HEMATOLOGY
&LYMPH SYSTEM

Microbiology sheet

Number	1
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Epstein-Barr Virus

-Please pay attention that the order of ideas in this sheet is a little bit different from that in the recording.

Review:

Viruses divide according to their genome into: DNA viruses and RNA viruses. The main DNA viruses include: adeno virus, hepatitis B, herpes viridae, polyoma virus, human papilloma virus, pox virus and parvovirus (1 a, 2 h, 4 p).

Our focus today is Epstein-Barr virus which is part of the family "herpes viridae".

Herpes viridae family includes eight genera: herpes simplex 1, herpes simplex 2, varicella zoster, **Epstein-Barr virus**, cytomegalovirus, herpes virus 6, herpes virus 7, and kaposi sarcoma virus

Epstein-Barr Virus

Epstein-Barr virus is an enveloped linear dsDNA virus, it has one of the largest genomes and expresses many proteins, characterized by Acute stage and latent stage.

Latency: the virus is present in the body but it is hidden somewhere (**dormant**), the latent virus is hidden in the form of **episome***, it **expresses the early proteins only**.

Note: the active Epstein-Barr virus expresses more than 200 proteins while the latent expresses 11 proteins only.

*(episome is an extrachromosomal circular dsDNA>> the main rule.
Exception: it can be integrated into the cellular genome [integration is more linked to malignancy]).

Epstein-Barr virus is associated with multiple malignancies yet it still an episome (extrachromosomal).

Epstein-Barr virus causes a disease called **infectious mononucleosis (IM) or called kissing disease** because it spreads by transfer of saliva (oral secretions) during kissing and other fluid secretions (blood, semen), so it is usually a disease of young adults and it spreads between colleagues (students) .

Epstein-Barr virus (EBV) belongs to gamma herpesvirinae subfamily (γ-herpesvirus).

EBV membrane is derived by budding of immature particles through cell membrane and is required for infectivity.

EBV uses the cell receptors CR2 and CD21 to enter into the B cell.

Epidemiology

Epstein-Barr virus infects any age group. By adulthood, more than 90% of individuals have been infected and have seroconversion (detectable antibodies in the blood). Most of the infections has occurred during childhood why? Children have poor hygiene, the habit of kissing children by adults.

Epstein-Barr virus is mainly transmitted by salivary secretions. Transmission by fomites contaminated with wet saliva is also feasible. In addition, it has been transmitted by transplanting organs or bone marrow transplantation.

if the patient has latent EBV, it will reactivate when you give him an immunosuppressive drug after the transplantation.

Pathogenesis

There are two sites of infection one is primary (symptomatic one) and the secondary (where the virus keeps dormant or hides).

ex. In HHV-1 &2 the primary site of infection is the skin then it becomes dormant in the dorsal nerve ganglia

The target cells for the Epstein-Barr virus:

Primary site of infections (acute period): epithelium of the oropharynx and the salivary glands

Secondary site of infections (latency period): in memory B cells

Note: **Cellular immunity is more important than humoral immunity in controlling EBV infection, why?**

Humoral immunity: production of antibodies, it can neutralize the circulating viruses within bloodstream only.

So, if the virus managed to enter the cell, it can replicate no matter how many antibodies circulating. So, we need cellular immunity to kill this infected cell in order to break the virus replicating cycle.

“cellular immunity is more potent in fighting EBV than the humoral immunity, because the antibodies are able to bind to the glycoprotein of the virus and neutralize them but when the virus become within the cell , it is more efficiently for T cells to work- mainly cytotoxic T cell”

What will happen if the t-cell immunity was compromised?

The virus will enter the B cell and start to proliferate and the cell becomes immortal, so the virus will continue proliferating and controlling over the cell, then all repair mechanisms in the cell will be impaired. Thus, the cell becomes malignant.

Presentation of the infection:

in children: is most of the time asymptomatic

in adults: it could be primary infection or reactivation.

Symptoms: divides into

•non-specific symptoms which occurs in the first week include: arthralgia (الم مفاصل), myalgia (الم عضلات) ,headache.

•specific symptoms start to appear after 2-3 weeks such as: fever (low grade may persist for 1 month.), enlarged lymph nodes (specifically in posterior cervical lymph nodes but may be generalized), **sore throat** (pharyngitis***), reversible hepatosplenomegaly*****. These are the presentation of IM.

***note: in general pharyngitis caused by viruses are non-exudative (i.e: no pus). However, Epstein-Barr virus and adeno virus both lead to exudative pharyngitis.

