Practical lesson 22: Microbiological diagnosis of viral hepatitis, HIV (human immunodeficiency virus)

Many viruses cause hepatitis. Of these, five medically important viruses are commonly described as "hepatitis viruses" because their main site of infection is the liver.

These five are hepatitis A virus (HAV), hepatitis B virus

(HBV), hepatitis C virus (HCV), hepatitis D virus (HDV, delta virus), and hepatitis E virus (HEV) (Tables 41-1 and

41-2). Other viruses, such as Epstein- Barr virus (the cause of infectious mononucleosis), cytomegalovirus, and yellow fever virus, infect the liver but also infect other sites in the body and therefore are not exclusively hepatitis viruses.

They are discussed elsewhere.

HEPATITIS A VIRUS (HAV)

Disease

HAV causes hepatitis A.

Important Properties

HAV is a typical enterovirus classified in the picornavirus family. It has a single-stranded RNA genome and a nonen-veloped icosahedral nucleocapsid and replicates in the cytoplasm of the cell. It is also known as enterovirus 72. It has one serotype, and there is no antigenic relationship to HBV or other hepatitis viruses.

Summary of Replicative Cycle

HAV has a replicative cycle similar to that of other entero-viruses **Laboratory Diagnosis**

The detection of IgM antibody is the most important test.

A fourfold rise in IgG antibody titer can also be used. Isolation of the virus in cell culture is possible but not available in the clinical laboratory.

Treatment & Prevention

No antiviral therapy is available. Active immunization with a vaccine containing inactivated HAV is available. The virus is grown in human cell culture and inactivated with formalin. Two doses, an initial dose followed by a booster 6 to 12 months later, should be given. No subsequent booster dose is recommended. The vaccine is recommended for travelers to developing countries, for children ages 2 to 18 years, and for men who have sex with men.

If an unimmunized person must travel to an endemic area within 4 weeks, then passive immunization (see later) should be given to provide immediate protection and the vaccine given to provide long-term protection. This is an example of passive-active immunization.

Passive immunization with immune serum globulin prior to infection or within 14 days after exposure can prevent or mitigate the disease. Observation of proper hygiene (e.g., sewage disposal and handwashing after bowel move-ments) is of prime importance.

HEPATITIS A

Hepatitis A virus

Capsid

RNA

VPG
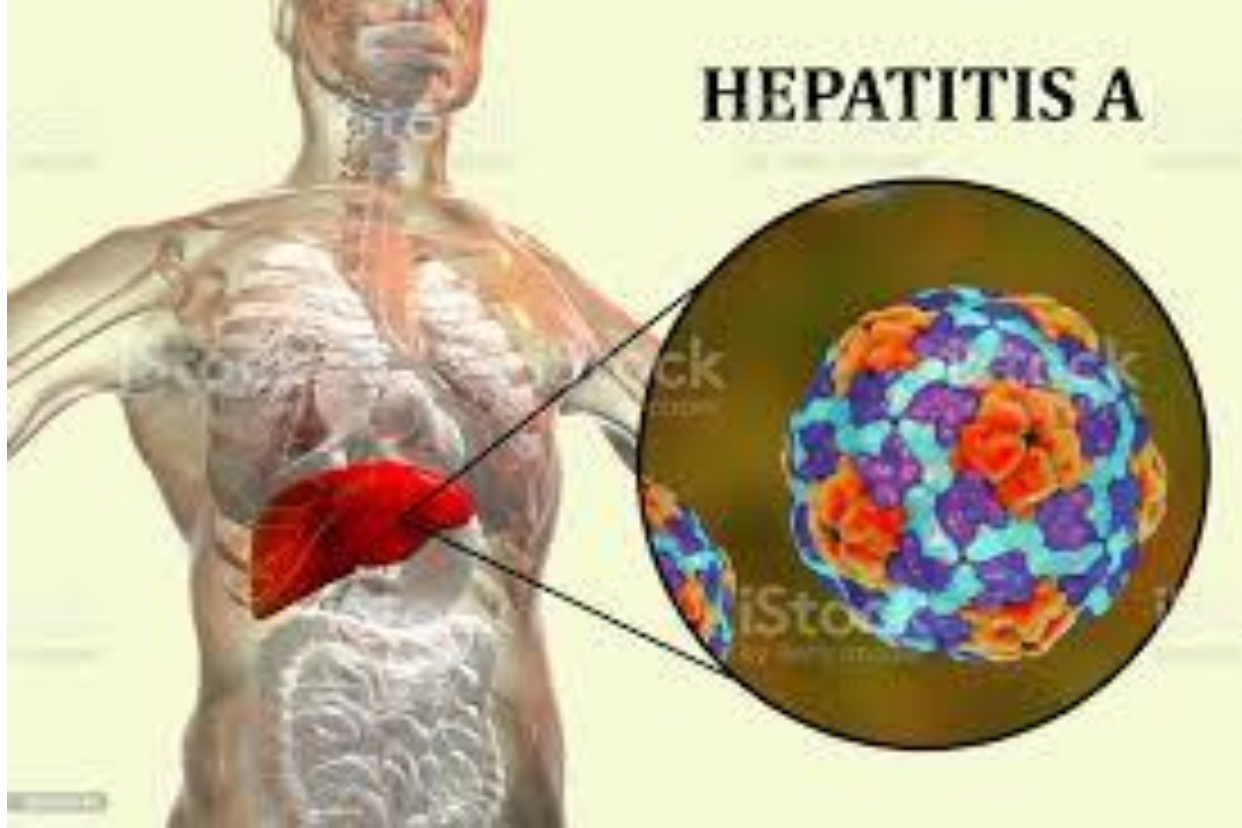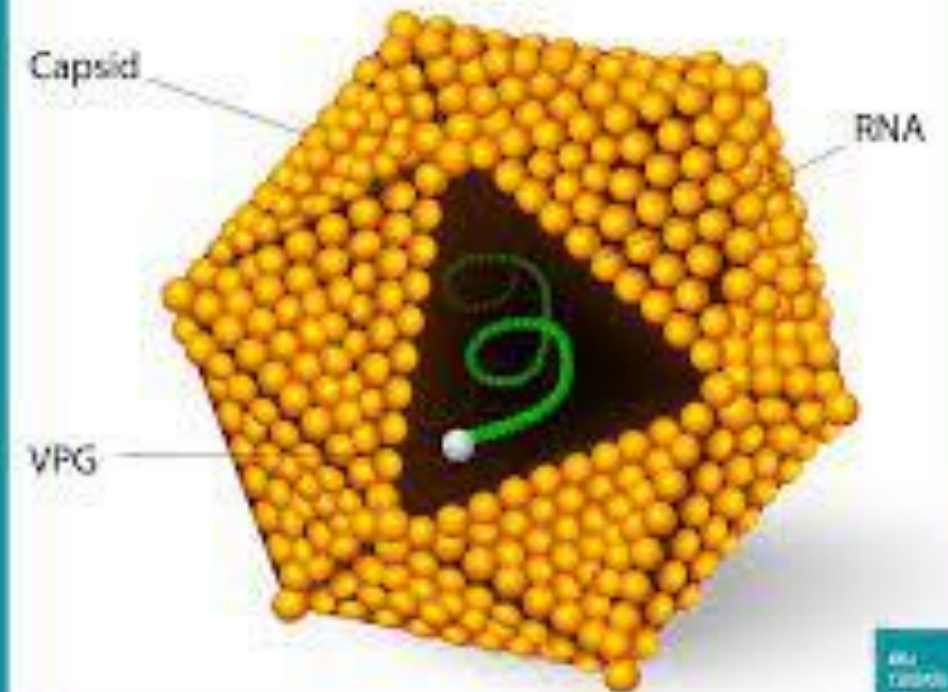
HEPATITIS B VIRUS (HBV)

