**LESSON 19.
Latent virus infections. Microbiology diagnosis of HIV infection (Human Immunodeficiency Virus). Oncogenic viruses. Prion infections**

**LESSON PLAN:**

1. Understanding slow viral infections.

2. Retroviruses. Human immunodeficiency viruses, classification. Virion structure, structural and non-structural genes. Continuity, ways of infection. Pathogenesis. Opportunistic infections. Microbiological diagnosis of HIV infection. Prevention problem and treatment preparations.

3. Oncogenic viruses. Historical development of the concept of the role of viruses in the etiology of malignant tumors. Modern theories of carcinogenesis. Mechanism of viral oncogenesis. Classification of oncogenic viruses.

4. Oncogenic viruses that cause tumors in humans:

 DNA-containing oncogenic viruses:

- Herpesviridae family: cytomegalovirus, Epstein-Barr virus, human herpes virus type 8.

- Hepadnaviridae family - hepatitis B virus

- Papillomaviridae family, general characteristics, types, pathogenetic characteristics of diseases caused by it, specific prevention.

- Poliomaviridae family. Merkel polyomavirus.

 RNA-containing oncogenic viruses:

- Retroviridae family: human T-lymphotropic viruses (Human T-lymphotropic virus, HTLV

- Family Flaviviridae Genus Hepacivirus – hepatitis C virus.

5. Prion infections.

***RETROVIRUSES***

**Trigger Words**

Reverse transcriptase, integration, syncytia

HIV: AIDS, CD4, chemokine co-receptor, opportunistic diseases

HTLV: leukemia, flower cell, CD4 T cell

**Biology, Virulence, and Disease**

ᑏ Virion: medium size, envelope, nucleocapsid, two copies of (+) RNA genome

ᑏᑏ Simple retroviruses have three genes: *gag, pol, env*

ᑏᑏComplex retroviruses (HIV, HTLV) have *gag, pol, env,* and other important genes

ᑏᑏ Encodes RNA-dependent DNA polymerase (RT), replicates in nucleus

ᑏᑏVirion carries RT, integrase, and protease enzymes

ᑏᑏ Replicates through DNA intermediate, integrates viral DNA into host chromosome

ᑏᑏCauses syncytia

ᑏᑏ Incapacitates and escapes immune control

ᑏᑏOncornaviruses may encode oncogene and have a short latency period before cancer

ᑏᑏHTLV-1, no oncogene, long latency period before leukemia

ᑏᑏ**HTLV:** acute T-cell lymphocytic leukemia, tropical spastic paraparesis

ᑏᑏ**HIV:** initially infects CD4/CCR5 macrophages, dendritic cells, and T cells; initial disease phase resembles mononucleosis followed by latent period; AIDS results when CD4 T cells drop below 200/μL

ᑏᑏ **Endogenous retroviruses:** integrated and approximately 8% of human genome

**Epidemiology**

ᑏᑏWorldwide

ᑏᑏ Transmitted in blood and semen

ᑏᑏHigh-risk groups: promiscuous individuals, IV drug users, infants of infected mothers

**Diagnosis**

ᑏᑏ RT-PCR, ELISA

**Treatment, Prevention, and Control**

ᑏᑏHIV treatment with nucleoside analogs, protease inhibitors, and other antiviral drugs

ᑏᑏ Prevention by screening of blood supply, safe sex, antiviral drug prophylaxis,education

**Classification of Retroviruses**



**Morphologic distinction of retrovirions. The morphology and position of the nucleocapsid core are used to classify the viruses. A-type particles are immature intracytoplasmic forms that bud through the plasma membrane and mature into B-type, C-type, and D-type particles.**



**Unique Characteristics of Retroviruses**

Virus has an **enveloped** spherical virion that is 80 to 120 nm in diameter and encloses a capsid containing **two** copies of the **positive-strand RNA** genome (≈9 kilobases for HIV and human T-cell lymphotropic virus).

