Hematology

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- lecture number :
  1- Viro, EBV
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EPSTEIN BARR VIRUS

EBV

Before you start :

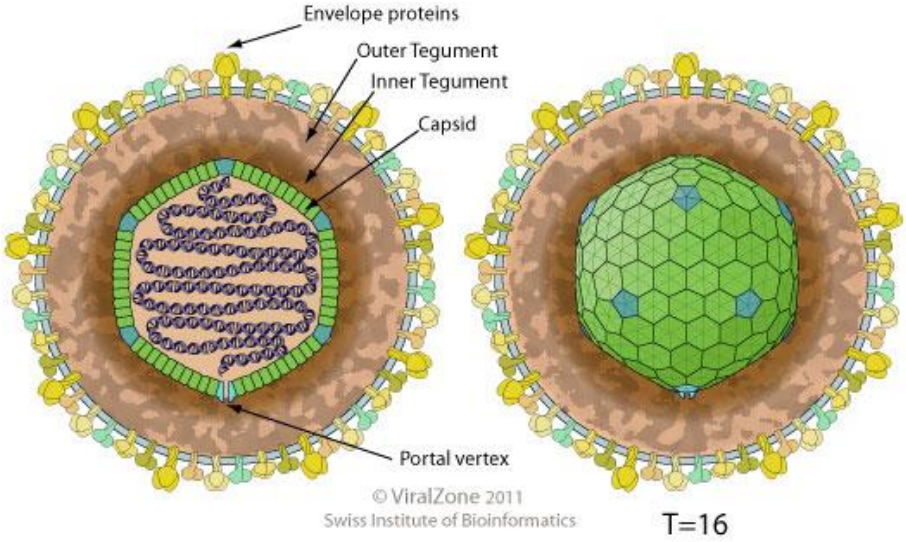
- **This sheet was made according to section 1 and 2**
- **There are some additional information from the slides but not all of them**
- **Some abbreviations (ag : antigen , Ab : antibody)**

Epv virus belongs to the herpesviridae family , it is linear double stranded DNA virus , it is enveloped virus .

There are eight genera in the herpesviridae family which are :

1- Human herpes virus 1 (HHV-1) : herpes simplex type 1
2- Human herpes virus 2 (HHV-2) : herpes simplex type 2
3- Human herpes virus 3(HHV-3) : varicella zoster which causes chickenpox in children and herpes zoster (shingles) in adults
4- **Human herpes virus 4(HHV-4) : Epstein – barr virus**
5- Human herpes virus 5(HHV-5) : cytomegalovirus
6- Human herpes virus 6 and 7(HHV-6 and HHV-7) : Human Roseolovirus which cause roseola infantum .
7- Human herpes virus 8 (HHV-): Kaposi virus

It has large genome , it encodes from 75-200 viral encoded proteins or more , and the similarity between different herpesviridae genera is between 50 – 70 % (the difference between them is in the genome but all of them have similar structure) .



The doctor points to the parts of the virus : it is an enveloped virus containing spikes of glycoproteins . the genome encodes the capsid which is icosahedral , this capsid is wrapped in a protein layer called the tegument containing viral proteins and enzymes which lies between the envelope and the genome .

HHV replicates in the nucleus .

General rule :

- All of the DNA viruses replicates in the nucleus except the pox virus which replicates in the cytoplasm due to its large size .

- All the RNA viruses replicates in the cytoplasm except the orthomyxovirus (ex. influenza virus) and the retrovirus (ex. HIV)

<u>There are 3 subfamilies in herpesviridae family :</u>

Subfamily: Alphaherpesvirinae : contain HHV-1 , HHV-2 , HHV-3.

Subfamily: Betaherpesvirinae : contain HHV-5, HHV-6, HHV-7

Subfamily: Gammaherpesvirinae : contain HHV-4, HHV-8

It is characterized by acute pattern of infection followed by latent period of infection , which means that the infection of HHV virus begins as acute infection which is mostly symptomatic (sometimes it could be asymptomatic and then it is called sub-clinical infection) , then at the latency period the virus goes and hides or seeks latency . this process differs between the eight genera .

