaction catalyzes by glutamate dehydrogenase. [51]

Glutamine would be degraded to glutamate by glutaminase in the mitochondria of the hepatocyte, releasing ammonia that would go into the urea cycle as well.

This may explain why in CFS patients with high severity they have high levels of glutamate and urea production along with low levels of glutamine and destruction of muscle mass.

The decrease in cysteine levels could be justified by the high oxidative stress of the infected cells, as this amino acid is the speed limiter for glutathione generation. Since the production of ROS is increased in cells with warburg effect, the formation of glutathione is constantly required. A specific transporter (Xc) is needed to introduce from the plasma to the cell.[57,58] Taking into

account the low cystine content inside the cells, the physiological direction of this exchange consists in the release of glutamate to favour the entry of cystine, which is rapidly reduced to cysteine. This increase in serum glutamate could be reused in the liver by exchanging it again for cysteine and using it for the formation of NAG (n-acetyl glutamate) from glutamate and coA by NAG synthase. NAG is an essential cofactor for the activation of carbamoyl phosphate synthase and therefore of hepatic ureagenesis. At the same time, the infected cells capture glutamine to restore the glutamate lost in the exchange.

KETONE BODIES

Acetyl-CoA formed in the oxidation of fatty acids only enters the citric acid cycle if the degradation of fats and carbohydrates is properly balanced. Entry into the acetyl-CoA cycle depends on the availability of oxalacetate, which will be decreased if there are no carbohydrates or if these are not used properly (if there is not enough pyruvate generated by glycolysis, oxalacetate cannot be generated by pyruvate carboxylase). In situations of starvation or diabetes, oxalacetate is consumed in glucose formation (gluconeogenesis) and is therefore not available for condensation with acetyl-CoA. Under these conditions, excess acetyl-CoA is diverted to form acetate and D-3-hydroxybutyrate. Acetate and 3-hydroxybutyrate are normal fuels in aerobic metabolism. The brain adapts to the use of acetate as a fuel in fasting or diabetes conditions. In prolonged fasting, acetate can provide up to 75% of the energy needs of the brain.

During prolonged fasting or in this case by the consumption of glucose by infected cells, a change in the use of fuels occurs. Tissues use less glucose than during a short fast and use predominantly fuels derived from the metabolization of fat tissue GADs (i.e. fatty acids and ketones). As a result, blood sugar does not drop dramatically and CFS patients develop cachexia with loss of muscle mass and fat.

EBV ALTERS THE SEROTONINERGIC SYSTEM OF THE INTESTINAL TRACT

Serotonin plays an important role in digestive regulation. We know that 95% of the body's total serotonin is generated in the enterochromophin (EC) cells of the intestinal epithelium. It is secreted from both intestinal light and interstitial fluid in response to mechanical and chemical stimuli, such as increased intraluminal pressure, changes in osmolarity and changes in luminal acidity. These changes are controlled by a number of receptors, which may be stimulants such as b-adrenergic, muscarinic, nicotinic, and 5-HT3, or inhibitors such as alpha2-adrenergic, gamma-aminobutyrate, H3 histaminergic, VIP-receptor, somastostatin, and 5-HT4.[59]

Enterocytes are responsible for serotonin reuptake by serotonin transporter expression (SERT). The metabolization of serotonin occurs at the intracellular level, mainly due to the activity of the enzyme monoamine oxidase (MAO) which produces an oxidative desamination, giving rise to the formation of an intermediate product, 5-hydroxy-indolacetaldehyde, which is subsequently oxidized by an aldehyde dehydrogenase to form 5-hydroxy-indolacetic acid (5-HIAA). When this system is saturated, the intermediate product is reduced in the liver, producing 5-hydroxytryptofol. In the digestive system, serotonin can also be catabolized by glucoronyl transferase and other intracellular enzymes, as can MAO and aldehyde dehydrogenase.[59]

The intestinal mucous membrane itself is rich in mast cells. Murine mast cells are capable of synthesizing and releasing 5-HT, but under normal conditions mast cells in the intestinal mucosa of humans do not appear to synthesize 5-HT. On the other hand, there is evidence that human enteric mast cells express TPH (tryptophan hydroxylase). Human mast cells may synthesize and release 5-HT under pathological conditions, contributing to conditions such as hypersensitivity.[60]

Not all cells containing serotonin synthesize it. Such is the case of platelets, which accumulate 8% of the total, capturing it from plasma through the serotonin transporter (SERT), a process in which intracellular calcium appears to play a regulatory role.59

Platelets that capture serotonin that has not been collected by epithelial cells at the intestinal level can promote haemostasis, influence bone development and contribute to allergic inflammation of the airways.[60] This accumulation of serotonin in platelets may increase their volume, as there is an increase in the average volume of platelets in patients with panic disorder due to the storage of serotonin in them.[61] Although we will discuss the behaviour of platelets during the pathology we describe later on.

There is evidence of alteration of the intestinal serotoninergic system and gastrointestinal dysfunction in pathologies such as Irritable Bowel Syndrome and the disease chronic inflammatory bowel disease[62]. It has also been observed that Irritable Bowel Syndrome (IBS) with predominance of diarrhea, causes an increase in serotonin concentration, possibly due to alteration in serotonin transporter functionality.[63,59]

On the other hand, extensive evidence suggests that serotonin plays a key role in the chronic intestinal inflammation of patients with Crohn's disease, ulcerative colitis or a history of diverticulitis. Thus, these processes have been associated with an increase in the number of enterochromophin[64,65] cells and a decrease in the transcription of the serotonin transporter gene, both of which have caused an increase in the availability of 5-HT in the intestinal mucosa. [59,65,66]

The pathophysiological consequences of excessive 5-HT release, including cholera toxin-induced diarrhea [67,68], nausea and vomiting, are known in the intestine. In fact, high serotonin levels in Irritable Bowel Syndrome have also been linked to a predominance of diarrhea. One of the causes of such an increase in serotonin concentration is the alteration in the functionality of the conveyor.[59,63]

