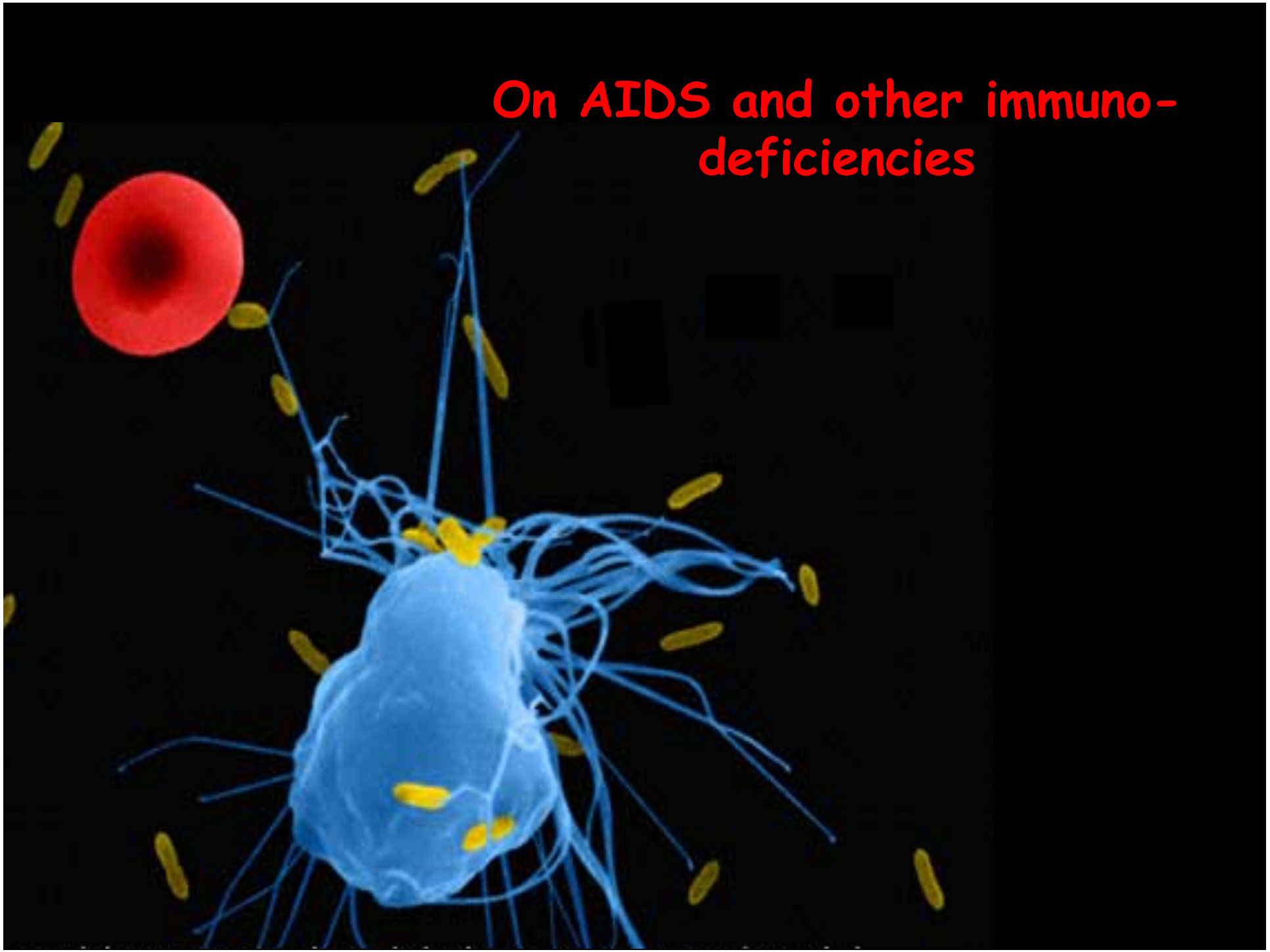
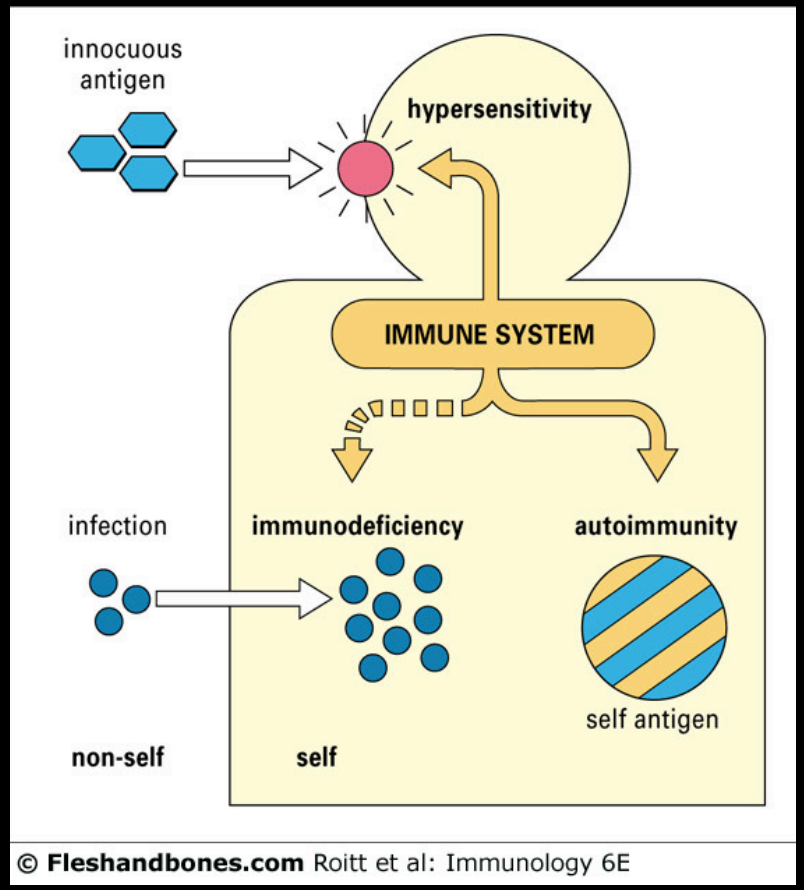
On AIDS and other immuno-deficiencies



Immunodeficiency, one of three major mechanisms of 'inappropriate' immunity



Topics to be discussed

Primary immunodeficiencies

- Progress in identifying the genetic defects that underlie these disorders
- Animal models of primary immunodeficiency
- Approaches for treatment, including innovative uses of gene therapy

Secondary immunodeficiencies

- AIDS
- Therapy and prevention strategies

Two major types of immunodeficiency

A primary immunodeficiency, which results from a genetic (inherited) or developmental defect in the immune system.
In such cases, defects are present at birth although they may not manifest themselves until later in life.

A secondary immunodeficiency, also termed, acquired immunodeficiency, is the loss of immune function and results from exposure to various agents.

The most common secondary immunodeficiency is the 'Acquired Immunodeficiency Syndrome', or AIDS, which results from infection with the 'Human Immunodeficiency Virus 1' (HIV-1).

Individuals with severe immunodeficiency are at risk of infection with opportunistic agents, *i.e.*, microorganisms that healthy individuals can harbor with no ill consequences but that cause disease in those with impaired immune function.

Primary immunodeficiency

May affect either the innate or the adaptive immune functions.

The immune system consists of two major lines of defense

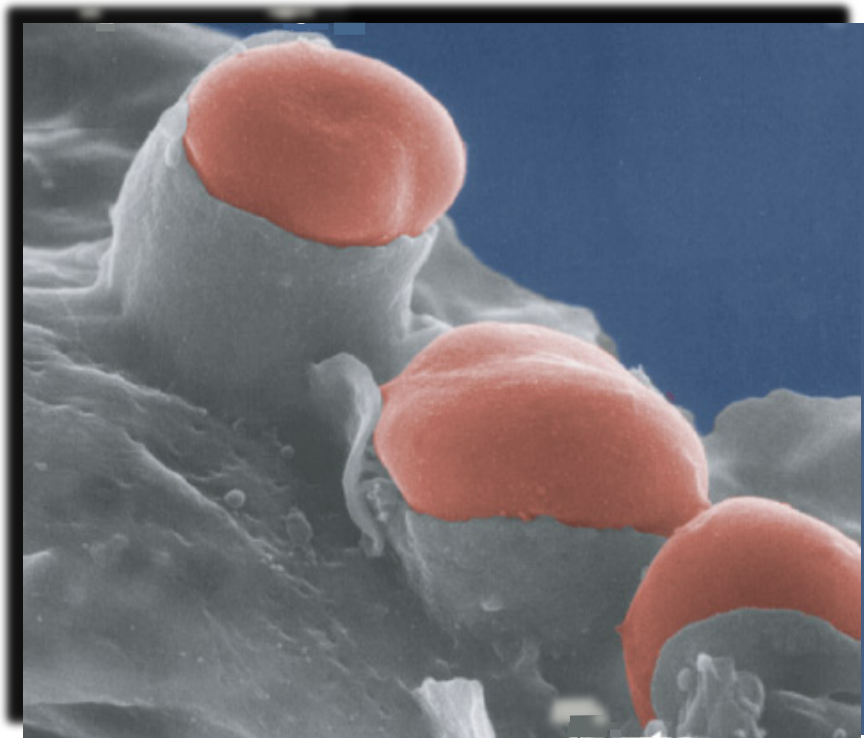
1. **Innate immune system** (Non-specific immunity)
   - Elie Metchnikoff (1908): Pathogens can be ingested and digested by phagocytic cells – macrophages
   - Combat microorganisms without prior exposure

2. **Acquired immune system** (Specific or adaptive immunity)
   - Only occurs after exposure to pathogens
   - Lifelong protective immunity to reinfection

Innate Immunity
(Vertebrates and Invertebrates)

- First line of defense - acting within a very short notice (minutes/hours) to protect the host.
- Resistance mechanisms are germline encoded - all elements exist throughout life span.
- Immunity which is not affected by prior exposure to infectious agents - No "memory"-stereotypic response.
- Antigen-"non-specific": Broadly recognize molecules possessed by various classes of microbes (bacterial cell wall components, bacterial DNA, glycoproteins/lipids).

One major mechanism of the innate immunity is the ability of phagocytic cells to phagocytose (engulf) foreign particles, such as bacteria, and dead or damaged cells

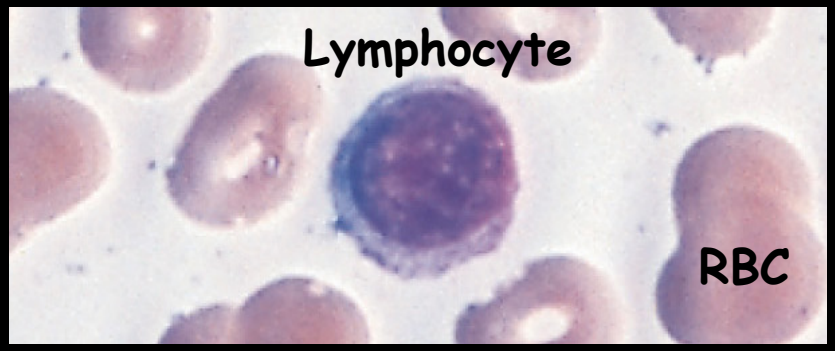


Macrophages phagocytose damaged red blood cells

Acquired (or Adaptive) Immunity

(Exists only in Vertebrates)

- A more specialized type of immunity, which supplements the innate system.
- Healthy individuals are born with the capacity to mount an immune response to a foreign antigen, but specific immunity is acquired by contact with the invader.
- Contact with a foreign agent (immunization) triggers a chain of events leading to lymphocyte activation, which directly or indirectly react against the foreign agent.
- Immunity can be induced against microorganisms, and their products, but also against enumerable natural and synthetic compounds.
- The compound against which the acquired immune system responsd is termed antigen (antibody generating).



