Casual Friday Presents

Epstein-Barr Virus Chronic and Reactivation

FM Strategies: Physiology, Pathology, Interventions.

A BIOGENETIX CLINICAL PRESENTATION

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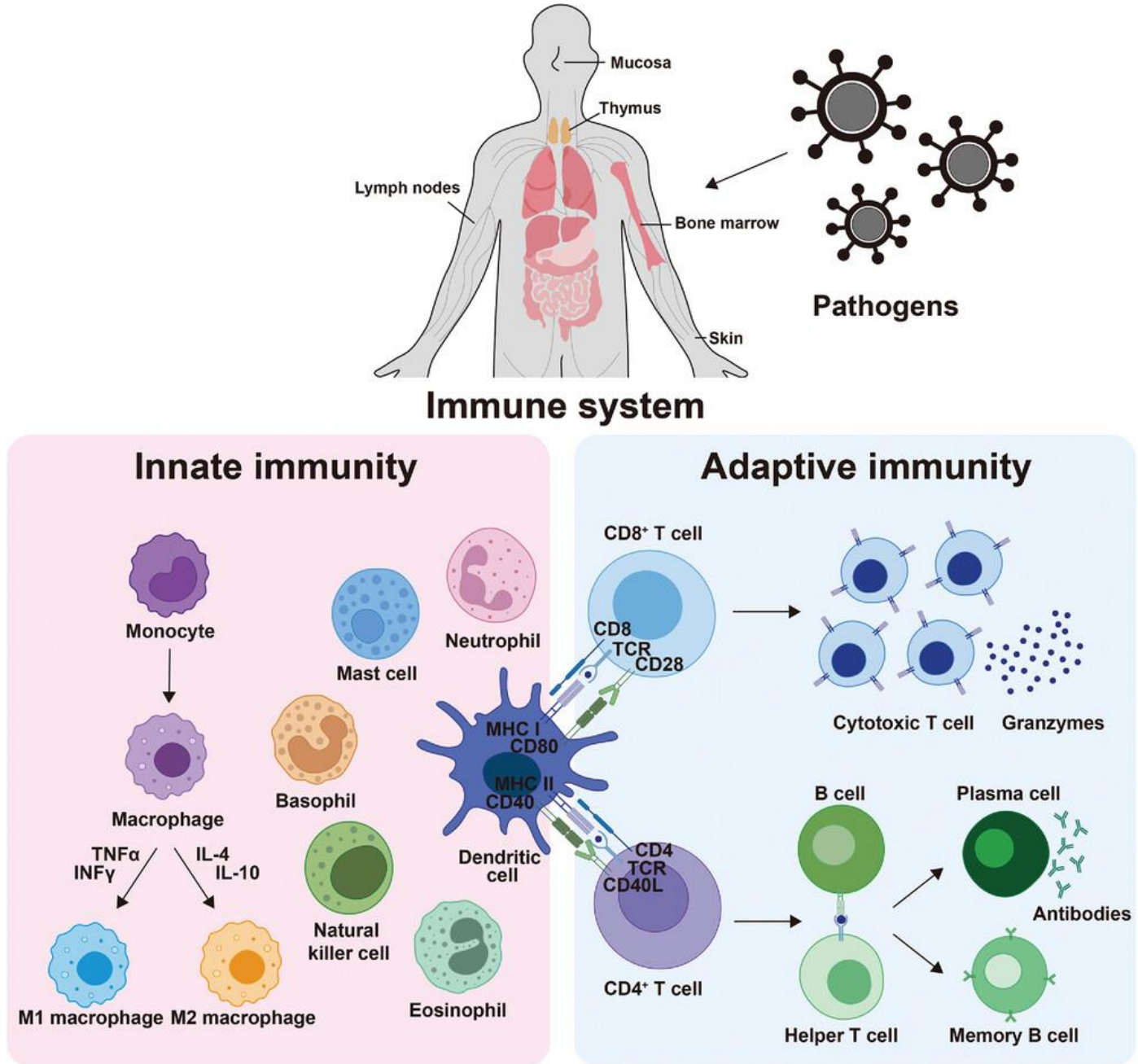