Therefore, circulating serotonin is often studied in gastrointestinal disorders as a reflection of the availability of 5-HT in the mucosa.[60] Post-meal 5-HT levels are elevated in platelet-poor plasma samples obtained from patients with IBS-D (Irritable Bowel Syndrome with predominance of diarrhea)[69,70] or post-infectious IBS,[70] but 5-HT levels have been reported to be reduced[70] or unchanged[71] in IBS-C (with constipation). In contrast, 5-HT levels in platelets are reduced in IBS-D [72], but doubled compared to healthy controls in patients with IBS-C.[71] Together, these results are consistent in the existence of a decrease in the uptake of 5-HT by the intestinal epithelium in both forms of IBS, as it appears that more 5-HT is ending up in circulation after food intake (especially carbohydrates). The ability to detect elevated postprandial levels of 5-HT in platelet-poor plasma in IBS-D but not IBS-C may reflect differences in SERT function in platelets in these disorders. The uptake of 5-HT by platelets appears to be altered in individuals with IBS-D. [72,73,74] This may explain the elevated postprandial 5-HT levels in platelet-poor plasma samples from patients with IBS-D, since both platelets and enterocytes express TLR3.[75,76] This receptor, when activated by viral infections such as EBV, causes a decrease in SERT activity, thus decreasing serotonin reuptake by both cells.[73]

In chronic constipation, the expression of SERT is not altered [77], but the content of 5-HT, the number of enterochromophin cells (EC), and the release of 5-HT are increased.[77, 78]

The intestinal epithelium, in addition to developing digestive and absorptive activity, constitutes an anatomical and immunological barrier between the light and the internal intestinal compartment. This epithelium is in close contact with a wide variety of commensal microorganisms and intake digestion products. Both pose a threat to the immune stability of the intestine.[59]

There are three mechanisms involved in the stability of the epithelium in its role as an intestinal barrier:[59]

1. The innate immune system, which is the first line of defense against resident intestinal microflora or luminous invading pathogens. TLR receptors are located in mononuclear, dendritic, macrophage and different epithelial cells and are an important mechanism in the innate immunity of the intestinal epithelium.
2. The transepithelial barrier in which the epithelium of the mucosa is the first line of physical defence against luminous aggressions. The integrity of this epithelial barrier is maintained by adhesion and intercellular bonding molecules that allow epithelial polarity to be established.
3. The adaptive intestinal immune system that is triggered when the balance between antigenic or pathogen-type stimulation and innate intestinal immune activity is broken.

The alteration of any of these three systems, i.e. an inappropriate regulation of the innate immune system, an increase in epithelial permeability or a defective regulation of the adaptive immune system, poses a risk for developing an inflammatory pathology in the intestinal mucosa.[59]

They showed that the extracellular increase of factors released in inflammation inhibits the activity of the serotonin transporter. Because the transporter internalizes serotonin, the results indicated that these same inflammatory factors increase the extracellular availability of serotonin, which may attempt to alleviate or contribute to the inflammatory situation.[59]

They concluded that by activating various toll receptors, the activity of the serotonin transporter was reduced. They observed that TLR3 was activated by viral double-stranded RNA, TLR4 by lipopolysaccharide from gram-negative bacteria, and TLR5 was activated by the flagellin protein from flagellated microorganisms present in the intestine such as Salmonella and Escherichia Coli.[59] EBV would activate TLR3 producing an increase in mucosal inflammation leading to an increase in the permeability of gram-negative bacteria that would also activate TLR4.
Therefore, the reduction of serotonin transporter activity and expression in the intestinal mucosa leads to an increase in the availability of 5-HT locally.[59,60]

As with EBV infection of HBMEC, infection of the epithelial cells of the intestinal mucosa with EBV leads to the rupture of the narrow junctions of the intestinal barrier, leading to the passage of bacteria and other substances. At the same time, EBV is able to infect plasma cells in the mucosa. Therefore, B cells with latent EBV infection release EBERs. The release of EBER1 from EBV-infected cells was shown to induce activation of TLR3 signaling, resulting in induction of type I IFNs and proinflammatory cytokines. Circulating EBER1 can induce dendritic cell activation and subsequent T cell activation, leading to systemic production of proinflammatory cytokines. Since CD8+ T cells and NK cells express TLR3, they would be activated by these signals from EBER1.[31] But EBV miRNAs would in turn inhibit CD8 lymphocyte response and CD4 lymphocyte activation, so there would only be an increase in proinflammatory cytokines without immune recognition of infected cells.

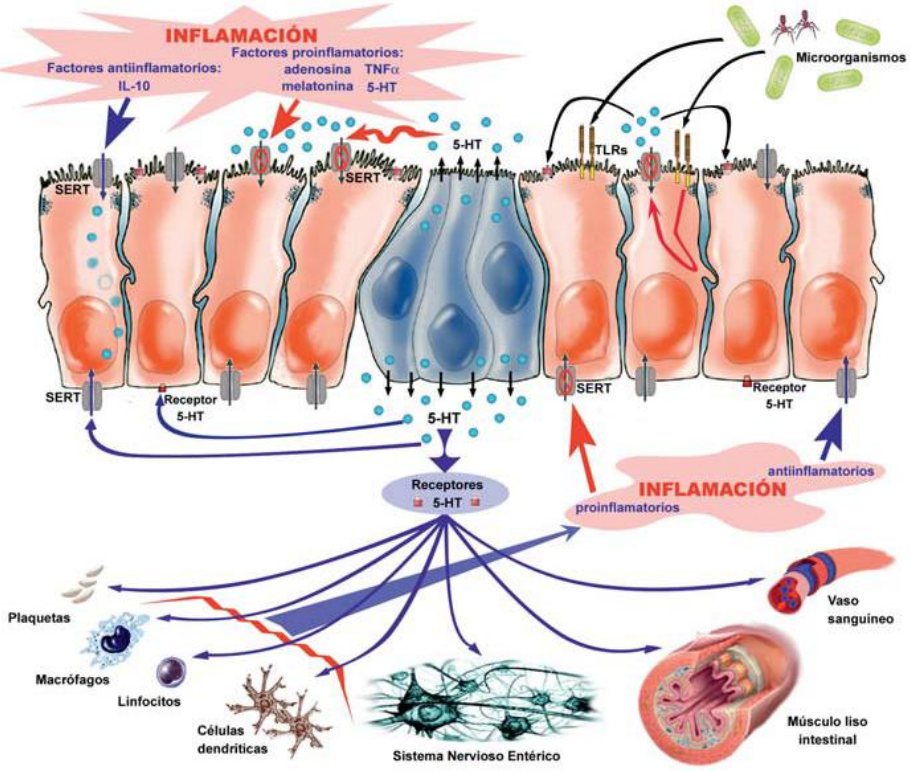

Figure 7: Scheme of the involvement of the intestinal serotoninergic system in inflammatory bowel pathologies. 5-HT released by enterochromophinic cells can act on a wide variety of tissues and cells that express some subtype of receptor for 5-HT. Among them are macrophages, dendritic cells and lymphocytes, which will activate the production of pro-inflammatory cytokines, affecting the immune system to activate an inflammatory process. SERT, as a regulator of extracellular 5-HT availability, is negatively regulated by the activation of different TLRs and various proinflammatory cytokines, while anti-inflammatory cytokines, such as IL-10, will increase its activity. In this way, the extracellular levels of 5-HT may increase or decrease during a process depending on the activated intermediate systems, thus making SERT essential in the initiation, development and maintenance of the inflammatory process. [79]

CONSEQUENCES OF EXTRACELLULAR SEROTONIN EXCESS

We will first focus on the effect of serotonin on the gastrointestinal system, as this is where it accumulates as a result of EBV infection. And then how the extracellular excess of serotonin, due to intestinal pathology, would affect the rest of the body.

