Sheet No. 3 & 4

HLS MICROBIOLOGY

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Hello there, in this sheet we are going discuss viruses that infect the hematolemphoreticular system: B19 parvovirus, Herpes viruses 4 and 8, and human T-lymphotropic virus

**Anything in grey and between brackets [ ] is in the slides but not mentioned by the doctor.

Take a quick look at this table to help you throughout this sheet, or you can use it for revision later on.

Virus and family	Properties	Transmission	Epidemiology	Pathogenesis	Manifestations	Diagnosis	Treatment
B19 parvovirus (parvoviridae)	Small ssDNA non-enveloped	respiratory droplets feco-oral blood and its products	Endemic Outbreaks in schools	Tropism for erythrocytes and their progenitors → Viremia and erythropoiesis inhibition	Mostly asymptomatic. Erythema infectiosum polyarthropathy Aplastic crisis Hydrops fetalis	Detecting igM, igG PCR Electron microscopy	5th disease: symptomatic TAC: symptomatic + blood transfusion pure red cell aplasia: IVig no vaccine
Epstein Barr virus (Herpesviridae)	dsDNA enveloped longlife infection with periodic reactivation	Oral secretions blood transfusion bone marrow transplant	Common worldwide 60-90% are carriers Biphasic; childhood and adolescence	Epithelium → B cells CD21 → bloodstream latency in B cells → neoplastic transformation	Adults→ IM children→ asymptomatic/mild associated tumors; mostly gastric carcinoma	serology: ELISA, monospot	Acyclovir stops only shedding no vaccine
Human herpesvirus- 8 (Kaposi sarcoma herpes virus) (Herpesviridae)	Same as above + associated with tumors	Oral secretions sexually vertically by blood			Kaposi sarcoma vascular tumors lymphomas	PCR (optimal) ELISA	Foscarnet, famciclovir
Human T-lymphotropic virus	RNA retrovirus latency for 30 years	Breastfeeding sexually by blood	Distributed worldwide	Tropism for helper T cells → flower cells	Asymptomatic but progress to Adult T cell leukemia lymphoma or tropical spastic paraparesis	ELISA real time PCR	No specific treatment IFα + zidovudine

1. Parvoviruses

Parvoviruses are members of the family **Parvoviridae**, and they share these characteristics:

a. Among the smallest viruses [diameter = 19-25 with a mean of ~22 nm]
b. Nonenveloped, Icosahedral
c. linear single-stranded DNA genome [of ~5000 nucleotides].
d. For replication, these viruses depend on either rapidly dividing host cells or helper viruses e.g. adenovirus and herpesvirus. The capsid formation happens in the nucleus of infected cells.

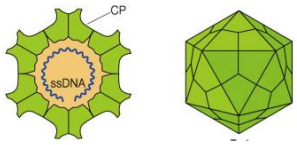


TABLE 31-1 Important Properties of Parvoviruses

Virion: Icosahedral, 18–26 nm in diameter, 32 capsomeres
Composition: DNA (20%), protein (80%)
Genome: Single-stranded DNA, linear, 5.6 kb, MW 1.5–2.0 million
Proteins: One major (VP2) and one minor (VP1)
Envelope: None
Replication: Nucleus, dependent on functions of dividing host cells
Outstanding characteristics: Very simple viruses Human pathogen, B19, has tropism for red blood cell progenitors One genus contains viruses that are replication-defective and require a helper virus

➢ This family has only a single human pathogen; **parvovirus B19.**

Parvovirus B19:

- Parvovirus B19 is the only human pathogenic parvovirus identified to date and is capable of autonomic replication, i.e., it requires no helper virus.
- It is the causative virus in erythema infectiosum (aka, slapped cheek syndrome or the 5th disease) in children and causes aplastic crisis in anemic patients.
- [The virus also contributes to joint diseases, embryopathies, and tissue rejection following renal transplants]. Replication is independent on the nucleus (not like other parvo viruses)
- Divided into 3 genotypes, but only **one antigenic type:**
  - Genotype 1 is the most common world-wide.
  - Genotype 2 is rarely associated with active infection.
  - Genotype 3 is the predominant in western Africa.

