

LET'S TALK ABOUT A DIFFERENT VIRUS...

REVIEWS AND UPDATES ON EBV:
EPSTEIN BARR VIRUS

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Spring CLPC 2022

1

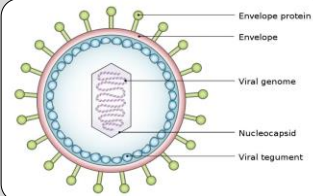
EBV Objectives:

- Summarize the pathogenesis and clinical characteristics of EBV infections
- Explain laboratory methods necessary to diagnose suspected EBV infections
- Discuss new discoveries and disease connections surrounding prior EBV infection

2

Description of EBV

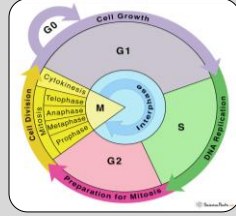
- Human Herpesvirus 4 (HHV-4)
- Enveloped
- Linear dsDNA
- Codes for >85 proteins



The diagram shows a cross-section of an EBV virion. It has a spherical shape with a pink outer envelope studded with green envelope proteins. Inside the envelope is a blue layer representing the viral genome, which is a linear double-stranded DNA molecule. The genome is surrounded by a white layer representing the nucleocapsid. The entire structure is surrounded by a thin layer representing the viral tegument.

3

EBV replication machinery



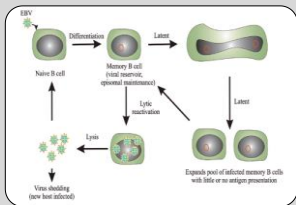
The diagram shows a circular cell cycle with phases G1, S, G2, and M. The S phase is highlighted in green and labeled 'DNA synthesis'. The G1 phase is highlighted in purple and labeled 'Cell Growth'. The G2 phase is highlighted in pink and labeled 'Preparation for Mitosis'. The M phase is highlighted in yellow and labeled 'Mitosis'. The diagram also shows 'Cell Division' and 'Cytokinesis'.

- Does not require host cell to be in S phase
- Carries its own replication machinery

4

EBV genome integration

- Portion of genetic material
- EBV tethers episome to host cell DNA
- Guarantees equal distribution of viral DNA in daughter cells



The diagram shows the process of EBV genome integration. It starts with a 'Naive B cell' which undergoes 'Differentiation' to become a 'Memory B cell (with memory, optimal environment)'. The 'Memory B cell' can enter a 'Latent' state or undergo 'Lysis' to release 'Virus shedding (new host infected)'. The 'Latent' state can also lead to 'Lysis' and 'Virus shedding'. The 'Latent' state is associated with 'Expands pool of infected memory B cells with little or no antigen presentation'.

5

DNA virus infection outcomes

Productive infection	Persistent infection	Transformation
<ul style="list-style-type: none"> • Active synthesis and release of new virions • Death of host cell 	<ul style="list-style-type: none"> • Virus remains in host cell without killing the cell • Chronic type • Latent type 	<ul style="list-style-type: none"> • Cellular communication and growth changes • Host cell transforms into a tumor cell


6

EBV virus infection outcomes		
Productive infection	Persistent infection	Transformation
<ul style="list-style-type: none"> Infectious mononucleosis (IM) 	<ul style="list-style-type: none"> "Every Body's Virus" Chronic IM 	<ul style="list-style-type: none"> Post-transplant disease Burkitt's lymphoma Nasal carcinoma

7

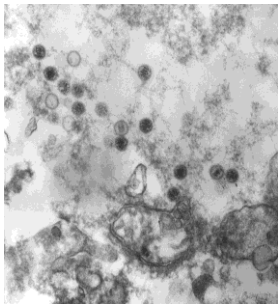
Discovery of EBV: 1963-4

• Isolated from biopsy samples of Burkitt's lymphoma patients



Michael "Tony" Epstein, Bert Achong, and Yvonne Barr

8




All because of a delayed flight...