At the peripheral level 5-HT participates in physiological processes including: hemostasis, regulation of the cardiovascular system, control of motility, secretion and intestinal epithelial absorption.

ACTIVATION OF SEROTONIN RECEPTORS

This increase in serotonin in the gastrointestinal tract causes relevant physiological responses due to the presence of various subtypes of receptors in various classes of myenteric neurons, smooth muscle cells and epithelial cells.[59]

The serotonin receptor subtypes 5-HT1, 5-HT2, 5-HT3, 5-HT4, and 5-HT7 are known to affect intestinal motor function. The 5-HT1A receptor is expressed in neurons of the myenteric plexus, submucosal and enterochromophin cells. It produces a rapid decrease in the amplitude of the

excitatory post-synaptic potentials. On the other hand, for 5-HT1B, 5-HT1D and 5-HT2A it has been shown to stimulate or inhibit smooth muscle contraction depending on the portion of the digestive tract and the species in question.
However, this type of receptor can perform other functions in humans such as mediating the contraction of the longitudinal smooth muscle. In addition, 5-HT2 receptors appear to be involved in regulating intestinal absorption of nutrients.[59] In addition, serotonin is known to be pro-inflammatory, acting through 5-HT2A receptors to increase extravasation of T-lymphocytes and eosinophils, and may also activate mast cells.[73]

The 5-HT3 and 5-HT4 receptors, when stimulated both centrally and peripherally, promote vomiting, gastric emptying, electrolyte secretion, serotonin secretion in enterochromophin cells, smooth muscle contraction and/or relaxation, and modulate the absorption of amino acids at the intestinal level.[59] The 5-HT3 receptor appears to be intimately involved in the signaling of the intestinal-brain system, particularly through the afferent (sensory) pathway of the vagus nerve. It is also involved in visceral hypersensitivity.[80]

With respect to the 5-HT7 receptor, its activation produces relaxation in the ileum and colon and it has also been postulated that its overstimulation may lead to exaggerated accommodation of the colon's circular smooth muscle with increased volume, a common symptom in many functional bowel disorders.[59]

Recent results have shown that both 5-HT1A and 5-HT7 are expressed in intestinal epithelial cells and modulate serotonin transporter activity.59

GASTROINTESTINAL TRACT

Serotonin secretion occurs both to the innermost portions of the wall of the intestinal tract (the lining of the intestinal tract) and to the intestinal lumen.[59]

If the serotonin secretion is in the direction of the own lamina, it may bind to receptors in the axons of the primary intrinsic submucosal afferent neurons that innervate the secretorial epithelium and are responsible for initiating the secretor and peristaltic reflexes, and/or it may bind to its receptors in the myenteric primary afferent neurons and participate in the regulation of peristalsis through the initiation of migratory contractions. In addition, serotonin has also been shown to regulate the activity of Cajal interstitial cells, with the corresponding regulation of smooth muscle electrical activity.[59]

On the other hand, extrinsic afferent neurons are activated directly by the serotonin released from the enterochromophin cells and indirectly by the intrinsic primary afferent neurons, bringing information from the intestinal tract to the central nervous system through the vagus nerve.59 That is, parasympathetic innervation in the stomach, small intestine and proximal colon is provided by the vagus nerve. A mixed nerve, containing both sensory and motor fibers. It contains approximately 70-80% of sensory fibers that transduce physiological events in the GI tract and transmit them to the CNS.80 This information is related to sensitivity to light content. The presence of carbohydrates and hyperosmotic stimuli strongly induce the release of 5-HT.[59]

The release of EBER by the plasma cells containing latent EBV at the level of the intestinal mucosa activates TLR3 causing a decrease in the activity of the serotonin transporter, which leads to an accumulation of this nerurotransmitter. As described above the presence of carbohydrates in the lumen would further increase their secretion and consequently their accumulation along with increased symptoms.

Also, the vagus nerve mediates inhibition of gastric emptying and duodenal motor responses, so it is related to nausea and vomiting. As well as the conduction of signals that lead to perceptions

of discomfort and pain in the gastrointestinal tract, a process carried out by spinal afferent pathway.[59]

In the intestine, serotonin has been shown to regulate the proliferation of epithelial cells and inhibit the intestinal absorption of monosaccharides and amino acids.[59]

At the stomach level, there are also 5-HT receptors where an increase in serotonin inhibits acid secretion. It decreases the release of gastrin by G cells and HCL (hydrochloric acid) as a consequence.[81,82]

In inflammatory bowel disorders, gastric hyposecretion with hypergastrinemia due to atrophic type A gastritis may also occur by autoimmune destruction of the parietal cells by autoantibodies.[83]

In both cases there is gastric hyposecretion which is one of the causes of bacterial overgrowth of the small intestine (SIBO).[84,85] And if we add changes in intestinal motility together with immunological alterations due to EBV infection, the probability of suffering from SIBO will increase even more.

