Laboratory Diagnosis of HIV Infection

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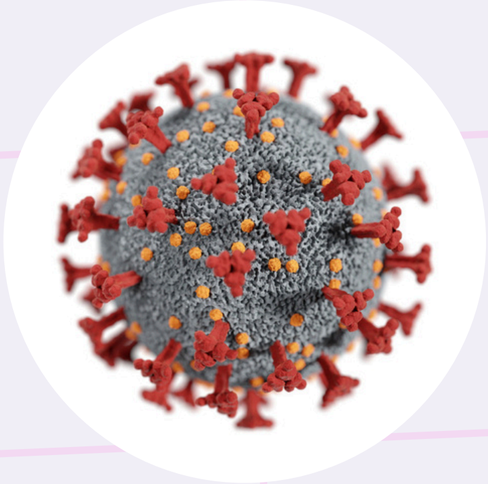
TOPICS

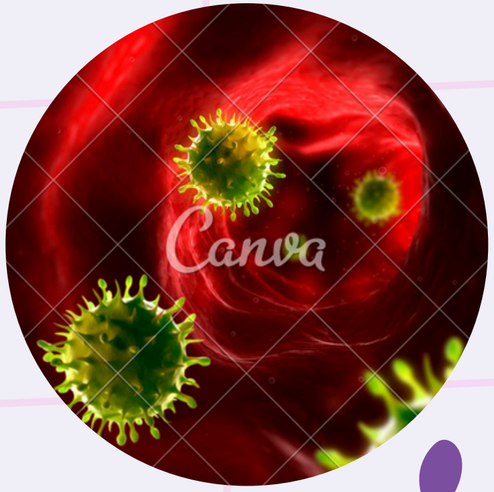
Structural genes