• Viral particles finally observed in cell culture via electron microscopy in February 1964

9


Epidemiology: "Every Body's Virus"



Estimated to be present in >90% of population worldwide	Transmits through oral secretions, organ transplant, transfusion
Primary infection in early childhood and teenage years	Seroconversion may be dependent on socioeconomic factors
Type 1: Europe, Americas, China, South Asia	Type 2: Africa, Papua New Guinea

10

Lytic and Latent genes of EBV

• EBV-EA (early antigen)	
• EBV-MA (membrane antigen)	
• VCA (viral capsid antigen)	
• EBNA-1	EBV nuclear antigens
• EBNA-2	
• EBNA-3	
• LMP-1	Latent membrane protein
• LMP-2	

11

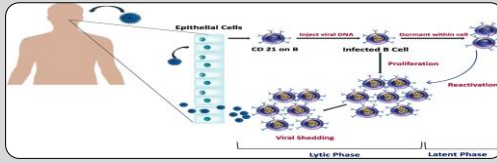
EBV antigens and antibodies

Early	Late	Latent
EA-D, EA-R	Viral capsid Ag (VCA)	EBV nuclear Ag (EBNA)
Anti-EA-D	IgM anti-VCA	LMP
Anti-EA-R	IgG anti-VCA	Anti-EBNA
		Anti-LMP

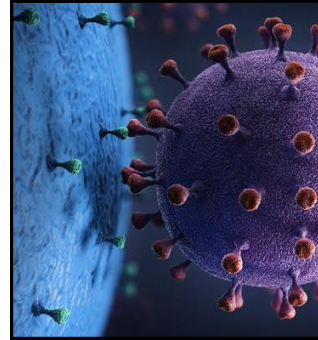
12

EBV pathogenesis

- Viral tropism for oropharyngeal epithelial cells and B-cells
- Begins life cycle in epithelial cells
- Virions spread to tonsils to infect B-cells



13



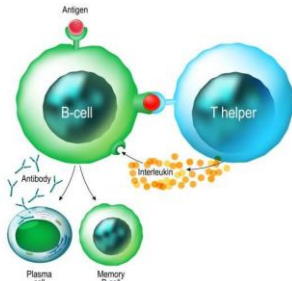
EBV really loves B-lymphocytes

- EBV enters B-cells by binding to CD21
- B-cells proliferate and differentiate
- Memory B-cell pools migrate to other lymphoid tissues

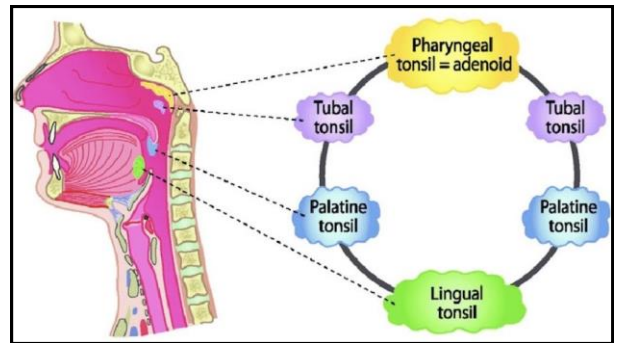
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The fate of EBV infected B-cells

- Infected cells killed by CD8+ T-cells and NK cells
- Plasma cells secreting various antibodies to EBV antigens
- Memory cells with life-long infection

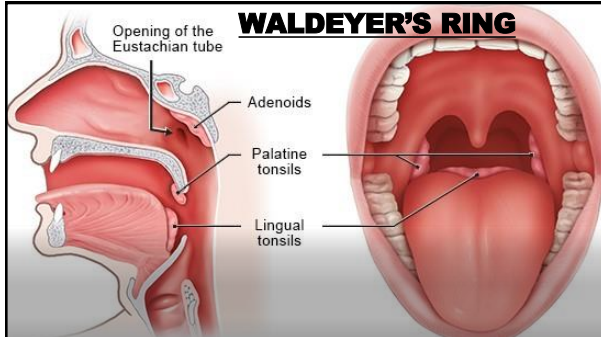


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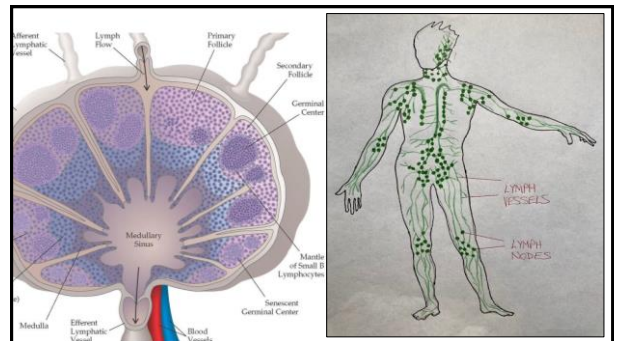


