Hepatitis C Virus and Human Immunodeficiency Virus: Pathogenesis, Immunity and Treatment

Unit 3

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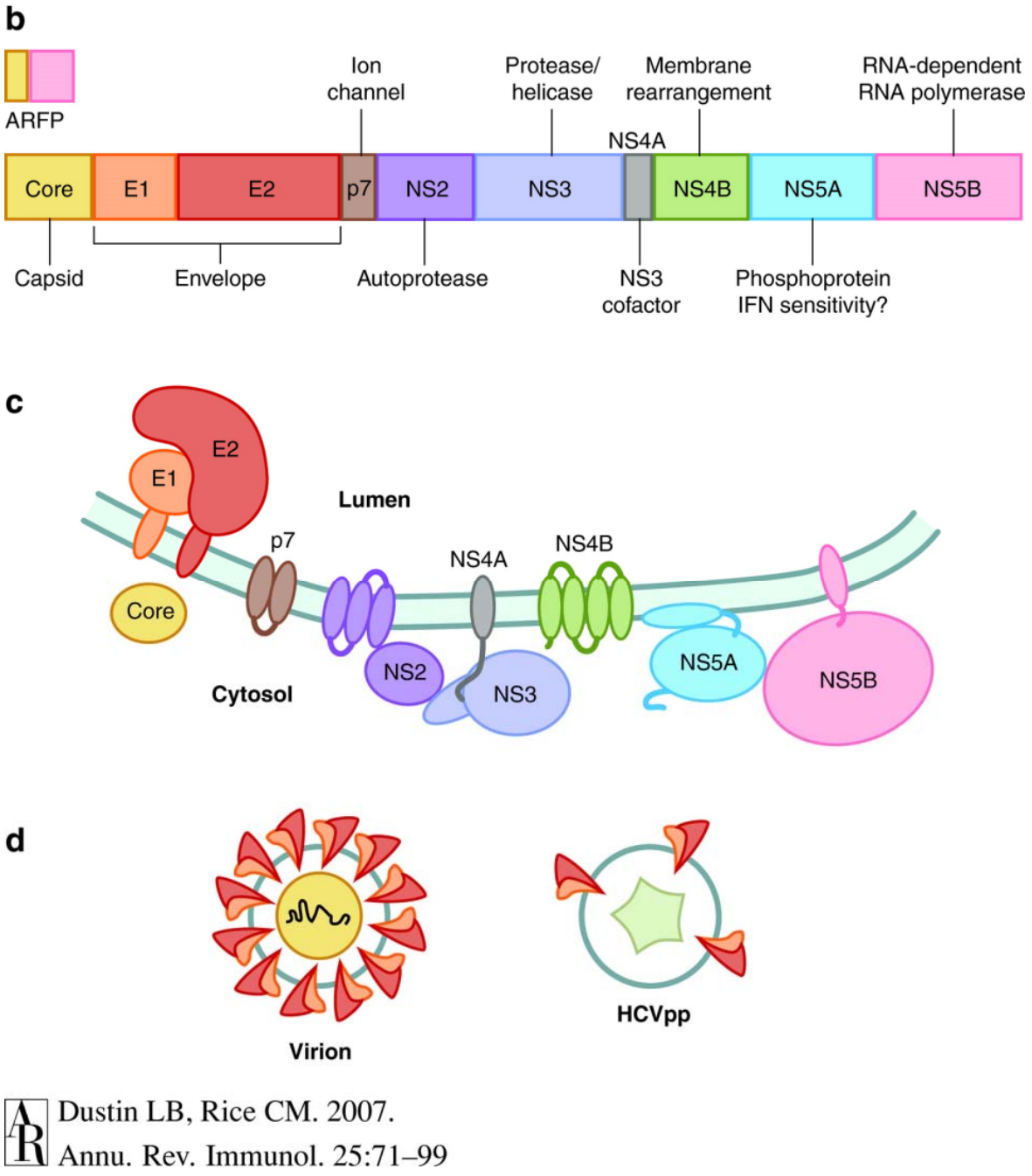
Hepatitis C virus

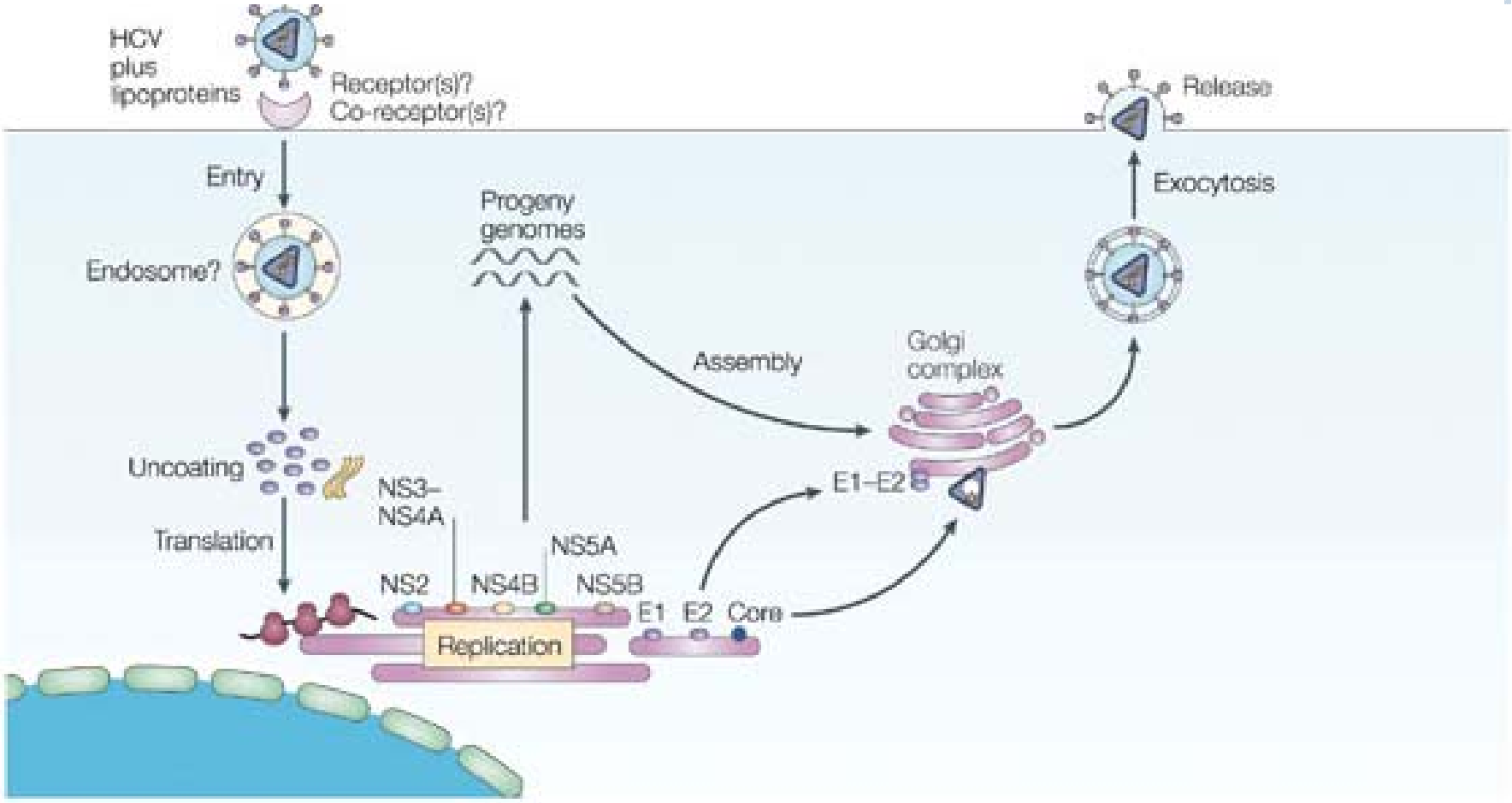
- Enveloped, positive strand RNA virus, *Flaviviridae*
- Isolated in 1989, treatments first emerged in early 1990s
- ~120 million-200 million infections worldwide, number one indication for liver transplant in the U.S.
- 10^{12} viral particles produced/day, ½ life 3 hours in circulation
- Six major genotypes, 3 dominate in the U.S. (1, 2, 3)
  - 30-50% genetic variation among genotypes
  - 1-5% variation among viruses within a single patient
- Replicates via negative-stranded RNA in membranous web in cytoplasm

HCV Structure

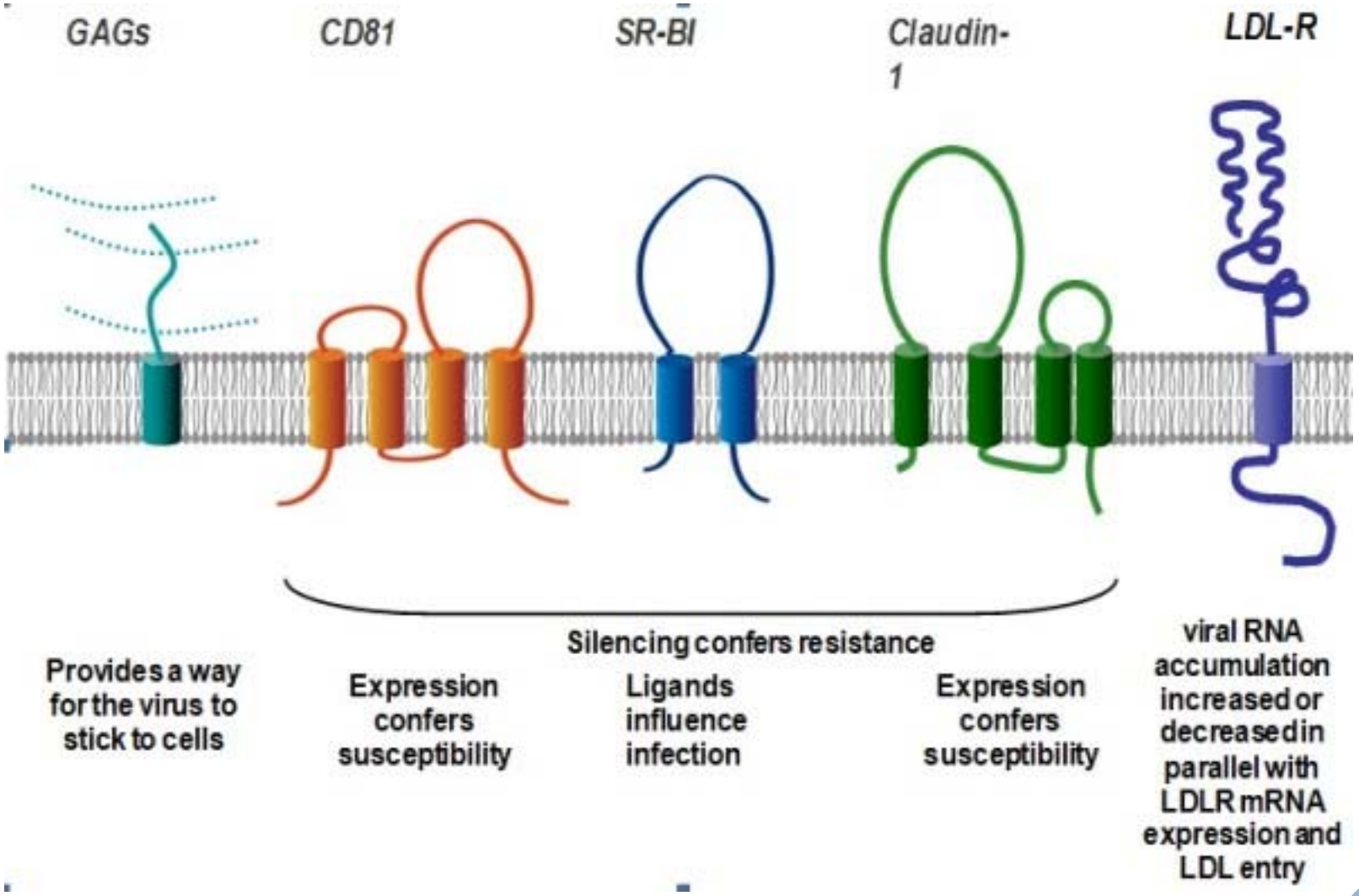


Dustin LB, Rice CM. 2007.
Annu. Rev. Immunol. 25:71–99

HCV LIFE CYCLE



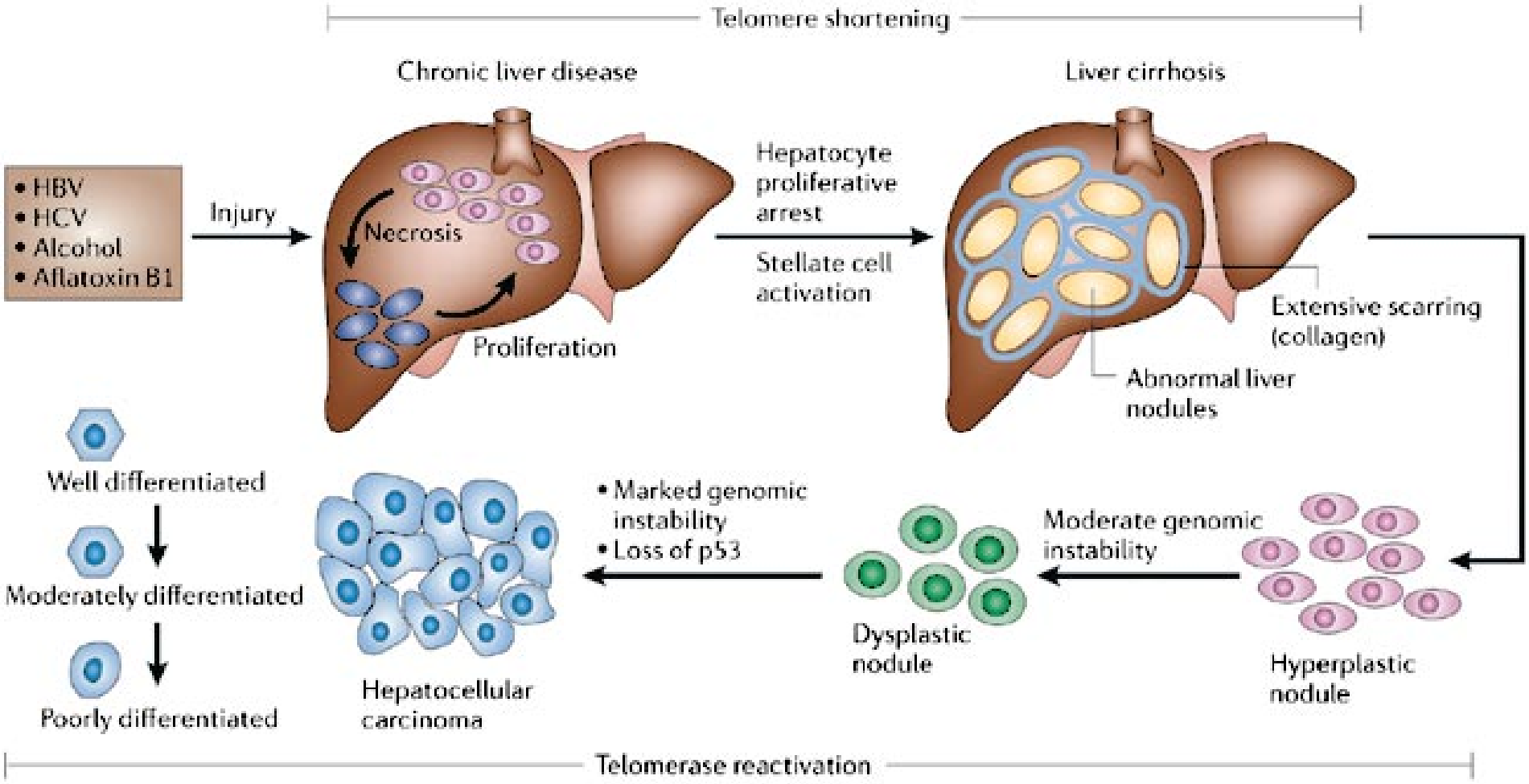
RECEPTORS FOR VIRAL ENTRY



HCV receptors for cell entry.
Ashfaq *et al. Virology Journal* 2011 **8**:161 doi:10.1186/1743-422X-8-161

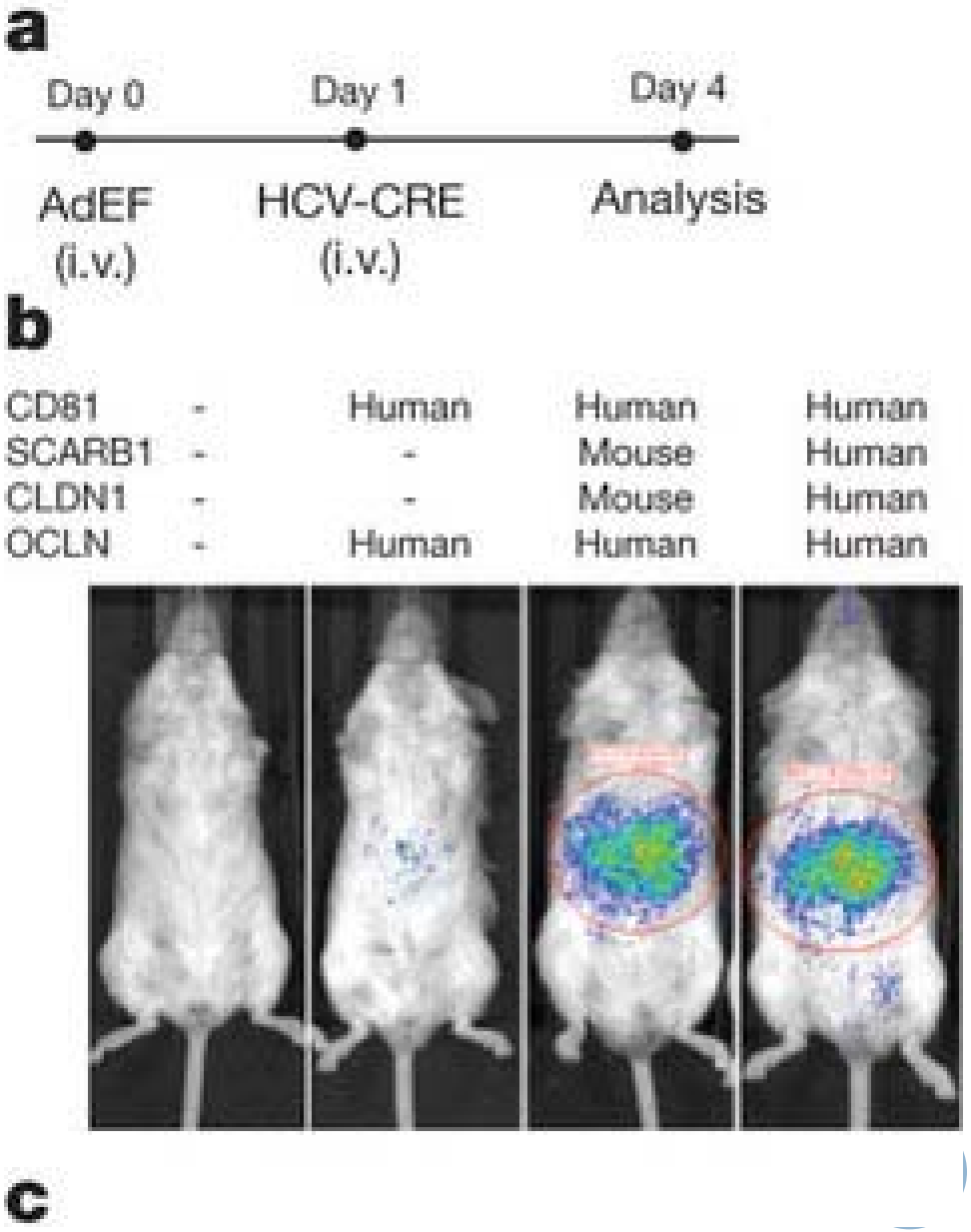
HCV Life Cycle 2

- HCV-associated disease results from viral persistence leading to long term inflammation and cell turnover



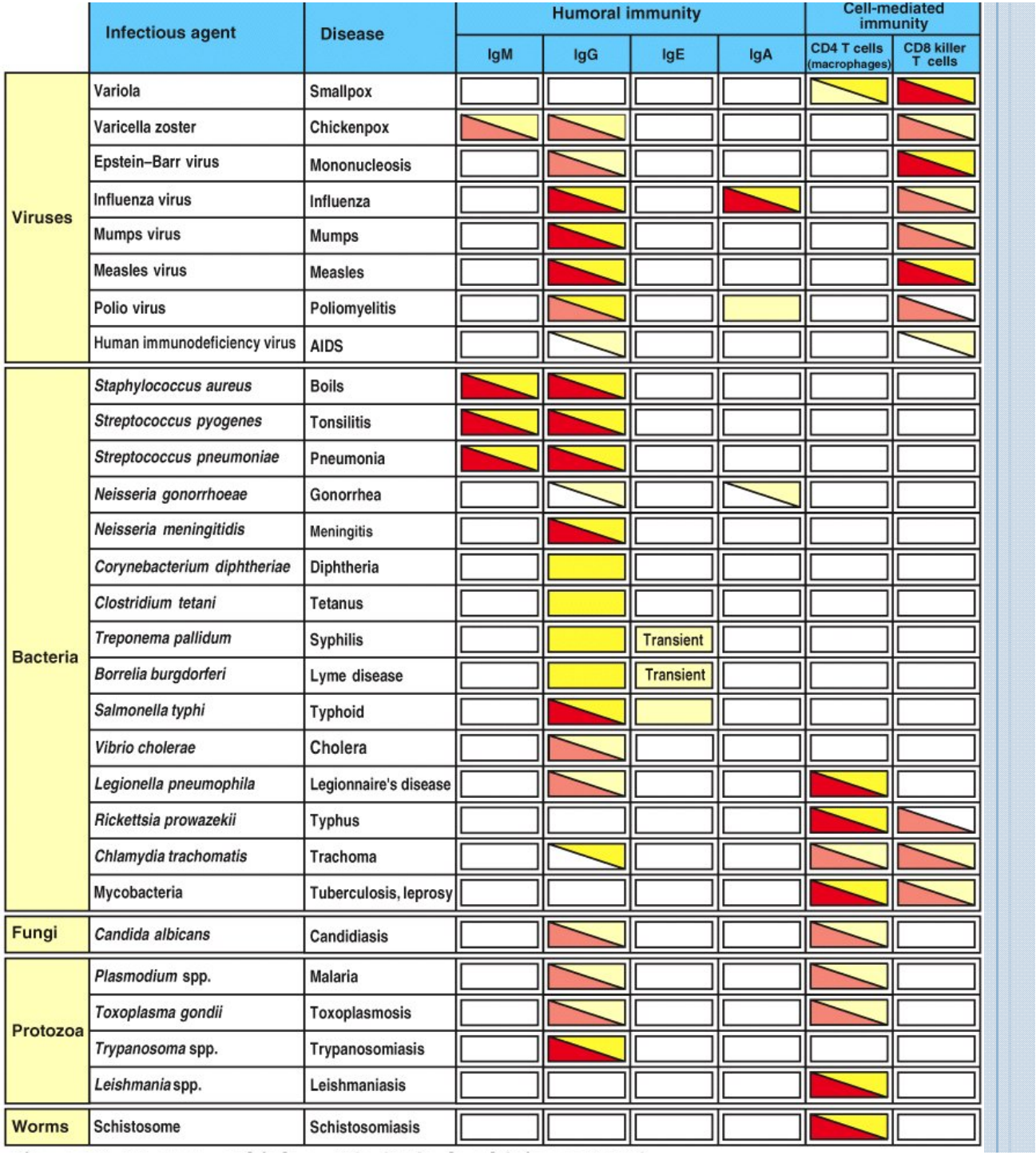
MOUSE MODEL OF HCV REPLICATION

- Previous models relied human liver transplant into immunodeficient mice—limited usefulness
- Transgenic approach using four known entry factors—Occludin, CD81, SCARB and claudin 1



A genetically humanized mouse model for hepatitis C virus infection
Nature 474, 208–211 (09 June 2011)

Specific Clearance Mechanisms For Pathogen Classes (keep in mind redundancy)