Viral Replication

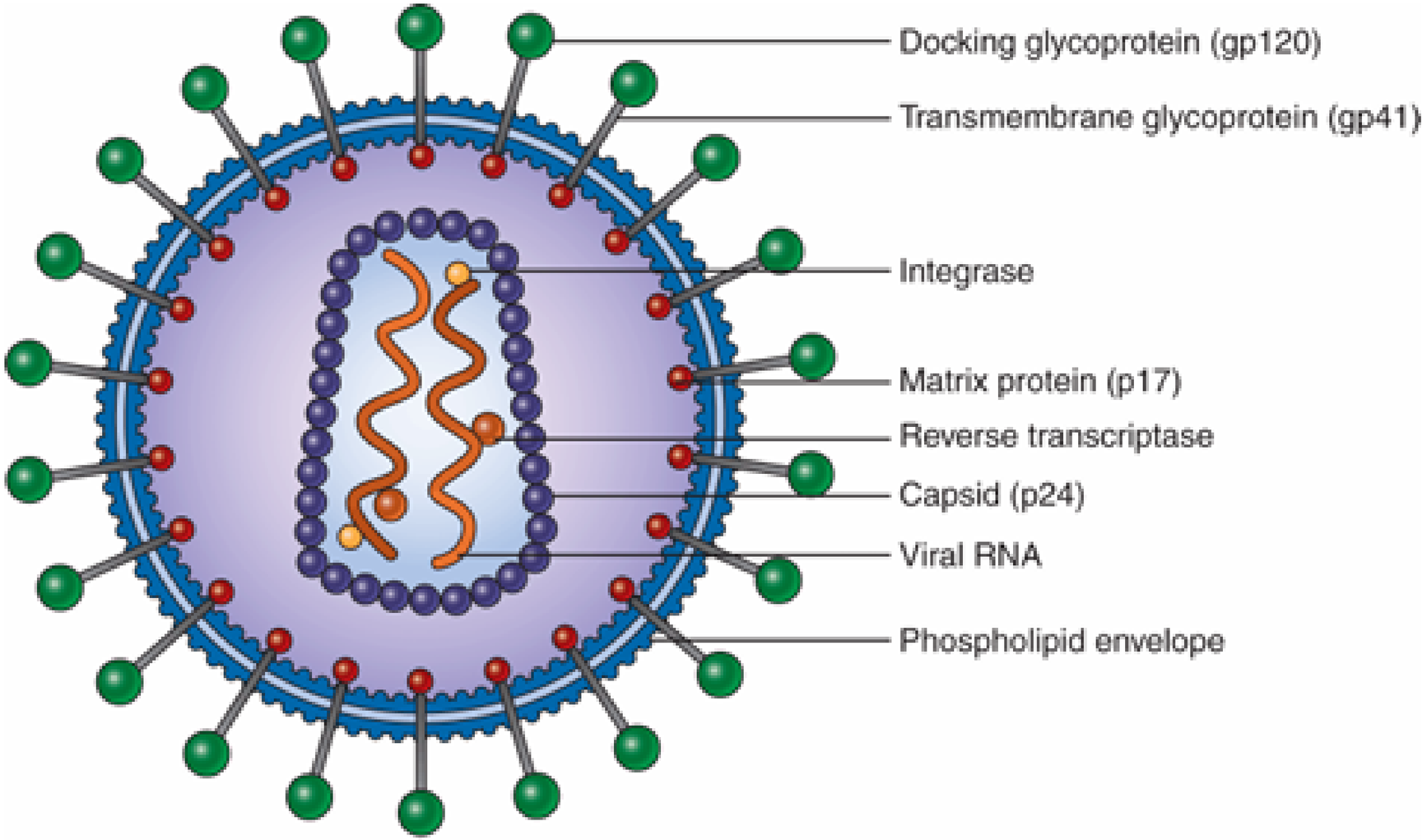


Immunologic Manifestations

Immune Responses to HIV



STRUCTURAL GENES



STRUCTURAL GENES

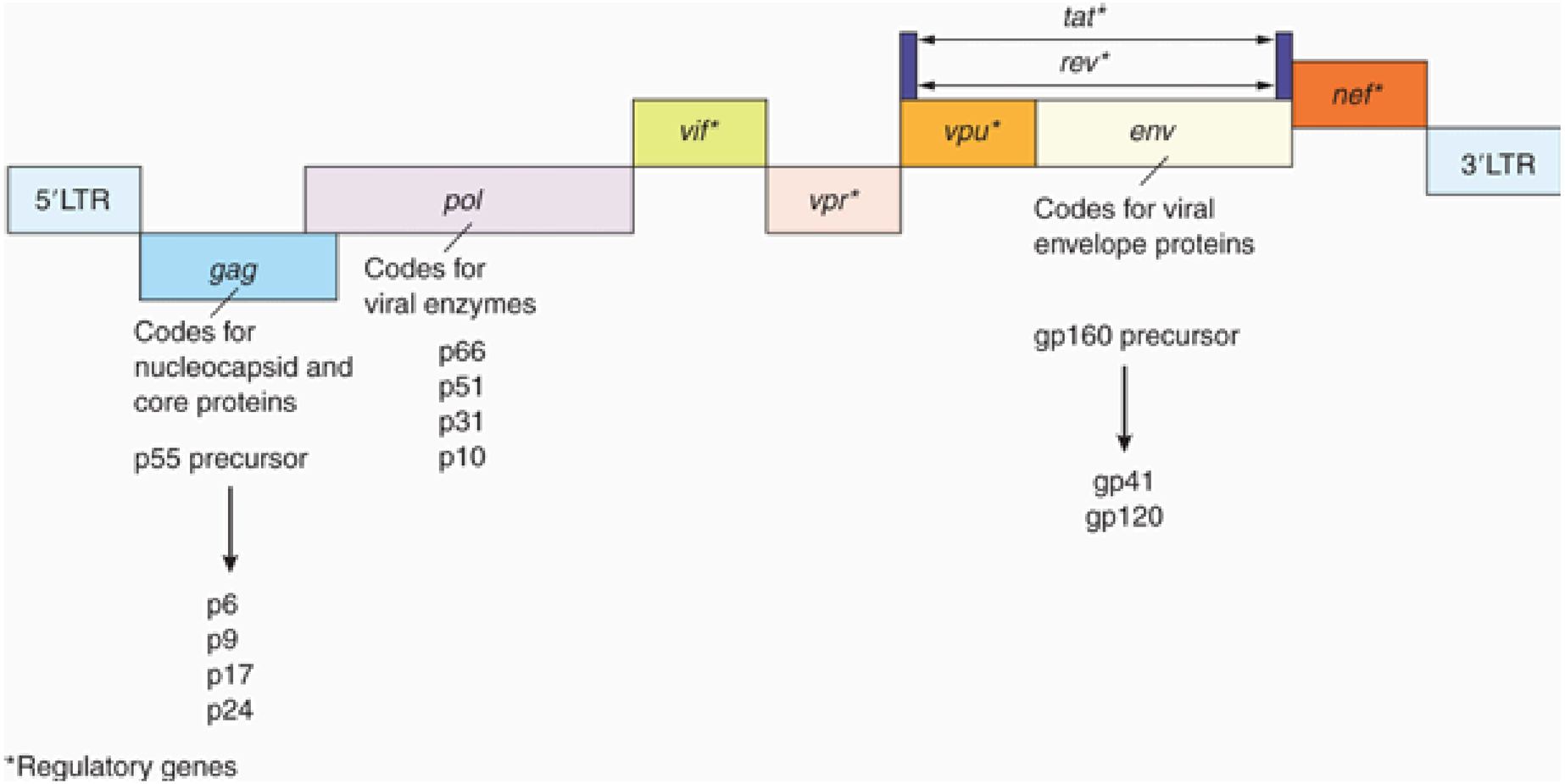