RNA-dependent DNA polymerase **(reverse transcriptase),** two copies of tRNA, protease, and integrase enzymes are carried in the virion.

Virus receptor is the initial determinant of tissue tropism.

Replication proceeds through a DNA intermediate termed the *provirus.*

The provirus **integrates** randomly into the host chromosome and becomes a cellular gene.

Transcription of the genome is regulated by the interaction of host transcription factors with promoter and enhancer elements in the long terminal repeat portion of the genome.

**Simple retroviruses** encode *gag, pol,* and *env* genes.

**Complex viruses** also encode accessory genes (e.g., *tat, rev, nef, vif,* and *vpu* for HIV). Virus assembles and buds from the plasma membrane.

Final morphogenesis of HIV *requires* protease cleavage of Gag and Gag-Pol polypeptides after envelopment.

**Cross section of HIV. The enveloped virion contains two identical ribonucleic acid *(RNA)* strands, RNA polymerase, integrase, and two transfer RNAs *(tRNA)* base-paired to the genome within the protein core. This is surrounded by proteins and a lipid bilayer. The envelope spikes are the glycoprotein *(gp)*120 attachment protein and gp41 fusion protein. *CA,* Capsid; *MA,* matrix; *NC,* nucleocapsid; *SU,* surface component; *TM,* transmembrane component of envelope glycoprotein. (Modified from Gallo, R.C., Montagnier, L., 1988. AIDS in 1988. Scientific American 259, 41–48. Copyright George Kelvin.)**



**Retrovirus Genes and Their Function**

**Life cycle of HIV. HIV binds to CD4 and chemokine coreceptors and enters by fusion. The genome is reverse transcribed into deoxyribonucleic acid *(DNA)* in the cytoplasm, enters the nucleus, and is integrated into the nuclear DNA. Transcription and translation of the genome occur as a cellular gene in a fashion similar to that of human T-cell lymphotropic virus (see Fig. 54.7). The virus assembles at the plasma membrane and matures after budding from the cell. *cDNA,* Complementary DNA; *mRNA,* messenger ribonucleic acid. (Modified from Fauci, A.S., 1988. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. Science 239, 617–622.)**

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**Target cell binding of human immunodeficiency virus *(HIV).* The CCR5 chemokine receptor is a co-receptor with CD4 on initial infection of an individual, and after mutation of the *env* gene, the CXCR4 receptor is also used. *RNA,* Ribonucleic acid. (Modified from Balter, M., 1988. New hope in HIV disease. Science 274, 1988.)**

**Pathogenesis of human immunodeficiency virus *(HIV).* HIV causes lytic and latent infection of macrophage, dendritic cells, and CD4 T cells and disrupts neuronal function. The outcomes of these actions are immunodeficiency and acquired immunodeficiency syndrome *(AIDS)* dementia. *CNS,* Central nervous system. (Modified from Fauci, A.S., 1988. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. Science 239, 617–622.)**

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**Disease Mechanisms of Human Immunodeficiency Virus**

HIV primarily infects CD4 T cells and cells of the myeloid lineage (e.g., monocytes, macrophages, alveolar macrophages of the lung, dendritic cells, and microglial cells of the brain).

Virus mutates during chronic infection and switches from myeloid/T-cell tropic to T-cell tropic based on co-receptor preference.

Virus causes lytic infection of activated permissive CD4 T cells and induces apoptosis-like death of nonpermissive CD4 T cells.

Virus causes persistent low-level productive and latent infection of myeloid lineage cells and memory T cells.

Virus causes syncytia formation, with cells expressing large amounts of CD4 antigen (T cells); subsequent lysis of the cells occurs.

Virus alters T-cell, dendritic cell, and macrophage cell function.

Virus reduces CD4 T-cell numbers and helper-cell activation of CD8 T-cell, macrophage, and other cell functions.

CD8 T-cell numbers and macrophage function decrease.