Disease

HBV causes hepatitis B.

Important Properties

HBV is a member of the hepadnavirus family. It is a 42-nm enveloped virion,' with an icosahedral nucleocapsid core containing a partially double-stranded circular DNA genome
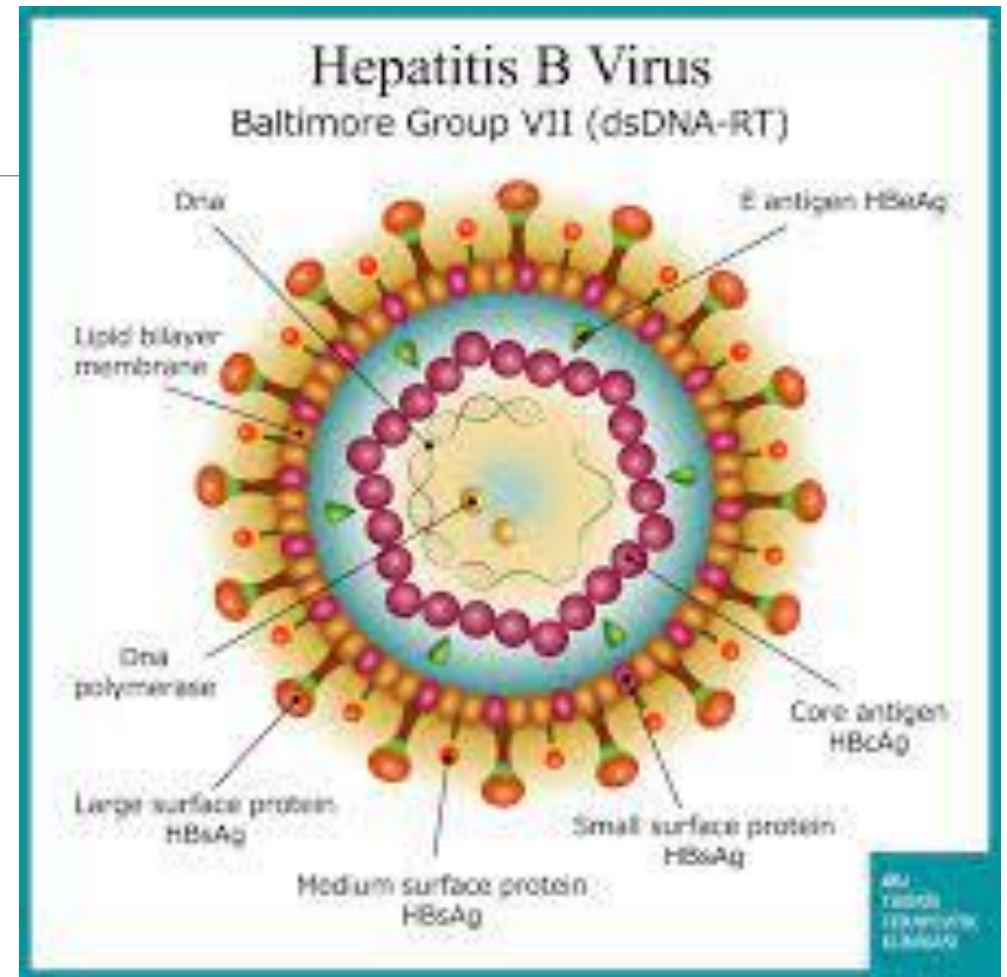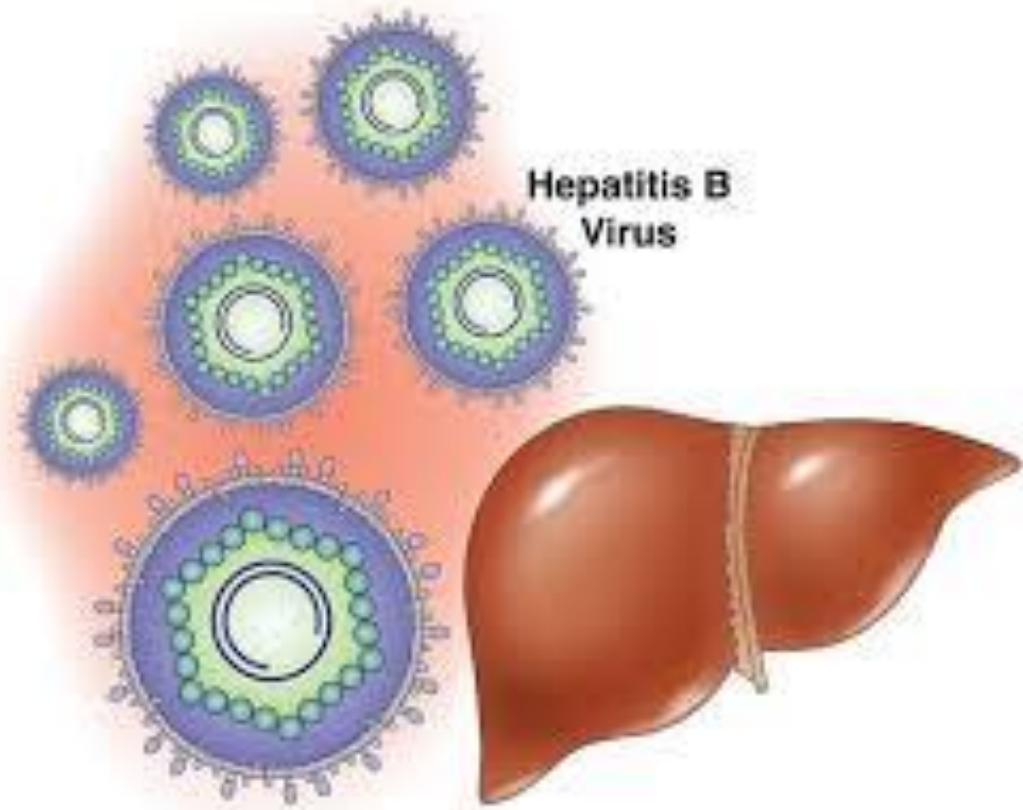
The envelope contains a protein called the surface antigen (HBsAg), which is important for laboratory diagnosis and immunization.Within the core is a DNA polymerase. The genome contains four genes (four open reading frames) that encode five proteins; namely, the S gene encodes the surface anti-gen, the C gene encodes the core antigen and the e antigen, the P gene encodes the polymerase, and the X gene encodes the X protein (HBx). HBx is an activator of viral RNA transcription and may be involved in oncogenesis because it can inactivate the p53 tumor suppressor protein (see Chapter 43). 'The DNA polymerase has both RNA-depen-dent (reverse transcriptase) and DNA-dependent activity.
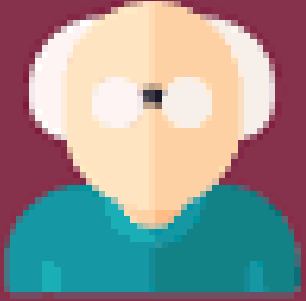
Electron microscopy of a patient's serum reveals three different types of particles: a few 42-nm virions and many