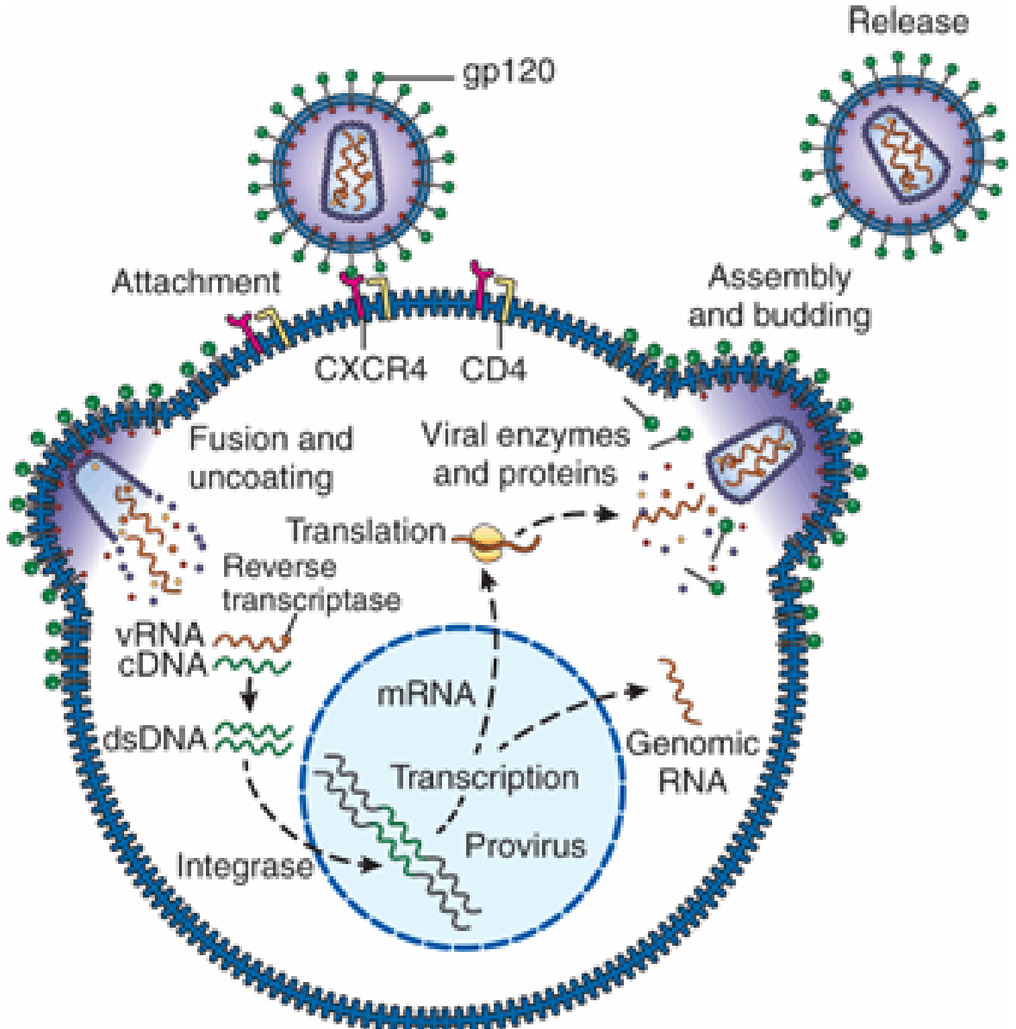
FIGURE 24-2 The HIV-1 genome. The relative locations of the major genes in the HIV-1 genome are indicated, as well as their gene products. * = regulatory genes.

