Defense Against Viruses/AIDS

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Micro 204, 11/30/18

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Recommended Reading:

Textbook:

- Janeway chapter 13.22-13.38.

Prof. V. Racaniello online material (Columbia University virology course)

- http://www.virology.ws/course/

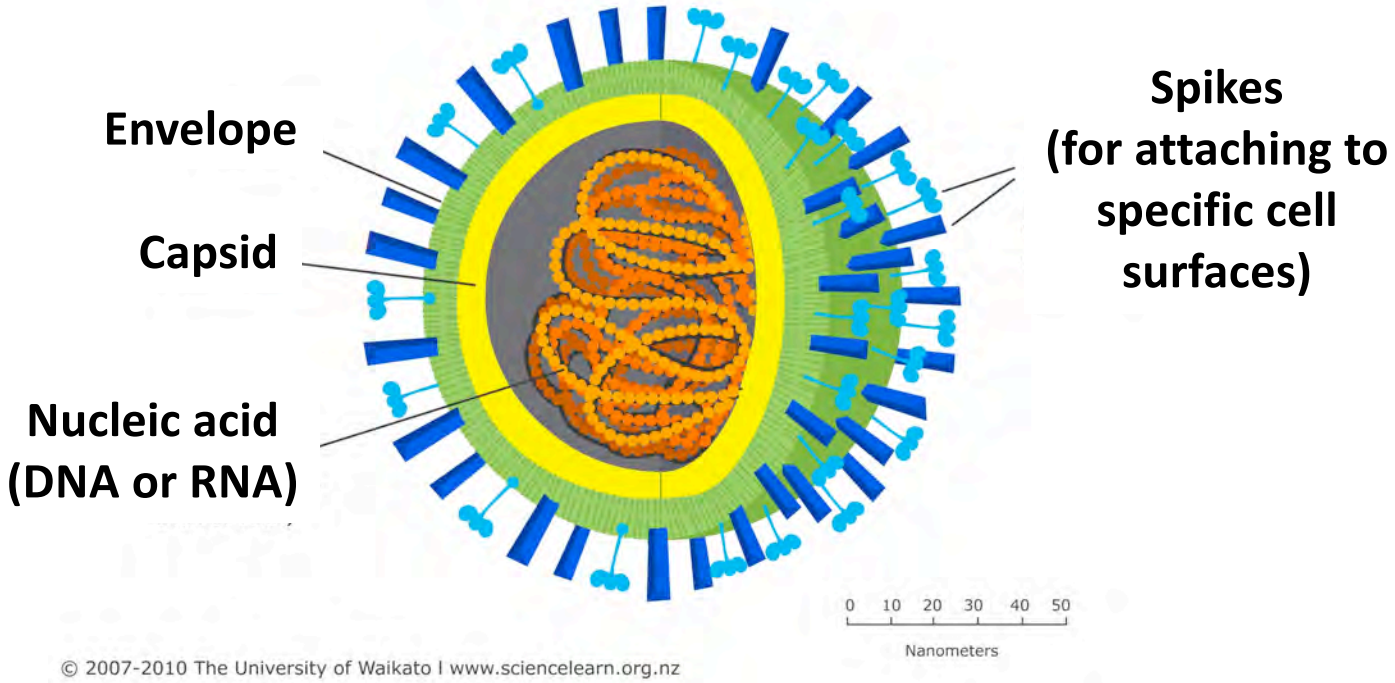
Reviews:

- Paludan SR. Innate antiviral defenses independent of inducible IFNα/β production. (2016) ***Trends in Immunology*** 37: 588
- Deeks et al. International AIDS Society global scientific strategy: towards an HIV cure. (2016) ***Nature Medicine*** 22: 839.
- Sengupta and Siliciano. Targeting the Latent Reservoir for HIV-1. (2018) ***Immunity*** 48: 872.
- Elong Ngono and Shresta. Immune Response to Dengue and Zika. (2018) ***Annul Review of Immunology*** 36: 279.

Primary literature:

- Yuan et al. A single mutation in the prM protein of Zika virus contributes to fetal microcephaly. (2017) ***Science*** 358: 933.
- Estes et al. Defining total-body AIDS-virus burden with implications for curative strategies. (2017) ***Nature Medicine*** 23: 1271.
- Buggert et al. Identification and characterization of HIV-specific resident memory CD8+ T cells in human lymphoid tissue. (2018) ***Science Immunology*** 3, eaar4526

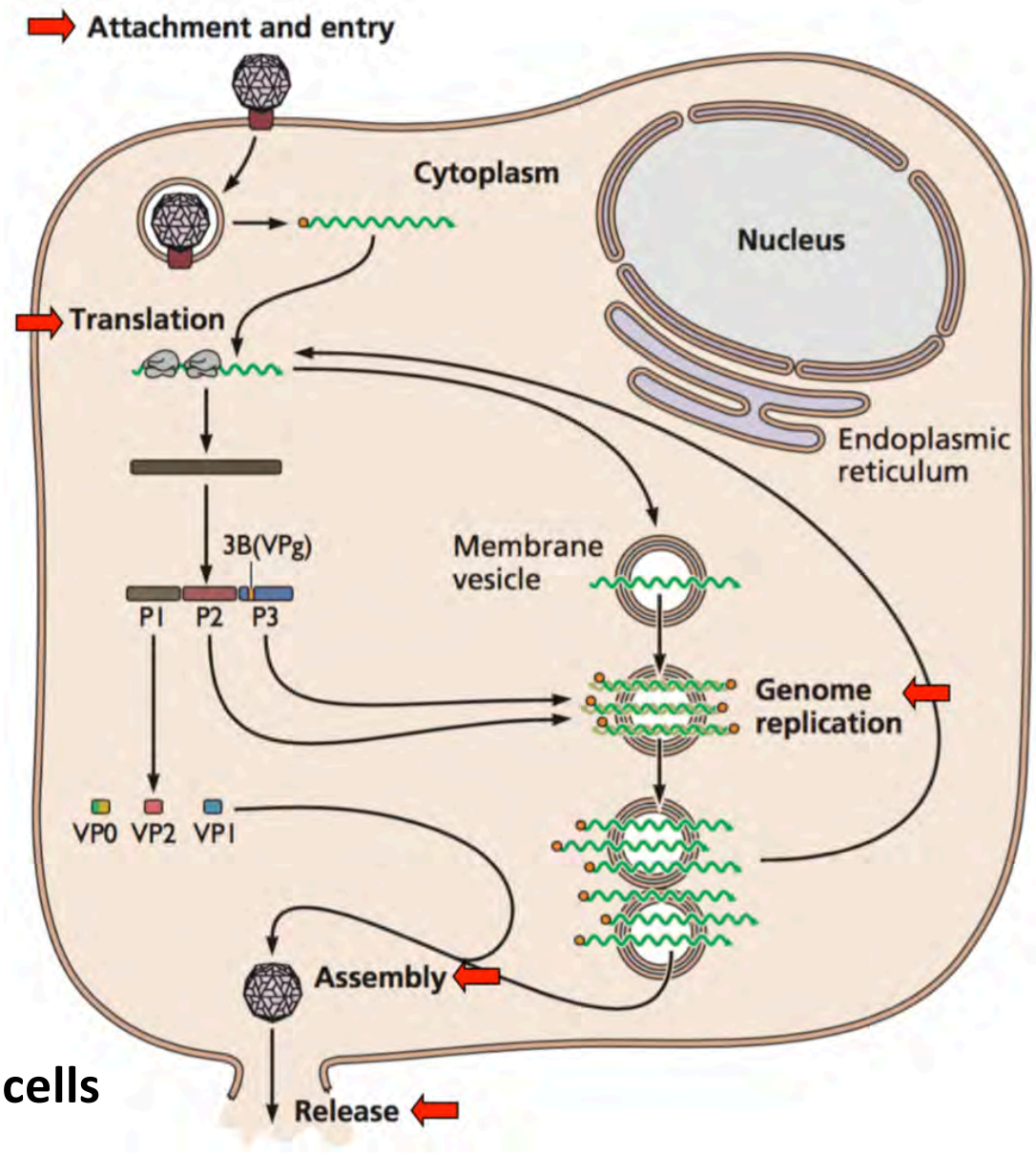
What is a virus?



- An infectious, obligate intracellular parasite comprising genetic material surrounded by a protein coat and/or an envelope derived from a host cell membrane.
- They are classified based on:
  - Nature and sequence of nucleic acid in virion (RNA/DNA)
  - Symmetry of protein shell (capsid)
  - Presence or absence of lipid membrane (+/- envelope)
  - Dimensions of virion and capsid

Generic Viral Life Cycle

1. Attachment and entry (cellular receptors)
2. Convert genome to mRNA
3. Translate mRNA to protein
4. Replicate genome
5. Assemble proteins around genome
6. Viral release from infected cell



Cytopathic effect (CPE):
Morphological alterations in infected cells caused by viral invasion.

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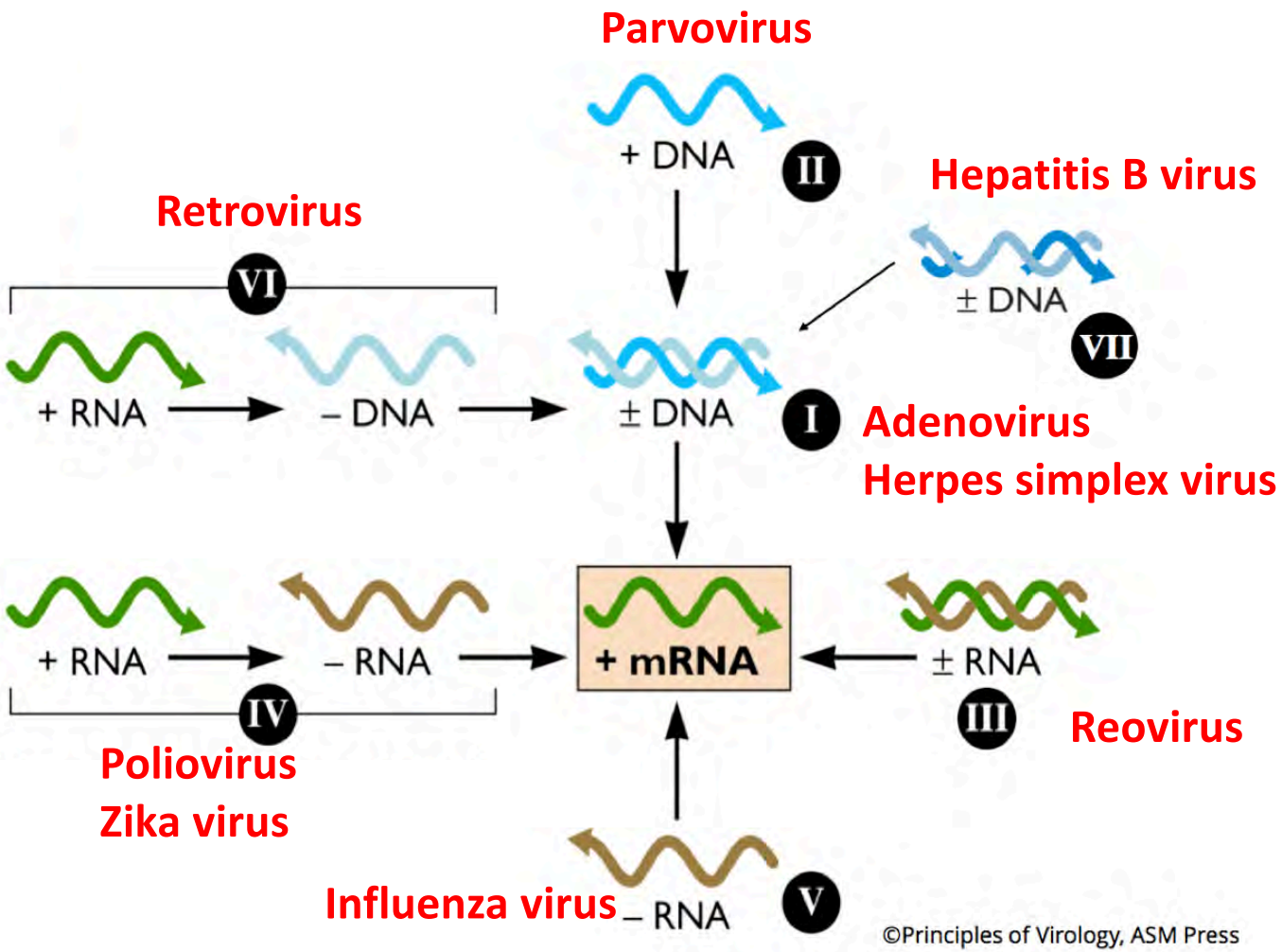
Baltimore System to Describe Viral Genomes

I. dsDNA
II. ssDNA
III. dsRNA
IV. ss (+) RNA
V. ss (-) RNA
VI. ss (+) RNA with DNA intermediate
VII. Gapped dsDNA

They all have to:
1. Make mRNA
2. Use host ribosomes to translate mRNA → protein



- **Replicating viruses produce large numbers of mutant genomes!**
- **Most DNA viruses evolve slower than RNA viruses as their genome replication is not as error prone as RNA viruses.**

Fundamental Questions of Viral Pathogenesis

- How does a virus particle enter the host? (transmission)
- What is the initial host response? (innate sensing)
- Where does primary replication occur? (tropism)
- How does the infection spread in the host? (viral dissemination)
- What organs and tissues are infected? (local vs. systemic infection)
- Is the infection cleared from the host or is a persistent infection established? (acute vs. chronic)
- How is the virus transmitted to other hosts? (viral shedding)

Viral Tropism is Determined by Susceptibility, Permissivity, Accessibility and Defense

- **A susceptible cell has a functional receptor for a given virus - *the cell may or may not be able to support viral replication***
- **A resistant cell has no receptor - *it may or may not be competent to support viral replication***
- **A permissive cell has the capacity to replicate virus - *it may or may not be susceptible***
- **A susceptible AND permissive cell is the only cell that can take up a virus particle and replicate it**

Two Components to Viral Pathogenesis

1. **Effects of viral replication on the host**

   → **Cytopathic vs. non-cytopathic effect**

2. **Effects of host response on virus and host**

   → **Immune protection vs. Immunopathology**

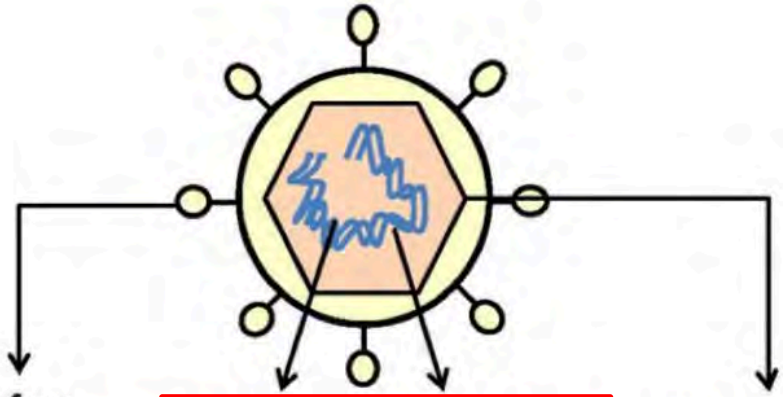
<u>Viral virulence is influenced by:</u>

- **Viral dose**
- **Route of infection**
- **Species**
- **Age**
- **Gender**
- **Susceptibility of host**

<u>3 Requirements for successful infection:</u>

- **Enough virus**
- **Cells accessible, susceptible, and permissive**
- **Local antiviral defense is absent or overcome**

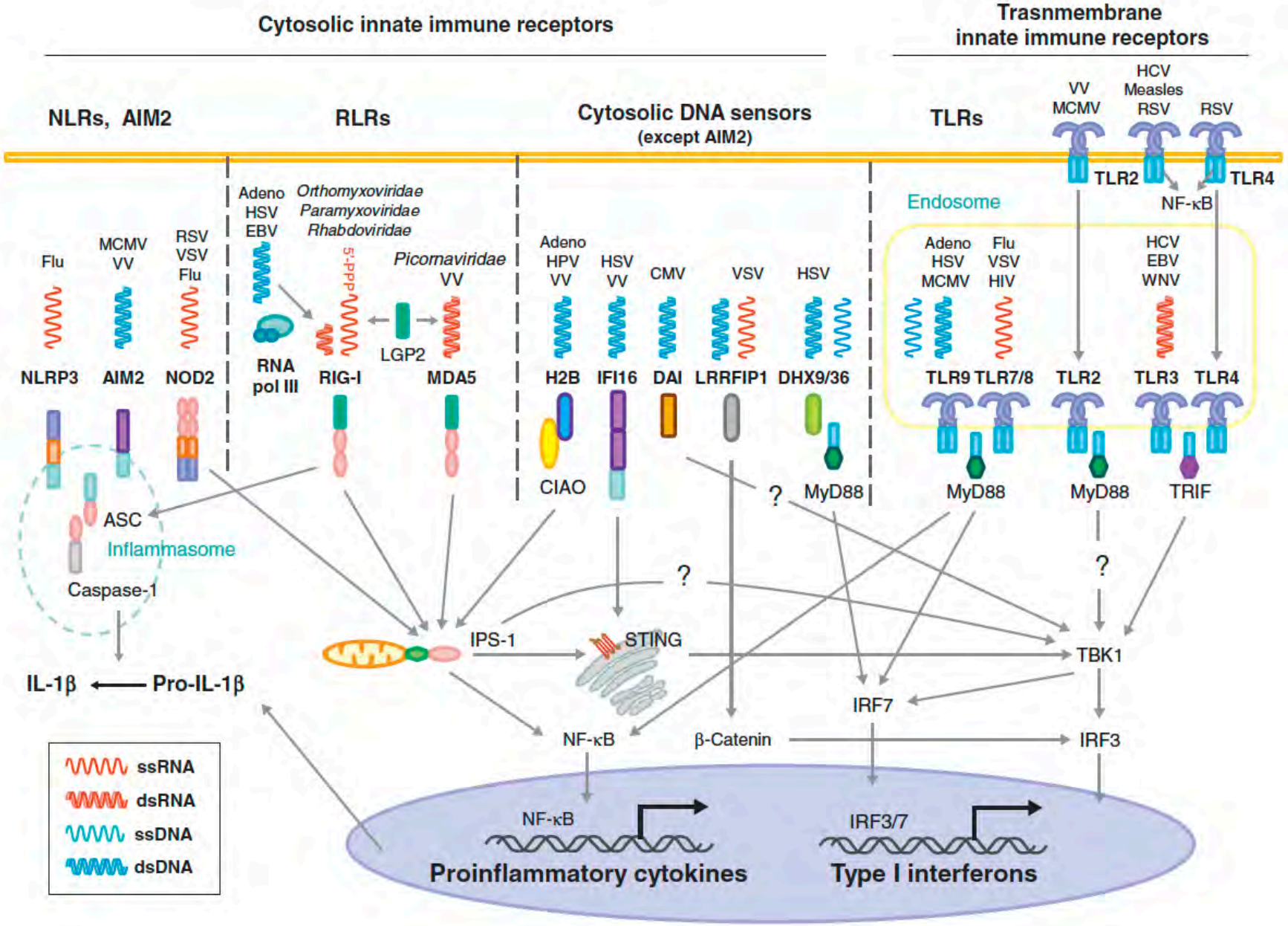
Immune Defense Begins by Viral Sensing



TLRs:	TLR2 TLR4	TLR9	TLR3 TLR7/8	-
RLRs:	-	-	MDA5 RIG-I LPG2	-
NLRs:	-		NLRP3 NOD2	-
ALRs:	-	AIM2 IFI16	-	-
Others:	-	DHX9 DHX36 Ku70 RNA pol III LRRFIP1 DDX41	LRRFIP1 PKR IFIT5	TRIM5 Cyclophilin A

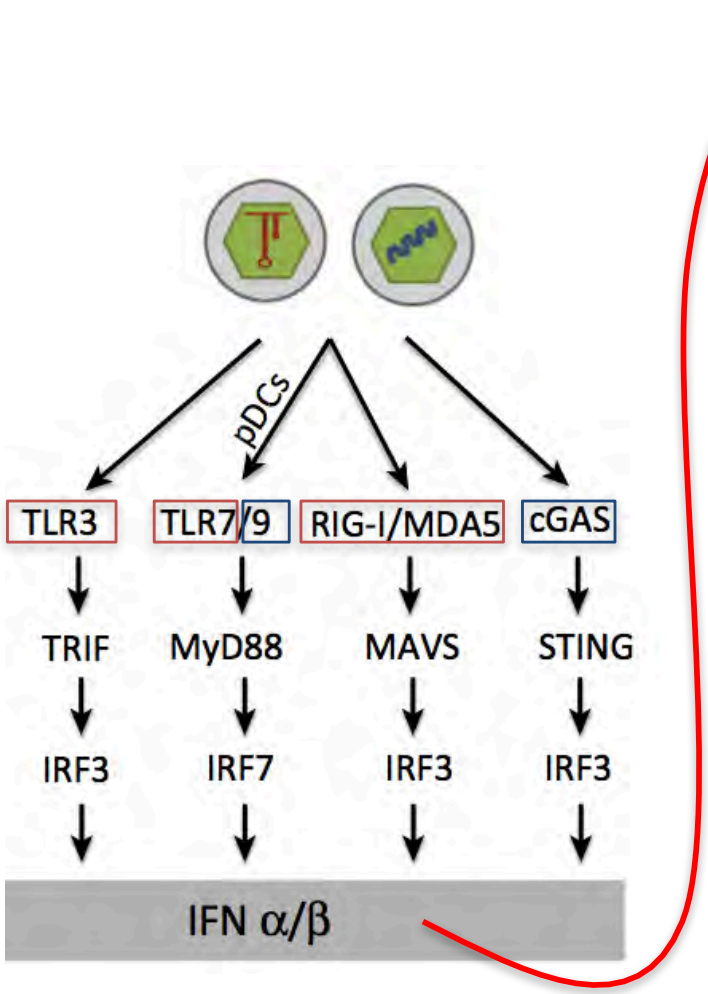
Melchjorsen 2013 ***Viruses***

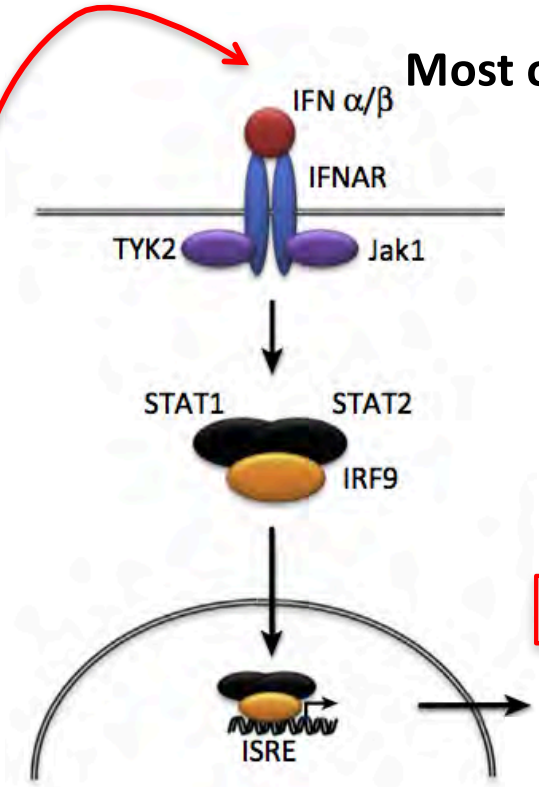
Specificity of Innate Immune Receptors for Viral Nucleic Acid



Aoshi et al. 2011 ***Current Opinion in Virology***

Viral Sensing Initiates Type I IFN Response





Most of our cells have IFN receptors

→ Antiviral activity to block infection!