Serotoninergic system disruption also occurs in patients with autism, who have been observed to have elevated serotonin levels due to serotonin transporter disruption (SERT).[86]

Autistic patients, as in the digestive pathologies described in this paper, have altered the lining of the intestinal mucosa with a decrease in the activity of digestive enzymes of carbohydrates and large proteins such as gluten, gliadin and casein leading to a malabsorption of these, which can cause inflammation and are believed to act as neuropeptides. Therefore, peptides derived from gluten and casein are not converted into amino acids. The increased intestinal permeability then allows these peptides to filter into the bloodstream, where they circulate and eventually cross the blood-brain barrier, which could lead to inflammation and impaired neurological functions. Neuropeptides have adverse effects on attention, brain maturation, social interactions and learning in these patients.[86,87]

Lau et al. conducted a study aimed at assessing gluten immune reactivity in pediatric patients diagnosed with autism with respect to healthy controls of the same age. Children with autism had significantly higher levels of IgG antibodies to gliadin compared to healthy controls, but did not reach statistical significance. There was no difference in IgA response to gliadin in all groups. The levels of serological markers specific to celiac disease, i.e. antibodies against gliadin desamidated and TG2, did not differ between patients and controls. No association was observed between increased anti-gliadin antibody and the presence of HLA-DQ2 and/or -DQ8. In conclusion, a subset of children with autism showed increased immune reactivity to gluten, the mechanism of which appears to be different from that of celiac disease. The increased anti-gliadin antibody response and its association with gastrointestinal symptoms points to a potential mechanism involving alterations in immune and/or intestinal permeability in affected children.88 This increased intestinal permeability resulting from damage to the intestinal epithelial barrier in people with autism may be responsible for increased exposure of the immune system to partially digested gluten fragments, resulting in a detected increase in the antibody response.8
Another study showed that specific elevated IgG-specific casein and gliadin titers were more common in patients with regular diet autism spectrum disorder (ASD) than in controls with casein- and gluten-free diet. These findings confirm previous articles on increased reactivity to milk proteins (casein). An increase in specific gliadin IgG titers was found in autistic patients that could be partly explained by the significant increase in AGA-IgG (IgG antigliadin antibodies) and IgG-IgG DPG (antibodies to the unloved gliadin Ig G peptide). In addition, the fact that the AGA-IgA and DPG-IgA titers were similar in ASD and controls indicates that the immune response of the mucosal surface was possibly not involved. Therefore, it is not considered celiac

disease because these IgA are not elevated but if there is a reaction against these proteins due to high AGA-IgG titres due to increased intestinal permeability.[89]

Due to damage to the intestinal mucosa, the absorptive capacity and/or expression of lactase is reduced, in addition to a possible significant increase in jejunal transit. We're looking at a hypolactásia secondary to EBV infection. When the activity of lactase is reduced, the lactose reaches the colon without hydrolysis, where it is fermented by the intestinal flora with the consequent production of short chain fatty acids (SCFA) and gas, mainly hydrogen (H2), carbon dioxide (CO2) and methane (CH4). Thus, undigested lactose accessing the large intestine can lead to osmotic diarrhea and the products of its bacterial digestion to secretory diarrhea and gas. But this type of intolerance, which also occurs in any small bowel disease such as celiac disease, Crohn's... is reversible as long as the mucosa is repaired (the same is true of intolerance to fructose in the presence of enteropathy).[90]

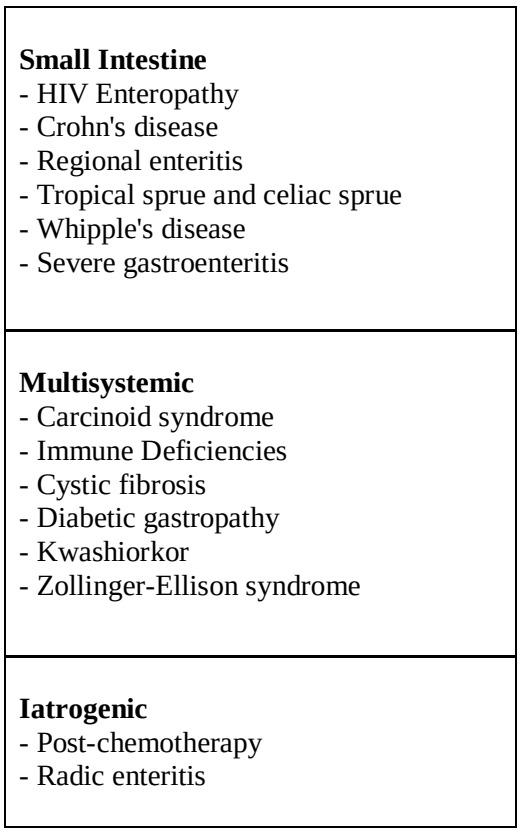

Figure 8: Causes of secondary hypolactasis.[90]

Bacterial fermentation in the colon of unabsorbed sugars such as fructose and/or sorbitol generates short-chain fatty acids (acetate, propionate, and butyrate), gases (hydrogen, carbon dioxide, and methane), and an osmotic charge in the intestinal lumen. Like the decrease in lactase, from inflammation caused by the infection (this would also be a secondary malabsorption). This type of malabsorption is not genetically coded and is due to the presence of an intestinal disease that temporarily damages the intestinal mucosa, although it can also be permanent. It is common in gastroenteritis, bacterial overgrowth, inflammatory bowel disease, radiation enteritis, and celiac disease.[90]

Carbohydrate malabsorption develops as a result of premature breakdown of sugars by bacteria along with decreased disaccharidase activity, secondary to interruption of the intestinal brush rim.[84]

The passage of undigested and properly absorbed food becomes a substrate for bacterial fermentation allowing overgrowth to occur along the small intestine. In the fructose and lactose intolerance tests, the levels of methane and hydrogen in breath are measured after the oral introduction of solutions with lactose or fructose. The point is that many digestives think that the positive results of these tests are not because of these intolerances but because of the presence of SIBO, since the metabolism of carbohydrates in the small intestine, in the presence of colonic bacteria, leads to changes in the concentrations of hydrogen and methane from fermentation. But the fact that this overgrowth is already indicating that there is a problem with the digestion/absorption of carbohydrates. So, if they are positive to these tests they will indicate malabsorptive problems (intolerances) and SIBO.

Due to inflammation, increased motility and malabsorption, patients are at risk for various deficiencies, especially of vitamins A, D, E, B12 and iron. (As is also the case in patients with CFS). They are also associated with weight loss and chronic diarrhoea.[84]

Fat malabsorption occurs as a result of bacterial deconjugation of bile salts. In addition, free bile acids are toxic to the intestinal mucosa, causing inflammation of the mucosa and malabsorption. The deconjugated bile salts are reabsorbed in the jejunum instead of the ileum, leading to altered micella formation, fat malabsorption, and fat-soluble vitamin deficiencies (A, D, E, and K). Fortunately, symptoms rarely develop; however, in severe cases, night blindness (vitamin A), osteomalacia and tetany due to hypocalcemia (vitamin D), prolonged prothrombin times (vitamin K), or neuropathy, retinopathy, and impaired T cell function may occur. [84]

A complication of bacterial overgrowth is a deficiency of cobalamin (vitamin B12). Patients with normal intestinal flora use gastric intrinsic factor to bind to vitamin B12 which allows its absorption into the ileum. An animal model of SIBO demonstrated that there is a competitive uptake of vitamin B12 by bacteria (especially aerobes). Human subjects with atrophic gastritis and bacterial overgrowth absorb significantly less B12 bound to the protein compared to controls, although this was reversed with antibiotic therapy. Folate levels may be normal, but are often elevated due to increased folic acid synthesis by bacteria in the small intestine.[84]

Another test that suggests poor digestion is the positivity of undigested food remains in feces.