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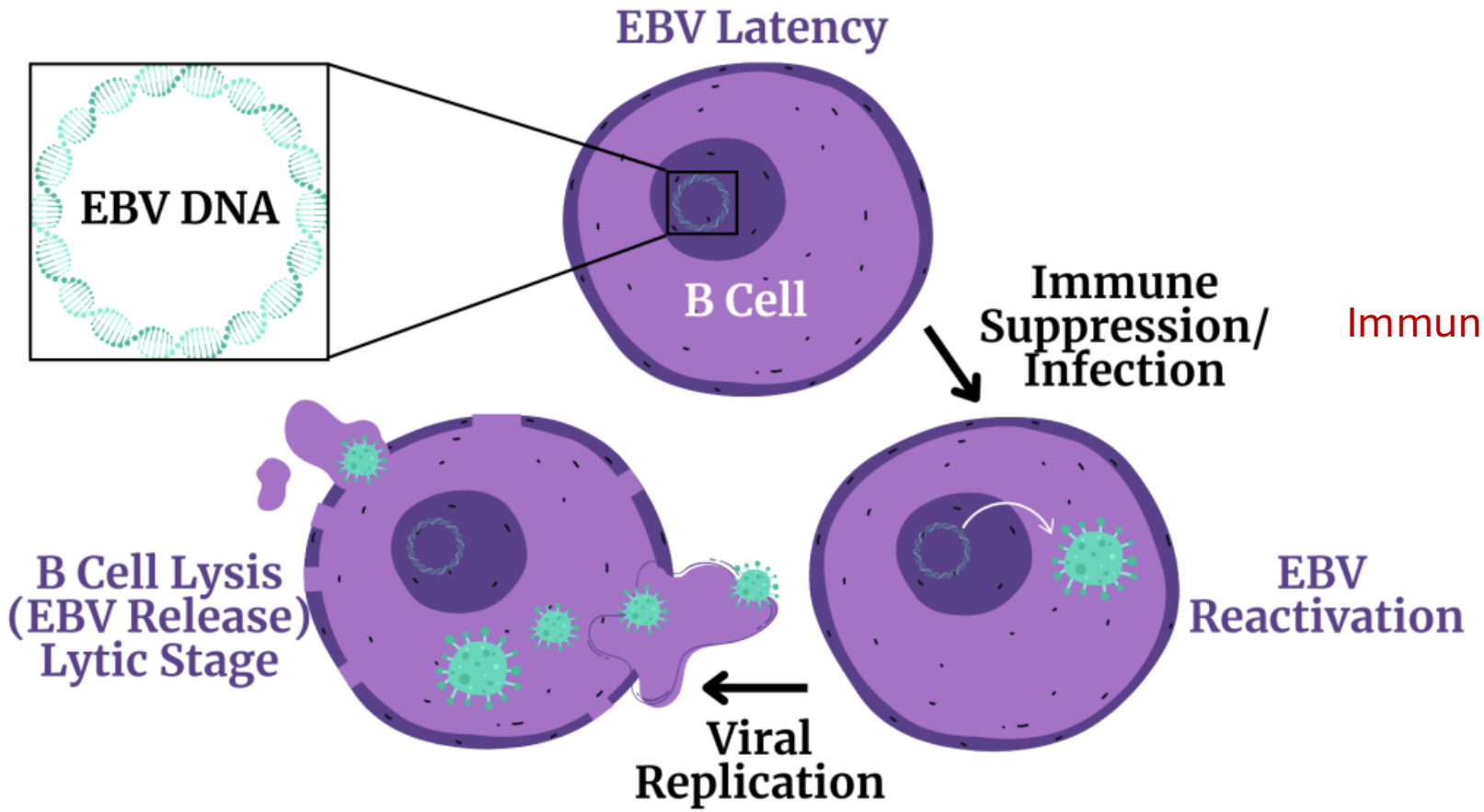

Immune Taxation?

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Front Immunol. 2024 Feb 7;15:1297994. doi: 10.3389/fimmu.2024.1297994

Epstein-Barr virus: the mastermind of immune chaos

Jean de Melo Silva [1,2], Carlos Eduardo de Castro Alves [1], Gemilson Soares Pontes [1,2,*]

Epstein-Barr virus (EBV) is a highly prevalent human herpesvirus worldwide, disseminated in 90-95% of the adult population (1–3). This ubiquitous virus was first identified in 1964 by Anthony Epstein and his team. Their discovery arose from observing herpesvirus-like particles in cultured tumor cells from African Burkitt lymphoma, a childhood cancer prevalent in Africa (4).

EBV belongs to the *Herpesviridae* family, *Gammaherpesvirinae* subfamily and is classified as human herpesvirus 4 (HHV-4) (5). It is an enveloped icosahedral virus, with an approximate size of 150nm in diameter and has a capsid formed by 162 capsomers (6). Its viral genome consists of a linear double-stranded DNA molecule of 172 kbp, which encodes approximately 100 viral proteins (6). EBV is mainly transmitted through direct contact with the saliva of infected individuals, such as kissing, sharing eating or drinking utensils. It can also be transmitted through blood transfusions and organ transplants (7).

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EBV has tropism for epithelial cells of the nasopharynx and oropharynx, as well as for B lymphocytes (8). Epithelial cells are infected when viral glycoproteins gHgL and gB interact with certain integrins (αvβ5, αvβ6, or αvβ8). B lymphocytes are infected when the viral protein gp350/220 binds to the cellular protein CD21. This is followed by the binding of three other viral proteins (gp85, gp25, and gp42) to the class II human leukocyte antigen (HLA II) (9, 10). In both cases, EBV can enter the host cell by endocytosis or fusion with the plasma membrane (8).

After insertion of the EBV genome into the cell nucleus, the virus can start its lytic cycle or enter a latency state, each with expression of different genes (11). The lytic phase begins with the expression of the *BZLF-1* and *BRLF-1* genes, which encode the transactivating proteins Zta and Rta, respectively, responsible for the course of the lytic phase of EBV (12). This cycle has three phases (early-immediate, early, and late) and generally occurs in epithelial cells, where virions are produced and the host cell is lysed (13, 14). In individuals with latent EBV infection, the lytic cycle can occur in tonsil plasma cells. These cells are mature B lymphocytes specialized in producing antibodies, also known as immunoglobulins (15).