IFN stimulated genes
(ISGs = IRF7, RIG-I, MDA5)

- Restriction of viral replication
- Recruitment of leukocytes
- Activation of leukocyte activities
- Priming of inflammatory responses
- Antigen cross-priming
- T cell proliferation

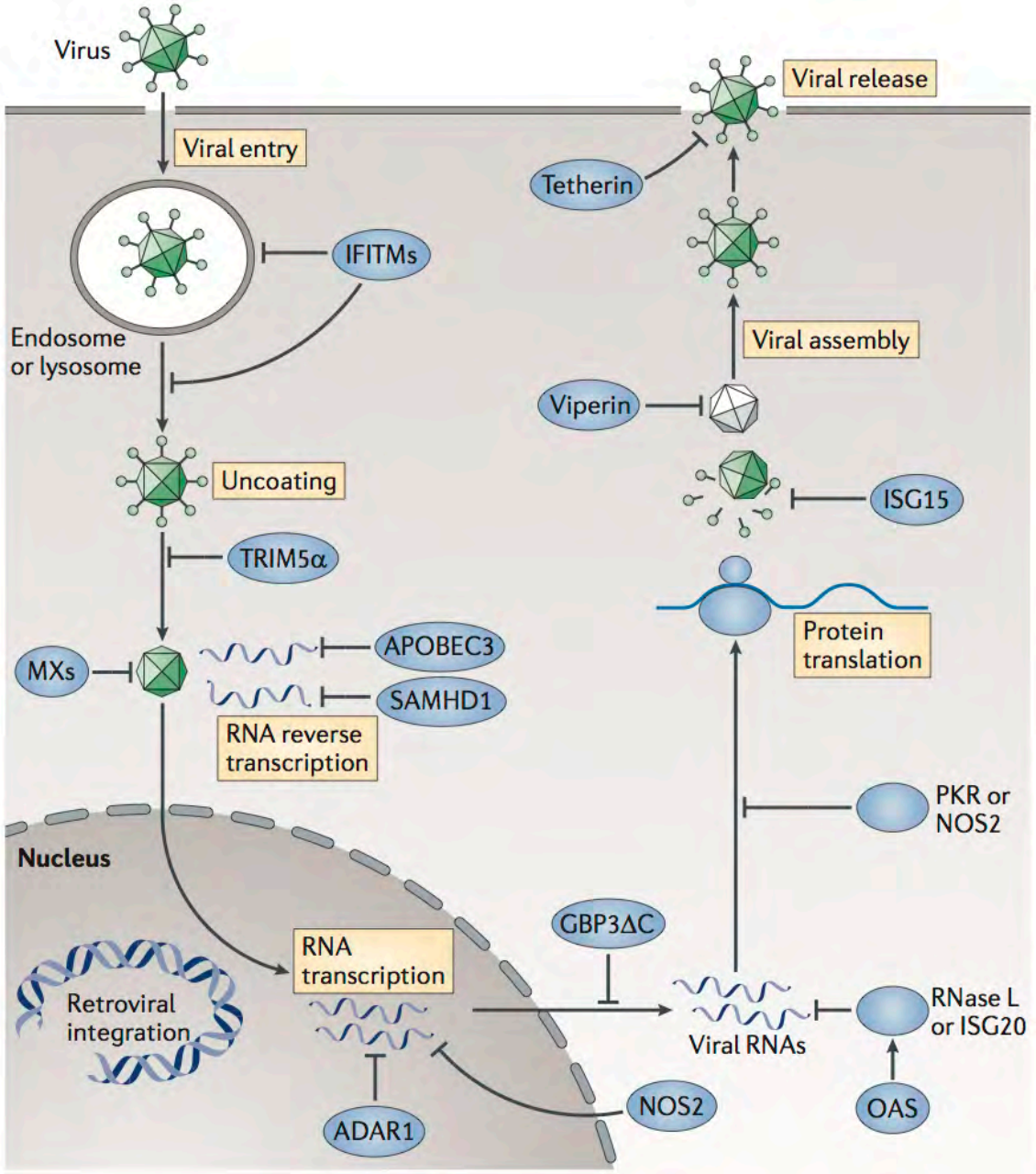
Large quantities of IFN have dramatic physiological consequences: fever, chills, nausea, malaise

→ 'flu-like' symptoms

Paludan 2016, ***Trends in Immunology***

Cell-autonomous Mechanisms Used by IFN-Induced Proteins Against Viruses

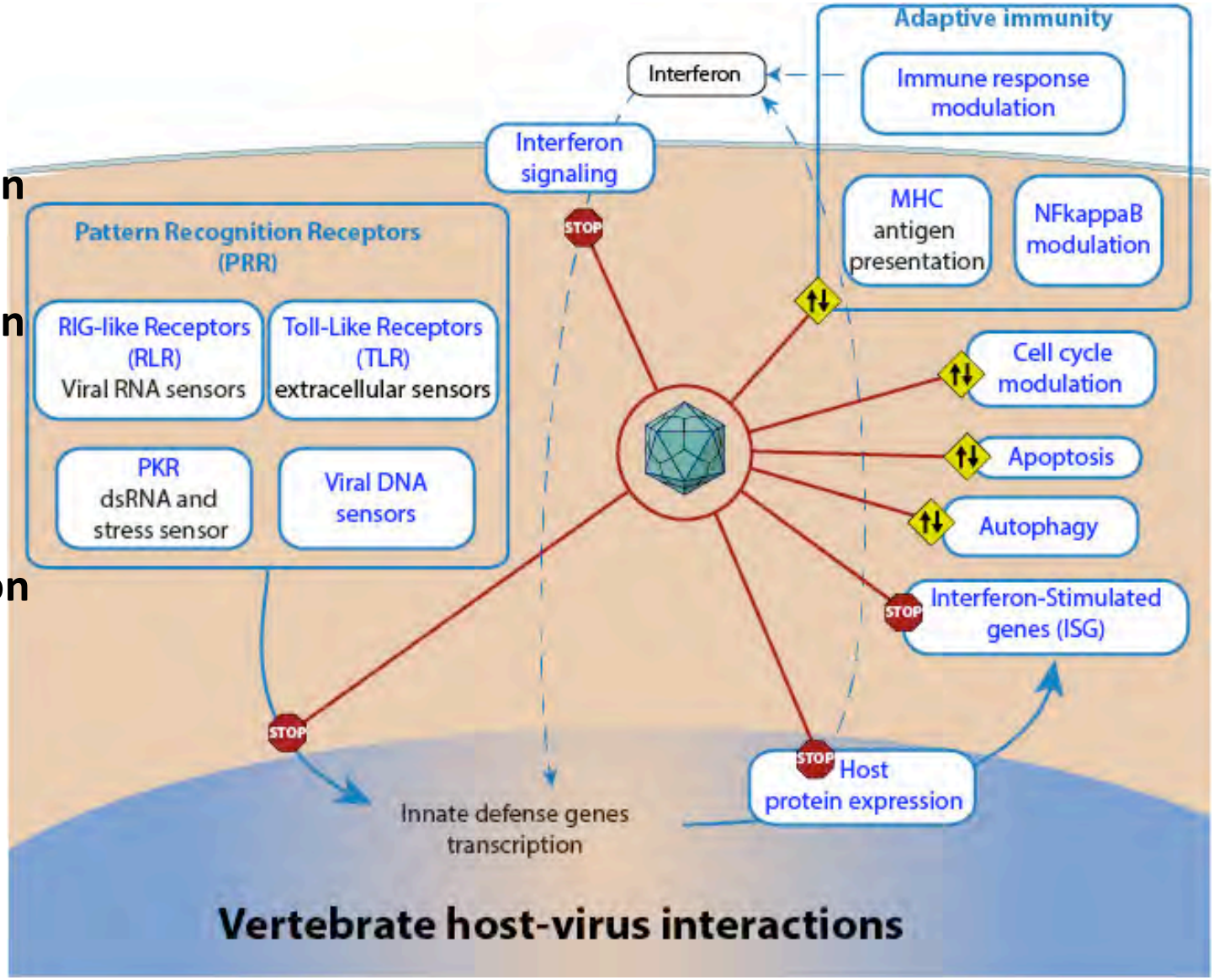
- **Viral entry and uncoating:**
  IFITMs, TRIM5α
- **Nucleocapsid transport:**
  MXs
- **Inhibition of RNA reverse transcription, protein translation and stability**
  APOBEC3, SAMHD1, ADAR1, NOS2, OAS, RNase L, ISG20, PKR, and ISG15
- **Prevent viral assembly and release**
  Viperin and Tetherin



MacMicking 2012 ***Nat Rev Immunol***

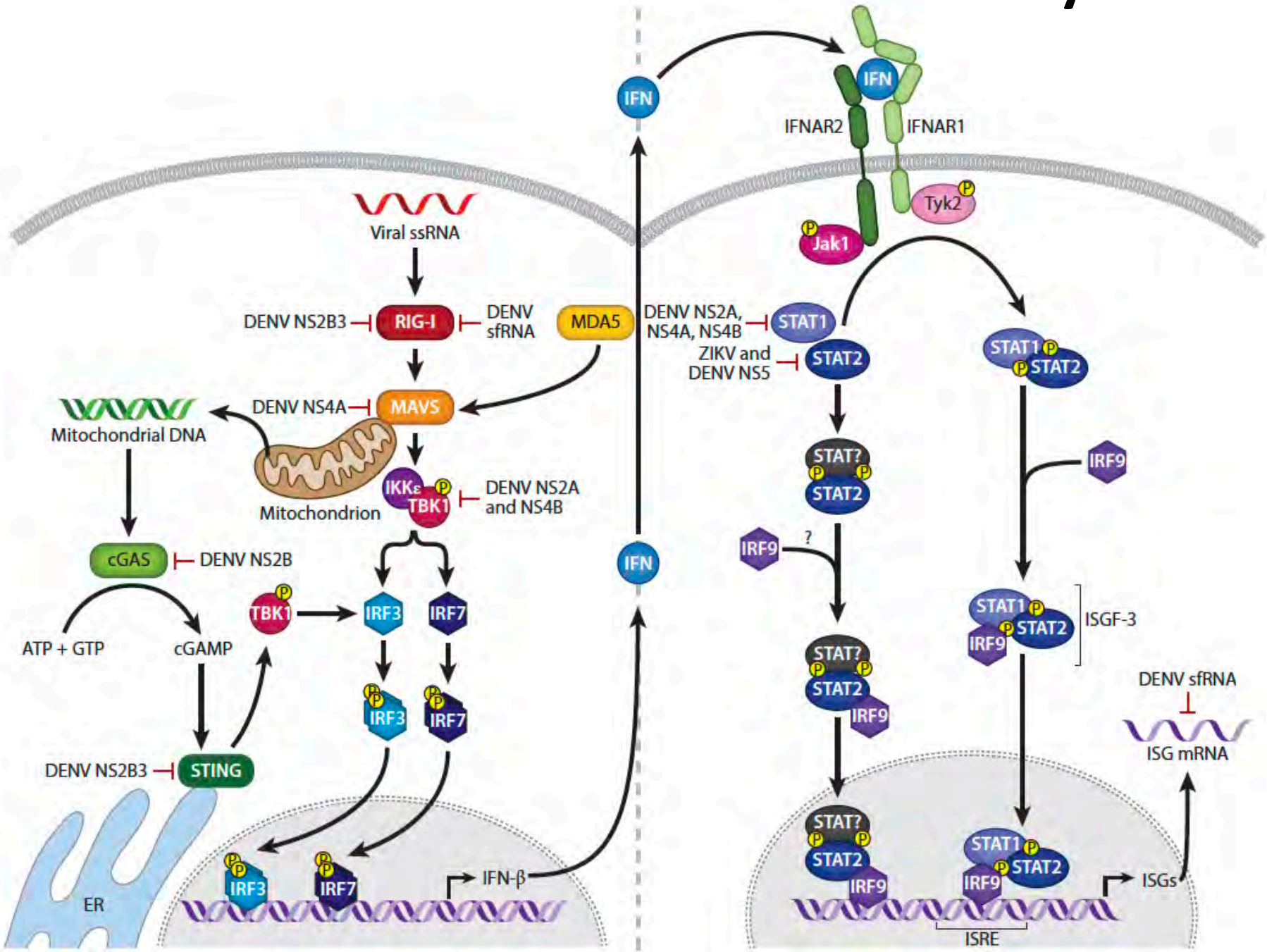
Viral Countermeasures Against Innate and Adaptive Immunity

- Sensing
- IFN production
- IFN signal transduction
- Cytokine production
- Chemokine production
- NK cell activation
- DC antigen presentation
- Complement inhibition



http://viralzone.expasy.org/all_by_protein/886.html

Flaviviral Inhibition of the Interferon System



Elong Ngono and Shresta 2018 ***Annu Rev Immunol***

Many Viruses Interfere with Antigen Presentation (Reason cross-presentation is important!)

Virus	Protein	Category	Mechanism
Herpes simplex virus 1	ICP47	Blocks peptide entry to endoplasmic reticulum	Blocks peptide binding to TAP
Human cytomegalovirus (HCMV)	US6		Inhibits TAP ATPase activity and blocks peptide release into endoplasmic reticulum
Bovine herpes virus	UL49.5		Inhibits TAP peptide transport
Adenovirus	E19	Retention of MHC class I in endoplasmic reticulum	Competitive inhibitor of tapasin
HCMV	US3		Blocks tapasin function
Murine cytomegalovirus (CMV)	m152		Downregulation of host MHC class I
HCMV	US2	Degradation of MHC class I (dislocation)	Transports some newly synthesized MHC class I molecules into cytosol
Murine gamma herpes virus 68	mK3		E3-ubiquitin ligase activity
Murine CMV	m4	Binds MHC class I at cell surface	Interferes with recognition by cytotoxic lymphocytes by an unknown mechanism

Figure 13.24 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Mechanisms to Subvert the Host Immune System (Reason we study viral virulence factors!)

Viral strategy	Specific mechanism	Result	Virus examples
Inhibition of humoral immunity	Virally encoded Fc receptor	Blocks effector functions of antibodies bound to infected cells	Herpes simplex Cytomegalovirus
	Virally encoded complement receptor	Blocks complement-mediated effector pathways	Herpes simplex
	Virally encoded complement control protein	Inhibits complement activation by infected cell	Vaccinia
Inhibition of inflammatory response	Virally encoded chemokine receptor homolog, e.g., β-chemokine receptor	Sensitizes infected cells to effects of β-chemokine; advantage to virus unknown	Cytomegalovirus
	Virally encoded soluble cytokine receptor, e.g., IL-1 receptor homolog, TNF receptor homolog, interferon-γ receptor homolog	Blocks effects of cytokines by inhibiting their interaction with host receptors	Vaccinia Rabbit myxoma virus
	Viral inhibition of adhesion molecule expression, e.g., LFA-3 ICAM-1	Blocks adhesion of lymphocytes to infected cells	Epstein–Barr virus
	Protection from NFκB activation by short sequences that mimic TLRs	Blocks inflammatory responses elicited by IL-1 or bacterial pathogens	Vaccinia
Blocking of antigen processing and presentation	Inhibition of MHC class I expression	Impairs recognition of infected cells by cytotoxic T cells	Herpes simplex Cytomegalovirus
	Inhibition of peptide transport by TAP	Blocks peptide association with MHC class I	Herpes simplex
Immunosuppression of host	Virally encoded cytokine homolog of IL-10	Inhibits T_H1 lymphocytes Reduces interferon-γ production	Epstein–Barr virus

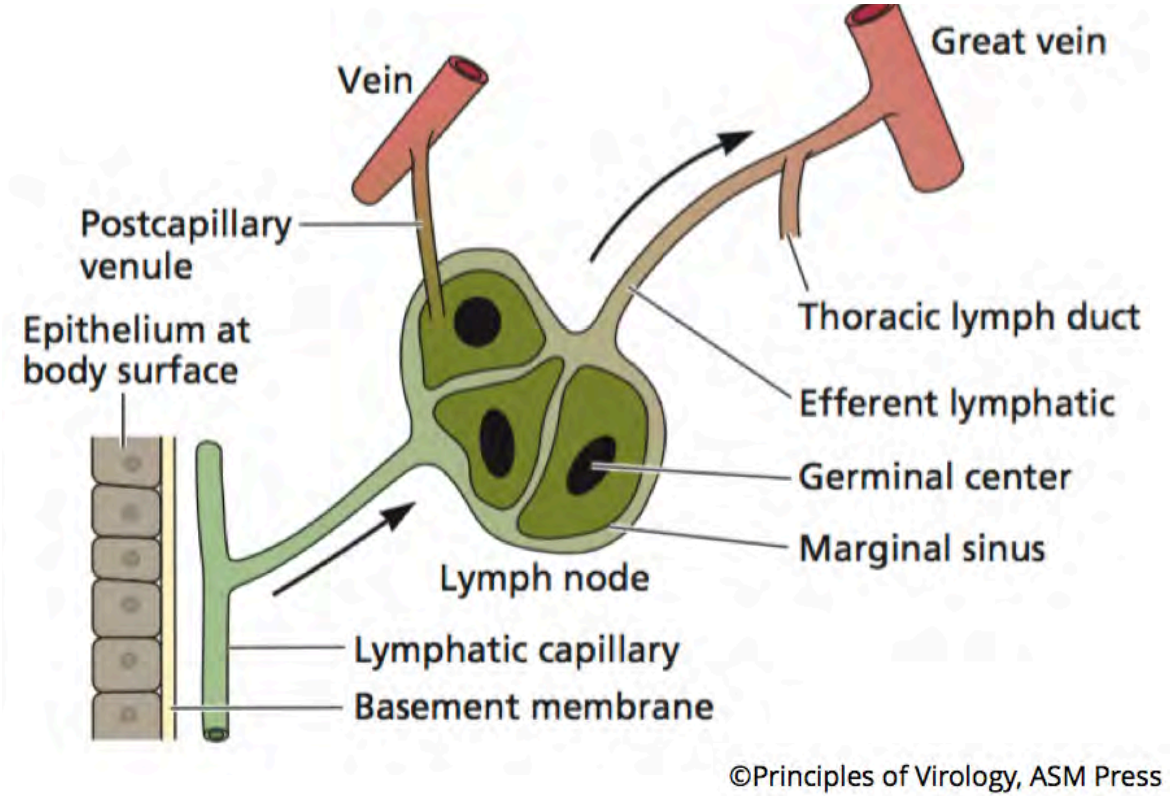
Figure 13.23 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Take Home Messages I:

- **Virus transmits through accessible (route matters), susceptible (cellular receptors) and permissive (cell has the right machinery) cells.**
- **Virus encounters host innate sensors.**
- **Type I IFN is produced.**
- **ISGs have antiviral and inflammatory activity.**
- **Virus counteracts innate and adaptive immunity.**

Localized Control versus Systemic Dissemination of Viruses

- If immune system prevails, viruses will remain localized.
- If physical and immune barriers are breached, virus will disseminate by spreading beyond the primary site of infection.
- If many organs are infected, virus has gone systemic.
- If virus enters blood system, it will hematogenously spread to other organs.



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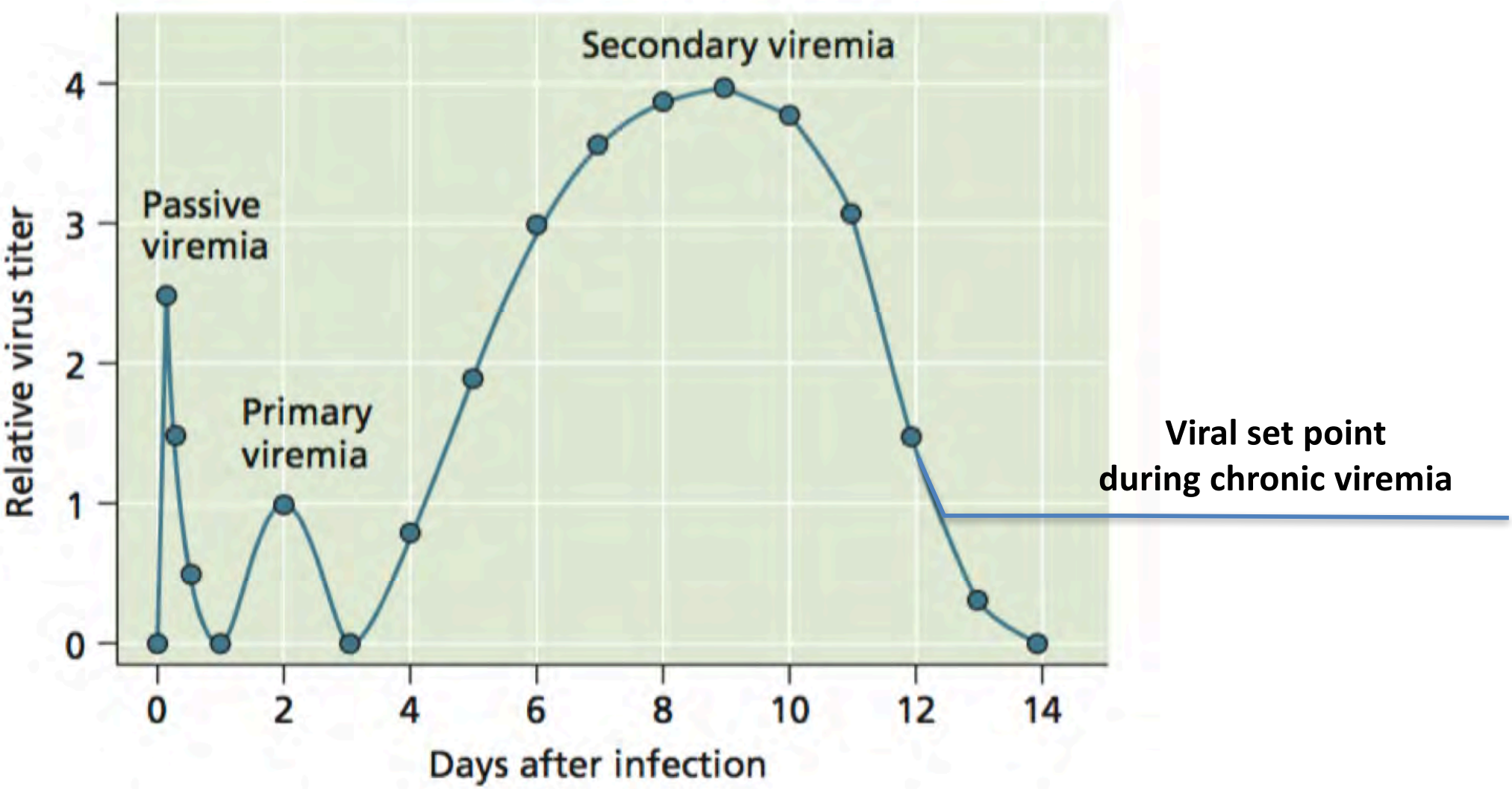
- Important to study viral pathogenesis via natural routes of transmission!

Acute vs Chronic Viremia



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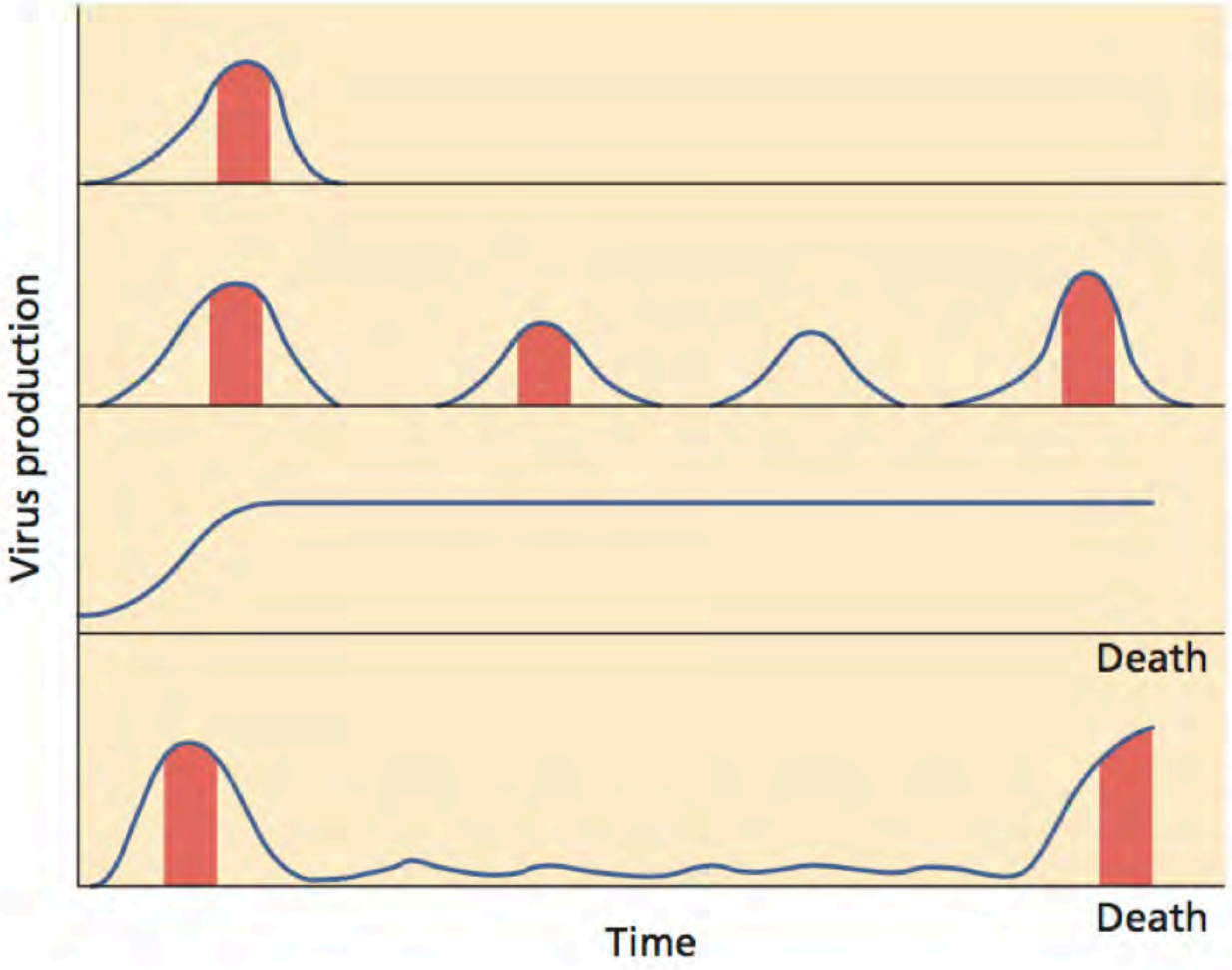
General Patterns of Viral Infection



Acute
- Rhinovirus
- Rotavirus
- Influenza virus

* LCMV (Armstrong)

Latent
- Herpes simplex virus

Persistent: asymptomatic

* LCMV (Clone 13)

* SIV-infected sooty mangabey

Persistent: pathogenic
- Human immunodeficiency virus
- Human T-lymphotropic virus
- Measles virus SSPE

* SIV-infected rhesus macaques

What are some of the reasons for these various patterns of viral infection?