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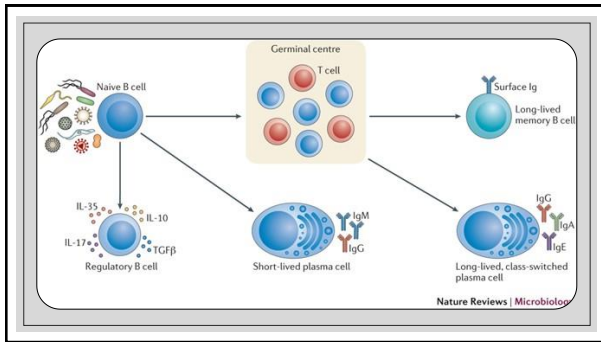
WALDEYER'S RING



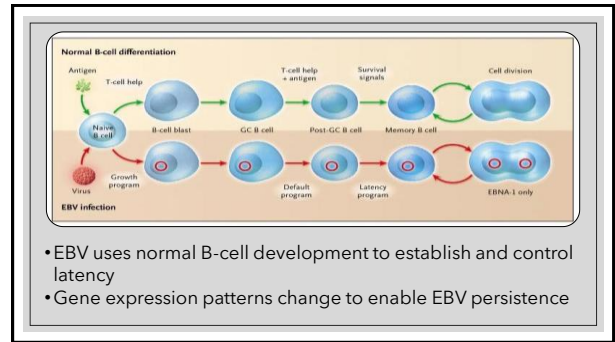
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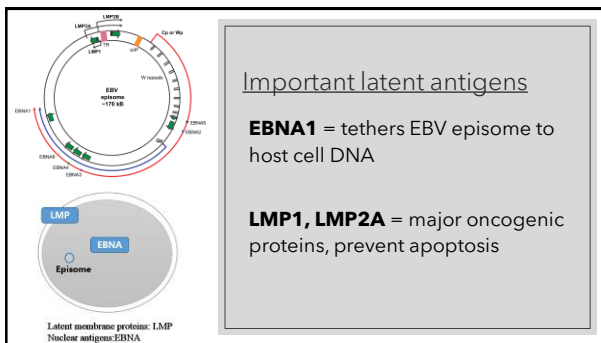
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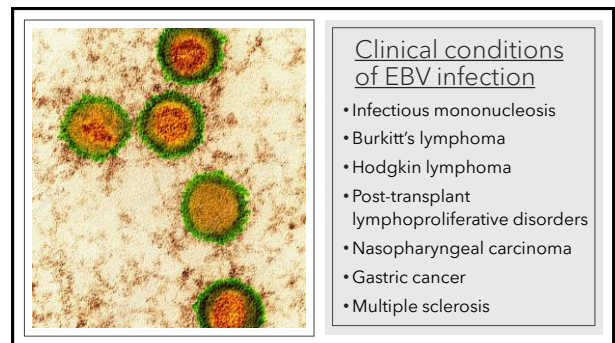
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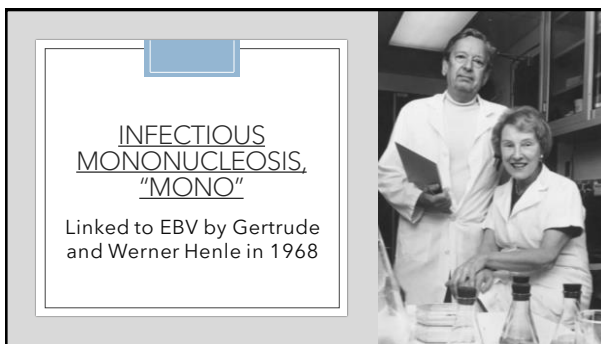
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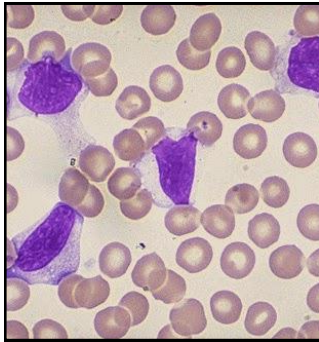


23

Infectious mononucleosis, "mono"

- Can occur during infancy, childhood, teens, young adult
- Classic symptoms of mono: **fever, lymphadenopathy, sore throat, extreme fatigue**
- Some liver involvement
- Symptoms last 2-4 weeks
- Fatigue, body aches can last for months



24





Important Lab features of acute infectious mono

- Absolute lymphocytosis
- 20% or more atypical lymphocytes (RR 0-5%)
- Heterophile antibody
- Anti-VCA IgM
- Anti-EA

25

Monospot test (New method)

- Testing for heterophile antibody
- Bovine RBC extract acts as Ag
- Rapid tests
- Ideal for POC