Acquired (or Adaptive) Immunity

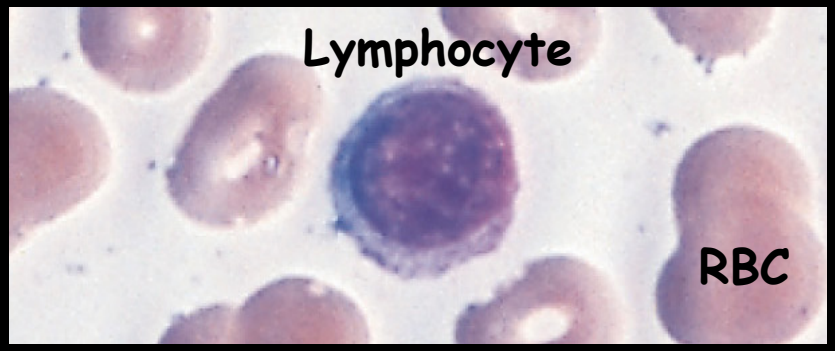
(Exists only in Vertebrates)

There are two major types of lymphocytes:

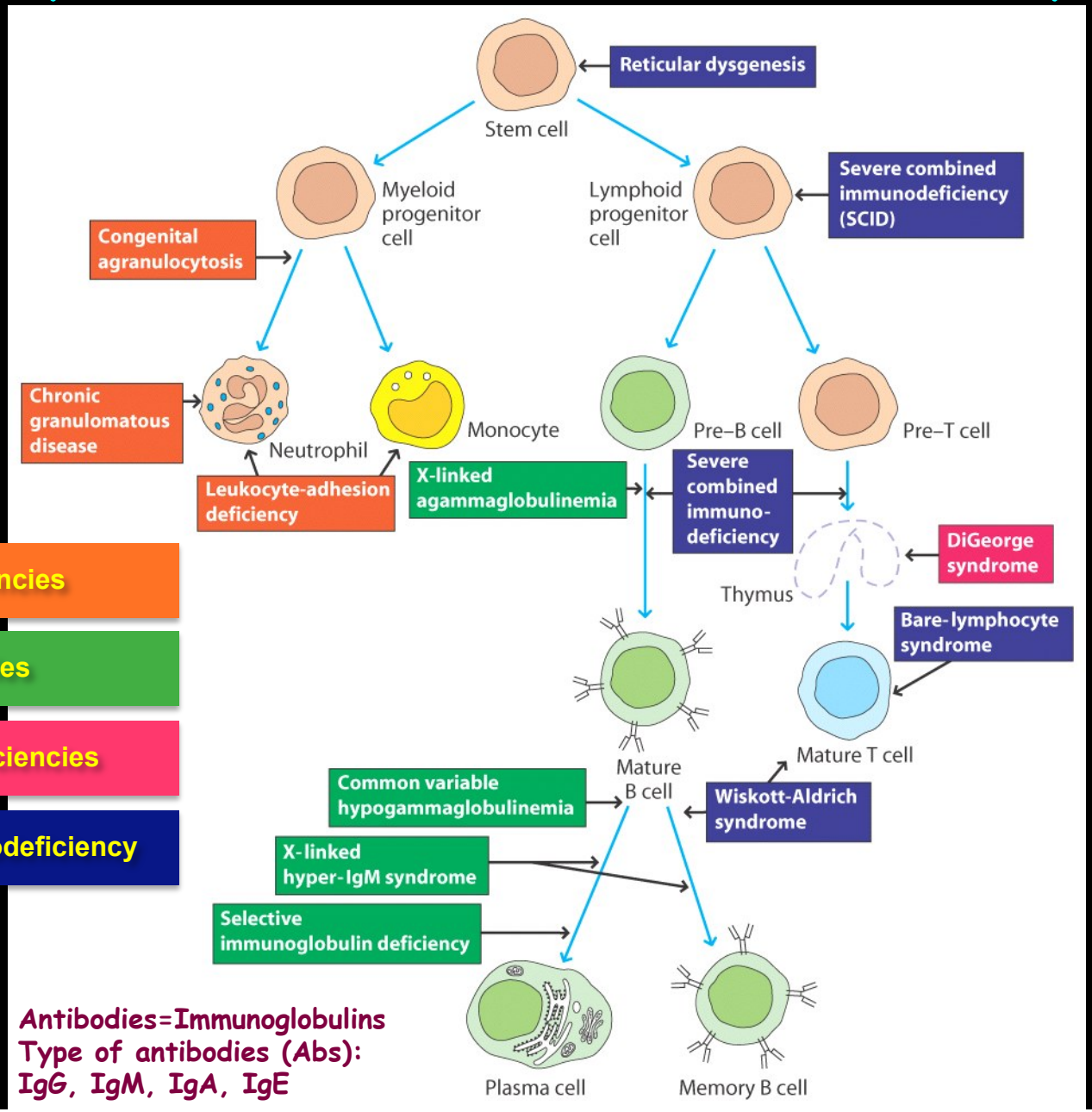
B lymphocytes (B cells) are in charge of antibody (Ab) production.

T lymphocytes (T cells) are in charge of killing virus infected cells, cancer cells and genetically incompatible cells of transplanted tissues and organs.

Immunological memory reside within the lymphocytes.



Inherited defects that interrupt hematopoiesis or impair functioning of immune-system cells result in various immunodeficiency diseases



Phagocytic deficiencies

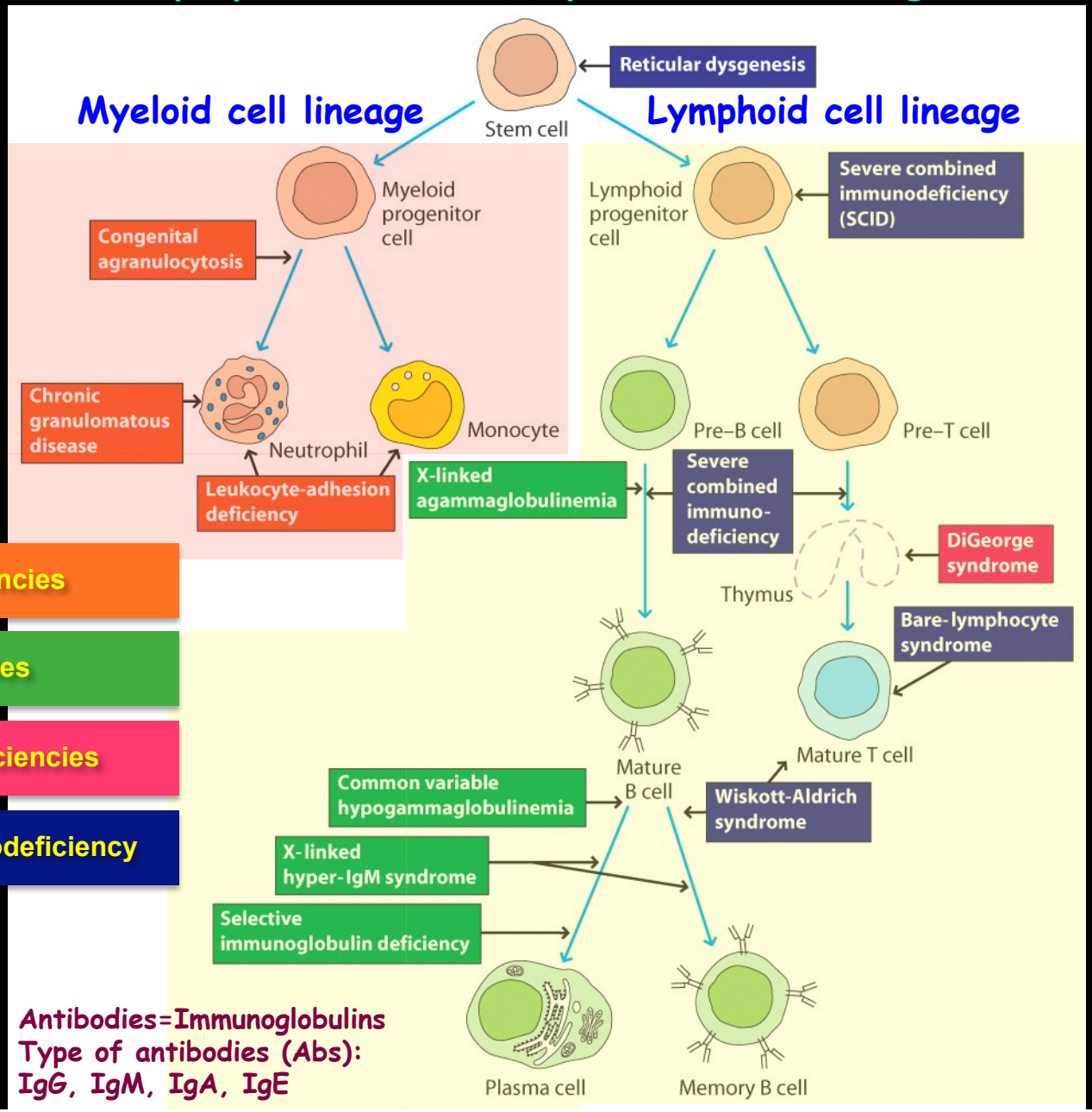
Humoral deficiencies

Cell-mediated deficiencies

Combined immunodeficiency

Antibodies=Immunoglobulins
Type of antibodies (Abs):
IgG, IgM, IgA, IgE

Most defects leading to primary immunodeficiency affect either the lymphoid or the myeloid cell lineage



Phagocytic deficiencies

Humoral deficiencies

Cell-mediated deficiencies

Combined immunodeficiency

Antibodies=Immunoglobulins
Type of antibodies (Abs):
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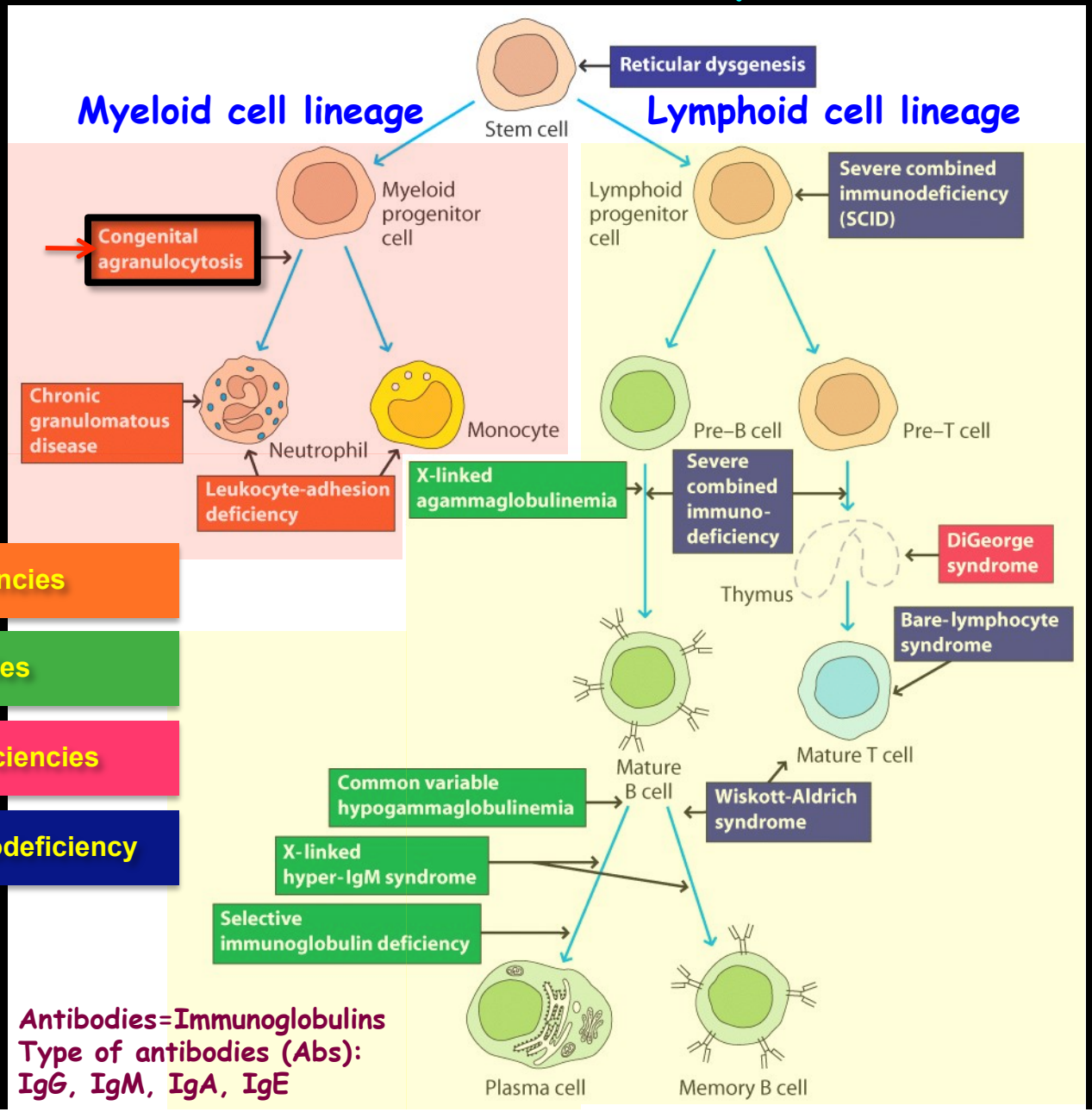
Congenital agranulocytosis results in the absence, reduced number or decreased function of myeloid cells