Principles of Virology, ASM Press

Cytopathic and Non-cytopathic Viruses Elicit Different Immune Responses

Cytopathic viruses

- **Cause high inflammation due to cell and tissue damage**
  - **Activate the innate response**

Non-cytopathic viruses

- **Typically cause low inflammation due to lack of cellular damage, apoptosis/necrosis**
  - **Low or ineffective innate immune response**
  - **Do not effectively activate adaptive immune response**
- **Dramatically different interactions with the host immune system**
  - **Persistent infections: rarely or inefficiently cleared**
- **Disease is usually a consequence of the immune response**
  - **Immunopathology**

Persistent (Chronic) Infections

- Occur when primary infection is not cleared by immune response
- Virions, protein, genomes continue to be produced
- Viral genomes may remain after proteins are not detected
- No single mechanism:
  - When cytopathic effects are absent and host defenses are reduced, persistent infection is likely
  - Reduced immune surveillance in immune privileged sites (CNS, eye…)
  - Viral immune modulation → immunosuppression
  - Latent infections → poor recognition by immune system

Immunopathology Caused by Antiviral Immunity

Mechanism	Virus
$CD8^+$ T cell mediated	Coxsackievirus B HIV-1 Hepatitis B virus
$CD4^+$ T cell mediated - Th1	Measles virus Herpes simplex virus
$CD4^+$ T cell mediated - Th2	Respiratory syncytial virus
B cell mediated (antibody)	Dengue virus

Principles of Virology, ASM Press

Viral Infection can be Blocked by Neutralizing Abs

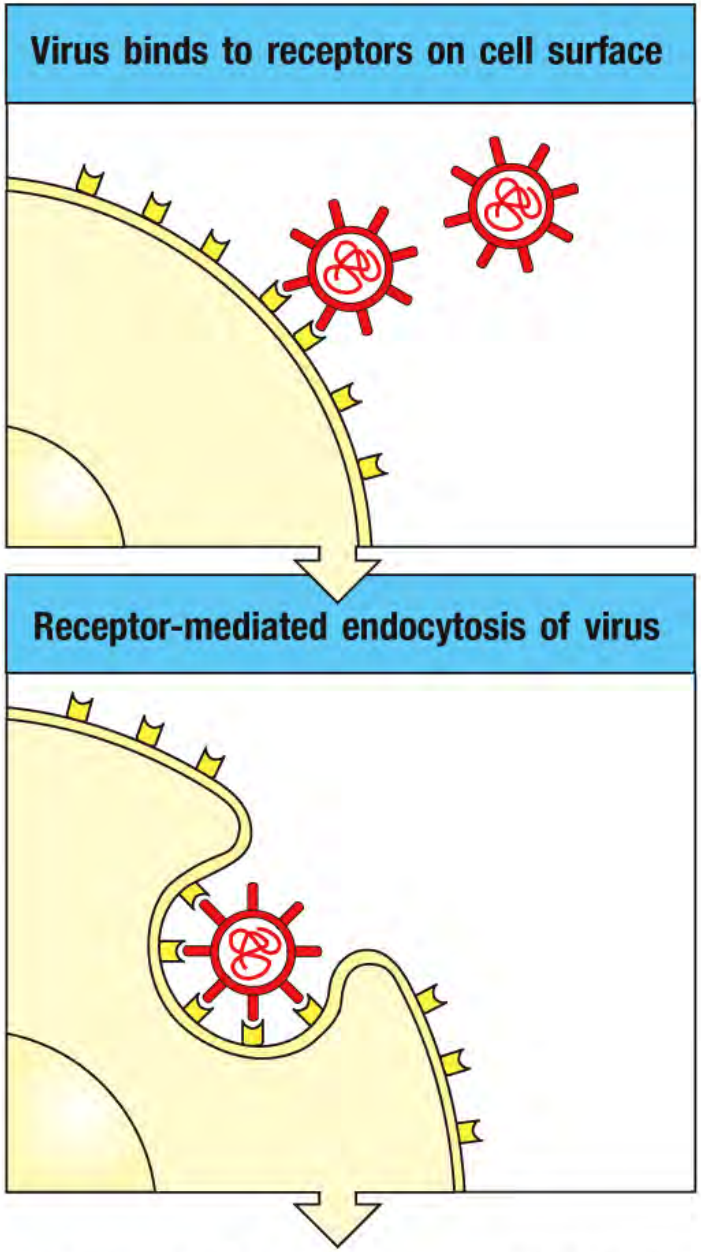


Figure 10.33 (part 1 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

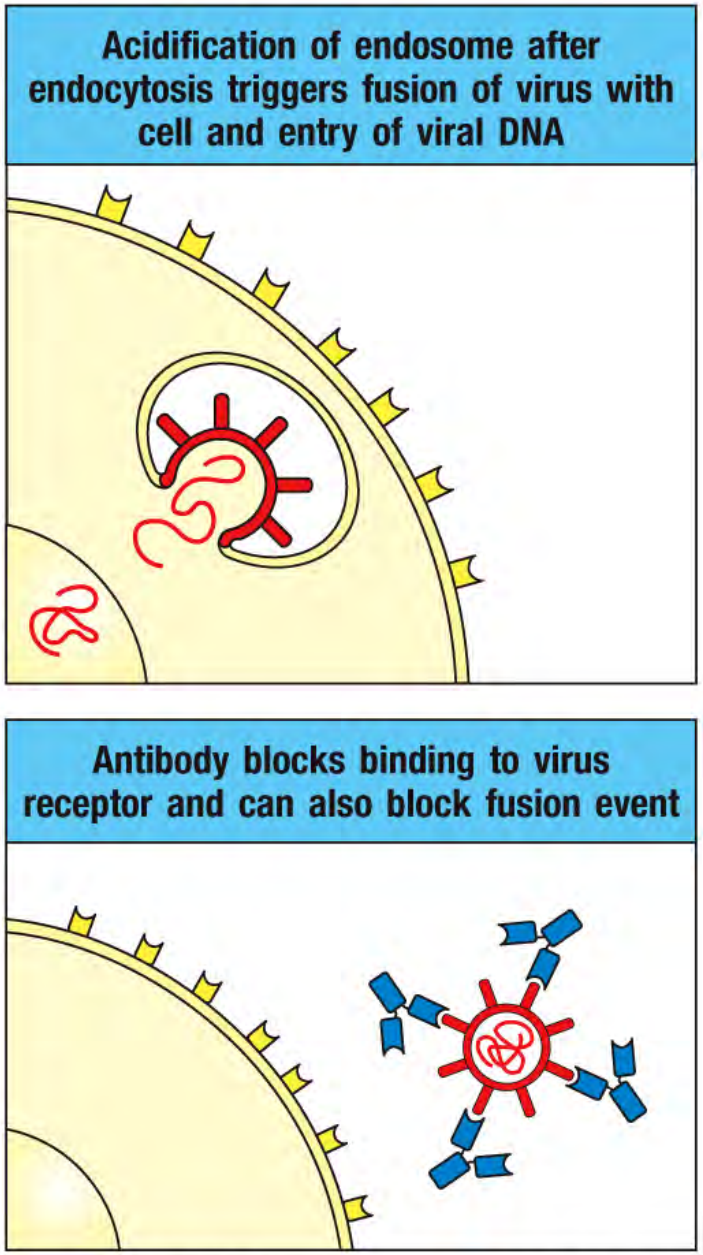
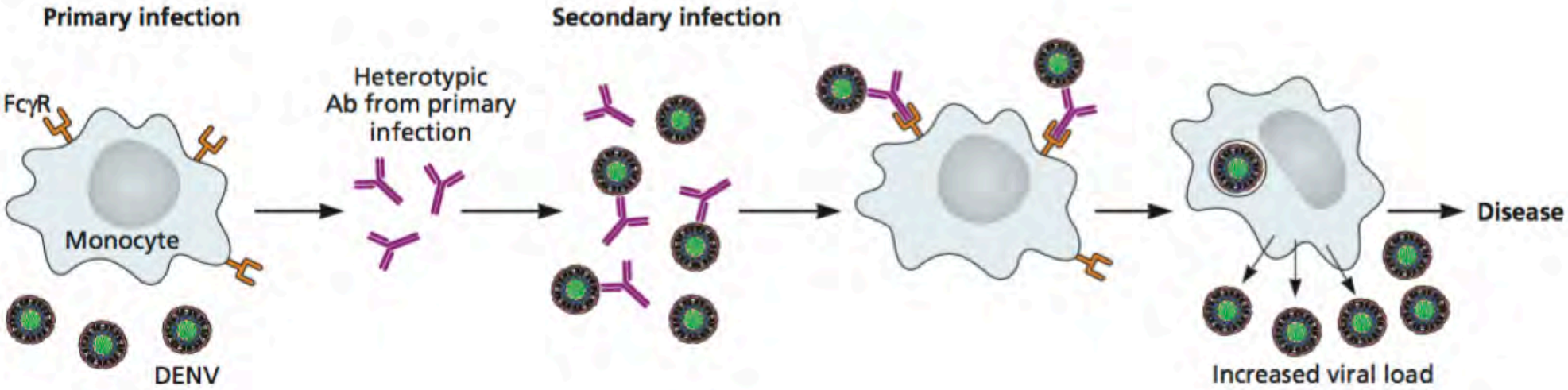


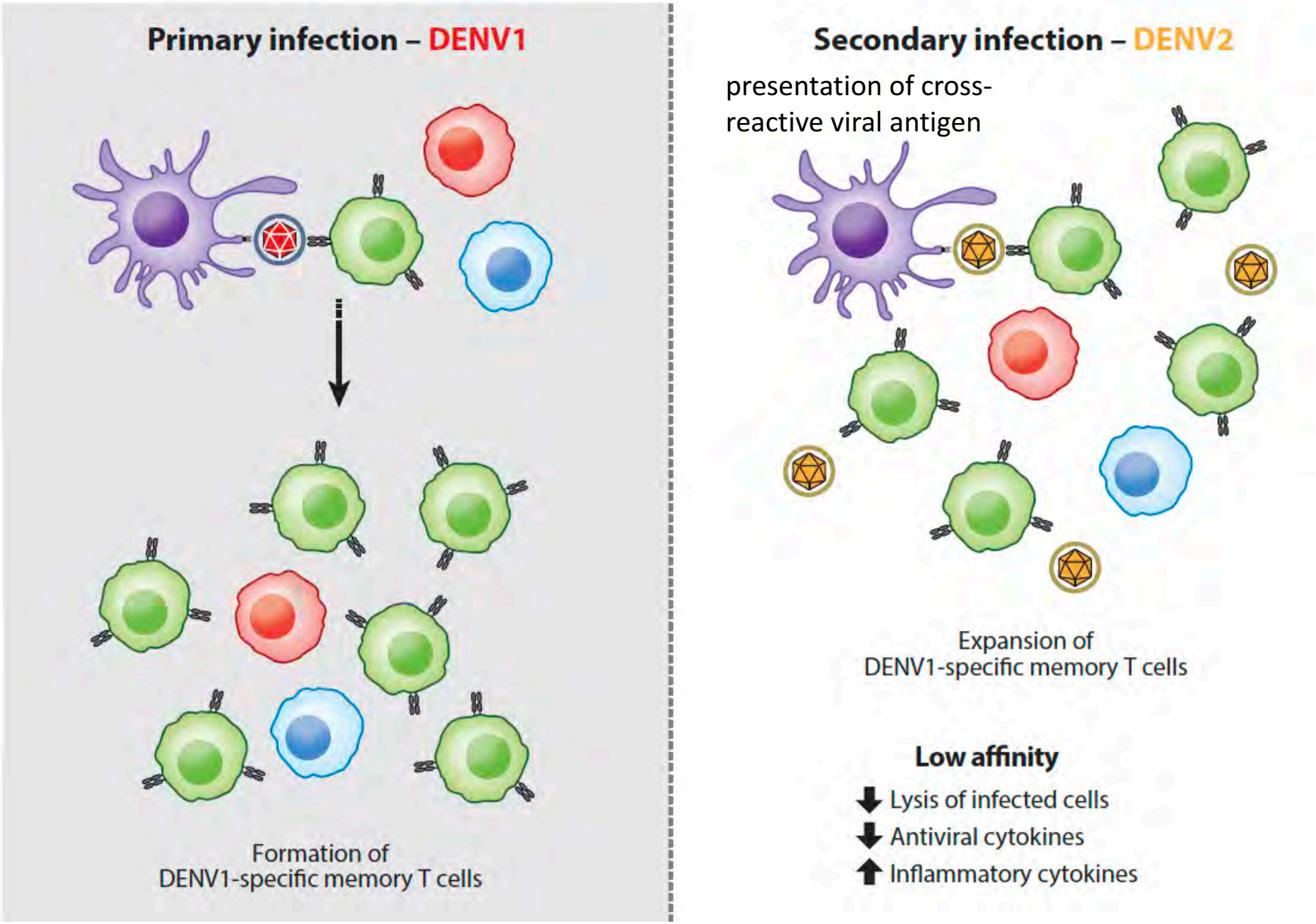
Figure 10.33 (part 3 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Antibody-dependent Enhancement (ADE) of Dengue Infection



- Primary dengue virus (DENV) infection generates Abs.
- Subsequent infection with a different serotype will bind these non-neutralizing Abs.
- Instead, the Ab–virus complex attaches to receptors called Fcγ receptors (FcγR) on circulating monocytes.
- The antibodies help the virus infect monocytes more efficiently.
- The outcome is an increase in the overall replication of the virus, more inflammation, and a higher risk of severe dengue fever.

T Cell Original Antigenic Sin



Elong Ngono and Shresta 2018 ***Annu Rev Immunol***

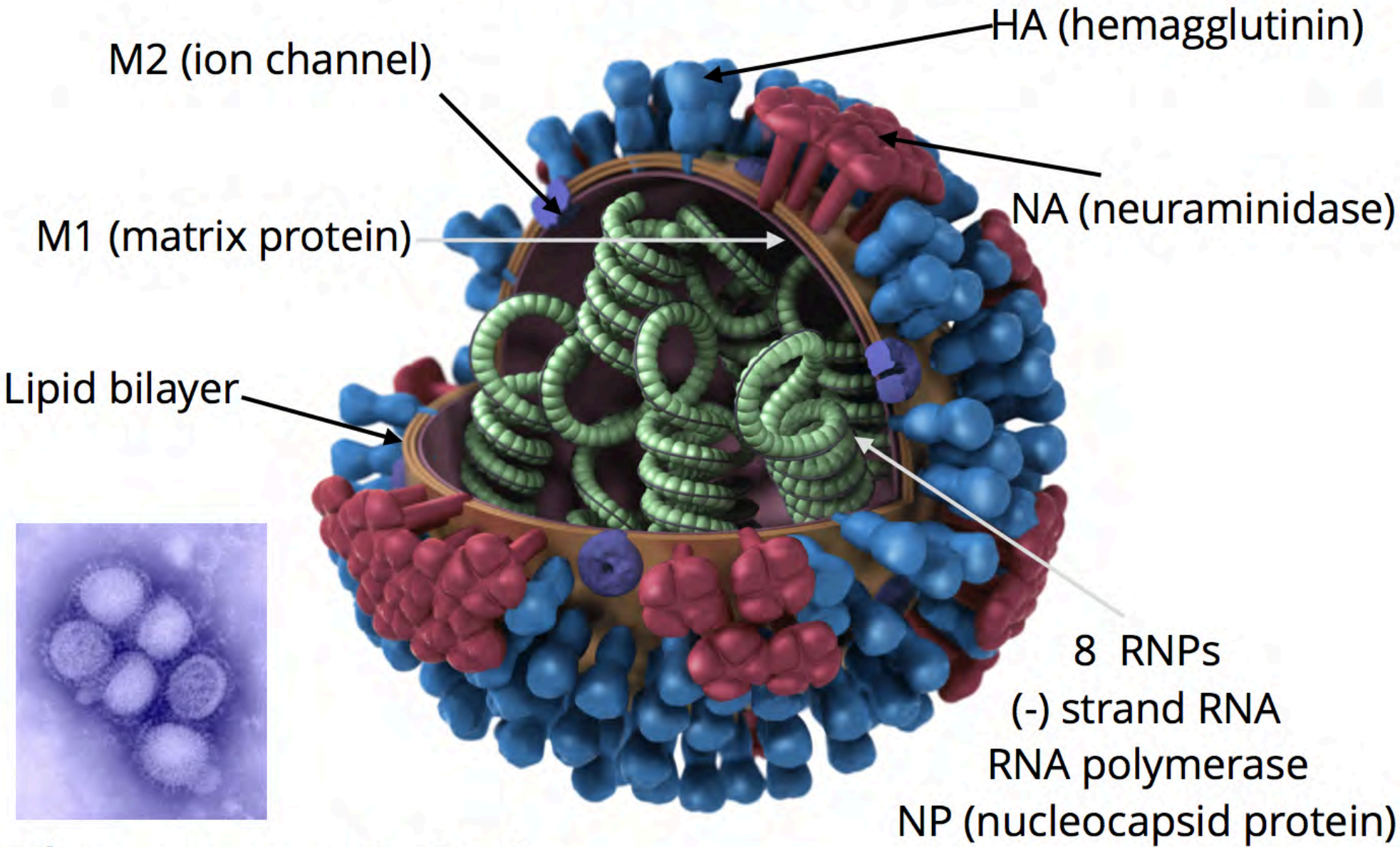
Viral Evolution: The Constant Change of a Viral Population in the Face of Selection Pressures

- **As host populations grow and adapt, virus populations are selected that can infect them**
  - *New viral populations emerge every day*
- **It also works the other way**
  - *Viral populations can be significant selective forces in the evolution of host populations*
- **If a host population cannot adapt to a lethal virus infection, the population may be exterminated**

- **Examples of viral evolution: Influenza, Zika virus, and HIV**

Note that these are all RNA viruses!

Influenza virus



Three types: A, B, C

Antigenic Drift versus Antigenic Shift

- **Drift** - diversity arising from copying errors and immune selection.
  - May occur each time a genome replicates
  - Cause of epidemics
- **Shift** - diversity arising after recombination or reassortment of segmented genomes.
  - Is relatively rare
  - Cause of pandemics

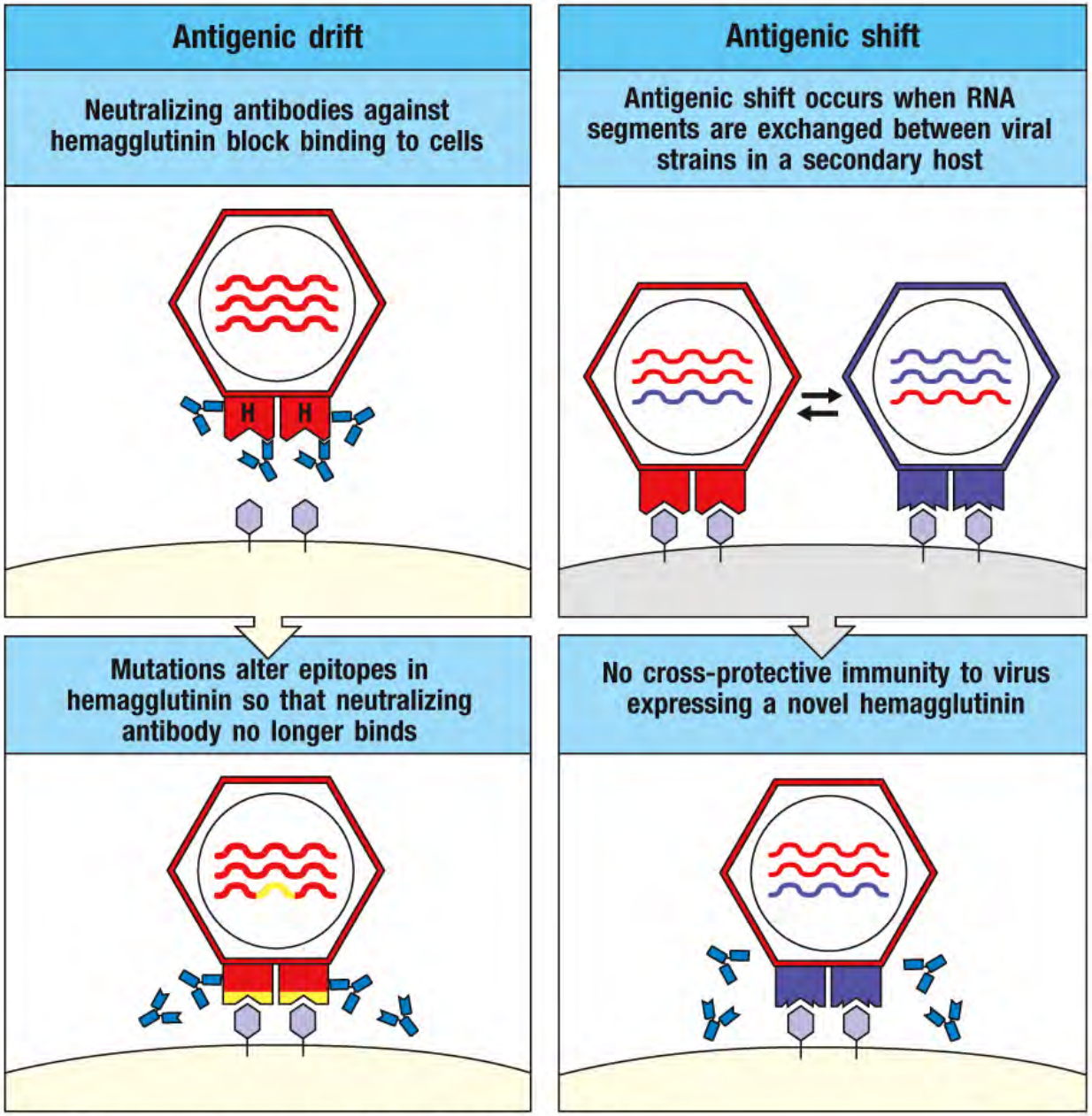
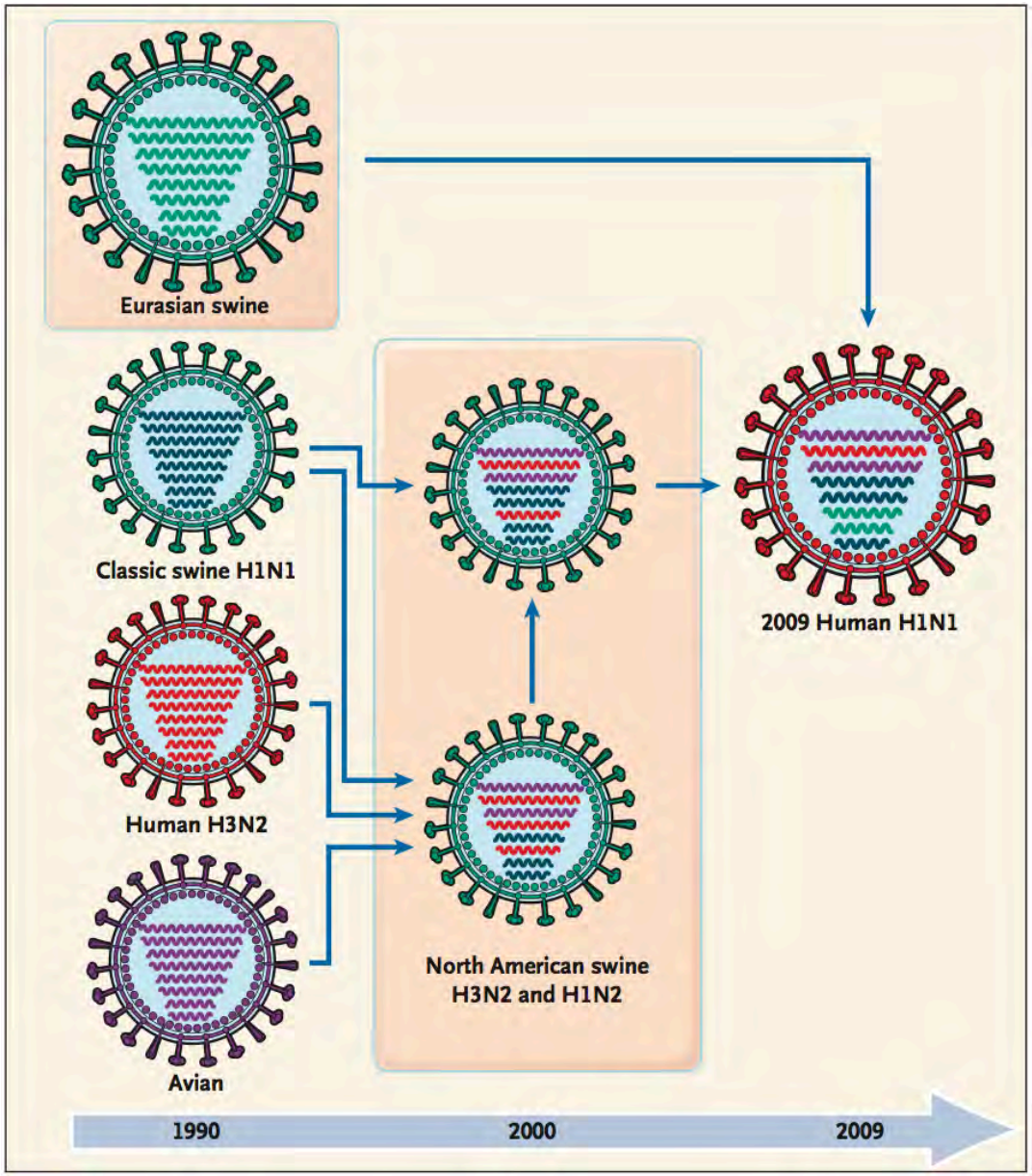


Figure 13.22 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

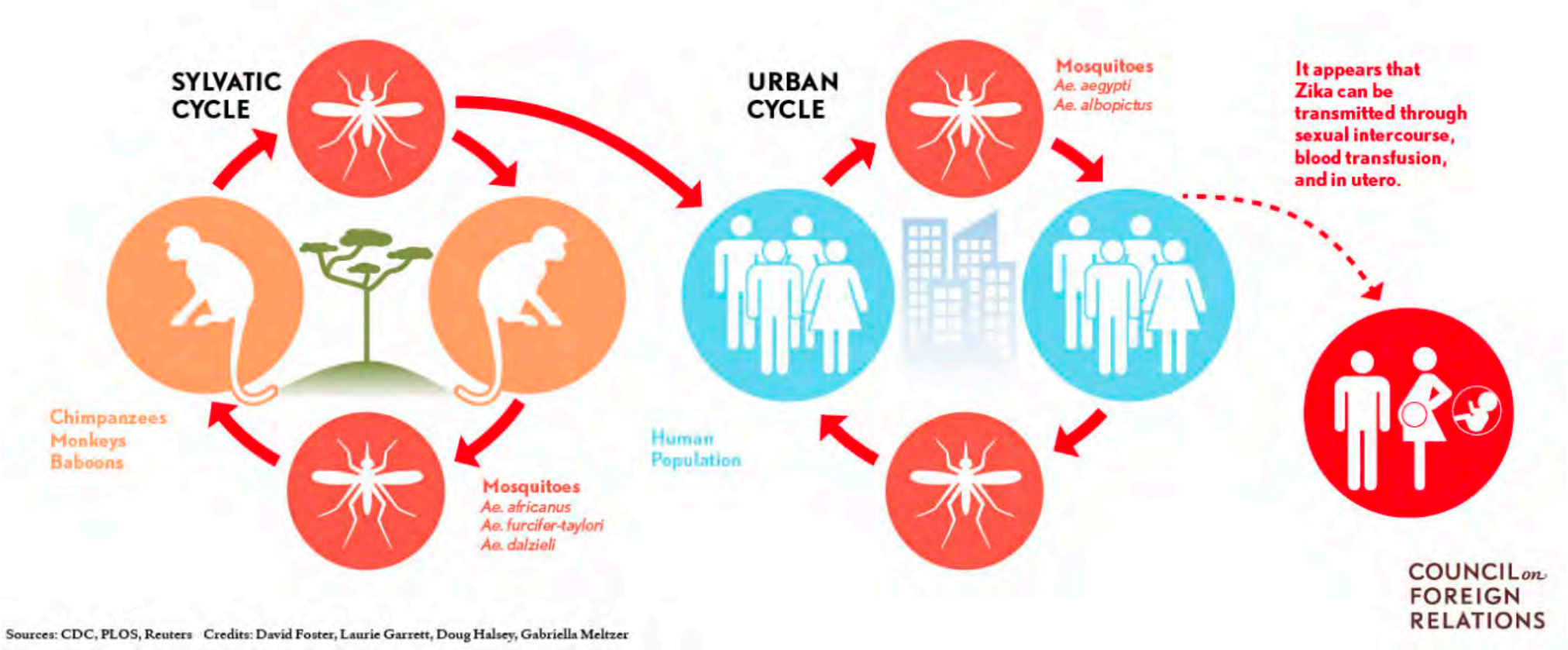
Reassortment Events Give Rise to Pandemic Influenza: The Evolution of the 2009 Influenza A (H1N1) Virus

- Influenza A viruses are classified by antigenic composition, by serologic testing of HA and NA.
- H1-17 can infect birds; H1-3 can infect and transmit between humans.
- The 2009 H1N1 pandemic strain is a reassortment of avian, human, and swine influenza viruses!