Infected microglial cells disrupt neuronal function.

**Time course and stages of human immunodeficiency virus *(HIV)*. A long clinical latency period follows the initial mononucleosislike symptoms. Initial infection is with the R5–M-tropic virus, and following mutation, the X4–T-tropic virus. The progressive decrease in the number of CD4 T cells, even during the latency period, allows opportunistic infections to occur. The stages in HIV disease are defined by the CD4 T-cell levels and occurrence of opportunistic diseases. HIV can be detected by the presence of p24, HIV genome, or antibodies to the virus. (Modified from Redfield, R.R., Burke, D.S., 1996. HIV infection: the clinical picture. Scientific American 259, 90–98; updated 1996.)**

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**CD4 T cells have a critical role in activating and regulating cell-mediated immune responses, especially toward intracellular pathogens. Human immunodeficiency virus *(HIV)–*induced loss of CD4 T cells results in loss of the functions activated and regulated by the indicated cytokines. *IFN,* Interferon; *IL,* interleukin; *NK,* natural killer; *TGF-\_,* transforming growth factor-β.**

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**Means of Human Immunodeficiency Virus Escape from the Immune System**

**Epidemiology of Human Immunodeficiency Virus Infections**

**Disease Viral Factors**

Enveloped virus is easily inactivated and must be transmitted in body fluids.

Disease has a long prodromal period.

Virus can be shed before development of identifiable symptoms.

**Transmission**

Virus is present in blood, semen, and vaginal secretions.

**Who Is at Risk?**

Intravenous drug abusers, sexually active people with many partners (MSM and heterosexual), prostitutes, newborns of HIV positive mothers, sexual partners of infected individuals.

Blood and organ transplant recipients and hemophiliacs treated before 1985 (before prescreening programs).

**Geography/Season**

There is an expanding epidemic worldwide.

There is no seasonal incidence.

**Modes of Control**

Antiviral drugs limit progression of disease.

Antiviral drugs for pre- and post-exposure prophylaxis.

No vaccines available.

Safe, monogamous sex helps limit spread.

Sterile injection needles should be used.

Circumcision.

Large-scale screening programs of blood for transfusions, organs for transplants, and clotting factors used by hemophiliacs.

*MSM,* Men who have sex with men.

**Transmission of Human Immunodeficiency Virus Infection**

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**Indicator Diseases of Acquired Immunodeficiency Syndrome a**

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a Manifestations of HIV infection—defining AIDS according to criteria of Centers for Disease Control and Prevention.

Modified from Belshe, R.B., Textbook of Human Virology, second ed. Mosby, St Louis, MO. *HPV,* Human papillomavirus.

**Clinical Summary**

A 32-year-old former heroin addict had a mononucleosis-like illness for 2 weeks. He recalled experiencing occasional night sweats and fever for 3 years and then presented with thrush, cytomegalovirus retinitis, and *Pneumocystis* pneumonia. His CD4 T-cell count was

 50/μL. He was started on highly active antiretroviral therapy.

**Laboratory Analysis for Human Immunodeficiency Virus**



**Potential Antiviral Therapies for Human Immunodeficiency Virus Infection**

**Nucleoside Analog Reverse Transcriptase Inhibitors**

Azidothymidine (AZT) (Zidovudine) [Retrovir]

3TC (Lamivudine) [Epivir]

Tenofovir disoproxil fumarate (adenosine class) [Viread]

ABC (Abacavir) [Ziagen]

FTC (Emtricitabine) [Emtriva]

**Nonnucleoside Reverse Transcriptase Inhibitors**

Nevirapine [Viramune]

Doravirine [Pifeltro]

Efavirenz [Sustiva]

Etravirine [Intelence]

Rilpivirine [Edurant]

**Protease Inhibitors (PIs)**

Tipranavir [Aptivus]

Darunavir [Prezista]

Ritonavir [Norvir]

Fosamprenavir [Lexiva]

Atazanavir [Reyataz]