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During the latent phase, EBV exists as an episome in the nucleus of host cells, especially B cells. This phase is divided into three latency forms (I, II, and III) and involves the expression of six nuclear antigens (EBNAs), two latent membrane proteins (LMPs), and non-coding RNAs (EBERs) (14). In the latency phase, EBV persists in the body using complex mechanisms to evade detection and elimination by the immune system, without causing symptoms (11). However, EBV can be reactivated in situations such as immunosuppression or stress (16).

EBV infection is associated with a variety of diseases, including infectious mononucleosis (IM), lymphomas (such as Burkitt lymphoma, Hodgkin lymphoma, and B-cell lymphomas in immunocompromised patients), some types of epithelial cell cancers (such as nasopharyngeal carcinoma), and certain types of gastric cancer (17–22). Evidence also suggests a link between EBV infection and certain autoimmune diseases, such as Sjögren syndrome and systemic lupus erythematosus, but the exact mechanisms of this relationship are still unknown (23).

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2.1. EBV's masterful manipulation of cell death

Apoptosis, or programmed cell death, is a cellular defense mechanism that can protect the body from infection. However, some viruses such as EBV have evolved mechanisms to inhibit apoptosis, which allows them to replicate and spread to new cells (26). EBV latent membrane proteins 1 and 2 (LMP1 and LMP2) are expressed in several types of cancer and interfere in several cell characteristics, such as cell signaling, differentiation, migration and growth, being therefore involved in tumorigenicity (27).

A recent study revealed the significant impact of EBV on B cell differentiation in the context of diffuse large B cell lymphoma (DLBCL) (28). EBV activates the gene *HLX* through the molecular conduits LMP1 and LMP2A, using the signal transducer and activator of transcription 3 (STAT3) pathway. The heightened activity of the *HLX* gene has a dual impact on key cellular processes. Firstly, it represses the expression of the pro-apoptotic gene *BCL2L11*, responsible for encoding the Bim protein—a crucial member of the Bcl-2 family. Bim protein plays a vital role in the cell death process. Additionally, the overexpression of *HLX* leads to the suppression of the *IL4R*, *NKX6-3*, and *SPIB* genes, all of which contribute to plasma cell differentiation. In the context of DLBCL, the inhibition of the *BCL2L11* gene facilitates the survival of infected B cells. This survival mechanism is pivotal in the progression of DLBCL. Simultaneously, the inhibition of *IL4R*, *NKX6-3*, and *SPIB* genes impedes B cell differentiation (28). These molecular events collectively highlight potential therapeutic targets for intervention in DLBCL. Understanding and manipulating these specific genetic pathways could offer novel approaches to combat this disease.

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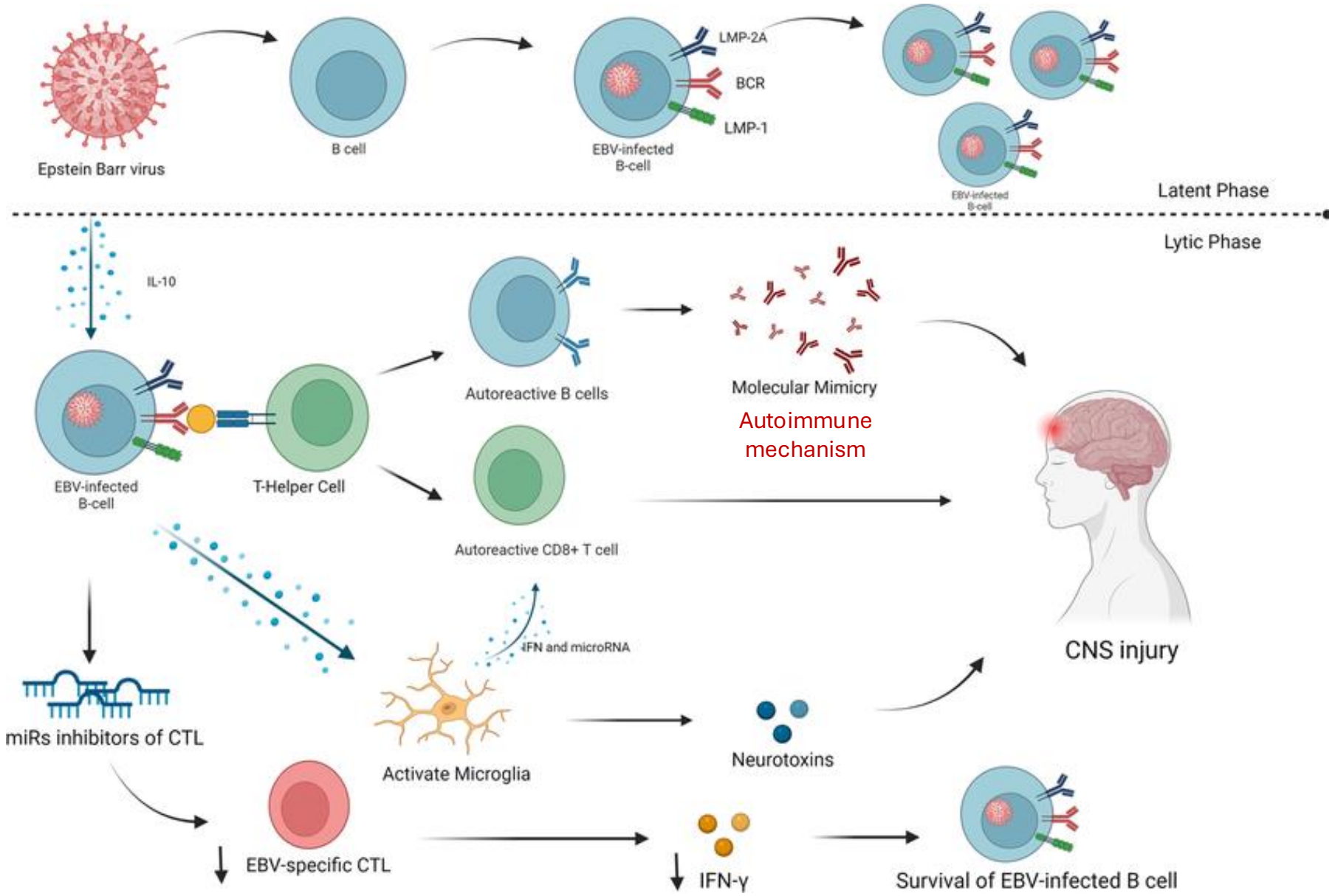