Phagocytic deficiencies

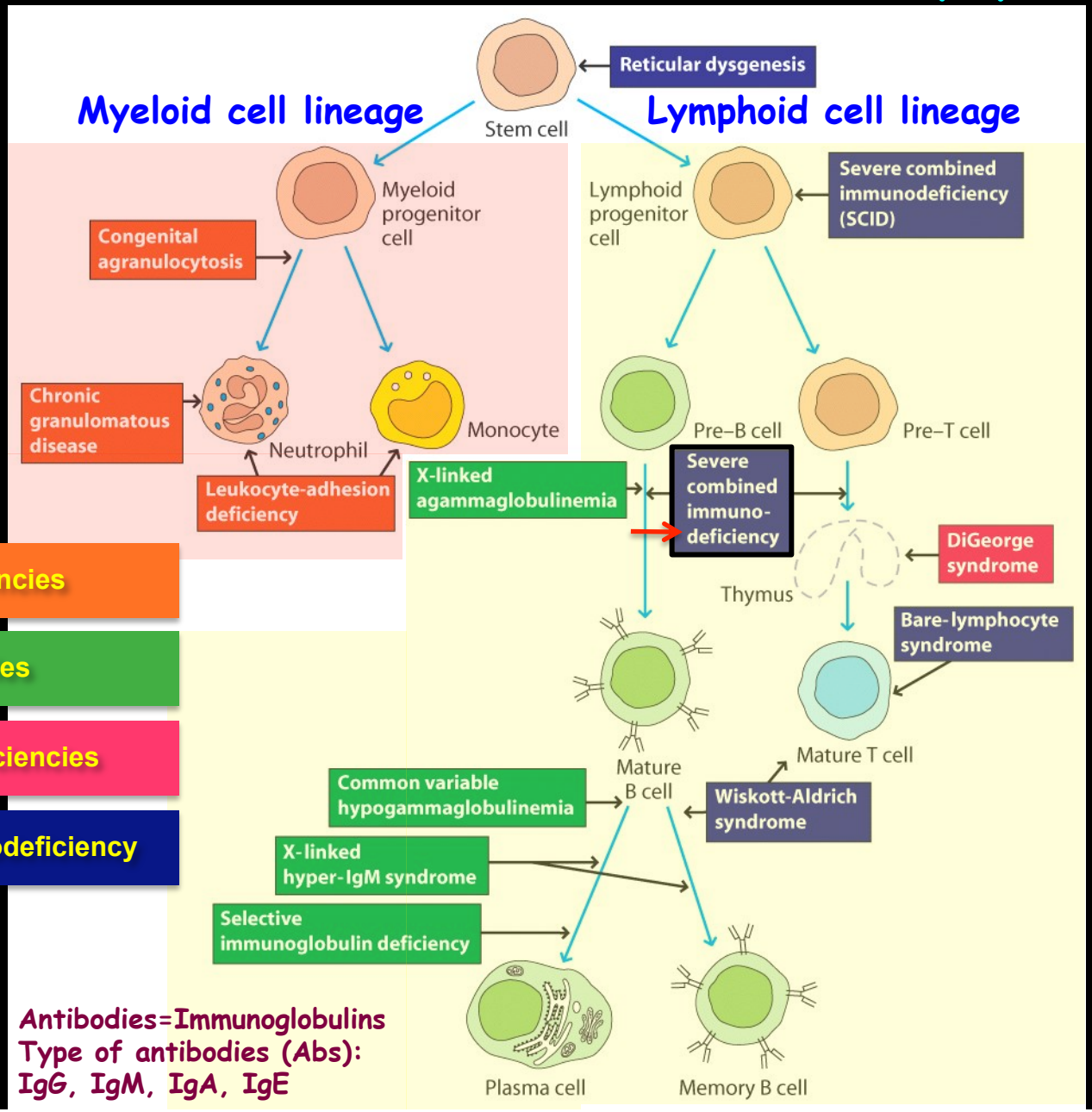
Humoral deficiencies

Cell-mediated deficiencies

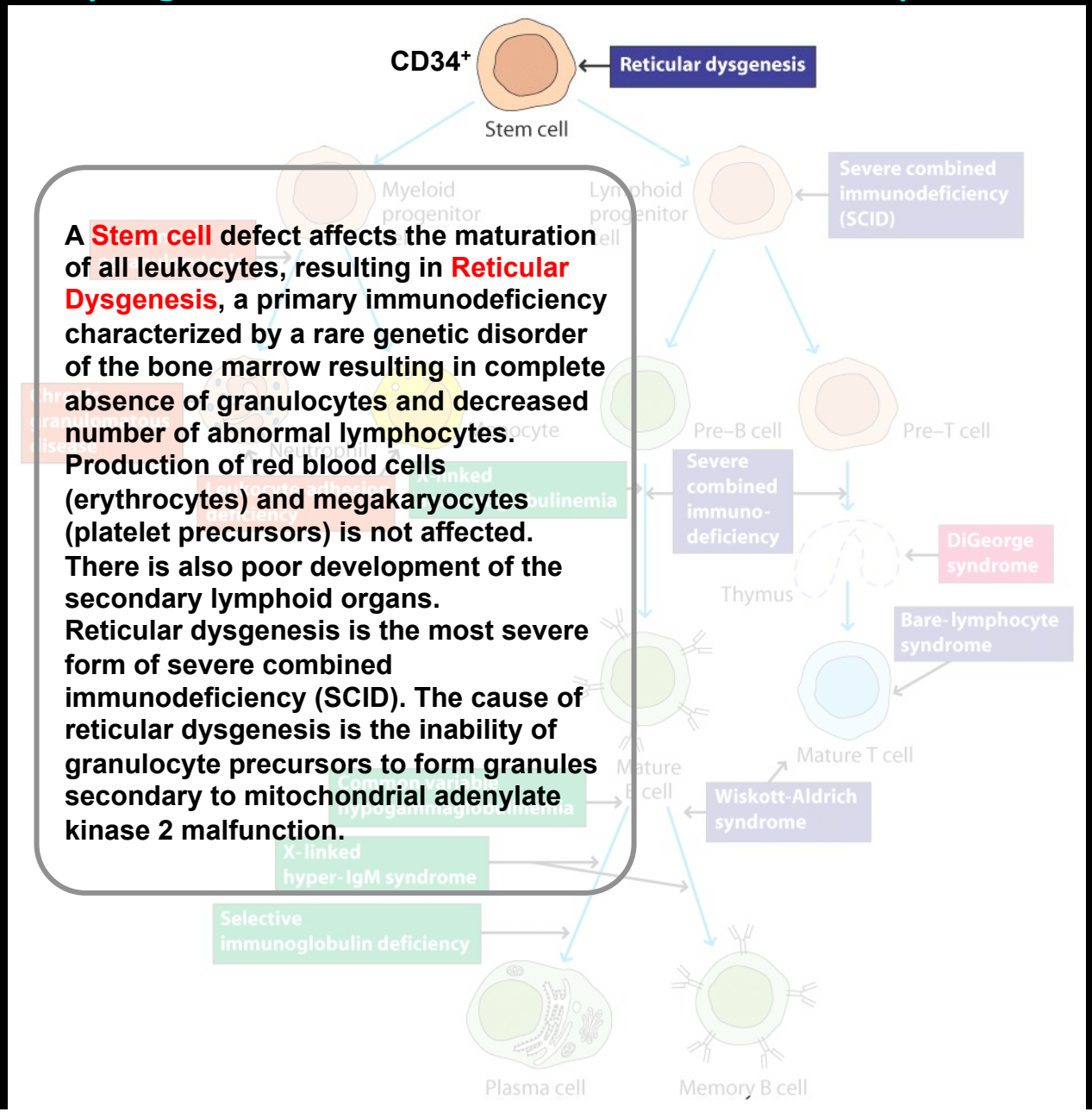
Combined immunodeficiency

Antibodies=Immunoglobulins
Type of antibodies (Abs):
IgG, IgM, IgA, IgE

Severe combined immunodeficiency (SCID) results in the absence, reduced number or decreased function of lymphocytes

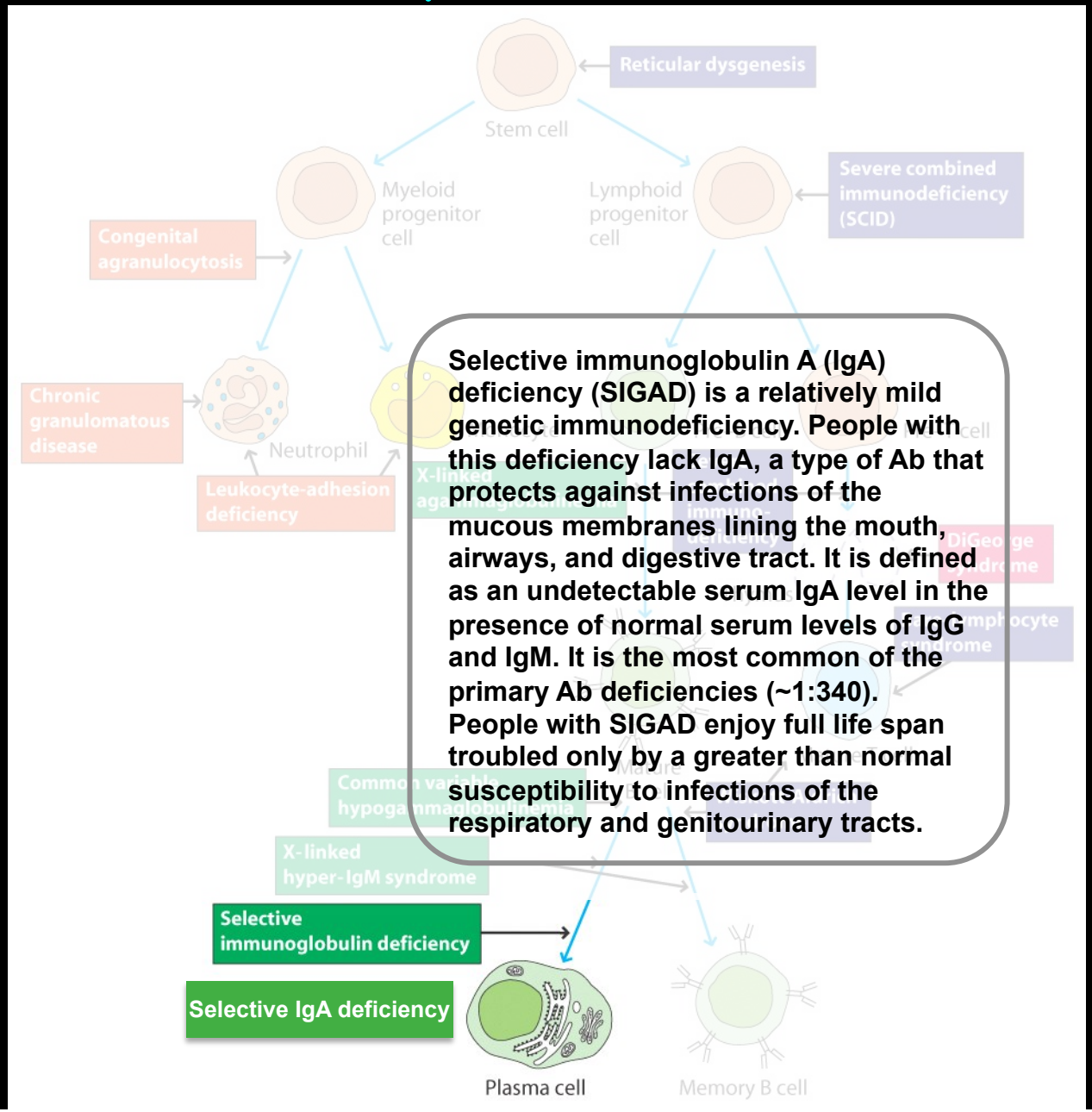


Defects in components early in the hematopoietic developmental program affect the entire immune system



A **Stem cell** defect affects the maturation of all leukocytes, resulting in **Reticular Dysgenesis**, a primary immunodeficiency characterized by a rare genetic disorder of the bone marrow resulting in complete absence of granulocytes and decreased number of abnormal lymphocytes. Production of red blood cells (erythrocytes) and megakaryocytes (platelet precursors) is not affected. There is also poor development of the secondary lymphoid organs. Reticular dysgenesis is the most severe form of severe combined immunodeficiency (SCID). The cause of reticular dysgenesis is the inability of granulocyte precursors to form granules secondary to mitochondrial adenylate kinase 2 malfunction.

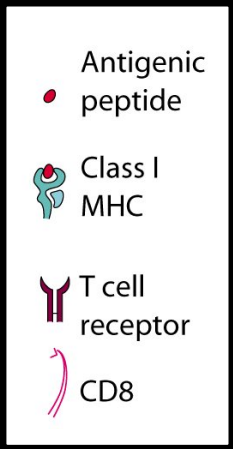
Defects in highly differentiated cells have consequences that are more specific and less severe