22-nm spheres and long filaments 22 nm wide, which are composed of surface antigen (Figure 41-2). HBV is the only human virus that produces these spheres and filaments in such large numbers in the patient's blood. The ratio of filaments and small spheres to virions is 1000:1.

In addition to HBsAg, there are two other important antigens both located in the core of the virus: the core antigen (HBcAg) and the e antigen (HBeAg). The core anti-gen, as the name implies, is located on the nucleocapsid protein that forms the core of the virion, whereas the e antigen is soluble and is released from infected cells into the blood. The e antigen is an important indicator of transmissibility.

The specificity of HBV for liver cells is based on two properties: virus-specific receptors located on the hepato-cyte cell membrane (facilitate entry) and transcription factors found only in the hepatocyte that enhance viral mRNA synthesis (act post-entry).

Hepatitis B Virus

Hepatitis B Virus
Baltimore Group VII (dsDNA-RT)

Dna

Lipid bilayer membrane

Dna polymerase

Large surface protein HBsAg

Medium surface protein HBsAg
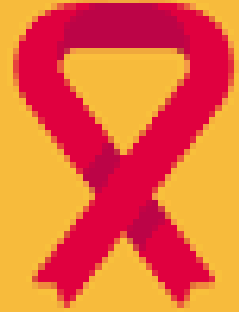
E antigen HBeAg

Core antigen HBcAg

Small surface protein HBsAg

İleri yaş

Kronik alkol tüketimi

Obezite ve diyabet

Hepatit B veya HIV enfeksiyonu

Summary of Replicative Cycle

The replicative cycle of HBV is depicted in Figure 41-3.