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2.2. EBV's secret weapon to promote cell proliferation

Although EBV-induced cell proliferation itself may not be considered a direct immune evasion mechanism, it becomes relevant due to its interplay with the virus's escape strategies. EBV's evasion tactics are elaborately linked to promoting cell proliferation, a process that safeguards cells transformed by the virus from elimination (24) One of EBV's secret weapons to stimulate cell proliferation is the viral protein EBNA1, capable of binding to the repeat family (FR) element in the oriP region of the viral genome. The binding of EBNA-1 to the FR element is a highly specific interaction that is essential for EBV's ability to replicate. This binding triggers a cascade of events that culminates in the expression of LMP1, a protein that promotes cell growth. Any disruption of this binding can lead to the inhibition of EBV replication (52, 53).

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PMCID: PMC10879370 PMID: 38384471

Throughout the latent phase of EBV infection, the expression of various viral genes defines distinct stages known as latencies, categorized from 0 to III based on observed patterns of gene expression (59). In this cycle, a limited number of proteins are produced, such as EBNAs and LMPs, which perform specific functions associated with EBV infection and its interaction with host cells (59, 60). These biological effects are unique and come to fruition through direct interactions with cellular proteins. For instance, in Burkitt lymphoma cells, EBERs exhibit resistance to IFN-α-induced apoptosis by inhibiting PKR phosphorylation through their direct binding (35).

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EBNA1 is a protein consistently expressed in all phases of EBV latency, except in latency phase 0 (16). Its primary role is closely associated with both the preservation of the viral genome and the control of genetic transcription (61). Furthermore, it plays a vital part in maintaining the viral genome, involving itself in DNA replication and the separation of viral chromosomes during cell division (62). Alongside these functions, EBNA1 employs various immune evasion strategies, primarily focused on suppressing MHC class I presentation in the host. This pivotal strategy substantially boosts the virus ability to evade detection by the immune system (63, 64).

EBNA2 assumes the role of a transactivating factor of viral and cellular gene expression. Predominantly expressed during the initial phases of latency, it plays a crucial role in the transition between latency I and latency III (16). Activation of viral and cellular genes triggers the transcription of a cascade of primary and secondary genes, essential for the conversion of normal B cells into immortalized cells (65). This subsequent development drives the proliferation of transformed cells, resulting in uncontrolled growth. Furthermore, EBNA2 demonstrated the ability to interact with other transcription factors involved in the Notch signaling pathway. This pathway, in particular, may be associated with the development of T-cell lymphoma in humans (66).

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LMP1 plays multiple roles in EBV pathogenesis. Notably, it downregulates pro-apoptotic genes, granting it anti-apoptotic potential and contributing to cell survival (99). Additionally, LMP1 disrupts host defenses by evading RIG-I-mediated immune responses, potentially promoting cancer progression (82). RIG-I is a crucial pattern recognition receptor in the innate immune response against viral infections. It induces cell death in virus-infected cells, triggers production of pro-inflammatory cytokines, and orchestrates other essential antiviral mechanisms (100, 101). LMP1 has been shown to upregulate the expression of PD-L1 as well. This was demonstrated in a study where EBV-positive nasopharyngeal carcinoma (NPC) cell lines displayed higher PD-L1 expression compared to EBV-negative counterparts. Notably, knocking down LMP1 in these EBV-positive cells suppressed PD-L1 expression, further solidifying the regulatory role of LMP1 in this process (102).