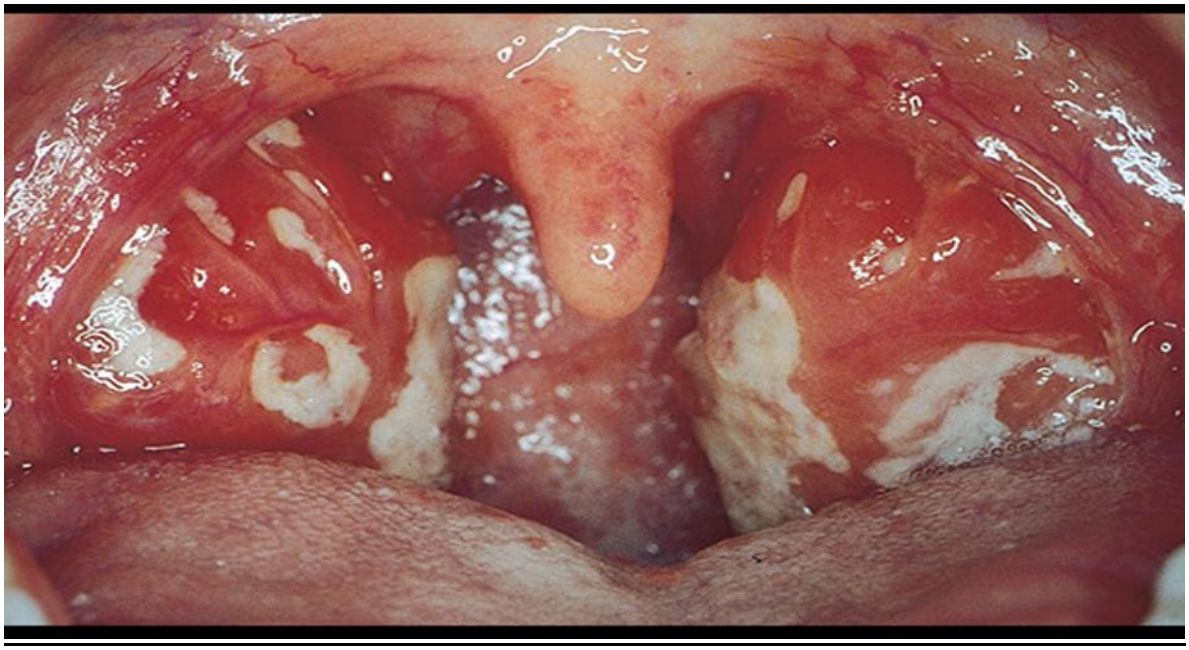
Note: 90-95% of the exudative pharyngitis is caused by bacteria

Exudative pharyngitis is seen as patches of white dots covering the tonsils and the pharynx

So, if a patient came to your clinic and you found that he has exudative pharyngitis, how are you going to deal with it?

The first thing you must do is to prescribe an antibiotic. **Why?** Because most of exudative pharyngitis is caused by bacteria (The most common and important bacterial cause of pharyngitis is Streptococcus pyogenes (group A

Streptococcus [GAS]), also we have to be aware of its complications especially in children which are: glomerulonephritis and rheumatic fever.

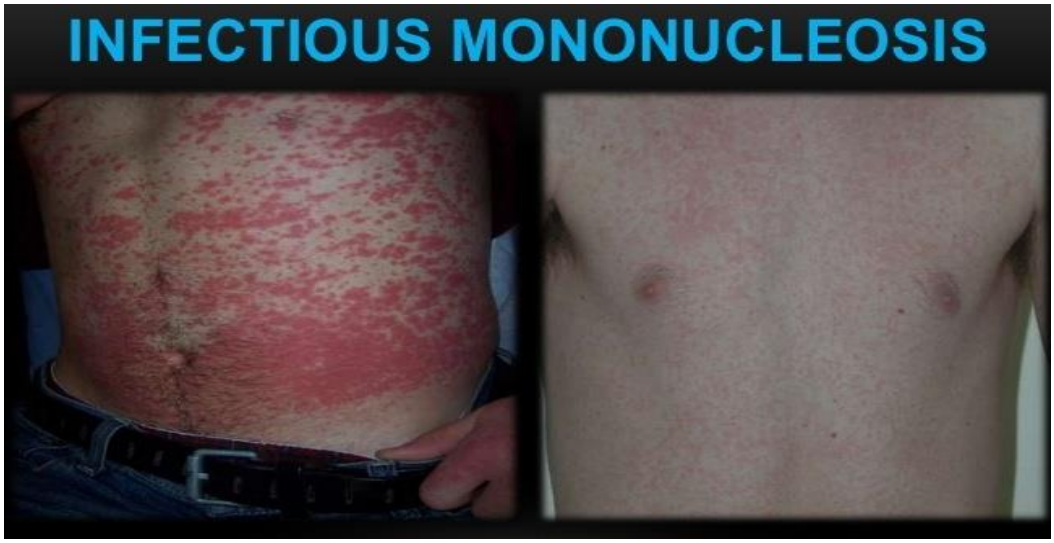


Exudative pharyngotonsillitis 1

A morbilliform or papular rash, usually on the arms or trunk, develops in 5% of cases after treatment with ampicillin, this rash isn't because of penicillin allergy (i.e. this rash is not predictive of future adverse reactions to penicillins), but it is a side effect of treatment with ampicillin. So, if this patient came to you again having follicular tonsillitis (bacterial) you should do penicillin allergy test for him before you prescribe it.

