EPSTEIN BARR VIRUS AND ITS ROLE ON BURKITT LYMPHOMA AND MULTIPLE SCLEROSIS

Andrea Aira Gomez, Grau de Microbiologia, Universitat Autònoma de Barcelona, 2015. (andrea_aira@hotmail.com)

Introduction

Epstein Barr virus (EBV) is one of the most common human viruses (affecting 95% of world population) and normally, it causes asymptomatic infections. However, EBV can cause infectious mononucleosis (IM) and other illnesses. It is known for its properties of causing deregulations of the host immune system and establishing latency in B lymphocytes.
Here, there is a compilation of results from diverse studies which debate the role of EBV in two diseases: **Burkitt lymphoma** and **Multiple sclerosis**, in order to comprehend its importance in these processes.

What is Epstein Barr Virus?[1,2]

Herpesvirus 4 (HSV-4), also known as **Epstein Barr virus** (EBV), is an enveloped γ-herpesvirus which was discovered in 1964 by Anthony Epstein in Burkitt lymphoma samples from children living in Uganda. It has been classified as **class I carcinogen virus** by Agency for Research on Cancer.

The virus contains 172kpb double strand DNA and it has **tropism for B lymphocytes** where it is able to establish **latency as an episome** [Figure 1].

EBV was the first found encoding **microRNAs (miRNAs)** which can down-regulate gene expression and inhibit the apoptotic response of the infected cell. Furthermore, miRNAs can inhibit viral genes in order to avoid immune response and establish a latent infection.

It also can drive B cells into **continuous proliferation** expressing:

- **Epstein-Barr nuclear antígens (EBNA):** EBNA1, EBNA2, EBNA3A, EBNA3C
- **Latent membrane proteins (LMP**): LMP1
- **Small non-coding RNAs**: EBER1, EBER2
- **miRNAs**

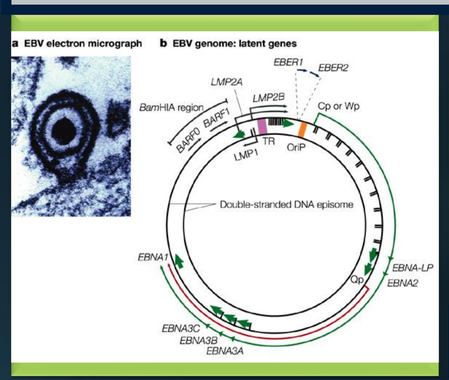

Figure 1. **a.** EBV virion electron micrograph **b.** Diagram showing the location of EBV latent genes on the genome.[3]

Understanding EBV infection[4,5,6]

EBV is mainly transmitted by **saliva** but also by other fluids contact.

In **primary infection** [Figure 2], the virus enters in buccal cavity and contact with naïve B cells in the tonsils through the union of viral gp350 and CD21 expressed in B cells. Then, a complex of viral glicoproteins (gH, gL, **gp42**) binds to HLA antigen class II to start the fusion of virus envelope and B cell membrane which produces an activation of B cells that become proliferating B blasts.

B blasts enter the follicles where they expand forming a germinal centre in which follicular dendritic cells and specific CD4+ T cells will check the ability of B cells to receive signals from antigen.

The cells leave the follicle as infected memory and latent B cells entering the peripheral circulation. Some of these cells will initiate replication of the virus to be dropped into saliva.

EBV **establishes latency** in memory B cells using only a limited number of genes to maintain its genome and evade the host immune reaction.

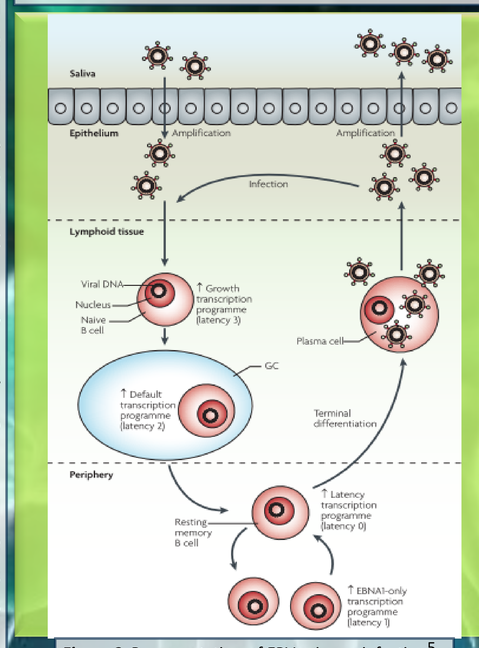

Figure 2. Representation of EBV primary infection[5]

- **Type III latency:** EBV expresses all EBNA, LP, 3 LMPs and EBER. This pattern is observed in IM and some post-transplantant lymphoproliferative disorders (PTLDs).
- **Type II latency:** EBNA1, LMP1, LMP2A and EBER expressed. It is observed in classical Hodgkin lymphoma and some PTLDs.
- **Type I latency**: Only EBNA1 and EBER are expressed. It is common of Burkitt lymphoma.
- **Type 0 or "true latency":** There is no viral gene expression except during cell division. These cells are not recognized by CTLs.