LMP2 is a gene expressed in various EBV-associated diseases, involved in the activation, proliferation and survival of tumor cells, and generates two transcripts: LMP2A and LMP2B (103). LMP2A is capable of reducing MHC class II expression through interference with the E47/PU.1-CIITA pathway (83). Class II transactivator (CIITA) is a master regulator of class II MHC genes and can upregulate class I MHC genes expression (104). By impairing the expression of MHC class II, EBV can avoid its recognition by CD4 T cells, allowing immune escape. EBV also encodes a lytic phase protein called viral interleukin-10 (vIL-10), which shares homology with human IL-10 and can suppress the production of pro-inflammatory cytokines (85).

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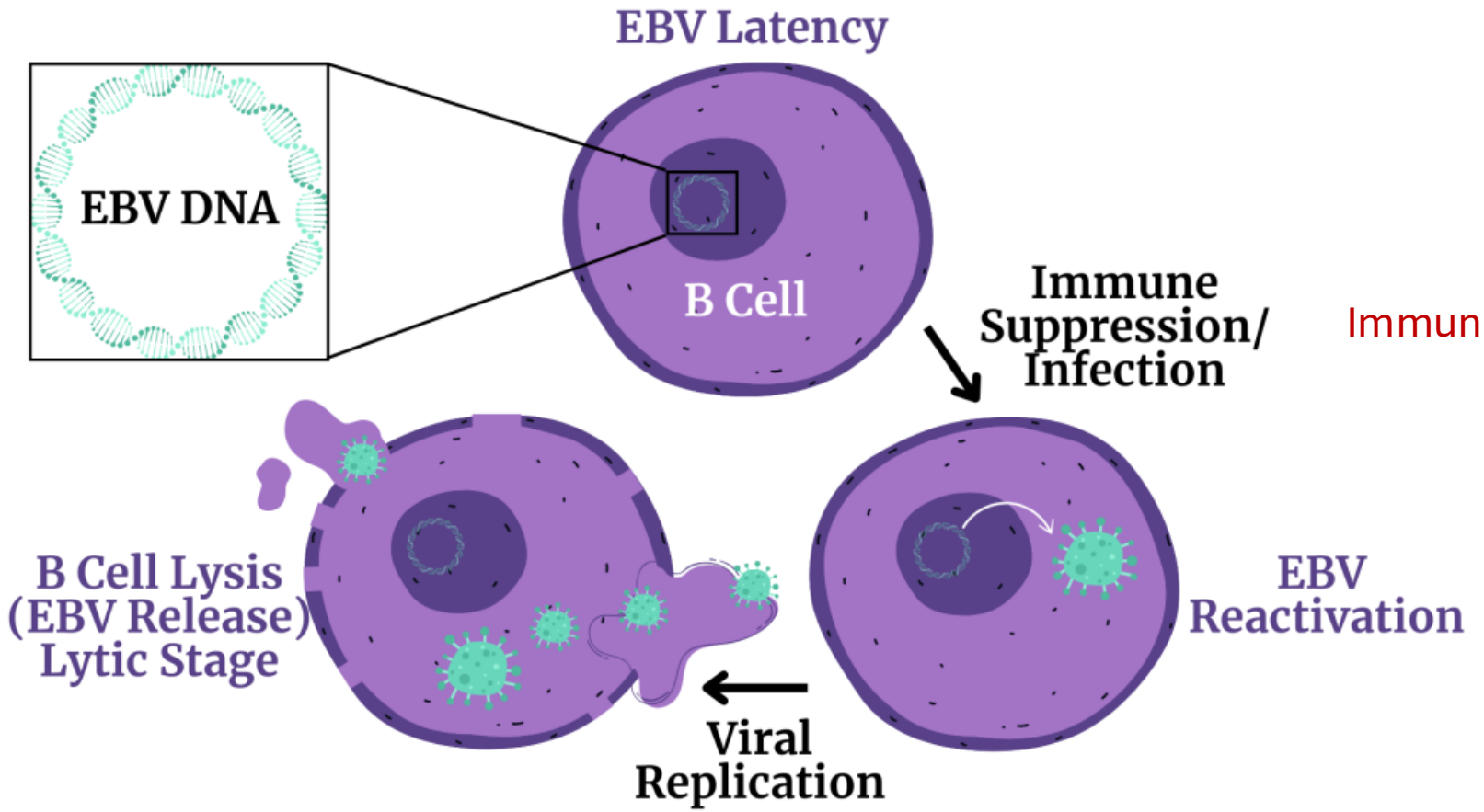

