Epstein Barr Virus (EBV):

Also known as Human Herpes Virus IV (HHV 4)

- It is widely disseminated in nature. 95% of adults are positive; however, only 5% have the related diseases. This means that most of the infections are subclinical in their course.
- Transmission is mainly through contact via saliva. However, it can happen via blood, transplantation, and unprotected sex.
- In developing countries, the infection onset is early (3-6 years of age). The infection is usually subclinical. In developed countries, the infection onset is later in life (10-30 years of age). The infection is usually clinically apparent.
    - In developing countries, the onset is earlier due to densely populated regions and low hygiene standards.
- EBV has a viral latency phase.
    - This viral latency is not to be confused with clinical latency. HIV has a clinically latent phase where the virus replicates, but it is asymptomatic. However, in EBV's case, the virus is not fully replicating. It produces a very small percentage of the viral genome (only about 10% of the whole genome). 10-11 proteins that are important for latency.

- **Host cells for the virus:**
    1) B-lymphocytes
    2) T lymphocytes
    3) Epithelial cells
- **Two subtypes of the virus:** (A and B or 1 and 2)
    - Type 1: developed countries
    - Type 2: developing countries
    - Co-infection with both subtypes has been reported in immune-compromised patients, especially HIV patients.
    - There is no difference in the lympho-proliferative capacity of the viruses between the two subtypes.
- EBV causes transformation in the cells.
- The diseases caused by this virus are all proliferative diseases except for two diseases; which are infectious mononucleosis (IM) and chronic active EBV infection.
- The other diseases include:
    - Lymphoproliferative disease of B cells
    - X-linked lymphoproliferative disease of B cells
    - Hodgkin lymphoma

- Burkitt lymphoma
- Nasopharyngeal carcinoma
- Gastric carcinoma
- Peripheral T cell lymphomas
- Nasal T/NK cell lymphomas
- Oral hairy leukoplakia (not malignant)
- Smooth muscle tumors in transplant patients

Molecular biology:

- It has a toroid shaped protein core
  - Toroid means donut shaped
- It has double stranded DNA (linear)
- Enclosed in a nucleocapsid.
- It has 20 faces (icosahedral)
- It has an amorphous protein tegument that contains proteins and enzymes that are important for viral replication.
- Outer envelope:
  - Membrane lipid bi-layer
  - Glycoprotein spikes: gp350 and gp220
    - They are required for infecting the cell. They are important for the process of identification and attachment to the host cell.
  - As this membrane comes from the cell itself, it can also contain normal membrane bound proteins.

Entrance (B cells and epithelial cells):

- **B cell**:
  - Complement receptor (CD21) identifies the GP spike on the virus and induces endocytosis. Then, there is a process of fusion of the viral membrane with the endocytotic vesicle releasing the nucleocapsid and the tegument into the cell. The nuclear capsid is taken by molecular motors, like microtubules, to be imported to the nucleus.
- **Epithelial cell:**
  - The difference between epithelial entry and B cell entry is that the receptors on the epithelial cell are integrins. Therefore, rather than inducing endocytosis, the virus fuses directly with the plasma membrane and gains entry into the cell.

- Once the virus is in the nucleus, the DNA circularizes to prevent it from degradation. This newly formed DNA molecule, called an episome, is more resistant to degradation than the linear form of DNA.

Fate of the virus upon reaching the nucleus:

- Latency (typical to B-cells)
- Replication

In both cases, the episome is attached to the nucleus via EBNA-1 (Epstein Barr Nuclear Antigen)

- In the case of latency, only 10% of the proteins are produced; however, if the virus goes into the replication phase, it produces all of the proteins (including the capsid. This produces a full virus, the cell lyses, and the virus is released. This happens in half of the epithelial cell

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General pathogenesis:

- Mostly happens through salivary contact. This will usually cause a lytic infection in the epithelial cells.
- After lyses of the epithelial cells, the virus reaches the resting B-cells. However, sometimes, access to B cells can be direct. This happens through the tonsillar crypts.
- After infecting the B-cells, the virus can dissemate to the local lymph nodes or the blood stream.
- B cell either:
    - Becomes latent: it cannot be detected by T cytotoxic cells and natural killer cells
    - Progresses into lytic phase
- Eventually the T cells and NK cells will recognize virally infected B cells and kill them. Here we can see that cellular immunity plays a very important role in combating the viral infection caused by EBV.
- After a period of latency viral cell might be reactivated; however, they will be recognized by CD4 and CD8 cells (specific)
- The important thing to know about EBV is that once this virus infects a B cell, it causes its proliferation. This leads to T cell activation and expansion. You have an expansion of both B and T lympohcytes. NK cells and T cells, although they play an important role, this does not mean that we do not produce any antibodies. Unfortunately, we might produce anti-self antibodies.
- Antibodies are produced to prevent IM, the disease. But, they cannot prevent the infection.

- We use antibodies for diagnosis and clinical follow up.

Types of antibodies:

- **Specific (IgM and IgG)**
    - Anti capsid antigen
    - Early antigen*
        - Diffuse
        - Restricted
    - Anti membrane
        - GP350
        - GP220and
        - Anti ENBA (Indicated end of disease)
- **Nonspecific antibodies**
    - Heterophile antibodies.
        - They are the hallmark of the EBV infection. IgM antibodies that are a result of polyclonal expansion. They can persist up to a year after the infection.

* The difference between restricted and diffuse antibody: Restricted anti EA is usually seen in Burkitt's lymphoma as well as active chronic EBV infection. Diffused, on the other hand, is seen in IM, chronic active EBV and nasopharyngeal carcinoma.

Serology(refer to the slides)

- **IM:**
    - Heterophile antibody, some of them may persist, IgM early infection against VCA (viral capsid antigen) later IgG
    - Post infection: is the EBNA and the IgG against VCA
    - In reactivation: all occur except for IgM
- **Burkitt's lymphoma**
    - IgG because they are proliferating
    - Anti EA-R
- **Nasopharyngeal carcinoma:**
    - Anti EA-D

- **What is unique about EBV?**
    - Latency and transformation. The virus can become latent by producing a small subset of its genome, and it can transform B cells.
    - Latently infected B cells are the body's main reservoir for the virus. They have minimal viral expression, thus they evade immune reactions. They get immortalized and proliferate indefinitely as lymho-blastoid cells.
    - The following subset of genes is important for latency and transformation:
        - EBNA-1 (attachment to nucleus)
        - LMP-1 (proliferation)
        - EBNA-2 (proliferation)
        - EBNA-3 (proliferation)
        - LMP-2 (prevents reactivation from latency.)

- **How does this transformation happen?**
    - **LMP-1 is oncogenic:**
        - It mimics TNF receptor in its active form. It looks like the receptor and it is constantly on.
        - It activates the pathway downstream.
        - These pathways are important in cell survival.
        - These pathways include:
            - Kinases
            - JAK
            - NFKB transcription pathway, which can transcribe bcl2. Bcl2 is an important anti-apoptotic factor
        - If the T-lymphocytes try to kill the infected cell by stimulating the death receptor, they can't. If they can bypass the intrinsic pathway, by directly activating the caspases via granzymes, which are proteins that bypass the intrinsic pathways and cleave the caspases directly. Granzymes are proteases that cleave right after an aspartate residue.
        - The main route for the virus to evade the immune is by not getting detected.
    - The virus increases adhesion molecules expression, which causes clumping of the lymphocytes during the early stages of the infections.
    - **LMP-2:**
        - Mimics the B cell receptor.
        - Downstream of the B cell receptor is:

        - RAS
        - PI3
        - AKT
    - All these converge to:
        - C-Abl (9,22 translocation)
        - mTor (important energetic pathway)
        - NKFB (bcl2 transcription)
        - Notch signaling (cell growth)
    - EBV latent membrane proteins (LMP-1 and 2) affect molecules that are important in cellular proliferation and functioning of the B cell. The virus activates a normal pathway in order to make the cells proliferate.