Different genes but the same antigen

Epidemiology:

- [The B19 virus is widespread. Infections can occur throughout the year in all age groups and as outbreaks or as sporadic cases].
- Infections are almost endemic in all parts of the world, [and most commonly seen as outbreaks in schools].

Transmission:

this route is a bit debatable

Through **respiratory droplets**. The feco-oral route, similar to other parvoviruses is suspected.

if one child get infected in school, all the children nearby will get infected (usually)

Blood and blood products are infectious, so patients with multiple transfusions and drug addicts are high incidence groups.

Fomites (common things between children) can also be a source of infection for B19

Pathogenesis

Therefore it causes anemia

Tissue tropism is the cells and tissues of a host that support growth of a particular virus.

- The virus has cell tropism for erythrocytes and erythroid progenitor cells. The specificity in tropism of B19 is due to the limited distribution of its receptor. This receptor is the **blood group P antigen**, expressed on mature erythrocytes, megakaryocytes, progenitor erythroid cells, and some other cells.

- [Parvovirus B19 replicates in the bone marrow in erythrocyte precursor cells, which are destroyed in the process].

- After inoculation, the virus causes a systemic infection and viremia, inducing cytotoxic effects leading to decreased RBC production.

**The outcomes are different in different individuals:

a. In healthy immunocompetent persons: normal erythropoiesis

Asymptomatic, or mild non-specific symptoms.

Viremia and erythropoiesis inhibition (low Hb) are transient and resolve as soon as antibodies igG and igM are mounted.

b. In children:

Harmless epidemic infection; erythema infectiosum ("slapped-cheek syndrome" or "5th disease").

c. Anemic patients (sickle-cell anemia, chronic hemolytic anemia): increased erythropoiesis

Such infections result in so-called aplastic crises in which the lack of erythrocyte resupply leads to a critical shortage.

d. Pregnant women:

Spontaneous abortions in early pregnancy and fetal damage in late pregnancy (hydrops fetalis).

We'll discuss each in detail

TABLE 31-2 Human Diseases Associated with B19 Parvovirus

Syndrome	Host or Condition	Clinical Features
Erythema infectiosum	Children (fifth disease) Adults	Cutaneous rash Arthralgia-arthritis
Transient aplastic crisis	Underlying hemolysis	Severe acute anemia
Pure red cell aplasia	Immunodeficiencies	Chronic anemia
Hydrops fetalis	Fetus	Fatal anemia

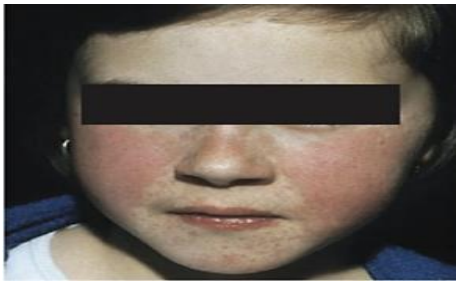
Before we start, you should know that most common B19 infections are asymptomatic..

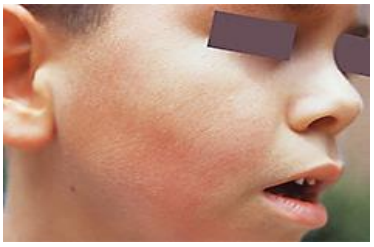
So many patients will have this infections, but they won't show any appearent symptoms