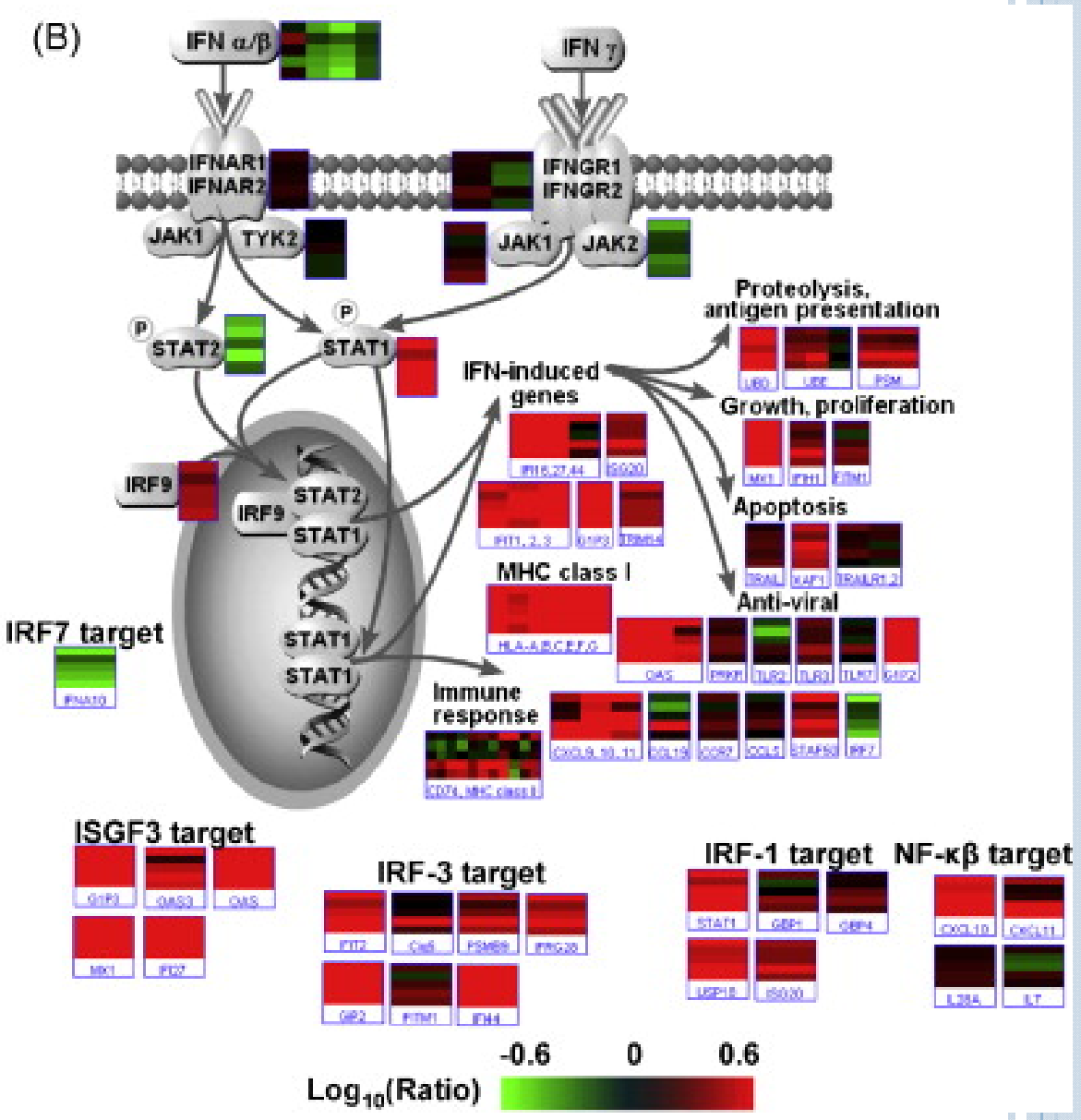
	Infectious agent	Disease	Humoral immunity				Cell-mediated immunity	
			IgM	IgG	IgE	IgA	CD4 T cells (macrophages)	CD8 killer T cells
Viruses	Variola	Smallpox						
	Varicella zoster	Chickenpox						
	Epstein–Barr virus	Mononucleosis						
	Influenza virus	Influenza						
	Mumps virus	Mumps						
	Measles virus	Measles						
	Polio virus	Poliomyelitis						
	Human immunodeficiency virus	AIDS						
Bacteria	*Staphylococcus aureus*	Boils						
	Streptococcus pyogenes	Tonsilitis						
	Streptococcus pneumoniae	Pneumonia						
	Neisseria gonorrhoeae	Gonorrhea						
	Neisseria meningitidis	Meningitis						
	Corynebacterium diphtheriae	Diphtheria						
	Clostridium tetani	Tetanus						
	Treponema pallidum	Syphilis			Transient			
	Borrelia burgdorferi	Lyme disease			Transient			
	Salmonella typhi	Typhoid						
	Vibrio cholerae	Cholera						
	Legionella pneumophila	Legionnaire's disease						
	Rickettsia prowazekii	Typhus						
	Chlamydia trachomatis	Trachoma						
	Mycobacteria	Tuberculosis, leprosy						
Fungi	*Candida albicans*	Candidiasis						
Protozoa	*Plasmodium* spp.	Malaria						
	Toxoplasma gondii	Toxoplasmosis						
	Trypanosoma spp.	Trypanosomiasis						
	Leishmania spp.	Leishmaniasis						
Worms	Schistosome	Schistosomiasis						

What Arms of the immune response are useful against HCV?

- Innate immunity
  - Antiviral effectors such as IFN that act on host cells, regulating key components of cell biology to limit viral growth and spread
- Antibody-mediated clearance
  - In principle, antibodies should be able to remove virus as it spreads from cell to cell
  - In practice, the correlation of antibody with HCV clearance and outcome is controversial or lacking
  - Patients with high levels of *neutralizing* antibodies nevertheless maintain chronic infection, indicating that neutralizing antibodies are not *sterilizing*
- Cell-mediated clearance
  - Infected cells can be killed before releasing progeny virions
  - Thought to be the primary means of long term control in HCV infection

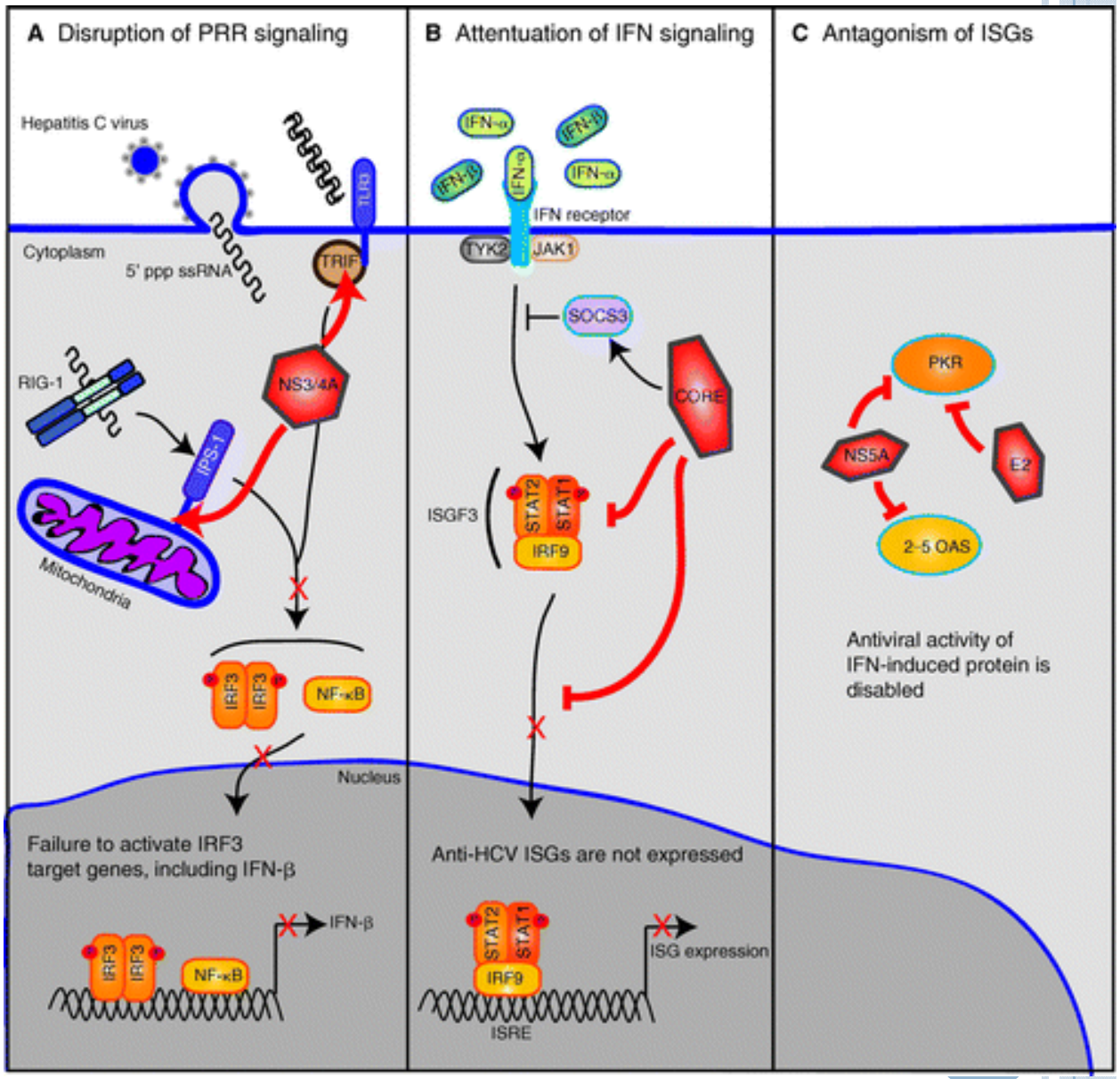
Induction of innate Immunity in Patients

- IFN-induced genes interfere with viral replication directly:
  - Reducing protein synthesis by inhibiting initiation factors (PKR, ISG56)
  - Targeting of viral RNA (OAS, RNAseL)
- Innate responses can enhance or initiate adaptive resposnes
  - MHC I expression
  - Chemokine secretion and recruitment of responder cells



Innate Recognition of HCV

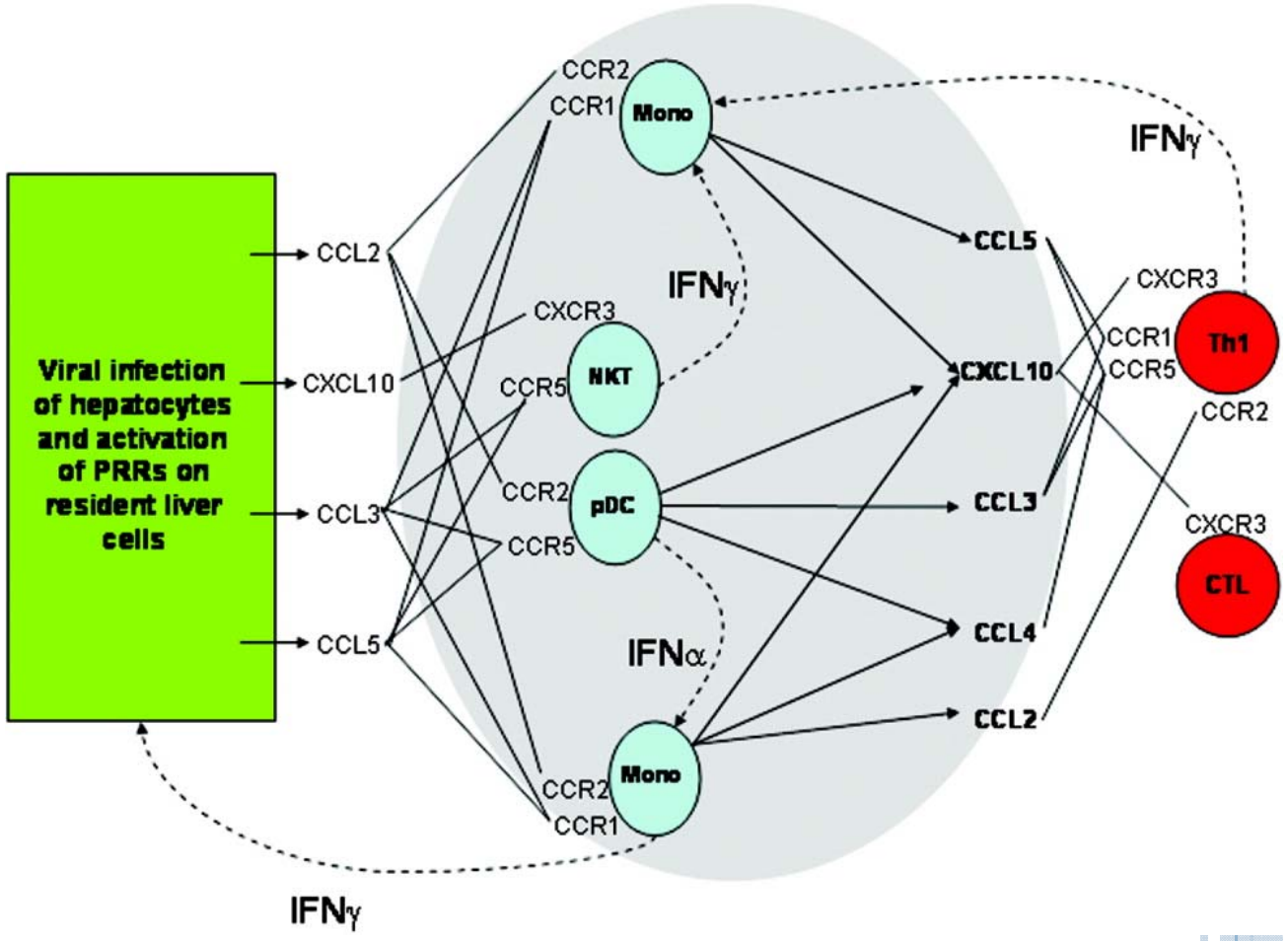
- The generation of dsRNA structures in HCV replication leads to recognition by multiple innate pathways
- HCV subverts these pathways by sequestering or cleaving key components of innate recognition
- The effects are both qualitative and quantitative on the ensuing innate response



Stacy M. Horner, Michael Gale. Journal of Interferon & Cytokine Research. September 2009, 29(9): 489-498

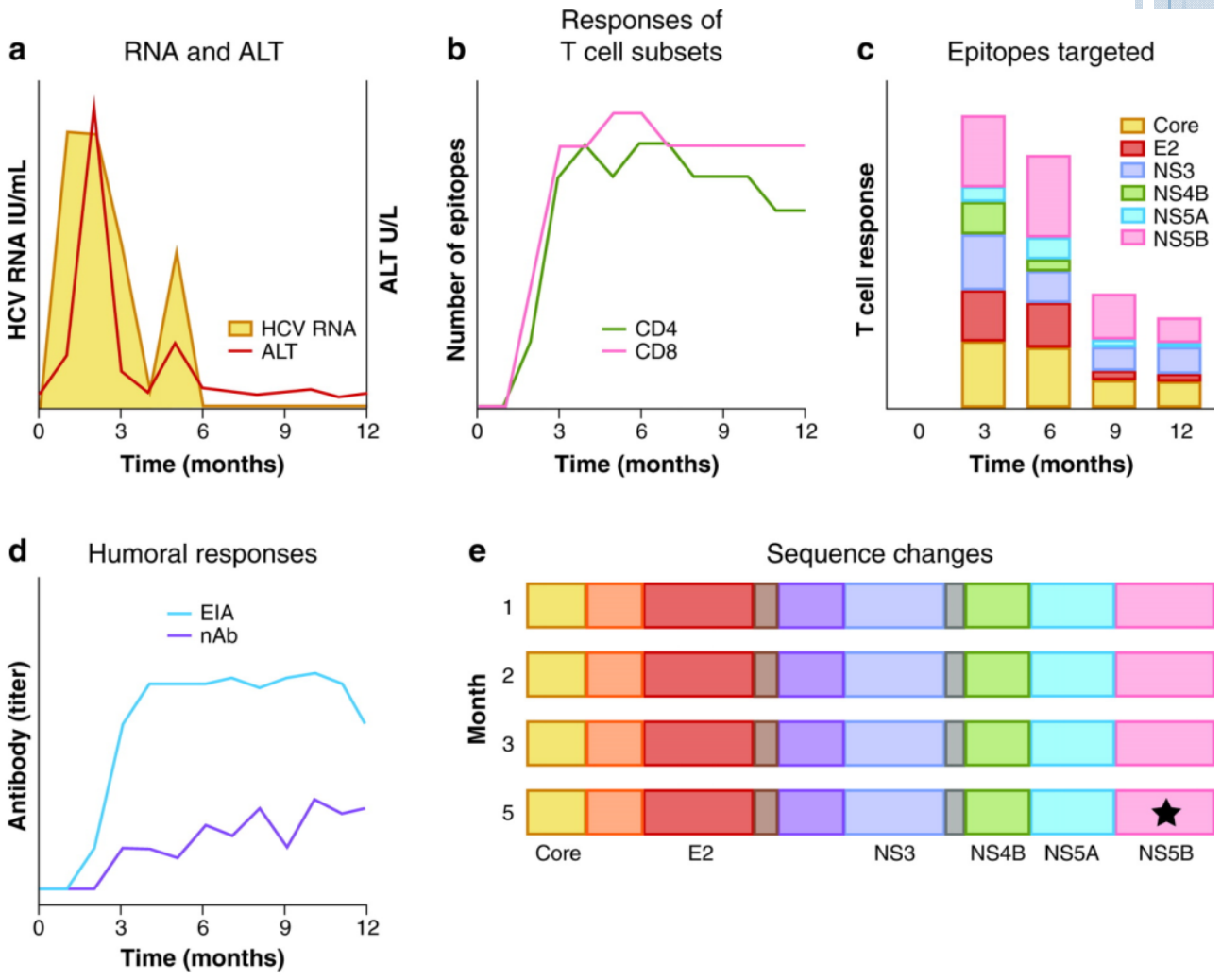
INNATE ACTIVATION OF ADAPTIVE RESPONSES

- The innate response results in the recruitment and "biasing" of key innate and adaptive cell types, including NK cells, NKT cells, antigen-presenting cells (monocytes/macrophages) and ultimately CD_4 T cells that will orchestrate the adaptive response



Successful HCV Control (Sustained Virological Response) is mediated by Robust Adaptive Immunity

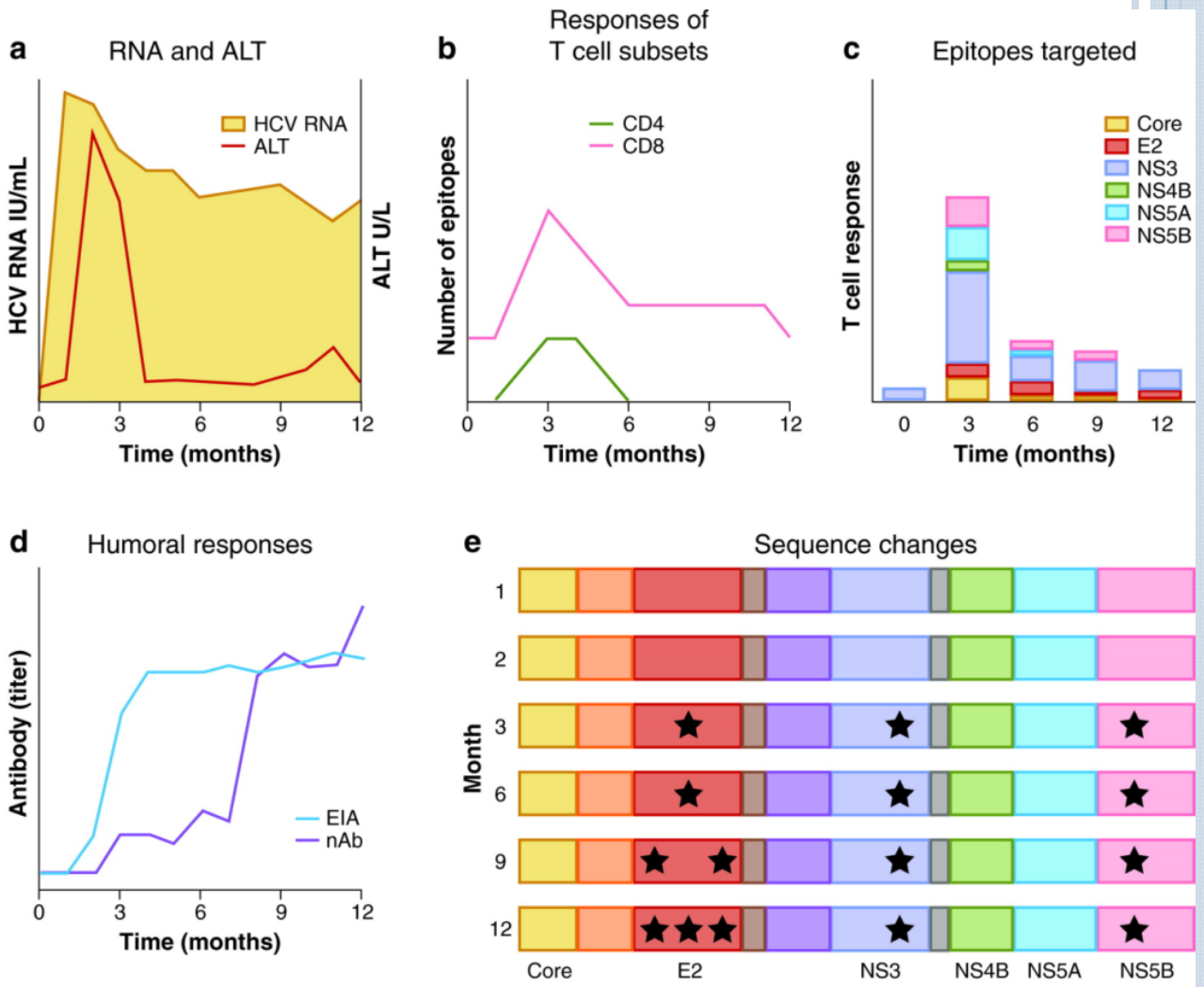
- Broad-based immunological repertoires (targeting multiple epitopes with diverse populations) control acute and prevent the development of chronic infections—particularly CD_4 and CD8 cells (the role of antibody is controversial)



Dustin LB, Rice CM. 2007. Annu. Rev. Immunol. 25:71–99

Chronic HCV infections result from poor T cell control, epitope escape and Limited Repertoires

- Limited TCR diversity, restricted epitope targets and dysfunctional T cell regulation result in weak T cell responses that are unable to avoid immunological escape



