Epstein-Barr virus in HIV-infected patients

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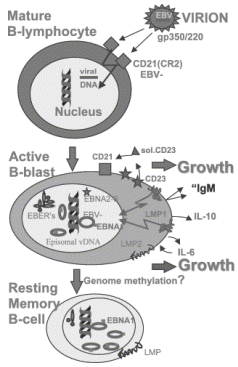
Epstein-Barr virus (EBV)

- ubiquitous human herpesvirus that remains in B-lymphocytes in a latent state
- can reactivate during immunosuppression

EBV maturation



EBV B-cell transformation:

1. Virus binding via CD21(CR2)
   - role C3d homology in gp350/220
2. Cellular pre-activation (0.5 hr)
3. Transport viral DNA to nucleus
4. Expression **EBNA2, LP** (>4 hr)
   - deregulation cell control (P53, Rb, Jk, c-fgr)
   - induction growth factors (sCD23, IL6, ...)
5. Expression **LMP1, EBNA3A,B,C** (>12 hr)
   - growth transformation (TRAF's, NF-kB)
   - block apoptosis (A20, bcl-2)
   - immunomodulation (IL10)
   - growth factor receptor induction (CD21)
6. **Circularization EBV-genome** (>20 hr)
7. Expression **EBNA1** (20 hr)
   - DNA replication

immortalisation completed!

8. Extra host cell modulation:
   - Expression LMP2A,B (TP1,2) (>24hr)
     - Inhibit cellular differentiation/ -activation
   - Expression EBER1-2, BARF0, ea.

Result:
- **permanent "living" EBV$^+$ B-cell.**

EBV antigens

- **six EBV-associated nuclear antigens**
  - EBNA 1,2,3a,3b,3c, -leader protein (LP)
- **Early antigen (EA)**
  - diffuse (D) and restricted (R) forms
- **Viral capsid antigen (VCA)**
- **EBV-induced membrane antigen (MA)**
- **Latent membrane proteins (LMP)**

EBNA

- **EBNA - 1**
  - important for maintenance of the plasmid viral DNA in latently infected cells and in activation of viral DNA replication
  - expressed in all EBV infected cells (but the other EBNAs are not)
- **EBNA - 2**
  - essential for the immortalization of lymphocites by EBV
  - the first gene expressed, in conjunction with **EBNA-LP**, and serves as a master switch in those cellular and viral genes involved in transformation
- **EBNA - 3a, 3b, 3c**
  - involved in B cells transformation

EA, VCA, MA

- **Early antigen (EA)**
  - appears before viral replication
  - Diffuse component is found in the nucleus
  - Restricted component is found in the cytoplasm
- **Viral capsid antigen (VCA)**
  - appears after viral replication and constitutes the virion
- **EBV-induced membrane antigens (MA)**
  - gp 350/220 – binds to CD21 facilitating entry into the B lymphocyte
  - gp 85 – homologous to the HSV viral protein; important in fusion of the virus to the cell membrane; causes virus neutralization in the presence of complement
  - gp 110 resides mainly in the nuclear membranes and ER of infected cells

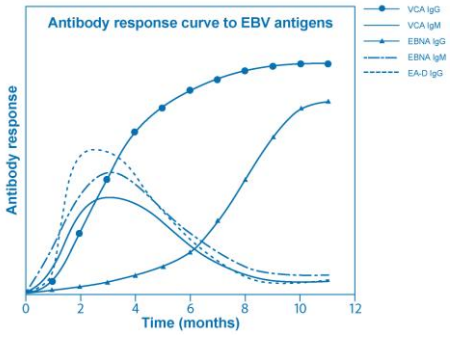
EBV diagnosis

- the diagnosis of EBV infection is mainly based on **serological determination** of specific antibodies to different antigens:
  - **viral capsid antigen (VCA)**
  - **diffuse component of early antigen (EA-D)**
  - **EBV nuclear antigens (EBNA)**