CARDIOVASCULAR SYSTEM

As mentioned above, there is communication between the intestinal tract and the CNS through the vagus nerve. Activation of the 5-HT3 receptors in these afferent vagal endings is associated with the Bezold-Jarisch reflex causing hypotension and bradycardia. When these fibers are activated, an abnormal or paradoxical autonomic response is produced, resulting in vasodilatation (through a decrease in sympathetic efference) and increased vagal tone, with subsequent reduction in cardiac filling and bradycardia, which can eventually lead to vasovagal syncope. This coincides with the fatigue and bradycardia that sometimes occurs in patients with CFS.91 Bradycardia and hypotension can lead to blurred vision due to syncope (inadequate cerebral blood flow), especially in the erect position (orthostatic hypotension).

LOCOMOTOR SYSTEM

Platelets examine blood vessels for endothelial damage and prevent loss of vascular integrity. However, there are circumstances in which vascular permeability is increased, suggesting that platelets sometimes fail to perform their expected function. Inflammatory arthritis is associated with tissue edema attributed to increased permeability of synovial microvasculature. Murine models have suggested that such vascular leakage facilitates the entry of autoantibodies and therefore may promote joint inflammation.[92]

Using an autoimmune arthritis model, the absence of platelets was found to decrease the patency of inflamed joints. This effect was mediated by platelet serotonin accumulating through the serotonin transporter.[92]

Platelets become the main cause of vascular permeability in arthritis, through the release of serotonin, which can promote the development of the disease. The critical role of platelets in vascular leakage may not be unique to arthritis. In models of acid-induced lung inflammation or abdominal sepsis, platelet depletion prevents inflammation and vascular loss, indicating a potential role of platelets in vascular patency during inflammation in the lung.[92]

It is important to note that they identified serotonin as a platelet-derived mediator capable of initiating the formation of voids in the vasculature during inflammation, compatible with the extravasation of MPs (platelet microparticles). However, holes formed in the joint vasculature during autoimmune arthritis can be harmful to the joint. Like platelet PM of platelets rich in IL-1, immune complexes, such as the etiologic agent in RA (rheumatoid arthritis), are also submicron in size, with diameters ranging from 0.1 m to approximately 1 m. It is therefore plausible that platelet gaps contribute to joint invasion by both immunocomplexes and PMs, which supports a double contribution to joint inflammation.[92]

Also indicate that absorption of serotonin by platelets is a prerequisite for permeability. Platelets get mainly 5-HT from the intestine, so the excess serotonin generated by the decreased activity of SERT from EBV infection in the intestinal mucosa should be collected and transported by the platelets. But the platelets do not get to collect all this excess because they also express TLR375,76 as enterocytes and when these receptors are activated by the infection, the reuptake of serotonin by these cells would decrease[73]. This allows for a build-up of serotonin in the intestinal mucosa.

Platelets contain different types of granules, mainly dense/delta granules, α granules and lysosomes. The activated platelets excrete the content of these granules.[93] Dense granules contain serotonin, so that when the platelets are activated via TLR3 they would be released. This released neurotransmitter would increase vascular permeability and may promote inflammation in joints[92] or other tissues where EBV-infected cells are present.

It would be logical to think that since the activity of the serotonin transporter (SERT) and its expression in the intestinal mucosa due to the viral infection is decreased, it would lead to an increase in the availability of 5-HT not only at local level but also at circulatory level. But in a study of mice modified not to express SERT, no 5-HT was found in blood. This indicates that these animals effectively prevent enteric 5-HT from reaching circulation by other means.[94] The absence of 5-HT plasma in SERT mice - / - indicates that alternative transporters expressed in the intestine and liver should completely remove 5-HT from portal blood (free 5-HT is absorbed into the portal vein and metabolized in the liver) and prevent it from reaching systemic circulation. But SERT mice stools - / - contained more water than those of their SERT littermates + / +, i.e. SERT mice -/- had watery diarrhea due to the accumulation of serotonin in the intestinal tract.[94] Add that SERT mouse platelets - / - are not loaded with 5-HT as they circulate through the intestine.[94] This is similarly true in patients with IBS-D and CFS. The decrease in SERT activity generated by EBV infection by activating TLR3 in platelets and enterocytes leads to a decrease in serotonin uptake and therefore an increase in extracellular serotonin at the intestinal level. Under fasting conditions, both patients with IBS-D[69] and CFS have the same plasma 5-HT levels as healthy patients. This occurs through alternative transport that removes free serotonin from the portal blood.[94] But in postprandial conditions by further stimulating the release of serotonin, transport systems are saturated, thus increasing the levels of free 5-HT in plasma, as has been seen in patients with IBS-D, along with an increase in plasma levels of their 5-HIAA metabolite compared to healthy subjects.[69] Hence, these patients' symptoms increase after meals.