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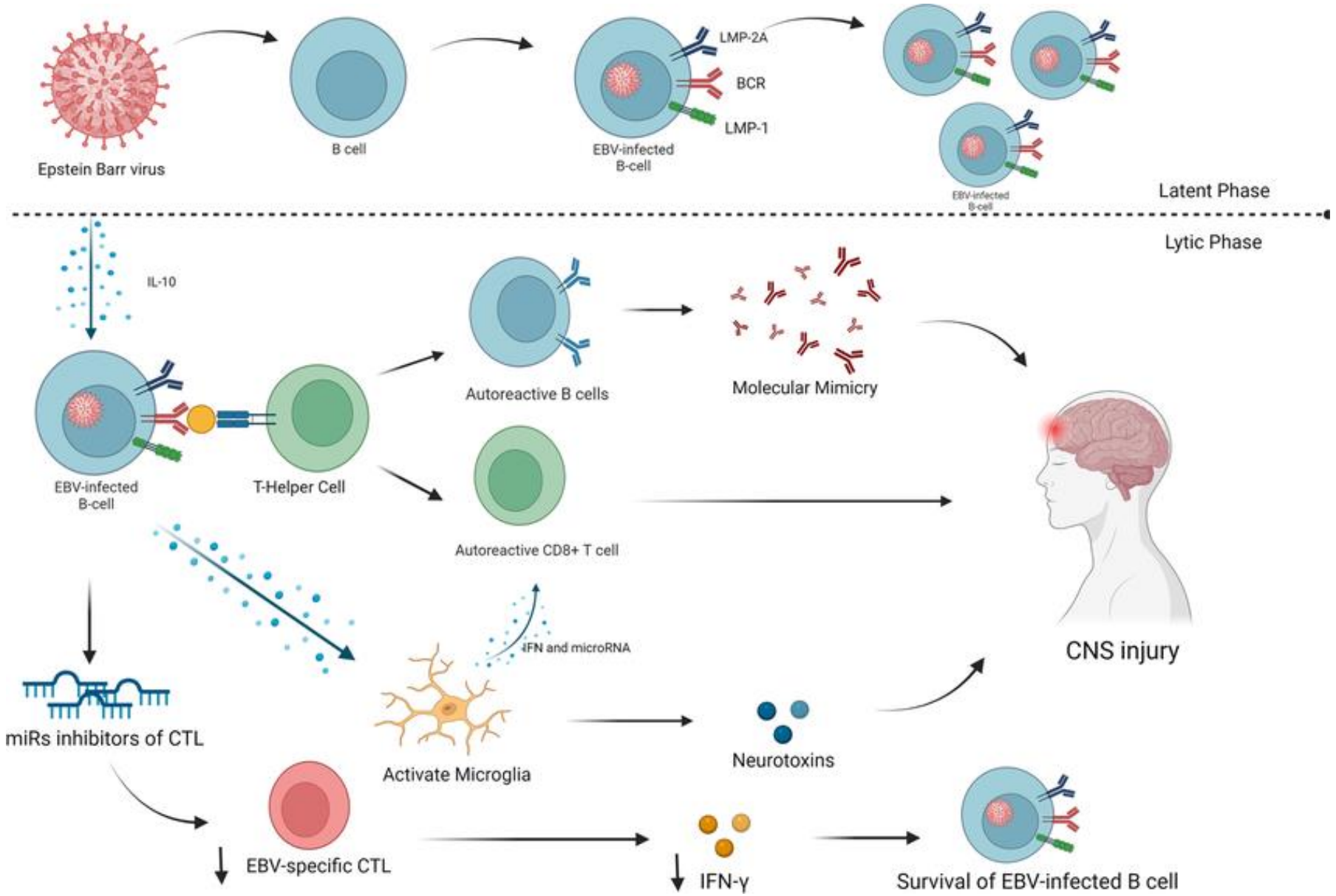

https://www.researchgate.net/publication/370980833/figure/fig2/AS:11431281160890175@1684895615926/The-mechanism-of-Epstein-Barr-Virus-induced-Nervous-System-damage-EBV-life-cycle-has-two.png

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Serology - What is being measured?

1. VCA (Viral Capsid Antigen)

- What it is: A structural protein that forms the outer shell (capsid) of the EBV particle. [1, 2]
- Antibody timeline:
    - VCA-IgM: Appears early during a primary or acute EBV infection and usually disappears within 4 to 6 weeks.
    - VCA-IgG: Develops during the acute phase of infection and typically persists for life, serving as a marker that a person has been infected with EBV at some point. [1, 2]

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Serology - What is being measured?

2. EBNA (Epstein-Barr Nuclear Antigen)

- What it is: A group of nuclear proteins expressed by the virus when it is in a latent (dormant) state inside host B cells. [1]
- Antibody timeline:
    - EBNA IgG: Does not appear immediately; it typically develops 2 to 4 months (or up to 6–8 weeks) after the initial infection starts and then persists for life. [1, 2, 3]
- Clinical meaning: The presence of EBNA antibodies alongside VCA IgG (with no VCA IgM) is the classic indicator of a past infection with established latency. [1]

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Serology - What is being measured?