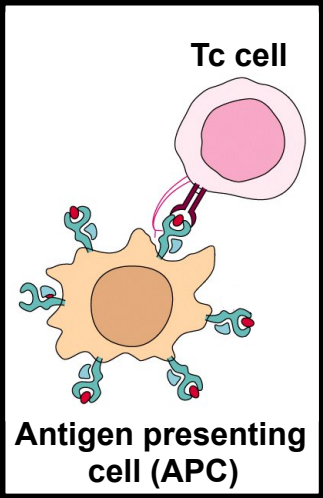


Some primary human immunodeficiency diseases and underlying genetic defects

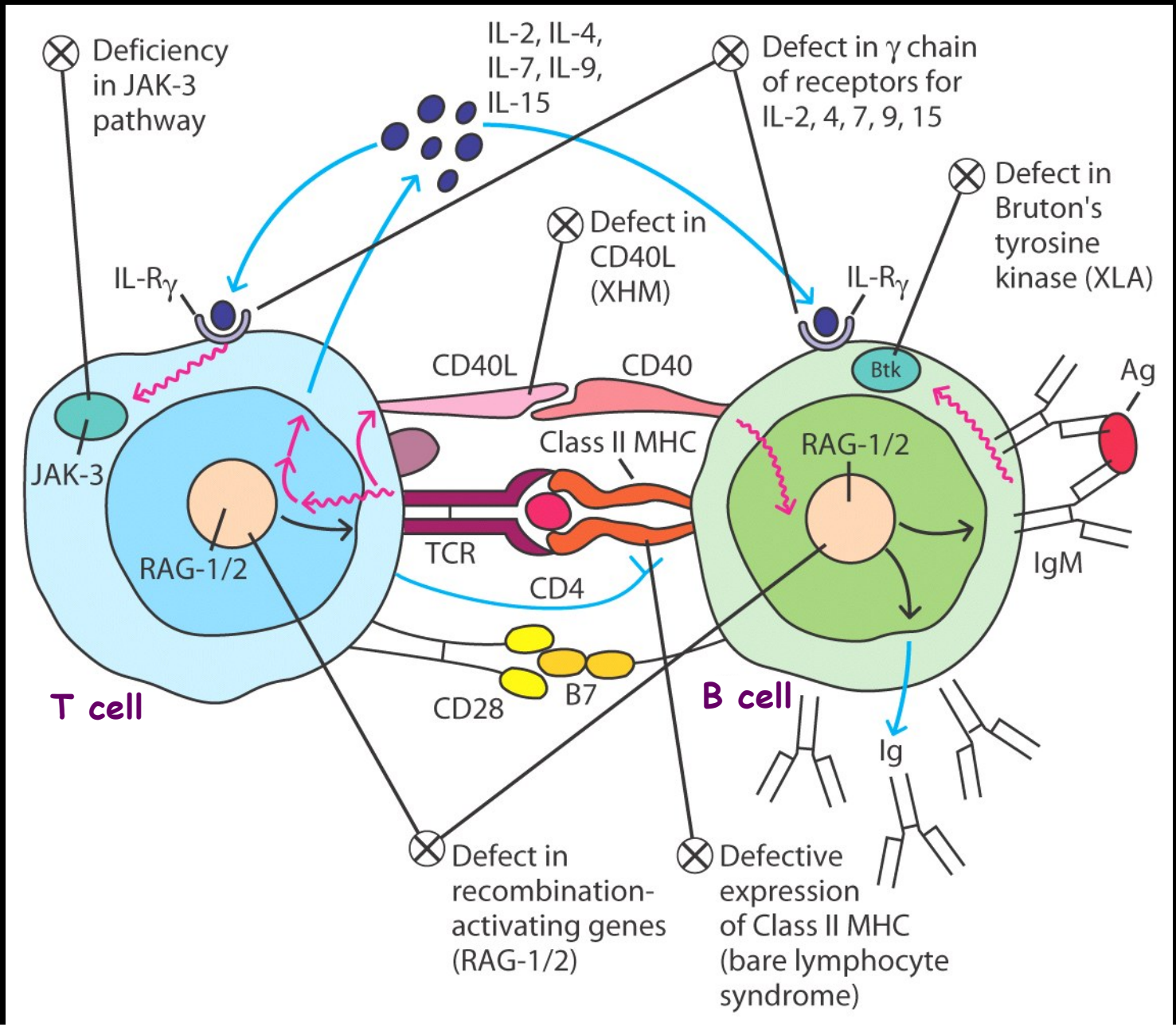
Immunodeficiency disease	Specific defect	Impaired function	Inheritance mode*	Chromosomal defect
Severe combined immunodeficiency (SCID)	RAG-1/RAG-2 deficiency	No TCR or Ig gene rearrangement	AR	11p13
	ADA deficiency	Toxic metabolite in T and B cells	AR	20q13
	PNP deficiency		AR	14q13
	JAK-3 deficiency	Defective signals from IL-2, 4, 7, 9, 15,	AR	19p13
	IL-2Rγ-deficiency		XL	Xq13
	ZAP-70 deficiency	Defective signal from TCR	AR	2q12
Bare lymphocyte syndrome	Defect in MHC class II gene promoter	No class II MHC molecules	AR	16p13
Wiskott-Aldrich syndrome (WAS)	Cytoskeletal protein (CD43)	Defective T cells and platelets	XL	Xp11
Interferon gamma receptor	IFN-γ–receptor defect	Impaired immunity to mycobacteria	AR	6q23
DiGeorge syndrome	Thymic aplasia	T- and B-cell development	AD	22q11
Ataxia telangiectasia	Defective cell-cycle kinase	Low IgA, IgE	AR	11q22
Gammaglobulinemias	X-linked agammaglobulinemia	Bruton's tyrosine kinase (Btk); no mature B cells	XL	Xq21
	X-linked hyper-IgM syndrome	Defective CD40 ligand	XL	Xq26
	Common variable immunodeficiency	Low IgG, IgA; variable IgM	Complex	
	Selective IgA deficiency	Low or no IgA	Complex	
Chronic granulomatous disease	Cyt $p91^{phox}$	No oxidative burst for bacterial killing	XL	Xp21
	Cyt $p67^{phox}$		AR	1q25
	Cyt $p22^{phox}$		AR	16q24
Chediak-Higashi syndrome	Defective intracellular transport protein (LYST)	Inability to lyse bacteria	AR	1q42
Leukocyte-adhesion defect	Defective integrin β2 (CD18)	Leukocyte extravasation	AR	21q22

*AR = autosomal recessive; AD = autosomal dominant; XL = X linked; "Complex" indicates conditions for which precise genetic data are not available and that may involve several interacting loci.

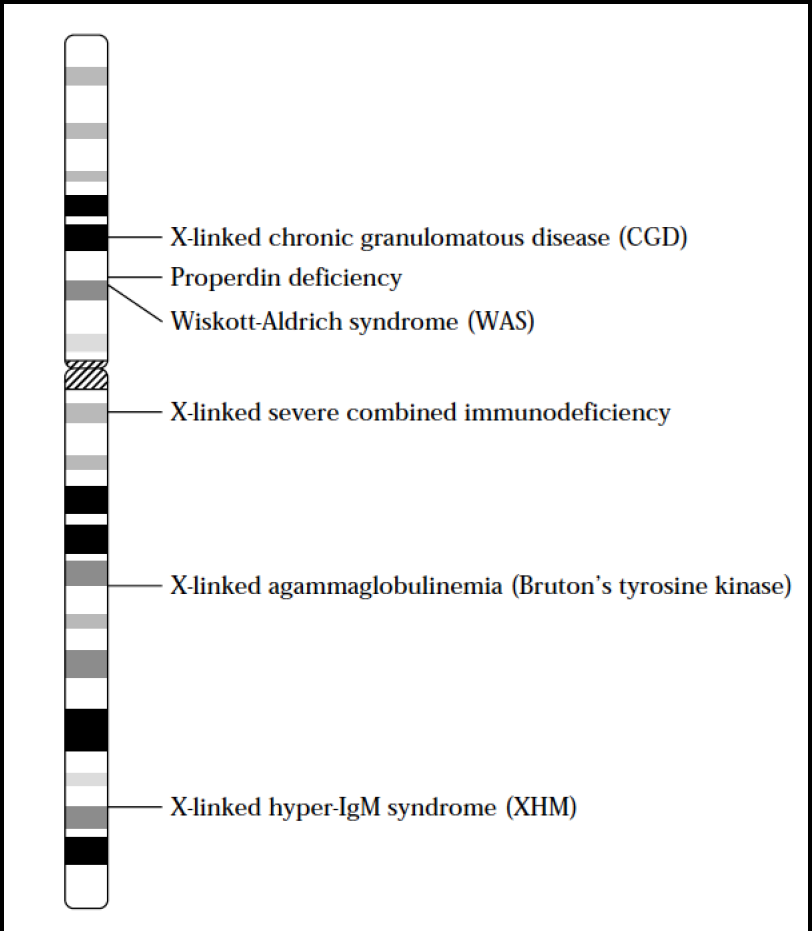




Defects in cell interaction and signaling can lead to severe immunodeficiency



Several X-linked immunodeficiency diseases result from defects in loci on the X chromosome



X-SCID is due to mutations occurring on the X-chromosome. Most often, these diseases affect males whose mother is a carrier (heterozygous) for the disorder. Because females have two X-chromosomes, the mother will not be affected by carrying only one abnormal X-chromosome, but any male children will have a 50% chance of being affected with the disorder by inheriting the faulty gene. Likewise, her female children will have a 50% chance of being carriers for the immunodeficiency.

Treatment of patients with immunodeficiency

There are no cures for immunodeficiency disorders.

Treatment includes:
1. Replacement of a missing protein
2. Replacement of a missing cell type or lineage
3. Replacement of a missing or defective gene

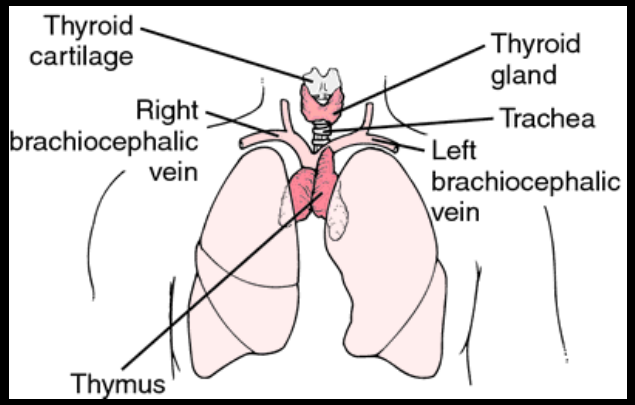
1. For disorders that impair Ab production – treatment by administration of the missing proteins. Pooled human gammaglobulin given *iv* or *sc* protects against recurrent infection. Administration of IFN-γ is effective in CGD patients, IL-2 partially restores immune functions in AIDS patients, recombinant adenosine deaminase has been successfully administered to ADA deficient SCID patients.

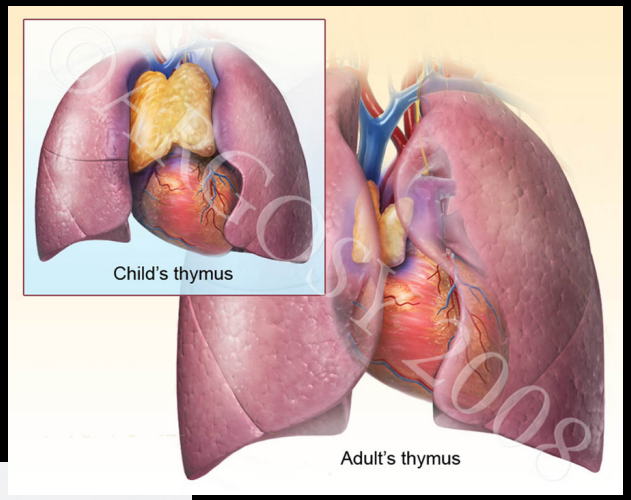
2. T-cell-depleted bone marrow transplantation, hematopoietic stem cell (CD34+) transplantation.

3. If a single gene defect has been identified, as in ADA deficiency or chronic granulomatous disease, replacement of the defective gene is an option. In clinical trials, CD34+ cells (from the patient) are isolated and transfected with a normal copy of the defective gene. The transfected cells are then returned to the patient.

Experimental models of immunodeficiency

The two best models for studying immunodeficiency in mice are the athymic nude mouse and the severe combined immunodeficiency (SCID) mouse.





Nude (athymic) mice

Mice homozygous for the 'nude' trait (nu/nu) are hairless and have a vestigial thymus. Heterozygotic (nu/+) litter mates have hair and a normal thymus. The hairlessness and the thymus defects are either caused by the same defective gene, or by closely linked defective genes, which, although unrelated, appear together in this mutant mouse.

Nude (athymic) mice

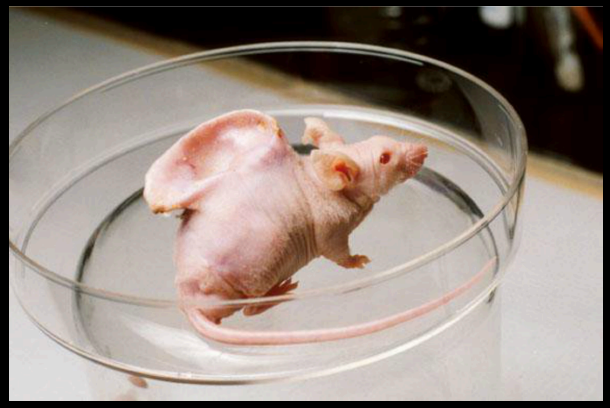
Nude mice lack cell-mediated immune responses, and they are unable to make antibodies to most antigens.

The immunodeficiency in the nude mouse can be reversed by a thymic transplant.

Homozygous 'nude' (nu/nu) mice are very sensitive to infections (50% die within the first two weeks after birth). Therefore, they are maintained in sterilized containers, and supplied with sterilized food, water, and bedding. The cages are protected by air filters fitted over the individual cages.

Because they can permanently tolerate allografts and xenografts, they have a number of practical experimental uses. For example, hybridomas or solid tumors from any origin (including human tumors) may be grown as ascites or as implanted tumors in a nude mouse.

CAN YOU EAR ME NOW ?



The nude mouse is valuable to research because it can receive many different types of tissue and tumor grafts, as it mounts no rejection response.

These xenografts are commonly used in research to test new methods of imaging and treating tumors.

The genetic basis of the nude mouse mutation is a disruption of the FOXN1 gene.

Severe combined immunodeficiency (SCID) mice

There are two strains of mouse SCID:

1. One that was discovered accidentally, where the precursor of T and B cells are unable to differentiate into mature functional lymphocytes. They cannot respond by Ab formation or cell mediated immunity and must be kept in a very clean environment.

2. A second mouse strain was developed in the lab by 'knock-out' of the Rag1 and Rag2 genes, which are responsible for the rearrangement of immunoglobulin or T-cell–receptor genes in both B- and T-cell precursors. Because cells with abnormal rearrangements are eliminated *in vivo*, both B and T cells are absent from the lymphoid organs of the 'RAG knockout' mouse.