Saquinavir [Invirase]

**Binding and Fusion Inhibitors**

CCR5 inhibitor (maraviroc) [Selzentry]

Fusion inhibitor (enfuvirtide) [Fuzeon]

**Integrase Inhibitor**

Raltegravir [Isentress]

Dolutegravir [Tivicay]

**Examples of Highly Active Antiretroviral Therapy**

Efavirenz/tenofovir/emtricitabine (EFV/TDF/FTC) [Atripla]

Abacavir/zidovudine/lamivudine [Trizivir]

Dolutegravir/abacavir/lamivudine [Triumeq]

Emtricitabine, rilpivirine, and tenofovir disoproxil fumarate [Complera]

Elvitegravir/cobicistat/tenofovir/emtricitabine [Stribild]

Emtricitabine/tenofovir disoproxil fumarate [Truvada]

Lamivudine/zidovudine [Combivir]

Lopinavir/ritonavir [Kaletra]

Modified from U.S. Department of Health and Human Services, 2018. FDA-approved HIV medicines. <https://aidsinfo.nih.gov/understandinghiv-> aids/fact-sheets/21/58/fda-approved-hiv-edicines

**Mechanisms of Retrovirus Oncogenesis**



**Representative Examples of Oncogenes**



**Oncogenic Viruses**

Some DNA viruses and retroviruses establish persistent infections that can also stimulate uncontrolled cell growth, causing **transformation** or **immortalization** of the cell.

*Characteristics of transformed cells include continued growth without senescence, alterations in cell morphology and metabolism, increased cell growth rate and sugar transport, loss of cell-contact inhibition of growth, and ability to grow in a suspension or pile up into foci when grown in a semisolid agar.*

Different **oncogenic** viruses have different mechanisms for immortalizing cells. Viruses immortalize cells by (1) activating or providing growth-stimulating genes, (2) removing the inherent braking mechanisms that limit DNA synthesis and cell growth, (3) preventing apoptosis, or (4) providing or inducing growth-stimulating cytokines. Immortalization by DNA viruses occurs in semipermissive cells, which express only selected viral genes but do not produce virus. Synthesis of viral DNA, late mRNA, late proteins, or virus leads to cell death, which precludes immortalization. Several oncogenic DNA viruses integrate into the host cell chromosome. Papillomavirus, SV40 virus, and adenovirus encode proteins that bind and inactivate cell growth–regulatory proteins, such as p53 and the retinoblastoma gene product, releasing the brakes on cell growth. Loss of p53 also makes the cell more susceptible to mutation.

Epstein-Barr virus immortalizes B cells by stimulating cell growth (as a B-cell mitogen) and by preventing programmed cell death (apoptosis).

Retroviruses (RNA viruses) use three approaches to oncogenesis. Some oncoviruses encode **oncogene** proteins (e.g., SIS, RAS, SRC, MOS, MYC, JUN, FOS) that are almost identical to the cellular proteins involved in cellular growth control (e.g., components of a growth-factor signal cascade [receptors, G-proteins, protein kinases], or growth-regulating transcription factors). The overproduction or altered function of these oncogene products stimulates cell growth. These oncogenic viruses *rapidly* cause tumors to form. *However, no human retrovirus of this type has been identified*.

**Human T-cell lymphotropic virus 1 (HTLV-1),** the only human oncogenic retrovirus identified, uses more subtle mechanisms of leukemogenesis. It encodes a protein **(TAX)** that **transactivates** gene expression, including genes for growth-stimulating cytokines (e.g., interleukin [IL]-2). This constitutes two approaches for oncogenesis.

The third approach is integration of the DNA copy of HTLV-1 near a cellular growth-stimulating gene, which can also cause the gene to be activated by the strong viral enhancer and promoter sequences encoded at each end of the viral genome (long terminal repeat [LTR] sequences). *HTLV-1–associated leukemias* ***develop slowly,*** *occurring 20 to 30 years after infection*. Retroviruses continue to produce the virus in immortalized or transformed cells. Some viruses may initiate tumor formation indirectly.