After entry of the virion into the cell and its uncoating, the nucleocapsid moves to the nucleus. In the nucleus, the virion DNA polymerase synthesizes the missing portion of DNA, and a double-stranded circular DNA is formed. This DNA serves as a template for mRNA synthesis by cellular RNA polymerase. After the individual mRNAs are made, a full-length positive-strand RNA is made, which is the template for the minus strand of the progeny DNA. The minus strand then serves as the template for the plus strand of the genome DNA. This RNA-dependent DNA synthesis catalyzed by reverse transcriptase encoded by HBV takes place within the newly assembled virion nucleocapsid core in the cytoplasm. The RNA-dependent DNA synthesis that produces the genome and the DNA-dependent DNA synthesis that fills in the missing portion of DNA soon after infection of the next cell are carried out by the same enzyme (i.e., the HBV genome encodes only one polymerase). Progeny HBV with its HBsAg-containing envelope are released from the cell by budding through the cell membrane.

Hepadnaviruses are the only viruses that produce genome DNA by reverse transcription with viral RNA as the template. (Note that this type of RNA-dependent DNA synthesis is similar to but different from the process in retroviruses, in which the genome RNA is transcribed into a DNA intermediate.)

Laboratory Diagnosis

The two most important serologic tests for the diagnosis of early hepatitis B are the tests for HBsAg and for IgM antibody to the core antigen. Both appear in the serum early in the disease. HBsAg appears during the incubation period and is detectable in most patients during the pro-drome and acute disease (Figure 41-4). It falls to undetectable levels during convalescence in most cases; its prolonged presence (at least 6 months) indicates the carrier state and the risk of chronic hepatitis and hepatic car-cinoma. As described in Table 41-4, HBsAb is not detectable in the chronic carrier state. Note that HBsAb is, in fact, being made but is not detectable in the laboratory tests because it is bound to the large amount of HBAg present in the blood. HBsAb is also being made during the acute disease but is similarly undetectable because it is bound in antigen-antibody complexes.

Note that there is a period

The detection of viral DNA (viral load) in the serum is strong evidence that infectious virions are present. Reduction of the viral load in patients with chronic hepatitis B is used to monitor the success of drug therapy.

Treatment

No antiviral therapy is typically used in acute hepatitis B.

For chronic hepatitis B, entecavir (Baraclude) or tenofovir (Viread) are the drugs of choice. They are nucleoside analogues that inhibit the reverse transcriptase of HBV. Interferon in the form of peginterferon alfa-2a (Pegasys) is also used. Other nucleoside analogues such as lamivudine (Epivir-HBV), adefovir (Hepsera), and telbivudine (Tyzeka) are used less frequently. A combination of tenofovir and emtricitabine (Emtriva) is also used.

HEPATITIS C VIRUS (HCV)

Disease

HCV causes hepatitis C.

Important Properties

HCV is a member of the flavivirus family. It is an enveloped virion containing a genome of single-stranded, positive-polarity RNA. It has no virion polymerase.

HCV has at least six genotypes and multiple subgeno-types based on differences in the genes that encode one of its two envelope glycoproteins. This genetic variation results in a "hypervariable" region in the envelope glyco-protein. The genetic variability is due to the high mutation rate in the envelope gene coupled with the absence of a proofreading function in the virion-encoded RNA poly-merase. As a result, multiple subspecies (quasispecies) often occur in the blood of an infected individual at the same time. Genotype 1 causes approximately 75% of infections in the United States.

Summary of Replicative Cycle

The replication of HCV is uncertain because it has not been grown in cell culture. Other flaviviruses replicate in the cytoplasm and translate their genome RNA into large poly-proteins, from which functional viral proteins are cleaved by a virion-encoded protease. This protease is the target of potent anti-HCV therapy (see treatment section). In addi-tion, HCV genome RNA encodes a protein called NS5A that cooperates with the RNA polymerase of the virus to synthesize progeny genome RNA's. The NS5A protein is also the target of potent anti-HCV therapy (see treatment section).

The replication of HCV in the liver is enhanced by a liver-specific micro-RNA called miR-122. This micro-RNA acts by increasing the synthesis of HCV mRNA. (Micro-RNAs are known to enhance cellular mRNA synthesis in many tissues.) In 2013, a clinical trial of an antisense nucleotide that bound to and blocked the activity of miR-122 showed prolonged reduction in HCV RNA levels in infected patients.

Laboratory Diagnosis

HCV infection is diagnosed by detecting antibodies to HCV in an enzyme-linked immunosorbent assay (ELISA). The antigen in the assay is a recombinant protein formed from three immunologically stable HCV proteins and does not include the highly variable envelope proteins. The test does not distinguish between IgM and IgG and does not distinguish between an acute, chronic, or resolved infection. If the result of ELISA antibody test is positive, a polymerase chain reaction-based test that detects the presence of viral RNA (viral load) in the serum should be performed to determine whether active disease exists. Reduction of the viral load in patients with hepatitis C is used to monitor the success of drug therapy. Isolation of the virus from patient specimens is not done. A chronic infection is characterized by elevated transaminase levels, a positive ELISA antibody test, and detectable viral RNA for at least 6 months.