STRUCTURAL GENES

Table 24-1 Major HIV Genes and Their Products

GENE	PROTEIN PRODUCT	FUNCTION
gag	p17	Inner surface of envelope
	p24	Core coat for nucleic acids
	p9	Core-binding protein
	p6	Binds to genomic RNA
env	gp120	Binds to CD4 on T cell
	gp41	Transmembrane protein associated with gp120
pol	p66	Subunit of reverse transcriptase; degrades original HIV RNA
	p51	Subunit of reverse transcriptase
	p31	Integrase; mediates integration of HIV DNA into host genome
	p10	Protease that cleaves *gag* precursor
tat	p14	Activates transcription of HIV provirus
rev	p19	Transports viral mRNA to the cytoplasm of the host cell
nef	p27	Enhances HIV replication
vpu	p16	Viral assembly and budding
vpr	p15	Integration of HIV DNA into host genome

VIRAL REPLICATION



VIRAL REPLICATION

The first step in HIV infection is when the virus attaches to a host cell using its gp120 glycoprotein, which binds to the CD4 antigen on the surface of T helper (Th) cells. These cells are the main targets because they have many CD4 molecules. Other cells like macrophages, monocytes, dendritic cells, Langerhans cells, and microglial brain cells can also be infected due to having some CD4. HIV strains that infect T cells are called T-tropic (X4), while those that infect both macrophages and T cells are M-tropic (R5).

VIRAL REPLICATION

HIV needs a second step to enter: binding to a co-receptor, which helps fuse the virus with the host cell membrane. These co-receptors are chemokine receptors, mainly CXCR4 (for T cells) and CCR5 (for macrophages). A mutation in the CCR5 gene can make people resistant to HIV. After this binding, the gp41 glycoprotein changes shape, allowing the virus to fuse with the cell.

VIRAL REPLICATION

Inside the cell, reverse transcriptase changes HIV's RNA into complementary DNA. Then double-stranded DNA is made and inserted into the host's genome by the HIV integrase enzyme, forming a provirus. This provirus can stay hidden for a long time. When the cell is activated by an antigen or cytokines, the virus becomes active. Its DNA is copied into genomic RNA and messenger RNA (mRNA), which are used to make viral precursor proteins and new virus parts. The new viruses bud out of the cell and get their envelope as they leave. Viral protease then cuts the precursor proteins in the new virus to make it mature and ready to infect more cells. This happens most in activated Th cells.

VIRAL REPLICATION

Because viral replication occurs very rapidly and the reverse transcriptase enzyme lacks proofreading activity, genetic mutations occur at a high rate, producing distinct isolates that exhibit an extraordinary level of antigenic variation. In fact, the level of HIV diversity in a single individual is greater than the diversity of al the influenza virus isolates throughout the world in a given year! This tremendous genetic diversity of HIV hinders the ability of the host to mount an e fective immune response.