The serotoninergic and 5-HT compounds stimulate the secretion of PRL (prolactin) from the anterior pituitary gland. With 5-HT3 receptors involved in both baseline and stress-induced PRL response regulation.[95]

The serotonergic neurons of the MRN (medial nucleus of the raphe) and DRN (nucleus of the dorsal raphe) project into the PVN (hypothalamic paraventricular nucleus) where they are in close contact with the CRH (corticotropin-releasing hormone) neurons. These three nuclei are important for 5-HT-mediated responses but are not essential, as injury to 5-HT neurons in NRP or NRD reduced but did not inhibit ACTH's response to stress.[95]
The serotoninergic system stimulates the HPA axis (hypothalamic-pituitary-adrenal axis) at both the hypothalamic and pituitary gland levels with increased levels of CRH mRNA in the PVN, POMC mRNA (proopiomelanocortin) in the anterior pituitary lobe, CRH in pituitary plasma, ACTH and corticosterone in plasma. Serotonin stimulates the secretion of ACTH in vitro from the anterior pituitary gland. The effect of 5-HT is mediated mainly through the 5-HT1A, 5-HT2A and 5-HT2C receptors, but the 5-HT3 receptor does not appear to be involved in serotonergic regulation of the HPA axis.[95]

In another study, serotonin injections in rats showed decreased secretion of LH (luteinizing hormone).[96] Serotonin was also found to be involved in the regulation of postmenopausal LH to a greater extent and to a lesser extent in the secretion of FSH (follicle-stimulating hormone.[97]

The neurohypophysiological system (vasopressin and oxytocin)

5-HT releases AVP (vasopressin) into extracellular tissue in the PVN. Peripheral AVP secretion mainly involves 5-HT2C, 5-HT4 and 5-HT7 receptors. OT (oxytocin) secretion is mainly mediated by 5-HT1A, 5-HT2C and 5-HT4 receptors and probably also 5-HT1B, 5-HT2A, 5-HT5A and 5-HT7 receptors. [95]

It can be concluded that 5-HT is involved in the basal and stress regulation of PRL, ACTH, AVP and oxytocin by inducing their increase through the 5-HT2A + 2C receptors mainly, but other receptors are also important, which differ between these hormones.

Temperature regulation

5-HT is an important neurotransmitter for thermoregulation through heat loss and heat production. The subtypes of 5-HT receptors participating in this regulation, especially 5-HT3 and 5-HT7, were discussed instead of 5-HT1A. In terms of the anatomical regions of the brain involved in this regulation, ATV (ventral tegmental area) or HMD (dorsomedial nucleus of the hypothalamus) are the main candidate areas in place of OP/HA.[98]

Selective 5HT1A receptor agonists produce a considerable hypothermic response. Activation of the 5-HT1A receiver was also shown to have effects on both heat loss and heat production. Not only 5-HT1A caused this, but also 5-HT3 and 5-HT7.[98]

Sleep regulation

Patients with autism have been observed to have elevated serotonin levels along with intestinal problems and insomnia. Serotonin being a precursor to melatonin would alter melatonin secretion. In animal models, it has been suggested that the level of serotonin in the brain controls sleep-wake behavior. An animal study showed that mice without 5-HT exhibit greater amounts of REM sleep than their wild counterparts. Mice without serotonin receptors also showed a significant increase in wakefulness and a reduction in slow-wave sleep. [86]

Subsequently it was observed that the variation in the SLC6A4 gene encoding the 5-HT transporter (SERT), especially the HTTLPR locus, has been associated with high blood serotonin levels and susceptibility to autism spectrum disorder. Therefore, due to the alteration of the serotonin transporter, serotonin is unable to enter the cells and perform its function, leading subsequently to a compensatory rise in the production of 5-HT. High levels of serotonin may work as an endogenous reaction to try to overcome the pathogenesis of autism. [86,99,100]

The consequences would be the same in CFS where low serotonin transporter activity, by activating TLR (would decrease 5-HT uptake), would lead to compensatory elevation. This, by influencing sleep-wake regulation, would cause insomnia.

Cognitive consequences

This disorder that we are describing would behave like patients with carcinoid syndrome since these types of tumors secrete this hormone.
Patients with CS (carcinoid syndrome) show greater cognitive difficulties than healthy controls in multiple domains, including measurements of visual scanning speed, verbal and visual memory measurements, visual perception and letter fluency. However, they showed no deficits in other measures of processing speed, semantic fluency, or their ability to use comments and alter responses. This study confirmed that patients with CS suffer from cognitive impairment.[101]

ALLERGIC CONSEQUENCES OF VIRAL INFECTIONS

Mast cells reside in tissues closely associated with blood vessels or on the surface of the body, particularly the skin and mucous membranes. Therefore, in addition to their roles as effector cells in IgE-mediated allergic diseases, mast cells are widely regarded as important innate immune cells.[102]

The roles of mast cells in immune surveillance and innate immunity against bacterial pathogens have been well defined. Innate host immune responses against various pathogens are usually initiated by the recognition of specific microbial components, such as lipopolysaccharides (LPS), lipoproteins, flagellins and nucleic acids, through pattern recognition receptors (PRRs). The recognition of microbial components by PRRs results in the coordinated activation of transcription factors, leading to the expression of inflammatory cytokines, chemokines and type I interferons (IFNs). They previously demonstrated that mast cells contribute to innate immune responses against invasive bacteria by expressing PRRs, such as TLRs-2,3,4,5,6,6,7 and 9, that respond to specific ligands by inducing the production of cytokines and chemokines or the release of cell granular content. Similarly, innate immune responses to viral infection begin with recognition of the virus by specific RRPs. As described above, viral nucleic acids are recognized by different types of sensors: TLRs, which detect double-stranded RNA (ds) or single-stranded RNA (ss) in the endosome; type I genetic receptors (RLRs) that can be reproduced in retinoic acid (RLRs), which recognize viral RNA in the cytoplasm; and DNA sensors, which detect cytoplasmic viral DNA.[102]

Human mast cells have previously been shown to express TLR3, a poly (I:C) and dsRNA receptor. Mast cell activation, including the specific production of antiviral cytokines such as interferon type I or the release of its granular content, has been observed at the time of stimulation with viruses, viral products or poly I:C. In addition, evidence has been accumulating regarding the role of mast cells in response to viral infections. Orinska et al. reported the functional consequences of mast cell activation in response to viral infection. They demonstrated that mast cells stimulated via TLR3 produced chemokines that mediated the recruitment of CD8+ T cells in vivo. In addition, TLR-induced activation of mast cells by LPS or poly I: C increased the ability of mast cells to activate CD8+ T cells. More recently, mast cells have been shown to play an important role in protecting the host from infection with herpes simplex virus 2 (HSV-2).[102]

Burke et al. found that poly I: C-exposed or reovirus-infected mast cells recruit NK cells in a CXCL8-dependent manner. It has been shown that mammalian reovirus, an RNA virus that is normally effectively controlled by the immune response, can infect human mast cells and induce the production of large amounts of CXCL8. These CXCL8 responses are sufficient to induce CD56+ NK cell chemotaxis, and CXCR1 expressed in NK cells plays an important role in this response. It has been suggested that NK cells express CXCL8, CXCR1 and CXCR2,17,19,21 receptors and this study confirmed these data, although there is some controversy in this area. Since CXCL8 is also a potent neutrophil chemo-attractor, the data from this study suggest a possible role for mast cells in the simultaneous recruitment of neutrophils that may contribute to the immune response against viral infection.[103]