Secondary immunodeficiencies
(Acquired immunodeficiencies)

- Acquired hypogammaglobulinemia – unknown origin
- Cytotoxic drugs or radiation given to treat various forms of cancer
- Drugs used to combat autoimmune diseases, such as corticosteroids
- Immunosuppressive drugs provided to allotransplantation patients, *e.g.*, Cyclosporin A and FK506
- Viral infections - Human Immunodeficiency Virus (HIV)

AIDS

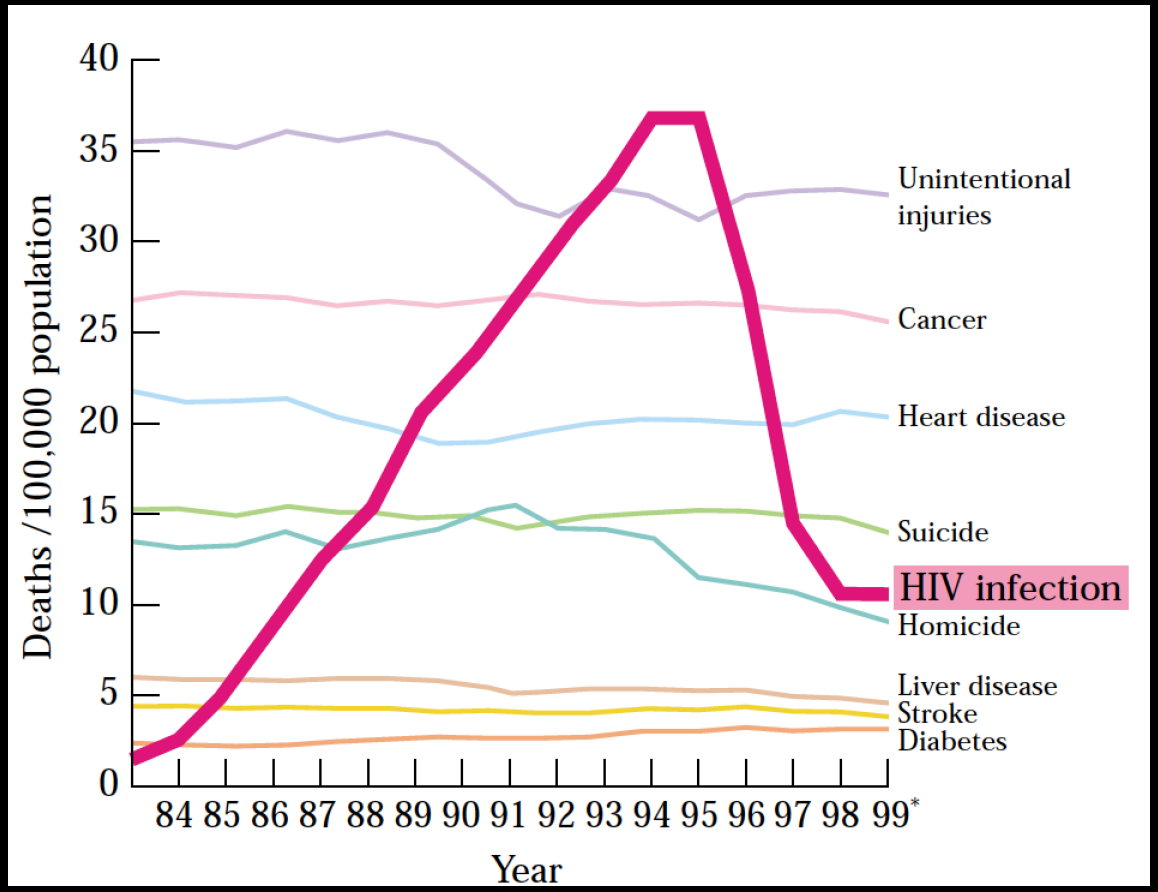
AIDS, Acquired Immunodeficiency Syndrome

The most common immunodeficiency, AIDS, first discovered in the US in 1981, is caused by the infectious agent called 'human immunodeficiency virus 1' (HIV-1).

The first patients displayed unusual infections, including the opportunistic fungal pathogen Pneumocystis carinii, which causes a pneumonia called PCP (P. carinii pneumonia) in persons with immunodeficiency. In addition to PCP, some patients had Kaposi's sarcoma, an extremely rare skin tumor.

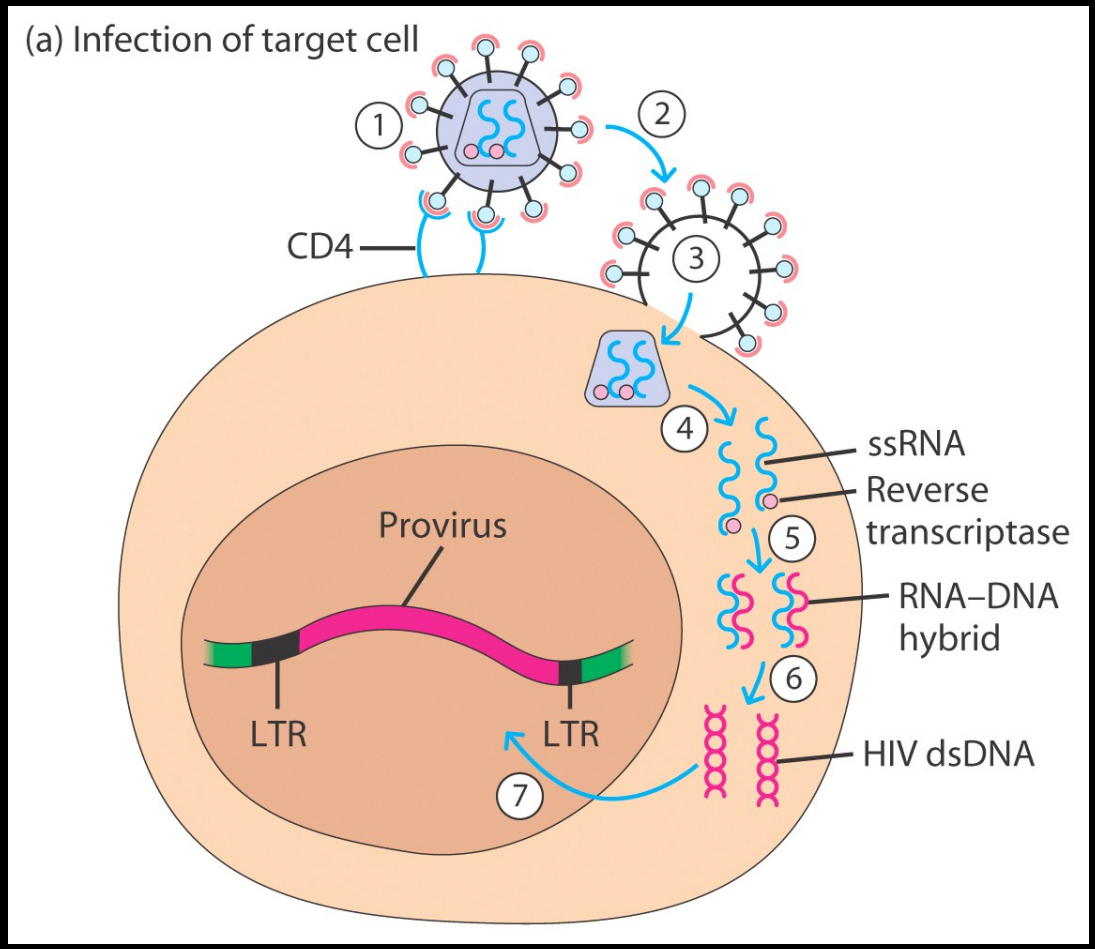
In the year 2000, AIDS killed approximately 3 million people.
HIV continues to spread to an estimated 15,000 individuals per day.

Rates of the leading causes of death in persons aged 25–44 in the US for the years 1982–99



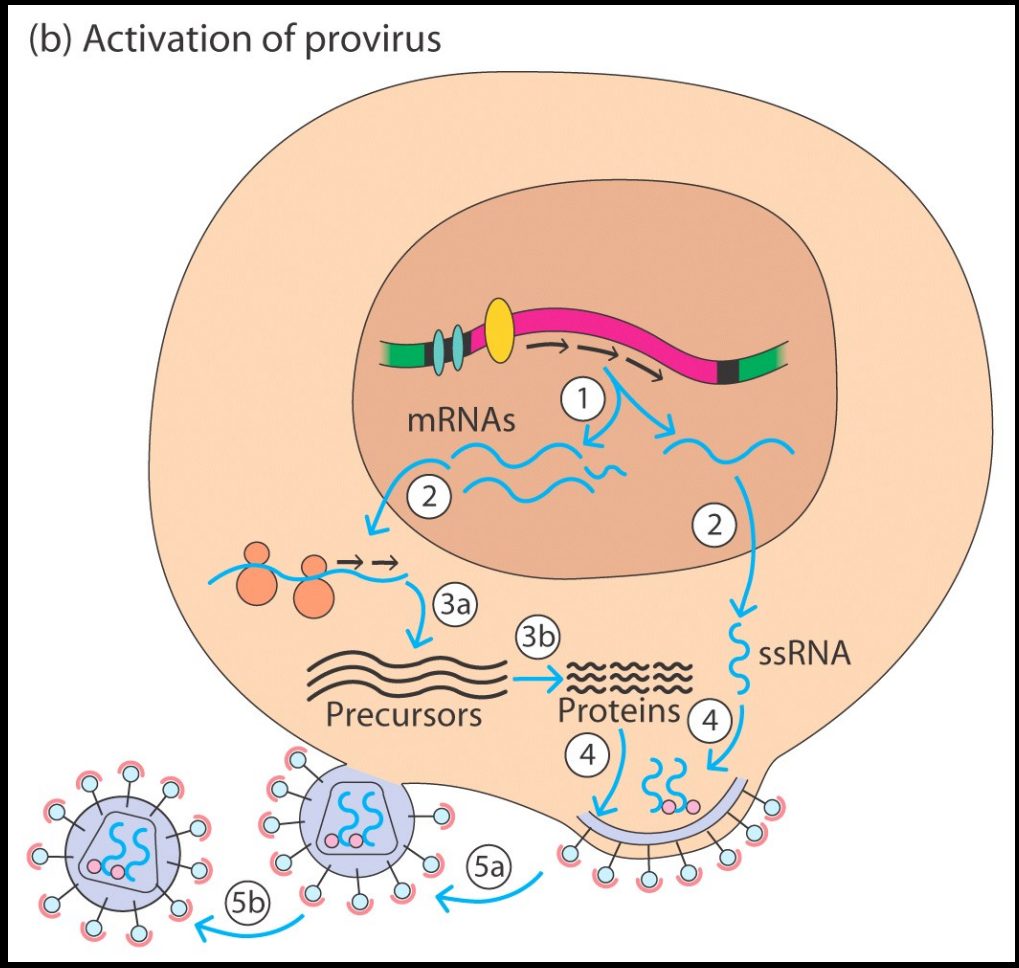
The heavy line shows that the death rate per 100,000 persons caused by AIDS surpassed any other single cause of death in this age range during the period 1993 to 1995. The recent decrease in AIDS deaths in the US is attributed to improvement in anti-HIV drug therapy, which prolongs the lives of patients.

Overview of HIV infection of target cells and activation of provirus



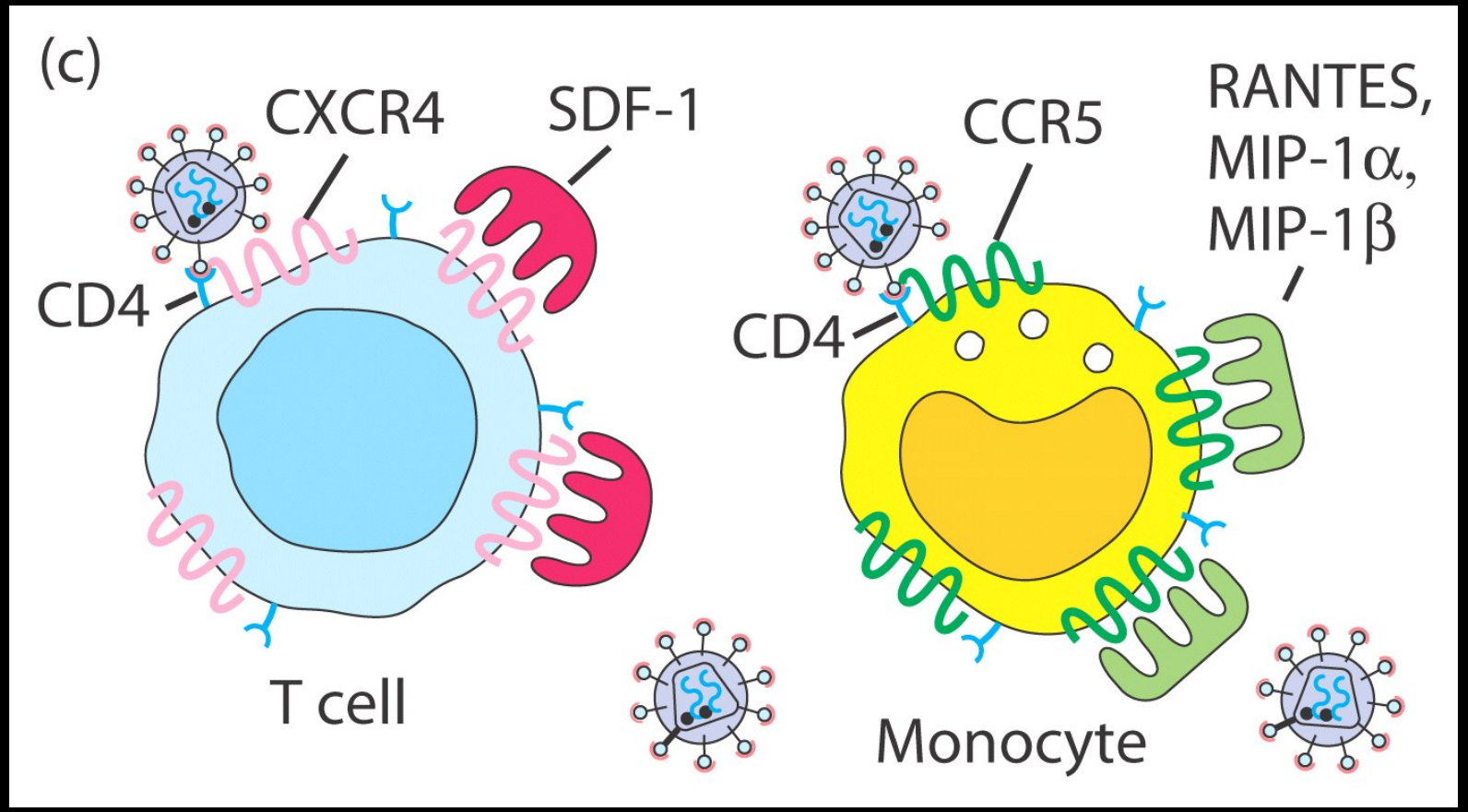
(a) Following entry of HIV into cells and formation of dsDNA, integration of the viral DNA into the host-cell genome creates the provirus.

