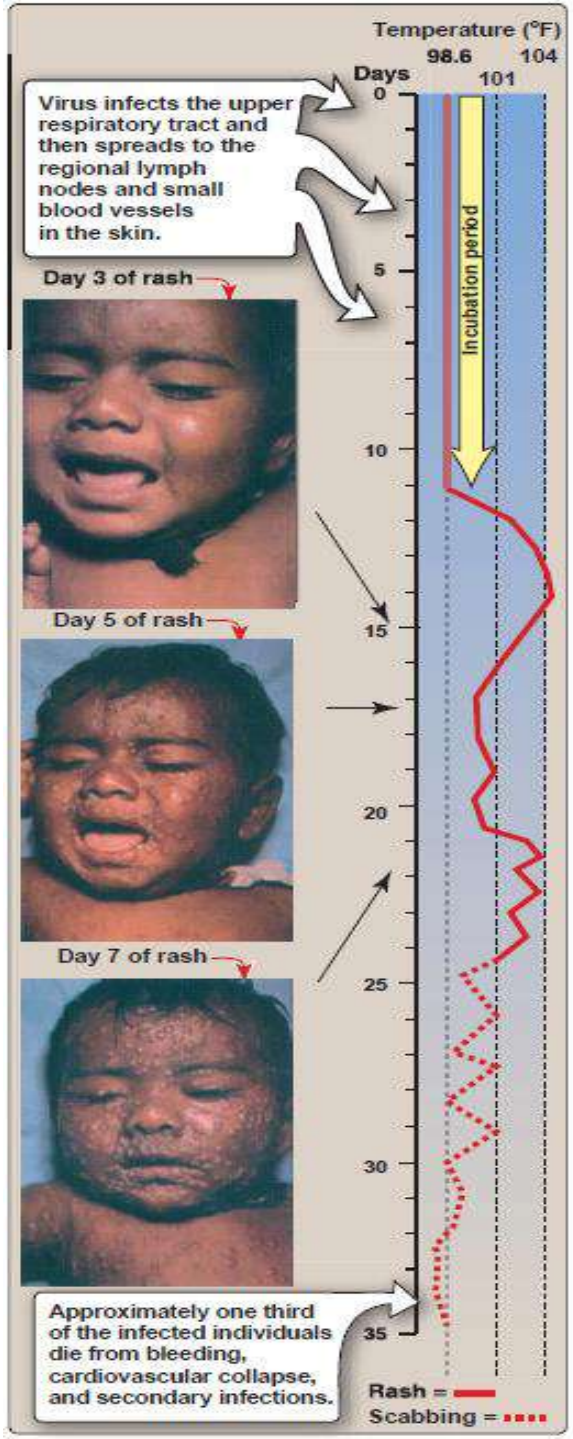
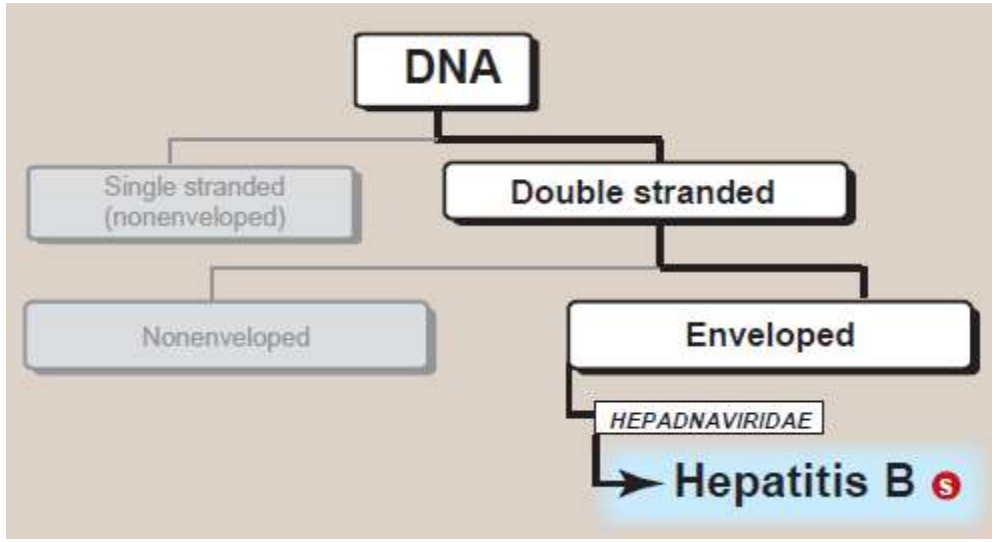
- **POXVIRIDAE**
- Poxviruses belong to a family of large, genetically complex viruses having no obvious symmetry.
- Members of this family are widely distributed in nature. The agent of previous medical importance to humans, variola virus, was the cause of smallpox, the first infectious disease to be declared eradicated from the Earth
- Among the factors that led to this success are:
- 1) the availability of an effective, attenuated vaccine;
- 2) variola's antigenic stability (that is, only a single antigenic type existed);
- 3) the absence of asymptomatic cases or persistent carriers;
- 4) the absence of an animal reservoir; and
- 5) the emotional effect of this highly lethal, disfiguring disease, which helped to galvanize public support of and cooperation in the eradication efforts.
- The highly effective poxvirus vaccine contains live vaccinia virus (which causes cowpox), and the viral genome is currently being used in attempts to construct vectors carrying immunizing genes from other infectious agents.
- Finally, the poxvirus, molluscum contagiosum virus (MCV), causes small, wartlike tumors

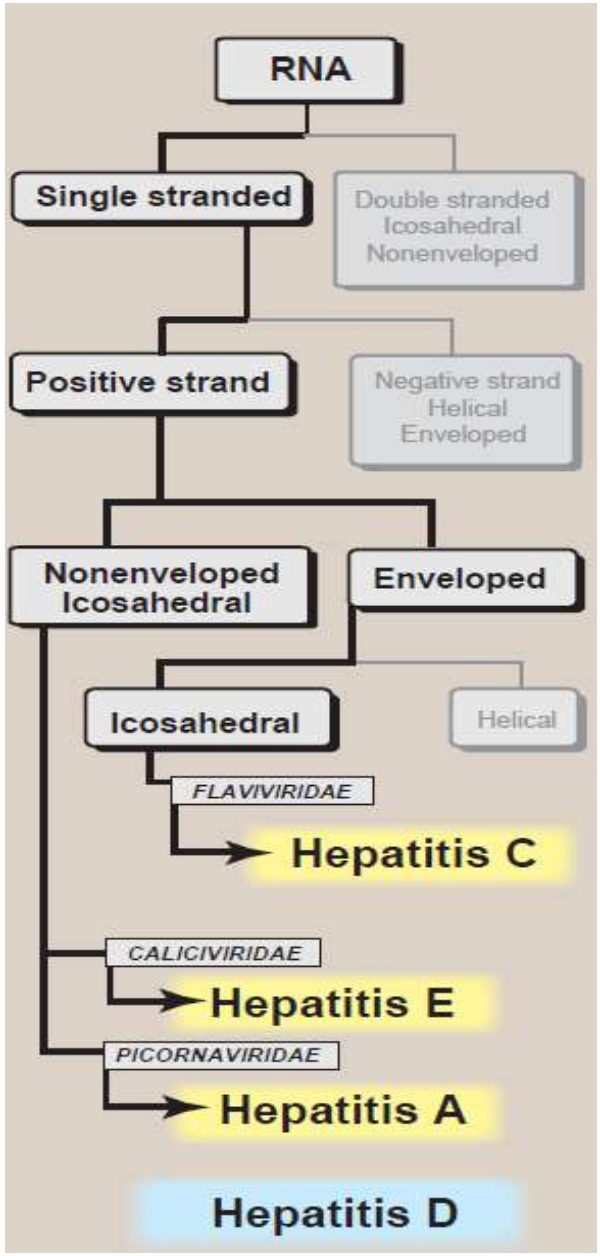
- **Replication of the poxviruses**
- Poxviruses follow the basic replication pattern for DNA viruses with a few notable exceptions.
- The most striking of these is that the entire replication cycle takes place in the cytoplasm, the virus providing all of the enzymes (including a viral DNA-dependent RNA polymerase) necessary for DNA replication and gene expression.
- **Laboratory identification**
- The unique cellular localization of poxvirus replication has enabled rapid diagnosis by observation of DNA-containing intracytoplasmic inclusion bodies in cells scraped from skin lesions
- **Smallpox as a biologic weapon**
- Smallpox is potentially a devastating biologic weapon because it is highly contagious and has a high case fatality rate—more than 30 percent among unvaccinated persons



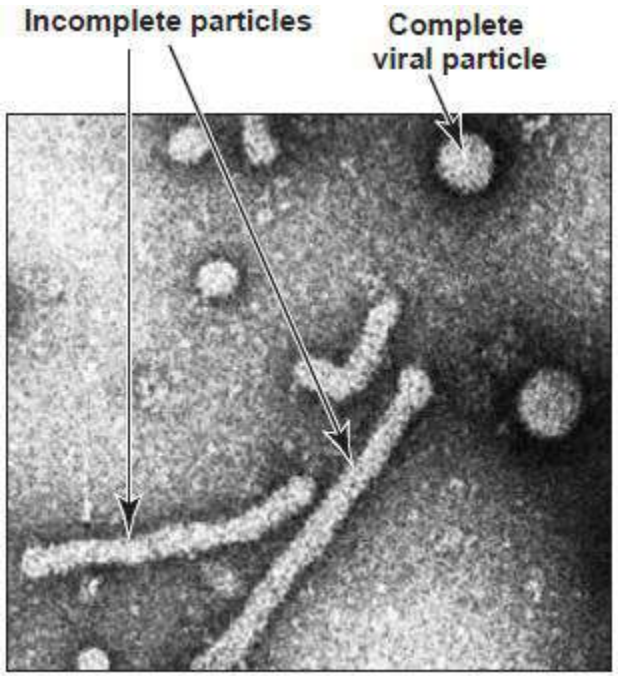
Hepadnaviridae

- With the exception of HBV, hepatitis viruses thus far identified (hepatitis A, C, D, and E viruses) contain RNA and belong to several different families but the acute disease produced by each is similar