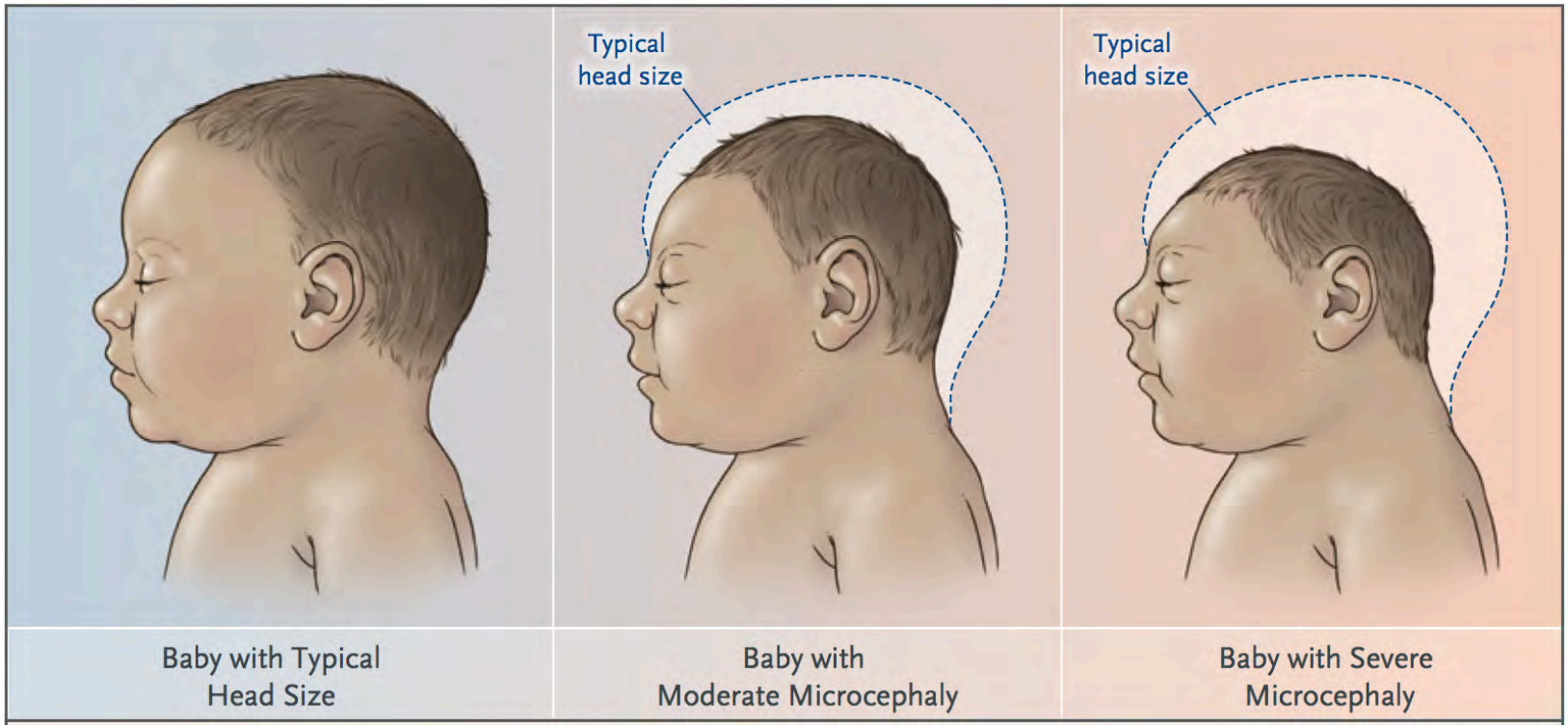
Trifonov et al. 2009 ***NEJM***

Zika Virus: The Evolving Epidemic



- Zika virus was discovered in 1947 in Zika Forest in Uganda.
- It was isolated from Rhesus Macaques.
- Arbovirus of the genus Flavivirus (Yellow Fever, Dengue, West Nile, Japanese encephalitis)
- First human case was detected in 1952.
- Sporadic infections reported in tropical Africa, Southeast Asia, and the Pacific Islands prior to 2014.

Zika Can Cause Microcephaly and Other Severe Brain Defects in Unborn Babies



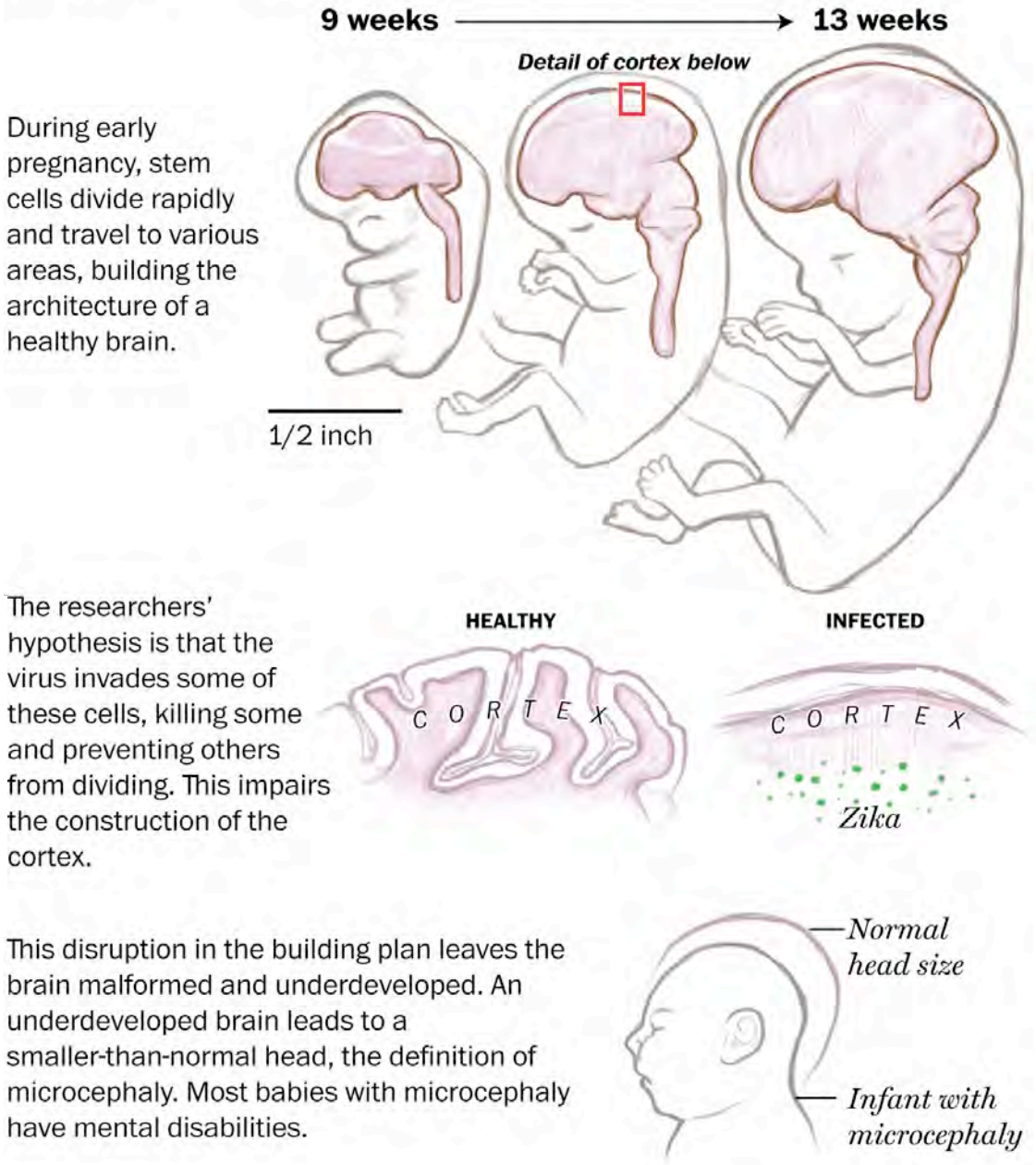
Petersen 2016 ***NEJM***

Historic number of cases of Microcephaly in Brasil 2010 - 2015

	2010	2011	2012	2013	2014	2015
Brasil	153	139	175	167	147	3,530

Source: Brasil Health Ministry

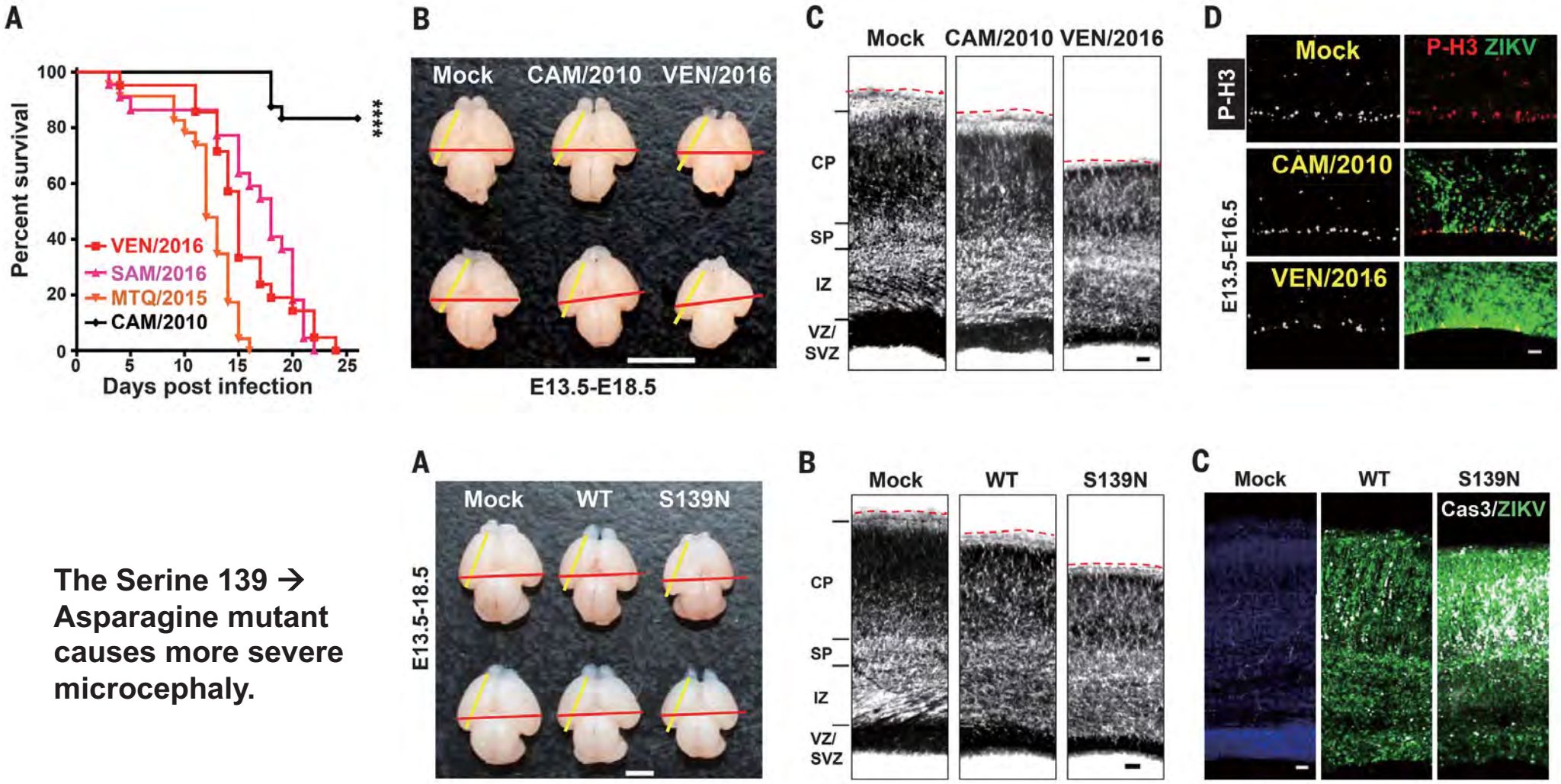
Link Between Zika and Microcephaly



Sources: Zhexing Wen, researcher at the Johns Hopkins Medical Institute; the Dana Foundation; BrainFacts.org, American Journal of Neuroradiology

BONNIE BERKOWITZ AND LAZARO GAMIO/THE WASHINGTON POST

Neurovirulence Phenotypes of the Contemporary ZIKV Strains and Their Ancestral Asian Strain



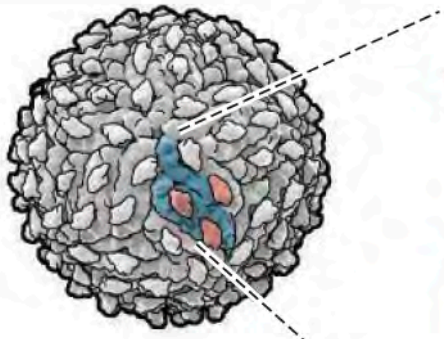
The Serine 139 → Asparagine mutant causes more severe microcephaly.

Yuan et al. (2017) ***Science*** 358: 933.

Mutation That Showed up around 2014 in ZIKV Forms a Receptor for Neural Progenitor Cells?

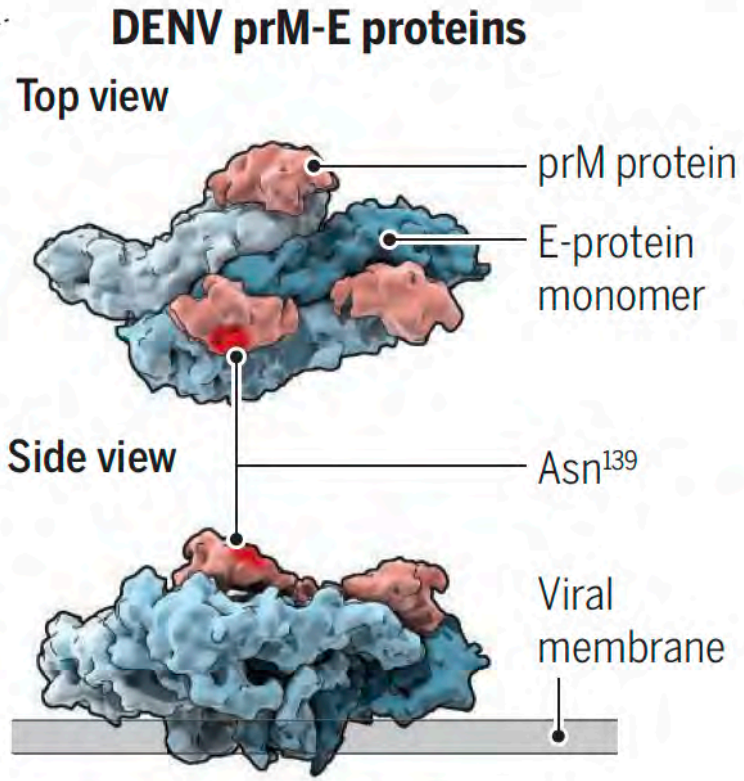
Gaining neurovirulence

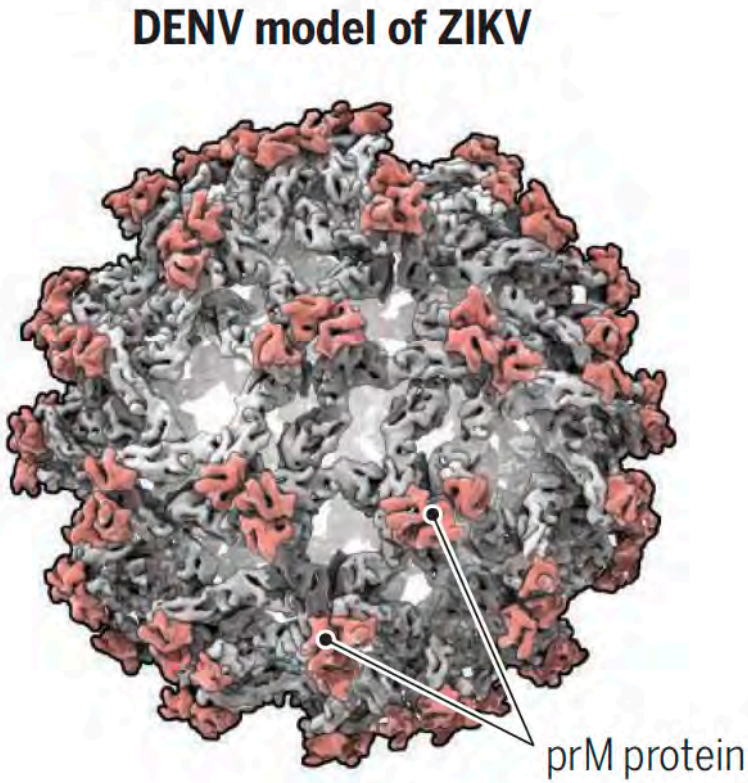
Structures of the immature DENV particle show heterohexameric spikes containing a trimer of prM protein above a trimer of E protein. prM is modeled onto the structure of mature ZIKV, also a flavivirus, to indicate how prM could fit over E-protein dimers when the virus passes through the Golgi apparatus.



Mutation

The S139N mutation introduces asparagine at position 17 of prM, which could form a new receptor for neural progenitor cells.





Screaton and Mongkolsapaya 2017 ***Science*** 358: 863

Phylogenetic Origins of HIV-1 and HIV-2

SIV

~1921: Patient zero

HIV

78,000,000 infections

39,000,000 deaths

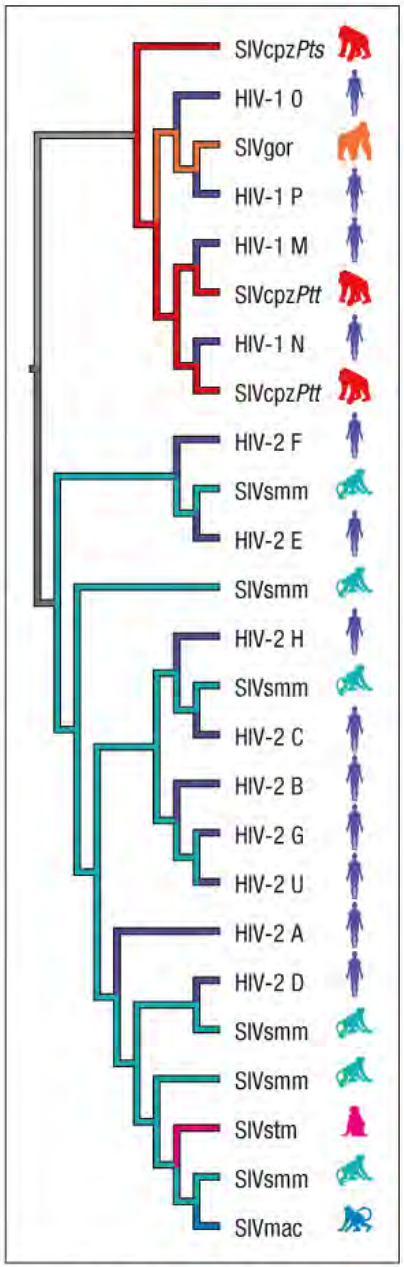
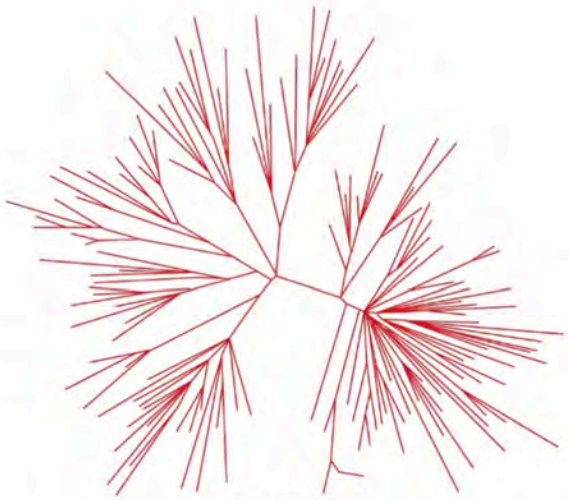


Figure 13.27 Janeway's Immunobiology, 9th ed. (

- **HIV-1 shows marked genetic variability.**
- **Classified on the basis of genomic sequence into four major groups:**
  - **M (main),**
  - **O (outlier),**
  - **N (non-M, non-O),**
  - **P (non-M, non-N, non-O),**
- **These are further diversified into subtypes, or clades, that are designated by the letters A to K.**
- **In different parts of the world, different subtypes predominate.**

Genetic variability of global influenza A virus (1996)



Genetic variability of HIV-1 V2-C5 (Congo, 1996)

HIV is a Member of the Lentivirus Family of Retroviruses

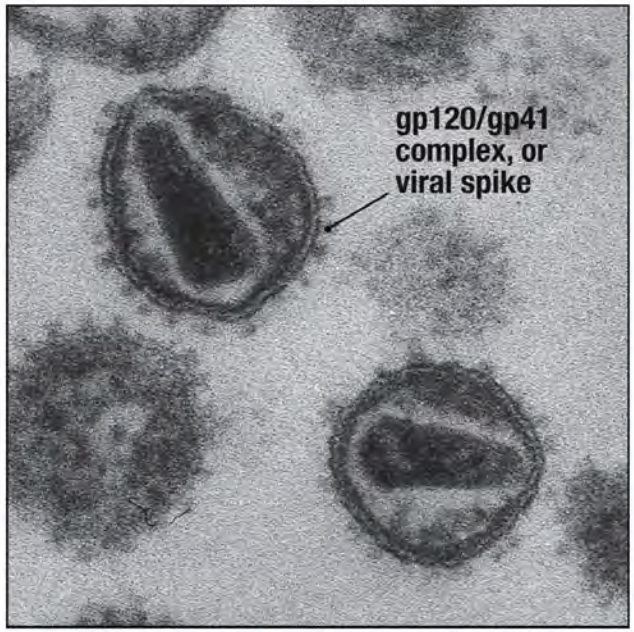


Figure 13.29 (part 1 of 2) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

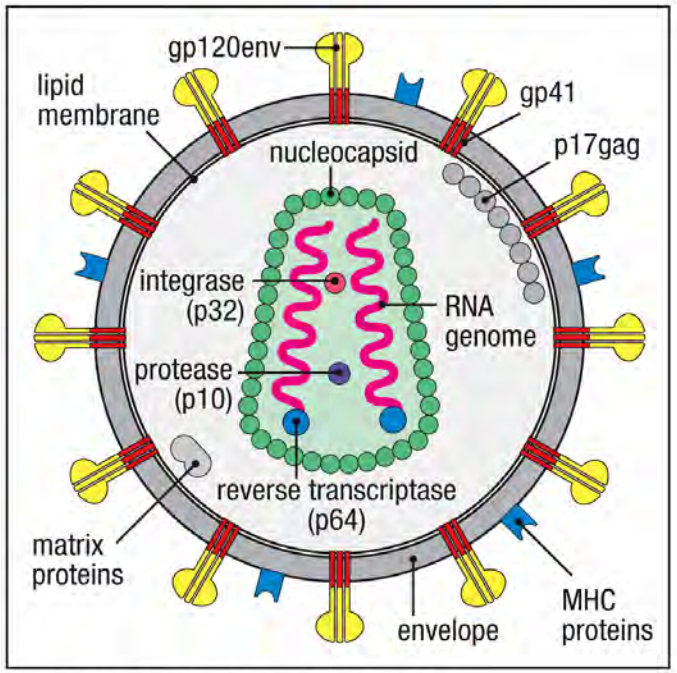
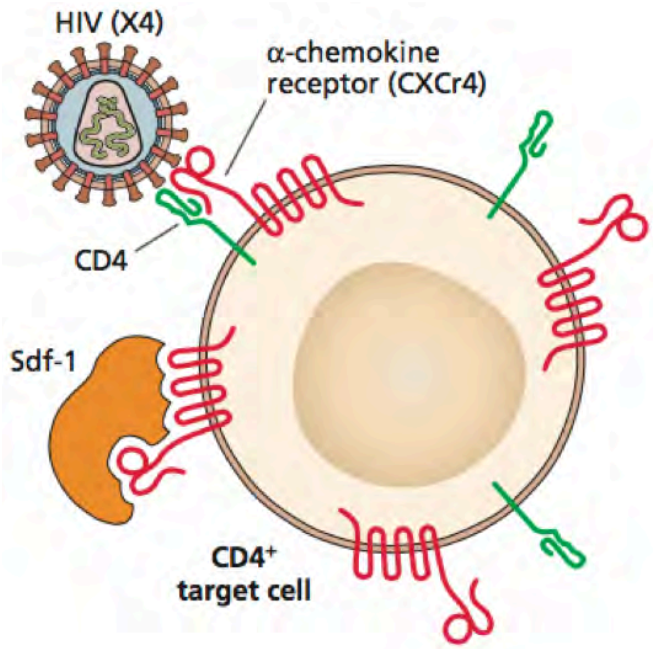
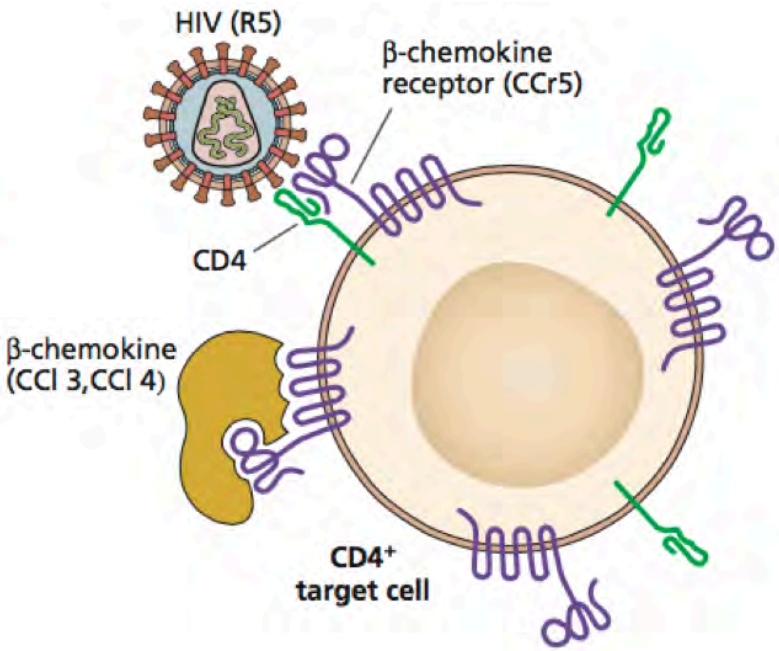


Figure 13.29 (part 2 of 2) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

- Lentiviruses can infect nondividing cells
- Replication driven from long terminal repeats
- RT makes a lot of mistakes!