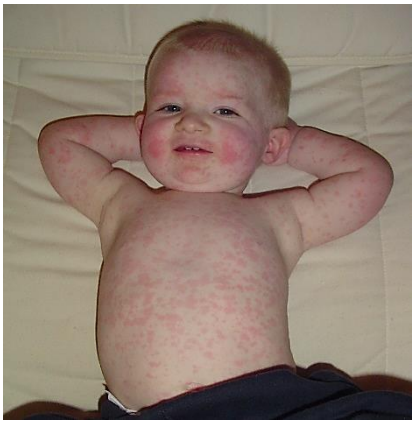
Clinical manifestations (if any):

1. Erythema Infectiosum (Fifth disease or slapped-cheek disease)

[Infection begins with a minor febrile prodrome ~7-10 days after exposure]. The classic exanthema facial rash develops several days later; after 2-3 days, the erythematous macular rash may spread to the extremities in a lacy reticular pattern.

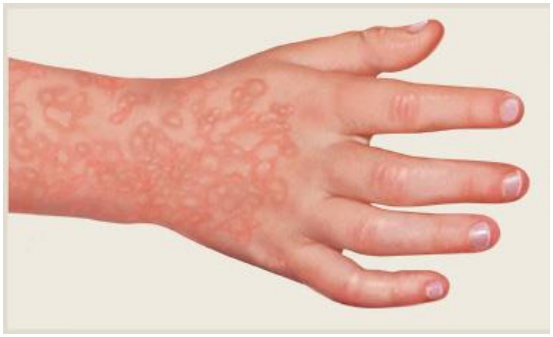






This rash is macular, it doesn't develop into vesicles

***Polyarthropathy Syndrome**



- Adults typically do not exhibit the "slapped-cheek" phenomenon but present with **arthralgia** (pain and swelling in joints), with or without the macular rash.
- Although uncommon among children, arthropathy occurs in ~50% of adults and is more common among women than among men.
- The distribution of the affected joints is often symmetrical, with arthralgia affecting the small joints of the hands, then the wrist, then it develops in the ankles.
- Resolution usually occurs within few weeks (about 3 weeks) without any long-term disability, but recurring symptoms can continue for months.

Note: PV B19 virus has an association with chronic arthropathies; it may mimic rheumatoid arthritis and may trigger rheumatoid disease. It also has been associated with juvenile idiopathic arthritis.

2. Transient Aplastic Crisis (TAC):

The host already has problems with RBCs erythropoiesis, so the problem will be even worse (recall that B19V affects mainly RBCs precursor)

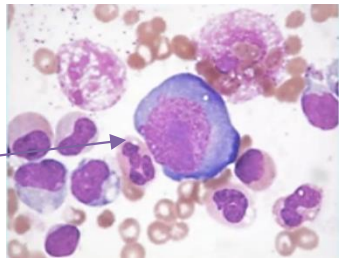
[In most individuals with B19V infection, asymptomatic transient reticulocytopenia occurs.] However, in patients who depend on continual rapid production of red cells, infection can cause ***transient aplastic crisis***.

Affected individuals include those with hemolytic disorders (e.g. sickle cell anemia), hemoglobinopathies (e.g. thalassemia), red cell enzymopathies, and autoimmune hemolytic anemias. Those people when infected with B19 virus, develop abrupt cessation

in RBCS synthesis in bone marrow, but the illness is transient, and anemia resolves with termination of the cytopathic effect in the erythroid progenitor cells.

Symptoms:

- Severe anemia (sometimes life-threatening)
- Low reticulocyte count.
- Bone marrow examination reveals an absence of erythroid precursors and characteristic giant pronormoblasts.



3. Pure Red-Cell Aplasia/Chronic Anemia

When individuals infected with B19 virus are immunodeficient, B19V leads to chronic suppression of bone marrow, causing severe chronic anemia.

[Chronic B19V infection has been reported in a wide range of immunosuppressed patients], including those with congenital or acquired immunodeficiencies e.g. AIDS, or lymphoproliferative disorders (especially acute lymphocytic leukemia), and patients on immunosuppressives for organ transplantation.