Hepatitis B virus (HBV) and HCV may have mechanisms for direct oncogenesis; however, both viruses establish persistent infections that cause inflammation and require significant tissue repair. Inflammation and continuous stimulation of liver cell growth and repair may promote mutations that lead to tumor formation. Human herpesvirus-8 (HHV-8) promotes the development of Kaposi sarcoma by means of growth-promoting cytokines encoded by the virus; this disease occurs most often in immunosuppressed patients, such as those with AIDS.

Viral transformation is the first step but is generally not sufficient to cause oncogenesis and tumor formation. Instead, over time, immortalized cells are more likely than normal cells to accumulate other mutations or chromosomal rearrangements that promote development of tumor cells. Immortalized cells may also be more susceptible to cofactors and tumor promoters (e.g., phorbol esters, butyrate) that enhance tumor formation. Approximately 15% of human cancers can be related to oncogenic viruses such as HTLV-1, HBV, HCV, human highrisk papillomaviruses, HHV-8, and Epstein-Barr virus.

**Chronic and Potentially Oncogenic Infections**

Chronic infections occur when the immune system has difficulty resolving the infection. The DNA viruses (except parvovirus and poxvirus) and the retroviruses cause latent infections with the potential for recurrence. CMV and other herpesviruses; hepatitis viruses B, C, G, and D; and retroviruses cause chronic productive infections. These “passengers” may influence the health of the individual in subtle ways.

HBV, HCV, EBV, HHV-8, HPV, and HTLV-1 are associated with **human cancers.** EBV, HPV, and HTLV-1 can immortalize cells; after immortalization, cofactors, chromosomal aberrations, or both enable a clone of virus-containing cells to grow into a cancer. EBV normally causes infectious mononucleosis but is also associated with African Burkitt lymphoma, Hodgkin lymphoma, lymphomas in immunosuppressed individuals, and nasopharyngeal carcinoma; HTLV-1 is associated with human adult T-cell leukemia. Many papillomaviruses induce a simple hyperplasia characterized by the development of a wart; however, several other strains of HPV have been associated with human cancers (e.g., types 16, 18, 33, 35, 58, and 68 are associated with cervical, anal, penile and oropharyngeal cancers.).

Direct viral action or the inflammation and chronic cell damage and repair in livers infected by HBV or HCV can result in a tumorigenic event leading to hepatocellular carcinoma. Immunosuppression in patients who have AIDS, patients undergoing cancer chemotherapy, or transplant recipients also allows the production of lymphoma by EBV. HHV-8 infection produces many cytokines to stimulate cell growth, and this growth can progress to Kaposi sarcoma, especially in persons with AIDS.

Vaccines are now available for HBV and high-risk HPV strains. Vaccination has reduced the spread of viral hepatitis, which will reduce the occurrence of primary hepatocellular carcinoma. Similarly, the HPV vaccines should also reduce the incidence of cervical and other HPV associated

cancers.

**PRIONS**

**Trigger Words**

Creutzfeldt-Jakob disease, spongiform encephalopathy, kuru, presenile dementia, myoclonus

**Biology, Virulence, and Disease**

ᑏ Prions are infectious protein aggregates resistant to inactivation

ᑏᑏ Prions consist of assembled subunits with an alternate conformation of normal host proteins (PrP)

ᑏᑏNormal PrP protein binds to the PrPSc or the multimeric PrPSc, which alters its conformation and binds and extends fibrils

ᑏᑏCollect in brain, where they cause spongiform vacuoles

ᑏᑏNo immune response, no inflammation

ᑏᑏAcquired, genetic, and sporadic forms of prion disease

ᑏᑏCreutzfeldt-Jakob disease (presenile dementia), kuru, Gerstmann-Sträussler-Scheinker disease, fatal familial insomnia

**Epidemiology**

ᑏᑏ Transmitted on contaminated surgical devices, by injection, in food, or genetic

**Diagnosis**

ᑏᑏ Symptomatology, MRI, indirect assays

**Treatment, Prevention, and Control**

ᑏᑏ Rigorous disinfection procedures

ᑏᑏNo means of prevention or control

*MRI,* Magnetic resonance imaging.