HIV Tropism Determined by Receptor/Co-receptor





CXCR4 is expressed primarily by naive and central memory CD4 T cells.

CCR5 is predominantly expressed on subsets of effector memory CD4 T cells (specially in the mucosa!), dendritic cells, and macrophages.

Dendritic Cells Transport HIV from Mucosal Surfaces to CD4 T Cells in Lymphoid Tissue

Transmission:
R5 Tropic virus (CCR5)

↓ **Mutates**

Chronic phase:
X4 Tropic virus (CXCR4)

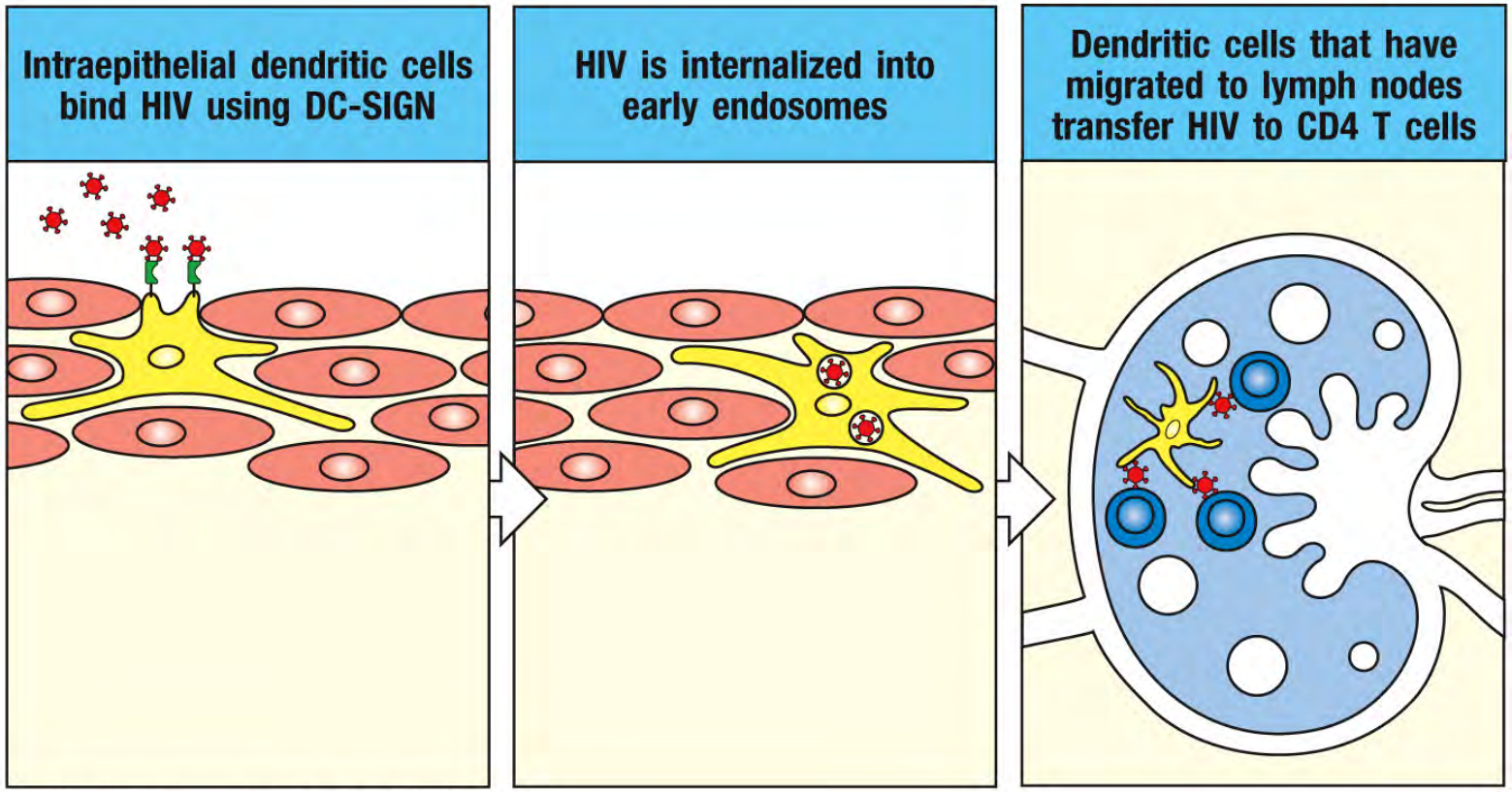
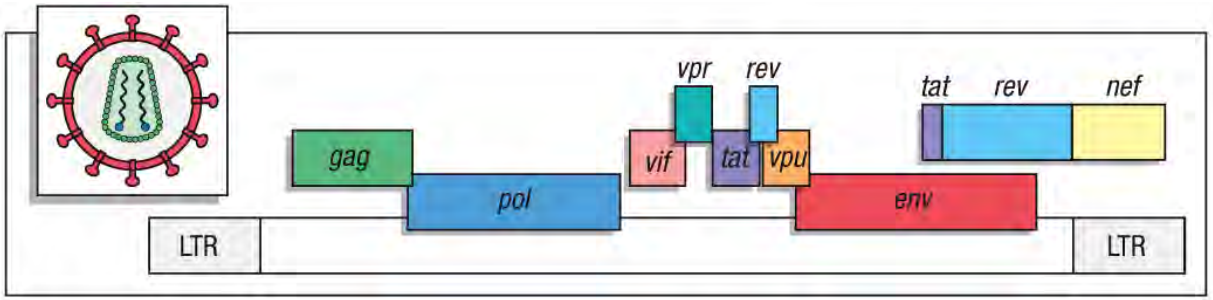


Figure 13.32 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

- HIV is a sexually transmitted pathogen that must cross mucosal barriers.
- Abrasion caused by sexual contact can promote transmission.
- Directly picked up by DCs that have protruded between epithelial cells to sample the external world.
- HIV virions are translocated back to the cell surface and transferred to T cells in secondary lymphoid tissue.

The genomic organization of HIV

- Genome is read in three frames, allowing the virus to encode many proteins in a small genome.
- Gene products of *gag*, *pol*, and *env* together with viral RNA are present in the mature viral particle.
- The mRNAs for Tat, Rev, and Nef proteins are produced by splicing of viral transcripts, so their genes are split in the viral genome. **(Basis of using multiply spliced HIV RNA (msRNA) for tat/rev genomic region in PCR assays to detect viral replication.)**
- Structural genes - gag, pol, env
- Regulatory genes - tat, rev
- Accessory genes - vif, vpr, vpu, nef



Gene		Gene product/function
gag	Group-specific antigen	Core proteins and matrix proteins
pol	Polymerase	Reverse transcriptase, protease, and integrase enzymes
env	Envelope	Transmembrane glycoproteins. gp120 binds CD4 and CCR5; gp41 is required for virus fusion and internalization
tat	Transactivator	Positive regulator of transcription
rev	Regulator of viral expression	Allows export of unspliced and partially spliced transcripts from nucleus
vif	Viral infectivity	Affects particle infectivity
vpr	Viral protein R	Transport of DNA to nucleus. Augments virion production. Cell-cycle arrest
vpu	Viral protein U	Promotes intracellular degradation of CD4 and enhances release of virus from cell membrane
nef	Negative-regulation factor	Augments viral replication *in vivo* and *in vitro*. Decreases CD4, MHC class I and II expression

Figure 13.31 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

HIV Life Cycle

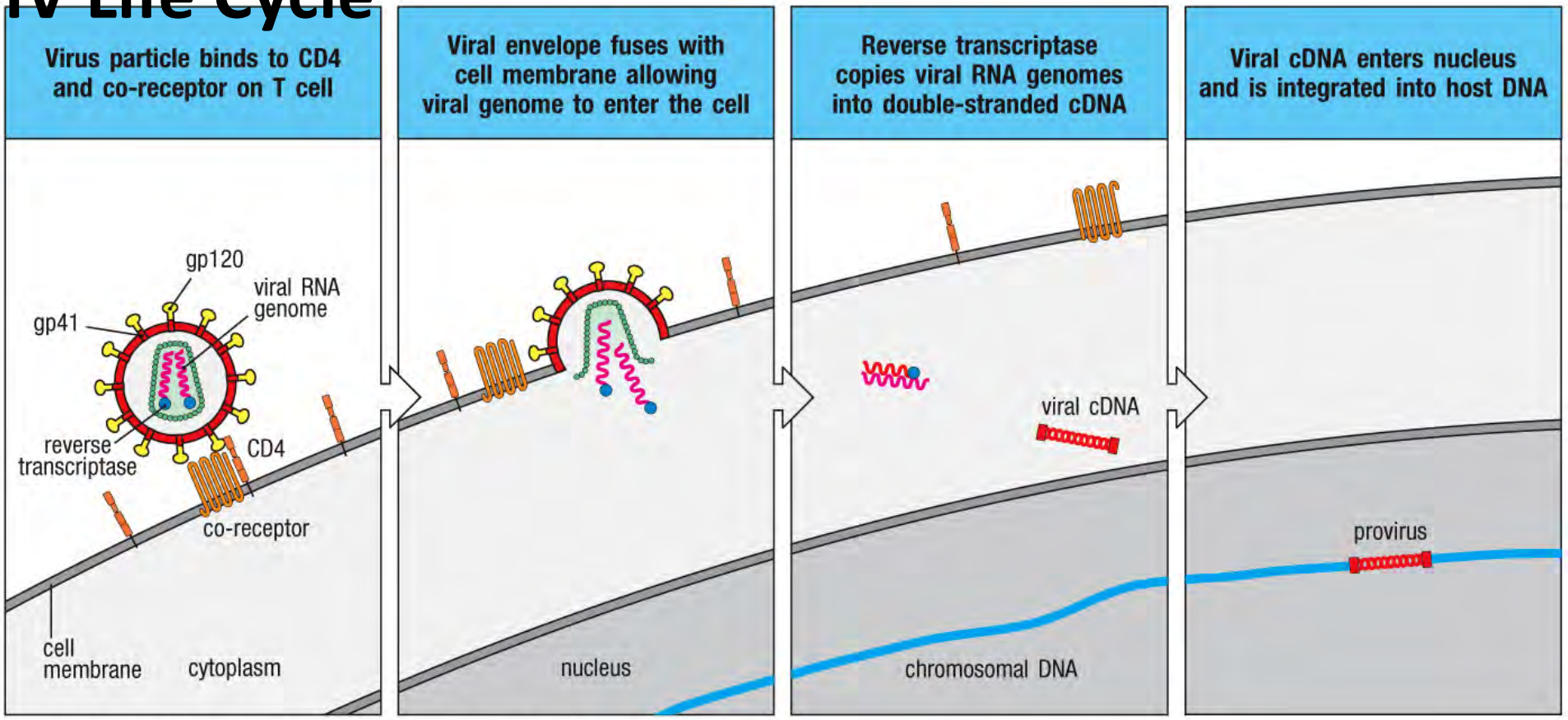


Figure 13.30 (part 1 of 2) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

- **Gp120 binds CD4 and undergoes a conformational change, exposing a high-affinity site that is bound by the co-receptor (CCR5/CXCR4).**
- **gp41 unfolds and inserts a portion of its structure into the plasma membrane.**
- **Fusion of viral envelope with the cell's plasma membrane occurs.**
- **Viral nucleocapsid, composed of the viral genome and associated viral proteins, enter the host-cell cytoplasm.**
- **RT transcribes the viral RNA into cDNA, encoding 9 genes.**
- **Viral integrase then inegrates viral cDNA into host genome, creating provirus.**

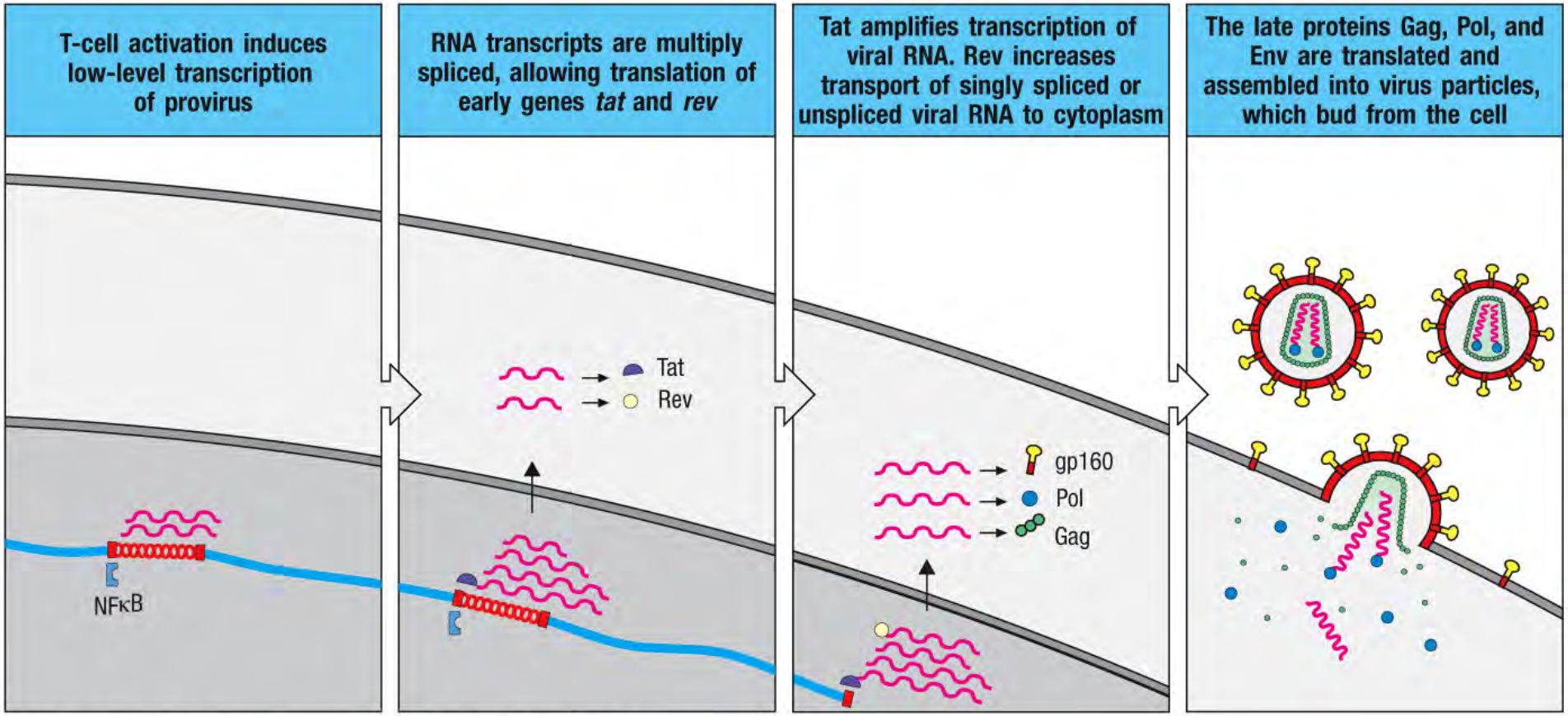
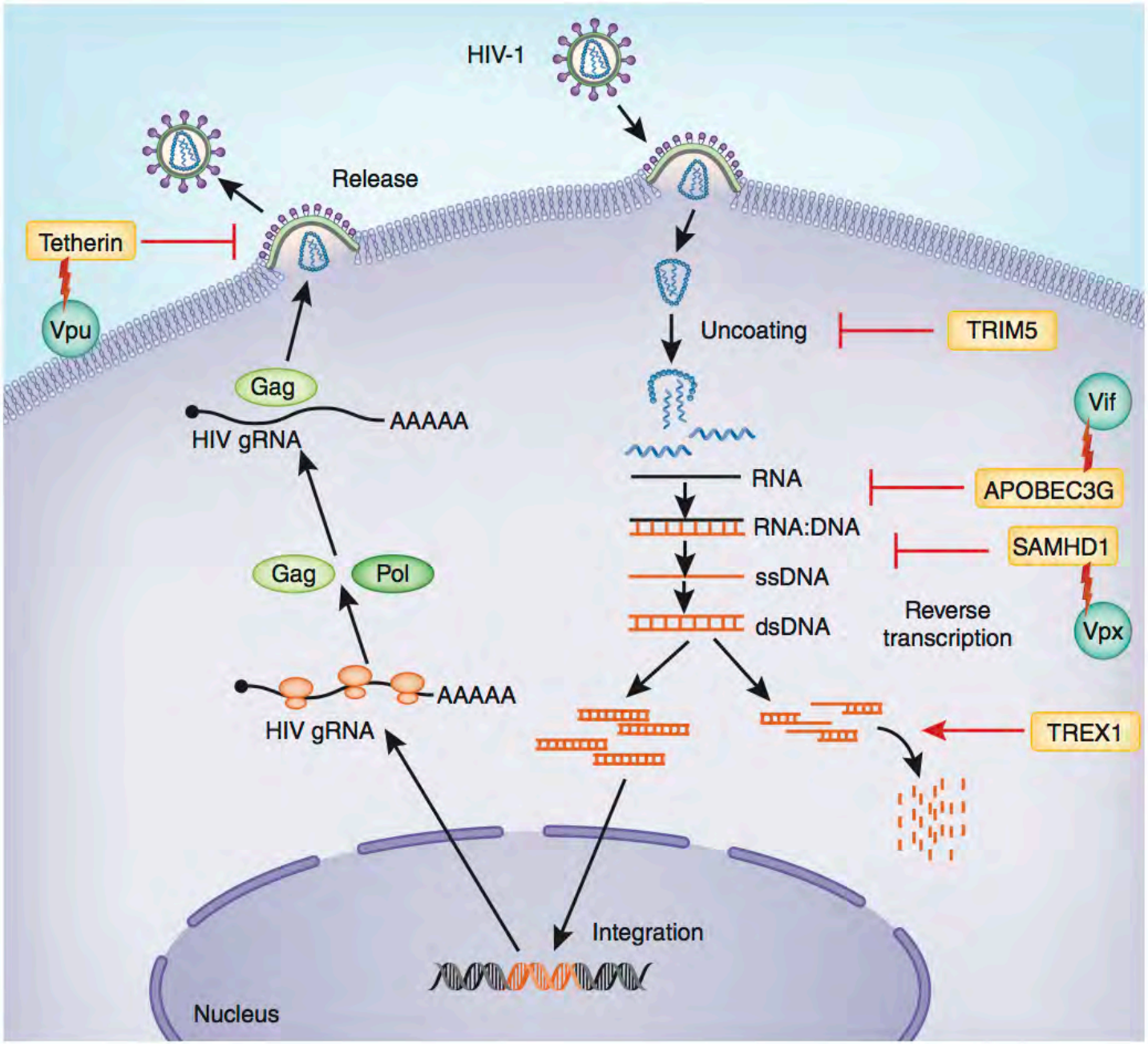


Figure 13.30 (part 2 of 2) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

- **In activated CD4 T cells, NF-κB and NFAT bind to LTR and initiate transcription of the HIV genome.**
- **Regulatory proteins, Tat and Rev, are produced upon extensive processing.**
- **Tat both enhances transcription from the provirus and binds to the RNA transcripts, stabilizing them in a form that can be translated.**
- **Rev binds the RNA transcripts and transports them to the cytosol.**
- **The singly spliced and unspliced transcripts encode the structural proteins of the virus.**
- **Unspliced transcripts, which are also the new viral genomes, are packaged with these proteins to form many new virus particles.**

HIV Counteracts Intrinsic Antiviral Factors



Yan and Chen 2012 ***Nature Immunology***

Typical Course of Untreated Infection with HIV

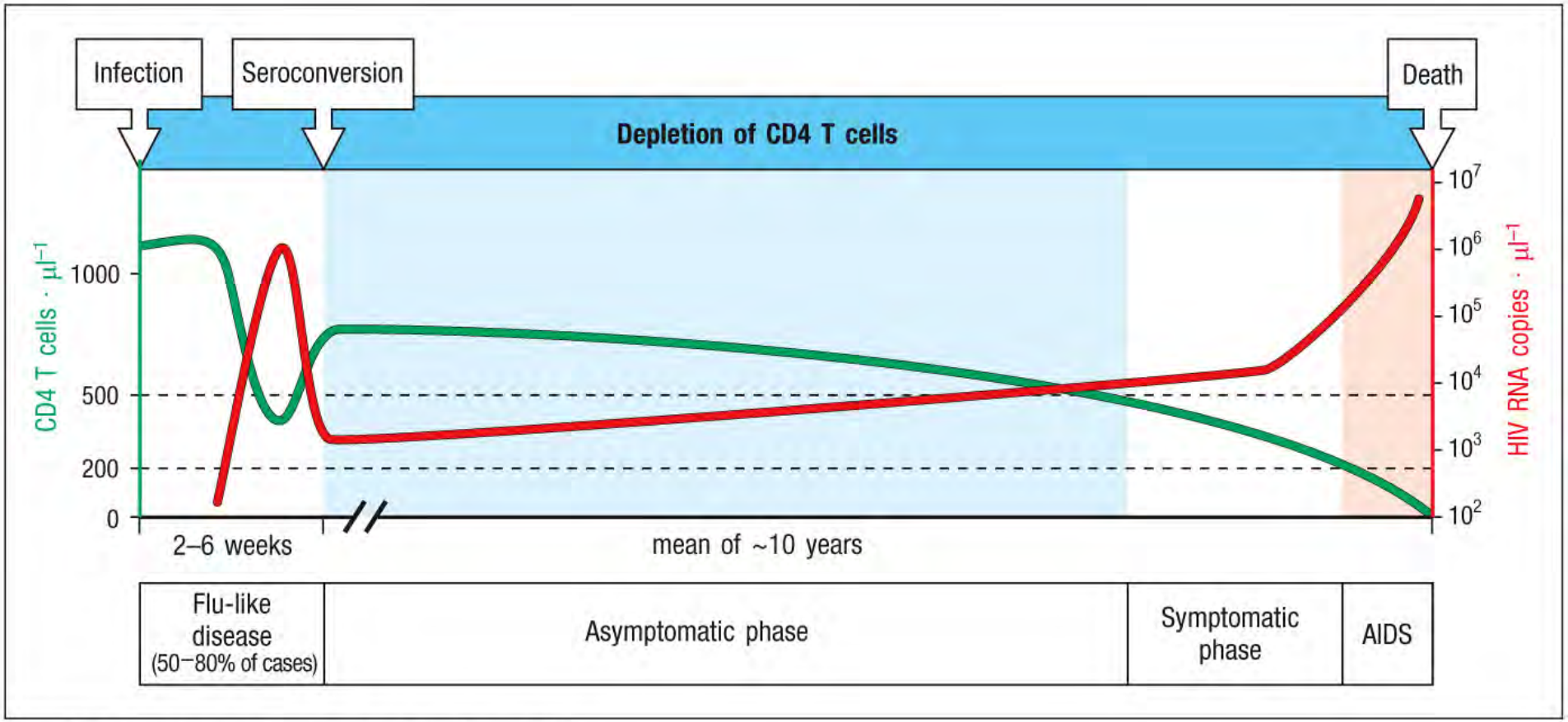


Figure 13.33 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

- **Acute flu-like disease, seroconversion disease, with high titers of virus in the blood.**
- **Adaptive immunity → controls acute illness, largely restores CD4 T cells, but does not eradicate the virus → asymptomatic phase (5–10 years)**
- **CD4 T cell numbers fall → opportunistic infections occur → symptomatic phase**
- **CD4 T-cell counts below 200 cells/µl , the patient is considered to have AIDS.**