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

Examples of viral tropisms (refers to which cell types Herpes viruses infect) :

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

(ex.in EBV the primary site of infection is the epithelium of the oropharynx and B lymphocytes but the secondary site of infection is B lymphocytes only)

What are the characteristics of the latency period ,and what are the conditions IN this period ?

- The virus becomes an **episome** (it resembles the plasmid of the bacteria which is extrachromosomal circular DNA)

- Controversial issue :about the transcription from episomes , some books say that there is only transcription of the early proteins from the episome and the others say that there is translation of the early proteins only . (here the doctor says that it is more probably that there's translation of the immediate early proteins) . (I think that the doctor means that there are 2 opinions the first one says that there is only transcription to form the RNA of the immediate early proteins AND the second opinion is that there is transcription and translation of the immediate early proteins)

- there are three stages of the production of the virus proteins : the **first one** : (immediate early phase) where DNA replicases and trascriptases are synthesized , the **second phase** : (early phase) is the phase of synthesis of other non structural proteins like : thymidine kinase and polymerases and the **third one** : (late phase) is the process of synthesis of structural proteins (ex : capsid)

How does the virus become reactivated (from the latent phase to the active phase) ?

Because the virus will not be totally removed from the body ,many factors can affect the immunity during the time at which the virus is latent which result in reactivation of the virus , these factors include : aging , sunlight , fever, infection and stress . All of these can cause reactivation of the dormant (latent) herpes virus , ex. in case of low immunity in young ages HHV-3 (varicella zoster) causes chicken pox but later on in the fifties and sixties it will return back again as shingles -الحزام الناري

There are two theories that illustrate the process of reactivation of the dormant virus :

- **the first one** : in the latency phase where the virus replicates at very low pace and when the cell becomes overcrowded with the virus it release them through the axons to the dermatomes (superficial innervations of the skin) (the doctor says : this is not the strongest theory because the virus become episome in the latent period and there is no replication in that phase we only have transcription and translation of the immediate early proteins) .

- **the second one** : in case of low immunity ,and due to changing in the biochemical environment inside the cell the virus can reactivate and replicate ,then the virus will be able to travel through the axons and reach the dermatomes .That include HHV 1,2,3 where the primary site of infection is the skin and the secondary is the dorsal nerve ganglia .

Now let's talk about EBV :

- **Primary site of infections :** epithelium of the oropharynx and B - lymphocytes
- **Secondary site of infections** (latency period) : B lymphocytes

The process of protein synthesis contains the immediate early , early and late which give the major structural proteins of the capsid .

The EBV virus can cause many kinds of diseases like : burkitt lymphoma , hodgkin lymphoma ,nasopharyngeal carcinoma and oral hairy leukoplakia , AIDS-Related cancers and Post-transplant Lymphoproliferative Disorders interstitial pneumonitis .

The virus genome encodes the enzyme thymidine kinase which is not found in humans , this enzyme plays a role in the replication of the genome of the virus and it is specific to that virus so when we find a specific viral enzyme then it is consider a target of antiviral drugs for treatment . for ex. Acyclovir (antiviral drug) it is prodrug that resemble the structure of the thymidine , once TK enzyme phosphorylate it , it will become active and blocks DNA synthesis of the virus , so our plan in viral infection treatment is to produce antiviral drugs that target viral components in the infected cells while sparing body cells that are not infected by the virus .

Epidemiology :

2 peaks of infection are seen : first peak in early childhood and the second peak is in late adolescence .

Higher rate in the developing compared to the developed countries .

In the developing countries : by the age of five 80-90 % of the children will have sero-conversion or become sero-positive (which means that the body sensitize the antigen and produce Ab against it by humoral immunity) .

Infectious mononucleosis , mononucleosis or mono(kissing disease) :

a disease is caused by EBV , usually it is a disease of young adults , is transmitted frequently from asymptomatic adults to the infants through the transmission of saliva during kissing (by oral secretion) , it spreads more between young adults or colleagues (students) , that happens because the primary site of infection of that disease is the epithelium of the oropharynx , which enable the virus transmission through the saliva after releasing the virus , EBV has been transmitted by blood transfusion and bone marrow transplantation , more than 90% of the asymptomatic seropositive patients shed the virus in the oropharynx secretion . Transmission by indirect contact is feasible – it has to be in a direct manner – so fomites rarely transmit the infection .