Structure of Hepatitis C Virus
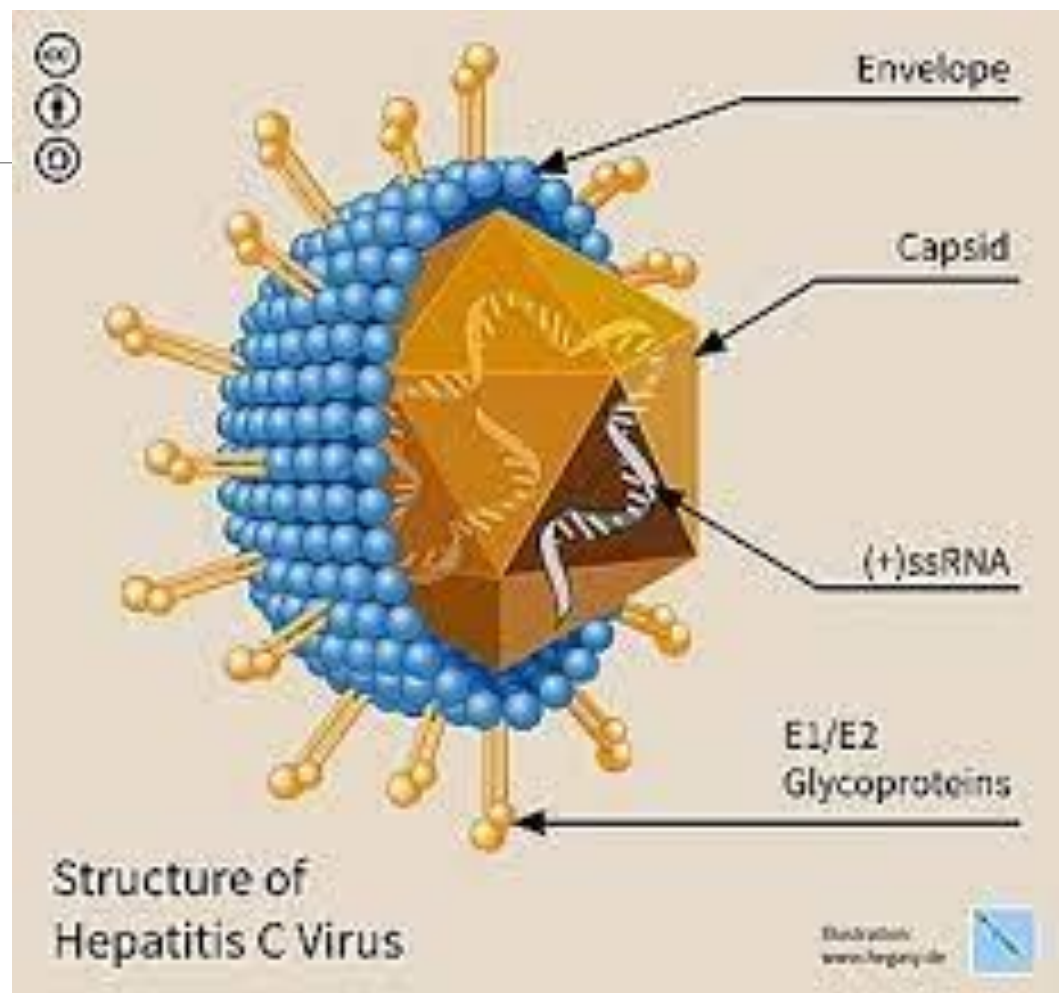
Treatment

Treatment of acute hepatitis C with peginterferon alfa significantly decreases the number of patients who become chronic carriers.

The treatment of choice for chronic hepatitis C is a combination of drugs from three classes: RNA polymerase inhibi-tors, NS5A inhibitors, and protease inhibitors .

These drugs are administered orally which is an improvement over the drugs in previous regimens that often included pegylated interferon-alpha which is administered parenter-ally and has significant adverse effects. One currently available combination is Harvoni which is a combination of sofosbuvir (RNA polymerase inhibitor) and ledipasvir (NS5A inhibitor). Another is Viekera which is a four-drug combina-tion: dasabuvir (RNA polymerase inhibitor), ombitsavir (NS5A inhibitor), paritaprevir (protease inhibitor) and rito-navir.

These drug combinations are very effective against genotype 1, the most common type in the United States. Daklinza, a combination of sofosbuvir (RNA polymerase inhibitor) and daclatasvir (NS5A inhibitor) is effective against genotype 3 HCV. These various drug combinations offer the prospect of a "cure" for chronic hepatitis C.

Prevention

There is no vaccine and hyperimmune globulins are not available. Pooled immune serum globulins are not useful

Patients with chronic HCV infection should be advised to reduce or eliminate their consumption of alcoholic beverages to reduce the risk of hepatocellular carcinoma and cirrhosis. Patients with chronic HCV infection and cirrhosis should be monitored with alpha-fetoprotein tests and liver sonograms to detect carcinoma at an early stage.

Patients with liver failure due to HCV infection can receive a liver transplant, but infection of the graft with HCV typically occurs.

Patients confected with HCV and HIV should be prescribed highly active antiretroviral therapy (HAART) with caution because recovery of cell-mediated immunity can result in an exacerbation of hepatitis (immune reconstitution syndrome, IRIS). Consideration should be given to treat the HCV infection prior to starting

HEPATITIS D VIRUS (HDV, DELTA VIRUS

Disease

HDV causes hepatitis D (hepatitis delta).

Important Properties & Replicative

Cycle

HDV is unusual in that it is a defective virus (i.e., it cannot replicate by itself because it does not have the genes for its envelope protein). HDV can replicate only in cells also infected with HBV because HDV uses the surface antigen of HBV (HBsAg) as its envelope protein. HBV is therefore the helper virus for HDV (Figure 41-5).