Symptoms:

- [Patients have persistent anemia]
- Reticulocytopenia
- Absent or low levels of B19V IgG because the patient is immunodeficient
- High titers of B19V DNA in serum
- In many cases, scattered giant pronormoblasts in bone marrow.]

4. Hydrops Fetalis

B19 infection during pregnancy can lead to hydrops fetalis and/or fetal loss.

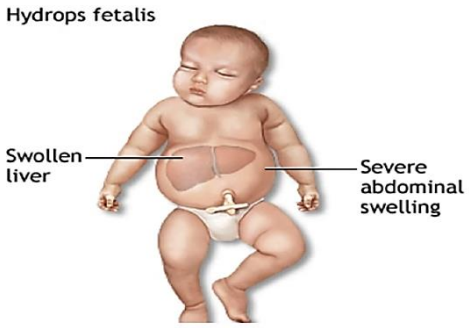
Although B19 virus is not teratogenic itself, a condition known as ***hydrops fetalis*** may develop, in which the fetus starts to accumulate fluid (edema). Hydrops mean edema

B19V results in anemia, so the heart needs to pump much greater volume of blood to deliver the same amount of oxygen, so the increased demand for cardiac output lead to heart failure and edema.

[The risk of transplacental fetal infection is ~30%, and the risk of fetal loss (predominantly early in the second trimester) is ~9%.]

In pregnant woman it doesn't lead to much further complications, unless she already has hemolytic anemia, the serious effect is on the BABY
It depends on what age the infection happened, if it's in the early trimester, spontaneous loss occurs if it's in the 3rd trimester, hydrops fetalis occurs (the fetus eventually dies ☹)

Remember that erythropoiesis happens in the liver during the 2nd trimester.



Diagnosis of B19 virus: (either we detect the virus itself or antigenic parts of the virus)

keep in mind that they're low in pure cell aplasia

- An enzyme immunoassay reveals **antibodies of the IgG and IgM classes**.
- During the viremic phase, at the onset of clinical symptoms, the virus can also be identified in the blood by means of electron microscopy or PCR.
- In-vitro culturing of the pathogen is not a standard procedure.

In immunocompetent individuals, the diagnosis of B19V infection rely on the B19V ***igM*** antibodies that are usually detected by the time of rash appearance in erythema infectiosum, and by the 3rd day of transient aplastic crisis.

B19 ***igG*** are detectable by the 7th day of illness and usually persist throughout life.

Treatment:

There's no specific antiviral treatment and no vaccine

- No antiviral is effective against the B19 virus infection and treatment is often ***symptomatic*** in the case of 5th disease.

Erythema infectiosum patients don't need treatment, they usually resolve spontaneously, but patients need supportive treatment

- Transient aplastic anemia & pure red cell aplasia frequently needs symptomatic treatment with blood transfusions.
- Commercial immunoglobulins (IVig) from healthy blood donors containing neutralizing antibodies to human B19 virus can sometimes be given to immunocompromised patients (Mainly given to patients with pure red cell aplasia) and those with anemia.
- There is **no available vaccine** to B19 virus infection.
- [Administration of IVig is not beneficial for erythema infectiosum or B19V- associated polyarthropathy.
- Intrauterine blood transfusion can prevent fetal loss in some cases of fetal hydrops.]

2. Herpesviruses

The herpes family of viruses include:

- The herpes simplex virus (HSV, 1&2 serotypes)
- The varicella-zoster virus (VZV) → chickenpox
- Cytomegalovirus (CMV) → fatal in immunocompromised
- The Epstein-Barr virus (EBV) → infectious mononucleosis

- Human herpesvirus 6 (HHV 6) (the disease is called roseola infantum)
- Human herpesvirus 8 (HHV 8) → AIDS-associated Kaposi sarcoma

- All members of the herpes family have an outstanding property to establish a ***lifelong infection*** in the host and reactivate after a period of latency.
- They are enveloped, contain viral glycoproteins as well as an Fc receptor, and they have a **dsDNA**.
- [The viruses in this family all feature a practically identical morphology but show little uniformity when it comes to their biology and the clinical pictures resulting from infections].
- [Replication of the DNA and the morphogenesis of the virus particle take place in the host-cell nucleus. The envelope (inner nuclear membrane) is then formed when the virus penetrates the nuclear membrane.]
- [Common to all herpesviruses is a high level of generalized contamination (60-90% carriers) and the ability to persist in a latent state in the body over long periods.]