For **reactivation** is necessary to replicate viral genomic DNA and the synthesis of specific proteins for packing the newly DNA into infectious virions.

Cytotoxic CD8+ T cells (CTL) are responsible for elimination of proliferating and lytically infected B cells causing symptomatology. CD4+ T cells and innate immune response also take part in host defense.

However, EBV will remain again in latent memory B cells that will return to the tonsils to have a massive replication to go to the saliva to be transmitted.

BURKITT LYMPHOMA [7,8]

Burkitt lymphoma (BL) is a neoplasic disease that affects human B lymphocytes and it is presented multifocal, but it is found typically in the area of the jaw [Figure 3]. BL is not frequent and usually affects to children aged 3-13 years with a peak of incidence at 6 years and it is more common in boys than girls. In untreated cases it provokes death.

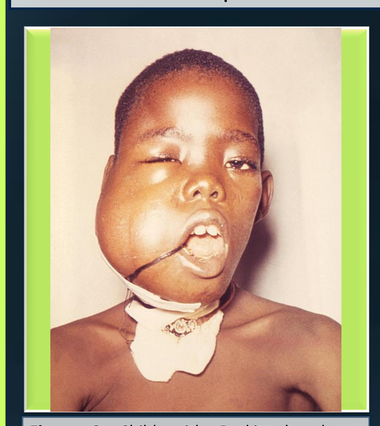

Figure 3. Child with Burkitt lymphoma typically located in the area of the jaw.[4]

There are three types of BL: **Endemic (or African) BL**, related with the presence of EBV; sporadic BL and immunodeficiency associated BL.

It is known that EBV can transform and drive B cells into unlimited proliferation *in vitro.* It has been observed that most tumour cells from endemic BL contains multiple EBV genomes and infectious virions can be found in tumour cell lines.

EBV-BART-6-3p miRNA specially plays an important role in BL pathogenesis, affecting the function of important signal transducers involved in regulating the cell cycle.

Although EBV has these abilities, it is not enough to cause per se BL and there are two possible cofactors that affect the regulation of apoptosis in BL: Malaria and MYC translocation.

Endemic BL occurs in areas where **MALARIA** is holoendemic whereas in places where malaria is eradicated, the BL incidence has decreased. Possibly:

- *Plasmodium falciparum* would lead an increase of EBV load because of the stimulation of the B cell system and rising B cells pool that can be infected.
- *P. falciparum* antigens and other antigens reactivate the virus from memory B cells increasing the viral load.

Both agents cause B cell hyperplasia, an essential component of lymphomagenesis in BL.

MYC TRANSLOCATION between the long arm of chromosome 8 and chromosomes 14,2 or 22 is a critical event in lymphoma's formation.

MYC protein is a potent inducer of proliferation causing apoptosis and its deregulation harm the immunogenicity of human B cells.

EBV infected B cells present typical somatic hypervariabe region mutations which suggests that they have passed through the germinal centre where it is found the activation-induced cytidine deaminase (AID) gene, responsible for MYC translocation.

MULTIPLE SCLEROSIS [6,9]

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the Central Nervous System (CNS) resulting in the formation of plaques. It causes progressive disability and affects more than 2.5 million people of the world, particularly young women.

EBV is the only agent infecting all MS patients and the only one which infects and modulates B cells, involved in the disease process. B cells from MS patients expresses EBV latent proteins showing that the virus displays the latency II transcription program.

There are **four hypothesis** to explain how EBV could provoke MS:

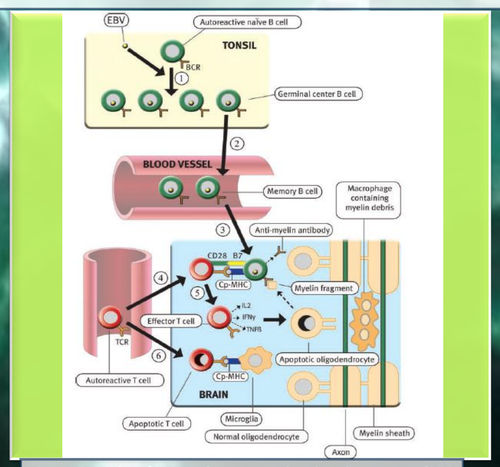

Figure 4. Role of EBV infection in the development of MS following "EBV infected autoreactive B cell hypothesis".[6]

1. EBV crossreactivity hypothesis

There is a crossreactivity between EBV and CNS antigens, consequently, EBV specific CD8+ T cells recognizes CNS antigens as EBV.