- **Structure and replication of hepatitis B virus**
- The HBV virion, historically referred to as the “Dane particle,” consists of an icosahedral nucleocapsid enclosed in an envelope



Organization of the hepatitis B virus genome: The short HBV DNA genome is unusual in that it is a partly single-stranded, partly double-stranded, noncovalently closed, circular DNA molecule (that is, one strand is longer than the other)

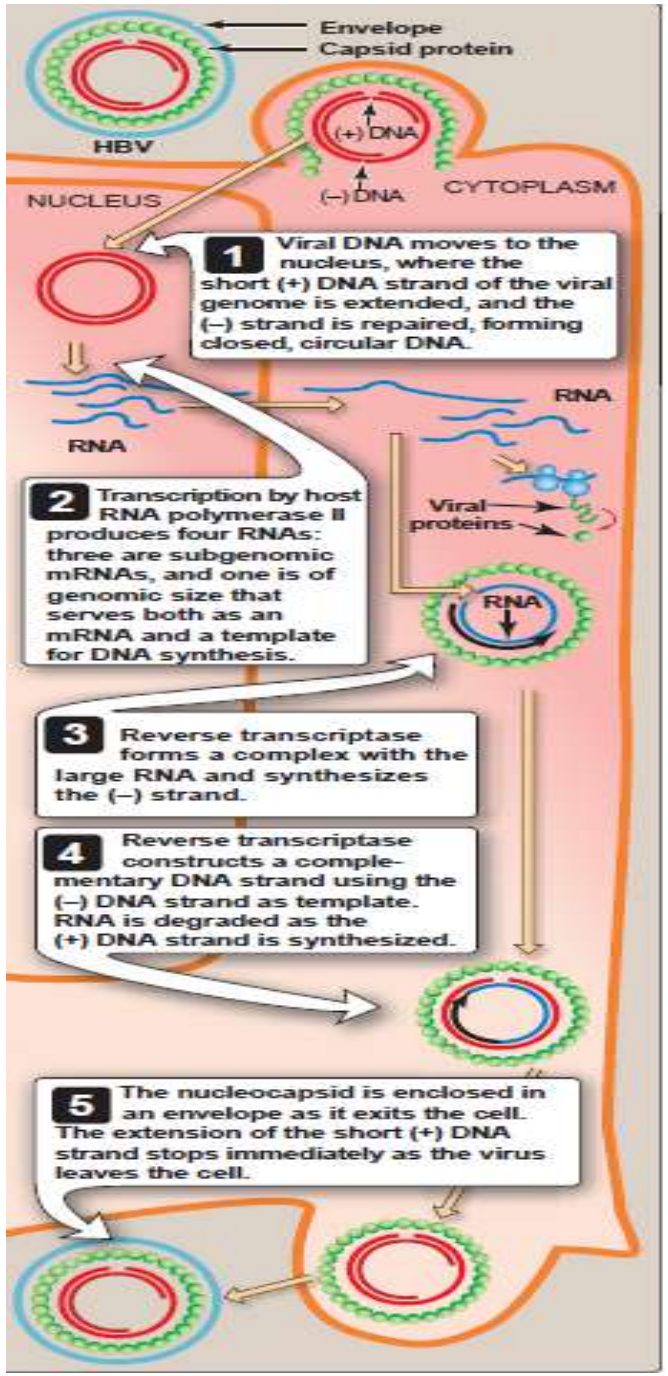
Viral proteins: The four proteins encoded by viral DNA are: 1) the core protein [hepatitis B nucleocapsid core antigen (HBcAg)];

2) envelope protein [a glycoprotein referred to as hepatitis B surface antigen (HBsAg)];

3) multifunctional reverse transcriptase/DNA polymerase, which is complexed with the DNA genome within the capsid; and

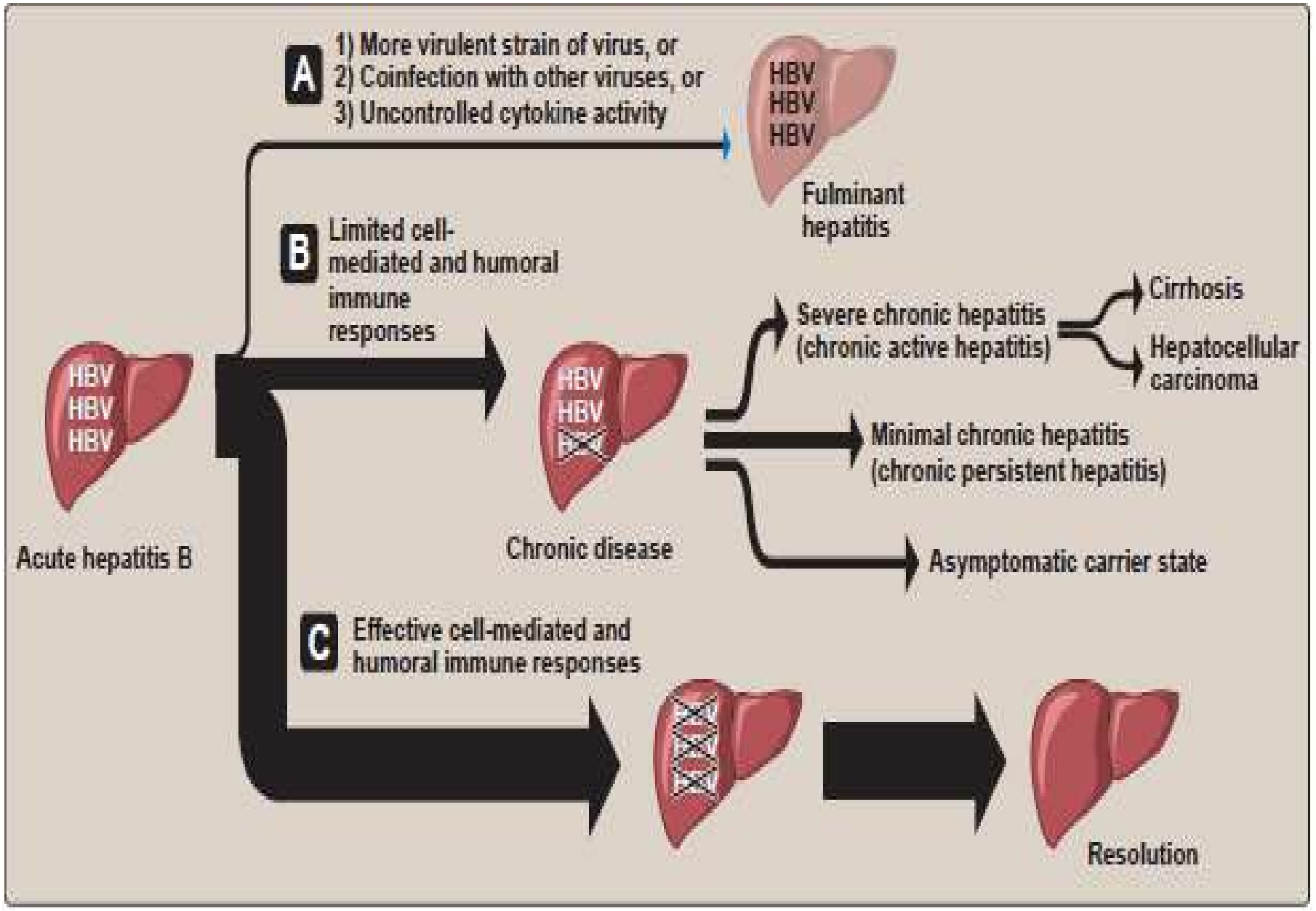
4) a nonstructural regulatory protein designated the “X protein.”

Replication of hepatitis B virus (HBV)

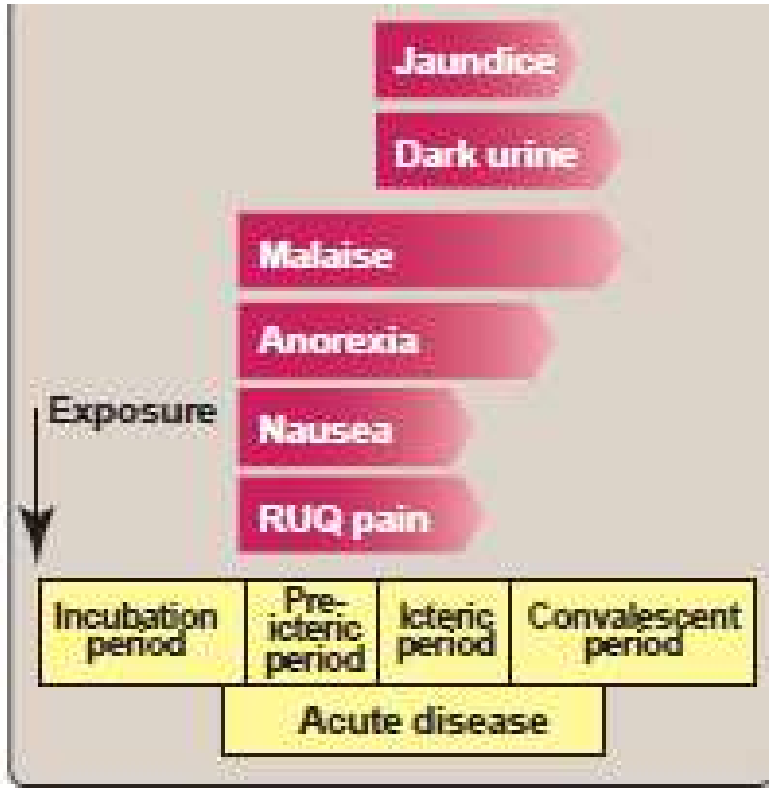


- **Pathogenesis**
- Fully differentiated hepatocytes are the primary cell type infected by HBV.
- The primary cause of hepatic cell destruction appears to be the cell-mediated immune response, which results in inflammation and necrosis.
- The cells involved are cytotoxic T cells, which react specifically with the fragments of nucleocapsid proteins (HBcAg and HBeAg), expressed on the surface of infected hepatocytes.
- This response also contributes to control of the infection by eliminating virus-producing cells.
- Enhanced natural killer cell activity, as well as production of interferon-γ also contributes to limiting the extent of infection.
- Anti-HBsAg antibody, which is the neutralizing antibody, does not appear until well into the convalescence period, when it may aid in clearing any remaining circulating free virus.
- More importantly, this antibody provides protection against reinfection. However, it is this same humoral antibody that is considered the source of extrahepatic damage seen in 10 to 20 percent of patients, through the formation and deposition of HBsAg/anti-HBsAg antibody immune complexes and the consequent activation of complement