VIRAL REPLICATION

- HIV mutates quickly because reverse transcriptase has no proofreading.
- This makes HIV very diverse, harder to fight with immune responses or vaccines

IMMUNOLOGIC MANIFESTATION

When HIV infects a healthy individual, there is typicaly an initial burst of viral replication folowed by a slowing down of virus production as the host's immune response develops and keeps the virus in check. This initial viral replication can be detected in the laboratory by the presence of increased levels of p24 antigen and viral RNA in the host's bloodstream (see discussion in the text that folows). As the virus replicates, some of the viral proteins produced within host cels form complexes with class I major histocompatibility complex (MHC) antigens and are transported to the cel surface, where they stimulate lymphocyte responses. HIV-specific CD4+ Th cels are generated and assist both humoral and cel-mediated immune responses against the virus. However, the interactions between HIV and the immune system are complex, and these responses are unable to eliminate HIV from the body

IMMUNOLOGIC MANIFESTATION

B lymphocytes are stimulated to produce antibodies to HIV, which can usualy be detected in the host's serum by 6 weeks after primary infection. The first antibodies to be detected are directed against the gp41 transmembrane glycoprotein, folowed by production of antibodies to the gag proteins such as p24, and, finaly, production of antibodies to the env, pol, and regulatory proteins. The most immunogenic proteins are in the viral envelope and elicit the production of neutralizing antibodies. These antibodies usualy appear 2 to 3 months after infection and prevent the virus from infecting neighboring cels. Antibodies to the envelope proteins have also been shown to bind to Fc receptors on natural kiler (NK) cels and participate in antibody-dependent celular cytotoxicity (ADCC)-mediated kiling of HIV-infected cels. However, these antibodies may also participate in the pathogenesis of HIV infection by facilitating Fc-receptor-mediated endocytosis of opsonized virus by uninfected cels. Furthermore, dense glycosylation of the env proteins can mask important epitopes, and the enormous diversity of the virus poses chalenges to the host in producing a protective antibody response