The Immune Response to HIV

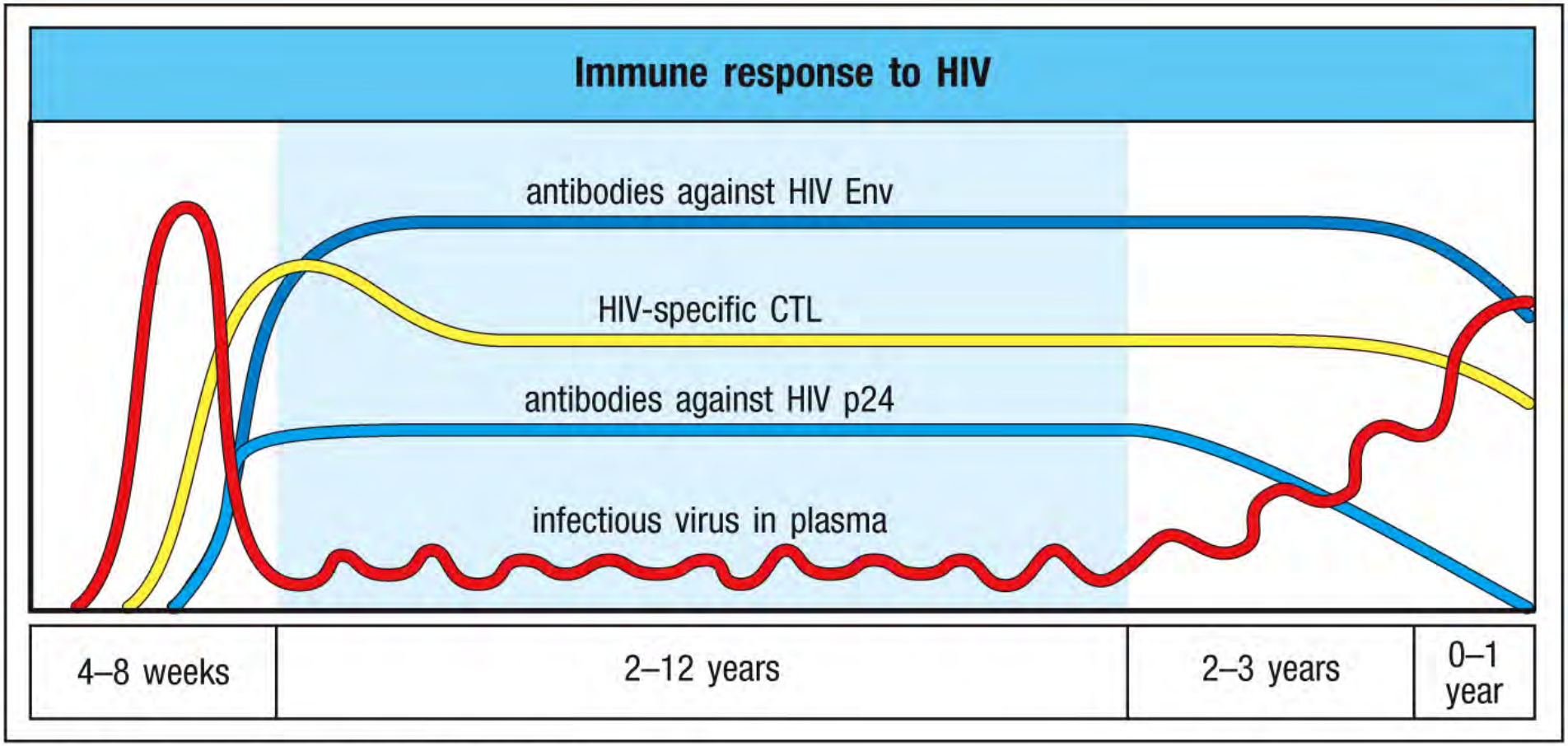
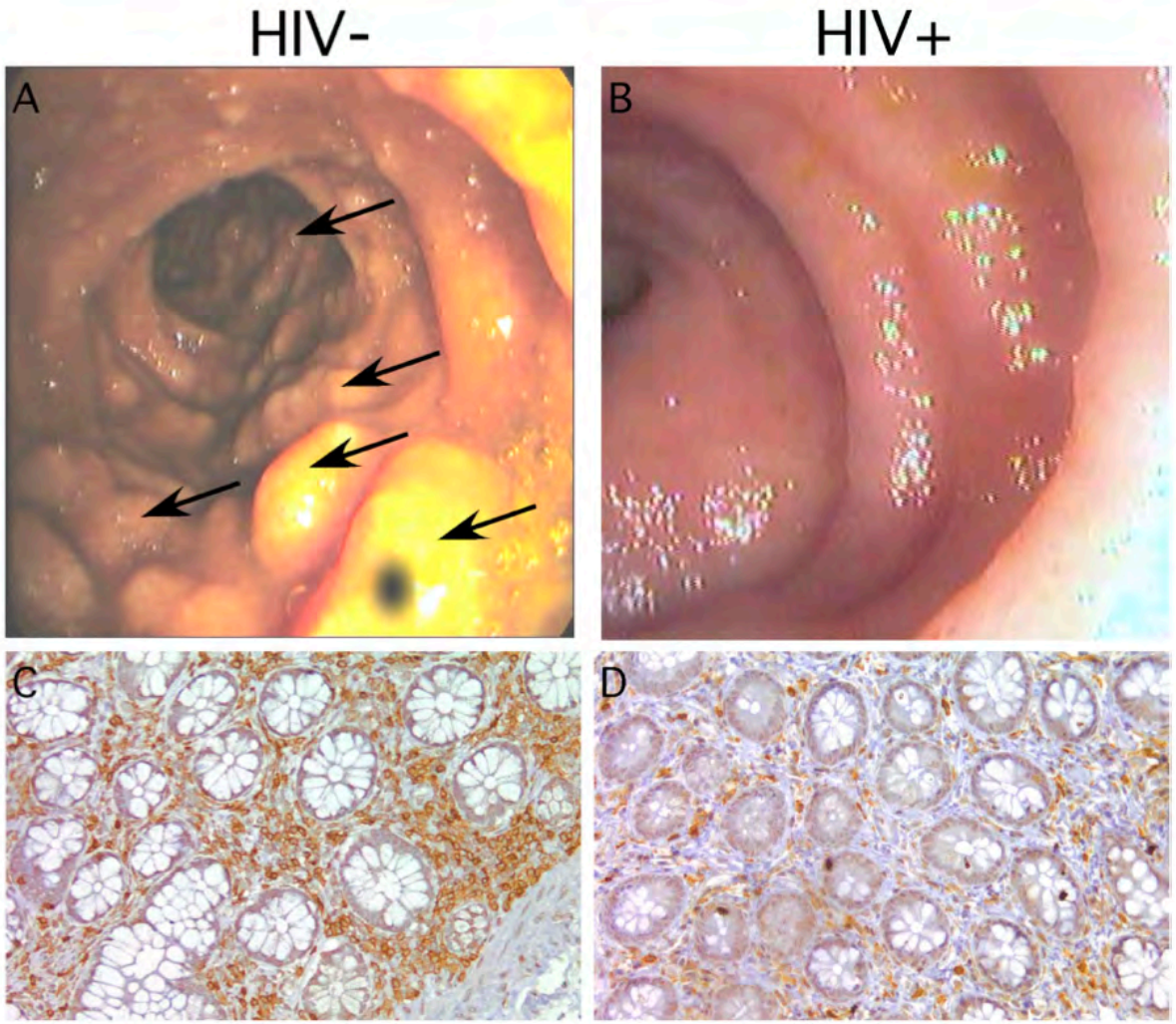


Figure 13.34 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

- **Asymptomatic phase → low level of virus in blood, but virus continues to replicate in lymphoid tissues.**
- **CD4 T-cell counts gradually decline, although antibodies and CD8 CTLs remain high.**
- **Once Ab and CTLs decline, infectious virus increases in blood.**

Massive Damage to the GI Associated Lymphoid Tissue Following Acute Infection



Absence of lymphoid cell aggregates in terminal ileum

↓

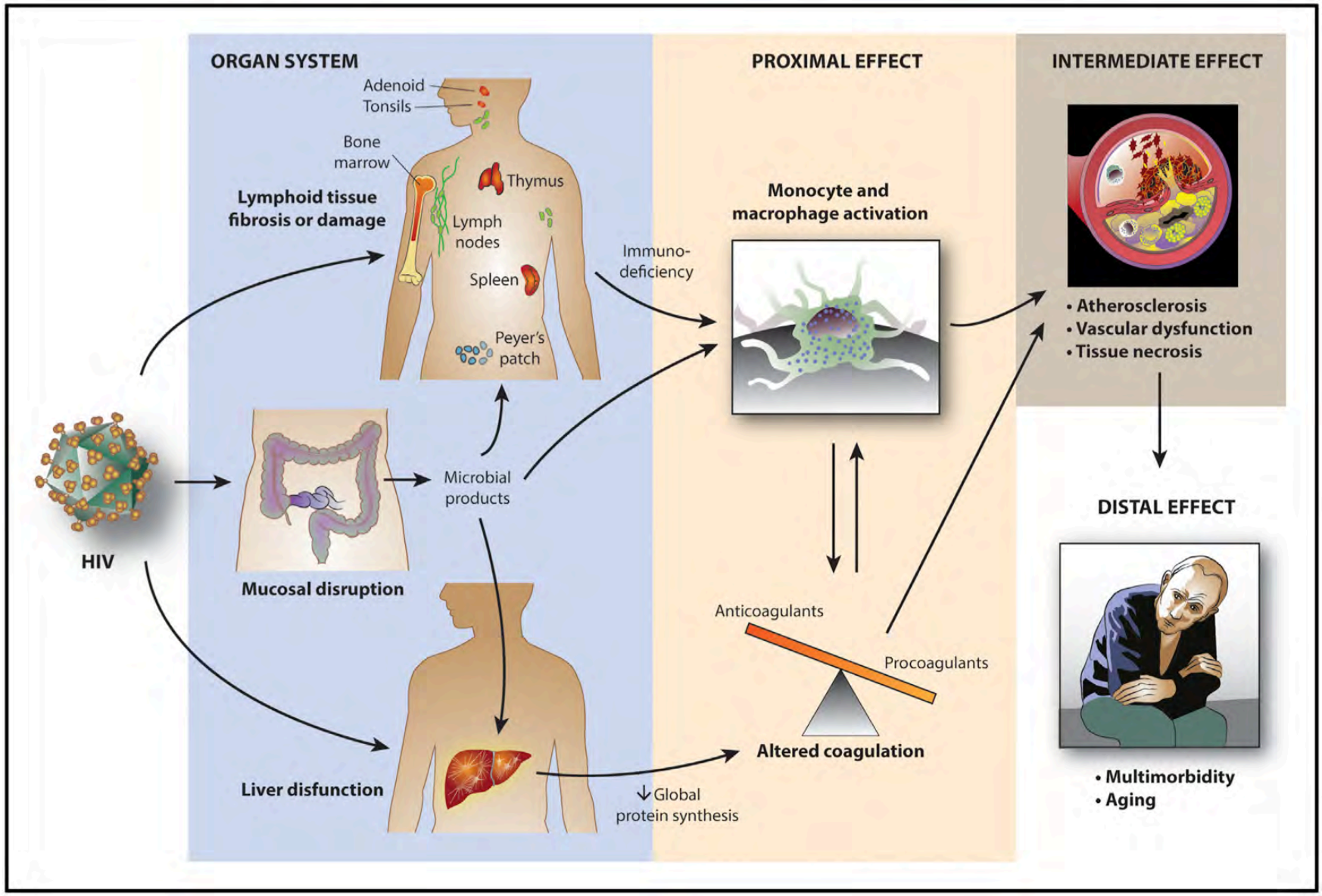
Microbial translocation

↓

Systemic inflammation

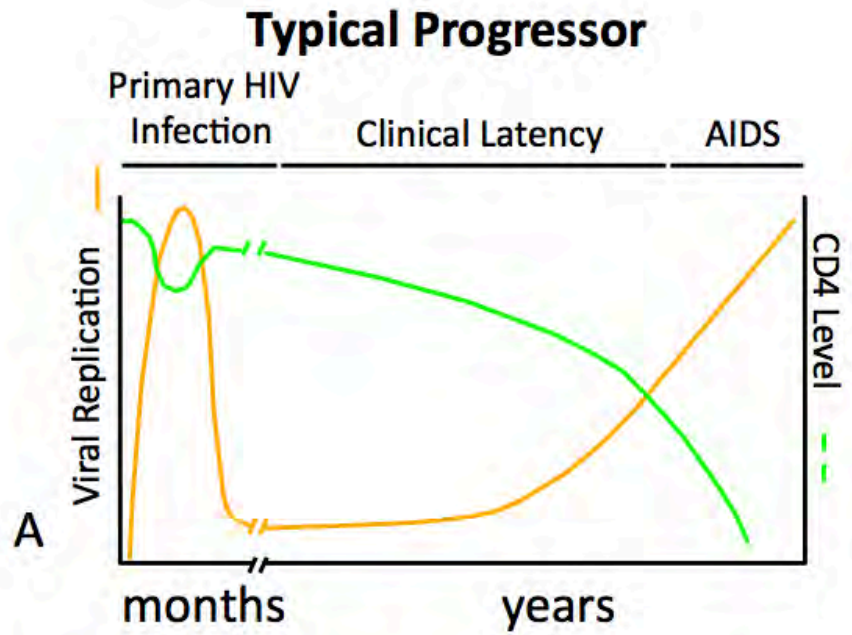
Brenchley et al. 2004 ***JEM***

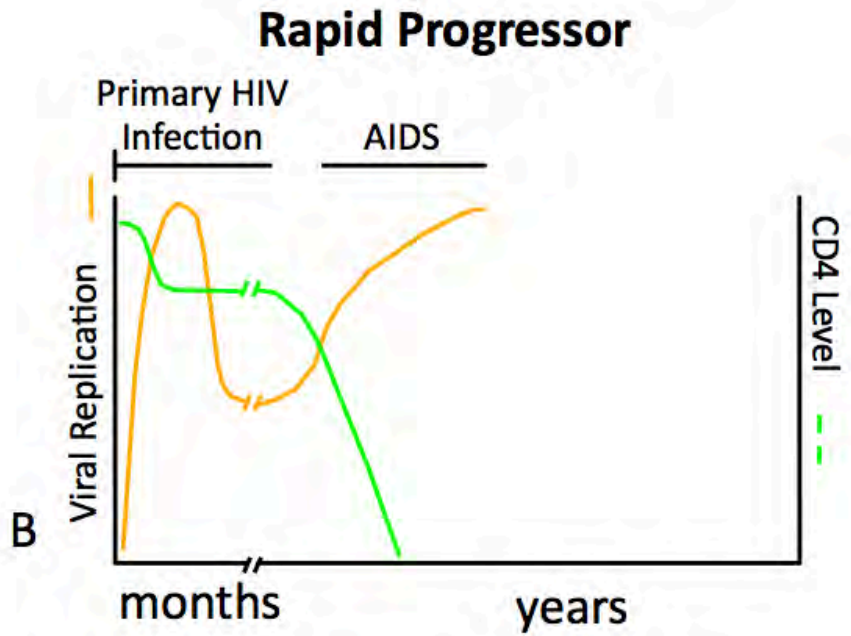
Pathogenesis of Inflammation-Associated Disease in HIV-Infected Adults

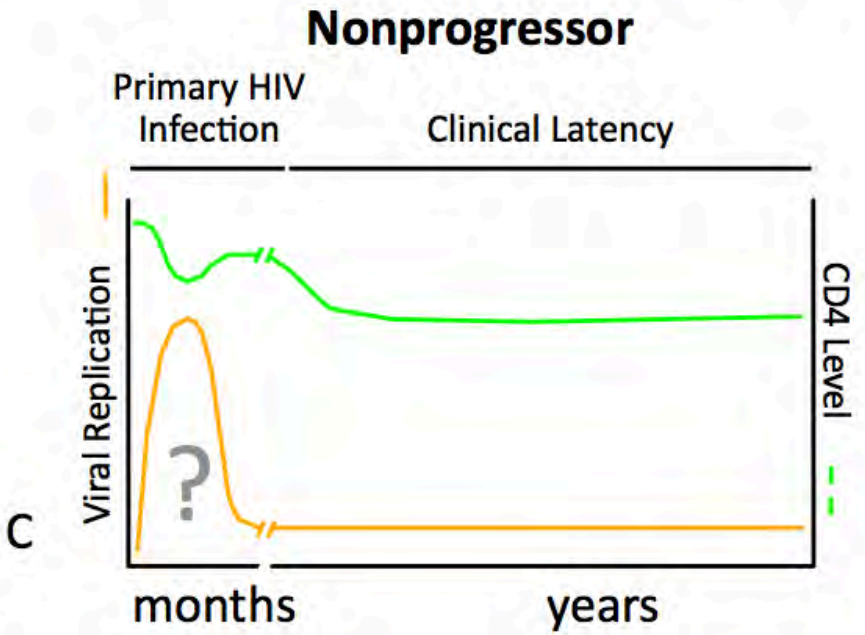


Deeks et al. 2013 ***Immunity***

The Variable Course of HIV-1 Infection







Elite controllers:

Individuals who maintain normal CD4 counts and undetectable viral loads (<50 copies HIV RNA/ml of plasma) for >10 years in the absence of antiretroviral therapy. Virus not defective.

Mechanism?

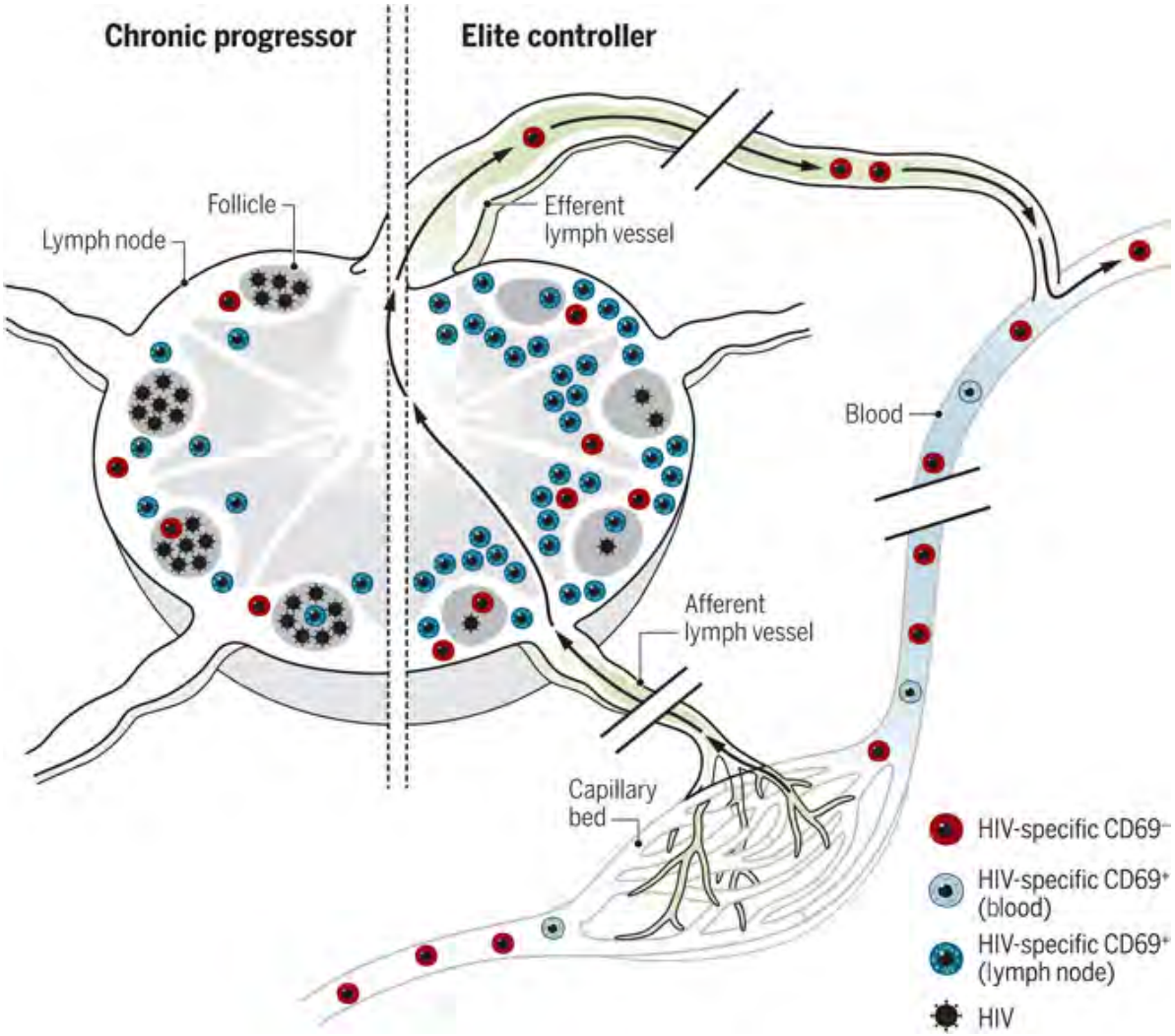
Host Genes that can Alter HIV-1 Progression

Genes that influence progression to AIDS				
Gene	**Allele**	**Mode**	**Effect**	**Mechanism of action**
Cytokine anti-HIV				
IL10	5′A	Dominant	Limits infection	Decreases IL-10 expression
			Accelerates AIDS	
IFNG	–179T	Dominant	Accelerates AIDS (E)	
Acquired immunity, cell-mediated				
	A, B, C	Homozygous	Accelerates AIDS	Decreases breadth of HLA class I epitope recognition
HLA	*B*27*	Codominant	Delays AIDS	Delays HIV-1 escape
	*B*57*			
	*B*35-Px*		Accelerates AIDS	Deflects CD8-mediated T-cell clearance of HIV-1
Acquired immunity, innate				
KIR3DS1	3DS1	Epistatic with HLA-Bw4	Delays AIDS	Clears HIV^+, HLA^- cells (?)

Figure 13.35 (part 2 of 2) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

*** These HLAs are also associated with autoimmunity!**

Resident Memory CD8+ T Cells in Lymphoid Tissues Provide Continuous Protection Against HIV in ECs



Roan, ***Sci. Immunol.*** (2018)
Buggert et al., ***Sci. Immunol.*** 3, eaar4526 (2018)

Host Genes that can Alter HIV-1 Progression

Genes that influence progression to AIDS				
Gene	**Allele**	**Mode**	**Effect**	**Mechanism of action**
HIV entry				
CCR5	Δ32	Recessive	Prevents infection	Knockout of CCR5 expression
		Dominant	Prevents lymphoma (L)	Decreases available CCR5
			Delays AIDS	
	P1	Recessive	Accelerates AIDS (E)	Increases CCR5 expression
CCR2	I64	Dominant	Delays AIDS	Interacts with and reduces CXCR4
CCL5	In1.1c	Dominant	Accelerates AIDS	Decreases CCL5 expression
CXCL12	3′A	Recessive	Delays AIDS (L)	Impedes CCR5–CXCR4 transition (?)
CXCR6	E3K	Dominant	Accelerates *P. jirovecii* pneumonia (L)	Alters T-cell activations (?)
CCL2-CCL7-CCL11	H7	Dominant	Enhances infection	Stimulates immune response (?)