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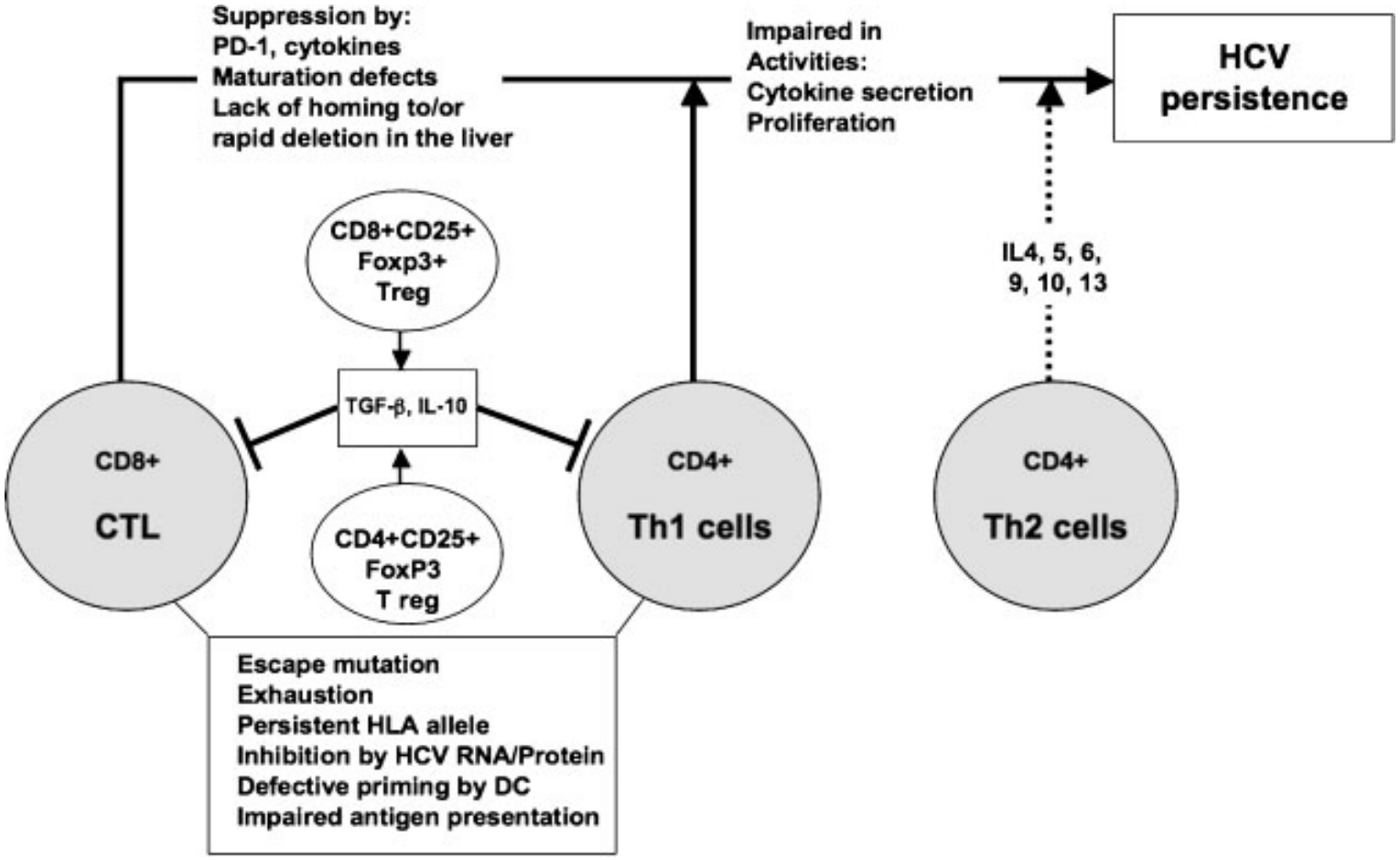
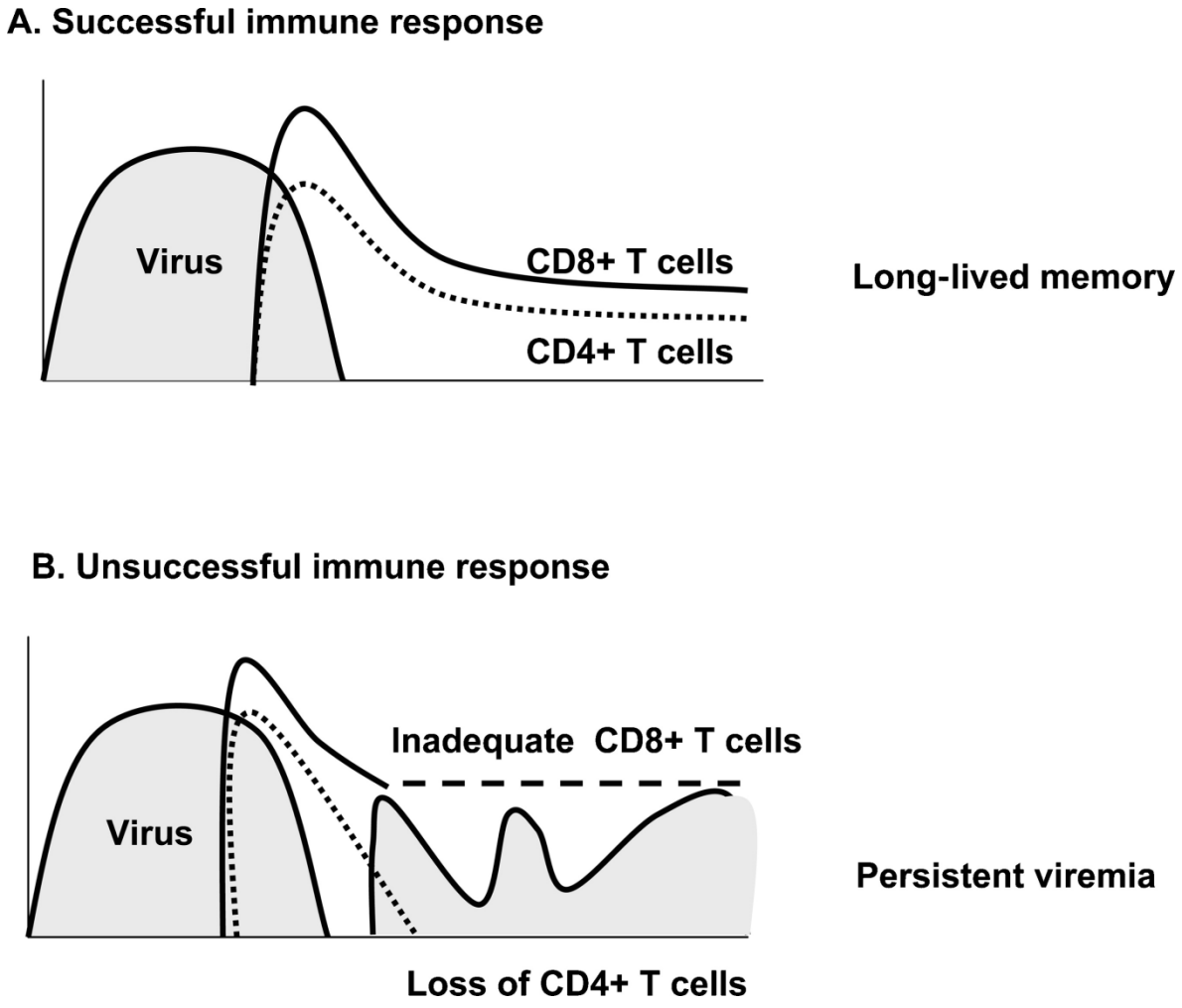
Chronic infections and Immunosuppression

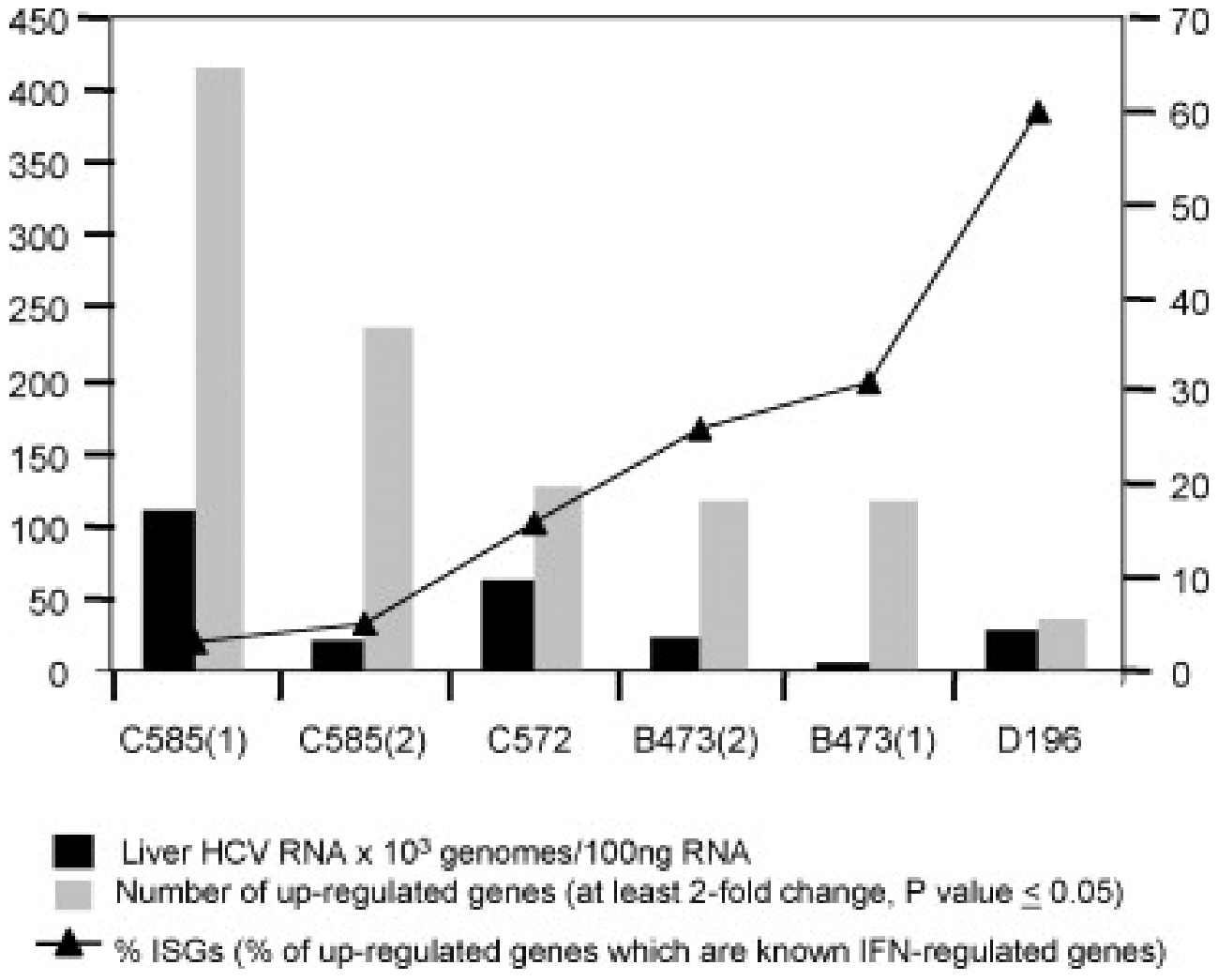


- Th2 biasing or immune senescence result in the downregulation of aggressive immunological control by CTL, providing the opportunity for viral escape and establishment of chronic infection

Sustaining an effective cellular Response is more important than Peak response numbers

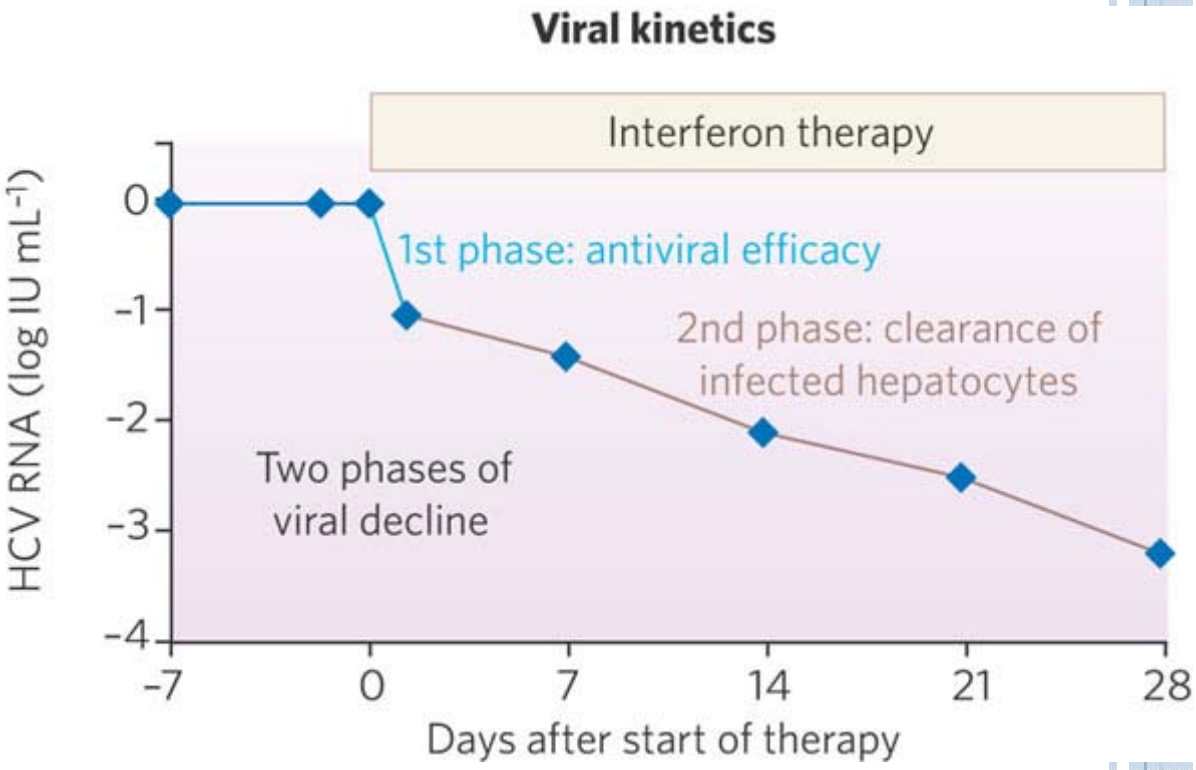


Control of Acute infection correlates with Interferon-Induced Genes



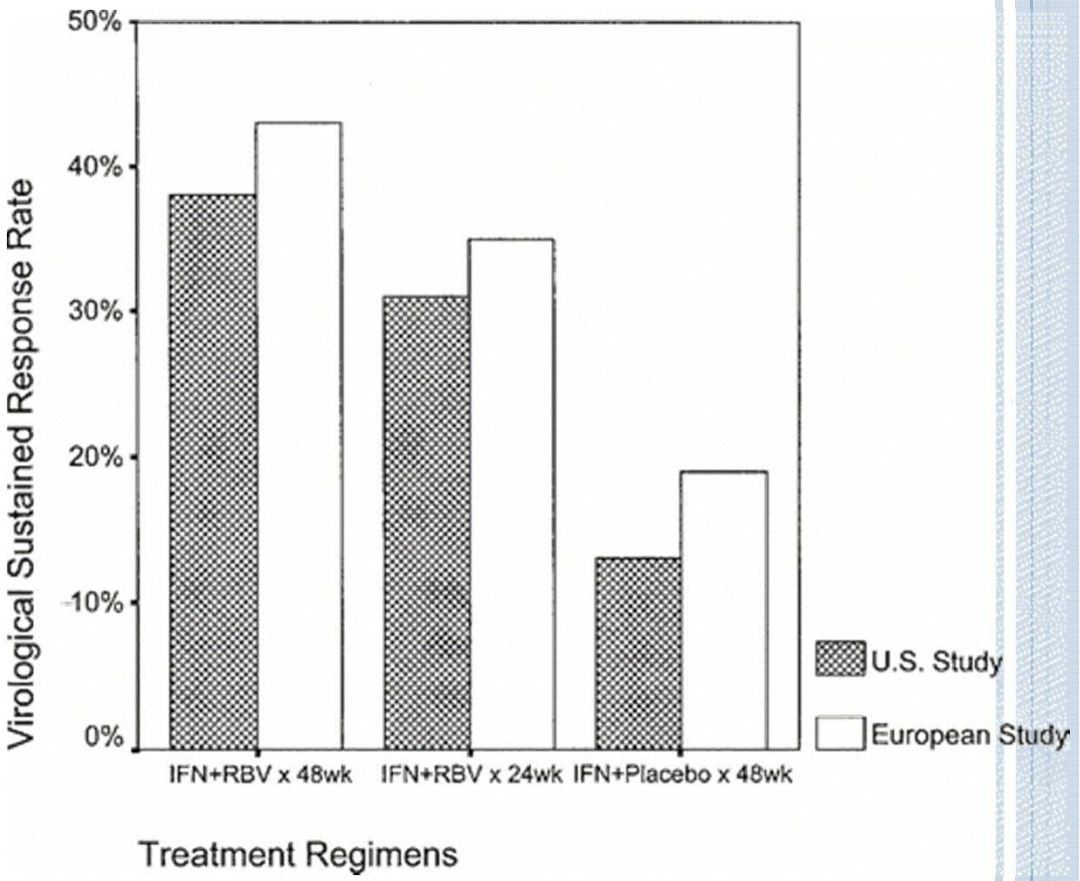
Treatment: Type I Interferon

- First therapy introduced for HCV
- Full mechanism of action unclear—presumably enhances the "normal" interferon response pathways
- Genotype of virus, low baseline levels of HCV RNA and stage of infection are the strongest correlates of efficacy
- Suggestions that immunomodulation may play a role and that high dose-inteferon may overcome some of the "regulatory" negative feedback loops active in the infected host
- Overall, the specific mechanism has not been clearly demonstrated biologically



Combination Therapy is significantly more effective

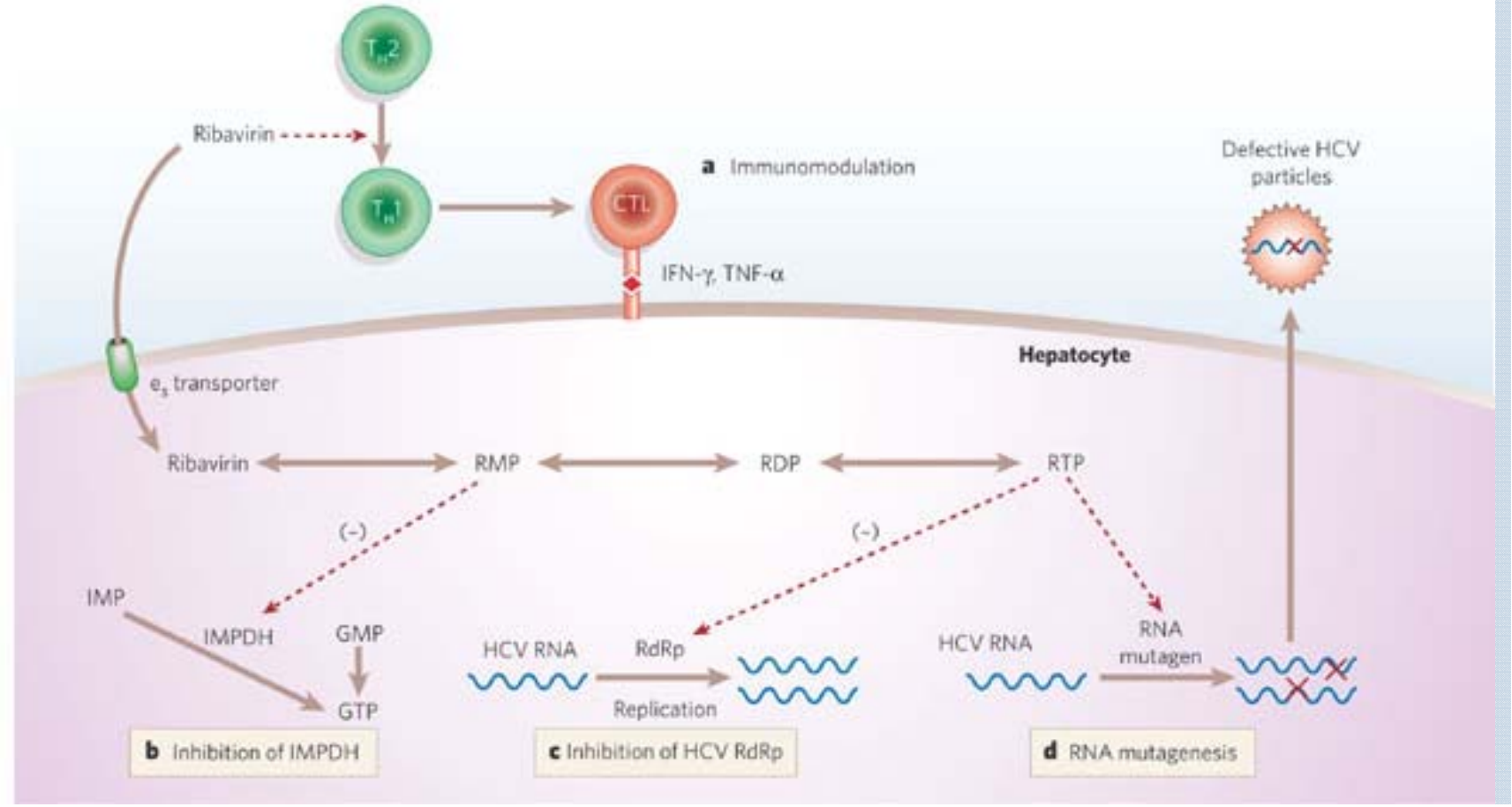
- Inteferon alone only yields a 20-25% response rate following a 12-18 month course
- Combination therapy with the "broad based" antiviral ribavirin results in 40% of individuals with SVR (30% genotype 1, 65% genotype 2 or 3)



How does ribavirin work against HCV?

- Ribavirin was initially designed as a nucleoside analog and developed as an anti-influenza drug, but failed to receive FDA approval or show significant efficacy in humans
- It has been used to treat hemorraghic fevers, RSV and is again under consideration as combination therapy for influenza
- Proposed Mechanisms:
  1) Immunomodulatory properties
  2) Inhibition of the inosine monophosphate dehydrogenase (IMPDH)
  3) Direct inhibition of the HCV-encoded NS5B RNA polymerase
  4) Induction of lethal mutagenesis
  5) Modulation of interferon-stimulated gene (ISG) expression

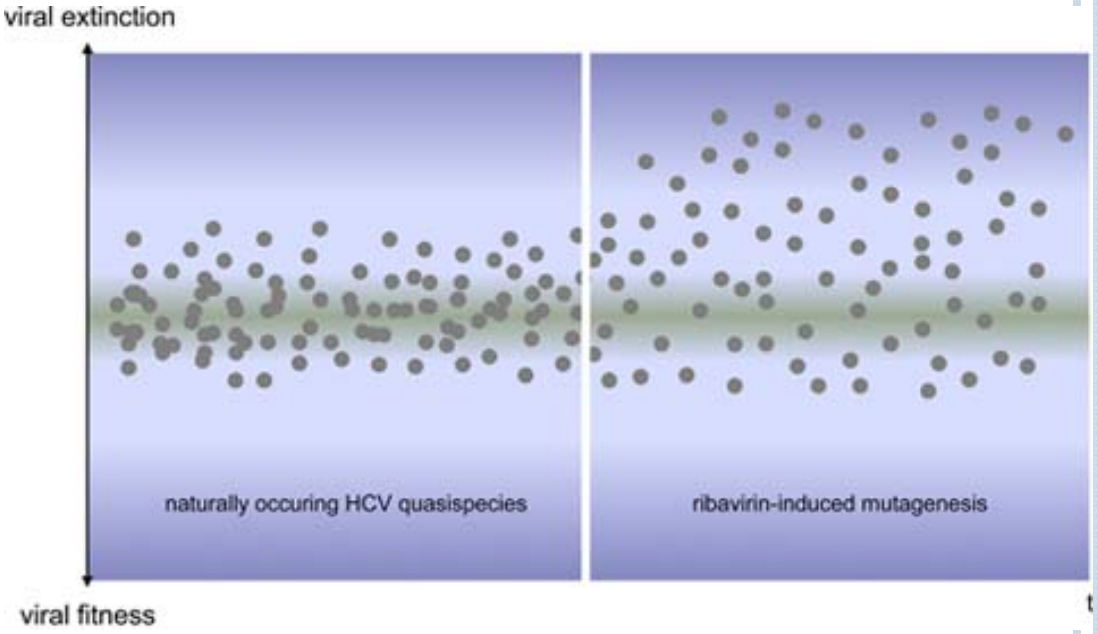
Possible Mechanisms for Ribavirin mode of action

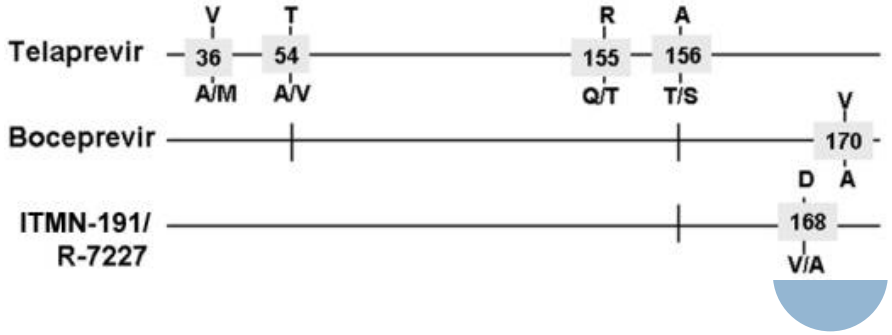


WHAT DATA WOULD HELP RESOLVE RIBAVIRIN'S MECHANISM?

Interferon reduces viral production--given the proposed mechanisms, how should ribavirin work?