- **Clinical significance: acute disease**
- HBV is important medically and in public health, not only as the cause of acute liver disease but also as the cause of chronic, persistent infections that can result in the eventual death of infected individuals from cirrhosis and liver cancer.
- Chronically infected people serve as the reservoir of transmissible virus in the population.
- In most individuals, the primary infection is asymptomatic and resolves as a result of an effective cell-mediated immune response


A
1) More virulent strain of virus, or
2) Coinfection with other viruses, or
3) Uncontrolled cytokine activity
HBV
HBV
HBV
Fulminant hepatitis
B
Limited cell-mediated and humoral immune responses
HBV
HBV
HBV
Acute hepatitis B
HBV
HBV
Chronic disease
Severe chronic hepatitis (chronic active hepatitis)
Cirrhosis
Hepatocellular carcinoma
Minimal chronic hepatitis (chronic persistent hepatitis)
Asymptomatic carrier state
C
Effective cell-mediated and humoral immune responses
Resolution

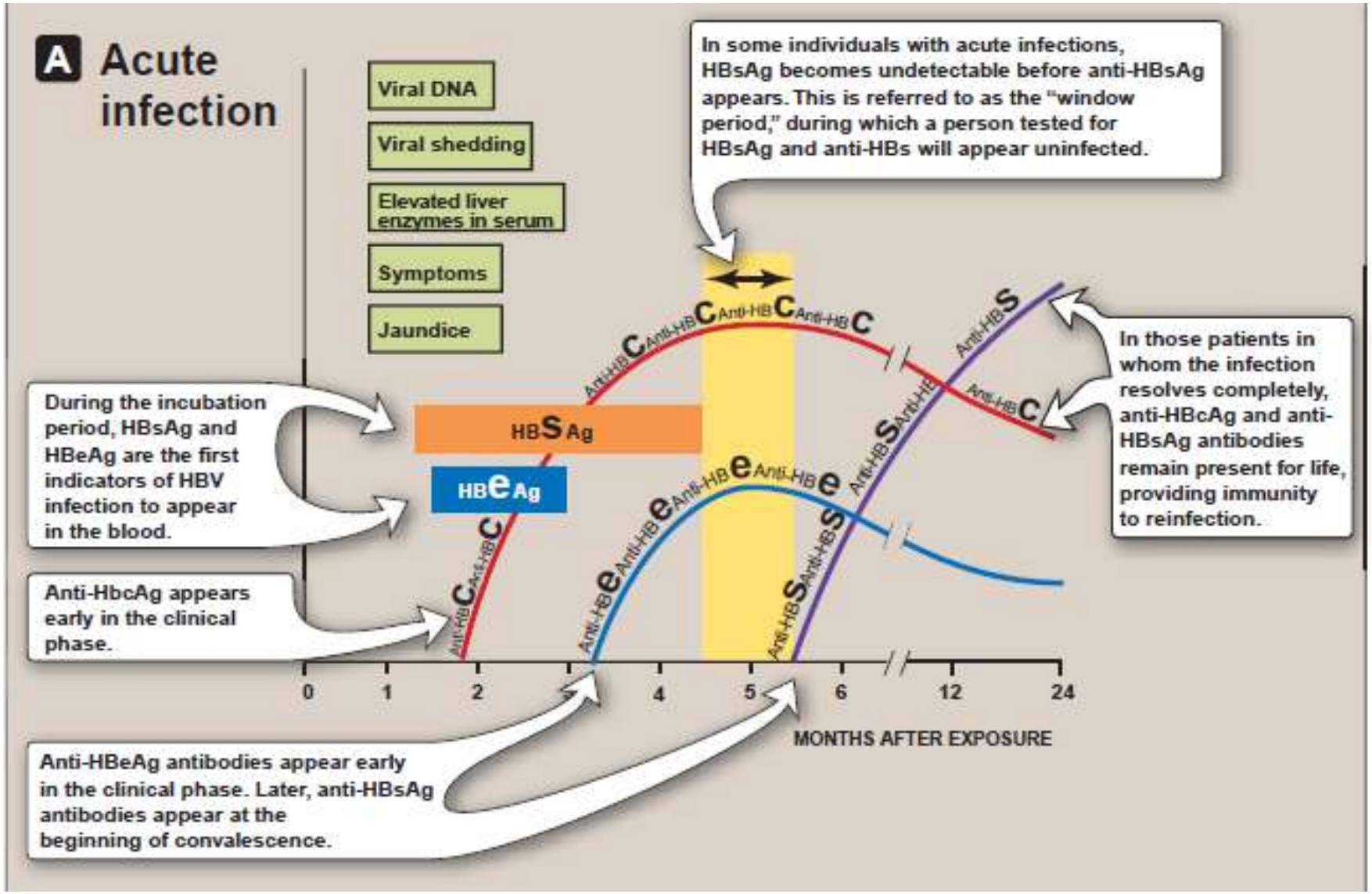
- **1. Phases in acute hepatitis B virus infections: Following infection,** HBV has a long but variable incubation period of between 45 and 120 days.
- Following this period, a pre-icteric (prejaundice) phase occurs, lasting several days to a week. This is characterized by mild fever, malaise, anorexia, myalgia, and nausea.
- The acute, icteric phase then follows and lasts for 1 to 2 months. During this phase, dark urine, due to bilirubinuria, and jaundice (a yellowish coloration of mucous membranes, conjunctivae, and skin) are evident.
- There usually is an enlarged and tender liver as well.
- In 80 to 90 percent of adults, a convalescent period of several more months is followed by complete recovery

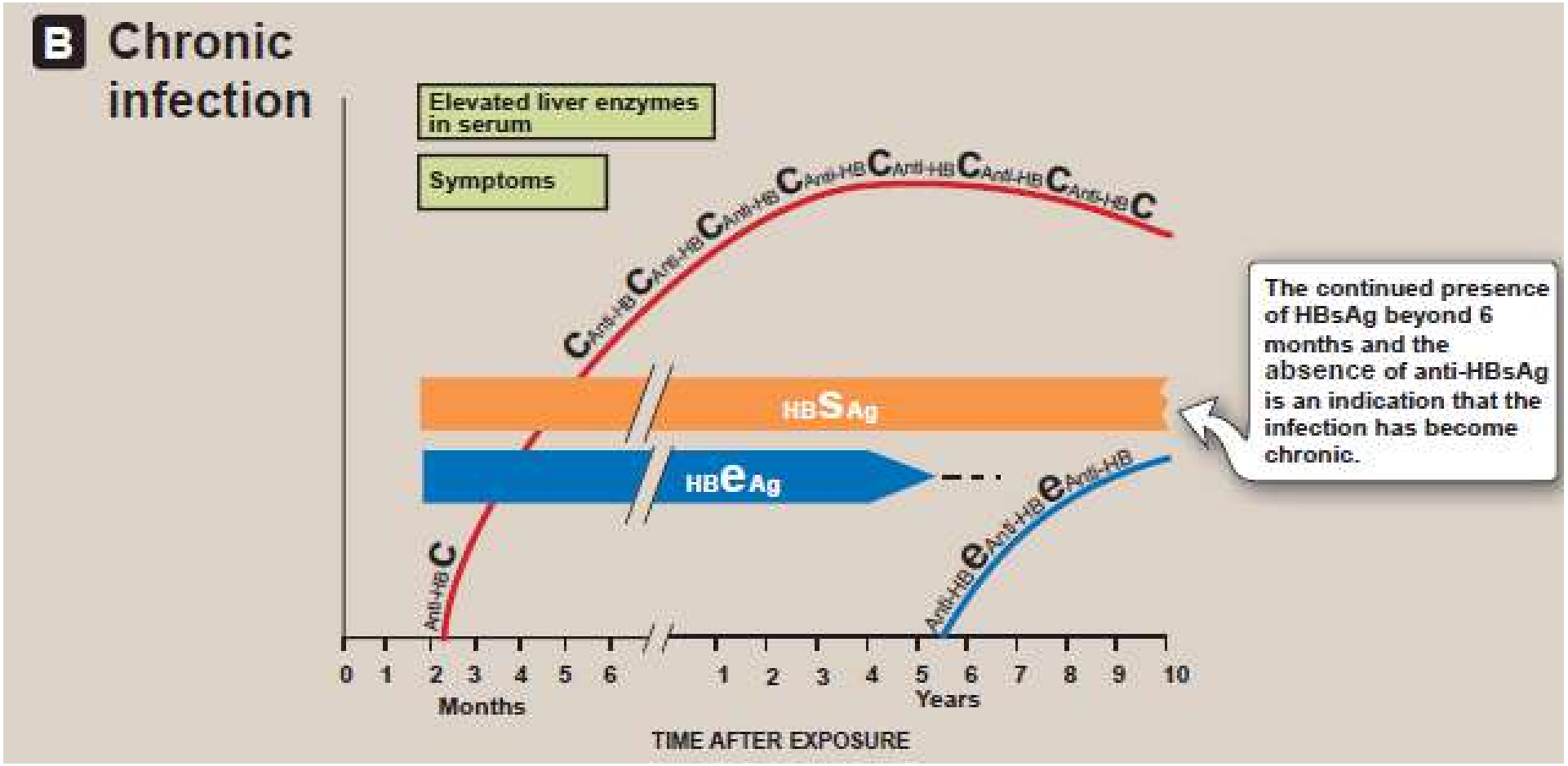


- **Monitoring the course of acute hepatitis B virus infection**

  a. Appearance of viral antigens: During the incubation period, HBsAg and hepatitis B e antigen (HBeAg) are the first indicators of HBV infection to appear in the blood
- Their presence indicates an active infection but does not distinguish between acute and chronic infections.
- Next, viral DNA, viral DNA polymerase, and complete virions become detectable.
- These continue to increase during the acute disease phase, when a patient's blood has the highest titer of infectious virus.