3. EA-D (Early Antigen-Diffuse)

- What it is: A non-structural protein produced during the active (lytic) replication phase of the virus before new viral particles are assembled; it has a diffuse distribution in both the nucleus and cytoplasm. [1, 2]

- Antibody timeline:

  EA-D IgG: Appears during the acute phase of primary infection. In most people, it declines to undetectable levels within 3 to 6 months, though roughly 20% to 30% of healthy people may retain detectable levels for years. [1, 2, 3]

- Clinical meaning: A positive EA-D IgG often signals acute or recent active infection, or occasionally virus reactivation, though it should always be interpreted alongside VCA and EBNA markers rather than on its own. [1, 2, 3]

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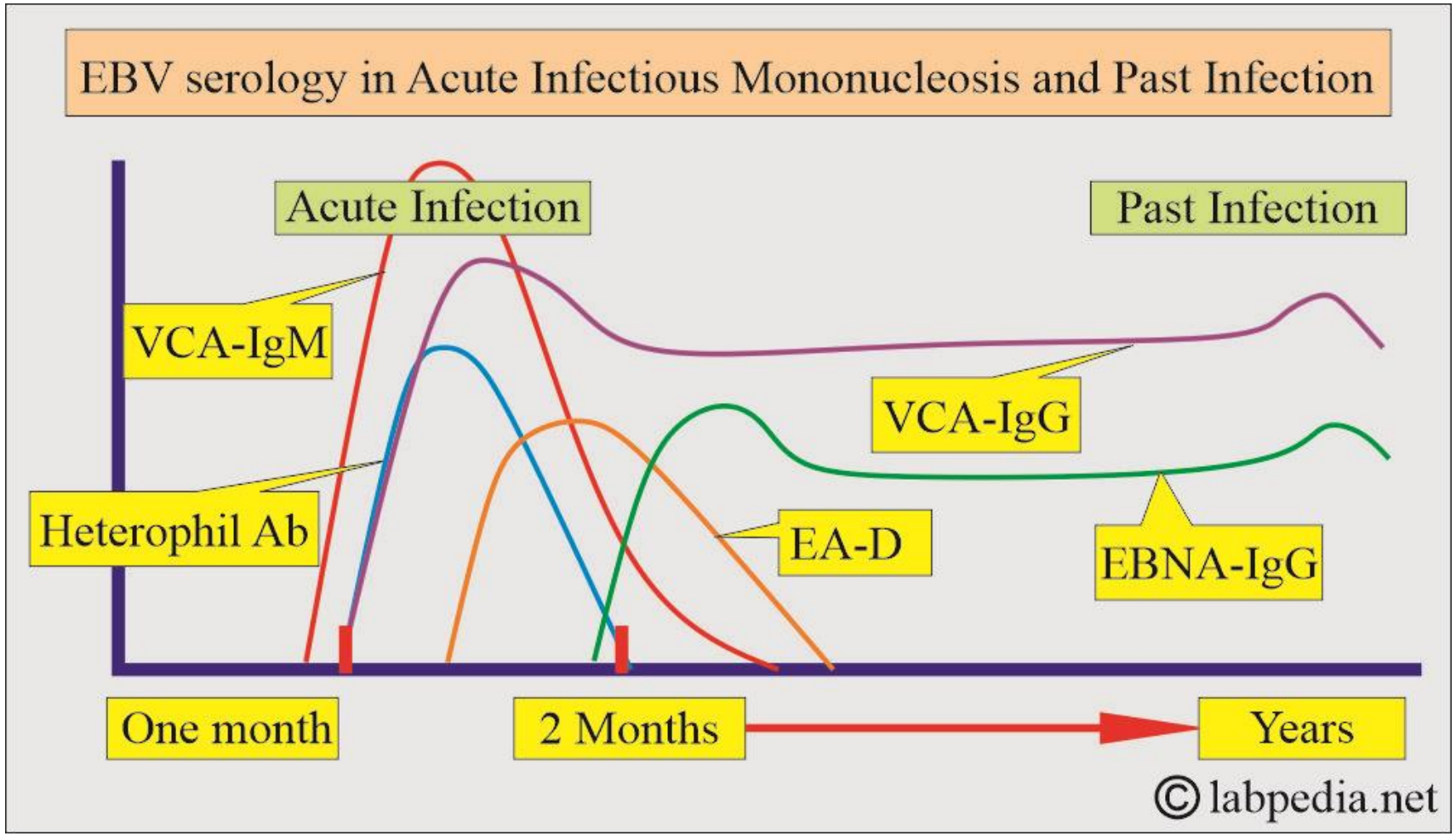
EBV serology in Acute Infectious Mononucleosis and Past Infection
Acute Infection
Past Infection
VCA-IgM
VCA-IgG
Heterophil Ab
EA-D
EBNA-IgG
One month
2 Months
Years
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Standard Interpretation Table (Mayo, CDC)

VCA IgM	VCA IgG	EBNA IgG	EA-D IgG	Most Likely Interpretation	Next Step / Notes
–	–	–	–	Susceptible (no prior infection)	Retest if symptoms are very early
+	±	–	±	Primary / recent acute infection	Classic mono pattern; supportive care
–	+	+	–	Past infection	Expected in most adults; no action if asymptomatic
–	+	+	+	Possible reactivation or persistent EA antibodies	Correlate with symptoms; EA can stay positive for years in some healthy people; consider PCR if clinically indicated
–	+	–	±	Indeterminate	Recent infection (pre-EBNA) or remote infection in the ~5–10% who never produce detectable EBNA. Repeat in 2–4 weeks or consider avidity/PCR
+	+	+	±	Indeterminate	Late primary infection, possible reactivation, or false-positive IgM. Clinical correlation ± PCR or avidity