1) Immunomodulatory properties—**Should act independently of interferon**
2) Inhibition of the inosine monophosphate dehydrogenase (IMPDH)**—Should reduce viral production, be guanosine dependent**
3) Direct inhibition of the HCV-encoded NS5B RNA polymerase—**Should reduce viral production, put pressure on polymerase to mutate**
4) Induction of lethal mutagenesis—**Viral production maintained, infected cell number maintained (clearance by decay), new cells infected at a lower rate**
5) Modulation of interferon-stimulated gene (ISG) expression—**Direct antiviral effects like interferon, should shift ISG expression from negative feedback pathways and be synergistic with poor interferon responders.**

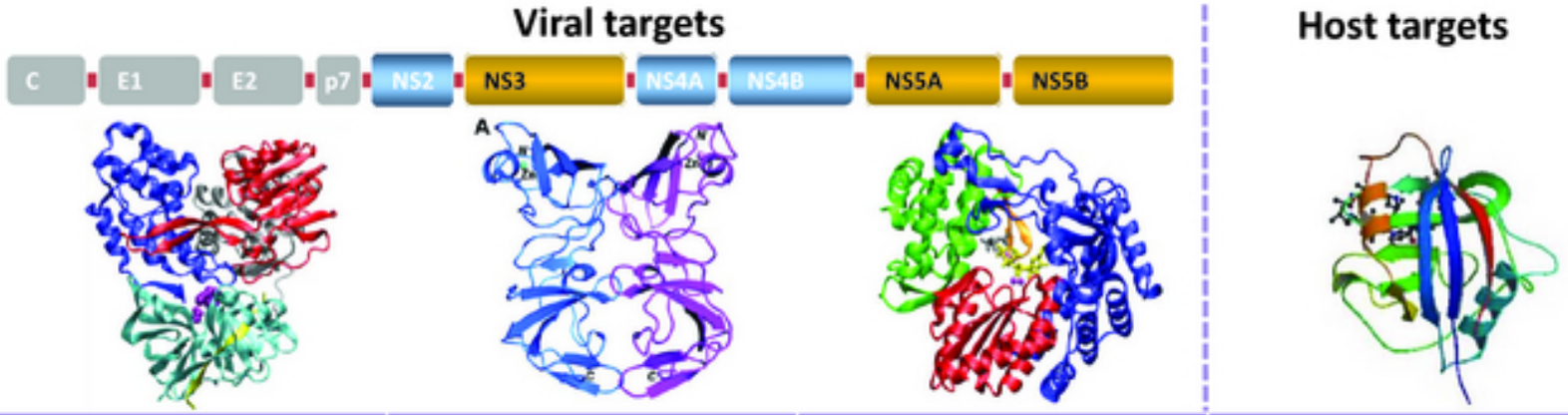




Determining an antivral treatment's mode of action

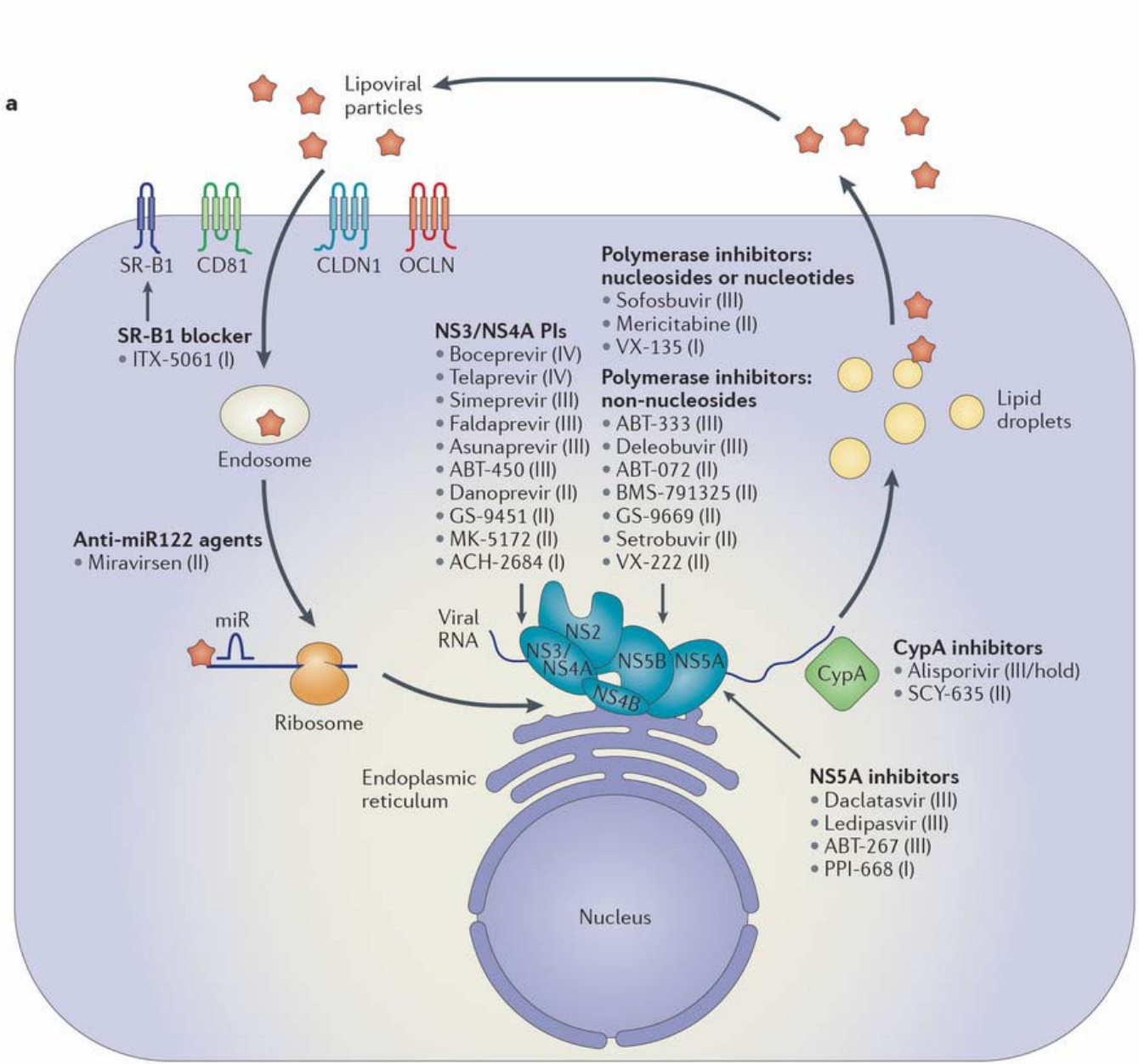
- Biological *in vitro* experiments with HCV have been difficult to perform as a result of the limited nature of developed culture systems
- Alternative drugs that perform a single "ribarvirin function" do not recapitulate ribarvirin efficacy, suggesting that multiple pathways may be acting together
- Biological mechanisms can often seem plausible, but can be difficult to prove conclusively that they play an important role (particularly when the drug is "reverse engineered" to the pathogen)
- Mathematical modeling from real infection data provides a compelling argument for the viral life cycle stage(s) that might be affected

New Drug Treatments for HCV



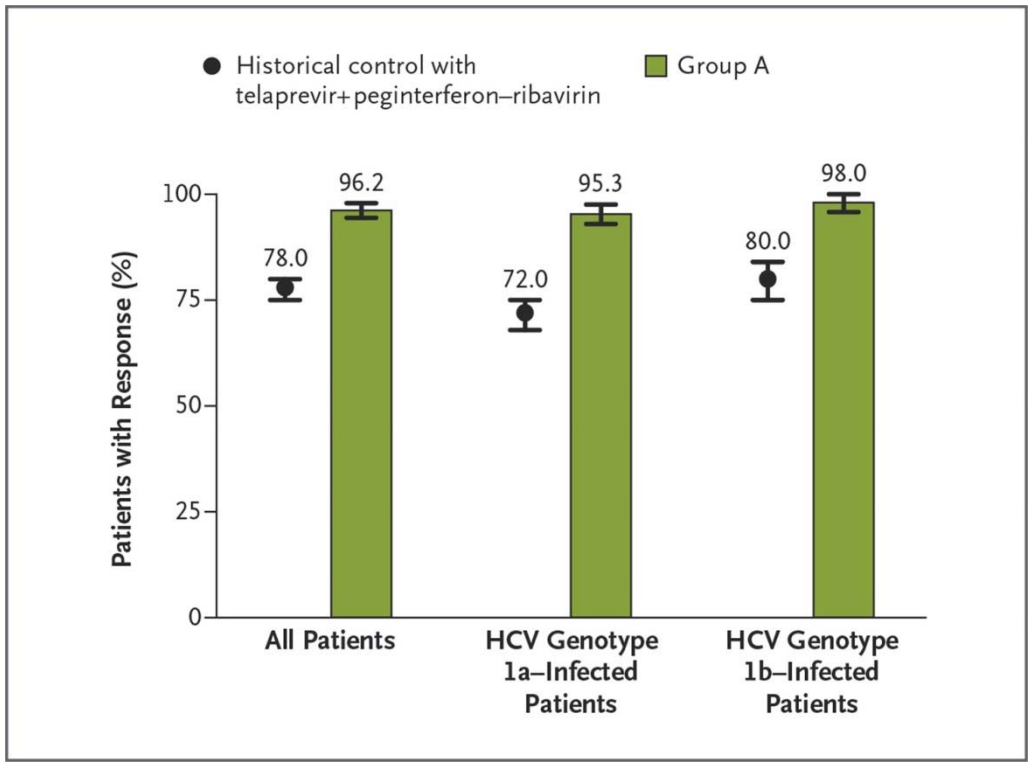
NS3	NS5A	NS5B	Cyclophilin A
The NS3/4A serine protease	Multifunctional phosphoprotein, component of the HCV-RNA replication complex	RNA-dependent RNA polymerase	Host protein interacting with NS5A and the NS5B
Boceprevir **Telaprevir** ABT-450/r, ACH-1625 Asunaprevir, TMC-435 (Simeprevir), BI-201335 Danoprevir/r, GS-9451 MK-5172	Daclatasvir GS-5885 ABT-267 PPI-668 MK	<u>Nucleos(t)ide analogue</u> GS-7977 (Sofosbuvir), Mericitabine, IDX-184 <u>Non-nucleoside analogue</u> BI-207127, ABT-333 ABT-072, BMS-791325 Tegobuvir, Setrobuvir VX-222, Filibuvir	Alisporivir SCY-635

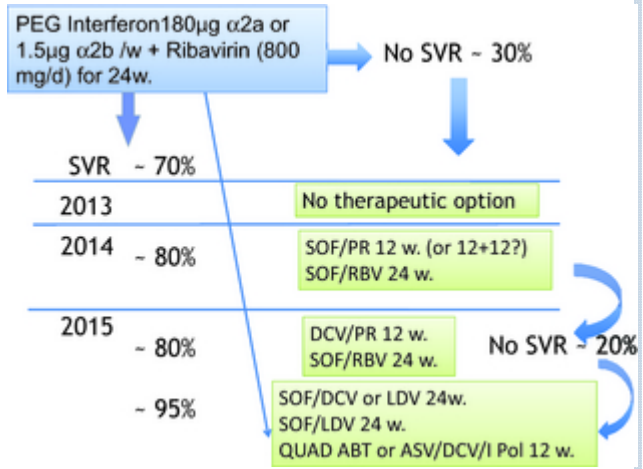
Liver International
Volume 34, Issue Supplement s1, pages 18–23, February 2014



Novel therapies for hepatitis C — one pill fits all?
Michael P. Manns & Thomas von Hahn
Nature Reviews Drug Discovery 12, 595–610 (2013) doi:10.1038/nrd4050

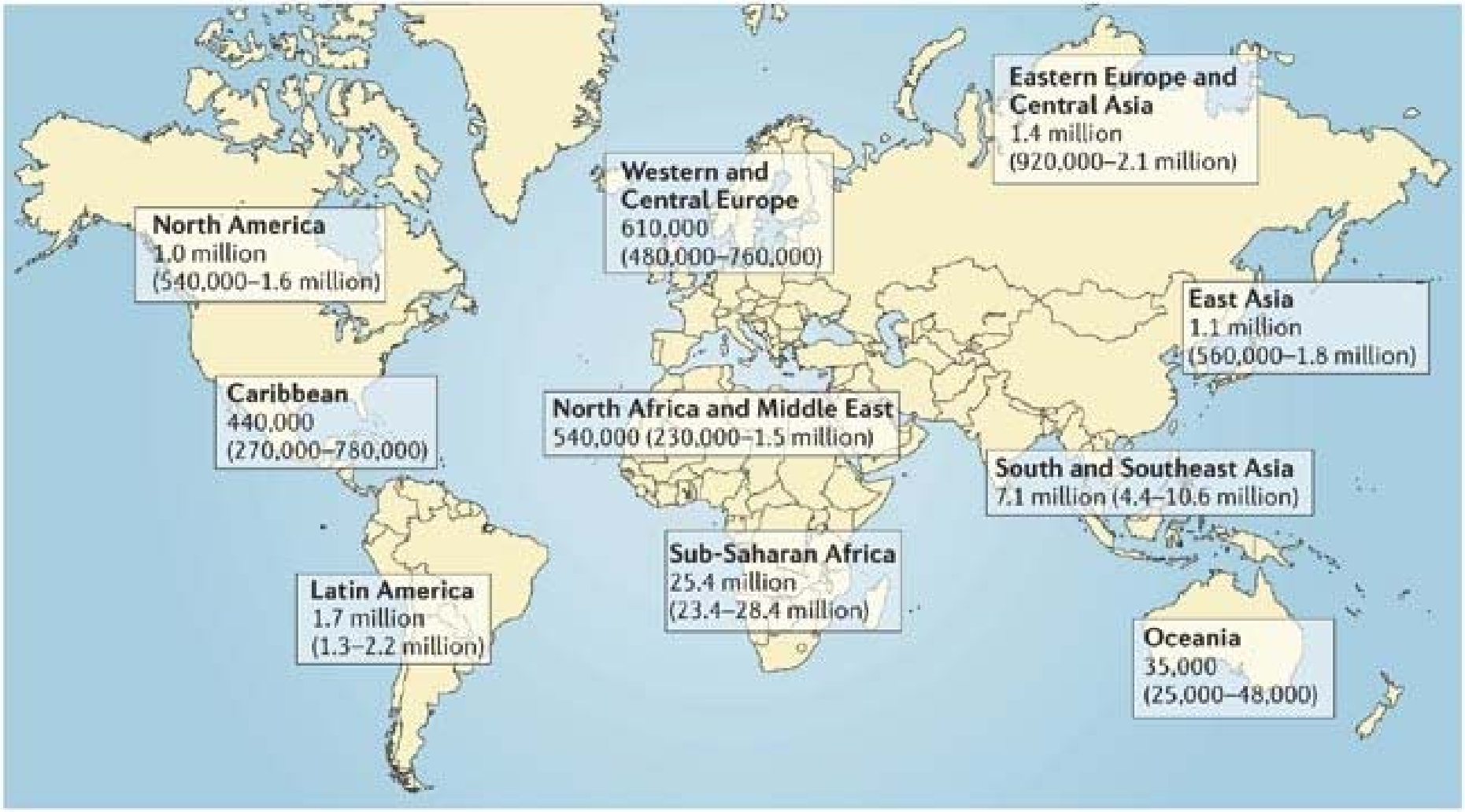
Rates of Sustained Virologic Response among All Patients and According to HCV Genotype in the Historical Control Group and in Group A.



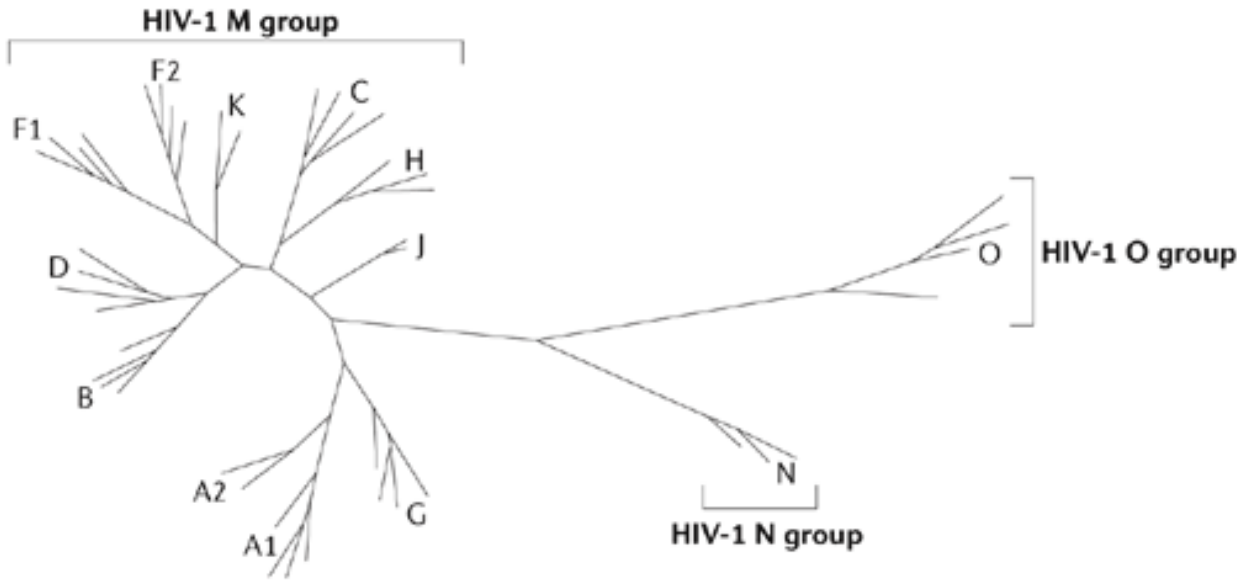


FELD JJ ET AL. N ENGL J MED 2014;370:1594-1603.

Prevalence of HIV Infection

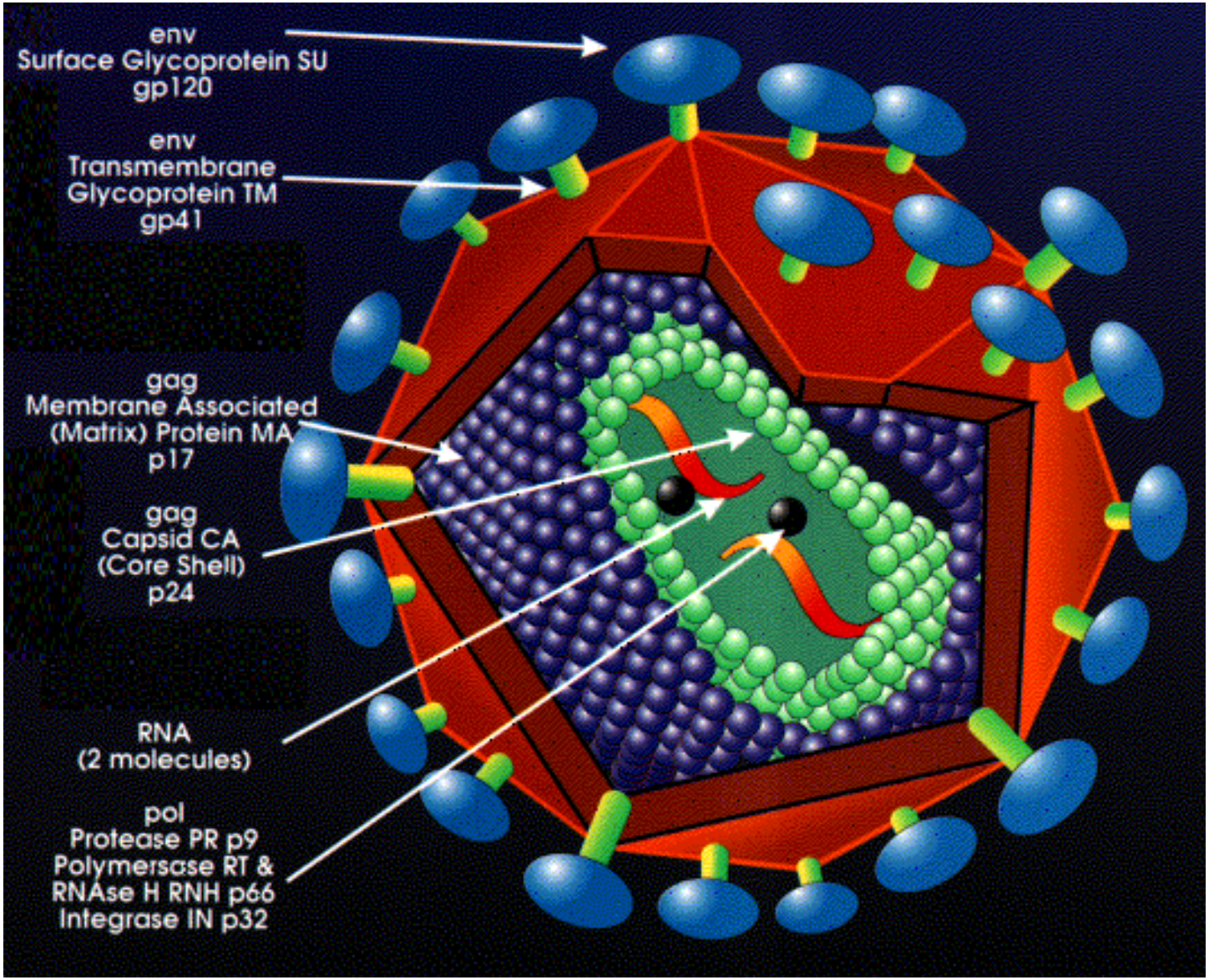


Genetic Diversity of HIV-1

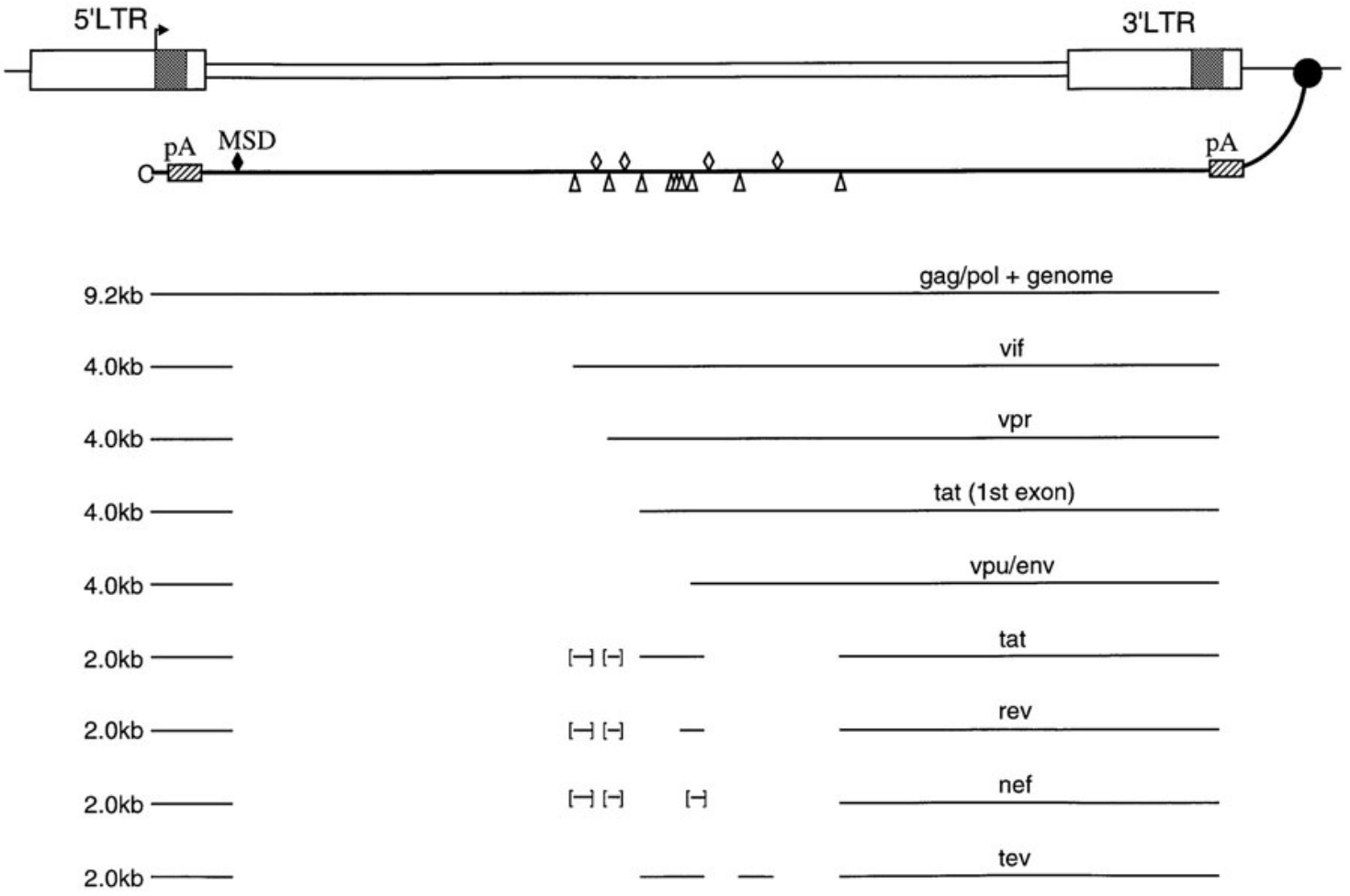


- Within HIV-1, a large sequence diversity exists with viral clades being geographically isolated
- Several studies have suggested that the clades have different biological characteristics, including disease pathogenicity and transmissibility