Overview of HIV infection of target cells and activation of provirus



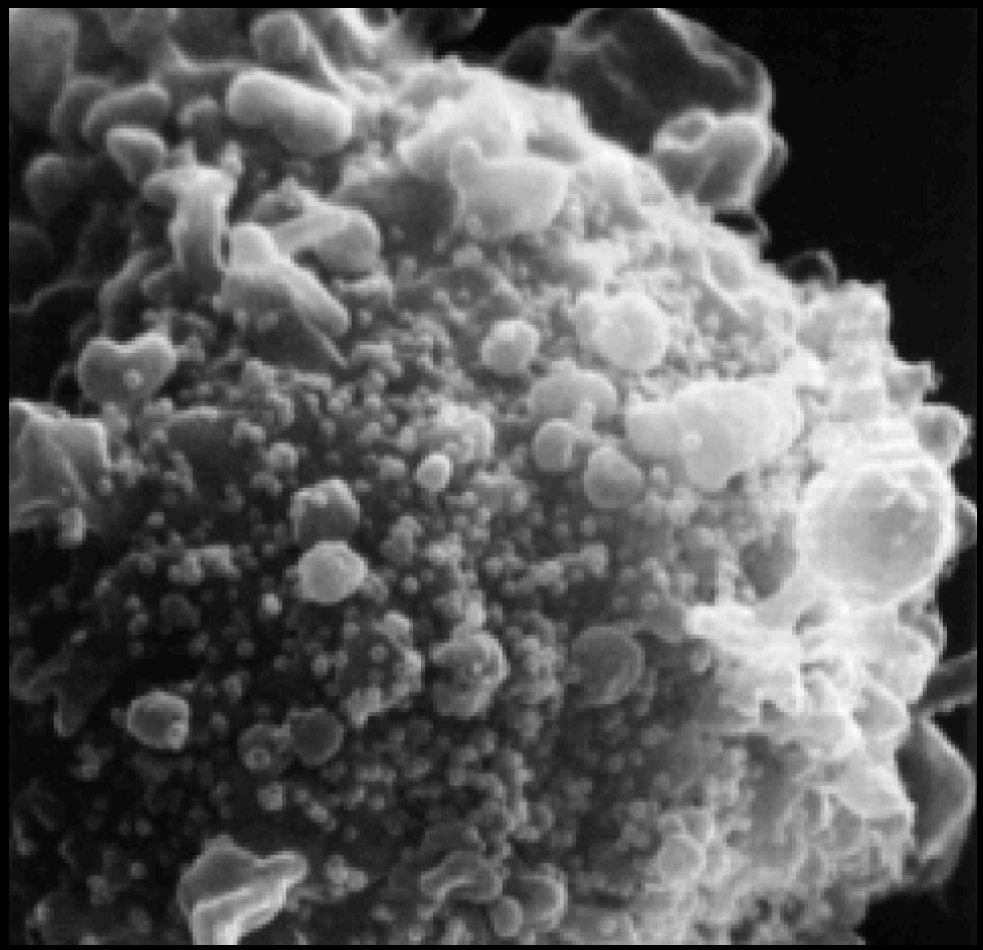
(b) The provirus remains latent until events in the infected cell trigger its activation (*e.g.*, inflammatory response), leading to formation and release of viral particles.

Overview of HIV infection of target cells and activation of provirus



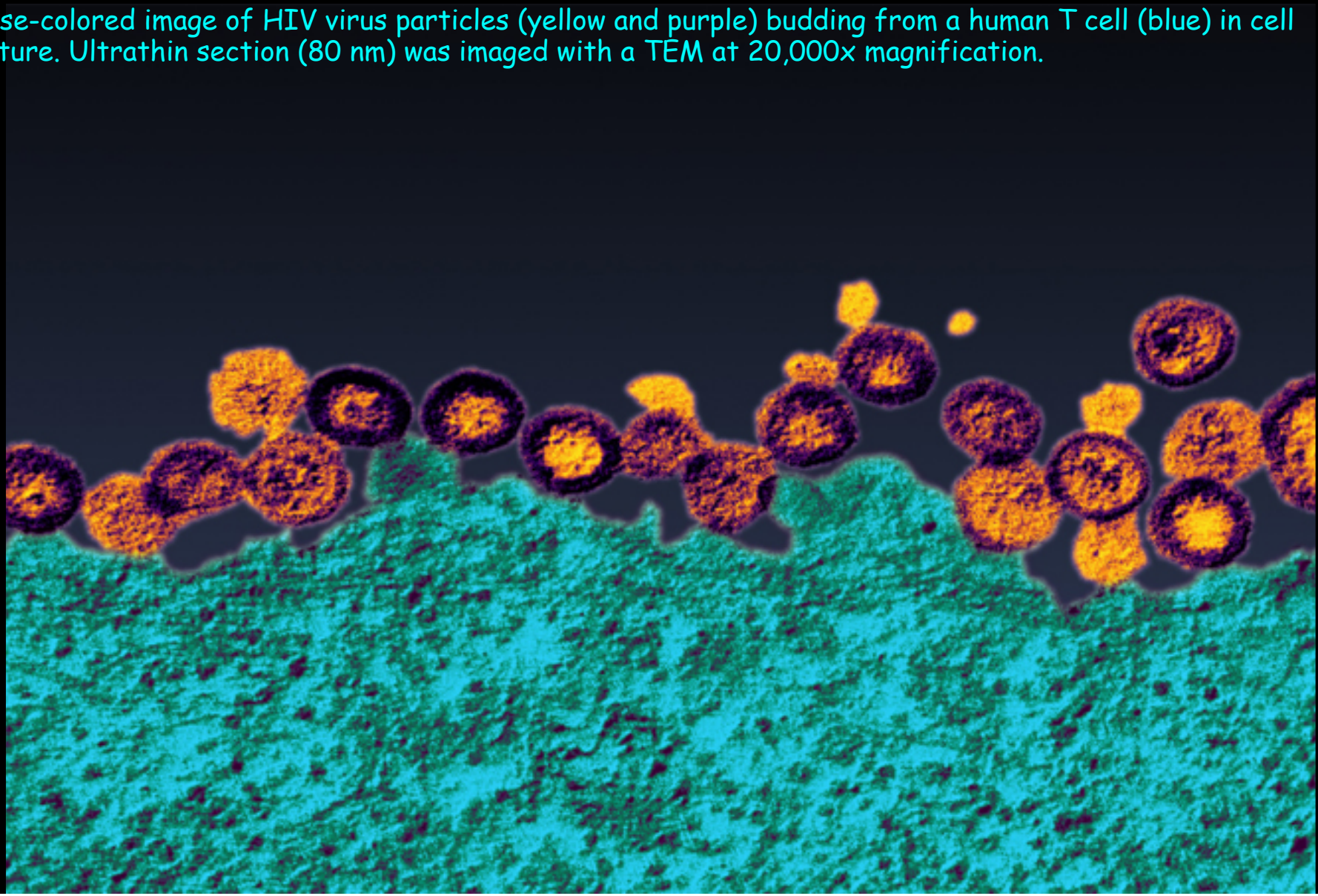
(c) Although CD4 binds to the envelope glycoprotein of HIV-1, a second receptor is necessary for entry and infection. The T-cell–tropic strains of HIV-1 use the co-receptor, CXCR4, while the macrophage-tropic strains use CCR5. Both are receptors for chemokines, and their normal ligands can block HIV infection of the cell.

Once the HIV provirus has been activated, buds representing newly formed viral particles can be observed on the surface of an infected T cell. The extensive cell damage resulting from budding and release of virions leads to the death of infected cells.



T cells counter HIV transmission using a surprisingly simple trick: they tie the virions to the cell membrane with an intermembrane protein, appropriately name "tetherin." When a virion buds from the cell surface, one tetherin domain inserts into the new viral membrane, while another domain stays embedded in the cell's plasma membrane, preventing the virus particle from diffusing away.

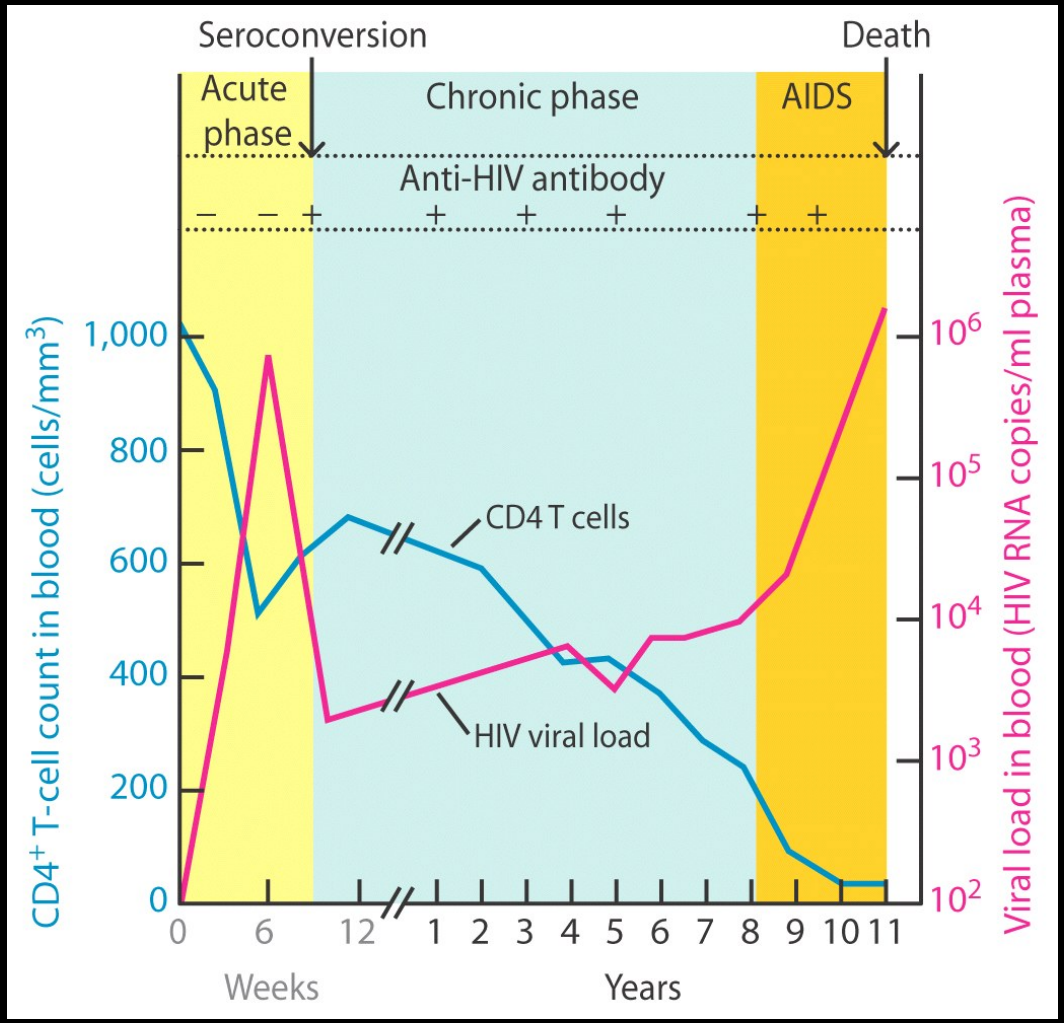
False-colored image of HIV virus particles (yellow and purple) budding from a human T cell (blue) in cell culture. Ultrathin section (80 nm) was imaged with a TEM at 20,000x magnification.



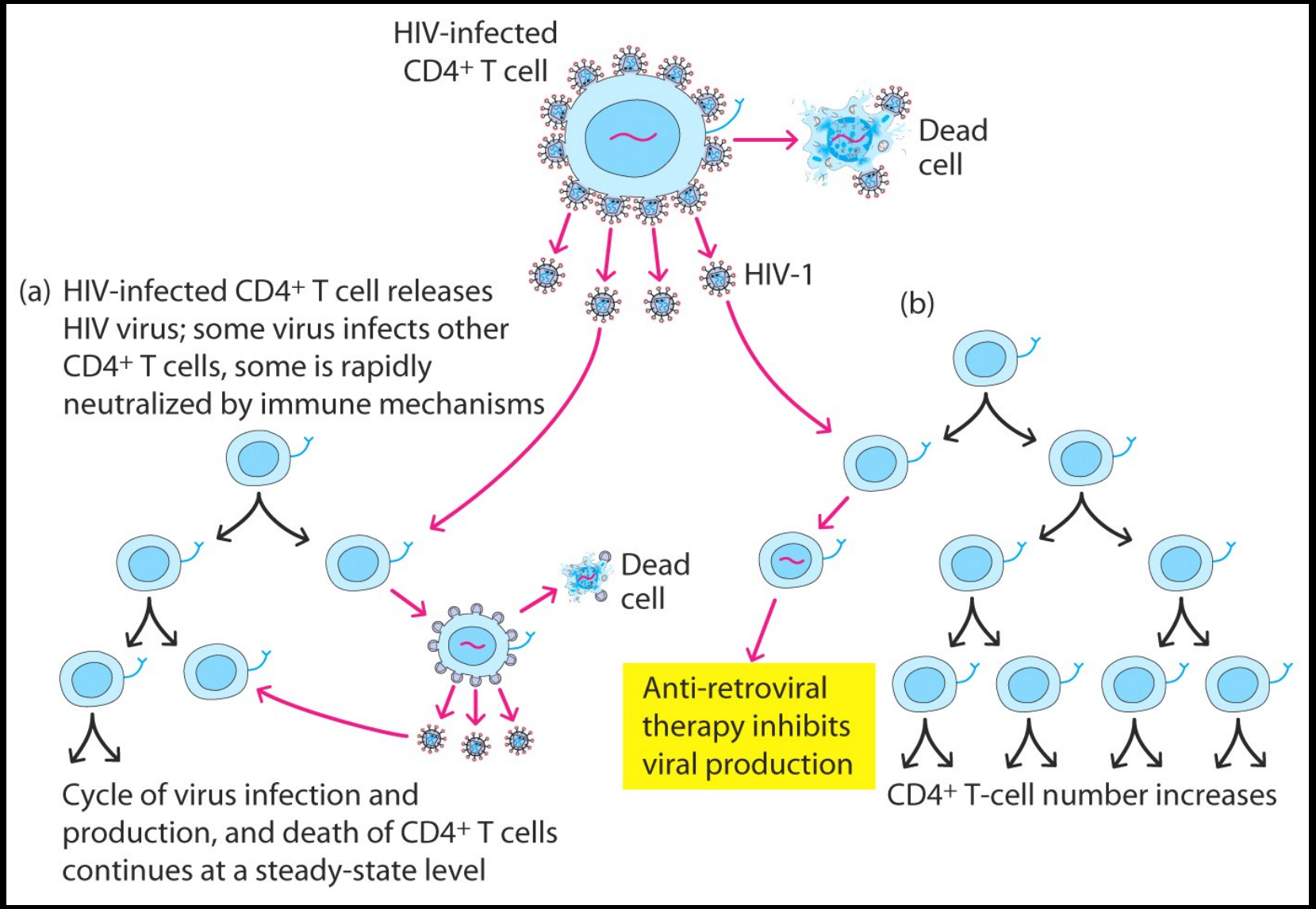
Serologic profile of HIV infection showing three stages in the infection process

Soon after infection, viral RNA is detectable in the serum. However, HIV infection is most commonly detected by the presence of anti-HIV antibodies after seroconversion, which normally occurs within a few months after infection. Clinical symptoms indicative of AIDS generally do not appear for at least 8 years after infection, but this interval is variable.