Clinical syndromes:

- **IM (glandular fever, Pfeiffer's disease, Filatov's disease, and the kissing disease)**
    - It is called the kissing disease because of adolescents' saliva exchange during kissing, which is a very common cause of the disease.
    - Nil Filatov and Emil Pfeiffer described the disease independently
        - Fever
        - Lymphadenopathy
        - Malaise
        - Hepatosplenomegaly,
        - Abdominal discomfort in adolescents and young adults.
    - 1920 IM term coined
    - 1923 detailed description of the lymphocyte morphology
    - 1932 discovery of the heterophile antibodies (Paul and Bunnel, there is a test named after them)
    - 1968 EBV designated as the causative agent for IM
    - **Symptoms:**
        - Very young:
            - Asymptomatic
            - Mild pharyngitis with or without tonsillitis, can be misdiagnosed and given antibiotics
        - Elderly:
            - Non-specific
            - prolonged fever
            - fatigue
            - Myalgia
            - malaise.

- Adolescents:
    - 75% of those infected get IM.
    - 4-6 weeks of incubation followed by 1-2 weeks of fatigue malaise and myalgia.
    - Fever
    - Headache
    - Pharyngitis
    - Tonsillitis
    - Lymphadenopathy
    - Splenomegaly
    - hepatomegaly (only 10%)

- EBV in the saliva:
    - Either direct access to B cell or through the epithelial cells in the pharynx. This leads to proliferation, polyclonal expansion, and antibody production.
- B cell proliferation leads to activation of T cells because they are abnormal B cells. Now, we can see abnormal lymphocytes called Downey cells (due to the huge activation). Because of this expansion of B cells and T cells, you will get: lymphadenopathy, hepatomegaly, and splenomegaly.
- **Clinical symptoms:**
    - <u>First two weeks</u>
        - Symmetrical, non fixed mildly tender lymphadenopahty (common in posterior cervical lymph nodes). The involvement of the epitrochlear lymph nodes is very suggestive of the disease, but not diagnostic
        - Phatyngitis is the most consistent physical finding, 1/3 of the cases are exudative.
        - Tonsillar enlargement may block the airway, but it is not common
    - <u>Weeks 2-3:</u>
        - Splenomegaly
        - Patient is at risk of spontaneous rupture, or for minor injury. Rest the patient and advice him/her not to participate in any contact sports or excessive physical activity.
        - Tender hepatomegaly and jaundice, which are very rare.
        - Abnormal liver function test.
        - Maculopapular rash happens in 15 % of the cases especially in younger children. It is usually associated with Ampicillin treatment. When given the Ampicillin treatment, the body might react by producing this rash.
- **Lab findings:**
    - Elevated WBC count (lymphocytes)

- Lymphocytosis with more than 10% abnormal cells with an enlarged abundant cytoplasm, vacuoles, and indentations of the cellular membranes.
- Most of the abnormal cells are CD 8+
- Low grade neutropenia and thrombocytopenia, which is most likely to happen during the first month
- Liver function test is abnormal in more than 90% of the cases
- Classic heterophile test: Paul and Bunnel. Heterophile antibodies can detect foreign antigen for example other animals' antigens. They used to bring sheep's red blood cells and the patient's serum in serial dilution and see when the agglutination happens. If more than 40 fold titer (you diluted your patient's serum 40 times and you still get agglutination), and the patient has typical symptoms and you see abnormal lymphocytes, this is definitely diagnostic of IM. 40% positive on the first week: if the patient presents early in the first week, you might get a negative Paul and Bunnel test; therefore, a misdiagnosis might happen. 80-90% are positive by third week
- A new test: Commercial monospot test
    - 75% sensitivity 90% specificity
    - More sensitive than the other test, but some false positives still exist:
        - CTD
        - Lymphoma
        - viral hepatits
        - malaria
- If these fail, we still have our EBV specific antibody testing and PCR