Virion Structure

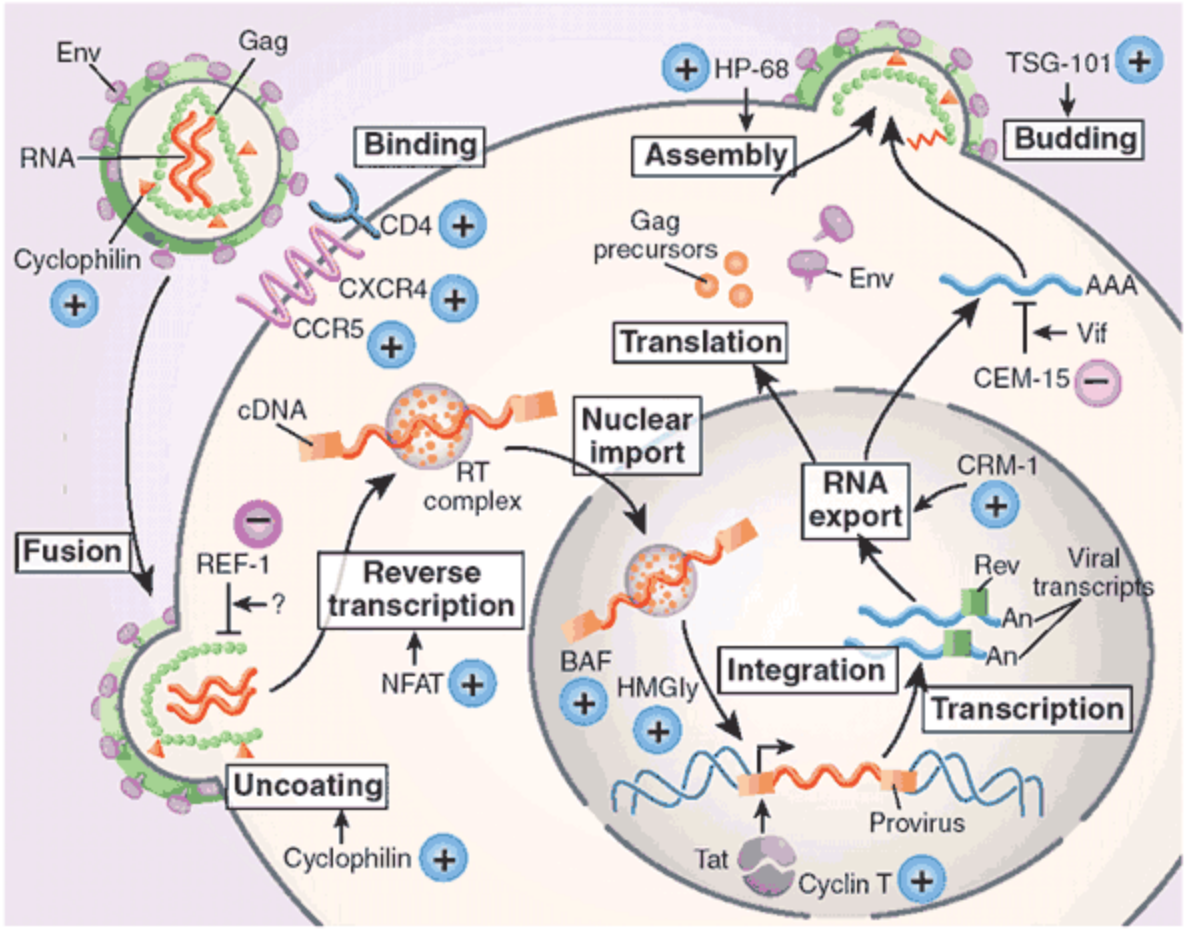


Single Stranded Genome, Multiple messages From alternative splicing



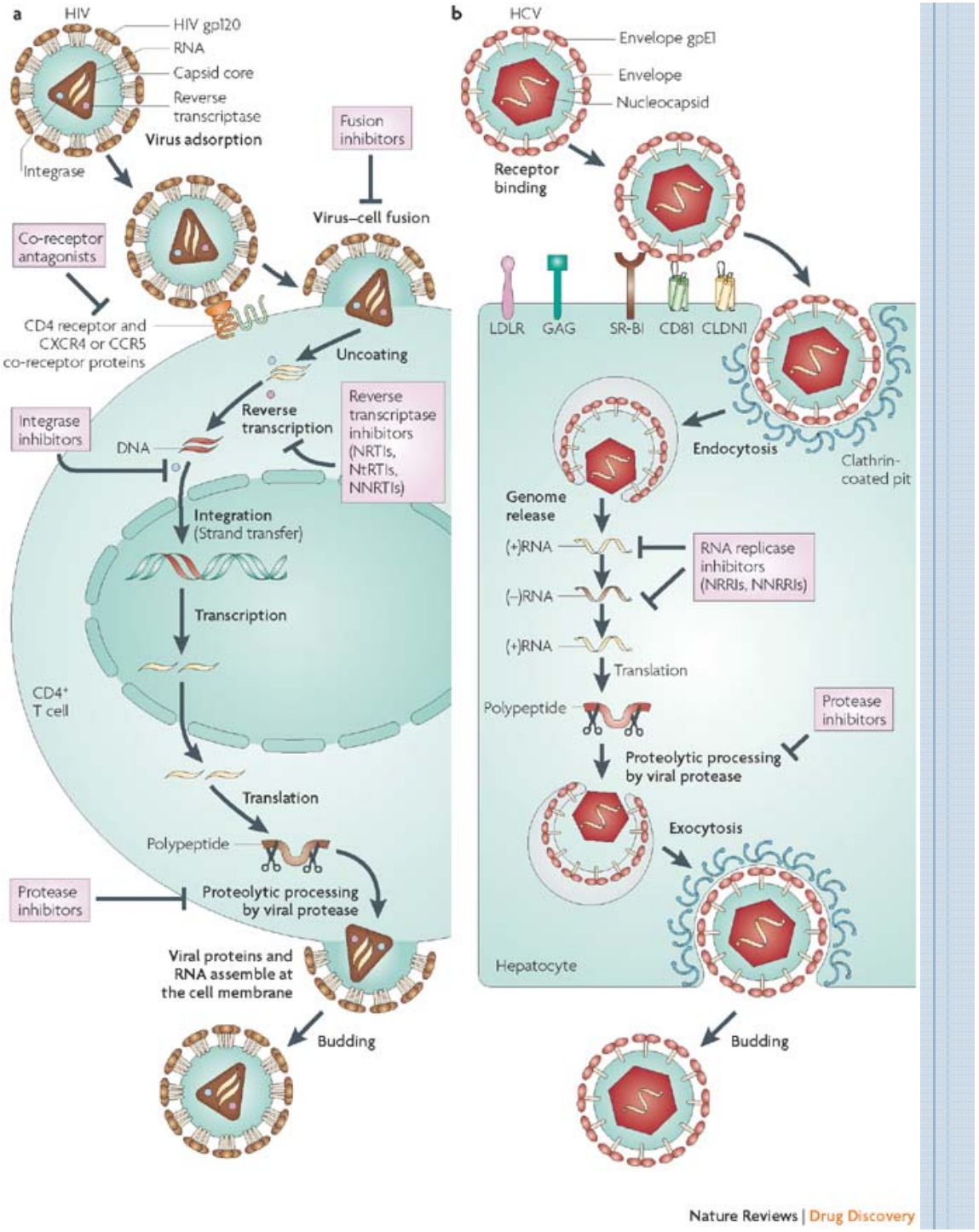
Viral Life cycle

- As a retrovirus, HIV replicates by making a DNA copy of itself that is inserted into the host genome
- Thus, an infected cell can become a stable reservoir for the long term production of viral particles

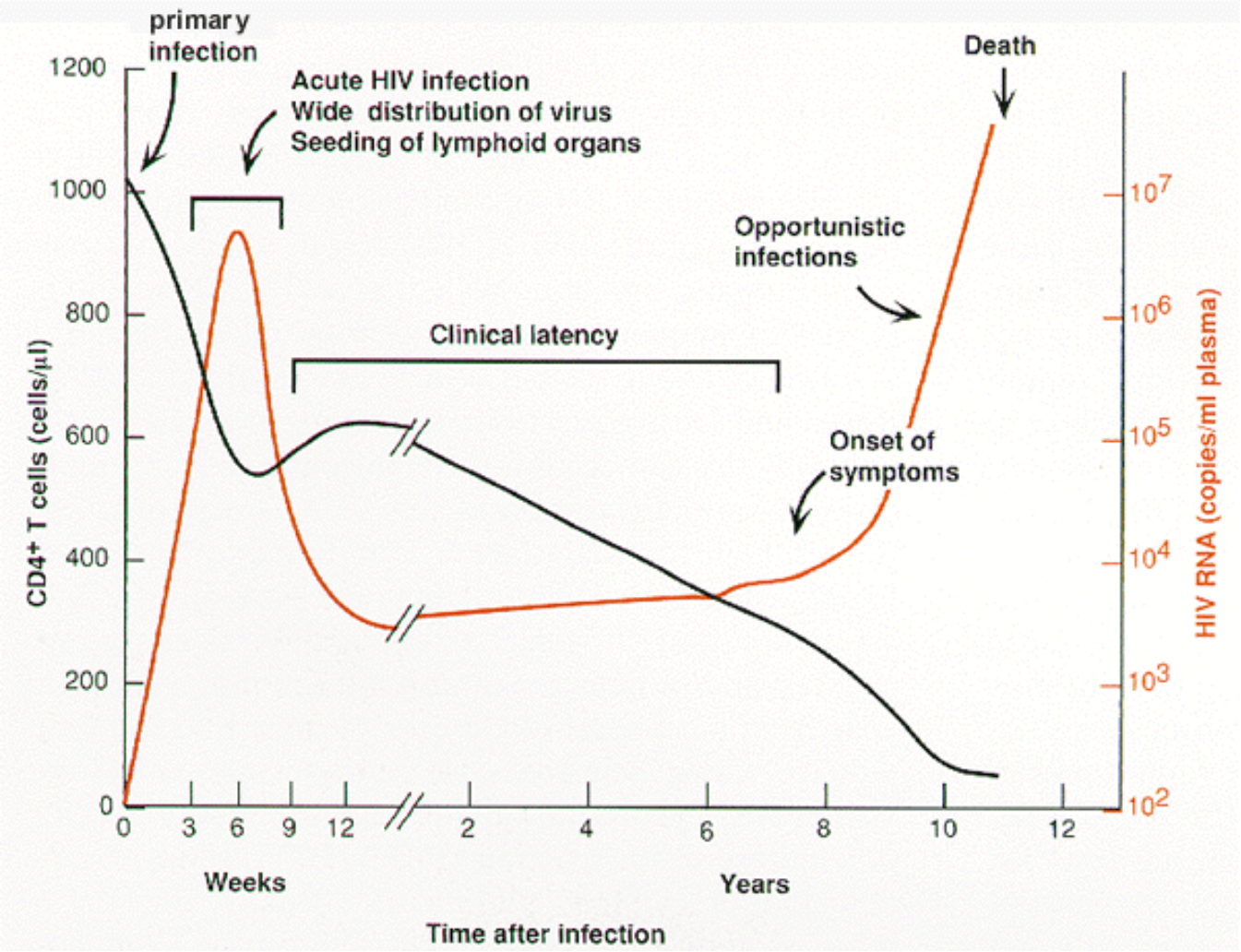


Comparison of HIV and HCV

- HIV and HCV both produce chronic infections, but are biologically very different viruses
- HIV has a DNA intermediate that become heritably integrated
- HCV is a purely RNA virus

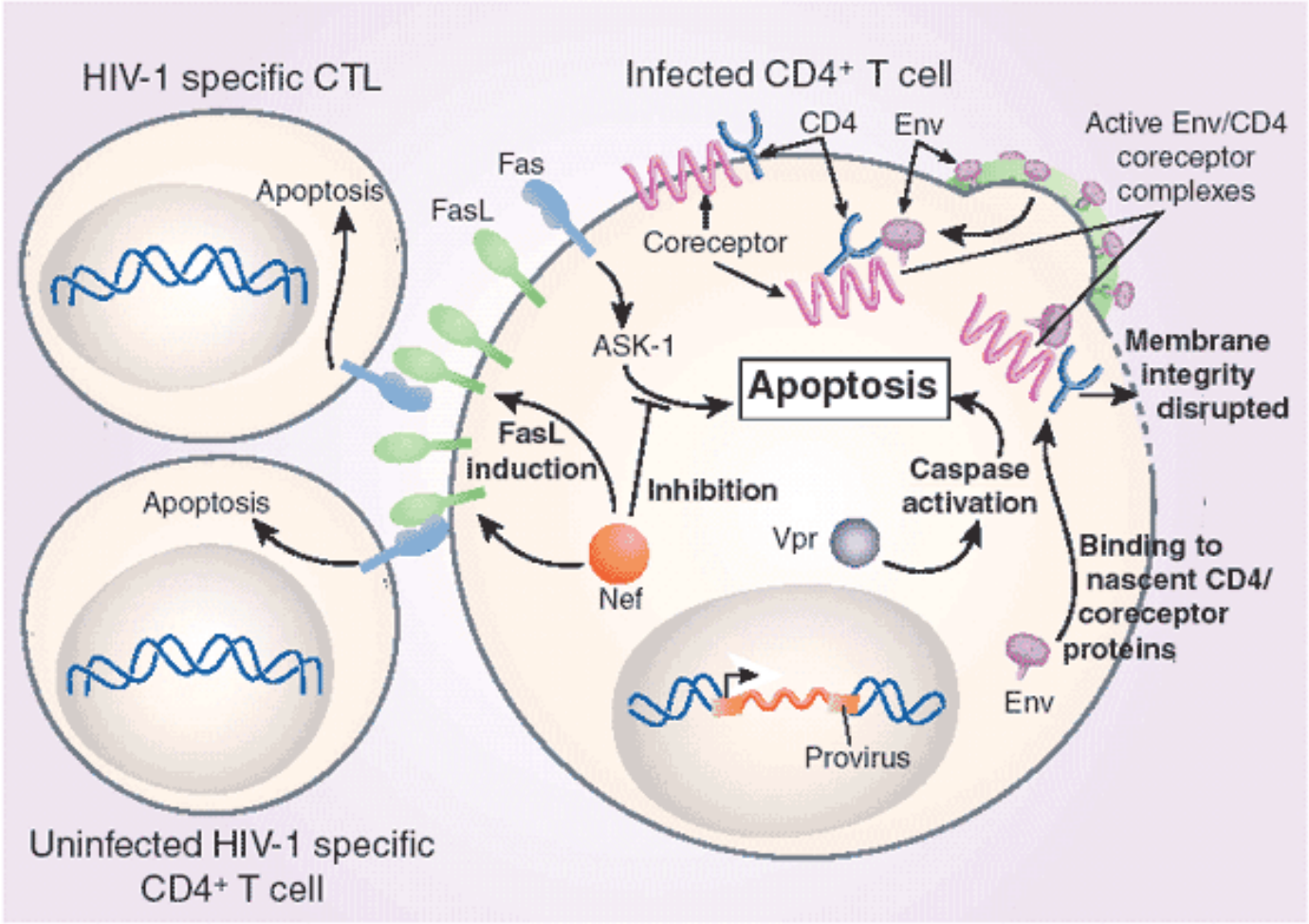


CLINICAL COURSE OF INFECTION

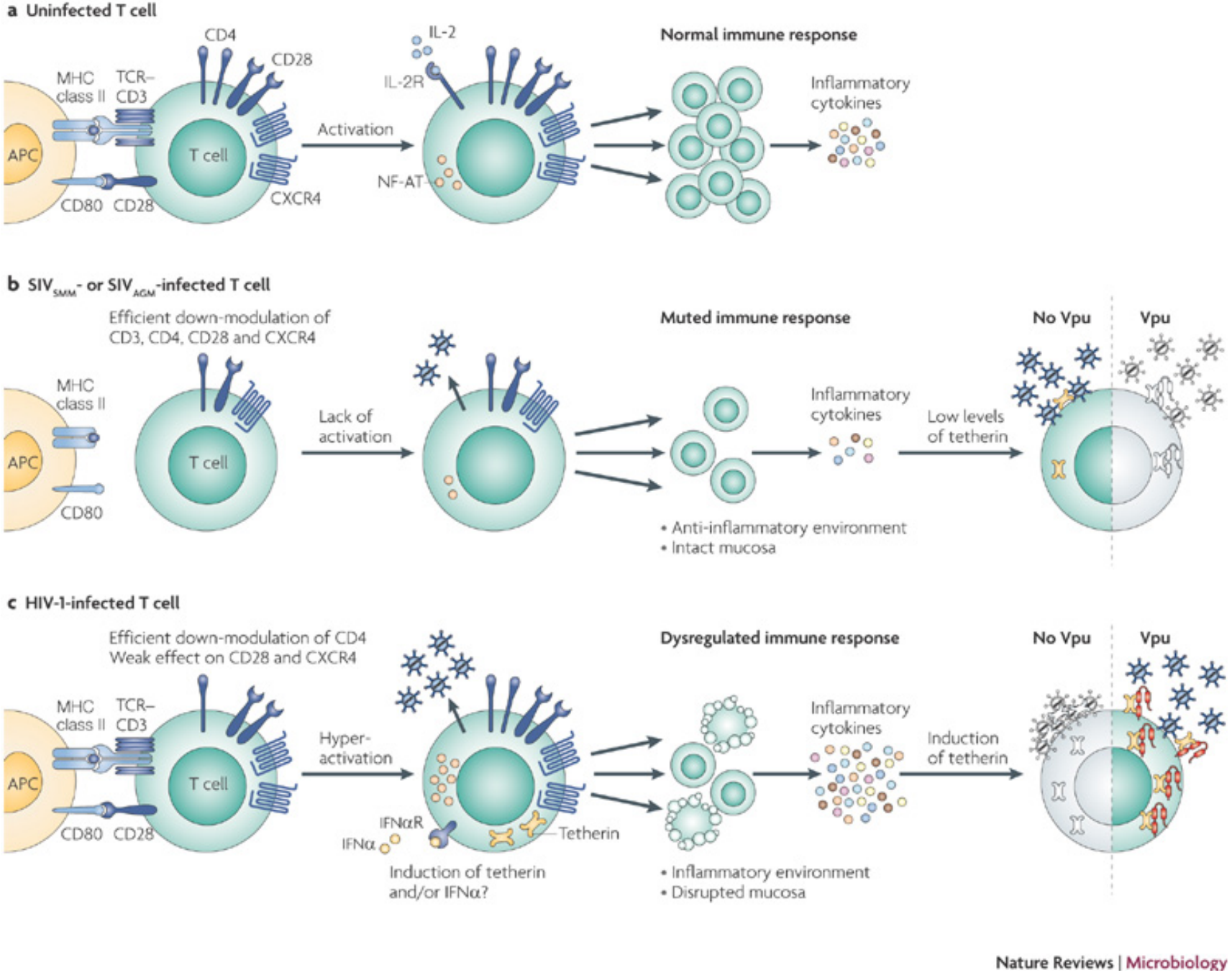


Mechanisms of Cytopathogenicity

- Viral envelope fusogenicity (ER compromised)
- Vpr activates caspases
- Nef contributes indirectly to apoptosis via FasL



Mechanisms of Immune Dysregulation



Nature Reviews | Microbiology

WHAT MAKES HIV LETHAL?

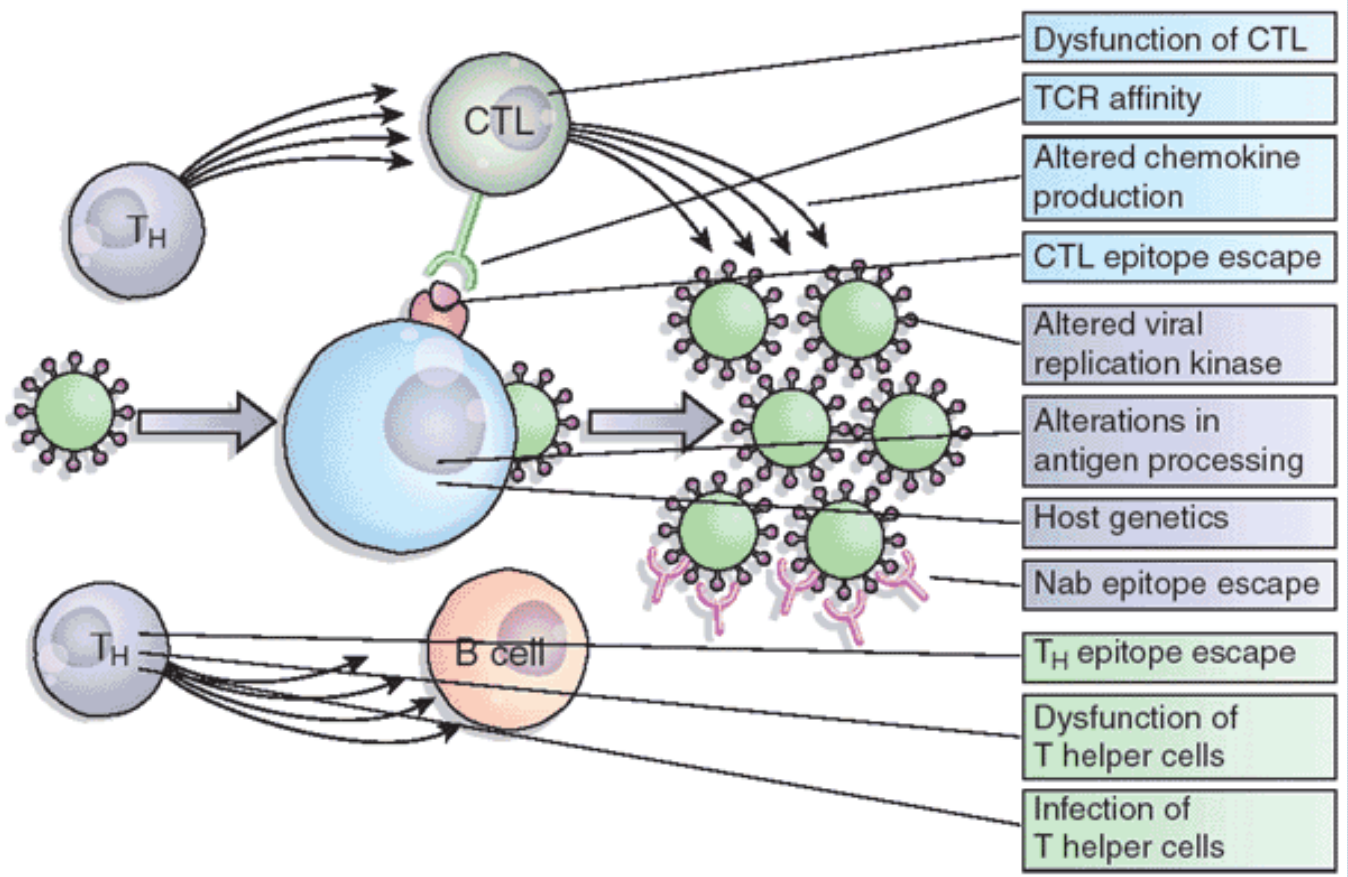
Infections	
Parasites	*Toxoplasma* spp. *Cryptosporidium* spp. *Leishmania* spp. *Microsporidium* spp.
Intracellular bacteria	*Mycobacterium tuberculosis* *Mycobacterium avium intracellulare* *Salmonella* spp.
Fungi	*Pneumocystis carinii* *Cryptococcus neoformans* *Candida* spp. *Histoplasma capsulatum*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