Note: 80-90 of tonsillitis and pharyngitis are caused by viruses. So, if a patient came to your clinic with tonsillitis or pharyngitis, we don't give antibiotics in this case, instead we treat the symptoms only.

*****note: because this virus infects young adults who are usually active all the time, if you found a patient with splenomegaly, you should advise them not to do intense sports and physical exercises because the spleen will rupture and the patient must get splenectomy.



rash after treatment with amoxicillin 1

Diseases Associated with EBV

In B Cell: Infectious mononucleosis , X-Linked Lymphoproliferative Disease, Hodgkin Disease, Burkitt Lymphoma, Lymphoproliferative disease, and *chronic active EBV.* *The last one is found in slides but the doctor didn't mention it.*

In other cells: Nasopharyngeal carcinoma, gastric carcinoma, Peripheral T cell lymphomas, Oral hairy leukoplakia, Smooth muscle tumors in transplant patients, *Nasal T/NK cell lymphomas.* *The last one is found in slides but the doctor didn't mention it.*

Infectious Mononucleosis

The incubation period for IM in young adults is 4 to 6 weeks.

Presentation of the disease is mentioned in page3.

Burkitt's Lymphoma

Burkitt's lymphoma (BL) occurs endemically in parts of Africa especially in areas where malaria is endemic. Therefore, it appears that malaria infection is a cofactor. So, if you treated malaria or decreased its incidence, you would decrease the incidence and prevalence of BL.

Multiple copies of EBV genome and some EBV antigens can be found in BL cells, and patients with BL have high titres of antibodies against various EBV antigens.

BL cells show a reciprocal translocation between the long arm of chromosome 8 and chromosomes 14, 2 or 22.

In theory BL can be controlled by the eradication of malaria or vaccination against EBV. No vaccines are available until now, but there are clinical trials targeting one of the EBV antigen "gp 350". It was found to be affective in dropping the number of IM cases but not in prevention against EBV infection.

Epidemiology: EBV isn't the only cause for Burkitt's lymphoma (BL). However, in the developing countries they found that 90% of BL cases were linked to EBV. In the developed countries they found that 20% of BL cases were linked to EBV.

AIDS also plays a role in Burkitt's lymphoma (BL).

Presentation: jaw tumors which leads to dysmorphic lesions.

Treatment: chemotherapy, with chemotherapy the dysmorphic features will be reversed.

B-Cell Lymphoma

In most individuals infected with EBV, the virus is present in the B-cells, which are normally controlled by T-lymphocytes.

When T-cell deficiency exists, one clone of EBV-infected B-lymphocytes escapes immune surveillance to become autonomously proliferating malignant cell.

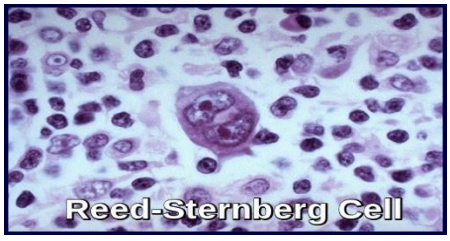
EBV induced B cell lymphomas are most prevalent in immunocompromised patients.

Hodgkin Disease

Hodgkin disease is linked to EBV in 60-70% of the cases in the developing countries and 35-50% in developed countries.

Therapy: Chemotherapy, radiation, Anti-EBV CTLs is effective in some cases.

EBV can be seen in Reed-Sternberg cells (so, it can be diagnosed histologically)



Nasopharyngeal Carcinoma

Nasopharyngeal carcinoma (NPC) is a malignant tumour of the squamous epithelium of the nasopharynx. It is very prevalent in Southern China.

Multiple copies of EBV genome and Epstein Barr nuclear antigen 1 (EBNA-1) antigen can be found in cells of undifferentiated NPC.

Patients with NPC have high titers of antibodies against various EBV antigens

Besides EBV there appears to be a number of environmental and genetic cofactors in NPC.

NPC usually is diagnosed in late stage of malignancy and thus the prognosis is poor.

In theory NPC can be prevented by vaccination.

Immunocompromised Patients

Please refer to slide 22, the doctor just mentioned “transplant recipients, AIDS patients, Duncan X-linked lymphoproliferative syndrome” without any explanation.