Figure 13.35 (part 1 of 2) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

- **CCR5-delta32 mutation is present in 4-16% of European descent.**
- **Stem cell therapy cured German AIDS patient – only person cured so far!**
- **Possible to disrupt *ccr5* with nucleases, CRISPR/Cas9 technology?**

Basic Research Led to Development of Drug Targets that Interfere with HIV Life Cycle

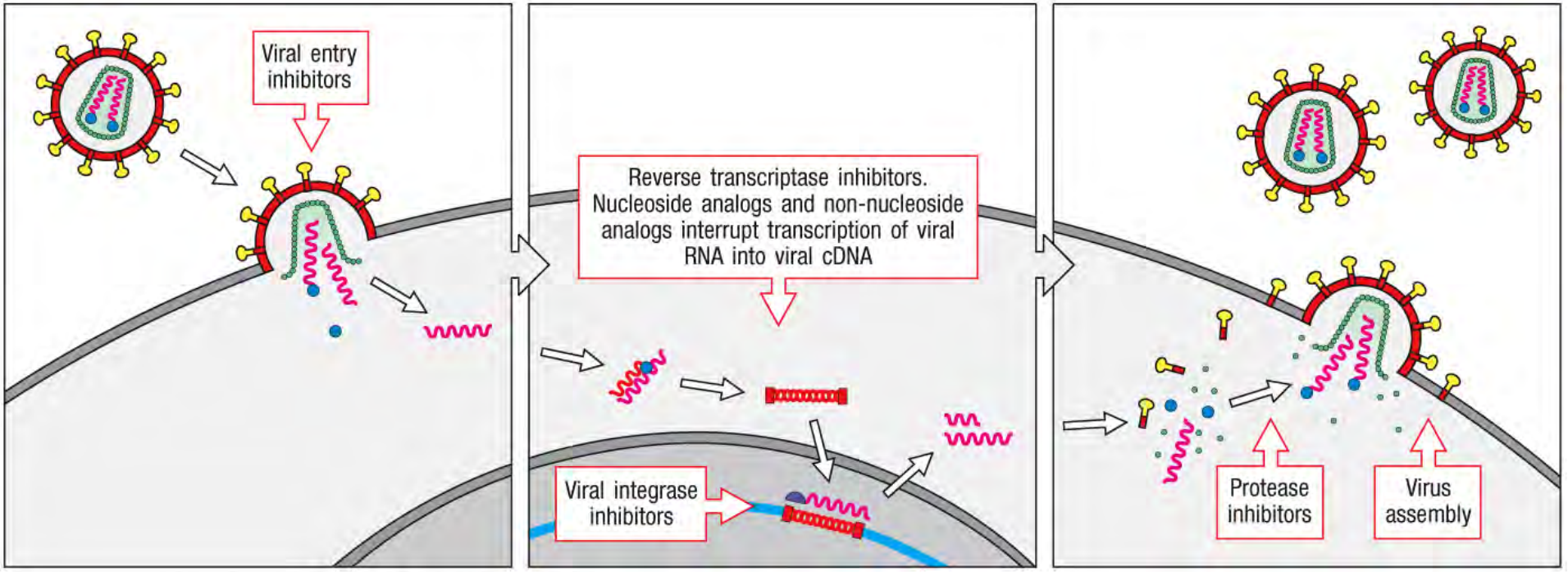


Figure 13.39 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

- **Due to high rate of viral mutations, combination therapy (HAART) is much more effective than using a single drug.**

Advent of Highly Active Antiretroviral Therapy (HAART)

Infections	
Parasites	*Toxoplasma* spp. *Cryptosporidium* spp. *Leishmania* spp. *Microsporidium* spp.
Intracellular bacteria	*Mycobacterium tuberculosis* *Mycobacterium avium intracellulare* *Salmonella* spp.
Fungi	*Pneumocystis jirovecii* *Cryptococcus neoformans* *Candida* spp. *Histoplasma capsulatum* *Coccidioides immitis*
Viruses	Herpes simplex Cytomegalovirus Herpes zoster

Malignancies
Kaposi sarcoma – (HHV8) Non-Hodgkin's lymphoma, including EBV-positive Burkitt's lymphoma Primary lymphoma of the brain

Figure 13.36 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

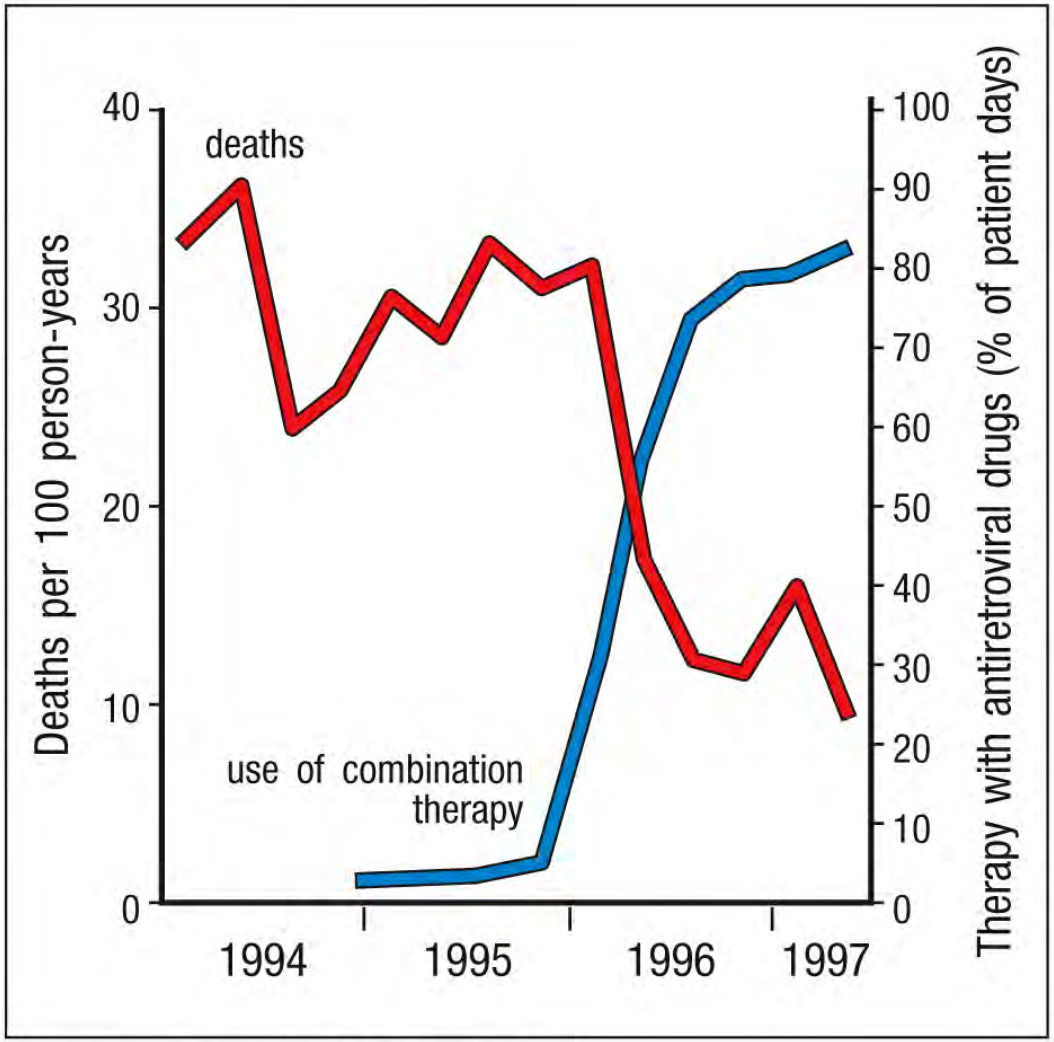
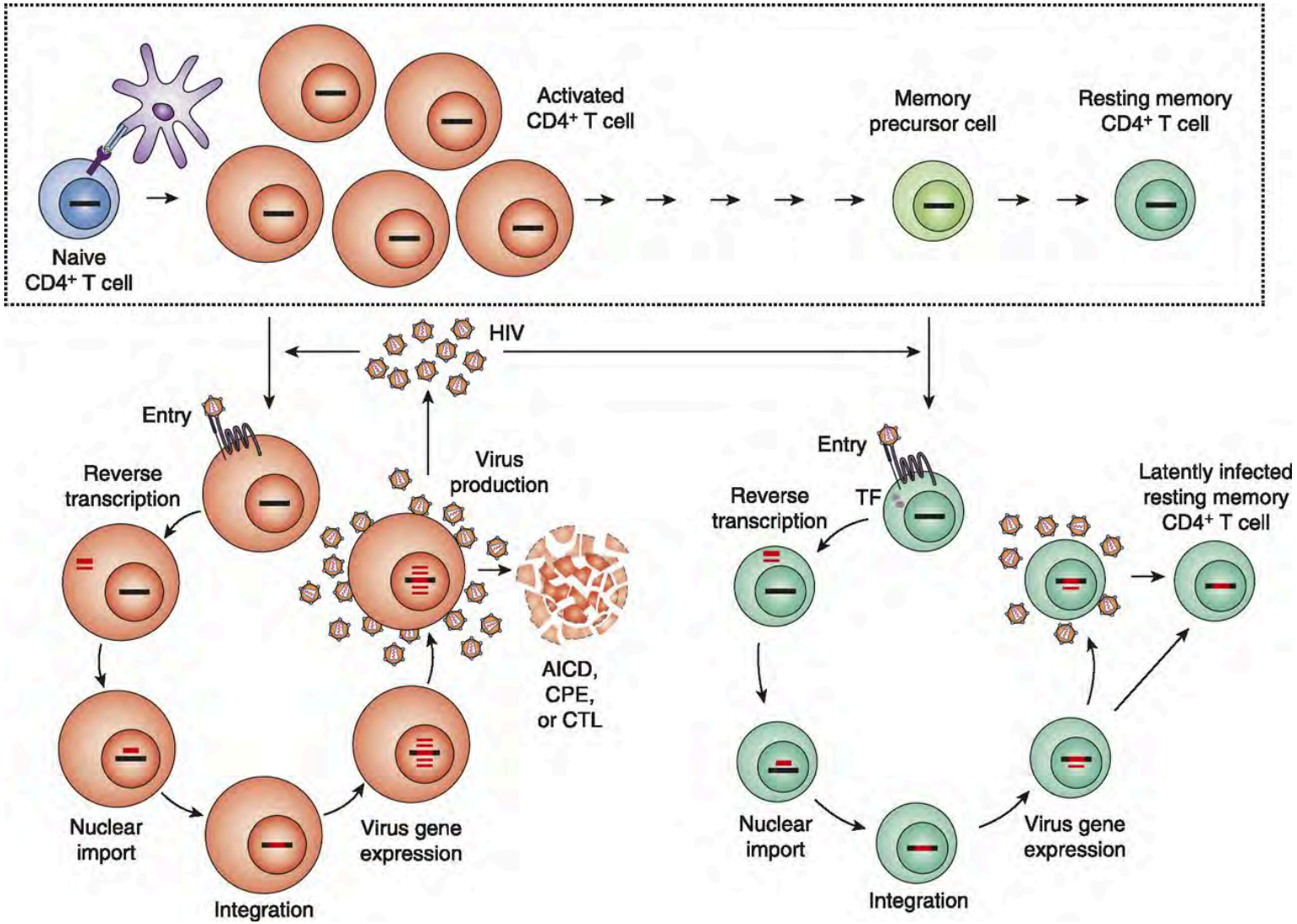


Figure 13.37 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

HIV Establishes Latency in Resting Memory CD4+ T Cells



Murray et al. J Immunol, 2016

HAART Does Not Cure the Patients!

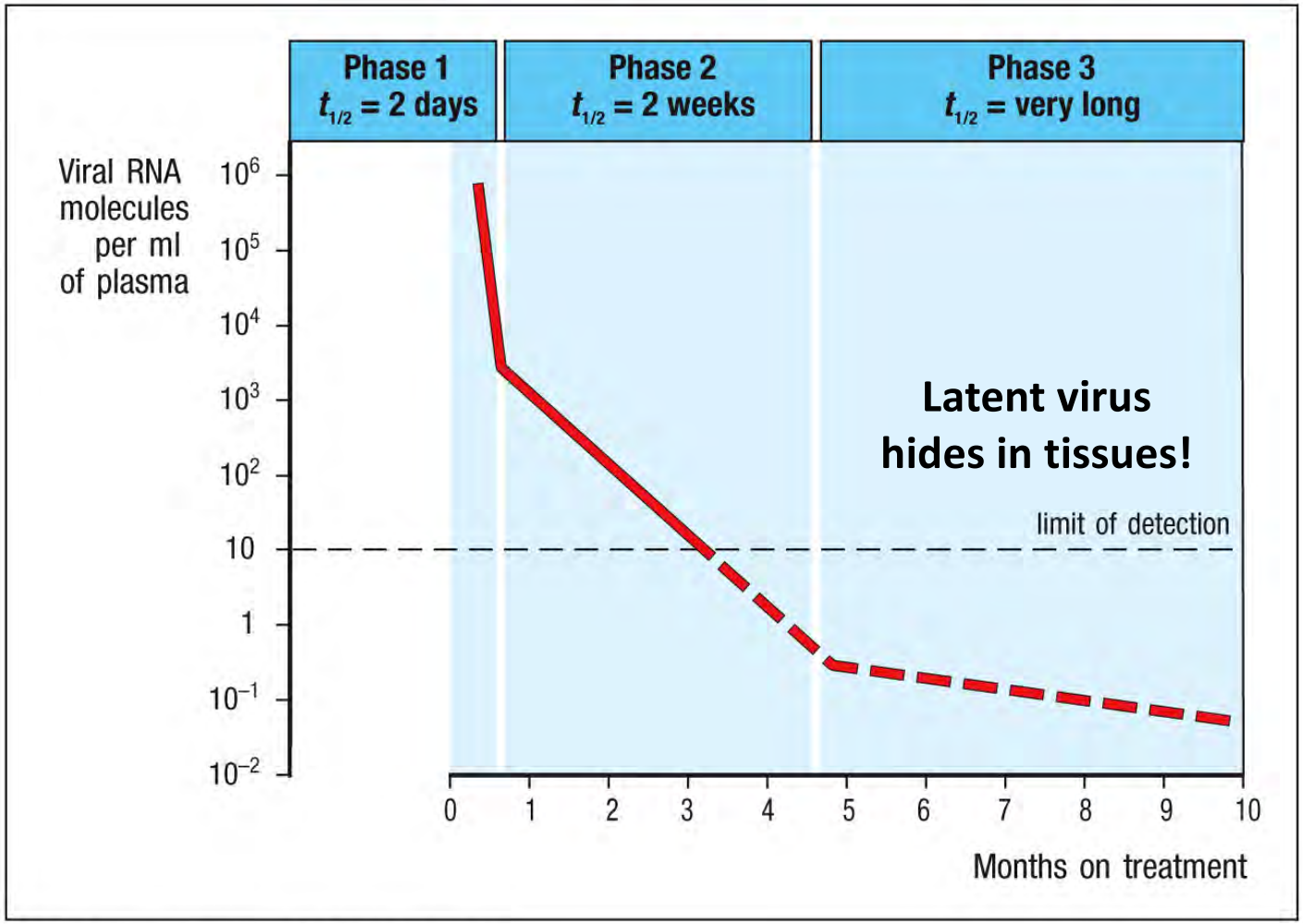
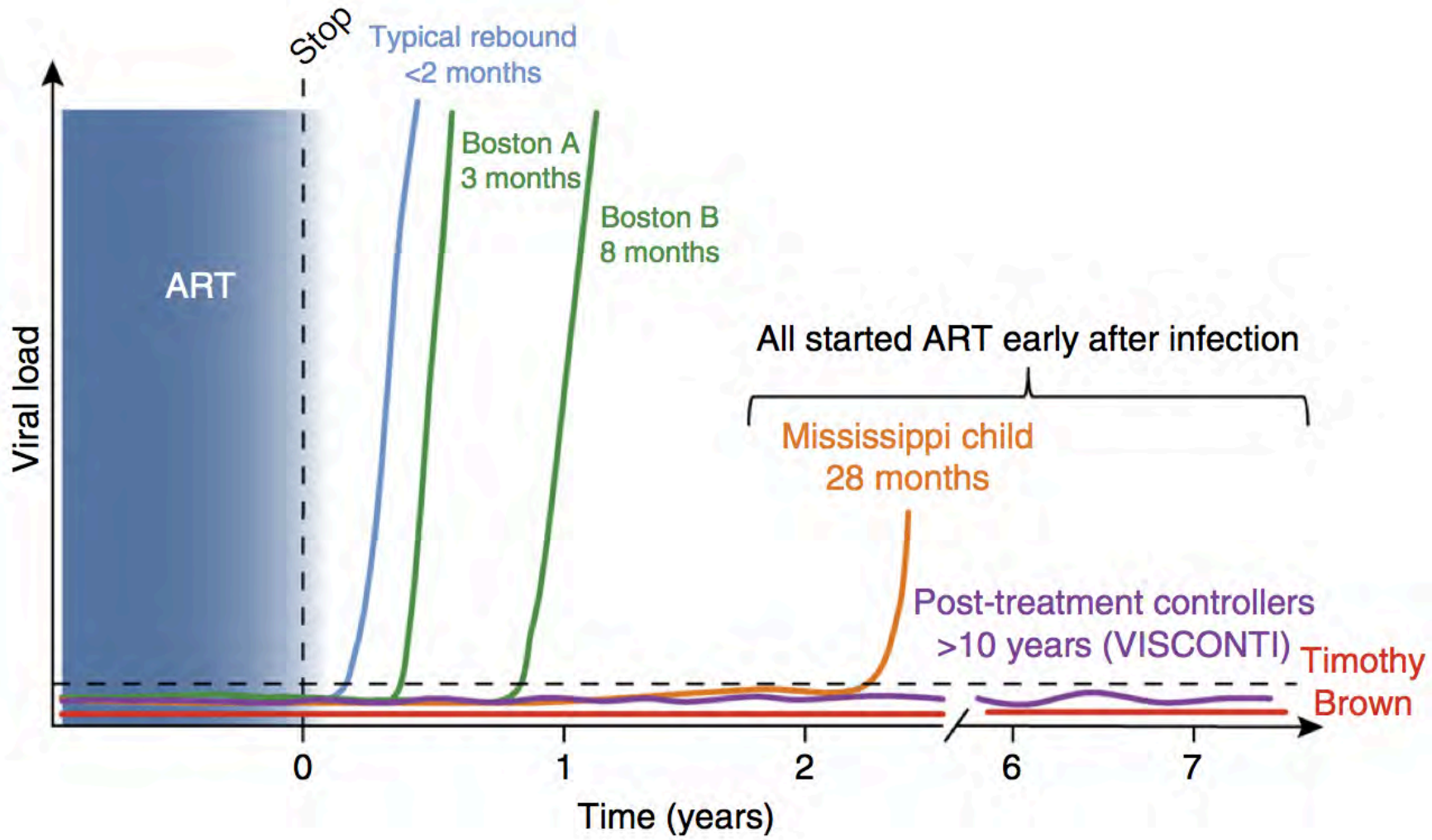


Figure 13.38 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

- **Latently infected CD4 memory T cells have a half-life of ~44 months.**
- **HAART needs to be administered for over 70 years to completely clear the virus.**

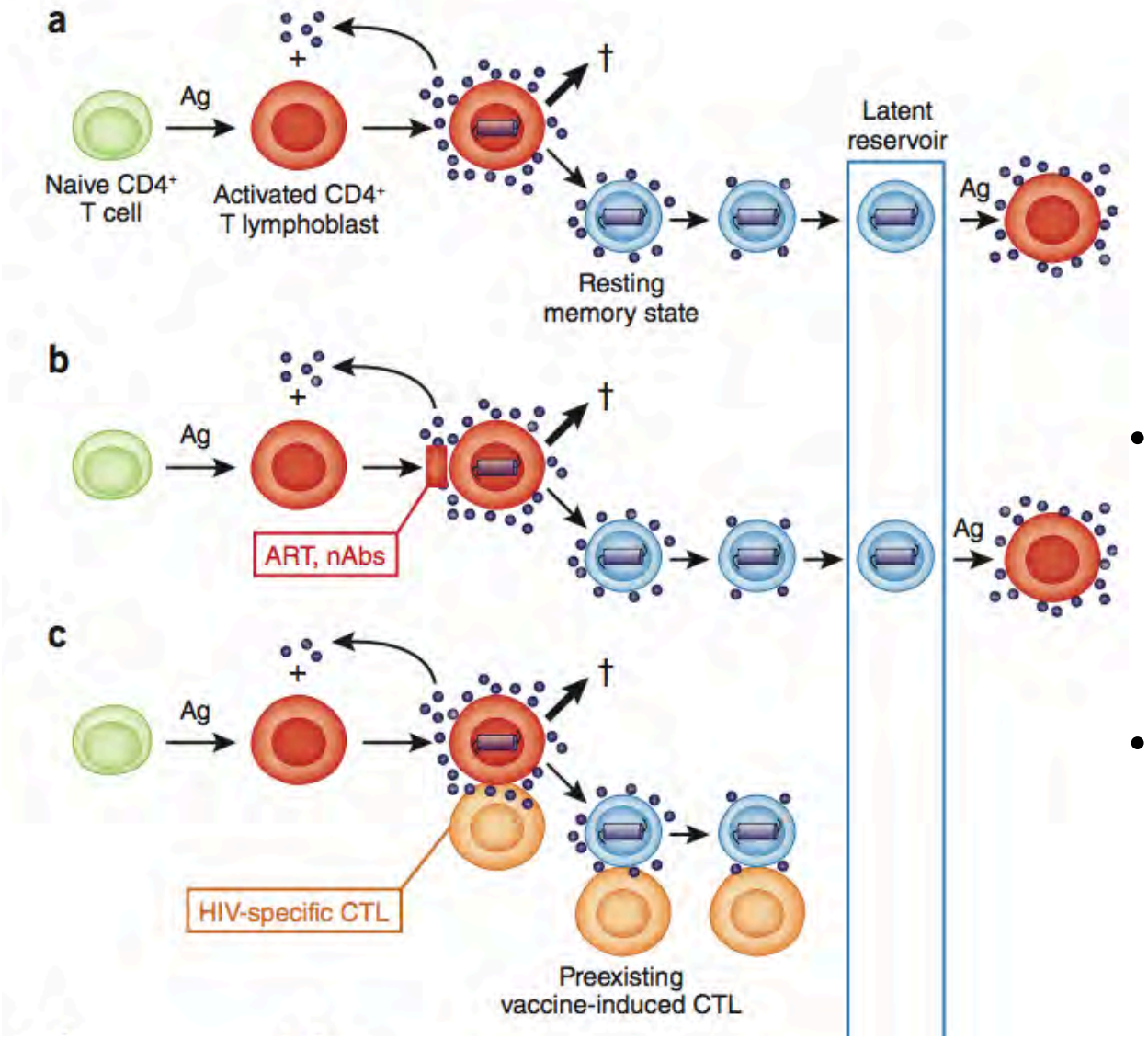
Only One Person so far Has Been Cured



How can we prevent or eliminate HIV?

Deeks et al. 2016 ***Nature Medicine***

Preventing or Eliminating Reservoirs to Cure HIV-1



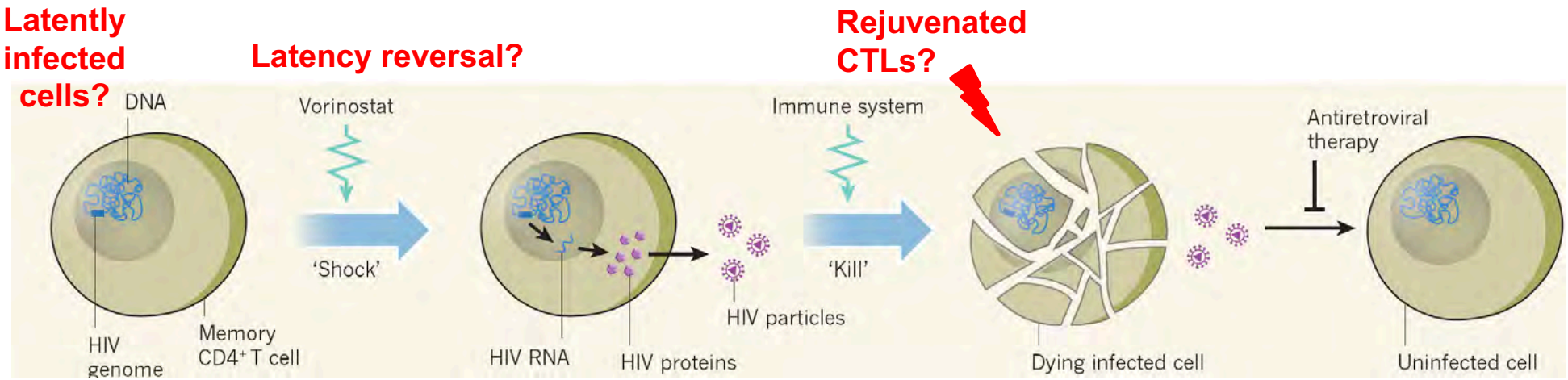
- **ART and neutralizing antibodies can stop new cells from becoming infected, but latent cells remain.**
- **Preexisting vaccine-induced CTLs can lyse infected cells before they can transition to a state of latent infection.**

Siliciano 2014 ***Nature Medicine***

How do we make latent cells visible to the immune system?

Latency is the Major Barrier to HIV Cure

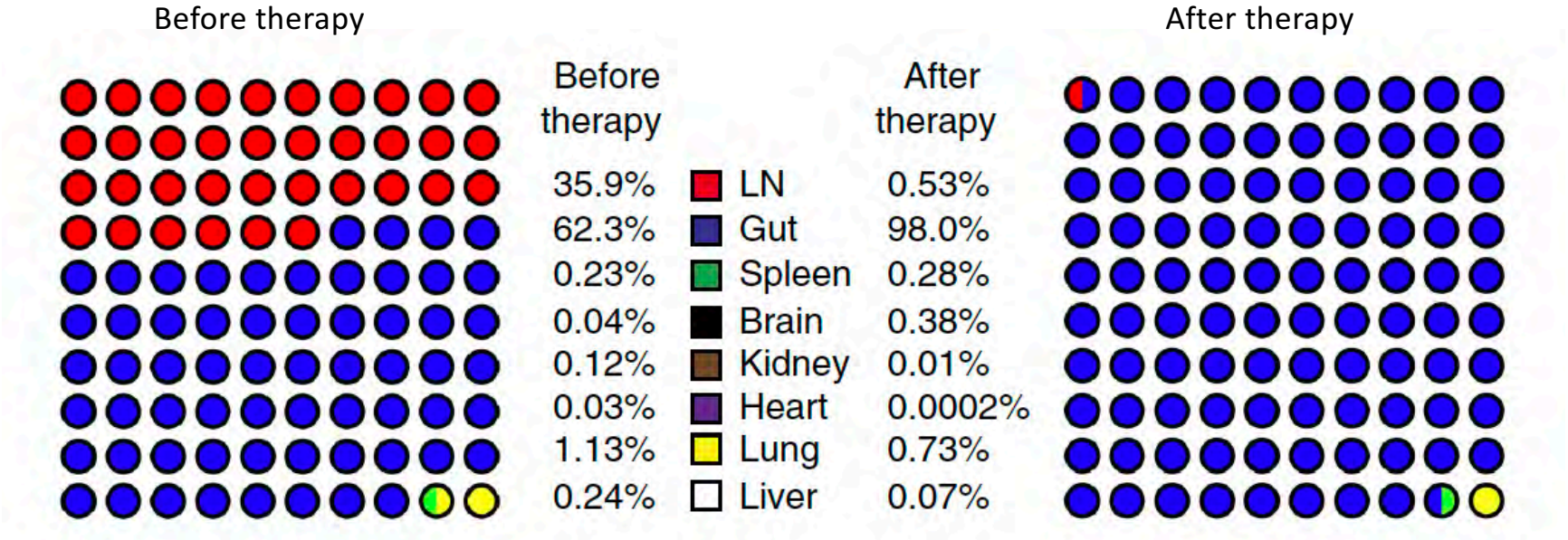
- "Shock and Kill"- Reactivate virus via immune activation and use of latency reversing agents, followed by CTL killing of virus-producing cells.