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

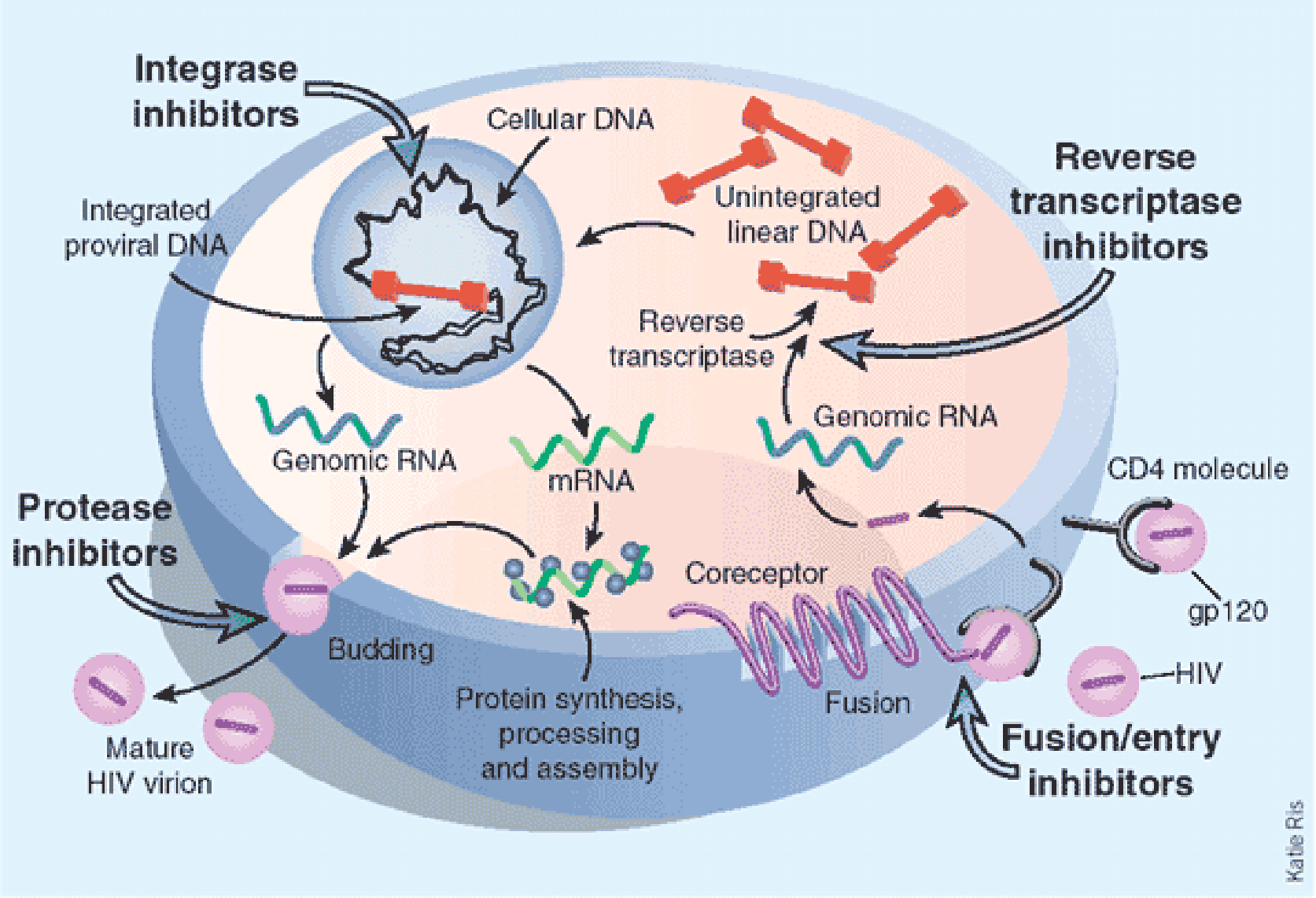
Figure 11-30 Immunobiology, 6/e. (© Garland Science 2005)

WHY IS HIV UNLIKE ANY OTHER CHRONIC INFECTION?

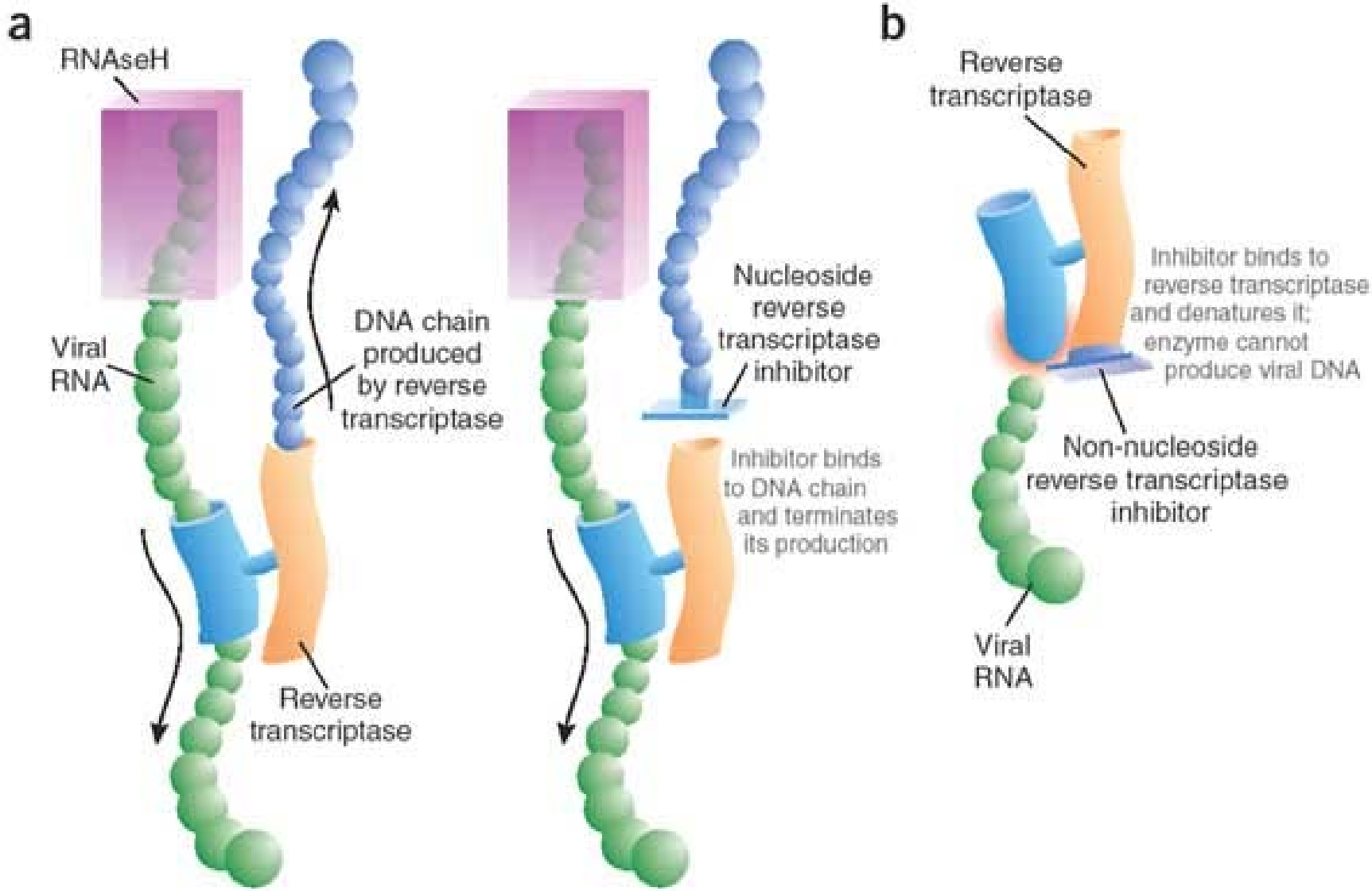
- A combination of "traditional" immune evasion mechanisms (CTL escape, antigen masking) and non-traditional (attacking immune function and cell compartments directly



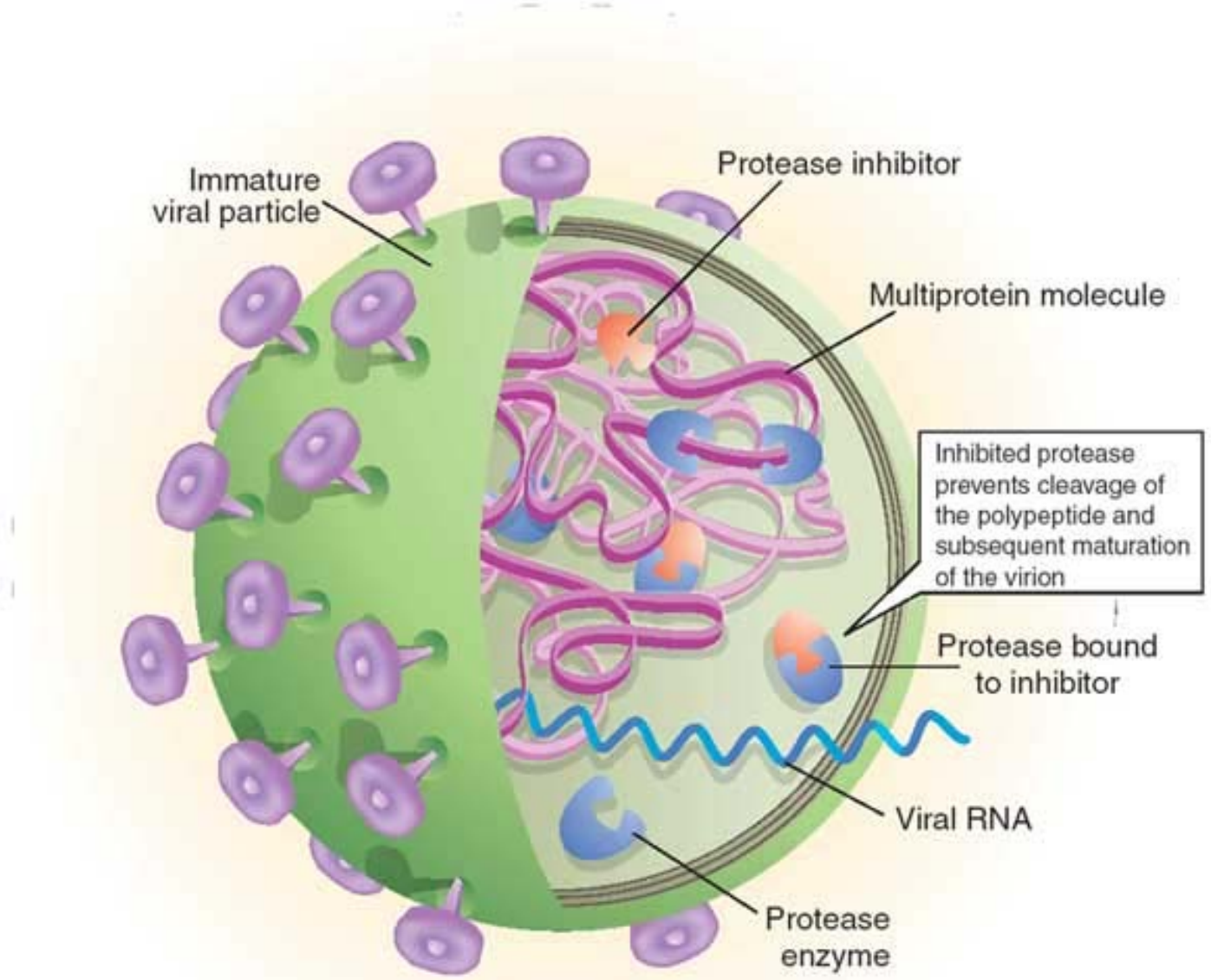
CAN INFECTION BE EFFECTIVELY CONTROLLED?



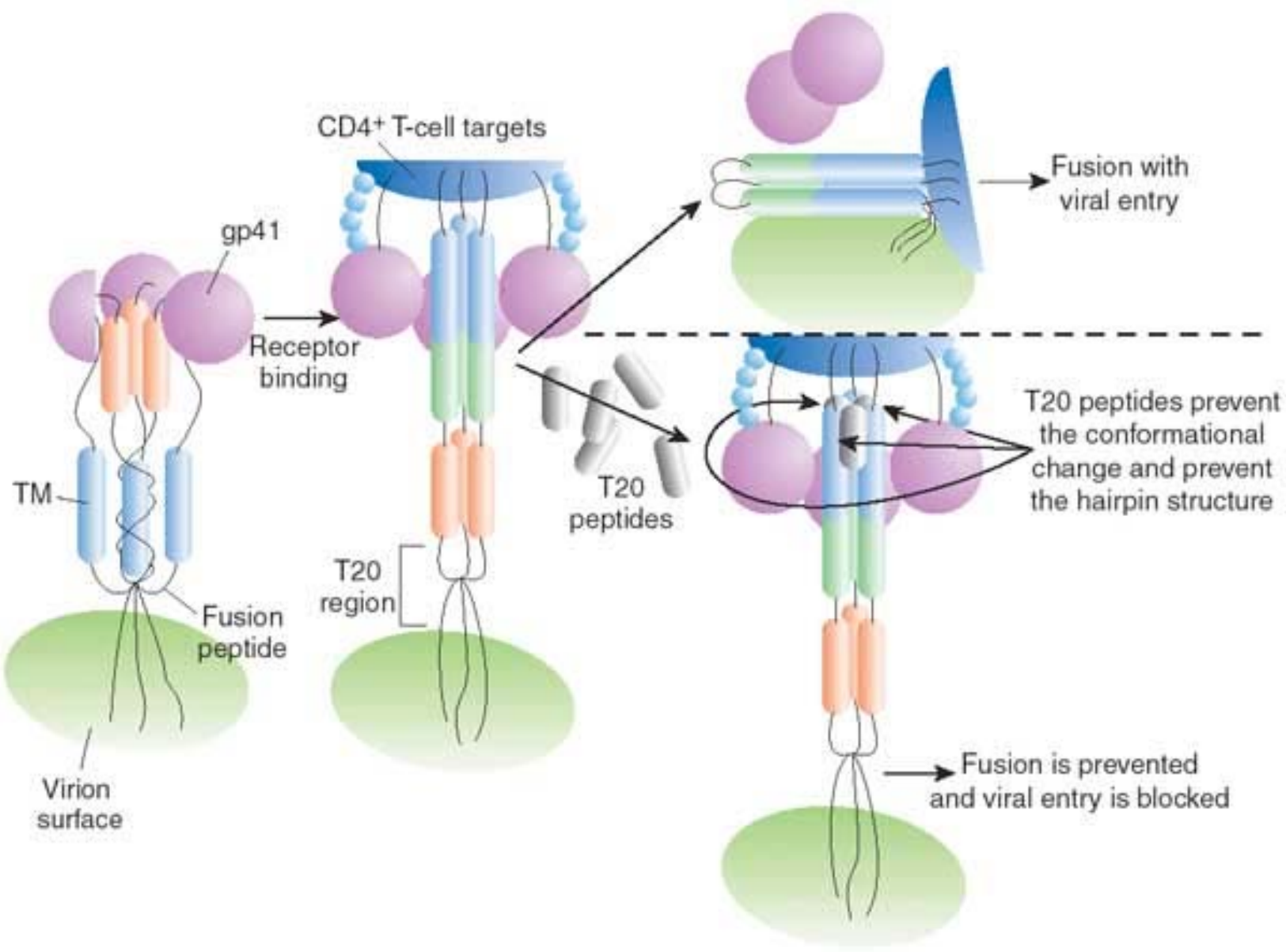
Mechanisms of RT Inhibitors



Mechanism of Protease Inhibitors

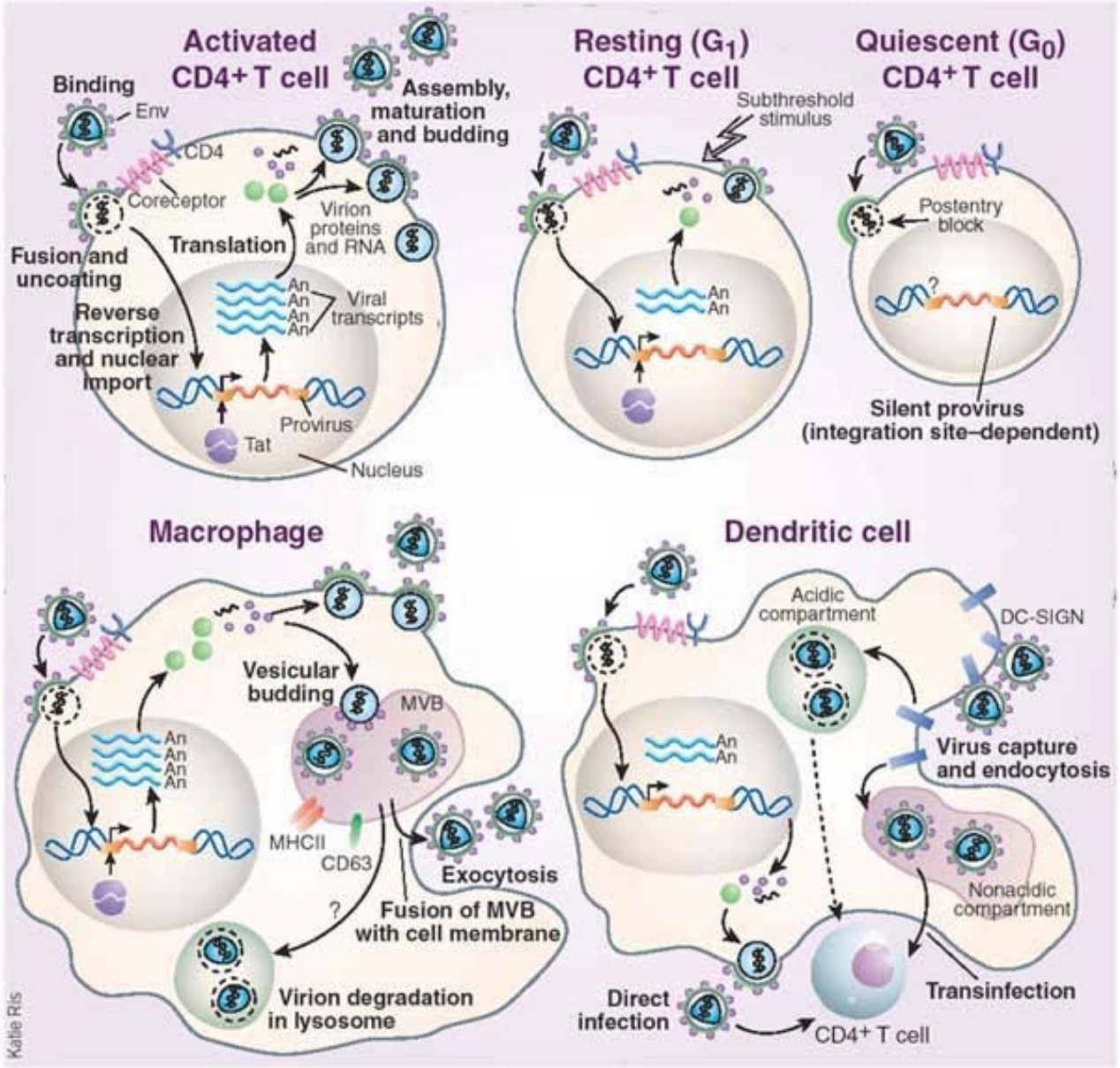


Fusion Inhibitors



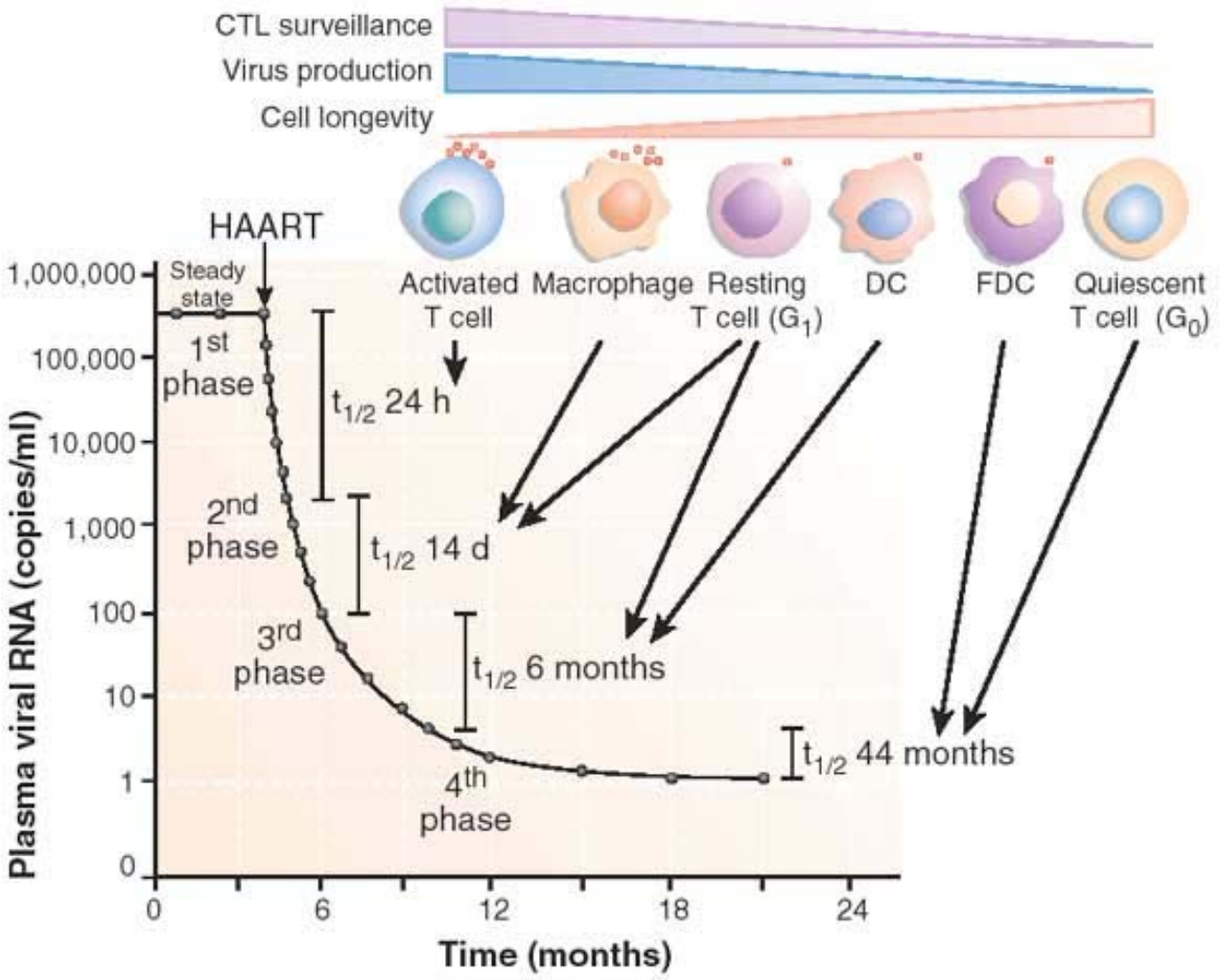
Latent Reservoirs of Virus

- Multiple cell types can serve as latent reservoirs
- "Quiescence" of infected cells constrains the possibility total viral elimination