Mast cells have also been shown to recruit many other types of effector cells into inflamed tissues, including blood monocytes and granulocytes (such as eosinophils) during infection or in models of allergic disease.[103]

The promotion of NK cell chemocytaxis mast cells enhances the recognized ability of mast cells to serve as sentinel cells in the recruitment of effector cells in bacterial, parasitic nematode infections and in the recruitment of NK cells in viral infections.[103]

It is important to clarify the virus recognition mechanisms that lead to mast cell activation in order to understand the role played by mast cells in viral infection, as mast cells function not only as innate immune cells for host protection, but also as exaggerators of allergic diseases associated with viral infection.[102]

Therefore, the current findings reinforce the role of mast cells as key responders to the immune response during the early stages of viral infection through their ability to directly recognize and respond quickly to a virus by rapidly producing cytokines and antiviral chemokines using RLRs and OAS-RNase L, in addition to TLR3. In particular, both allergens and microbial antigens can trigger mast cell activation, and allergic or autoimmune diseases, where mast cells play an important role in their pathogenesis, which are often exacerbated by viruses such as rhinoviruses.[102] As shown in our article, EBV also activates TLR3 and can generate this response from mast cells.

MUSCLE CONSEQUENCES OF EBV INFECTION

Recently, abnormal lactate responses to exercise in patients with chronic fatigue syndrome (CFS) were correlated with the detection and characterization of enterovirus sequences in skeletal muscle, using the same techniques successfully used to demonstrate such sequences in the heart muscle. Enteroviral RNA was detected in 10 of 48 muscle biopsies (20.8%) of CFS patients, but not in 29 control tissue samples from normal subjects or patients with a variety of muscle diseases, showing that the presence of enterovirus sequences in muscle is not typical of the general population. An abnormal lactate response to exercise occurred nine times more commonly in CFS patients with enterovirus sequences in the muscle than in enterovirus-negative cases. PCRs were more closely related to Coxsackie B virus but were not made for herpesviruses. It may be that all other CFS patients with muscle problems tested positive for CRP in muscle samples for other viruses that were not tested in this study. Hence, CFS is heterogeneous depending on the infection that caused it in each patient.[104]

The EBV virus has also been linked to dermatomyositis/polymyositis. The results of the Der-Yuan Chen study showed a positive association of Epstein-Barr virus (EBV) with dermatomyositis/polymyositis and nasopharyngeal carcinoma. Patients with dermatomyositis or polio who have antiEBNA-1 positive IgA or increased EBV DNA loads should be highly suspected of having occult nasopharyngeal carcinoma. However, other diagnostic markers that include antibodies to early antigen (EA) may provide additional data to serological tests for the presence of nasopharyngeal carcinoma.[105]

This association allows us to believe that a muscle biopsy to determine the presence of dermatomyositis or polymyositis, together with a PCR to EBV in this tissue could help in the diagnosis, in the event that the pathogen causing CFS was this virus and had muscular problems.

DISCUSSION

Chronic fatigue syndrome or myalgic encephalomyelitis (ME/CFS) is currently a disease of unknown etiology, which appears suddenly in a previously active person and whose onset appears to be related to an acute infection in most cases. Until now, CFS patients have been studied without classification into pathogen subgroups. The aim of this study is to show how the viral cycle of the Epstein-Barr virus and its mechanism of immune evasion can generate CFS and what the metabolic and physiological consequences are that could be responsible for the symptoms of chronic fatigue.

The EBV expresses at least 44 miRNAs, most of them with unknown function, and two non-coding RNAs (EBERs). EBV-coded miRNAs control the expression of various cellular genes with antiapoptotic functions, but also interfere with innate immune responses and inflammation. Some EBV miRNAs act by suppressing, in infected B lymphocytes, the release of pro-inflammatory cytokines such as IL-12, which resulted in suppression of differentiation of CD4 naive + T-cells to Th1 cells (important antiviral effectors that activate macrophages and NK lymphocytes to kill intracellular pathogens).[10]

Several EBV miRNAs modulate the immune recognition of newly infected B cells (preferably EBV target cells). Viral miRNAs, in infected B cells, control the gene expression of HLA class II and three lysosomal enzymes important for proteolysis and epitope presentation to CD4+ T cells. This allows them to interfere with peptide processing and class II HLA antigenic presentation. As a result of the decrease in HLA II antigenic presentation, the activation of EBV-specific cytotoxic effector CD4+ T cells and the death of infected B cells is reduced.[10]

Also, to avoid detection of EBV-specific CD4 T cells, EBV latent membrane protein 2A (LMP2A) was found to play a critical role in the negative regulation of the expression of MHC class II molecules in infected B cells. Functionally, LMP2A mimics constitutively activated BCR signaling; however, the LMP2A-activated PI3K pathway mediates suppression of MHC class II and CD74 in EBV-infected B cells. Previous studies have revealed that CIITA is a major regulator of the expression of MHC class II and CD74 molecules. They demonstrated that LMP2A mediated the reduction of IUPAC levels by decreasing the expression of PU.1 and E47.[11]

EBV-infected B-lymphocytes generate an IL-10 homologue (vIL-10), encoded by the EBV BCRF1 gene during the prelatent and latent phases.9,17 vIL-10 can act on multiple cell types and inhibit cytokine synthesis in T cells (inhibits production of IL-2 and IFN-g by Th1 cells) and NK.19 cells. This allows the antiviral functions of effector CD4+ T-cells to be suppressed and the NK-cell-mediated death of infected B-cells to be reduced.21 It is also a potent inhibitor of antigenic presentation, reducing the expression of MHC II and the accessory co-stimulation molecules CD80 and CD86 in dendritic cells.[18]

Other miRNAs interfere with the recognition and destruction of EBV-infected cells by CD8+ T cells. First, miRNAs directly target TAP2, negatively regulate the entire TAP complex, and reduce HLA class I allotypes that preferentially have TAP-dependent epitopes. Second, they repress EBNA1, a protein expressed in most forms of EBV latency and a target of EBV-specific CD8+ T-cells. Third, miRNAs decrease the release of IL-12 by infected B cells, as IL12B is directly suppressed by these miRNAs in infected cells. This repression of IL12B not only can reduce the differentiation of CD4+ T-cells, it can also regulate the functions of effector T-cells, decreasing the activity of CD8+ T-cells specific to EBV.[10,13]

EBV can infect the CNS through HBMEC infection. This leads to the rupture of the adhesion molecules or narrow BBB bonds, leading to the passage of leukocytes (including EBV-infected B cells) through the capillaries into the surrounding tissue.[30] B cells with EBV latent infection are able to release EBERs (two non-coding RNAs). Where the release of EBER1 induces the activation of TLR3[31] signaling resulting in an increase in proinflammatory cytokines (inflammation is generated in the tissue).