Latency is the most remarkable feature of Hepresviruses

a. Epstein-Barr virus (EBV)

- EBV is a member of the family ***Herpesviridae***.
- It's an ubiquitous herpes virus that is the causative agent of heterophile-positive infectious mononucleosis (IM), [which is characterized by fever, sore throat, lymphadenopathy, and atypical lymphocytosis].
- [The two types of EBV that are widely prevalent in nature are not distinguishable by conventional serologic tests.]
- **Cell tropism of EBV is B lymphocytes**

Infectious mononucleosis is called kissing disease because it's transmitted through the saliva

Epidemiology:

- EBV infections are common worldwide. These infections are biphasic; most common in early childhood, with a second peak during late adolescence. By adulthood, more than 90% of individuals have been infected and are seropositive (have antibodies to the virus).
- [IM is usually a disease of young adults. in adults it's more non specific (like any viral infection). In lower socioeconomic groups and in areas of the world with deficient standards of hygiene (e.g., developing regions), EBV tends to infect children at an early age, and IM is uncommon.]
- [In areas with higher standards of hygiene, infection with EBV is often delayed until adulthood, and IM is more prevalent.]

Transmission:

This is the main route, that's why it's called kissing disease

– EBV spreads by **contact with oral secretions**, i.e. transmission by non-intimate contactis rare. However, transmission through Intimate contact (sexual contact), blood products, blood transfusion, sharing needles, IV drug abuses and bone marrow transplant have been reported.

– Like all herpes viruses, EBV general contamination is high (60-90% carriers); The virus is frequently transmitted from asymptomatic adults to infants and among young adults by transfer of saliva during kissing. → (EBV aka kissing disease)

– [More than 90% of asymptomatic seropositive individuals shed the virus in oropharyngeal secretions. Shedding is increased in immunocompromised patients and those with IM.]

Pathogenesis:

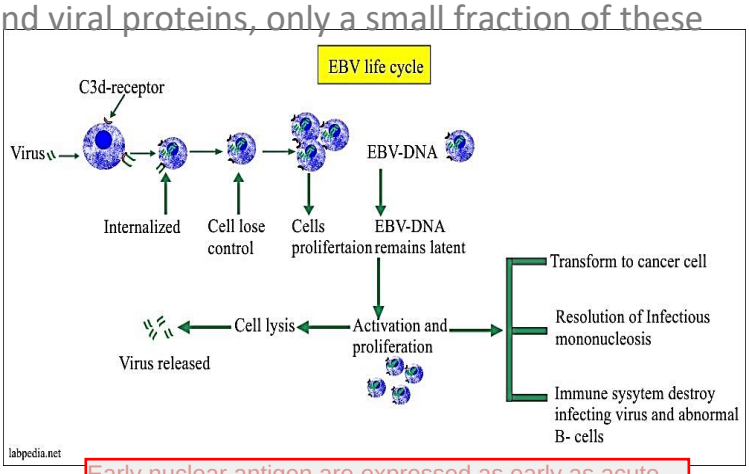
- When EBV is transmitted by salivary secretions, the virus infects the epithelium[1] of the oropharynx and the salivary glands and is shed from these cells. B cells[2] may become infected after contact with epithelial cells, then EBV spreads through the bloodstream[3] and continue to proliferate.