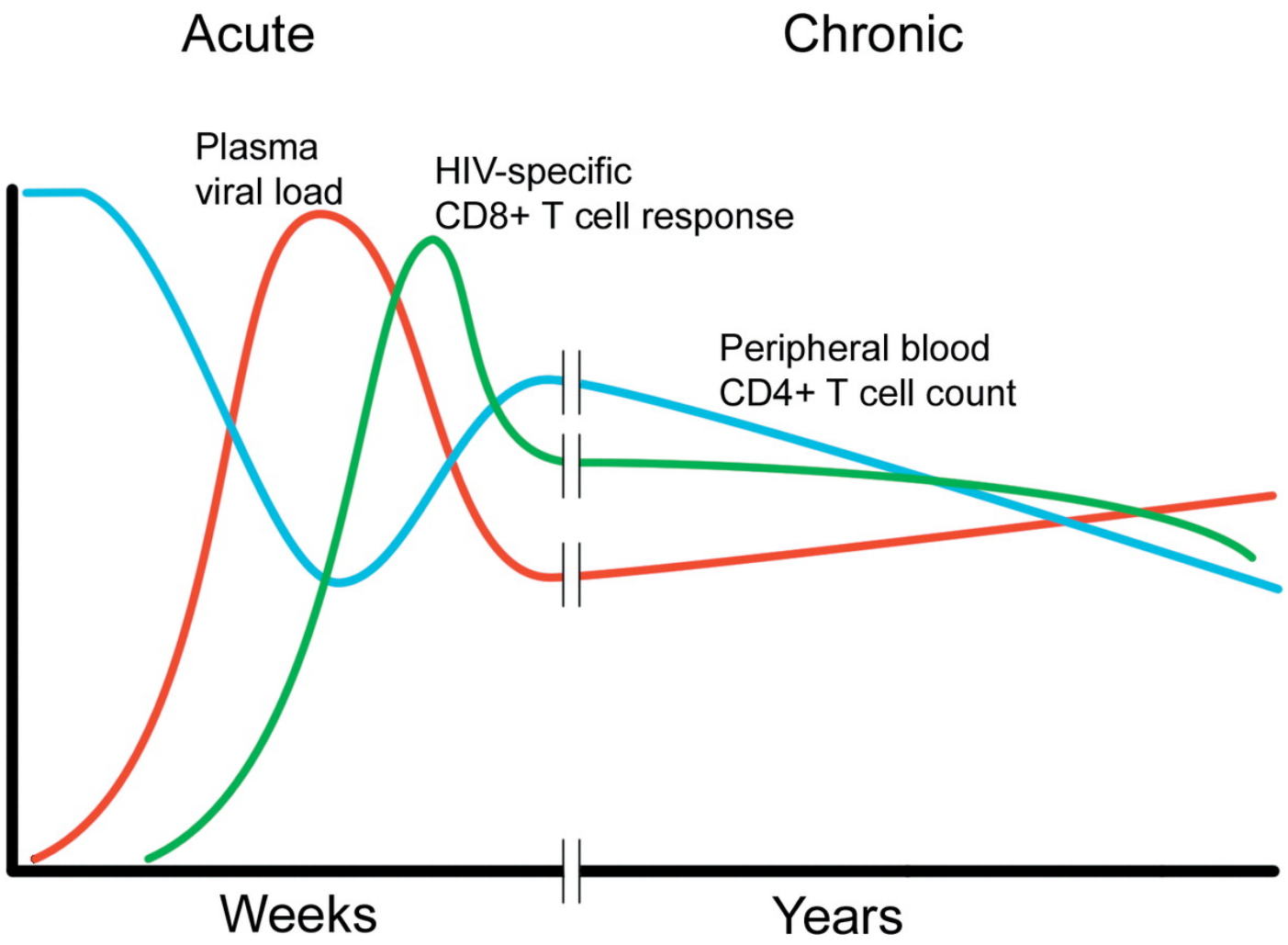


Contribution of Individual Reservoirs

- Steady-state virus levels result from the relative contributions and turnover of each reservoir compartment
- After viral inhibition by HAART, plasma viral RNA decays in four distinct phases allowing a dissection of each reservoir's individual contribution

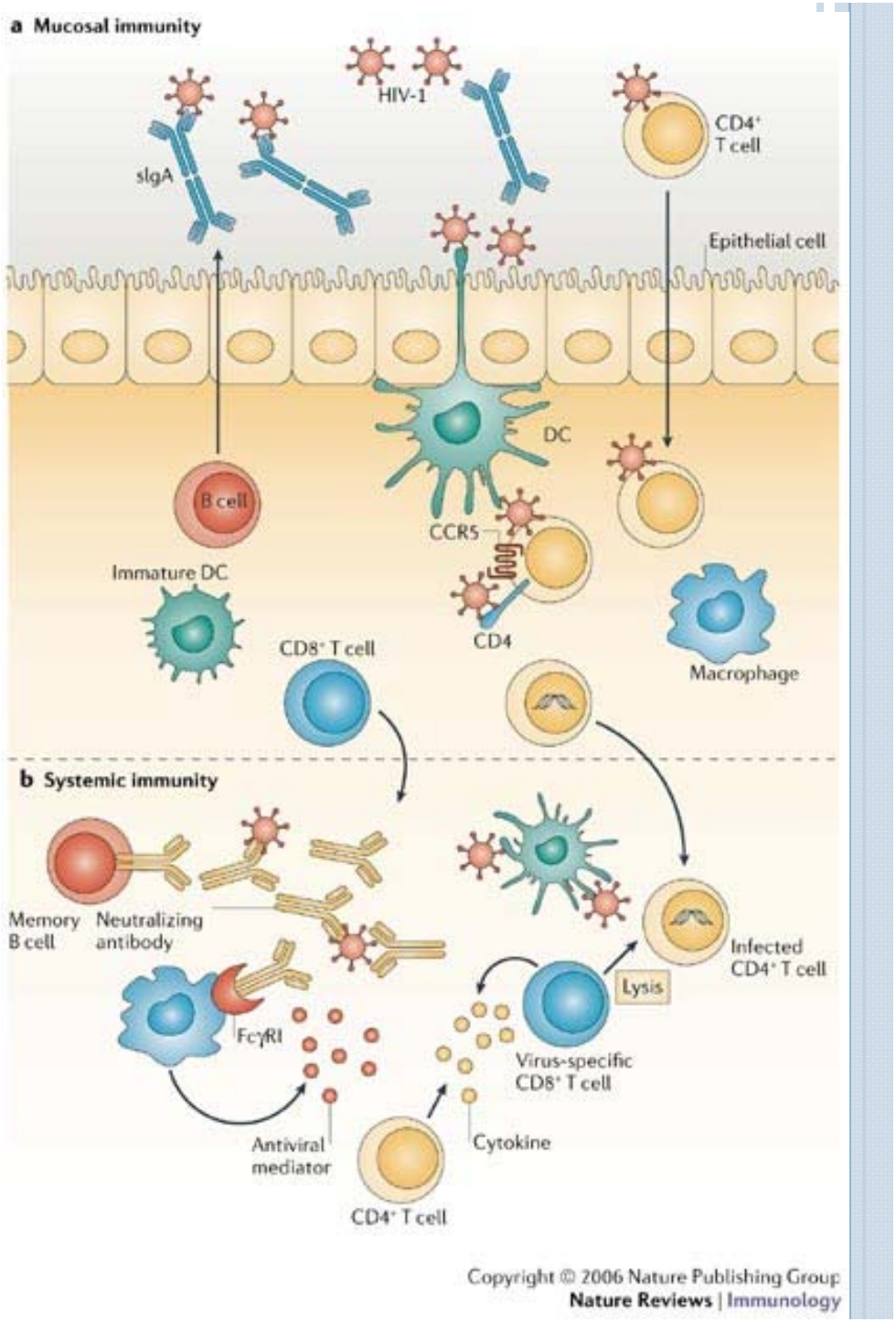


Can the Immune system be used to Prevent or Clear infection?



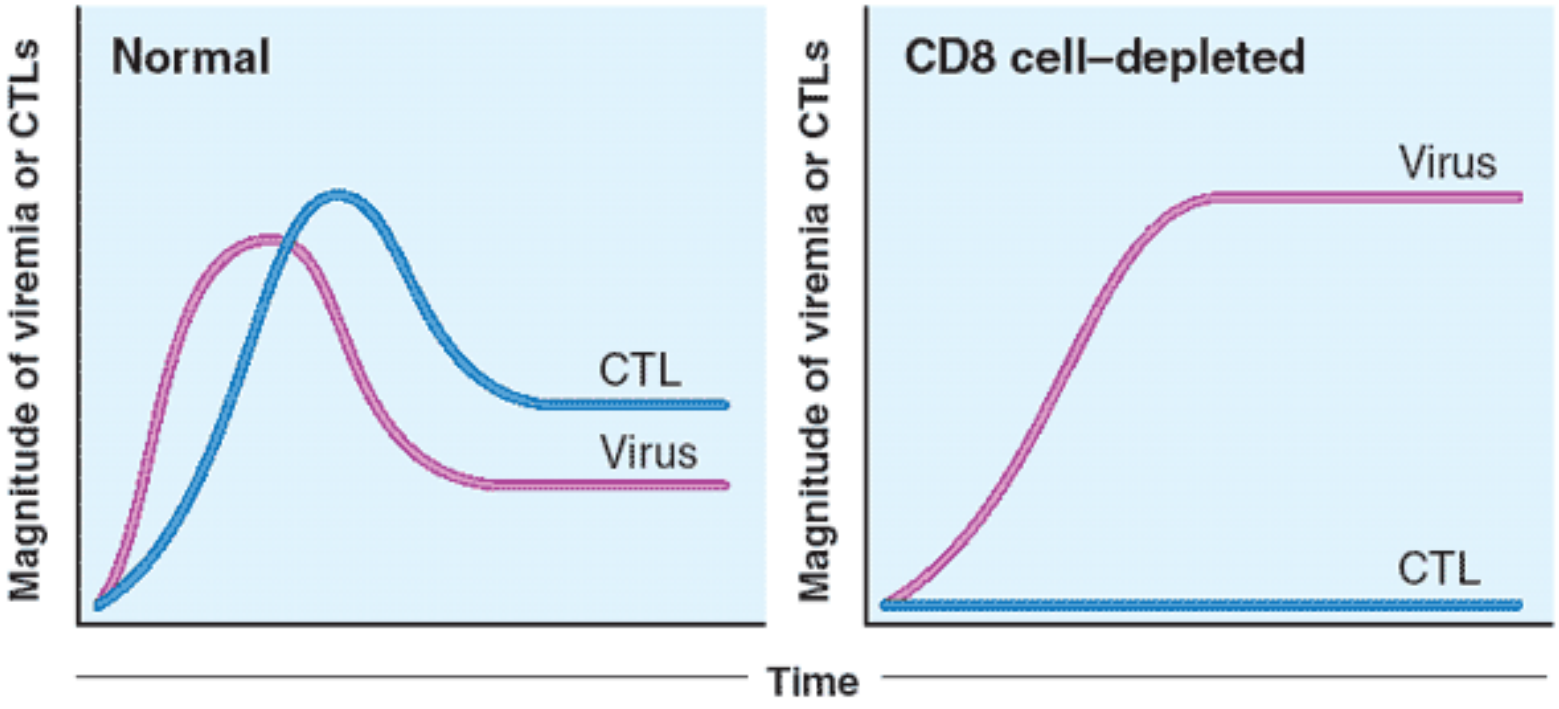
Mechanisms of Immune protection

- "Standard" immunological protection mechanisms, including antibody, clearance by phagocytic cells and Fc receptors, and cytotoxic killing of infected cells all function to limit infection and control long-term viral loads
- The loss of effective immune control is what leads to the development of AIDS, therefore the immune response in principle is an effective tool for viral control and clearance



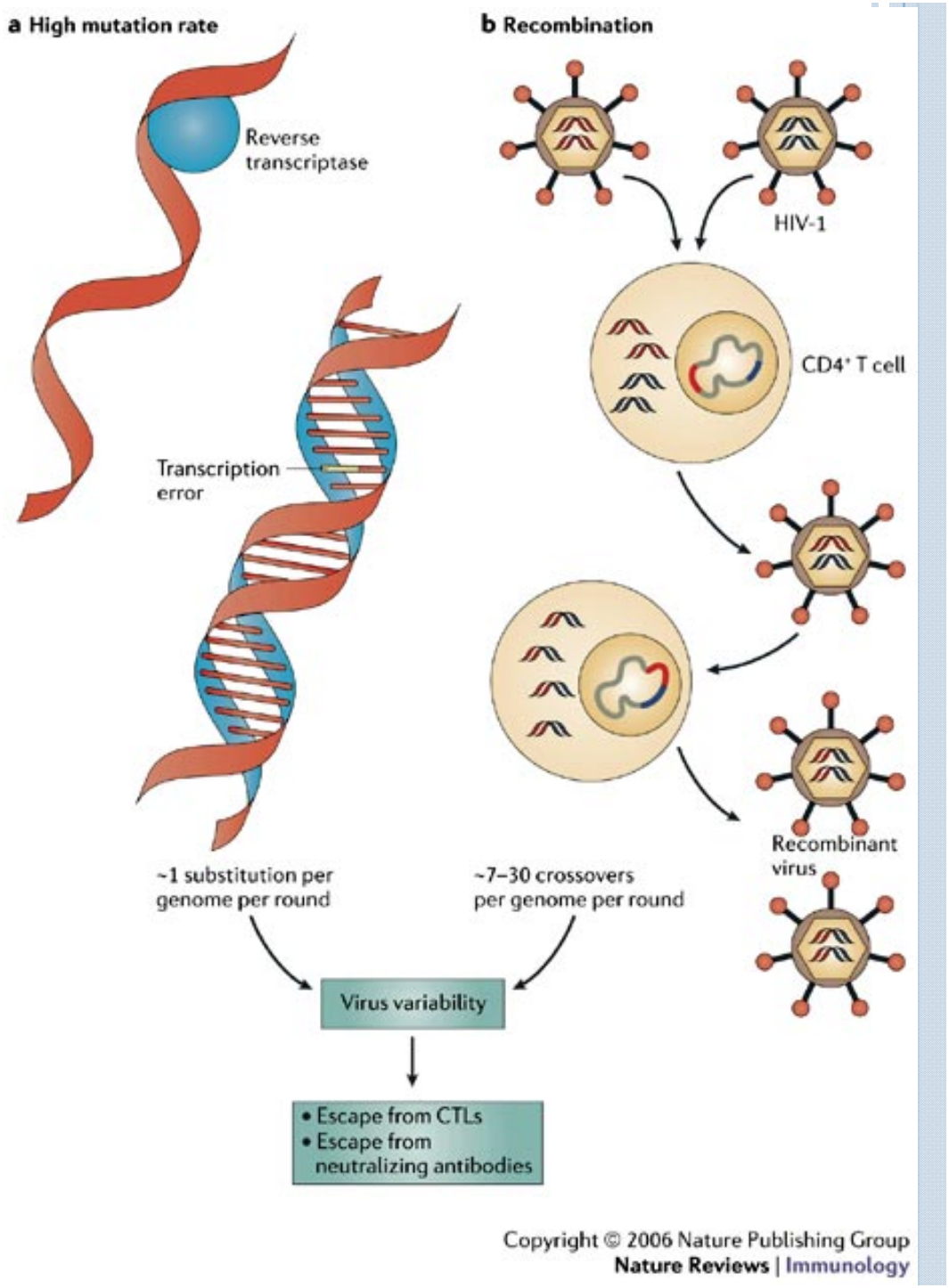
CD8 T CELLS PROVIDE SIGNIFICANT VIRAL CONTROL DURING THE CHRONIC PHASE OF INFECTION

- CD8 depletion in SIV-infected animals leads to rapid increase in viral titers and pathogenesis of disease

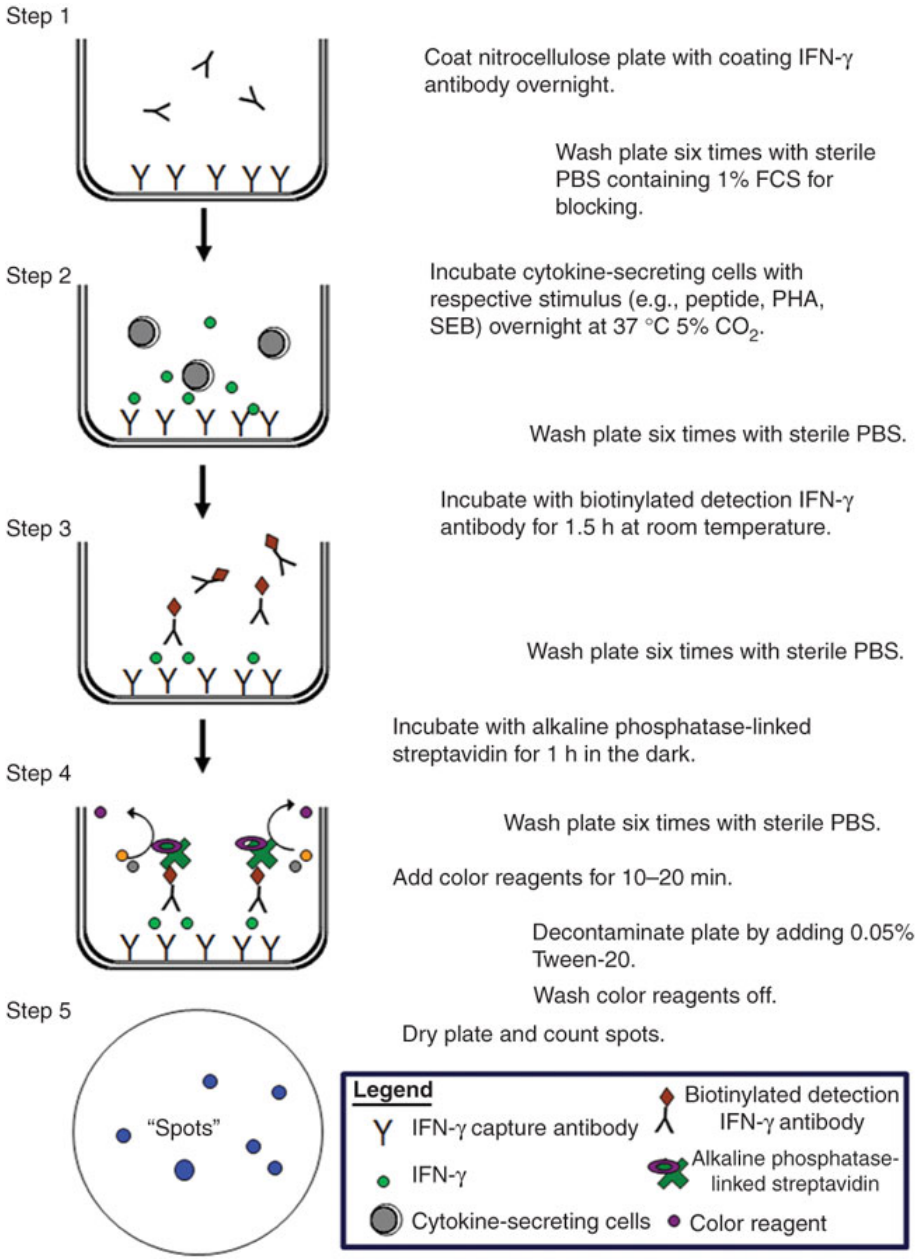


Viral Immune Escape mechanisms

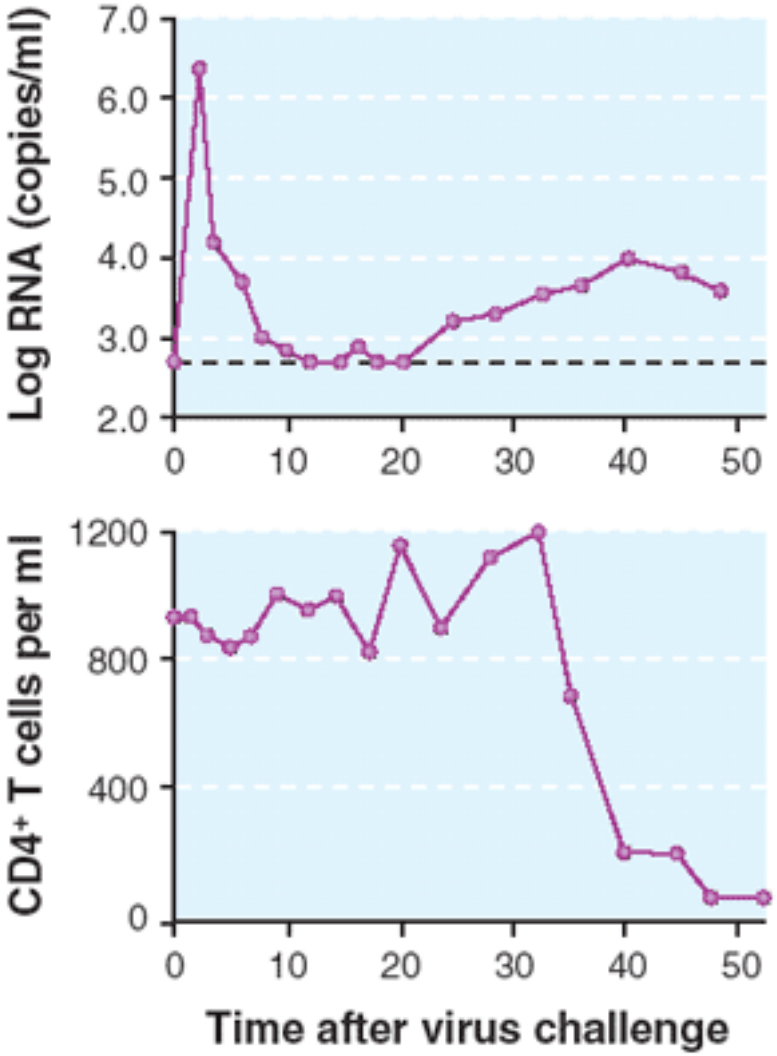
- "Antigenic drift" from the very high rate of mutation of the RT enzyme allows rapid escape from individual antibody and CTL responses
- Epitopes are constrained by structural/functional requirements



How do we assay for T cell responses in HIV infected individuals?