The onset of clinical AIDS is usually signaled by a decrease in CD4+ T-cell numbers and an increase in viral load.



Production of virus by CD4+ T cells and maintenance of a steady state of viral load and T cell number



Can mice serve as a model system for studying infection by HIV, the causative agent of AIDS?

Infection of cells by a wide range of viruses occurs predominantly via receptor-mediated endocytosis, the same mechanism used by cells to endocytose essential nutrients, hormones, growth factors, neurotransmitters and antigens.

Receptor mediated endocytosis

A mechanism by which selected macromolecules are taken up by cells following their specific binding to complementary transmembrane cell surface receptors.

Example:

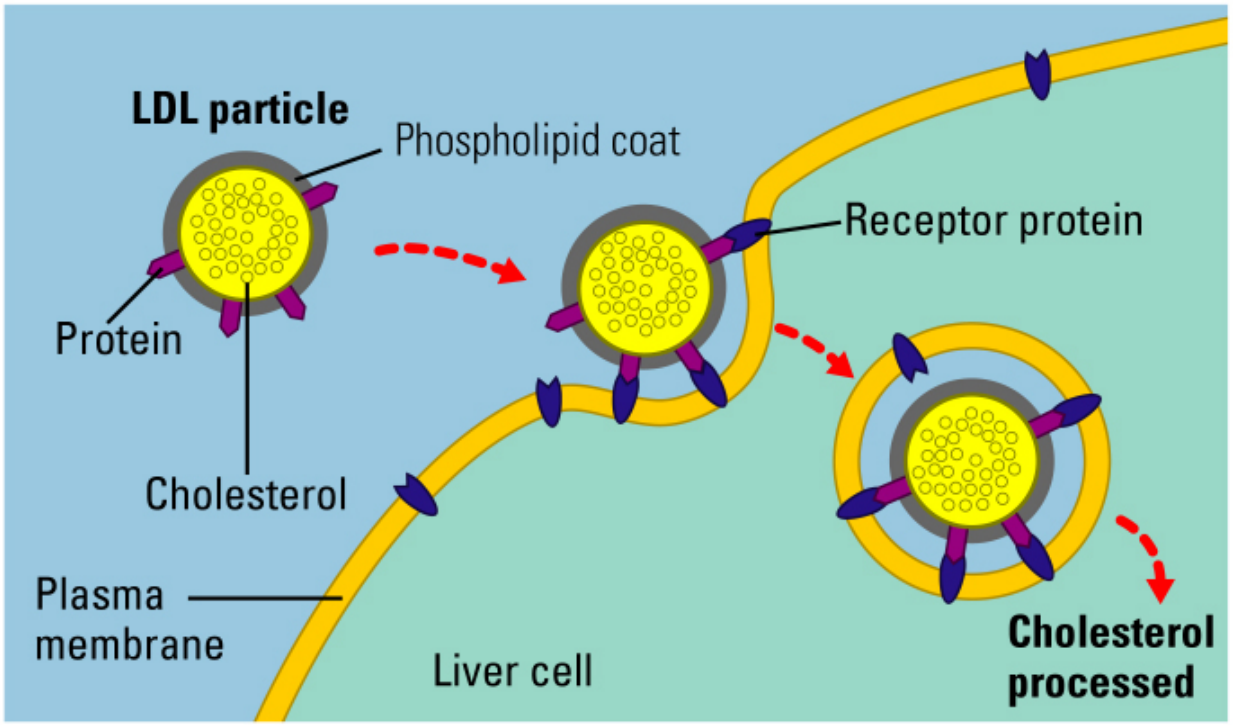
1. The cholesterol is transported in blood complexed to protein in low density lipoprotein (LDL) particles.

2. LDL binds to receptors on cell surface.

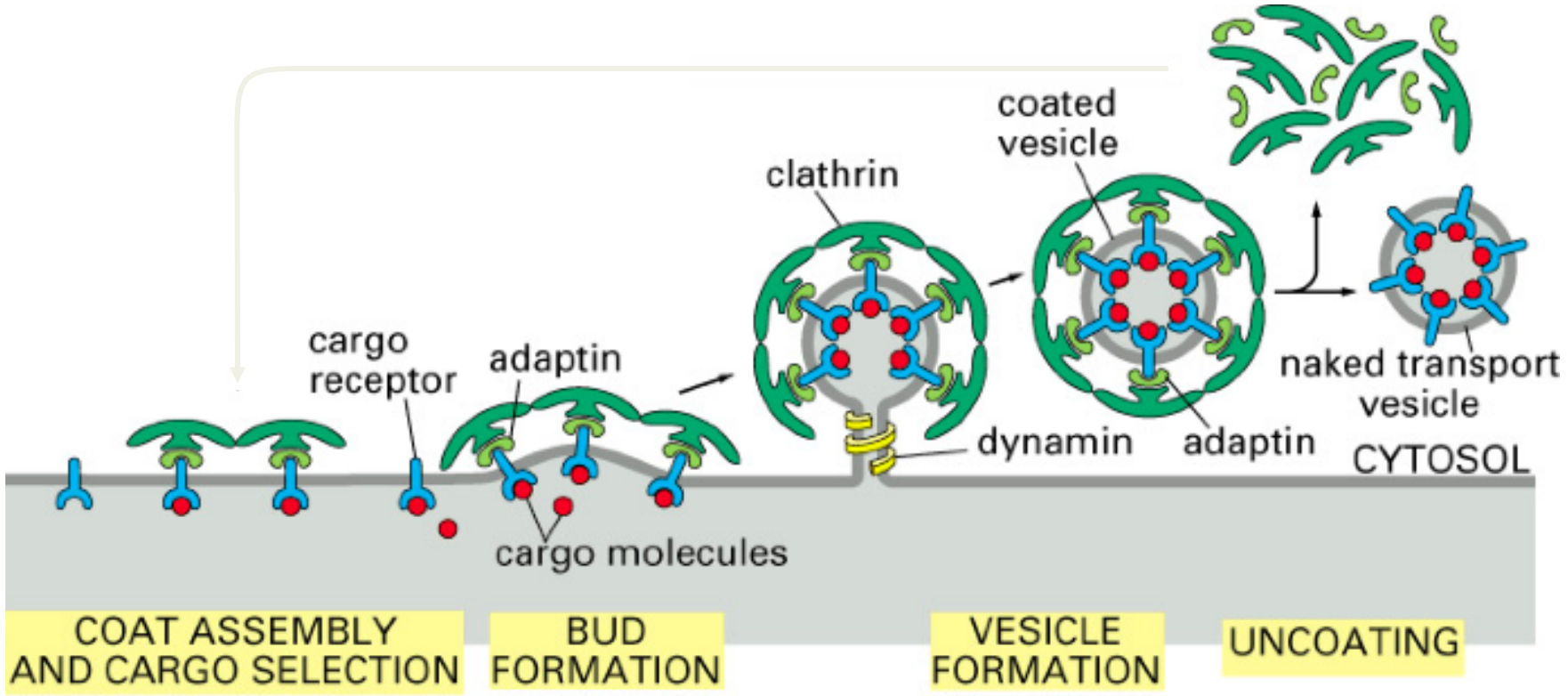
3. Complexes of LDL plus receptor are taken up by endocytosis and delivered to endosomes.

4. Within endosomes, LDL and receptor dissociate.

5. LDL is transferred to lysosome, degraded and cholesterol is released into cytosol.

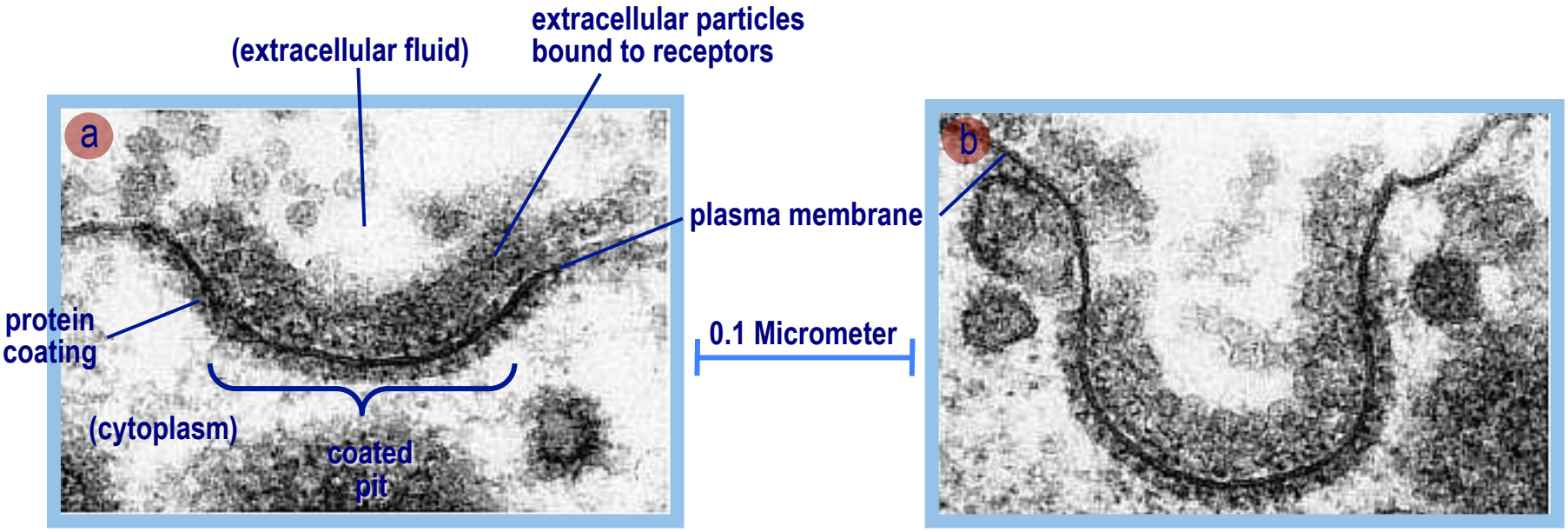


The assembly and disassembly of a clathrin coat



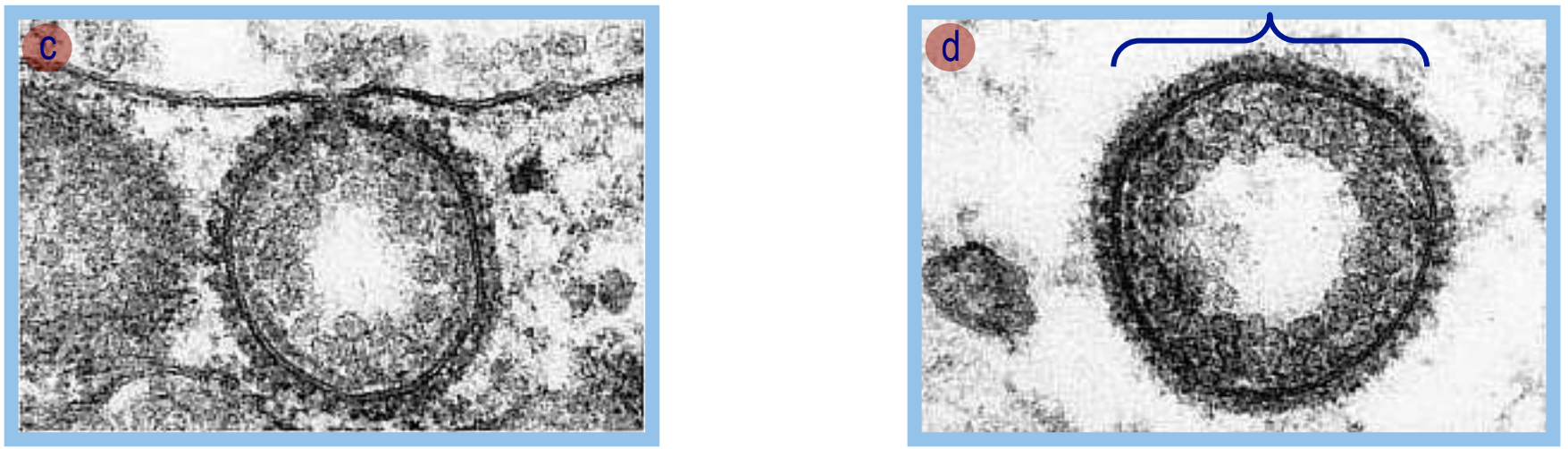
The assembly of the coat introduces curvature into the membrane, which leads in turn to the formation of uniformly sized coated buds.
The adaptins bind both clathrin triskelions and membrane-bound cargo receptors, thereby mediating the selective recruitment of both membrane and cargo molecules into the vesicle. The pinching-off of the bud to form a vesicle involves membrane fusion; this is helped by the GTP-binding protein dynamin, which assembles around the neck of the bud. The coat of clathrin-coated vesicles is rapidly removed shortly after the vesicle forms.