Those B cells will become immortalized (They don't die)
Immortalized & uncontrollable growth = the definition of cancer/tumor

- B cells have a receptor called CD21 or CR2 for binding the C3d complement component. **EBV binds to this receptor** leading to neoplastic changes.

complement receptor 2

- [The proliferation and expansion of infected B cells along with reactive T cells result in enlargement of lymphoid tissue. Polyclonal activation of B cells leads to the production of antibodies to host-cell and viral proteins, only a small fraction of these cells produces the virus.]
- EBV persists in a latent state in the B lymphocytes and can lead to their immortalization as well as tumor transformation on the long-term; If T cell immunity is compromised, EBV-infected B cells may begin to proliferate, virus-induced proliferation is but one step in a multistep process of neoplastic transformation.

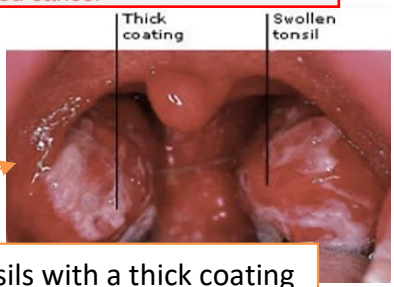


Early nuclear antigen are expressed as early as acute infection.
While the latent membrane proteins antigen are only expressed once developed cancer

B cells that are infected with EBV start to secrete immunoglobulins (They're called plasmacytooid B cells).The B cell itself doesn't do virus shedding

Clinical manifestations:

- Most EBV infections in young children are either asymptomatic or present as mild pharyngitis with or without tonsillitis.

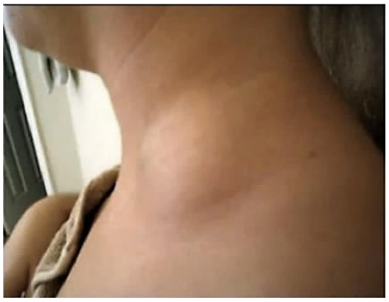


Pharyngeal congestion and enlargement of the tonsils with a thick coating

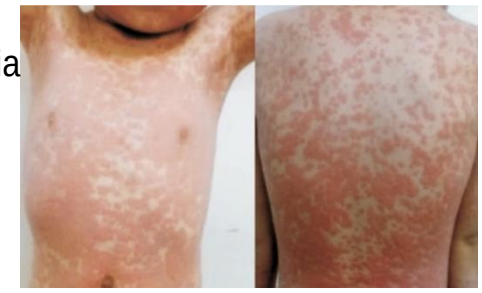
if we do CBC,we'll notice that WBCs is very high....lymphocytes are abnormal
Specifically, they'll have a certain type of T lymphocytes called Downey cells

- ***Infectious mononucleosis:**

In contrast, 75% of adults present with the classic syndrome of ***infectious mononucleosis*** which usually starts after an incubation period of one to two months (~4-6 weeks).



First symptoms are fatigue, malaise, and myalgia before the onset of fever, sore throat, rash, and lymphadenopathy, enlargement of spleen and liver and atypical lymphocytes.
Young patients will have poor feeding.
Old patients will suffer from fatigue,malaise,arthralgia,myalgia
A morbilliform rash or papular rash appears usually on the arms and trunk (Chest, abdomen and back mainly).



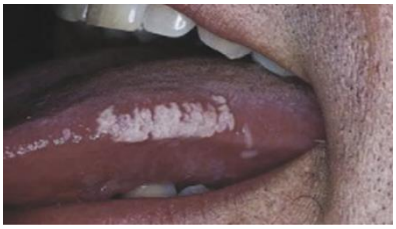
The typical illness is **self-limited** and usually lasts 2-4 weeks.

EBV-associated diseases other than IM:

***BUT remember, it's an association relationship not a causative one.

In patients with congenital or acquired immunodeficiencies, EBV has been reported to be associated with several malignancies and tumors including:

- [B cell hyperplasia or poly- or monoclonal lymphoma.
- X-linked lymphoproliferative disease]
- Oral hairy leukoplakia
- Burkitt's lymphoma
- Anaplastic nasopharyngeal carcinoma
- Gastric carcinoma (most common)
- Hodgkin's and non-Hodgkin's disease



Mechanism of neoplastic transformation:

There are characteristic chromosome translocations that involve immunoglobulin genes and result in dysregulation of expression of the c-myc proto-oncogene.