HDV is an enveloped virus with an RNA genome that is a single-stranded, negative-polarity, covalently closed circle. The RNA genome of HDV is very small and encodes only one protein, the internal core protein called delta antigen. HDV genome RNA has no sequence homology to HBV genome DNA. HDV has no virion polymerase; the genome RNA is replicated and transcribed by the host cell RNA polymerase. HDV genome RNA is a "ribozyme" (ie., it has the ability to self-cleave and self-ligate-properties that are employed during replication of the genome). HDV replicates in the nucleus, but the specifics of the replicative cycle are complex and beyond the scope of this book.

HDV has one serotype because HBsAg has only one serotype. There is no evidence for the existence of an animal reservoir for HDV.

Laboratory Diagnosis

The diagnosis of HDV infection in the laboratory is made by detecting either delta antigen or IgM antibody to delta antigen in the patients serum.

Treatment & Prevention

Peginterferon alfa can mitigate some of the effects of the chronic hepatitis caused by HD but does not eradicate the chronic carrier state. There is no specific antiviral therapy against HDV. There is no vaccine against HDV, but a person immunized against HBV will not be infected by HDV because HDV cannot replicate unless HBV infection also occurs.

HEPATITIS E VIRUS (HEV)

HEV is a major cause of hepatitis transmitted by the fecal-oral route. It is thought to be more common than HAV in many developing countries. It is a common cause of waterborne epidemics of hepatitis in Asia, Africa, India, and Mexico but is uncommon in the United States. HEV is a nonenveloped, single-stranded RNA virus classified as a member of the hepevirus family.

Clinically the disease resembles hepatitis A, with the exception of a high mortality rate in pregnant women.

Chronic infection resulting in chronic hepatitis and cirrhosis but not hepatocellular carcinoma, occurs in immuno-compromised individuals such as HIV-infected patients, those who are receiving cancer chemotherapy, and patients who are receiving immunosuppressive drugs to prevent reiection of solid-organ transplants.

The diagnosis is typically made by detecting IgM antibody to HEV. A PCR assay that detects HEV RNA in patient specimens is available. There is no antiviral drug available for acute infection in immunocompetent patients.

In immunocompromised patients, ribavirin cleared HEV viremia in solid organ transplant recipients. There is no

HEPATITIS G VIRUS (HGV)

In 1996, hepatitis G virus (HGV) was isolated from patients with posttransfusion hepatitis. HGV is a member of the flavivirus family, as is HCV. However, unlike HCV, which is clearly the cause of both acute hepatitis and chronic active hepatitis and predisposes to hepatocellular carcinoma, HGV has not been documented to cause any of these clinical findings. The role of HGV in the causation of liver disease has yet to be established, but it can cause a chronic infection lasting for decades. Approximately 60% to 70% of those infected clear the virus and develop antibodies.

HG V is transmitted via sexual intercourse and blood. It is carried in the blood of millions of people worldwide. In the United States, it is found in the blood of approximately 2% of random blood donors, 15% of those infected with HCV, and 35% of those infected with HIV. Patients coin-fected with HIV and HGV have a lower mortality rate and have less HIV in their blood than those infected with HIV alone. It is hypothesized that HGV may interfere with the replication of HIV. (HGV is also known as GB virus C.)

HİV

Disease

Human immunodeficiency virus (HIV) is the cause of acquired immunodeficiency syndrome (AIDS).

Both HIV-1 and HIV-2 cause AIDS, but HIV-1 is found worldwide, whereas HIV-2 is found primarily in West Africa. This chapter refers to HIV-1 unless otherwise noted.

Important Properties

HIV is one of the two important human T-cell lympho-tropic retroviruses (human T-cell leukemia virus is the other). HIV preferentially infects and kills helper (CD4) T lymphocytes, resulting in the loss of cell-mediated immunity and a high probability that the host will develop opportunistic infections. Other cells (e.g., macrophages and monocytes) that have CD4 proteins on their surfaces can be infected also.

The natural host range of HIV is limited to humans, although certain primates can be infected in the laboratory.

HIV is not an endogenous virus of humans (i.e., no HIV sequences are found in normal human cell DNA). The origin of HIV and how it entered the human population remains uncertain. There is evidence that chimpanzees living in West Africa were the source of HIV-1. If chimpanzees are the source of HIV in humans, it would be a good example of a virus "jumping the species barrier.