As with EBV infection of HBMEC, infection of epithelial cells of the intestinal mucosa by EBV leads to the breakdown of the narrow junctions of the intestinal barrier, leading to the passage of bacteria and other substances.[59] At the same time, EBV is able to infect plasma cells in the mucosa. Therefore, B cells with latent EBV infection release EBERs. EBER1 activates TLR3 signaling of enterocytes, resulting in the induction of type I IFNs and proinflammatory cytokines.[31] This activation of TLR3 at the intestinal level reduces the activity of the serotonin transporter (SERT) in enterocytes, thus reducing serotonin uptake and causing an increase of extracellular 5-HT in this tissue.[59] This excess serotonin should be collected and transported by platelets, as platelets get 5-HT mainly from the intestine. But the platelets do not get to collect all this excess, as they also express TLR3[75,76] as enterocytes. When these receptors are activated by the infection, the serotonin reuptake of these cells decreases.[73] This eventually allows for a buildup of serotonin in the intestinal mucosa. Platelets activated via TLR3 also excrete the content of their granules (dense granules contain serotonin).[93] The serotonin released by platelets would increase vascular permeability and may promote inflammation in tissues[92] where EBV-infected cells are present. All this results in a decrease of 5-HT levels in platelets.

Add that in fasting conditions the 5-HT plasma levels of these patients are the same as those of healthy patients. This occurs thanks to alternative transports that remove free serotonin from the portal blood[94] (prevents excess 5-HT at the intestinal level from reaching the systemic circulation). But in postprandial conditions by further stimulating the release of serotonin (especially with carbohydrate consumption), transport systems are saturated, thus increasing the levels of free 5-HT in plasma, as has been seen in patients with IBS-D, along with an increase in plasma levels of their 5-HIAA metabolite compared to healthy subjects.[69]

In addition, by breaking down the narrow junctions of the intestinal barrier, bacteria and other harmful substances can pass from the lumen into the bloodstream, activating TLR4, which also decreases SERT activity.[59] Activation of the various serotonin receptors would lead to increased intestinal motility[59], malabsorption problems along with vitamin deficiencies (vitamin A, E, D, K and B12)[84], diarrhea[59], dysautonomia by communication of the vagus nerve between the enteric and cardiovascular systems, significant increase in wakefulness and a reduction in slow wave sleep[86] along with cognitive problems. In addition to problems with temperature regulation[98] and hormone secretion[95].

It should be noted that the environmental factor (EBV infection) not only influences the onset of CFS, but also the age at which the primary infection occurs and the genetic susceptibility to this infection. That is, patients with genes from MHC class I and II molecules that are susceptible to developing EBV-related diseases will have difficulty fighting EBV infection. As most of these diseases have numerous polymorphisms of these susceptibility genes, there is a great genetic heterogeneity among patients who develop one of these diseases, which is manifested as a great phenotypic variability among different patients suffering from the same disease. This is because in all vertebrate species the MHC molecules are highly polymorphic. This polymorphism reflects an immune system strategy to prevent the evasion of immune system pathogens. Having different MHC molecules, individuals deal with microbes in a different way, with individuals in a given population being more susceptible and more resistant to a given disease.[12]

For future research, it would be interesting to start classifying patients into subgroups according to the possible pathogens involved based on understanding and focusing on a possible treatment. Rituximab is currently being studied for this disease and the explanation of why it works in some

patients and not in others may be due to the type of pathogen involved. On the other hand, common markers must be found based on a diagnosis of the disease. At the metabolic level, it behaves in a similar way to cancer, all the antioxidants (vitamin C, Q10, E....) are reduced to compensate for the high oxidative stress and the appearance of cachexia and constitutional syndrome is common due to the high energy consumption caused by the Warburg effect. In more advanced disease there may be a decrease in the levels of glutamine, cysteine along with high production of urea and glutamate, this could be a marker of severity and a key point to consider based on supplementing them before implementing any therapy. It should be noted that the role of NK is key to viral infection and therefore key to CFS. Above all, it is essential to evaluate which pathogen is involved in the clinical picture, how it circumvents the immune system and therapeutic strategies to reverse the process and return the system to its initial state. For example, in this case Epstein Barr infection, rituximab could be crucial, since the virus acts by generating latency mainly in B cells. So if the infected B-lymphocytes were lysed, we would reduce the Warburg effect and consequently the chronic fatigue, as well as the immunological problems (there would no longer be a deficit of expression of MHC class II molecules). But rituximab treatment may have to be given with antivirals, because by immunosuppressing the patient the EBV could reactivate and continue to infect. There is now a more promising treatment without significant adverse effects, Epstein-Barr virus-specific adoptive immunotherapy. Where the death of EBV-infected B cells is achieved by adoptively transferred CD8+ T cells. This treatment has had promising preliminary results in progressive multiple sclerosis due to EBV, with improvements in the patient's symptoms and signs.

For all these reasons, several markers are proposed to be used in patients with CFS post-infection with EBV:

1. Activated T lymphocytes (CD3+, DR+), (CD4+, DR+): A low level of activated T lymphocytes indirectly indicates a decrease in HLA-II antigenic presentation. This decrease in CD4+ DR+ T-lymphocytes can be seen in other EBV-related diseases, such as children with EBV-associated hemophagocytic lymphohistiocytosis.
2. To verify in laboratory the decrease of the antigenic presentation HLA-II on the part of the antigen-presenting cells.
3. Molecular typing of the HLA system: to verify the existence of certain HLA alleles with a predisposition to develop EBV-related diseases.
4. IgG antibodies to nuclear antigen (antiEBNA IgG): presence of a high number, as in multiple sclerosis.

Tests 1 and 2 should be present in most patients with CFS, as other pathogens also evade the immune system in this way. These pathogens are able to generate an acquired functional immunodeficiency through the deficit of expression of major histocompatibility complex class II molecules. Although some also manage to decrease the MHC class I molecules, as has been mentioned in this article.

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