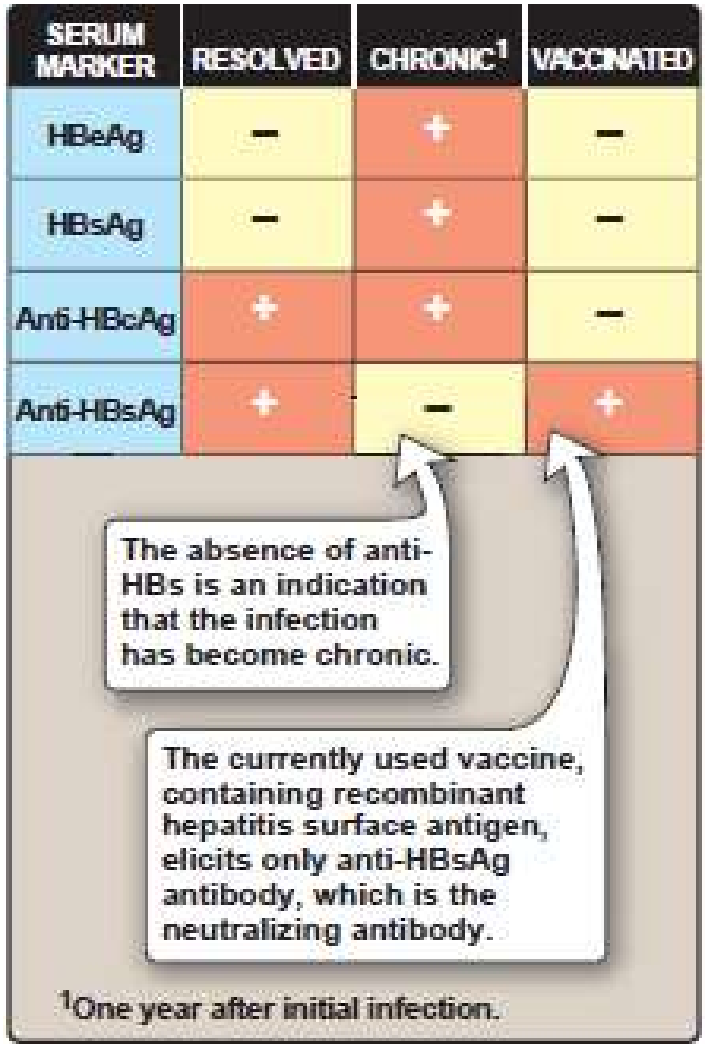
  b. Appearance of antiviral antibodies: Antibodies to HBcAg rise concurrently with liver enzymes in the serum, whereas anti-HBeAg antibodies and, still later, anti-HBsAg antibodies do not appear until the beginning of convalescence (generally after the respective antigens have disappeared from the blood).
- In those patients in whom the infection resolves completely, anti-HBcAg and anti-HBsAg antibodies remain present for life, providing immunity to reinfection.
- Continued presence of HBsAg beyond 6 months and absence of anti-HBsAg indicates that the infection has become chronic.
- A patient suffering chronic HBV infection is capable of eliciting an immune response against HBsAg but the anti-HBs antibody levels are too low to be detectable. All of the antibody that develops is complexed with circulating HBsAg.


A Acute infection
Viral DNA
Viral shedding
Elevated liver enzymes in serum
Symptoms
Jaundice
In some individuals with acute infections, HBsAg becomes undetectable before anti-HBsAg appears. This is referred to as the "window period," during which a person tested for HBsAg and anti-HBs will appear uninfected.
During the incubation period, HBsAg and HBeAg are the first indicators of HBV infection to appear in the blood.
Anti-HbcAg appears early in the clinical phase.
Anti-HBeAg antibodies appear early in the clinical phase. Later, anti-HBsAg antibodies appear at the beginning of convalescence.
In those patients in whom the infection resolves completely, anti-HBcAg and anti-HBsAg antibodies remain present for life, providing immunity to reinfection.
HBSAg
HBeAg
Anti-HBC
Anti-HBe
Anti-HBS
0
1
2
4
5
6
12
24
MONTHS AFTER EXPOSURE


B Chronic infection
Elevated liver enzymes in serum
Symptoms
Anti-HBc
HBsAg
HBeAg
Anti-HBe
The continued presence of HBsAg beyond 6 months and the absence of anti-HBsAg is an indication that the infection has become chronic.
0 1 2 3 4 5 6
Months
1 2 3 4 5 6 7 8 9 10
Years
TIME AFTER EXPOSURE

3. **Fulminant hepatitis: In 1 to 2 percent of acute symptomatic** cases, much more extensive necrosis of the liver occurs during the first 8 weeks of the acute illness.

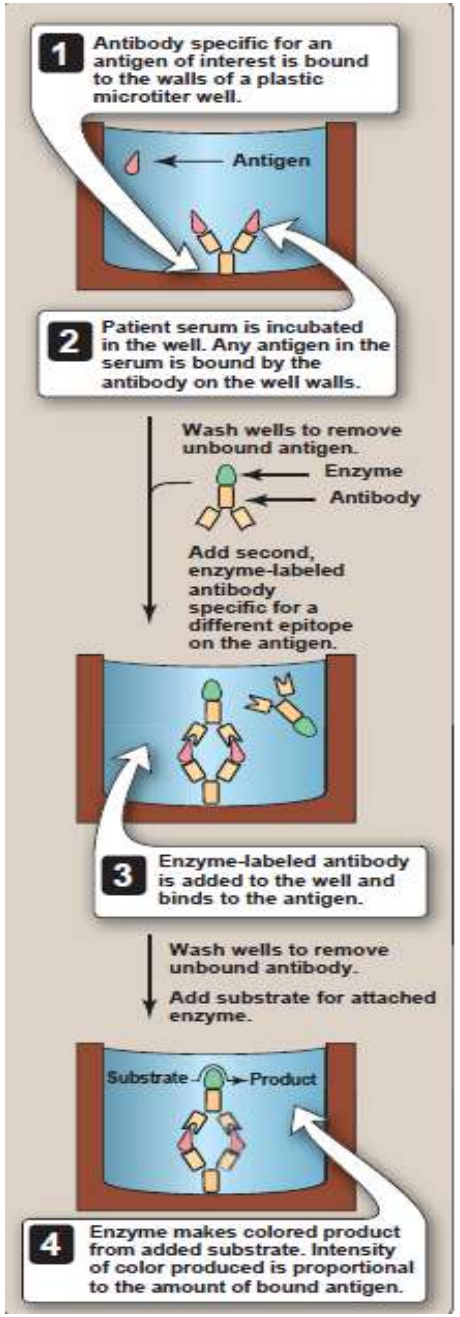
- This is accompanied by high fever; abdominal pain; and eventual renal dysfunction, coma, and seizures.
- Termed fulminant hepatitis, this condition is fatal in roughly 8 percent of cases.
- Although it is not clear why the acute disease takes this course, a more highly virulent strain of HBV, coinfection with HDV or another hepatitis virus (for example, HCV), and/or perhaps an uncontrolled immune response by the patient, are thought to play a role



Interpretation of serologic markers of hepatitis B infection.
HBeAg = hepatitis B e antigen; HBsAg = hepatitis B surface antigen; anti- HBcAg, and anti-HBsAg each refer to antibodies to the corresponding antigen.

- **Types of chronic carriers: The asymptomatic carriers of HBsAg** are the most common type of persistently infected individuals.
- They usually have anti-HBeAg antibodies and little or no infectious virus in their blood
- Later progression of liver damage or recurrence of acute episodes of hepatitis is rare in such patients.
- Those carriers with minimal chronic hepatitis (formerly, “chronic persistent hepatitis”) are asymptomatic most of the time but have a higher risk of reactivation of disease, and a small fraction does progress to cirrhosis
- **2. Development of hepatocellular carcinoma (hepatoma):** Hepatocellular carcinoma (HCC) is fairly uncommon and more frequent in areas of high HBV endemicity. In all populations, males experience a higher rate of chronic HBV infections; a higher rate of progression to cirrhosis; and, ultimately, a higher rate of HCC, for which the male-to-female ratio is 6:1.
- HBV gene product X is actively involved in tumor formation, following integration of the gene into the host's chromosome.