Box 1.1 Structure of the HIV-1 virus (adapted from reference 6). This figure is schematic and somewhat simplified for clarity. For example, the structures of the envelope glycoproteins gp120 and gp41 are actually trimers in reality, not the monomers as shown here.
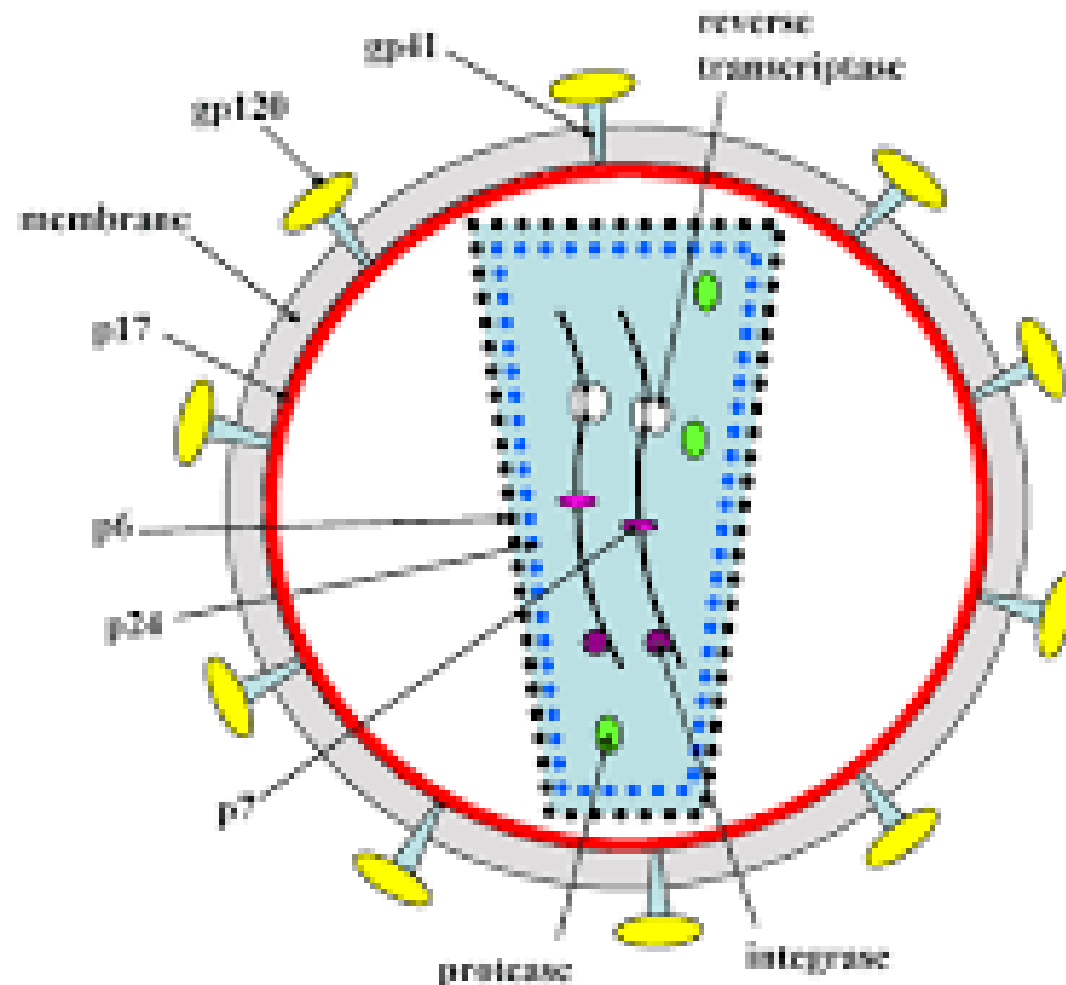
In addition to HIV-1, two other similar retroviruses are worthy of comment:

Human immunodeficiency virus type 2 (HIV-2) was isolated from AIDS patients in West Africa in 1986. The proteins of HIV-2 are only about 40% identical to those of the original HIV isolates. HIV-2 remains localized primarily to West Africa and is much less transmissible than HIV-1.

Simian immunodeficiency virus (SIV) was isolated from monkeys with an AIDS-like illness. Antibodies in some African women cross-react with SIV. The proteins of SIV resemble those of HIV-2 more closely than they resemble those of the original HIV isolates.

Summary of Replicative Cycle

In general, the replication of HIV follows the typical retro-viral cycle (Figure 45-4). The initial step in the entry of HIV into the cell is the binding of the virion gp120 envelope protein to the CD4 protein on the cell surface. The virion gp120 protein then interacts with a second protein on the cell surface, one of the chemokine receptors. Next, the virion gp41 protein mediates fusion of the viral envelope with the cell membrane, and the virion core containing the nucleocapsid, RNA genome, and reverse transcriptase enters the cytoplasm. Chemokine receptors, such as CXCR4 and CCR5 pro-teins, are required for the entry of HIV into CD4-positive cells. The T cell-tropic strains of HIV bind to CXCR4, whereas the macrophage-tropic strains bind to CCR5. Mutations in the gene encoding CCR5 endow the individual with protection from infection with HIV. People who are homozygotes are completely resistant to infection, and heterozygotes progress to disease more slowly. Approximately 1% of people of Western European ancestry have homozygous mutations in this gene, and about 10% to 15% are heterozygotes. One of the best-characterized mutations is the delta-32 mutation, in which 32 base pairs are deleted from the CCR5 gene. In the cytoplasm, reverse transcriptase transcribes the genome RNA into double-stranded DNA, which migrates to the nucleus, where it integrates into the host cell DNA. The viral DNA can integrate at different sites in the host cell DNA, and multiple copies of viral DNA can integrate. Integration is mediated by a virus-encoded endonuclease (integrase). Viral mRNA is transcribed from the integrated (proviral) DNA by host cell RNA polymerase (augmented by virus-encoded Tat protein) and translated into several large polyproteins. The Gag and Pol polyproteins are cleaved by the viral protease, whereas the Env polyprotein is cleaved by a cellular protease. The Gag polyprotein is cleaved to form the main core protein (p24), the matrix protein (p17), and several smaller proteins. The Pol polyprotein is cleaved to form the reverse transcriptase, integrase, and protease. The immature virion containing the precursor polyproteins forms in the cyto-plasm, and cleavage by the viral protease occurs as the immature virion buds from the cell membrane. It is this cleavage process that results in the mature, infectious virion.
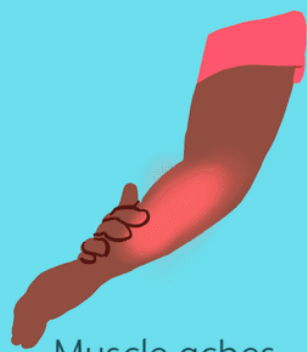
Symptoms of Acute HIV