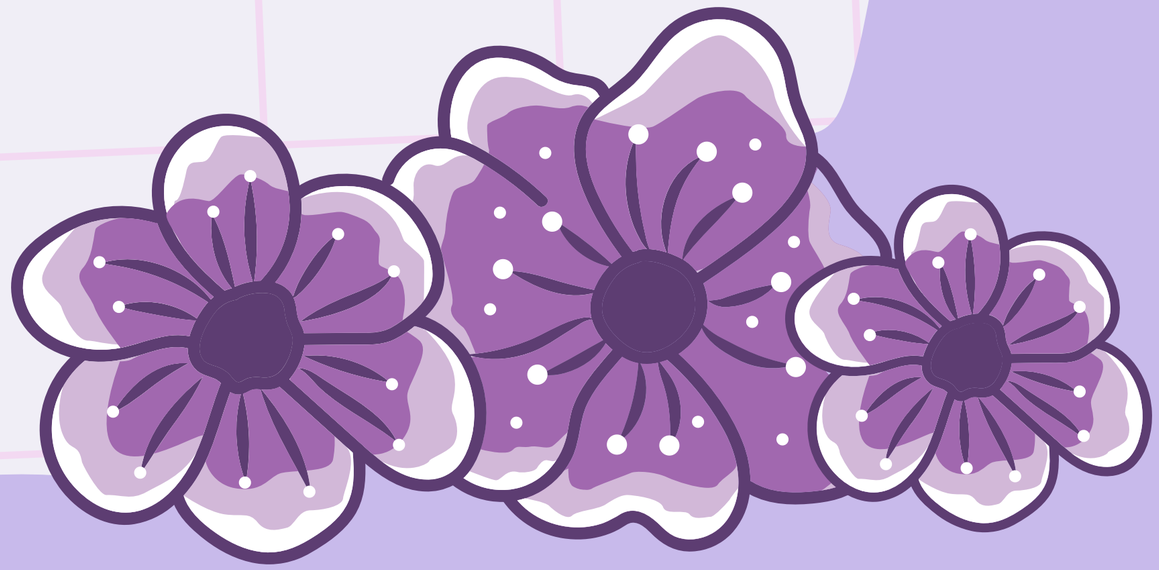
IMMUNOLOGIC MANIFESTATION

T-celmediated immunity is thought to play an important role in the immune response to HIV, as it does in other viral infections. CD8+ cytotoxic T lymphocytes, also known as cytolytic T ce ls (CTLs), appear within weeks of HIV infection and are associated with a decline in the amount of HIV in the blood during acute infection. CTLs a tack HIV-infected host cels by binding to HIV proteins associated with class I MHC molecules on the surface of infected cels. HIV-specific CTLs are stimulated to develop into mature, activated clones through the e fects of cytokines released by activated CD4 Th cels, a process that is common to immune responses against other viruses (see Chapter 4 for details). After the CTLs bind to HIV-infected host cels, cytolytic enzymes are released from their granules and destroy the target cels. Free virions are released from the damaged cels and can be bound by antibodies. CTLs can also suppress replication and spreading of HIV by producing cytokines such as interferon (IFN)-γ, which have antiviral activity Innate immune defenses may play a role in responding to HIV in the early stages of the infection. In particular, NK cels become activated during acute HIV infection and can mediate cytolysis of host cels infected with the virus. In addition, dendritic cels recognize viral components through pa tern-recognition receptors, resulting in the release of proinflammatory cytokines that have antiviral e fects and can activate other cels of the immune system.

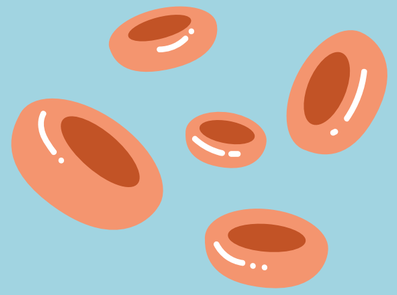
CTL Versus Antibody Responses to HIV

That antibodies can attach only to virions circulating freely outside of host ce ls. In contrast, CTLs can attack host ce ls harboring viruses interna ly

THANK YOU



EFFECTS OF HIV INFECTION ON THE IMMUNE SYSTEM



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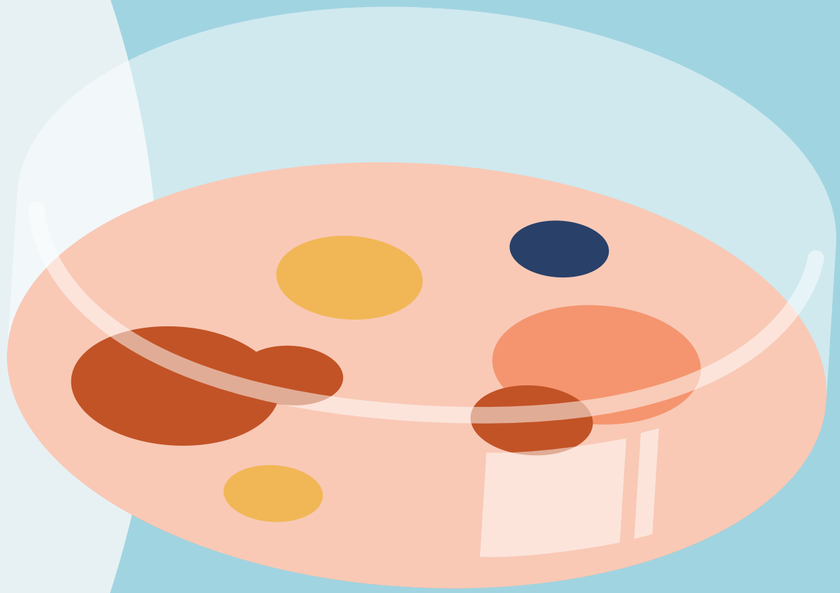
HIV has developed several mechanisms by which it can escape immune responses. Although the humoral and cell-mediated immune responses of the host usually reduce the level of HIV replication, they are generally not sufficient to completely eliminate the virus



HIV IN THE IMMUNE SYSTEM

HIV evades immune responses through rapid genetic mutations that create escape mutants and by downregulating **class I MHC** molecules, preventing effective **CTL** recognition.

Numerous cells in the body can harbor HIV as a silent provirus for extended periods, including resting **CD4 T cells, dendritic cells, monocytes, macrophages, and microglial cells in the brain**. In this proviral state, HIV remains shielded from immune attack until cell activation triggers viral replication and antigen expression.