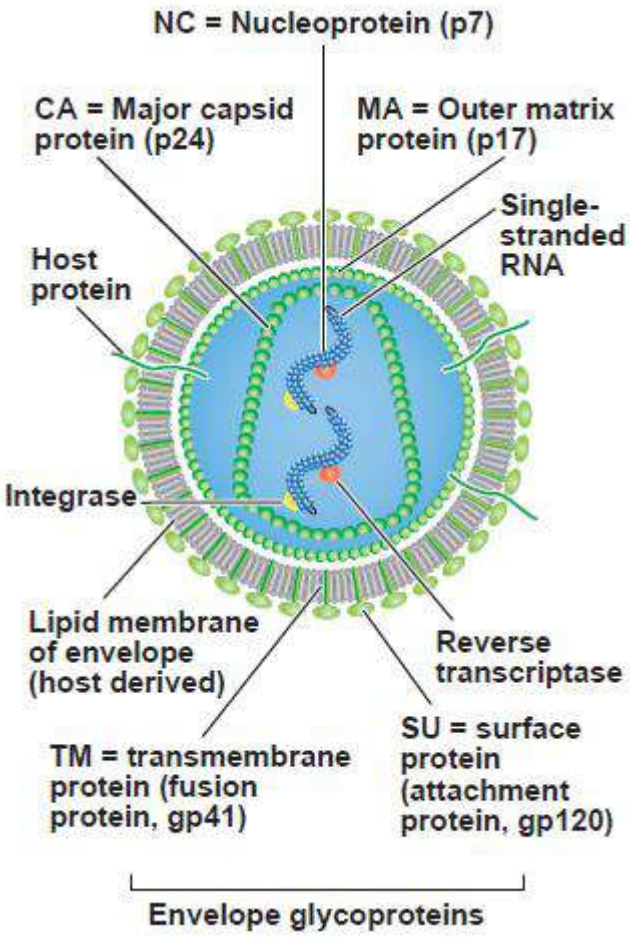
- **Laboratory identification**
- The purpose of diagnostic laboratory studies of patients with clinical hepatitis is to, first, determine which hepatitis virus is the cause of the illness and, second (for HBV), to distinguish acute from chronic infections.
- The diagnosis of hepatitis is made on clinical grounds, coupled with biochemical tests that evaluate liver damage.
- Elevations of aminotransferases, bilirubin, and prothrombin time all contribute to the initial evaluation of hepatitis.
- Commonly known as ELISA, enzyme-linked immunosorbent assay, and other immunologic techniques for detection of viral antigens and antibodies are the primary means to distinguish among HAV, HBV, HCV, and HDV.
- In addition, identification of the presence or absence of specific antiviral antibodies and viral antigens permits differentiating between acute and chronic HBV infections



Retroviridae ()

- They are named “retroviruses” because they reverse the usual order of transcription. They contain an enzyme called **reverse transcriptase (RT) that catalyzes** the replication of double-stranded DNA from single-stranded RNA.
- The association of retroviruses with their hosts can be so intimate that viral genes are permanently integrated into the host genome.
- Not only can this retroviral DNA be incorporated into the host genome as a provirus that can be passed on to progeny cells, but some retroviruses also transform cells and regulate certain host genes.
- The most prominent human retroviruses are HIV and the T-cell lymphotropic viruses I and II (HTLV-I and HTLV-II).
- The two types of HIV are HIV-1 (family lentivirus) which is the dominant form in most of the world, and HIV-2, found mainly in parts of Africa.
- HTLV type I is associated with leukemia and lymphoma
- HIV and other retroviruses display structural features typical of enveloped RNA viruses

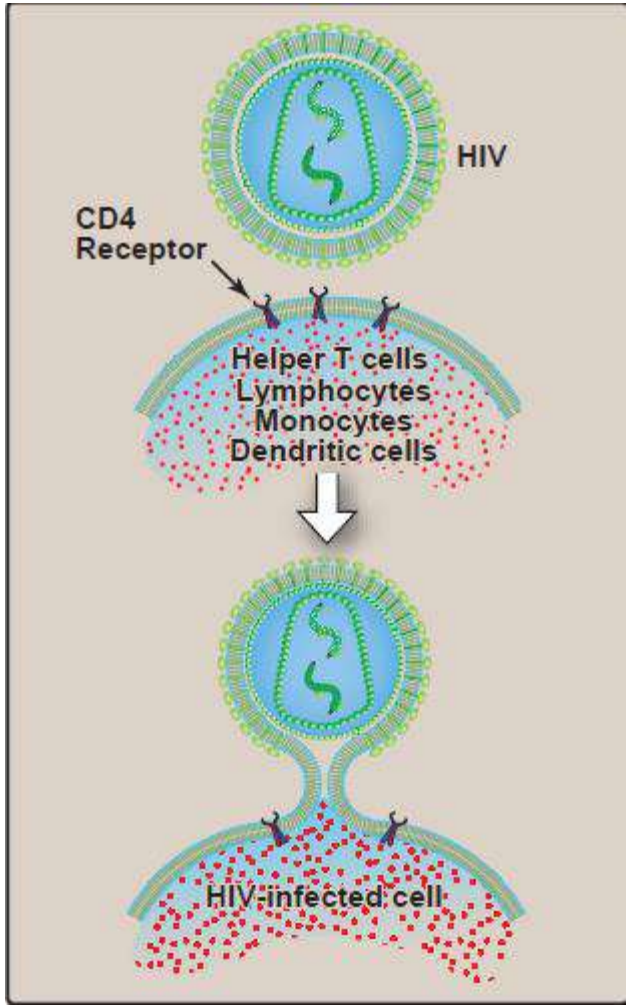
- **Organization of the HIV genome**
- The HIV RNA genome contains three major genes: gag, pol, and env
- The gag gene encodes p17 (MA), p24 (CA), and p7 (NC) (core and matrix proteins).
- The pol gene encodes reverse transcriptase, protease, integrase, and ribonuclease.
- Finally, the env gene encodes gp41 (TM) and gp120 (SU) (transmembrane and surface proteins).



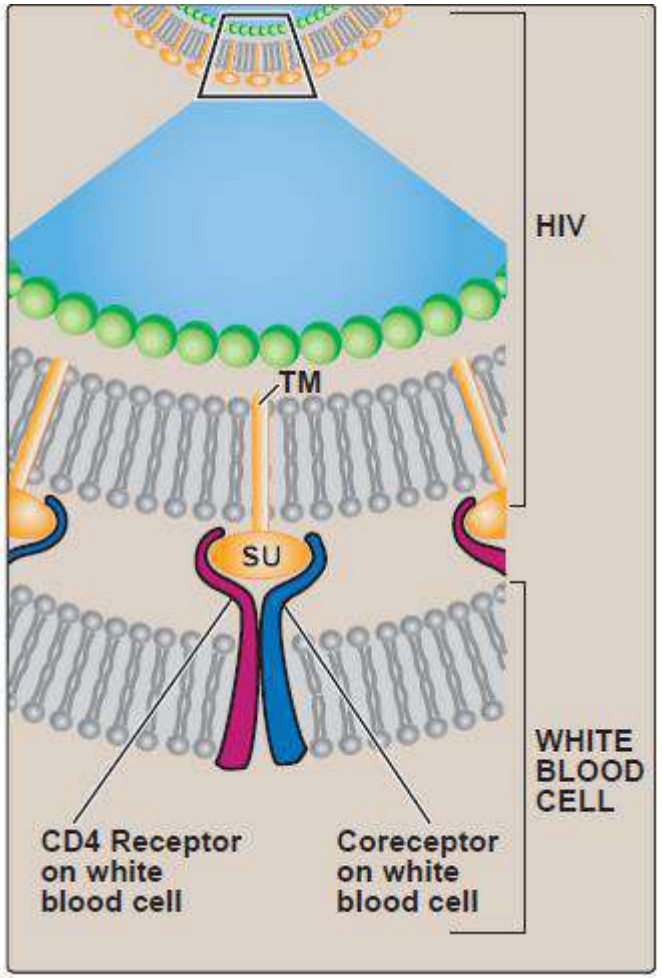
- **B. HIV replication**
- The first phase of HIV replication, which includes viral entry, reverse transcription, and integration of the virus into the host genome, is accomplished by proteins provided by the virus.
- The second phase of replication, which includes the synthesis and processing of viral genomes, mRNAs, and structural proteins, uses the host cell machinery for transcription and protein synthesis.
- The end result of HIV replication in most cell types is cell death.

Attachment to a specific cell surface receptor: Attachment is accomplished via the gp120 fragment of the env gene product on the HIV surface, which preferentially binds to a CD4 receptor molecule

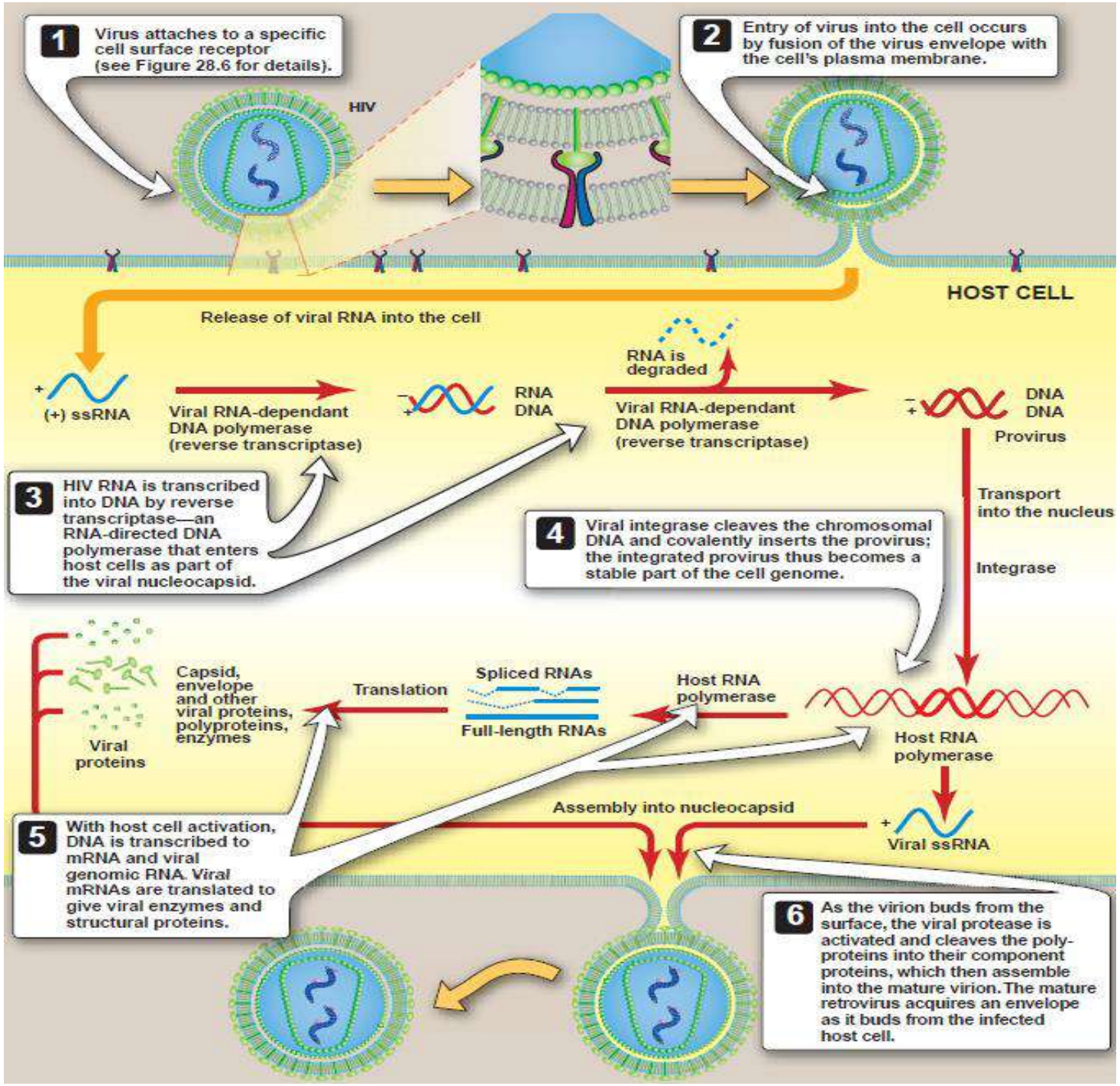
Thus, the virus infects helper T cells, lymphocytes, monocytes, and dendritic cells, which contain this protein in their cell membranes