Diagnosis:

- Patients with EBV usually present with **lymphocytosis** with more than 10% **atypical** lymphocytes; they are enlarged, have abundant cytoplasm, vacuoles, and indentations on their cell membrane.

- Molecular Assays for Identification of Virus

Nucleic acid hybridization is the most sensitive means of detecting EBV in patient materials. However, this method is laborious and time consuming and needs special facilities.

You should know that viral culture are not like bacterial cultures, as they're not made based on proteins.. they have specialized laboratories

- Isolation of Virus

[EBV can be isolated from saliva, peripheral blood, or lymphoid tissue by immortalization of normal human lymphocytes, usually obtained from umbilical cord blood.]

Diagnosis in viral infection is serology (you look for either antigen or antibody)

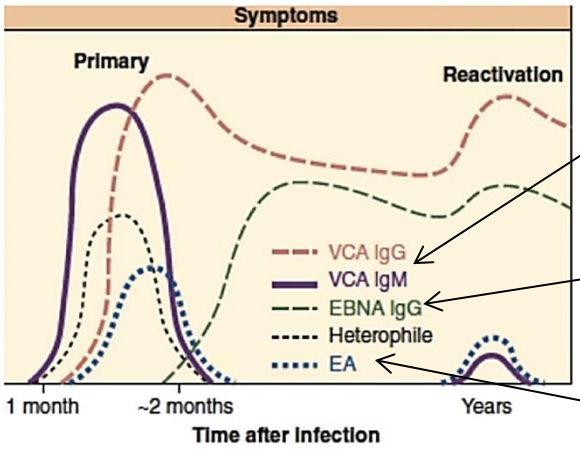
- Serology (more practical):

We usually look for antibodies (So we use western blot or ELISA
If we are looking for antigen we will use PCR)

1. Enzyme-linked immunosorbent assays (ELISA), immunoblot assays, and [indirect immunofluorescence tests using EBV-positive lymphoid cells.]
2. The heterophil agglutination test **(Monospot):**

Transient heterophilic antibodies develop and can agglutinate sheep cells. It is a rapid test and is confirmatory when the physician suspects EBV infection.

During latent infection of B cells, several patterns of latent viral gene expression are recognized and expressed in vitro (used in diagnosis), including the EBV nuclear antigens (EBNAS), latent membrane proteins (LMPS), and EBV encoded small RNAS (EBERS).



Typical pattern of antibody response to EBV specific antigens:

Patients with recent infection develop igM and igG antibodies to the **viral capsid antigens only** and only the igG persists for years.

Several weeks after infection, antibodies to EBV **nuclear antigen** and membrane antigens are mounted and persist for life.

Antibodies to early antigens persist for months.

-VCA IgM is raised almost in three days from the onset of symptoms then drops significantly
- At the same VCA IgG is raised, but it stays persistent

- EBNA IgG starts appearing late (after 2 months) then it stays positive

- EA (early antigen) appear directly after VCA (IgM & IgG) then drops after VCA IgM

Treatment:

Acyclovir reduces EBV shedding from the oropharynx during the period of drug administration, but it does not affect the number of EBV-immortalized B cells nor the
symptoms of mononucleosis and is of no proved benefit in the treatment of EBV-associated lymphomas in immunocompromised patients.

There is no EBV vaccine available.

b. Human herpesvirus 8 (aka Kaposi sarcoma herpesvirus)

[A new herpesvirus, designated HHV-8 also called KSHV, was first detected in 1994 in Kaposi sarcoma specimens. KSHV is lymphotropic and is more closely related to EBV]

The KSHV genome [~165 kbp] contains numerous genes related to cellular regulatory genes involved in cell proliferation, apoptosis, and host responses [cyclin D, cytokines, chemokine receptor] that presumably contribute to viral pathogenesis.