Routs of transmission of the viral infections :

- Feco-oral : by contamination of the food by fecal matter containing the virus and accidental ingestion of that food such as : rota virus
- Inhalation : through droplets
- Direct : through blood
- Fomites : direct contact with patient belongings : mugs , towels, teeth-brushes .

EBV get its envelope from the **nucleus** in CONTRAST WITH all of the other viruses that they take their envelope from the membrane of the cell .

EBV can integrate its genome with the cellular DNA ,integration of the genome means that it becomes a part of the cellular DNA . HIV can integrate to the human genome too .

there are 2 strains of the virus : strain A and strain B .The cellular receptors of the virus are : CR2 and CDR21

how does the immune system deal with the virus ?

B memory cells are the main reservoir of the virus , cellular immunity is more potent in fighting EBV than the humoral one , because the ab mainly work in the extracellular compartment and they are able to bind to the glycoprotein of the virus but when the virus become inside the cell , it is more efficiently for T cells to work- mainly cytotoxic T cell which can sense it by CD4 which recognize MHC1 that is complexed with the viral antigens and the TCR which recognize the Ag ,then it will secret enzymes and perforins to kill these cells , in case of low immunity the virus start to reactively proliferate due to metabolic changes in the infected cell especially if there is deficiency in cellular immunity, so B cells will become immortal in vitro and in vivo .

pathogenesis : (addition from the slides)

- EBV is transmitted by salivary secretions.

• The virus infects the epithelium of the oropharynx and the salivary glands and its shed from these cells.

• The virus then spreads through the bloodstream.

• Data suggest that memory B cells, not epithelial cells, are the reservoir for EBV in the body.

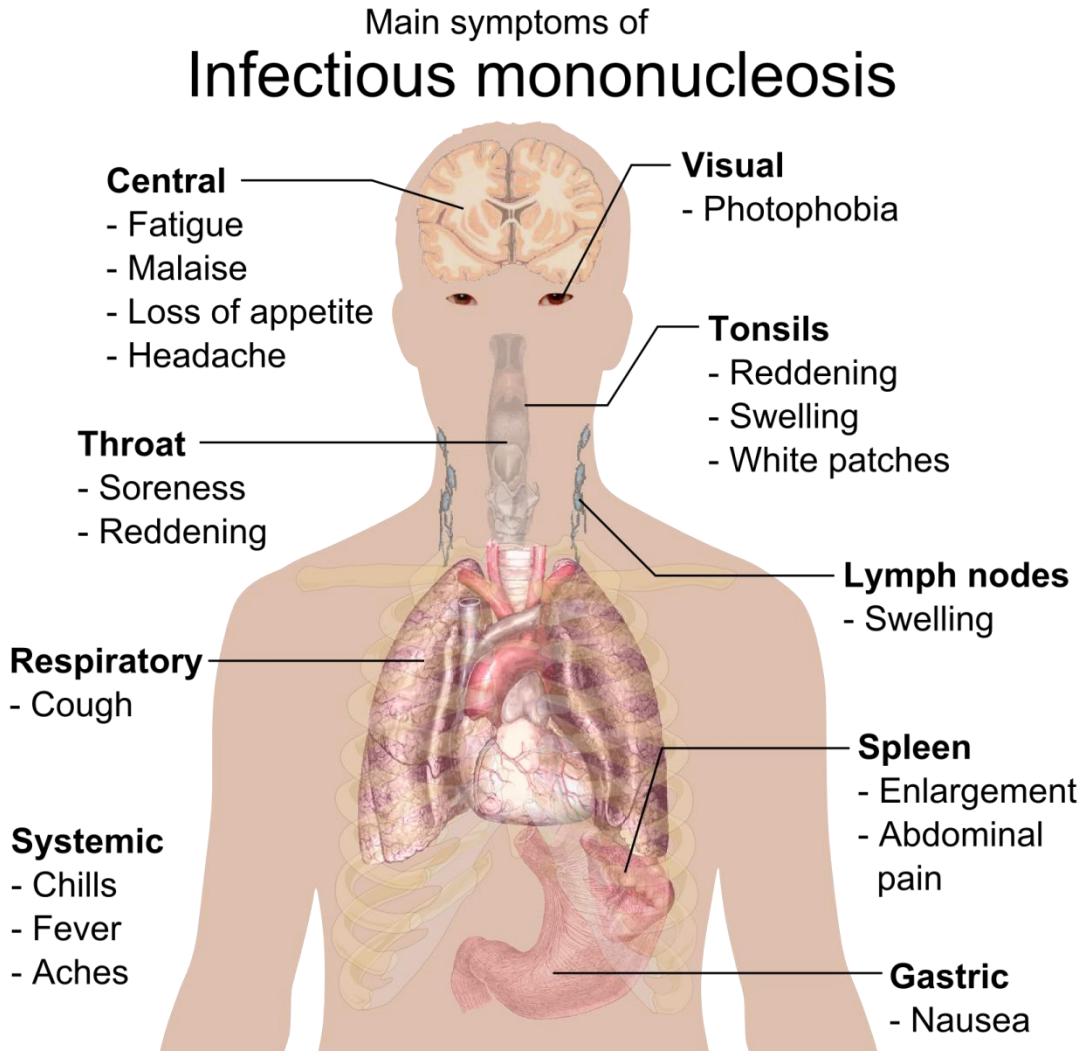
• Cellular immunity is more important than humoral immunity in controlling EBV infection.