**Comparison of Classic Viruses and Prions**

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**Prion Diseases**

**Human**

Kuru

Creutzfeldt-Jakob disease

Variant CJD

Gerstmann-Sträussler-Scheinker syndrome

Fatal familial insomnia

Sporadic fatal insomnia

**Animal**

Scrapie (sheep and goats)

Transmissible mink encephalopathy

Bovine spongiform encephalopathy (BSE [mad cow

**Comparison of Scrapie Prion Protein and (Normal) Cellular Prion Protein**

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**Pathogenic Characteristics of Prions**

No cytopathologic effect in vitro

Long doubling time of at least 5.2 days

Long incubation period

Cause vacuolation of neurons (spongiform), amyloid-like plaques, gliosis

Cause loss of muscle control, shivering, tremors, dementia

Lack of antigenicity

Lack of inflammation

Lack of immune response

Lack of interferon production

**Epidemiology of Disease Caused by Prions**

**Disease/Viral Factors**

Agents are impervious to standard microbial disinfection procedures.

Diseases have very long incubation periods, as long as 30 years.

Disease acquisition may be infectious, genetic, or sporadic (random occurrence).

**Transmission**

Transmission is via **infected tissue,** or syndrome may be **inherited.**

Infection can occur by ingestion,through cuts in skin, transplantation of contaminated tissues (e.g., cornea), and use of contaminated medical devices (e.g., brain electrodes).

**Who Is at Risk?**

Members (especially women and children) of the Fore tribe in New Guinea were at risk for kuru because of ritual cannibalism.

Surgeons, transplant and brain-surgery patients, and others are at risk for CJD and GSS syndrome.

**Geography/Season**

GSS syndrome and CJD have sporadic occurrence worldwide.

There is no seasonal incidence.

**Modes of Control**

No treatments are available.

Cessation of ritual cannibalism has led to the disappearance of kuru.

Elimination of animal products from livestock feed to prevent vCJD development and transmission

For GSS syndrome and CJD, neurosurgical tools and electrodes should be disinfected in 5% hypochlorite solution or 1.0 M sodium hydroxide or autoclaved at 15 psi for 1 hour.

*CJD,* Creutzfeldt-Jakob disease; *GSS,* Gerstmann-Sträussler-Scheinker; *vCJD,* variant Creutzfeldt-Jakob disease.

**Template-mediated protein refolding model for proliferation of prions. PrPC is a normal cellular protein that is anchored in the cell membrane by phosphatidylinositol glycan. PrPSc is a hydrophobic globular protein that aggregates with itself and with PrPC on the cell surface *(1)*. PrPC acquires the conformation of PrPSc *(2).* The cell synthesizes new PrPC *(3),* and a chain is built along cell surface anionic glycosaminoglycans *(4).* The chain breaks on phagocytosis or from shear forces and releases PrPSc aggregates that act like seed crystals to start the cycle over. A form of PrPSc is internalized by neuronal cells and accumulates *(5)*. Other models have been proposed.**

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**Progression of transmissible Creutzfeldt-Jakob disease.**

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**Creutzfeldt-Jakob disease:** A 63-year-old man complained of poor memory and difficulty with vision and muscle coordination. Over the course of the next year, he developed senile dementia and irregular jerking movements, progressively lost muscle function, and then died.

**Variant Creutzfeldt-Jakob disease:** A 25-year-old is seen by a psychiatrist for anxiety and depression. After 2 months, he has problems with balance and muscle control and has difficulty remembering. He develops myoclonus and dies within 12 months of onset.