2. EBV bystander damage hypothesis

The immune system attacks EBV which is found in CNS and it results in bystander damage to the CNS.

3. The αβ-crystallin or "mistaken self" hypothesis

The αβ-crystallin is a small heat-shock protein and an immunodominant antigen of CNS that is expressed in oligodendrocytes and myelin in early MS lesions.

Peripheral EBV infection induces the expression of this protein in HLA class II molecules in B cells. Consecuently, CTLs are prepared against microbial antigens and also to new expressed αβ-crystallin.

4. EBV-infected autoreactive B cell hypothesis

If after primary infection there is a defect in the defense mechanism of the host, the number of EBV infected B cells will not be controlled and surviving autoreactive B cells could enter the CNS. There, they could produce oligoclonal IgG and pathogenic autoantibodies, which would attack myelin and other components of the CNS. Autoreactive T cells receive costimulatory signals to destruct myelin and oligodendrocytes [Figure 4].

Conclusions

- EBV is able to regulate the host immune response in lytic and latent infections and remain invisible for long periods. That difficult the control and possible eradication of this virus.
- EBV latent genes and miRNAs are essential for inducing B cells proliferation and tumours generation.
- EBV infection is a prerequisite for the development of BL and MS but it is not enough by itself to cause these diseases.
- The association between malaria, EBV infection and MYC translocation is strongly related with BL development. The eradication of malaria could decrease the incidence of BL.
- Controlling EBV infection could be important to decrease BL and MS incidence.
- More studies with comparisons between EBV infection and other viruses infections are needed to have convincing results.

Treatment [4,10]

- There is no vaccine to protect against EBV.
- There is no treatment for EBV but there are advices for relieving symptoms in IM.
- In sporadic BL cases there is a treatment with antineoplasic agents which produces an improvement in 80% of cases.
- MS has no defined treatment but sunlight and vitamin D protect against that disease.

Research [11]

- Understand the switch from latency to lytic replication cycle is a critical step in virus spreading. **Zta** regulates this change and it is a possible target for future vaccines.

References

1. National Academy of Science. The royal society [webpage]. London, UK [consulted 18th March 2015]. Available in http://www.royalsociety.org
2. Barth, S., Meister, G., & Grässer, F. A. (2011). EBV-encoded miRNAs. *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms*, *1809*, 631–640.
3. Young, L. S., & Rickinson, A. B. (2004). Epstein-Barr virus: 40 years on. *Nature Reviews. Cancer*, *4*, 757–768.
4. Centers for Disease Control and Prevention [webpage]. Georgia, USA [consulted 21st March 2015]. Available in http://www.cdc.gov
5. Thorley-Lawson, D. A., & Allday, M. J. (2008). The curious case of the tumour virus: 50 years of Burkitt's lymphoma. *Nature Reviews. Microbiology*, *6*, 913–924.
6. Pender, M. P. (2011). The essential role of Epstein-Barr virus in the pathogenesis of multiple sclerosis. *The Neuroscientist : A Review Journal Bringing Neurobiology, Neurology and Psychiatry*, *17*, 351–367.
7. Magrath, I. (2012). Epidemiology: Clues to the pathogenesis of Burkitt lymphoma. *British Journal of Haematology, 156,* 744-756.
8. Grömminger, S., Mautner, J., & Bornkamm, G. W. (2012). Burkitt lymphoma: The role of Epstein-Barr virus revisited. *British Journal of Haematology*, 156, 719-729
9. Lossius, A., Johansen, J. N., Torkildsen, Ø., Vartdal, F., & Holmoy, T. (2012). Epstein-barr virus in systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis-association and causation. *Viruses*. 4, 3701-3730
10. Virology-online [webpage], Derek Wong. Hong Kong, China [consulted 27th November 2014]. Available in http://www.virology-online.com
11. Ramasubramanyan, S., Osborn, K., Al-Mohammad, R., Naranjo Perez-Fernandez, I. B., Zuo, J., Balan, N., Sinclair, A. J. (2015). Epstein–Barr virus transcription factor Zta acts through distal regulatory elements to directly control cellular gene expression. *Nucleic Acids Research*, *43*(7), 3563–3577.