- **Entry of virus into the cell: An additional coreceptor, a chemokine** receptor, is required for entry of the viral core into the cell
- [Note: A chemokine is a cytokine with chemotactic properties, produced by lymphocytes and macrophages]
- Macrophages and T cells express different chemokine receptors that fulfill this function.
- Two chemokine receptors that are employed by HIV as coreceptors are CCR5 and CXCR4, which are expressed differentially on different cell types.
- Binding to a coreceptor activates the viral gp41 gene product, triggering fusion between the viral envelope and the cell membrane



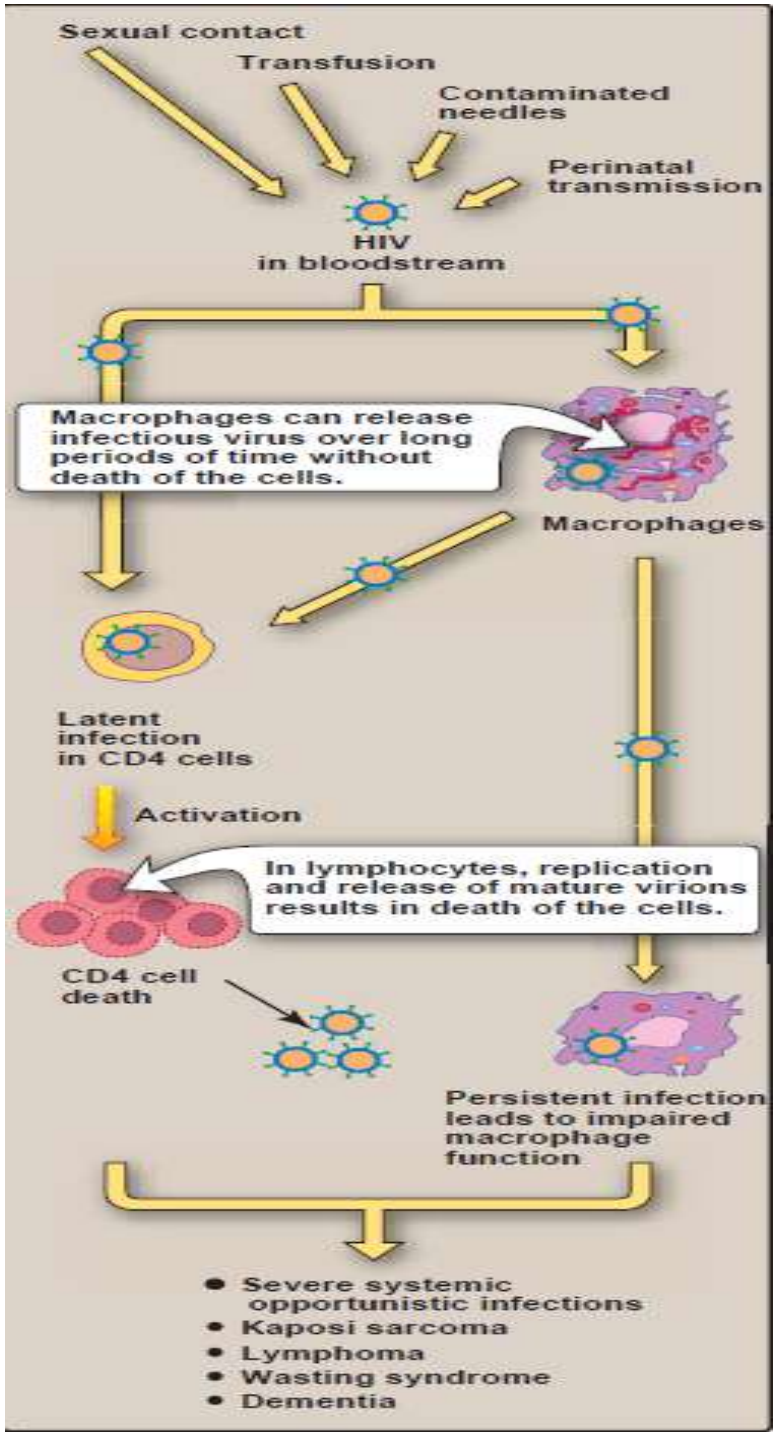
- **Reverse transcription of viral RNA: After entering the host cell,** the HIV RNA is not translated. Instead, it is transcribed into DNA by reverse transcriptase, an RNA-directed DNA polymerase that enters host cells as part of the viral nucleocapsid
- This process takes place in the cytoplasm. The viral reverse transcriptase first synthesizes a DNA-RNA hybrid molecule, and then its RNase activity degrades the parental RNA molecule while synthesizing the second strand of DNA.
- **Integration of the provirus into host cell DNA: The provirus, still** associated with virion core components, is transported to the nucleus with the aid of p17 (MA).
- In the nucleus, viral integrase cleaves the chromosomal DNA and covalently inserts the provirus. The integrated provirus, thus, becomes a stable part of the cell genome and can never be eliminated
- The insertion is random with respect to the site of integration in the recipient DNA.
- Therefore, HIV has two genomic forms: namely, single-stranded RNA present in the extracellular virus and proviral double-stranded DNA within the cell.


1 Virus attaches to a specific cell surface receptor (see Figure 28.6 for details).
HIV
2 Entry of virus into the cell occurs by fusion of the virus envelope with the cell's plasma membrane.
HOST CELL
Release of viral RNA into the cell
(+) ssRNA
Viral RNA-dependant DNA polymerase (reverse transcriptase)
RNA
DNA
RNA is degraded
Viral RNA-dependant DNA polymerase (reverse transcriptase)
DNA
DNA
Provirus
Transport into the nucleus
Integrase
3 HIV RNA is transcribed into DNA by reverse transcriptase—an RNA-directed DNA polymerase that enters host cells as part of the viral nucleocapsid.
4 Viral integrase cleaves the chromosomal DNA and covalently inserts the provirus; the integrated provirus thus becomes a stable part of the cell genome.
Capsid, envelope and other viral proteins, polyproteins, enzymes
Viral proteins
Translation
Spliced RNAs
Full-length RNAs
Host RNA polymerase
Host RNA polymerase
Assembly into nucleocapsid
Viral ssRNA
5 With host cell activation, DNA is transcribed to mRNA and viral genomic RNA. Viral mRNAs are translated to give viral enzymes and structural proteins.
6 As the virion buds from the surface, the viral protease is activated and cleaves the poly-proteins into their component proteins, which then assemble into the mature virion. The mature retrovirus acquires an envelope as it buds from the infected host cell.

Transmission of HIV

1. **Sexual contact**
2. **Transfusions: HIV has been transmitted by transfusion with whole** blood, plasma, clotting factors, and cellular fractions of blood.
3. **Contaminated needles: Transmission can occur by inoculation** with HIV-contaminated needles or syringes among drug users or accidentally if a contaminated needle punctures the skin of a health care worker
4. **Perinatal transmission: An HIV-infected woman has a 15 to 40** percent chance of transmitting the infection to her newborn, either transplacentally, during passage of the baby through the birth canal, or via breastfeeding. Because of the high rates of HIV-1 infection in women of childbearing age in developing countries, perinatally acquired HIV-1 infection is responsible for approximately 20 percent of all AIDS cases in these areas

- **Pathogenesis and clinical significance of HIV infection**
- The pathology of HIV disease results from either tissue destruction by the virus itself or the host's response to virus-infected cells.
- In addition, HIV can induce an immunodeficient state that leads to opportunistic diseases that are rare in the absence of HIV infection.
- The progression from HIV infection to AIDS develops in 50 percent of HIV-infected individuals in an average of 10 years, and, if untreated, it is uniformly fatal, generally within 2 years of diagnosis.
- However, there is a significant fraction (about 10 percent) of HIV infected individuals who have not developed AIDS after 20 years



- **Initial infection: After the acquisition of HIV, the initially infected** cells are generally macrophages within the genital tract.
- From this initial localized infection, HIV disseminates via the blood, and virus may then localize in dendritic cells throughout the lymphoid tissue.
- From the surface of dendritic cells, HIV can then infect CD4+ lymphocytes moving through the germinal centers of lymph nodes.
- This process creates a reservoir of chronically HIV infected cells within the lymphatic tissue throughout the body.
- Some individuals are resistant to some variants of HIV-1 due to a deletion in the gene that encodes the coreceptor (C-C chemokine receptor type 5, or CCR5) for the virus.