HIV IN THE IMMUNE SYSTEM

HIV evades the immune response, leading to persistent infection and immune system destruction, primarily by **depleting CD4 Th cells**. The **gastrointestinal immune system (GALT) is severely affected**, with early **loss of CCR5+ CD4+ memory T cells and Th17 cells**. This depletion compromises the intestinal barrier, allowing microbial leakage and causing systemic immune activation.

HIV depletes CD4 Th cells through viral budding, CTL-mediated destruction, and apoptosis, leading to rapid cell turnover that outpaces replacement. Beyond reducing T-cell numbers, HIV also disrupts **Th-cell function and impairs memory responses**.

HIV IN THE IMMUNE SYSTEM

The **destruction of CD4 T cells** by **HIV impairs both antibody**- and **cell-mediated immune responses**, weakening defenses against HIV and other antigens. HIV also disrupts **immune regulation by inducing polyclonal B-cell activation**, leading to functional defects, **elevated immunoglobulin levels, and autoantibody production**. However, despite this **hyperactivation, B cells exhibit a diminished ability** to generate specific antibody responses due to reduced T-cell support.

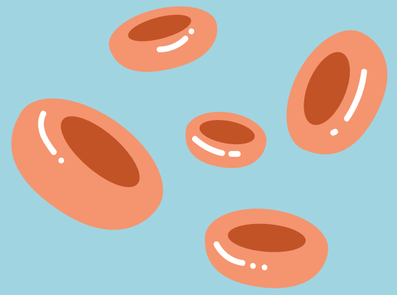
HIV IN THE IMMUNE SYSTEM

HIV-induced loss of **CD4 Th cells weakens cell-mediated immunity**, reducing CTL activity and delayed-type hypersensitivity responses. Early disease stages show increased cytokine levels, but as infection progresses, **IL-2, IFN-γ, and antibody-dependent cytotoxicity decline**, shifting the immune profile from Th1 to Th2. Late-stage infection causes severe lymphoid tissue damage, impairing T and B cell activation. Additional immunologic abnormalities include defective **antigen presentation, impaired monocyte and macrophage function, and reduced NK-cell activity**.

HIV IN THE IMMUNE SYSTEM

- **Immune Evasion & Latency** – HIV mutates rapidly, downregulates MHC I, and remains latent in CD4 T cells, dendritic cells, and macrophages.
- **CD4 T Cell Depletion** – Targets and destroys CD4 Th cells, especially in GALT, leading to immune activation and microbial leakage.
- **Cell Destruction** – CD4 T cells die due to viral budding, CTL attacks, and apoptosis, outpacing replacement.
- **Immune Dysfunction** – Reduced antibody and CTL responses, B-cell dysregulation, and impaired antigen presentation.
- **Cytokine Imbalance & Tissue Damage** – Shift from Th1 to Th2, cytokine decline, lymphoid tissue destruction, and reduced NK-cell activity.
- **Progression to AIDS** – Severe immunodeficiency, opportunistic infections, and immune system collapse.

CLINICAL SYMPTOMS OF HIV INFECTION



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HIV IN THE IMMUNE SYSTEM

HIV infection progresses through three stages: **acute infection, clinical latency, and AIDS**.

ACUTE PHASE

The **acute stage features a rapid rise in viral replication and viremia, spreading HIV to lymphoid organs**. CD4 T-cell counts drop initially but may partially recover. About **50–70% of patients experience flu-like symptoms within 3–6 weeks**, lasting up to a few weeks, while others remain asymptomatic.

In this stage, high levels of **circulating virus, or viremia,** can be seen in the blood of infected individuals; therefore, HIV begins to disseminate to the lymphoid organs.

HIV IN THE IMMUNE SYSTEM

CLINICAL LATENCY

After the acute phase, HIV enters clinical latency, where viral replication slows, viremia decreases, and symptoms are minimal or absent. Though the virus persists in plasma and lymphoid tissues, **CD4 T-cell counts remain stable before gradually declining**.

A rare subset of patients, elite controllers, maintain low viral loads and normal CD4 counts without treatment, likely due to genetic factors like HLA-B57-01 and HLA-B27-05, which enhance immune control of the virus.