EBV antibodies

- **VCA (*viral capsid antigen*)**
  - VCA IgM appear in 2-3. week
  - VCA IgG appear 4-7 weeks after clinical signs; persists lifelong
- **EA-D (*early antigen diffuse*)**
  - EA-D IgG pick in 3. week
  - Sign of viral replication
- **EBNA (*EBV nuclear antigen*)**
  - Appear approximately 8 weeks after clinical signs
  - Mostly persists lifelong

Epstein-Barrov virus (EBV)



EBV infection

- **ACUTE (PRIMARY) INFECTION**
  - ACUTE PHASE
  - TRANSIENT SUBACUTE PHASE
  - CONVALESCENT PHASE
- **PAST (LATENT) INFECTION**
- **REACTIVATION**

EBV serological profiles

EBV infection	VCA IgM	VCA IgG	EA-D IgG	EBNA IgG
EBV negative	-	-	-	-
Past	-	+	-	+
Primary (acute)	+ +	+ -	+ + / -	- -
Primary (transient)	+ -	+ +	+ -	+ -
Primary (convalescence)	- -	+ +	- -	- +
EBV reactivation	-	+	+	+

The aim of study
EBV in HIV-infected patients

- to determine the EBV serological profiles in HIV-infected adult patients from Croatia in a cross sectional study
- to compare HIV-infected patients with healthy adults findings

Definition of different EBV serological profiles

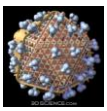
EBV infection	EBV antibodies			
	VCA IgM	VCA IgG	EA-D IgG	EBNA-1 IgG
Latent (Past)	-	+	-	+
Reactivated	-	+	+	+
Transient phase	-	+	-	-
Non-active with IgM response	+	+	-	+
Acute infection	-/+	+/-	+/-	-
No infection	-	-	-	-

Specific antibodies to EBV antigens in 166 HIV-infected adults and 219 blood donors

Antibodies to EBV	HIV-infected patients N (%)	Blood donors N (%)	OR (95% CI)*	P
IgM anti-VCA	4 (2.4)	25 (11.4)	0.19 (0.05-0.57)	0.001
IgG anti-VCA	166 (100.0)	212 (96.8)	ND	0.02
IgG anti-EA-D	74 (44.6)	30 (13.7)	5.07 (3.02-8.58)	< 0.0001
IgG anti-EBNA	153 (92.2)	196 (89.5)	1.38 (0.65-3.07)	0.37

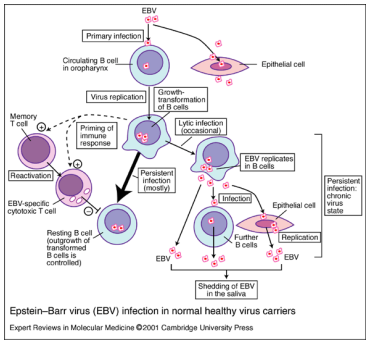
EBV infection according to serological profiles in HIV-infected patients and blood donors

EBV infection	HIV-infected patients N = 166 (%)	Blood donors N= 219 (%)	OR (95% CI)	P
Latent (past)	83 (50.0)	146 (66.7)	0.50 (0.32-0.77)	0.0011
Reactivated	66 (39.8)	28 (12.8)	4.55 (0.15-7.69)	<0.0001
Transient phase	6 (3.6)	16 (7.3)	0.48 (0.15-1.32)	0.12
Non-active with IgM response	4 (2.4)	25 (11.4)	0.19 (0.05-0.57)	<0.0001
Acute infection	7 (4.2)	1 (0.5)	10.0 (1.20-500.00)	0.02



CONCLUSION

- HIV-infected patients had different EBV serological profiles than blood donors.
- Serological diagnostics may be useful in the EBV reactivation diagnostics although EBV viral load has to be defined with molecular diagnostics methods for the diagnosis confirmation.



Epstein–Barr virus (EBV) infection in normal healthy virus carriers