• If T cell immunity is compromised, EBV-infected B cells may begin to proliferate.

• EBV is able to immortalize B-lymphocytes in vitro and in vivo

Symptoms :

malaise , myalgia , fatigue , fever , sore throat , nausea , headache, pain , swelling of the lymph nodes and photophobia so all of these symptoms are generalized non specific symptoms ; they are common in many other viral infections , these symptoms can persist from 2 weeks to one month .



infection in children : it is most of the time subclinical but they could be symptomatic (general symptoms)

infection in adults : it is more pronounced and there are more specific symptoms such as : pharyngitis, fatigue , lymphoadenopathy(swollen glands) most commonly appear around the back of the neck (mainly posterior cervical lymph nodes) and sometimes throughout the whole body, enlargement of the liver and spleen (those patients with EBV-associated splenomegaly should reframe from direct contact with animals ,otherwise they will end up with splenic rupture) . The most common associated symptom is pharyngitis which can be accompanied by tonsillitis with exudate (pus) (exudative pharyngo-tonsillitis) similar to streptococcus pharyngitis . since the infection can mimic streptococcus pharyngitis

doctors can sometimes prescribe amoxicillin , EBV-infected people experience rash if they are treated with amoxicillin .

associated diseases :

burkitts lymphoma :

occur in a high rate in Africa , it is restricted to the areas where malaria is endemic , some EBV Ags can be present in burkitt lymphoma patients . the tumor appear in the jaw in 90% of the cases in the developing countries, 20% of US cases are children with abdominal tumors and tumors of the lymph nodes which occurs in immunocompromised patients

this tumor is very sensitive to chemotherapy and within a short period of time the patient can recover (4 – 6 days of chemotherapy)

B-cell lymphoma :

In most individuals who are infected with EBV , the virus is present in the B lymphocytes and is controlled by T –lymphocytes .

When T-cell deficiency exist , one clone of the B-infected cell will escapes immune surveillance to become autonomously proliferating

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

Hodgkin disease :

60-70% of cases occur in developing countries , treatment is through chemotherapy or radiotherapy .

Nasopharyngeal carcinoma :

It is a malignant tumor of the epithelium of the nasopharynx , rare in most parts of the world , multiple EBV parts or nuclear Ag of the virus can be found in the undifferentiated cells of NPC . those patients have high titer of Abs against many Ags of the virus .Can be prevented by vaccination.

In Immunocompromised patients ,after the infection with EBV , we can see steady increase in the infection , and that will shift the patient to have lymphoproliferative lesions , which tend to develop to an extent that lead to extra-nodal lesions in GI & CNS .

Latency followed by immunosuppression leads to activation of the virus:

- transplant recipients: renal- EBV would be affected by lymphoma ,
- AIDS patients :EBV is associated with oral leukoplakia and non-Hodgkin lymphoma .