Receptor-mediated Endocytosis



Shallow pit in plasma membrane, coated with clathrin

Pit deepens



Pit deepens further and begins to pinch off

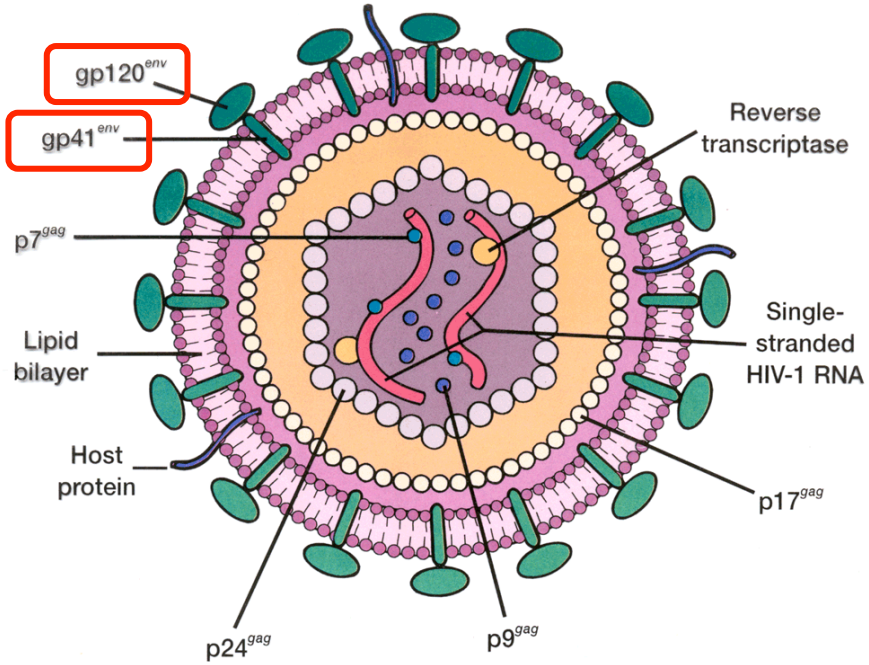
The pit becomes a coated vesicle

Molecular Biology of the Cell, Alberts, *et al*., 4th Ed., Fig. 13.41

Viral entry into cells by a mechanism of receptor-mediated endocytosis

In order for a virus to successfully infect a host cell, the cell must contain a specific receptor to which the virus binds in the process of initiating an infection. The part of the virus that binds to the receptor is called ligand. The ligand is on the capsid of naked viruses and on the envelope of enveloped viruses. Virus binding to a surface receptor is followed by entry into the cell by a mechanism of receptor-mediated endocytosis.

Human Immunodeficiency Virus (HIV)



The HIV possesses two envelope glycoproteins that serve as ligands: gp120 (1st ligand) binds CD4 (receptor) and gp41 (2nd ligand) binds CXCR4 or CCR5 (co-receptors).

Therefore, HIV can infect only human cells that express CD4 and CXCR4 or CCR5 receptors on their surface (predominantly T cells).

Human Immunodeficiency Virus (HIV)

In order for a virus to successfully replicate in a host cell, the host cell must contain a surface receptor for the virus, and must have a cellular machinery that is required for the viral replication.

If the virus successfully replicates in the host cell, the infection is productive and the host cell is said to be permissive for the virus.

When a cell lacks some components required for viral replication, the infection is abortive or non-productive and the host cell is considered to be non-permissive for the virus.

Apparently, the CD4 receptor on the surface of mouse Th cells is not permissive for HIV binding. Furthermore, heterologous expression of the human CD4 on mouse T cells enables HIV entry into the cells, but the cells are not permissive for viral replication.

SCID MICE

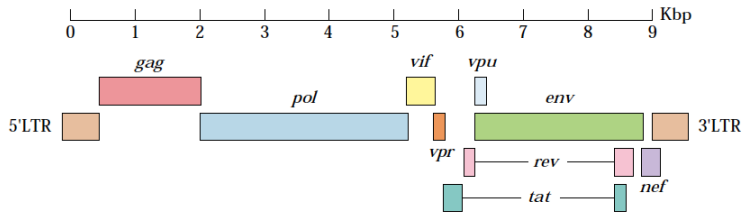
SCID mice are very useful in many types of studies, including the infection by HIV.

Immune precursor cells from human sources may be used to reestablish the SCID mouse's immune system. These human cells can develop in a normal fashion and, as a result, the SCID mouse circulation will contain T and B lymphocytes and immunoglobulins of human origin. In one important application, these SCID mice are infected with HIV-1.

Although normal mice are not susceptible to HIV-1 infection, the SCID mouse reconstituted with human lymphoid tissue (SCID-Hu mouse) provides an animal model in which to test therapeutic or prophylactic strategies against HIV infection of the transplanted human lymphoid tissue.

Genetic organization of HIV and functions of encoded proteins

(a)

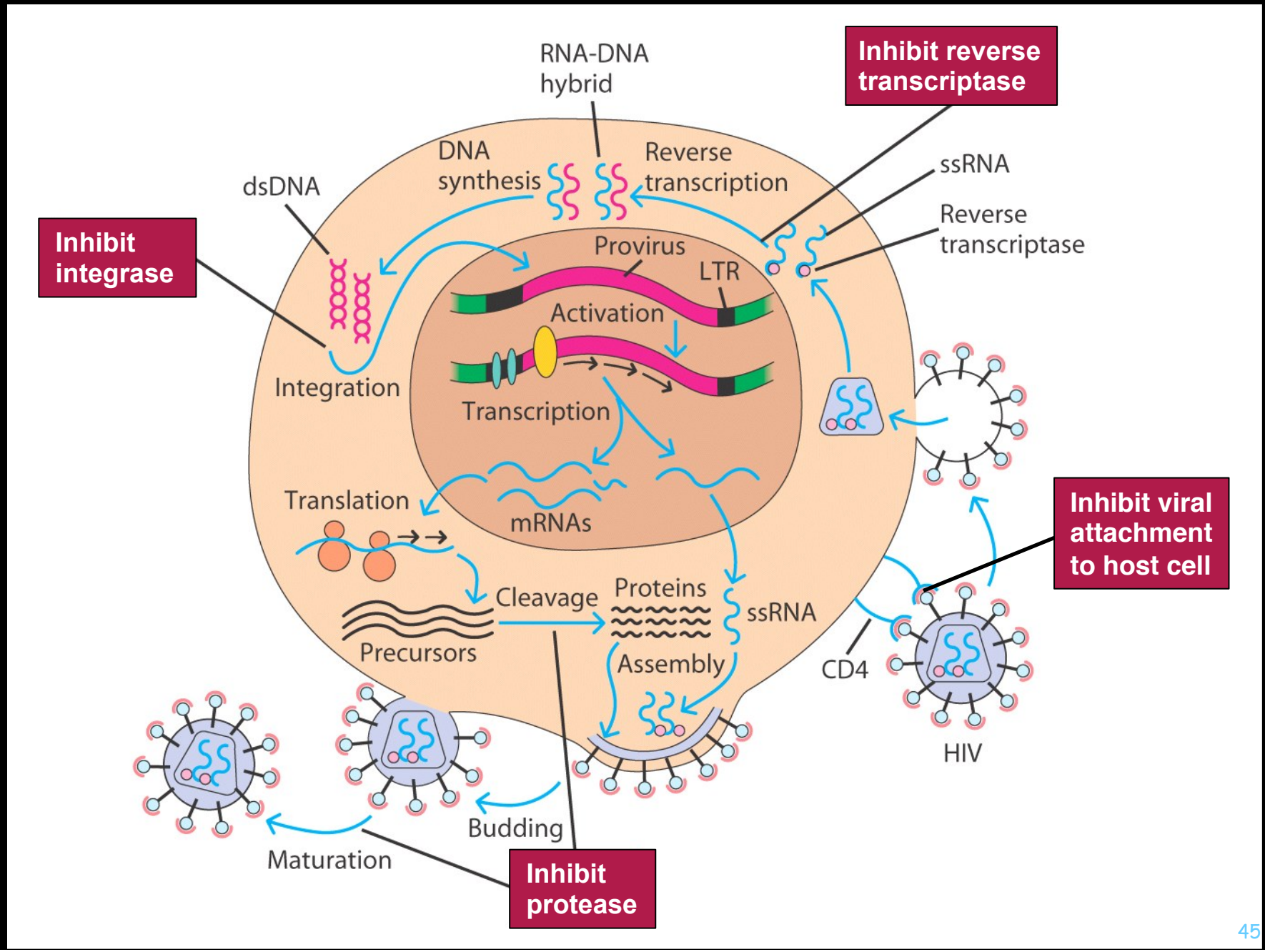


(b)

Gene	Protein product	Function of encoded proteins
gag	53-kDa precursor	*Nucleocapsid proteins*
	↓	
	p17	Forms outer core-protein layer
	p24	Forms inner core-protein layer
	p9	Is component of nucleoid core
	p7	Binds directly to genomic RNA
env	160-kDa precursor	*Envelope glycoproteins*
	↓	
	gp41	Is transmembrane protein associated with gp120 and required for fusion
	gp120	Protrudes from envelope and binds CD4
pol	Precursor	*Enzymes*
	↓	
	p64	Has reverse transcriptase and RNase activity
	p51	Has reverse transcriptase activity
	p10	Is protease that cleaves *gag* precursor
	p32	Is integrase
		Regulatory proteins
tat	p14	Strongly activates transcription of proviral DNA
rev	p19	Allows export of unspliced and singly spliced mRNAs from nucleus
		Auxiliary proteins
nef	p27	Down-regulates host-cell class I MHC and CD4
vpu	p16	Is required for efficient viral assembly and budding. Promotes extracellular release of viral particles, degrades CD4 in ER
vif	p23	Promotes maturation and infectivity of viral particle
vpr	p15	Promotes nuclear localization of preintegration complex, inhibits cell division

GURE 19-10 Genetic organization of HIV-1 (a) and functions of sion; two (*vif* and *vpu*) encode proteins required for virion matur

Strategies for inhibition of HIV infection and replication



Some anti-HIV drugs in clinical use

Generic name (other names)	Typical dosage	Some potential side effects
REVERSE TRANSCRIPTASE INHIBITORS: NUCLEOSIDE ANALOG		
Didanosine (Videx, ddI)	2 pills, 2 times a day on empty stomach	Nausea, diarrhea, pancreatic inflammation, peripheral neuropathy
Lamivudine (Epivir, 3TC)	1 pill, 2 times a day	Usually none
Stavudine (Zerit, d4T)	1 pill, 2 times a day	Peripheral neuropathy
Zalcitabine (HIVID, ddC)	1 pill, 3 times a day	Peripheral neuropathy, mouth inflammation, pancreatic inflammation
Zidovudine (Retrovir, AZT)	1 pill, 2 times a day	Nausea, headache, anemia, neutropenia (reduced levels of neutrophil white blood cells), weakness, insomnia
Pill containing lamivudine and zidovudine (Combivir)	1 pill, 2 times a day	Same as for zidovudine
REVERSE TRANSCRIPTASE INHIBITORS: NONNUCLEOSIDE ANALOGUES		
Delavirdine (Rescriptor)	4 pills, 3 times a day (mixed into water); not within an hour of antacids or didanosine	Rash, headache, hepatitis
Nevirapine (Viramune)	1 pill, 2 times a day	Rash, hepatitis
PROTEASE INHIBITORS		
Indinavir (Crixivan)	2 pills, 3 times a day on empty stomach or with a low-fat snack and not within 2 hours of didanosine	Kidney stones, nausea, headache, blurred vision, dizziness, rash, metallic taste in mouth, abnormal distribution of fat, elevated triglyceride and cholesterol levels, glucose intolerance
Nelfinavir (Viracept)	3 pills, 3 times a day with some food	Diarrhea, abnormal distribution of fat, elevated triglyceride and cholesterol levels, glucose intolerance
Ritonavir (Norvir)	6 pills, 2 times a day (or 4 pills, 2 times a day if taken with saquinavir) with food and not within 2 hours of didanosine	Nausea, vomiting, diarrhea, abdominal pain, headache, prickling sensation in skin, hepatitis, weakness, abnormal distribution of fat, elevated triglyceride and cholesterol levels, glucose intolerance
Saquinavir (Invirase, a hard-gel capsule; Fortovase, a soft-gel capsule)	6 pills, 3 times a day (or 2 pills, 2 times a day if taken with ritonavir) with a large meal	Nausea, diarrhea, headache, abnormal distribution of fat, elevated triglyceride and cholesterol levels, glucose intolerance

SOURCE: J. G. Bartlett and R. D. Moore, 1998, Improving HIV therapy, *Sci. Am.* 279(1):87.

Vaccine development against HIV is difficult to make:

- Unlike other common vaccines, an HIV vaccine cannot consist of attenuated, actively replicating (live) HIV, due to possible reactivation.

- Killed whole virus (like the polio vaccine) might also be dangerous because some viral particles may still remain alive. Furthermore, a killed whole virus vaccine worked poorly in animal studies.

- Classical vaccines mimic natural immunity. They are efficient against reinfection and are observed in recovered individuals. However, there are no individuals who recovered from AIDS.

- Most vaccines provide long-term protection from viruses that change very little over time. The HIV mutates at a very rapid rate and therefore newly formed mutants can successfully evade immunity.

- We have never before attempted to develop a vaccine against a retrovirus like HIV. Retroviruses, by integrating their genome into ours, are able to hide completely from immune surveillance within quiescent lymphocytes. This means that any vaccine would have a small 'window' of opportunity to prevent infection and would have to be extremely effective, repelling all attempts by HIV to attach to and infect host cells.

False-colored image of two HIV virus particles budding from a cultured human T-cel (green). Image acquired with a TEM at 20,000x magnification. The mature particle (right) has a condensed core inside the virus shell, whereas the capsid protein is still associated with the viral membrane in the immature particle (left).