- **Acute phase viremia: Several weeks after the initial infection with** HIV, one-third to two-thirds of individuals experience an acute disease syndrome (also referred to as the primary infection) similar to infectious mononucleosis.
- During this period, there is a high level of virus replication occurring in CD4+ cells.
- Large amounts of virus and capsid protein (CA antigen) are present in the blood, but circulating antibody does not appear until 1 to 10 weeks after the initial infection
- During this window of time, antibody tests will not identify HIV-infected people.
- Lymph nodes also become infected during this time and later serve as the sites of virus persistence during the asymptomatic period

- **Latent period: The acute phase viremia is eventually reduced significantly** with the appearance of a HIV-specific cytotoxic T-lymphocyte response, followed by a humoral antibody response.
- A clinically asymptomatic or “latent” period lasting from months to many years follows the acute infection.
- During this latent period, the majority (90 percent) of HIV proviruses are transcriptionally silent, so that only 10 percent of the cells containing integrated HIV DNA also contain viral mRNA or viral proteins.
- A constant level of virus and virus-infected cells is maintained by a combination of replacement of the CD4+ cells killed by HIV infection with cells newly produced in lymphoid organs and the subsequent infection of these new cells with progeny virus.
- Although there is continuous loss of those CD4+ cells in which HIV is replicating, active replacement through stem cell multiplication compensates for this loss, and the CD4+ count declines only slowly over a period of years. In addition, the host immune response is still sufficiently effective to maintain a relatively stable, low level of virus production.
- Virus isolated during this period is also less cytopathic for CD4+ cells and replicates more slowly than does virus isolated later during symptomatic AIDS.
- Despite the nearly normal levels of CD4+ cells, however, impairment of T-cell responses to specific antigens is evident. The infection remains relatively clinically asymptomatic as long as the immune system is functional

- **Clinical complications of HIV infection during the latent period:**
- During this period (of variable length but lasting on average about 10 years), there are multiple, nonspecific conditions, such as persistent, generalized lymphadenopathy (swollen lymph nodes); diarrhea; chronic fevers; night sweats; and weight loss.
- The more common opportunistic infections, such as herpes zoster and candidiasis, may occur repeatedly during this period as well as when patients progress to AIDS.
- **Progression to AIDS: The progression from asymptomatic infection** to AIDS is not sudden but, in fact, occurs as a continuum of clinical states.
- A number of virologic and immunologic changes occur that affect the rate of this progression.
- For example, coinfection with a number of the herpesviruses, such as human herpesvirus type 6 can transactivate transcription from the silent HIV provirus, increasing HIV replication.
- Any stimulation of an immune response causing activation of resting T cells also activates HIV replication.

- T cell precursors in the lymphoid organs are also infected and killed, so the capacity to generate new CD4+ cells is gradually lost.
- The capacity to contain the infection is further compromised by the appearance of HIV mutants with altered antigenic specificity, which are not recognized by the existing humoral antibody or cytotoxic T lymphocytes.
- The eventual result of these accumulating, interacting factors is an increasingly rapid decline in CD4+ count, accompanied by loss of immune capacity.
- With the CD4+ count falling below 200/µl and the appearance of increasingly frequent and serious diseases and opportunistic infections (“AIDSdefining illnesses”), the patient is said to have AIDS.

- **End-stage AIDS: Nearly all systems of the body can be affected** as a result of HIV infection, either by HIV itself or by opportunistic organisms. The weakening immune system leads to many complications, including malignancies such as Kaposi sarcoma

  a. Spread of HIV to additional body sites: Cell types other than CD4+ lymphocytes can be infected by HIV. For example, microglia are the HIV-infected cells present in brains of patients with AIDS encephalopathy, which typically evolves over a period of 1 year, with gradual deterioration resulting in severe dementia.

  b. Opportunistic infections in AIDS: Multiple recurrent bouts of infections with fungi, bacteria, and viruses occur as the CD4+ cell count declines. The eye can be infected with HIV, but also with opportunistic agents, the most prominent of which is cytomegalovirus (CMV), a cause of retinal destruction.
- P. jirovecci pneumonia being one of the most common. Mycobacterial infections are also a common problem in the lung. For example, currently, 30 percent of AIDS patients die from tuberculosis
- Recurrent infections by Epstein-Barr virus (EBV), varicella zoster virus, human papillomavirus, and herpes simplex virus are common. Mucocutaneous candidiasis (oral, esophageal)

- **Laboratory identification**
- **Demonstration of virus or virus components: Amplification of** viral RNA or DNA proviruses by the polymerase chain reaction(PCR) technique is the most sensitive method for early detection of virus in blood or tissue specimens.
- For purposes of initial screening of the blood supply, ELISA (for enzyme-linked immunosorbent assay) testing for the CA (p24) antigen in serum can detect otherwise undetectable infection in individuals who are infectious by screening for anti-HIV antibodies
- Although the ELISA test is highly specific, there are false-positives, so any positive result is confirmed using the Western blot technique

COVID 19

- Coronaviruses are enveloped non-segmented positive sense RNA viruses belonging to the family Coronaviridae and the order Nidovirales and broadly distributed in humans and other mammals.
- Although most human coronavirus infections are mild, the epidemics of the two betacoronaviruses, severe acute respiratory syndrome coronavirus (SARS-CoV) 2–4 and Middle East respiratory syndrome coronavirus (MERS-CoV), have caused more than 10 000 cumulative cases in the past two decades, with mortality rates of 10% for SARS-CoV and 37% for MERS-CoV

- **Replication cycle of 2019-nCoV**
- The recently reemerged 2019-nCoV is an enveloped positive-sense RNA virus.
- It is characterized by club-like spikes projecting from its surface (Phan, 2020).
- Coronavirus is a complex pathogen with a high capability to infect multiple host species (Fung et al., 2019).
- The main steps involved in the replication cycle are

(1) binding and 2019-nCoV entry to the host via membrane fusion or endocytosis mechanism,

(2) upon entrance to the host, release of viral genome,

(3) translation of viral polymerase protein,

(4) RNA replication,

(5) sub-genomic transcription,

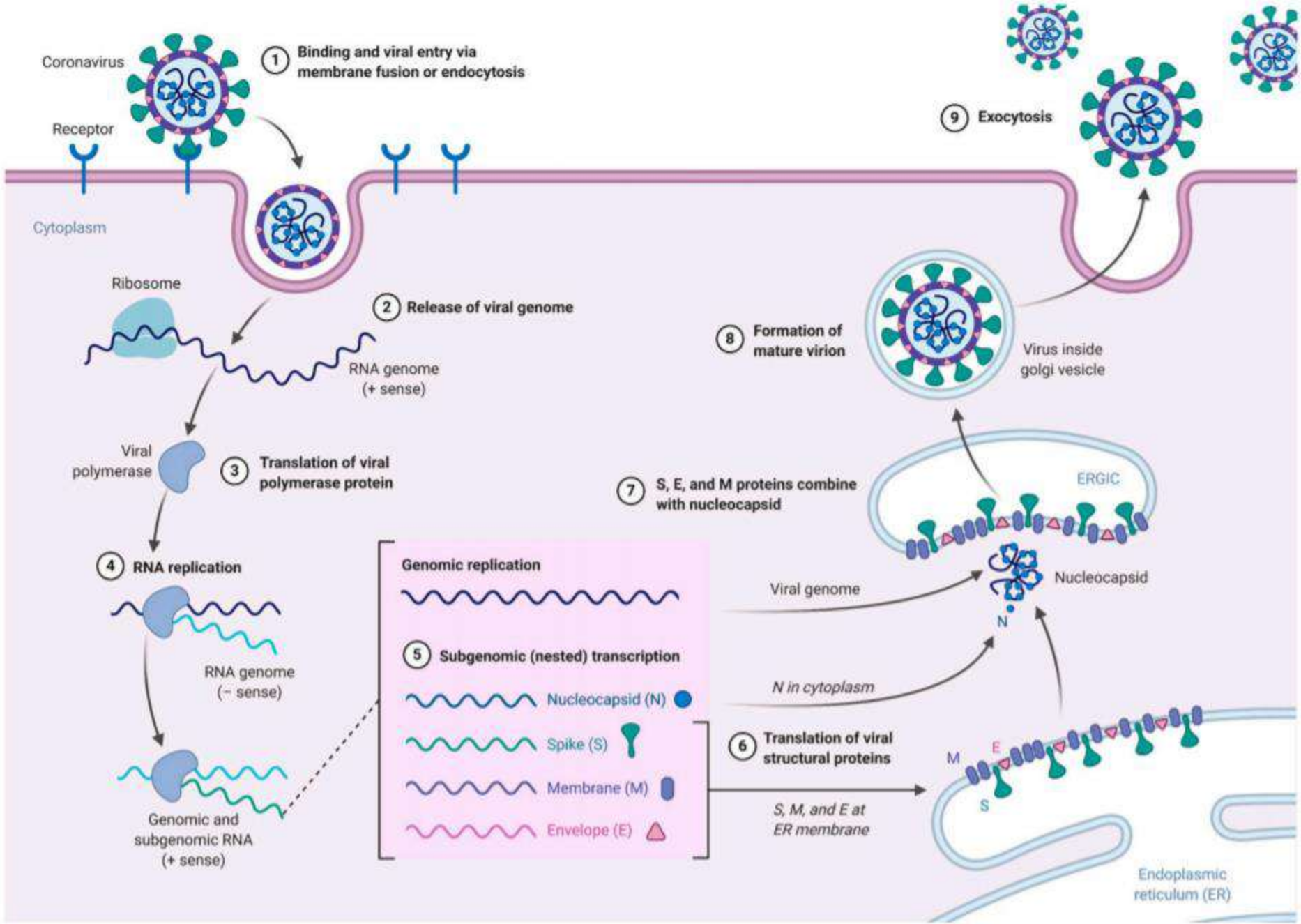
(6) translation of viral structural protein,

(7) viral structural proteins combine with nucleocapsid,

(8) formation of mature virion, and

(9) release of mature virion via exocytosis mechanism.