Deeks *Nature* 2012; Archin *et al. Nature* 2012

Where are the latent cells located?

HIV Remains in the Gut Even After Antiretroviral Therapy

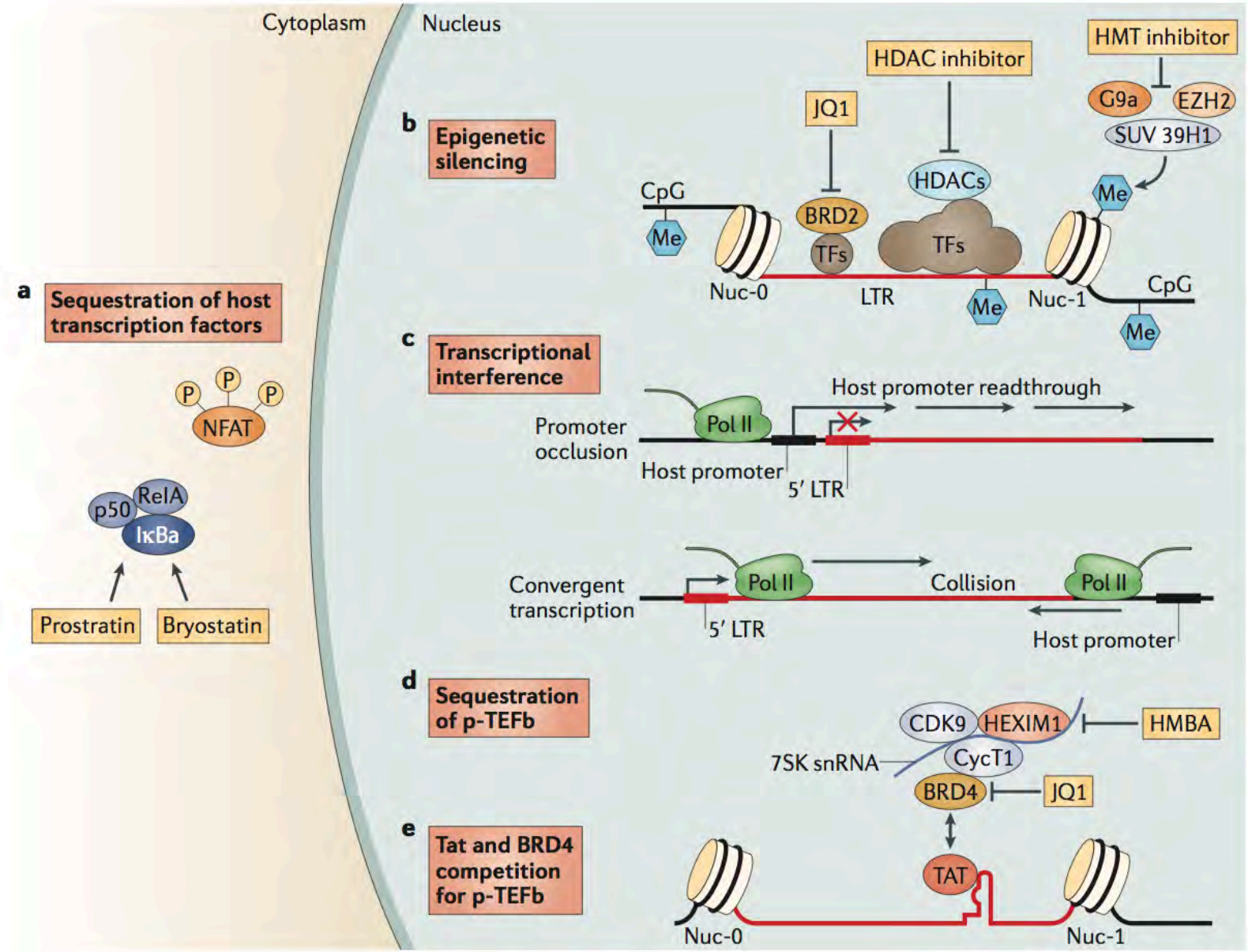


Estes et al., 2017, ***Nature Medicine***

Despite high levels of proviral DNA, low levels of HIV transcriptional activity is observed in the large bowel, suggesting a large fraction of latent HIV resides in the gut. (Yukl et al. 2010 & 2013 ***J Infect Dis***)

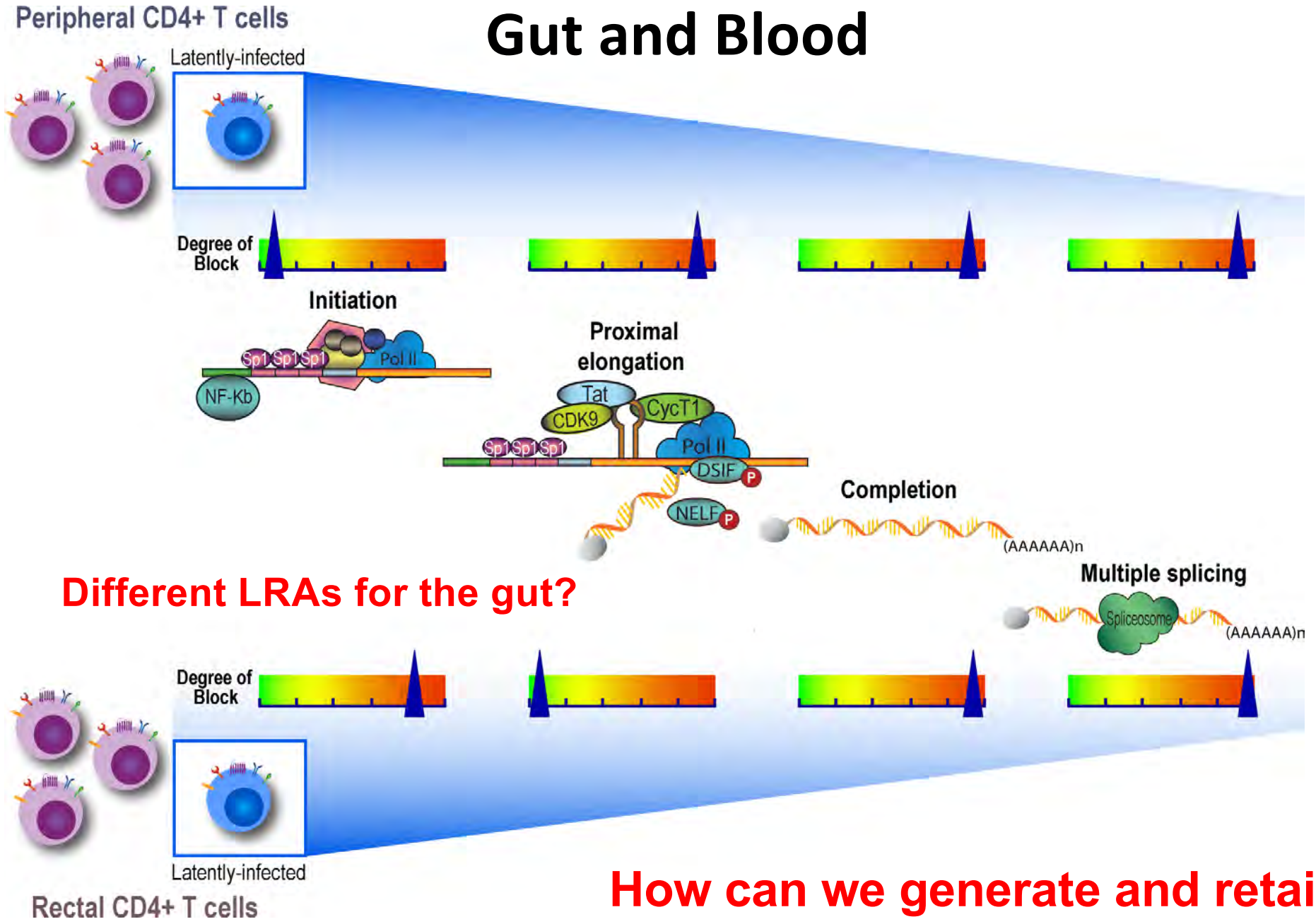
How can we activate the latent reservoir?

Strategies to Disrupt HIV Latency by Using Latency-Reversing Agents (LRAs)



Archin 2014 ***Nat Rev Microbio***

Blocks to HIV Transcription Differ in the Gut and Blood



Different LRAs for the gut?

How can we generate and retain HIV-specific CTLs in the gut?

Telwatte et al 2018 ***PLOS Pathogens***

Why Don't We Have an HIV Vaccine?

1. Escape variants/altered peptide ligands - virus operates near mutational threshold
2. Neutralizing antibodies low-affinity, arise late
3. Loss of CD4 help required for CD8, antibody responses
4. Immune exhaustion with PD-1 expression on CD4 and CD8 anti-HIV T cells
5. Prolonged time required to develop broadly neutralizing protective antibodies (bnAbs)
6. Need to learn how to generate protective TRMs through vaccination.

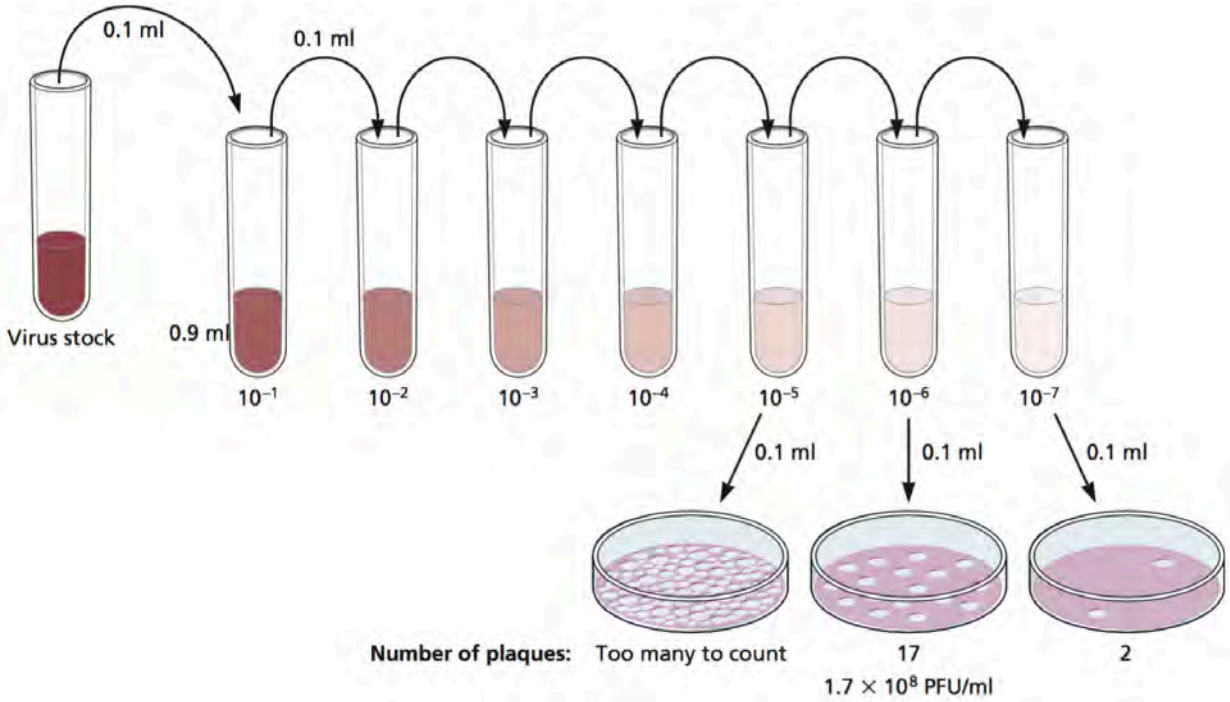
If we cure people of their HIV, will they be protected against re-infection?

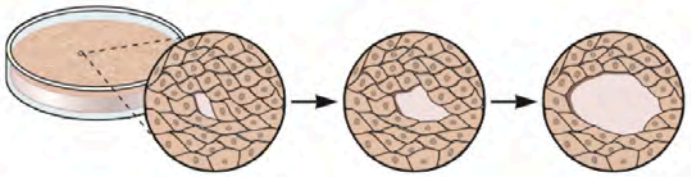
Methods to Measure Viral Titers

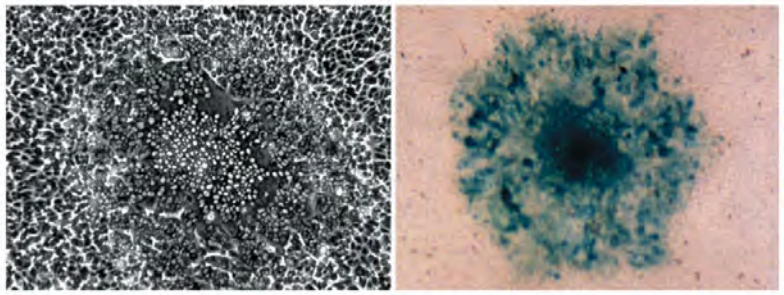
- Plaque assay
- Focus Forming Assay (FFA)
- Endpoint dilution assay
- Enzyme-Linked Immunosorbent Assay (ELISA)
- Quantitative Polymerase Chain Reaction (qPCR)/ddPCR
- Viral Outgrowth Assay (VOA)

Plaque Assay/Focus Forming Assay

1. Serial dilution of virus is made.
2. A confluent monolayer of host (susceptible and permissive) cells is infected with varying dilution of the virus.
3. Cells are covered with a semi-solid medium, such as agar.
4. A Plaque is formed when a virus infects a cell within the fixed cell monolayer, lyses that cell and infects neighboring cells. Infection-to-lysis cycle is repeated.
5. The plaques are seen visually (with help of dead cell staining) or with an optical microscope.
6. For FFA, plaques are visualized using immunostaining of viral antigens.







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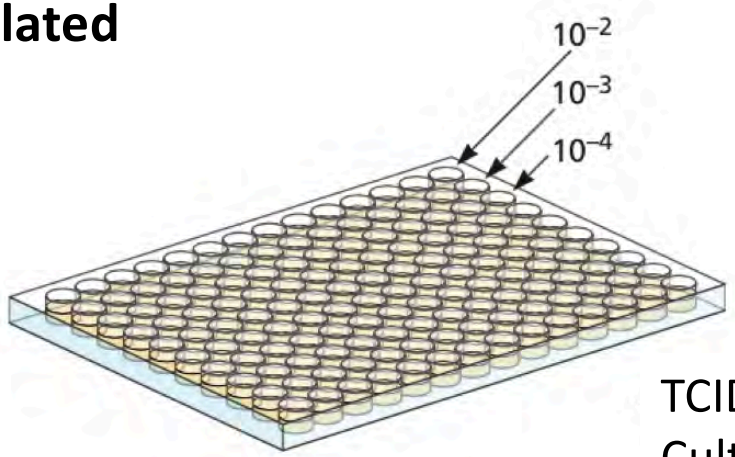
Endpoint Dilution Assay

Quantifies the amount of virus required to kill 50% of infected hosts or to produce a cytopathic effect in 50% of inoculated tissue culture cells.



$TCID_{50}$= 50% Tissue Culture Infective Dose

Virus dilution	Cytopathic effect									
10^{-2}	+	+	+	+	+	+	+	+	+	+
10^{-3}	+	+	+	+	+	+	+	+	+	+
10^{-4}	+	+	−	+	+	+	+	+	+	+
10^{-5}	−	+	+	−	+	−	−	+	−	+
10^{-6}	−	−	−	−	−	−	+	−	−	−
10^{-7}	−	−	−	−	−	−	−	−	−	−

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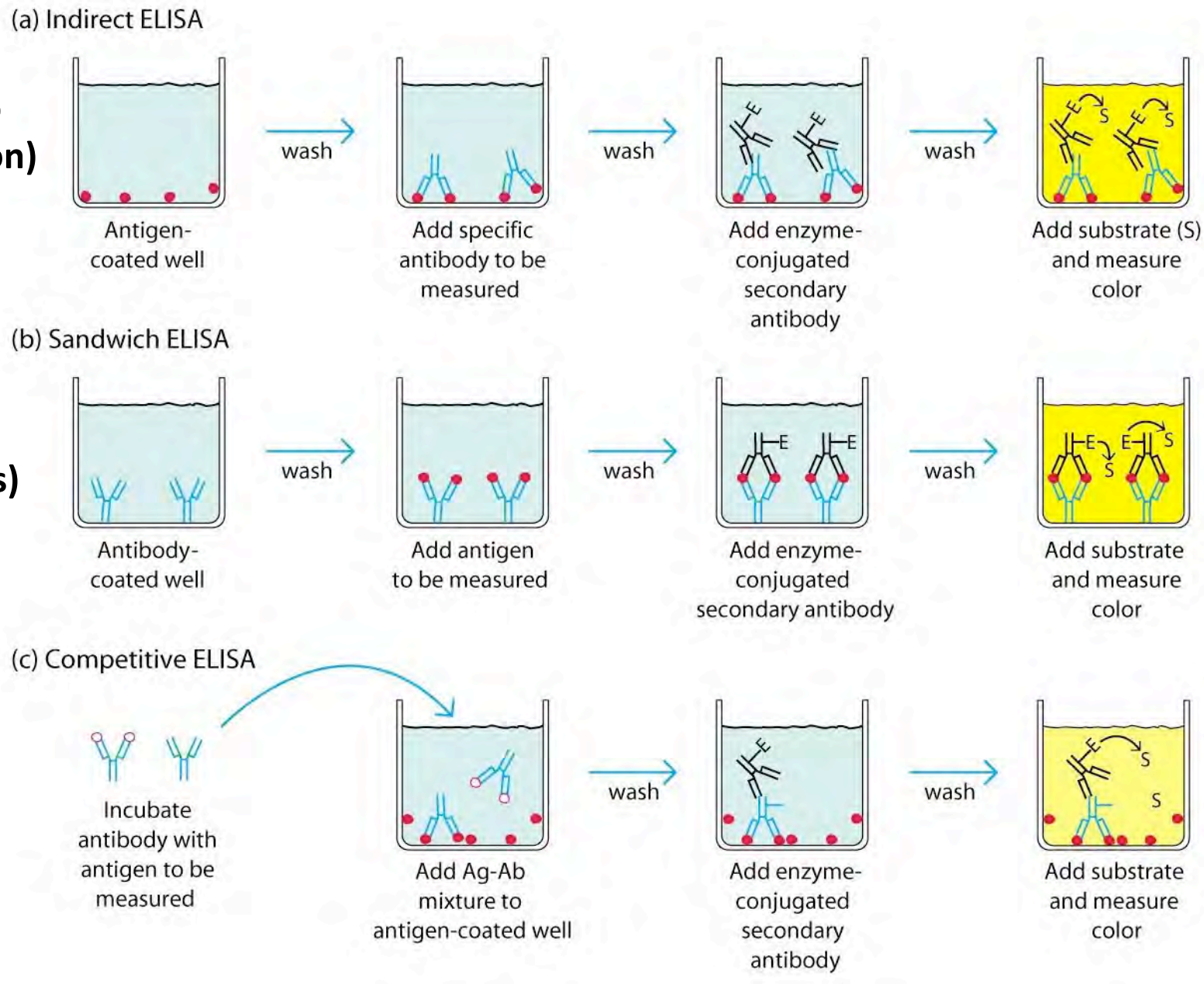
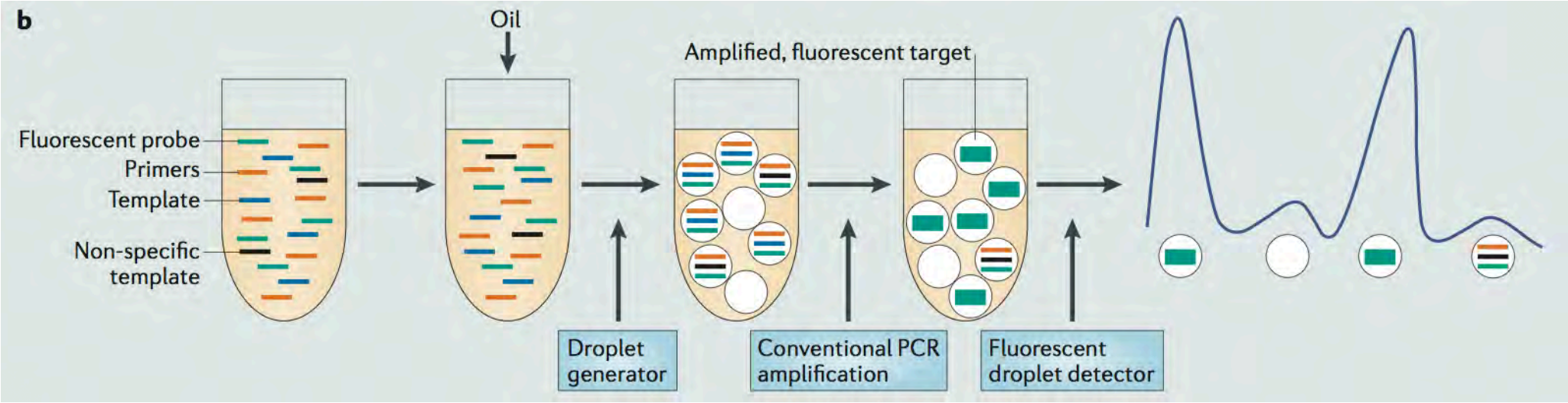
Enzyme-Linked Immunosorbent Assay (ELISA)



Quantitative/Droplet Digital PCR

PCR reaction mixture is partitioned into thousands of individual droplets such that each contains a single copy of the target, which facilitates precise endpoint quantification.

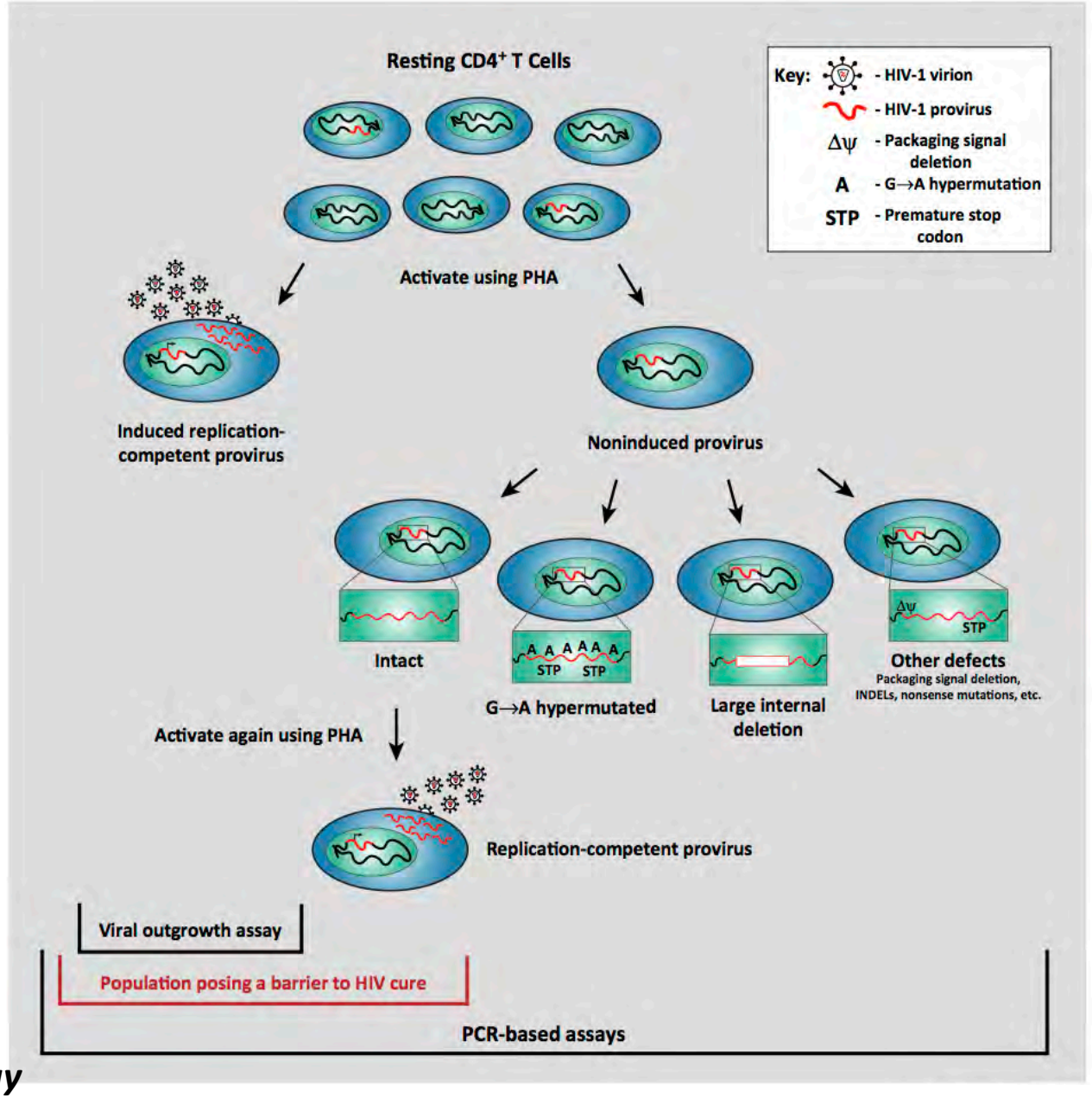


PCR-based assays cannot distinguish defective virus from intact replication-competent ones!

Archin 2014 ***Nat Rev Microbio***

Viral Outgrowth versus PCR-based Assays to Measure Latent HIV

- **Culture-based assays detect induced replication-competent proviruses only, while PCR-based assays detect all types of proviruses.**
- **Only induced replication-competent proviruses and intact, noninduced proviruses (INPs) pose a barrier to an HIV-1 cure.**



Bruner et al. 2015 ***Trends in Microbiology***