- **Rare complications:**
    - Death is rare, but most commonly happens due to CNS complications (meningitis, encephalitis, and Guillian Barre syndrome). Guillian Barre syndrome: Self antibodies that cause polyneuropathy. It is fatal if it reaches the respiratory muscles
    - Splenic rupture
    - Airway obstruction
    - Bacterial super-infection: your immune system is too occupied to fight bacteria
    - Other complications:
        - Hepatitis
        - hemolytic anemia
        - Thrombocytopenia
        - autoimmune diseases

- **Treatment:**
  - Supportive
  - Rest
  - Analgesics
  - avoid heavy physical activity
  - Glucocorticoids are only indicated for severe airway obstruction, hemolytic anemia, or thrombocytopenia. Do not use them for uncomplicated cases because you increase the risk for a bacterial infection
  - No role for acyclovir.
- More than 90% of mononucleosis is caused by EBV, 5-10% of the cases are caused by CMV. CMV is the most common cause of heterophile negative mononucleosis.

- **Chronic active EBV:**
  - It is very rare and does not cause chronic fatigue syndrome, but they share similar symptoms.
  - It is defined as an illness that lasts for more than six months.
  - You have very high level EBV DNA in the blood
  - Very high titer of anti-EBV antibodies
  - Many organs are involved:
    - Hepatosplenomegaly
    - Lymphadenopathy
    - Pneumonitis
    - Uveitis
    - Neurological involvement
  - Patients who have chronic active EBV usually have an underlying abnormality in their CD8 cells.

- **EBV associated transformations:**
  - Refer to slide 25 (the professor read the slide)
  - Africa's Burkitt's Lymphoma and Asia's Nasopharyngeal Carcinoma:
    - For Africa, these areas are endemic with malaria. This causes impaired cellular immunity. Then, the virus can proliferate more easily causing B cell expansion.

        - In South East Asia the association is vaguer. But the theory says that it might be due to dietary habits of the inhabitants of those areas. People from an Asian descent and live in the US have a higher incidence rate of NPC. Dietary habits in South East Asia have changed in the past years, and NPS has decreased.

- **EBV associated lympho-proliferative diseases:**
    - Occur in patients with congenital or acquired immunodeficiency:
        - SCID
        - AIDS
        - Transplant patients receiving immunosuppressive drugs (PTLD, post transplant lympho-prilferative disease)
        - RA patients on methotroxate
    - For the last two, they is easy to treat. Reduce immunosuppressant dose. Mount immune response, treat disease. Return to normal dose.
    - With impaired T-Cell function, you are not able to control EBV infected B cells
    - Proliferating EBV-infected B-cells can infiltrate lymph nodes and organs.
    - The pathology of the disease can range from a lympho-proliferative disease to a large B cell lymphoma which is quite aggressive.
    - Most of the patients are immune-compromised, but this does not mean that immune-competent patients are immune against these diseases.
- **X-linked lymphoproliferative disease:**
    - Recessive disease in males.
    - These people respond normally to other infections. They mount a normal immune response and the disease convulses. However, they usually die because of a lympho-prolifeative disease caused by EBV
    - The most common mutation is in a protein called SAP (SLAM associated protein). It is absent.
    - This protein is important in B cells and T cells interactions.
    - In other interactions, SAP's sufficiency is not a problem. For example, a SAP deficient dendritic cell can still perform its function normally. However, when it comes to T and B cell interactions, we will have a decreased contact time.
    - This leads to:
        - Decreased T cell help
        - decreased recruitment to and retention in germinal centers
        - decreased T follicular helper development.
        - B cells will be infected, no immunity to stop them.
        - T cells are not working properly as well.

- **Oral hairy leukoplasia:**

- Not malignant, not painful, non removable hyperplasia.
- No treatment is required.
- Although it is very responsive to acyclovir, the minute you stop giving the patient acyclovir, it will come back.
- Early manifestation of HIV infection.