Ebv has certain Ag that the immune system produce Ab against them :

- Viral capsid Ag (VCA) : which is the Primary capsid protein that is formed in replicating cells .
- EBV early Ag (EA)
- EBV nuclear Ag (EBNA)

Diagnosis :

Acute EBV infection is usually made by heterophil antibody test and/or by detection of IgM antibodies against EBV VCA

Burkitt lymphoma : histological diagnosis

Nasopharyngeal carcinoma : histological diagnosis

- The DETERMINATIN OF THE TITER OF anti-EBV VCA IgA in screening for early lesions of NPC and also for monitoring treatment .

Dx of infectious mononucleosis :

in case of mononucleosis that is associated with EBV the incubation period is from 4-6 weeks .

Patient with non specific symptoms could be consider as EBV infected person .

Infectious mononucleosis findings : increase (WBCs) count /**atypical** lymphocytosis/low grade thrombocytopenia and neutropenia / abnormal liver function test .

-Heterophile-Ag is always related to EBV.

- Heterophile antibody titers rise up to half or more during the first two or three weeks with highest rise during the first week of illness. (50% in first week of illness)

- 60-90% in the second or third weeks

- The level of antibody gradually declines and usually disappears in eight to twelve weeks following the onset .

- Elevated titers sometimes lingers for four to six months up to a year or more.

- Heterophile-Ag is commonly used in serological test , early Ag is rising at the beginning of symptoms up to 3-4 weeks, VCA- IgM peaks at 2-3 weeks while VCA- IgG peaks at 2-3 months of the symptoms onset .

- EPV nuclear Ag (EPNA) which is seen in convalescence(during recurrence stage) and remains present throughout life.

According to this table :

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

VCA IgG indicates immunoglobulin (Ig) G class antibody to viral capsid antigen; VCA IgM, IgM class antibody to VCA; EA (D), early antigen diffuse staining; and EBNA, EBV nuclear antigen.

--No previous infection : means no Ab at all, once there is infection there will be sero conversion,

- IN acute infection :we will see VCA-IgM AND VCA-IgG, and we might see early –Ag this means that the virus is present and is replicating, VCA-IgM AND VCA-IgG are seen in acute infection ,nuclear Ag is not seen in acute infection but they appear in recent infection which means after 2-3 months so the patient is no longer symptomatic.

- IN recent infection IgG is positive and IgM is either declines or lost .if the virus does not replicate we will not see the early Ag .Nuclear Ag maybe present to undetectable level and need more than 2-3 month to elevate to a detectable level.

- IN past infection IgG is high and there is no IgM OR EARLY Ag, but in some patients the early Ag may still high up to 2-3 years, and nuclear Ag tends to be present after past infection.

so this is how we are able to differentiate between acute ,latent or previous Ag.

Treatment:

in case of mononucleosis ,the treatment consists of supportive measures which includes rest and analgesics , specific physical activity should be avoided and splenectomy is required after splenic rupture, Acyclovir can be given.

Post-transplantation EBV symptoms doesn't respond to antiviral , so they should decrease the doses of immunosuppressant therapy

We can also give interferons and Ab against CD21 to inhibit action of the receptors .

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

Infusions of EBVspecific cytotoxic T cells

- Infusion of autologous EBV-specific cytotoxic T lymphocytes
- The isolation of patients with IM is unnecessary.

Summary :

In addition to clinical signs and symptoms, laboratory testing is necessary to establish or confirm the diagnosis of IM. This can provide important information for both the diagnosis and management of EBV-associated disease.

- If the classic signs and symptoms of IM are absent, a diagnosis of IM is more difficult to make. A definite diagnosis of IM can be established by serologic antibody testing. The antibodies present in IM are heterophile and EBV antibodies.
- EBV is widely disseminated. It is estimated that 95% of world's population is exposed to the virus, which makes it the most ubiquitous virus known to man.
- EBV is only a minor problem for immunocompetent patients, but it can become a major one for immunologically compromised patients.
- After primary exposure a person is considered to be immunized and generally no longer susceptible to overt reinfection.

Praises to Allah

Sorry for any mistake

Dedicated to : Tamara ayasrah , Haneen Walied and Hiba alatrash

Sura Bassam