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Type of antibodies	When detected first	Peak level	When disappearing from the blood
EA-D	5 days after S/S appear	14 to 21 days after the S/S	9 weeks after the appearance
VCA-IgM	At the start of S/S	During the first week of S/S	2 to 3 months after S/S onset
VCA-IgG	3 days after the onset of S/S	2nd week after onset of S/S	Decline and then persists for life
EBNA-IgM	At the start of S/S	During the first week of S/S	2 to 3 months after S/S onset
EBNA-IgG	3rd week after onset of S/S	8 months after the appearance	Persists lifelong
Heterophil Ab	3 to 5 days after the onset of S/S	During the 2nd week of the onset of S/S	2 to 3 months after the onset of S/S

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EBV VCA/EA Ab, IgG

Test	Current Result and Flag	Previous Result and Date	Units	Reference Interval
▲ **EBV Early Antigen Ab, IgG** [01]	**12.2** **High**		U/mL	0.0-8.9
Hepatitis A, Hepatitis C and HIV antibodies may cross-react with this assay.				
		Negative < 9.0		
		Equivocal 9.0 - 10.9		
		Positive >10.9		
▲ **EBV Ab VCA, IgG** [01]	**>600.0** **High**		U/mL	0.0-17.9
		Negative <18.0		
		Equivocal 18.0 - 21.9		
		Positive >21.9		

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Standard Interpretation Table (Mayo, CDC)

VCA IgM	VCA IgG	EBNA IgG	EA-D IgG	Most Likely Interpretation	Next Step / Notes
–	–	–	–	Susceptible (no prior infection)	Retest if symptoms are very early
+	±	–	±	Primary / recent acute infection	Classic mono pattern; supportive care
–	+	+	–	Past infection	Expected in most adults; no action if asymptomatic
–	+	+	+	Possible reactivation or persistent EA antibodies	Correlate with symptoms; EA can stay positive for years in some healthy people; consider PCR if clinically indicated
–	+	–	±	Indeterminate	Recent infection (pre-EBNA) or remote infection in the ~5–10% who never produce detectable EBNA. Repeat in 2–4 weeks or consider avidity/PCR
+	+	+	±	Indeterminate	Late primary infection, possible reactivation, or false-positive IgM. Clinical correlation ± PCR or avidity

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⚠ EBV VIRAL CAPSID AG (VCA) AB (IGG)

Analyte	Value	
⚠ EBV VIRAL CAPSID AG (VCA) AB (IGG)	323.00 H	U/mL

U/mL	Interpretation
<18.00	Negative
18.00-21.99	Equivocal
>21.99	Positive

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Standard Interpretation Table (Mayo, CDC)

VCA IgM	VCA IgG	EBNA IgG	EA-D IgG	Most Likely Interpretation	Next Step / Notes
–	–	–	–	Susceptible (no prior infection)	Retest if symptoms are very early
+	±	–	±	Primary / recent acute infection	Classic mono pattern; supportive care
–	+	+	–	Past infection	Expected in most adults; no action if asymptomatic
–	+	+	+	Possible reactivation or persistent EA antibodies	Correlate with symptoms; EA can stay positive for years in some healthy people; consider PCR if clinically indicated
–	+	–	±	Indeterminate	Recent infection (pre-EBNA) or remote infection in the ~5–10% who never produce detectable EBNA. Repeat in 2–4 weeks or consider avidity/PCR
+	+	+	±	Indeterminate	Late primary infection, possible reactivation, or false-positive IgM. Clinical correlation ± PCR or avidity

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+	819	Epstein-Barr Virus (EBV) Antibodies to Early Antigen, IgG (096248)	**$16.00**	☆
+	1105	Epstein-Barr Virus (EBV) Antibodies to Viral Capsid Antigen (VCA), IgM (096735)	**$16.00**	☆
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+	5131	Epstein-Barr Virus (EBV), Quantitative, PCR (138230)	**$167.00**	☆
+	6619	Epstein-Barr Virus (EBV) Antibody Profile (240610)	**$52.00**	☆

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EPSTEIN-BARR VIRUS (EBV) ANTIBODY PROFILE (EBV-VCA, IGG; EBV-VCA, IGM; EPSTEIN-BARR NUCLEAR ANTIGEN ANTIBODIES (EBNA))

DESCRIPTION | COMPONENTS | COLLECTION DETAILS

Clinician Pricing: $52.00

Turnaround Time: 1-2 Days

CPT Code: 86665,86664

Specimen Type: 2 mL Serum

Stability:

Temperature	Period
Room temperature	14 days
Refrigerated	14 days
Frozen	14 days
Freeze/thaw cycles	Stable x4

Overview:
Aid in determining past or present infection with EBV as well as susceptibility to future infection.

FEATURED
Individual LabCorp Tests
Individual Quest Diagnostics Tests
Evexia Panels
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