26

Limitations of Monospot

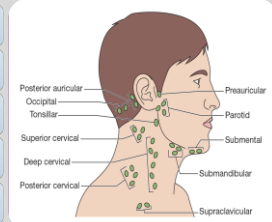
• Screening test only

- 10% of adults will test negative
- 50% of children will test negative (≤ 4 years old)
- Not as sensitive as EBV-specific antibody testing
- False positives can occur due to lymphoma, viral hepatitis, malaria, autoimmune disease

27

Lymphoproliferative disorders

- Burkitt's Lymphoma
- Hodgkin Lymphoma
- Nasopharyngeal carcinoma
- Gastric cancer
- Post-transplant lymphoproliferative disorder (PTLD)



28

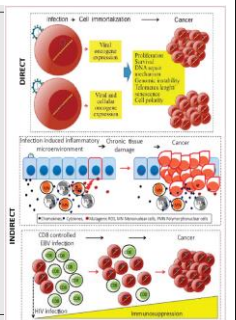
EBV Serology

Condition	Viral Capsid Ag			Early Ag	EBNA	Heterophile Ab
	IgM	IgG	IgA	Anti-EA	Anti-EBNA	IgM
Uninfected	-	-	-	-	-	-
Acute IM	+	++	+/-	+/-	-	+/-
Past infection IM	-	+	-	-	+	-
Post-transplant dz	-	++	+/-	+	+/-	-
Burkitt's	-	+++	-	+/-	+	-
NP carcinoma	-	+++	+	++	+	-

29

How EBV *causes* cancer

- Immortalizes B-cells
- High rate of cellular turnover
- Genetic mutations occur
- c-MYC gene deregulated
- Gene expression changes
- Cells get stuck in proliferation
- Chronic inflammation and immunosuppression



30

NEW DISCOVERIES:

DOES EBV INFECTION CAUSE MULTIPLE SCLEROSIS?

REPORT

MULTIPLE SCLEROSIS

Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis

Kjetil Bjørnæs^{1,2}, Mariana Cortes^{1,2}, Brian G. Healy^{2,3,4}, Jens Kuhle⁵, Michael J. Mina^{6,7}, Yuxin Long⁸, Stephen J. Dudgeon⁹, David W. Nebeker¹⁰, Ann L. Scher¹¹, Kassandra L. Munger¹², Alberto Ascherio^{13,14}

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system of unknown etiology. We tested the hypothesis that MS is caused by Epstein-Barr virus (EBV) in a cohort comprising more than 10 million young adults on active duty in the US military, 955 of whom were diagnosed with MS during their period of service. Risk of MS increased 32-fold after infection with EBV but was not increased after infection with other viruses, including the similarly transmitted cytomegalovirus. Serum levels of neurofilament light chain, a biomarker of neuronal degeneration, increased only after EBV seroconversion. These findings cannot be explained by any known risk factor for MS and suggest EBV as the leading cause of MS.

31

What is Multiple Sclerosis?

- Demyelinating disease of central nervous system
- Inflammation and destruction of myelin sheath surrounding axons
- Plaques form in brain and spinal cord
- Immune mediated process

32

Multiple Sclerosis (MS)

- T-cells and macrophages in the plaques predominate and orchestrate demyelination
- Stimulates macrophages and phagocytic microglial cells
- Inflammation, injury to axons, post-inflammatory neurodegeneration

33

MULTIPLE SCLEROSIS

Important Diagnostics for MS

- Lesions on MRI
- Increased IgG in CSF
- Oligoclonal bands on serum IPE
- Increased IgG index
- Young-middle aged adults
- Weakness, sensory problems

34

EBV has been "sus" for a long time!

Immunological control of Epstein-Barr virus-transformed lymphocytes in multiple sclerosis

J C Craig, M Haire, J H Miller, K B Fraser
PMID: 6309449 DOI: 10.1016/0090-12

Abstract

T-cell control of Epstein-Barr virus (EBV) multiple sclerosis (MS) patients and in

Epstein-Barr virus infection and antibody synthesis in patients with multiple sclerosis

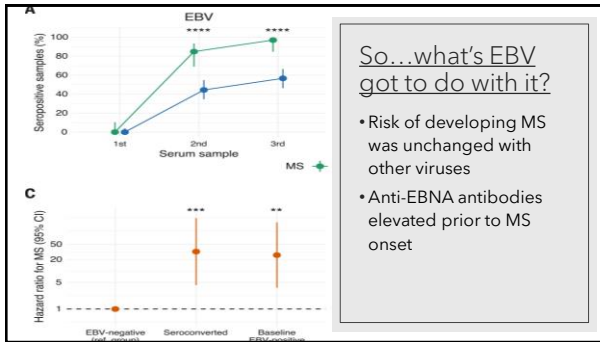
P F Bray, L C Bloomer, V C Salmon, M H Bagley, P D Larsen
PMID: 6860175 DOI: 10.1001/archneur.1983.04050070036006