HIV IN THE IMMUNE SYSTEM

AIDS

Without treatment, HIV progresses to AIDS, **marked by severe immunosuppression, low CD4 T-cell counts, high viremia, and life-threatening opportunistic infections and malignancies**. The median time to AIDS in untreated i**ndividuals is about 10 years**, though antiretroviral therapy significantly slows progression. HIV can also affect the brain, causing neurological symptoms ranging from cognitive decline and apathy in early infection to severe **dementia, seizures, and motor impairments in advanced disease**.

HIV IN THE IMMUNE SYSTEM

In **infants**, AIDS symptoms include **failure to thrive, persistent oral candidiasis, hepatosplenomegaly, lymphadenopathy, recurrent diarrhea, and bacterial infections, with possible neurological abnormalities**. Progression varies based on immune maturity, viral dose, and infection route.

The CDC initially defined AIDS as a disease indicating cell-mediated immunity defects with no other known cause. Over time, definitions evolved, incorporating new HIV tests.

HIV STAGING

- **Stage 0** – Early HIV infection with an initial positive test followed by a negative/indeterminate test within 6 months. Patients can be reassigned after 180 days.

- **Stages 1, 2, 3** – Based on CD4 T-cell count/percentage.

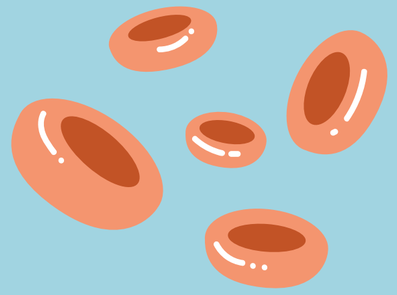
- **Stage 3 (AIDS)** – Defined by severely low CD4 T-cell counts or the presence of opportunistic infections.

STAGING CRITERION

Table 24-2 CD4 T-Cell Parameters Used in HIV Staging

	AGE ON DATE OF CD4+ T-LYMPHOCYTE TEST					
	YOUNGER THAN 1 YEAR		1 TO 5 YEARS		6 YEARS OR OLDER	
STAGE	CELLS/mL	PERCENT	CELLS/mL	PERCENT	CELLS/mL	PERCENT
1	1,500	≥34	≥1,000	>30	≥500	≥26
2	750–1,499	26–33	500–999	22–29	200–499	14–25
3	<750	<26	<500	<22	<200	<14

TREATMENT AND PREVENTION



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TREATMENT

HIV treatment includes supportive care for infections and malignancies and **antiretroviral therapy (ART)** to suppress viral replication. ART consists of multiple drug classes targeting different stages of the HIV life cycle:

- **NRTIs & NNRTIs** – Inhibit reverse transcription
- **Protease Inhibitors** (PIs) – Block viral protein processing
- **INSTIs** – Prevent viral DNA integration
- **Fusion Inhibitors & CCR5 Antagonists** – Block viral entry
- **CD4 Post-Attachment Inhibitors** – Prevent viral fusion

New drugs continue to emerge, with guidelines provided by the U.S. Department of Health and WHO.

Laboratory Testing for HIV Infection

Laboratory testing is essential for **HIV diagnosis and monitoring ART response.** Increased screening recommendations by the U.S. Preventative Task Force and CDC have improved awareness among high-risk individuals.
Key diagnostic and monitoring tests include:

- **HIV Antibody Detection** – Identifies immune response to HIV
- **HIV Antigen Detection** – Detects viral proteins
- **Viral Nucleic Acid Testing (NAT)** – Confirms active infection
- **CD4 T-Cell Enumeration** – Assesses immune status

Virus culture is definitive but impractical due to cost, complexity, and biohazards.

Screening and Diagnosis

Serological tests for HIV antibodies are crucial for early diagnosis, blood donor screening, and epidemiological studies. They help **identify infections within 1–2 months of exposure**, preventing transmission and guiding public health interventions.

Serological testing for **HIV includes automated immunoassays** (ELISA, CLIA) for screening and rapid tests for quick detection. **Confirmatory testing** distinguishes true from false positives, formerly using **Western blot, now replaced by newer methods. p24 antigen** testing and **NAT for HIV RNA improve early and accurate detection**.