Molecular Biology

During replication

• Viral capsid antigens (VCAs), which include VCA igM and VCA igG.

• EBV early antigens (EAs): consist of more than 15 proteins codes by genes distributed throughout the genome.

• EBV nuclear antigen (EBNA): corresponds to six virally encoded proteins found in the nucleus of an EBV-infected cell.

•More than 100 gene products may be expressed during active viral replication

During latency:

Latently infected B cells are the primary reservoir of EBV in the body.

Only 11 gene products are expressed during viral latency.

Diagnosis

•Acute EBV infection is usually made by the **heterophile antibody test**, and/or detection of anti-EBV VCA IgM.

The concept of heterophile antibodies test: you bring the serum of the patient which has the antibodies and then add a horse RBCs, if it agglutinates (the horse RBCs) then it is positive for heterophile antibody.

Heterophile antibody: are IgM antibodies which agglutinates sheep/horse red blood cells. generated by the human immune system against EBV.

• Cases of Burkitt's lymphoma and Nasopharyngeal Carcinoma should be diagnosed by histology.

• **The determination of the titre of anti-EBV VCA IgA in screening for early lesions of NPC and also for monitoring treatment**.

Note: IgA is seen in secretions, so the secretions of EBV's patient have elevated levels of IgA against EBV capsid.

• A patient with non-specific ENT (ear, nose, and throat) symptoms - especially in areas like S. China (where there are high numbers of NPC) - represented with positive heterophile antibodies; you refer him to ENT for further check up (Nasopharyngeal carcinoma possibility).

Infectious Mononucleosis Lab Findings

• increase in the white blood cell count (10,000 to 20,000/L).

• Lymphocytosis with >10% atypical lymphocytes.

Atypical lymphocytes are enlarged lymphocytes that have abundant cytoplasm, vacuoles, and indentations of the cell membrane.

• Low-grade neutropenia and thrombocytopenia

• Liver function is abnormal.

• Serum levels of aminotransferases and alkaline phosphatase are usually mildly elevated, which indicates hepatocellular injury.

• heterophile antibodies titres rise during the first week (50% chance to see them in the first week) and rise more in the second and third week (60-90%

chance). The level of antibody gradually declines and sometimes it might linger for four to six months up to a year or more (in case of elevated titres).

• Heterophile antibody most commonly used in the serological diagnosis of IM.

Time course of antibody production

• early antigens (EA) is rising at symptom onset: rise for 3-4 weeks, then quickly decline to undetectable levels by 3-4 months.

• VCA-IgM usually is measurable at symptom onset, peaks at 2-3 weeks, then declines by 3-4 months.

• VCA-IgG rises shortly after symptom onset, and persists for life.

Note: IgG is the one responsible for long term immunity. So, when you give any vaccine, your target is to elevate the IgG to a protective level.

• EBNA rises when the patient enters the recovery state or convalescence state, and remain present for life.

Q: a patient has been previously infected a year ago, what are the main markers (antibodies) that we can find? VCA-IgG and EBNA.

Note: if the patient came to you at the beginning of the symptoms, you might not find the VCA-IgG, EBNA or the EA (because it rises at 3-4 weeks), but you'll **surely** find VCA-IgM.

Note: VCA-IgG and EBNA are seen in recovery state.

Note: VCA-IgG might rise with symptoms.

Infection	VCA IgG	VCA IgM	EA(D)	EBNA
No previous infection	-	-	-	-
Acute infection	+	+	+/-	-
Recent infection	+	+/-	+/-	+/-
Past infection	+	-	+/-	+

• No previous infection: we have seronegative (No antibodies against EBV)

• in Acute infection: definitely you will find VCA IgM . The EA might not be seen, but later (3-4 weeks) it's going to be there. VCA IgG also rise at the time of infection so you can find it. EBNA is not found.

• in Recent infection (about 2 months after): you'll find VCA IgG. EBNA, VCA IgM and EA might be found or might not.

• in Past infection: VCA IgG and EBNA are positive, VCA IgM are negative. EA might be found or might not depends on the stage.

Treatment

• Therapy for IM consist of supportive treatment. Rest and avoidance of intense sports (because we are afraid of splenic fractures).

• Acyclovir has been effective for the treatment of oral hairy leukoplakia

• post transplantation EBV lymphoproliferative syndrome generally does not respond to antiviral therapy. When possible, therapy should be directed toward reduction of immunosuppressive drugs. ("i.e: balancing the dosage of the immunosuppressive drugs in a way not to cause reactivation of the virus or give the immune cells the opportunity to kill transplanted cells")

• Interferon

• antibody to CD20

• Infusions of donor lymphocytes are often effective for stem cell transplant recipients.

• Infusions of EBV specific cytotoxic T cells.

• The isolation of patients with IM is unnecessary.