35

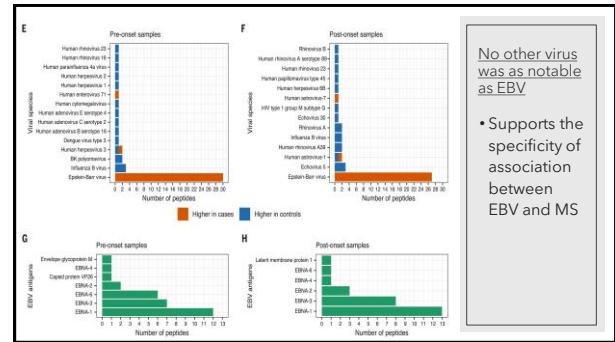
Results of Harvard study

- Cohort of > 10 million active-duty military personnel from 1993 to 2013
- Using serum samples from DoD Serum Repository
- Identified 955 adults diagnosed with MS during service
- Risk of MS increased 32-fold after infection with EBV
- Serum levels of neurofilament light chain increased only after EBV infection

36



37



38

So...what's EBV got to do with it?

nature

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Article | Published: 24 January 2022

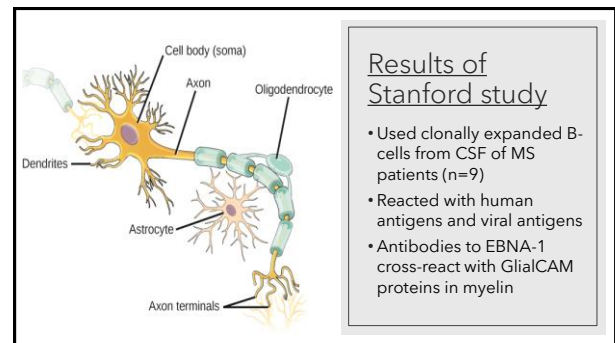
Clonally expanded B cells in multiple sclerosis bind EBV EBNA1 and GlialCAM

Tobias V. Lanz, R. Camille Brewer, ... William H. Robinson

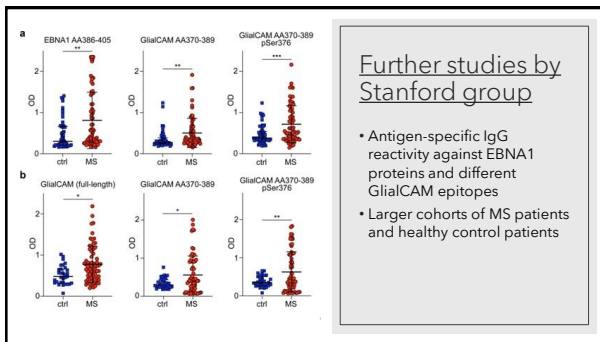
Nature 603, 321–327 (2022) | Cite this article

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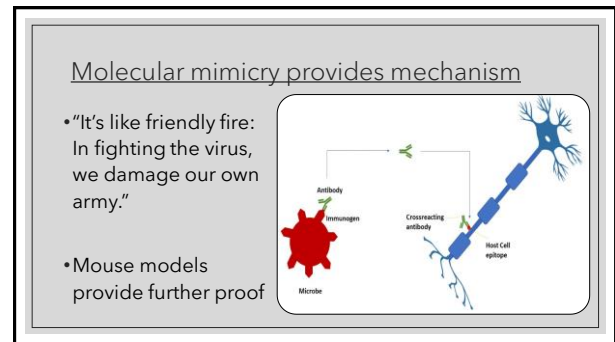
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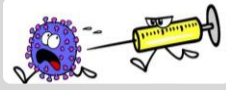
41



42

Will there ever be a vaccine?

- Many ongoing clinical trials
- EBV envelope protein (gp350)
- Virus-like Particle (VLP)
- mRNA vaccines - Moderna
- Viral vectors - adenovirus, vaccinia
- T-cell immunotherapies: adoptive T-cells, EBV-specific T-cell receptors, CAR T-cell therapy



43

In summary...



- We've likely all had it...and still have it...
- Not a safe virus just because it goes dormant
- Could yield helpful info for treating other autoimmune diseases
- Vaccine may eliminate MS and lessen burden of lymphoma

44

References

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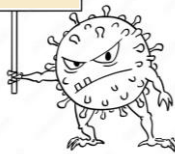
45

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46

THANK
YOU!!!



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47