Fever

Chills

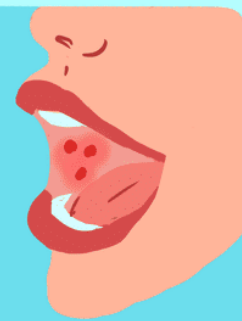Headaches

Night Sweats

Sore throat

Muscle aches and pains

Joint pain

Fatigue

Swollen lymph nodes, mainly on the neck

Mouth ulcers

*Many patients experience no symptoms

verywell

Transmission & Epidemiology

Transmission of HIV occurs primarily by sexual contact and by transfer of infected blood. Perinatal transmission from infected mother to neonate also occurs, either across the placenta, at birth, or via breast milk. It is estimated that more than 50% of neonatal infections occur at the time of delivery and that the remainder is split roughly equally between transplacental transmission and transmission via breast feeding. There is no evidence for airborne, water-borne, or insect transmission of HIV. Infection occurs by the transfer of either HIV-infected cells or free HIV (i.e., HIV that is not cell-associated).

Although small amounts of virus have been found in other fluids (e.g., saliva and tears), there is no evidence that they play a role in infection. In general, transmission of HIV follows the pattern of hepatitis B virus (HBV), except that HIV infection is much less efficiently transferred (i.e., the dose of HIV required to cause infection is much higher than that of HBV). People with sexually transmitted dis-eases, especially those with ulcerative lesions such as syphi-lis, chancroid, and herpes genitalis, have a significantly higher risk of acquiring HIV. Uncircumcised males have a higher risk of acquiring HIV than do circumcised males.

Transmission of HIV via blood transfusion has been greatly reduced by screening donated blood for the presence of antibody to HIV. However, there is a "window" period early in infection when the blood of an infected person can contain HIV but antibodies are not detectable.

Blood banks now test for the presence of p24 antigen in an effort to detect blood that contains HIV.

Laboratory Diagnosis

The presumptive diagnosis of HIV infection is often made by the detection of antibodies in the patient's serum to the p24 protein of HIV using the enzyme-linked immunosor-bent assay (ELISA) test. Because there are some false-positive results with this test, the definitive diagnosis is made by Western blot (also known as Immunoblot) analysis, in which the viral proteins are displayed by acryl-amide gel electrophoresis, transferred to nitrocellulose paper (the blot), and reacted with the patient's serum. If antibodies are present in the patient's serum, they will bind to the viral proteins (predominantly to the gp41 or p24 protein). Enzymatically labeled antibody to human IgG is then added. A color reaction reveals the presence of the HIV antibody in the infected patient's serum.

HIV infection.

OraQuick is a rapid, screening immunoassay for HIV antibody that uses an oral swab sample in an ELISA-type test that can be done at home. Results are available in 20 minutes. Positive results for HIV antibody require confirmation by a Western blot test.

The PCR test is a very sensitive and specific technique that can be used to detect HIV DNA within infected cells.

Treatment

The treatment of HIV infection has resulted in a remarkable reduction in mortality and improvement in the quality of life of infected individuals. The two specific goals of treatment are (1) to restore immunologic function by increasing the CD4 count, which reduces opportunistic infections and certain malignancies, and (2) to reduce viral load, which reduces the chance of transmission to others.

There is evidence that starting drug therapy as soon as possible after making the diagnosis of HIV infection is the best way to achieve these goals.

Unfortunately, no drug regimen results in a "cure" (i.e., eradicates the virus from the body), but long-term suppression can be achieved. However, if drugs are stopped, the virus resumes active replication, and large amounts of infectious virus reappear.

Treatment of HIV infection typically involves multiple antiretroviral drugs. The use of a single drug (monother-apv) for treatment is not done because of the high rate of mutation to drug resistance.

The choice of drugs is complex and depends on several factors (e.g., whether it is an initial infection or an estab-ished infection, the number of CD4 cells, the viral load, the resistance pattern of the virus, and whether the patient is pregnant or is confected with HBV or hepatitis C virus

Prevention

No vaccine is available. Multiple trials of a variety of experimental vaccines have failed to induce protective antibodies, protective cytotoxic T cells, or mucosal immu-nity. Prevention consists of taking measures to avoid exposure to the virus (e.g., using condoms, not sharing needles, and discarding donated blood that is contaminated with HIV).

Postexposure prophylaxis (PEP), such as that given after a needle-stick injury or a high-risk nonoccupational exposure, employs three drugs: the preferred regimen consists of the combination of tenofovir and emtricitabine (given as Truvada) plus raltegravir. Three alternative drug regimens are available. PEP should be given as soon as possible after exposure and continued for 28 days. Truvada can also be used for preexposure prophylaxis (PrEP) in individuals at high risk of infection, such as men who have sex with men.

Two steps can be taken to reduce the number of cases of

HIV infection in children: antiretroviral therapy should be given to HIV-infected mothers and neonates, and HIV-infected mothers should not breast feed. The choice of antiretroviral drugs is dependent on several factors, so current guidelines should be consulted. In addition, the risk of neonatal HIV infection is lower if delivery is accomplished by cesarean section rather than by vaginal delivery. Circumcision reduces HIV infection. Several drugs are commonly taken by patients in the advanced stages of AIDS to prevent certain opportunistic infections (Table 45-4). Some examples are trimethoprim-sulfamethoxazole to prevent Pneumocystis pneumonia, flu-conazole to prevent recurrences of cryptococcal meningitis, ganciclovir to prevent recurrences of retinitis caused by cytomegalovirus, and oral preparations of antifungal drugs, such as clotrimazole, to prevent thrush caused by C. albicans.