At the end of the cycle, the newly released mature virion can further infect a new target, and it continues over…


Coronavirus
Receptor
1 Binding and viral entry via membrane fusion or endocytosis
Cytoplasm
Ribosome
2 Release of viral genome
RNA genome (+ sense)
Viral polymerase
3 Translation of viral polymerase protein
4 RNA replication
RNA genome (– sense)
Genomic and subgenomic RNA (+ sense)
Genomic replication
5 Subgenomic (nested) transcription
Nucleocapsid (N)
Spike (S)
Membrane (M)
Envelope (E)
6 Translation of viral structural proteins
S, M, and E at ER membrane
7 S, E, and M proteins combine with nucleocapsid
Viral genome
N in cytoplasm
ERGIC
Nucleocapsid
N
M
E
S
Endoplasmic reticulum (ER)
8 Formation of mature virion
Virus inside golgi vesicle
9 Exocytosis

- **<u>Symptoms and warning signs</u>**
- The incubation period of COVID-19 is 1-14 days (mean duration of 5-7 days), with peak viraemia occurring before the onset of symptoms.
- This underlines the transmission potential of asymptomatic or minimally symptomatic patients
- The most common presenting features of COVID-19 infection are fever (80-90%), cough (60-80%) and dyspnoea (18-46%).
- Other symptoms at presentation include myalgia or fatigue, sore throat, nasal congestion, headache, nausea, vomiting and diarrhoea
- On examination, findings of pneumonia may be present in a minority
- Warning signs or red flag signs that can assist in triage, indicating the need for urgent care, are summarized →→→

Table I. Comorbid illness and case fatality rates in high-risk groups

Age, yr (case fatality rate, %)	Comorbid illness (case fatality rate, %)
60-70 (4)	Cardiovascular disease (10.5)
>70-80 (8)	Diabetes mellitus (7.3)
>80 (15)	Chronic respiratory disease (6.3)
	Systemic hypertension (6.0)
	Cancer (5.6)

Table II. Symptoms and warning signs

Symptoms (frequency in %)	Warning signs (needs hospitalization)
Fever (80-90)	Fever and upper respiratory symptoms lasting for >5 days and any of the following:
Cough (60-80)	
Breathlessness (18-46)	
Fatigue (38)	Breathlessness/respiratory rate >24/min
Body ache/joint pain (15)	
Sore throat (11-14)	Oxygen saturation (SpO_2) <95% in room air
Headache (6-14)	
Chills (12)	Fatigue with heart rate of >110/bpm
Body ache/joint pain (15)	
Running nose (5)	Systolic blood pressure <90 mmHg
Nausea/vomiting (5)	

Table III. Laboratory abnormalities and complications

Laboratory abnormalities	Complications
CBC: Lymphopenia	Pneumonia
Creatinine↑	ARDS
AST/ALT/bilirubin ↑	Hypotension
CRP ↑, LDH ↑, ferritin ↑	Myocarditis
CXR: Interstitial infiltrates/ARDS	Acute kidney injury

AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; CRP, C-reactive protein; ARDS, acute respiratory distress syndrome; CXR, chest X-rays; CBC, complete blood count

Table IV. Categorization of probable coronavirus disease 2019 (COVID-19) severity, testing and admission strategy

Clinical category of COVID-19	Features	Testing strategy	Level of care
Mild	Fever with upper respiratory symptoms Mild sore throat and GI symptoms Testing may be considered in select individuals in the high-risk group	Low priority	Home care
Moderate	Breathlessness/respiratory rate >24/min Oxygen saturation (SpO_2) <95% in room air Fatigue with heart rate of >110/bpm Systolic blood pressure <90 mmHg	High priority	Inpatient care
Severe	SpO_2 <90% in room air Hypotension requiring ionotropic support ARDS/myocarditis	High priority	Intensive care

- Sample collection and laboratory confirmation Sample collection for the detection of SARS-CoV-2 infection should be carried out as early as possible in patients with suspected COVID-19 falling into the moderate and severe groups.
- The high upper respiratory viral load in COVID-19 makes nasopharyngeal swab the recommended sample for confirmatory testing.
- Sputum collection and testing should be considered only for patients with productive cough, while the induction of sputum is not advised as aerosols produced by the procedure can facilitate disease transmission.
- For intubated patients, a lower respiratory tract aspirate or bronchoalveolar lavage sample is preferred.
- Swabs are to be placed in a viral transport medium (ones with a surfactant (e.g. Guanidine thiocyanate) or molecular grade Ethanol.) and transported on ice to the laboratory

- The recommended diagnostic test is the reverse transcription-polymerase chain reaction (RT-PCR) on respiratory samples.
- Though initial studies revealed low sensitivities of 30-60 per cent, newer assays show improved results
- Based on the CoV grouping criterion, RdRp (RNA-dependent RNA polymerase) gene assay is considered the reference standard PCR
- The U.S. Food & Drug Administration (FDA) has recently approved a rapid diagnostic test capable of providing the results within 45 minutes
- Cepheid's COVID-19 test, a molecular PCR-based assay, has succeeded in demonstrating high accuracy using the company's GeneXpert machine
- **Antibody detection may reveal positivity about a week after the onset of illness and hence has no role in the diagnosis in the first week of illness**

- General treatment measures
- The theoretical concern that non-steroidal anti-inflammatory drugs (NSAIDs) worsen outcomes in COVID-19 infections as these upregulate angiotensin-converting enzyme 2 (ACE-2) levels in the lung, the entry receptor for the virus, remains yet to be proven
- The mechanism of lung injury produced by COVID-19 also appears to be through its effect on ACE-2, though this has not yet been confirmed.
- This has in turn led to the hypothesis that patients with cardiac diseases, hypertension or diabetes being treated with ACE inhibitors or angiotensin receptor blockers are at higher risk for severe COVID-19 infection as they upregulate increased ACE-2 receptor expression
- There is no clear clinical evidence for the same and hence, cessation or a change in medication for the general population on regular treatment with ACE inhibitors or angiotensin II receptor blockers (ARBs) for the underlying comorbid disease is not recommended

- Specific treatments and treatment strategy

- **Hydroxychloroquine and chloroquine**
- One of the earliest trials conducted in China in an attempt to discover the role of the existing drugs against COVID-19 infection revealed that chloroquine has in vitro activity against SARS-CoV-2
- The 4-aminoquinolone, commonly used as an antimalarial and anti-inflammatory agent, possesses broad antiviral activity.
- While the exact mechanisms are unknown, it is considered to gain its antiviral effects through alkalinization of the phagolysosome as well as inhibition of viral entry by blocking receptor binding and membrane fusion.
- With a similar mechanism of action, hydroxychloroquine (HCQ) has demonstrated more potent in vitro inhibition of SARS-CoV-2 virus compared to chloroquine.
- Its fewer side effects, safety in pregnancy and inexpensive nature makes it more preferable to chloroquine
- While HCQ has been suggested as an option for prophylaxis for healthcare workers who are taking care of COVID-19 patients and household contacts of laboratory-confirmed patients, the potential benefit must be weighed against the increased risk of life-threatening arrhythmias.
- The QT interval must be monitored with frequent electrocardiographs (ECGs).

Lopinavir/ritonavir

Lopinavir/ritonavir, a boosted protease inhibitor combination, while commonly used in the treatment of HIV-1 infection, came into spotlight during the SARS outbreak in 2003 when it was proved to have in vitro activity against the causative SARS-CoV

Remdesivir

Remdesivir, an adenosine analogue and RNA polymerase blocker, is a novel drug developed for the treatment of Ebola virus infection.

Favipiravir

Favipiravir, a RNA polymerase inhibitor, has shown modest activity against SARS-CoV-2 virus with pronounced cytopathy in Vero cell studies24 The drug has been used in China for the treatment of COVID-19 and is being studied in a clinical trial for mild SARS-CoV-2 disease and also as an adjunct agent in moderate and severe diseases

Interleukin-6 (IL-6) inhibitors

- A subgroup of patients with COVID-19 develop severe cytokine activation and secondary haemophagocytic lymphohistiocytosis (HLH), leading to rapid-onset hypoxemia, shock and multiorgan dysfunction
- A higher neutrophil count and elevated C-reactive protein may predict this subgroup of patients.
- Interleukin-6 (IL-6) is a key cytokine in the cytokine storm, and tocilizumb, a humanized anti-IL-6 receptor antibody, is proposed as a therapeutic agent in severe SARS-CoV-2 disease.

Corticosteroids

- Corticosteroids are generally not useful against similar severe respiratory viral illnesses such as SARS or Middle East respiratory syndrome (MERS)-CoV disease.
- A recent retrospective review showed decreased likelihood of death among patients with SARS-CoV-2-related acute respiratory distress syndrome (ARDS) who received methylprednisolone
- Steroids can be used if indicated for another reason such as accompanying severe asthma and septic shock

Convalescent plasma from COVID-19 survivors

- Studies during the SARS epidemic showed that convalescent plasma therapy decreased hospital stay and mortality when used in the critically ill48. Convalescent plasma therapy was attempted with some benefit in MERS, Ebola and H1N1 pandemic influenza

- **Cardiovascular and shock management in SARS-CoV-2 disease**
- Acute cardiac injury (ACI) diagnosed by elevated troponins or abnormal ECG findings is commonly encountered in severe SARS-CoV-2 disease.
- Up to one-fifth of the hospitalized patients show ACI, typically late into disease onset with prognostic significance.
- The mechanism of cardiac injury is not clear, but direct viral-mediated mechanisms appear less likely
- A significant proportion of critically ill SARS-CoV-2 patients develop shock during the hospital stay
- The common causes of this include cardiogenic shock, secondary infections, sepsis as well as cytokine storm.
- In patients with cytokine storm, cytokine blockade therapies such as IL-6 blockers may be beneficial in a select group of patients
- Early recognition of a secondary infection leading to septic shock, followed by initial resuscitation with crystalloids and then continuation with vasopressors and dobutamine administered through a central line, is recommended.
- Fluid overloading should be avoided.