KSHV is the cause of Kaposi sarcomas, vascular tumors of mixed cellular composition, and is involved in the pathogenesis of body cavity-based lymphomas occurring in AIDS patients.

Transmission:

Contact with **oral secretions** is likely the most common route of transmission.

vertically = from the mother to the fetus through the placenta

The virus can also be transmitted sexually, vertically, by blood, and through organ transplants. Viral DNA has also been detected in breast milk samples in Africa.

Diagnosis:

- Viral DNA can be detected in patient specimens using PCR assays. (optimal way)
- Serologic assays are available to measure persistent antibodies to KSHV using indirect immunofluorescence, Western blot, and enzyme-linked immunosorbent assay (ELISA).
- [Direct virus culture is difficult and impractical.]

Treatment:

Foscarnet, famciclovir, [ganciclovir, and cidofovir] have activity against KSHV replication.

3. Human T-Lymphotropic Viruses (HTLV)

Human T-lymphotropic viruses are **RNA retroviruses**.

Retrovirus family contains 2 famous subfamilies: Delta viruses with the HTLVs being part of it and Lentiviruses, HIV is a famous virus in this family.

Human T-lymphotropic virus 1

- HTLV-1 has been established as the causative agent of ***adult T-cell leukemia-lymphomas*** (ATLL), it's a very aggressive tumor with a very poor prognosis (five-year survival rate <5%).
  Also, a nervous system degenerative disorder called ***tropical spastic paraparesis*** (HTLV-1-associated myelopathy (HAM)).
- The human lymphotropic viruses have a marked affinity for mature T cells. (cell tropism)
- HTLV1 has a long **latency period** (incubation period); between the infection and the emergence of the adult T cell lymphoma-leukemia. (usually between 20 to 30 years).
- [The virus is distributed worldwide, with an estimated 20 million infected individuals.]

Transmission:

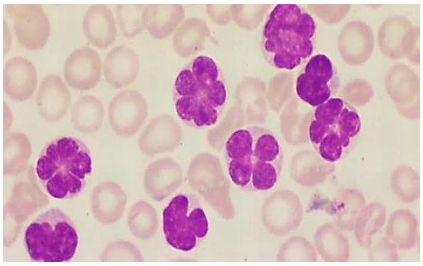
[Transmission of HTLV-1 seems to involve cell-associated virus.] → Virus particles remain attached to or within the host cell after replication.

One of the most important routes is through breast milk

- Mother-to-child transmission via breast feeding is an important mode.
- Blood transfusion is an effective means of transmission, as are sharing blood contaminated needles (drug abusers).
- Sexual transmission.

Clinical symptoms: Incubation period is veeery long, averaging from 20 years to develop ATLL

HTLV infection is usually asymptomatic but can progress to ATLL in approximately 1 in 20 persons over a period of 30 years. (a slow oncogenic process over 20-30 years).



ATLL caused by HTLV-1 is a neoplasia of the **CD4 (helper) T cells** that can be acute or chronic. The malignant cells have been termed ***"flower cells"*** because they are pleomorphic and contain lobulated nuclei.

[ATLL is usually fatal within a year of diagnosis, regardless of treatment.]

Diagnosis:

- Serology: ELISA (to detect viral antigens or viral antibodies), Western blot
- Viral real time PCR

Treatment:

For the small number of patients who develop HTLV-1 related diseases, therapies are not curative. i.e. no specific antiviral therapy.

However, the combination of interferon α and zidovudine may extend survival .

Prevention:

- Women in endemic areas should not breast-feed their children
- Blood donors should be screened for serum antibodies to HTLV-1.
- And as in the prevention of HIV infection, the practice of safe sex and the avoidance of needle sharing are important.