IMMUNODOMINANT EPITOPE ESCAPE CAN LEAD TO LOSS OF VIRAL CONTROL



Gag p11C (181–189) sequences

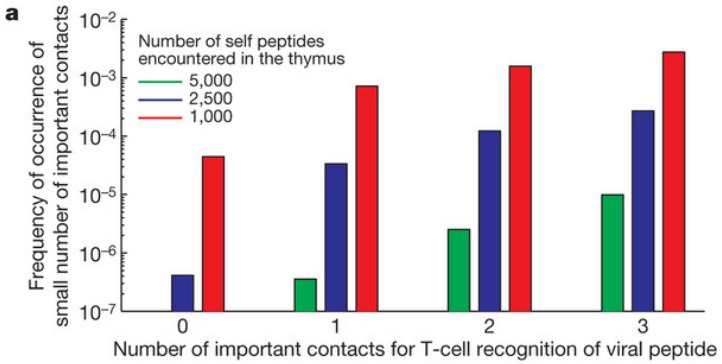
	C	T	P	Y	D	I	N	Q	M	
Week 0	-	-	-	-	-	-	-	-	-	(15/15)
Week 14	-	-	-	-	-	-	-	-	-	(8/8)
Week 20	-	I	-	-	-	-	-	-	-	(10/10)
Week 24	-	I	-	-	-	-	-	-	-	(11/11)
Week 28	-	I	-	-	-	-	-	-	-	(11/11)
Week 36	-	I	-	-	-	-	-	-	-	(11/11)
Eeek 44	-	I	-	-	-	-	-	-	-	(10/10)

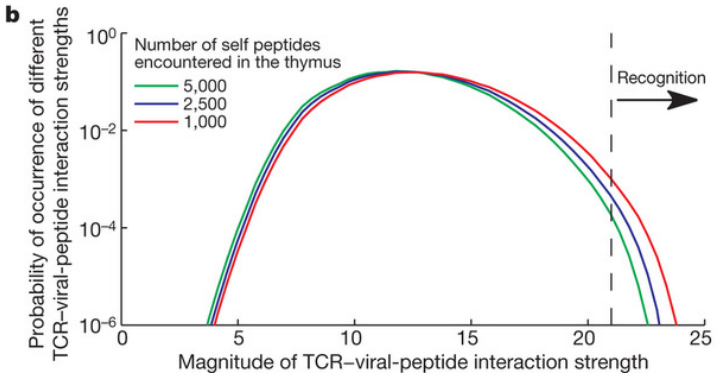
Recent Reports Relating MHC Haplotype to HIV control

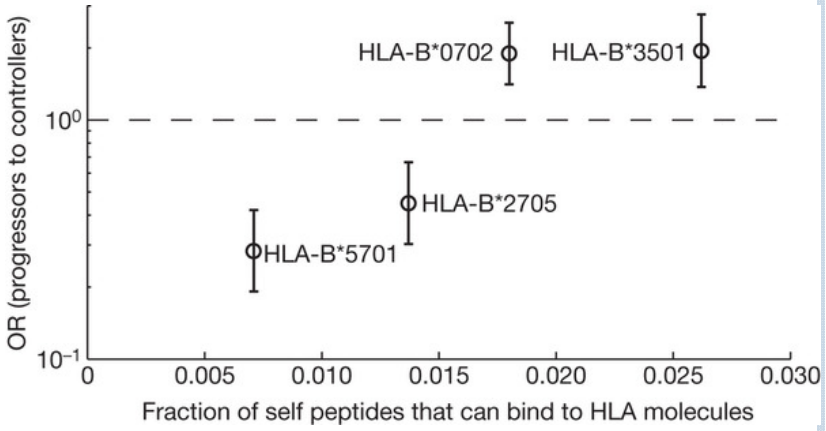
Nature 465, 350–354 (20 May 2010) Effects of thymic selection of the T-cell repertoire on HLA class I-associated control of HIV infection

Andrej Košmrlj, Elizabeth L. Read, Ying Qi, Todd M. Allen, Marcus Altfeld, Steven G. Deeks, Florencia Pereyra, Mary Carrington, Bruce D. Walker & Arup K. Chakraborty

- Relating the breadth of the TCR repertoire (how many different T cell receptors does the body make?) to the MHC haplotype (the more self peptides available for negative selection, the narrower (and less "cross-reactive" the TCR repertoire)
- Less cross-reactive TCR repertoires are then associated with poor control

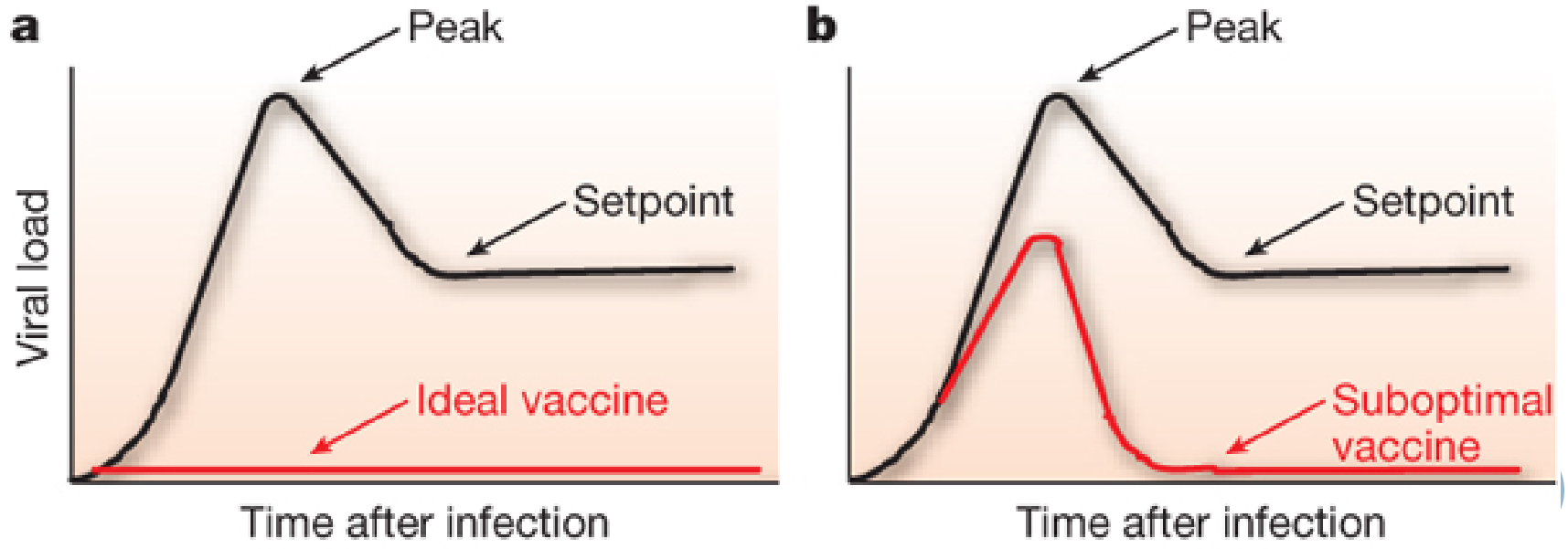






VACCINE EXPECTATIONS

- Since viral load "set point" is a key predictor of disease progression and pathogenesis, even a suboptimal vaccine could be of use in highly endemic areas to protect against disease and spread (we'll talk more about this when we get to malaria)



Summary of Vaccine Trials in 2006

Vaccine candidate	Antigen (HIV-1 clade)	Manufacturer	Trial start date	Question being addressed
Prime with canarypox vector expressing HIV-1 genes	*env* (B, E), *gag*/*pol* (B)	Sanofi-Pasteur	October 2003	Will a gp120 protein vaccine that did not confer protection when used alone be useful in combination with a live, recombinant pox vector prime?
Boost with gp120 protein	gp120 (B, E)	Vaxgen		
Replication-defective adenovirus serotype 5 expressing HIV-1 genes	*gag*, *pol*, *nef* (B)	Merck	December 2004	Will an adenovirus-based vector vaccine confer a clinical benefit in individuals who become infected after vaccination?
Prime with plasmid DNA encoding HIV-1 genes	*gag*, *pol*, *nef* (B), *env* (A, B, C)	Vical, VRC	September 2005	Will a prime–boost strategy using DNA- and adenovirus-based vaccines encoding envelope proteins from three HIV-1 clades, as well as viral structural proteins, confer a benefit?
Boost with replication-defective adenovirus serotype 5 expressing HIV-1 genes	*gag*, *pol* (B), *env* (A, B, C)	GenVec, VRC		

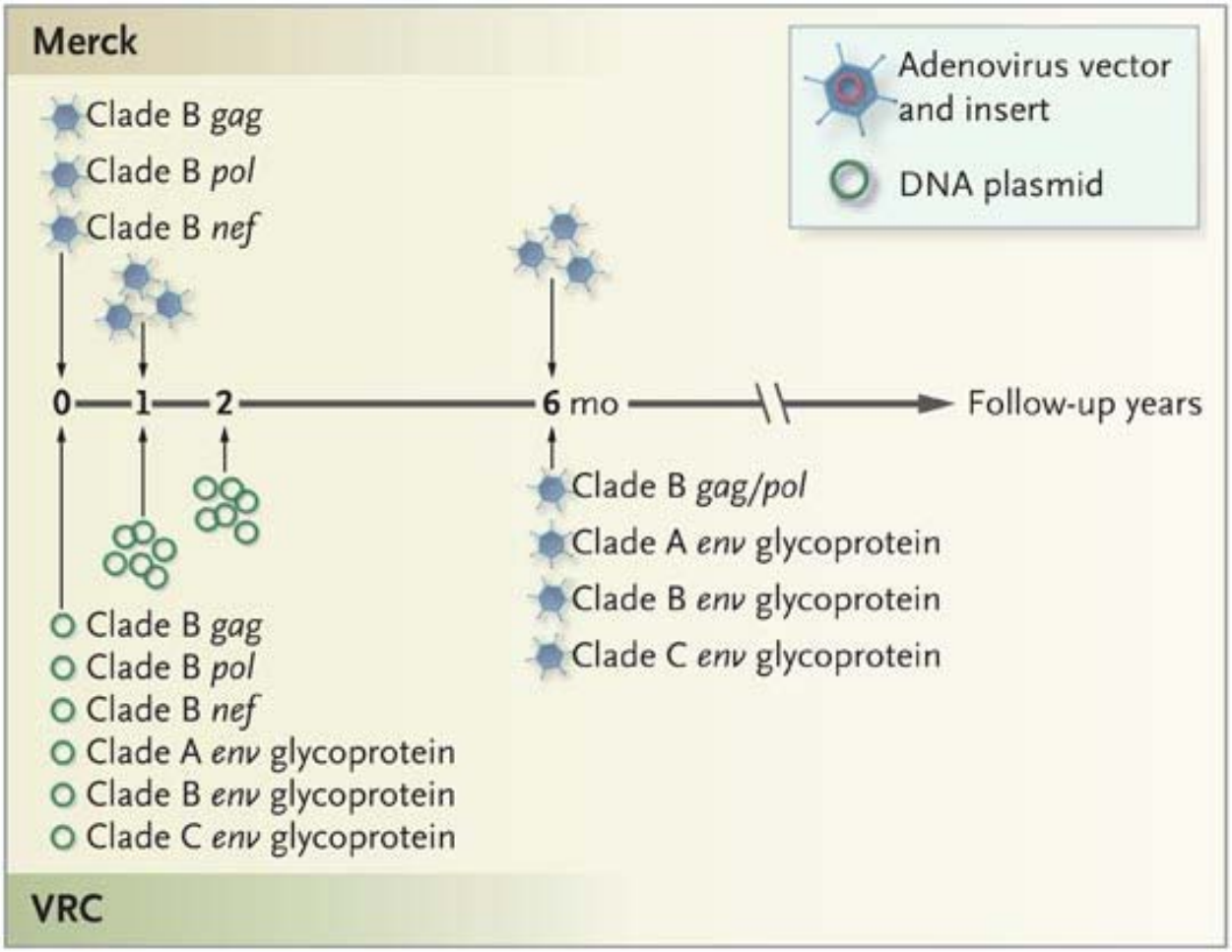
Further information on ongoing trials of preventative AIDS vaccines can be found in the 2006 International AIDS Vaccine Initiative report. *env*, envelope; *gag*, group-specific antigen; gp120, glycoprotein 120; *nef*, negative factor; *pol*, polymerase; VRC, Vaccine Research Center, National Institutes of Health, Maryland, USA.

Letvin *Nature Reviews Immunology* **6**, 930–939 (December 2006) | doi:10.1038/nri1959

nature REVIEWS IMMUNOLOGY

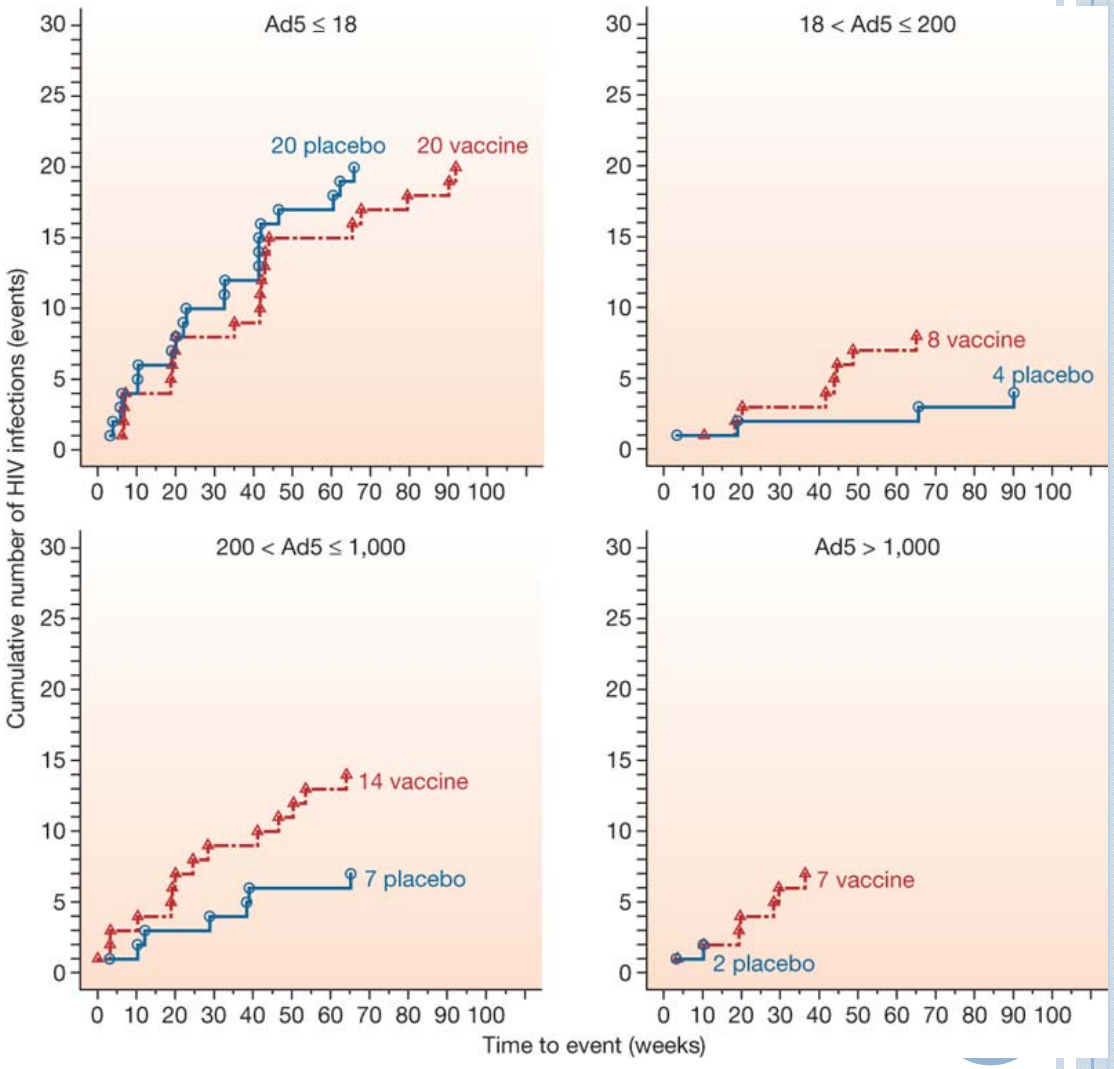
The Merck Vaccine

- Use of a viral vector has been shown experimentally to boost cellular responses, by delivering more antigen with the proper innate/PAMP signals



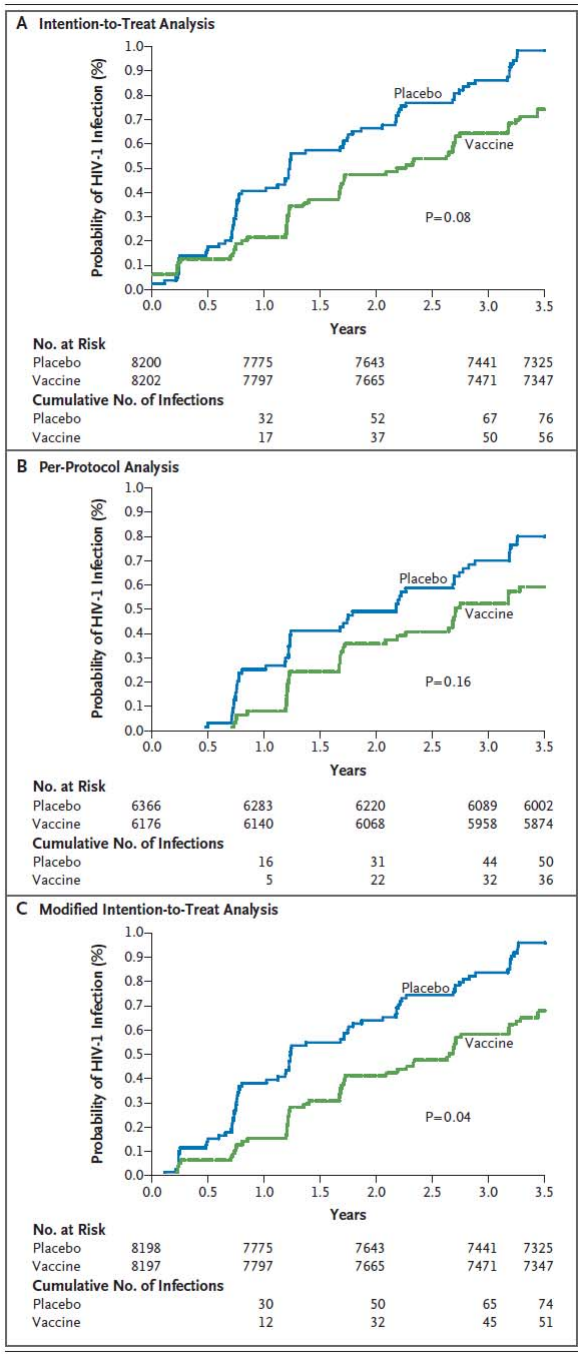
Merck Vaccine Failure

- Not only did the Merck Vaccine fail to protect, there appeared to be an enhancement of infection in vaccinees who had relatively higher pre-existing antibody titers to the viral vector
- This failure led to the cancellation of other vaccine trials based on a similar approach
- HVTN-505 just halted in April 2013—also Ad5 based (41 vacc inf, 30 placebo)

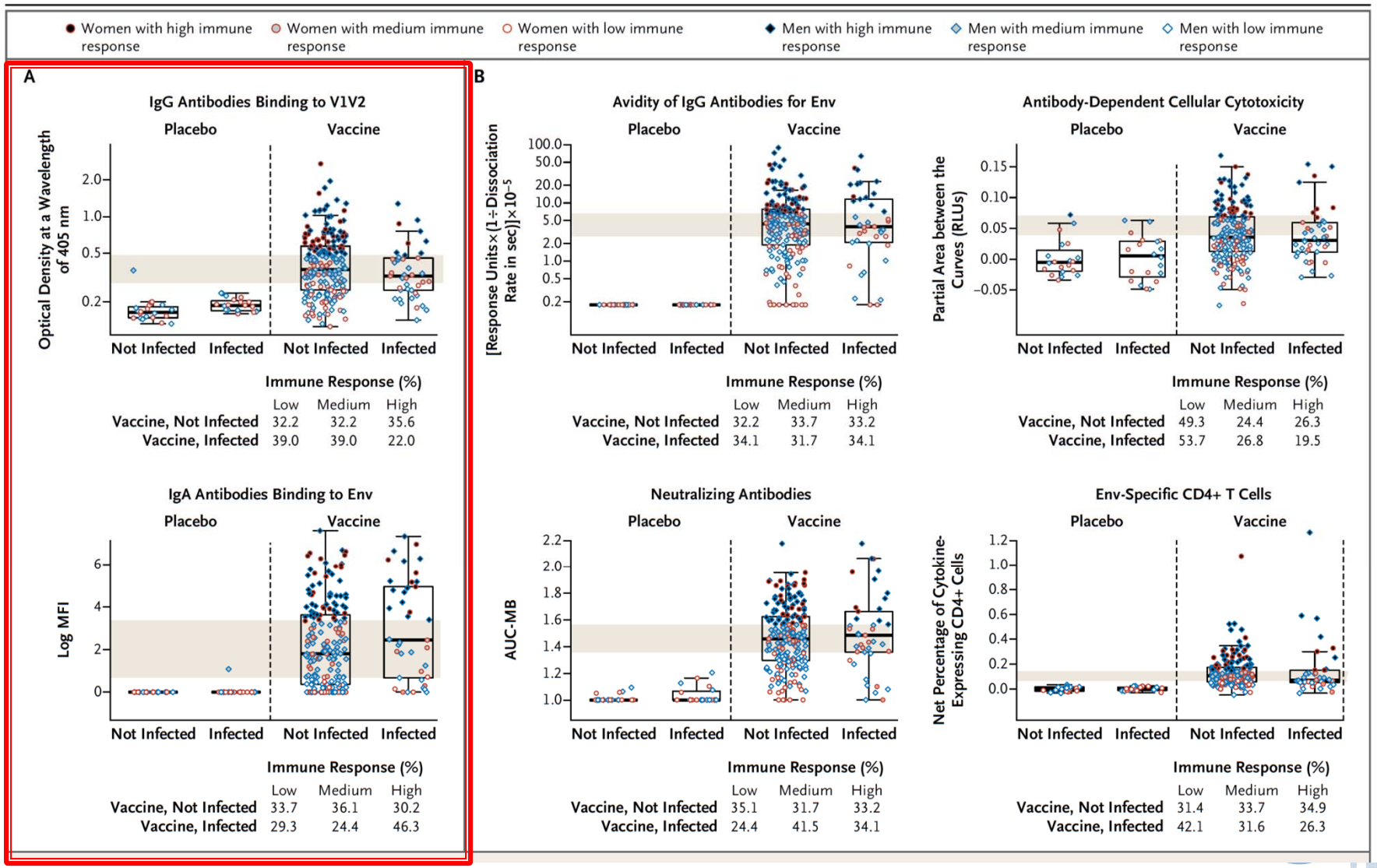


A Protective Vaccine? RV144 Trial

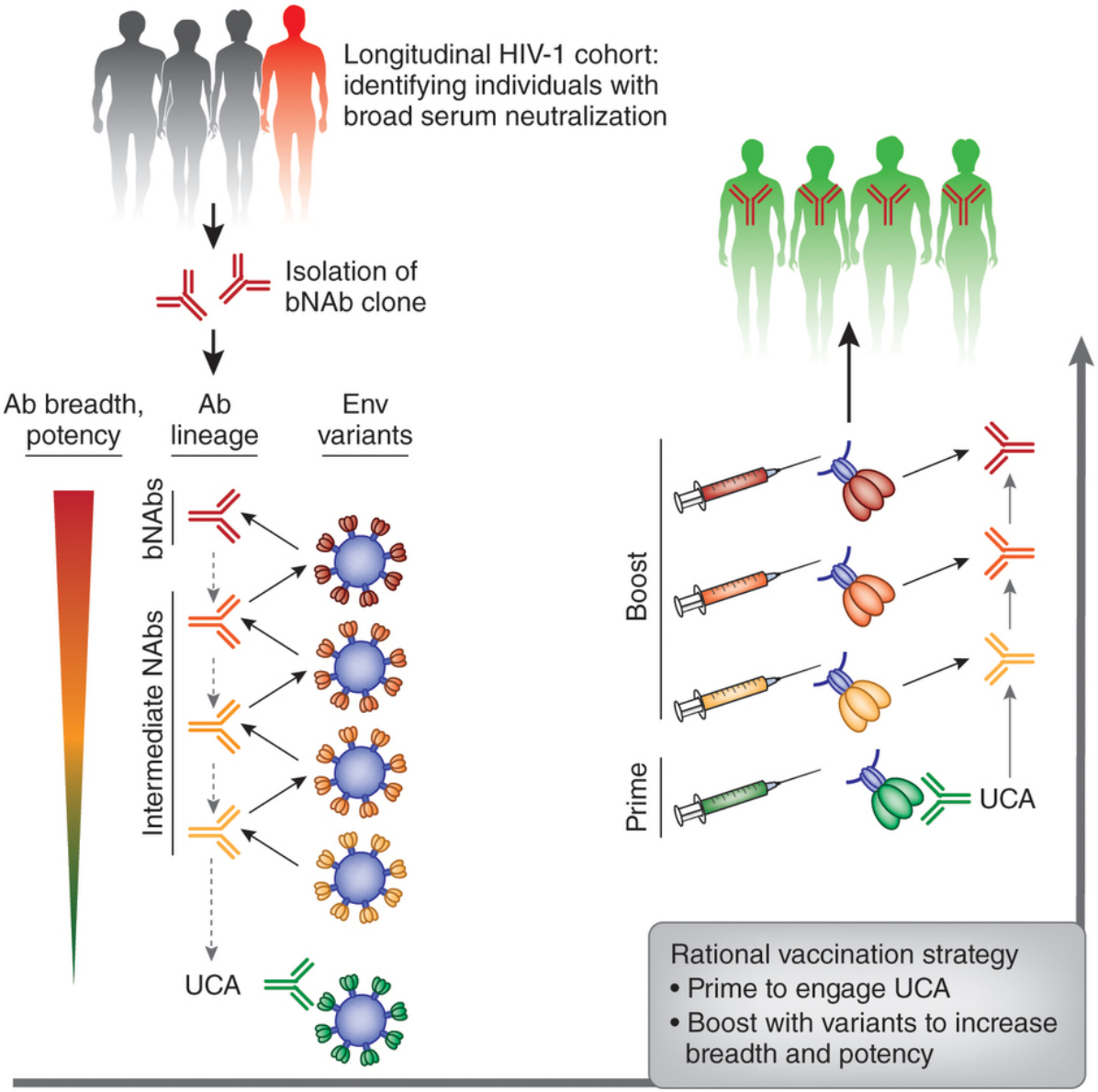
- ALVAC/AIDSVAX Prime boost-boost vaccine (canarypox followed by protein boost, gp120 based)
- 16,402 vaccinees
- Vaccine efficacy was 31.2%
- No mitigation of viral load in those that did become infected



IMMUNE CORRELATES OF HIV RISK



Broadly Neutralizing Antibody approaches for HIV Vaccine

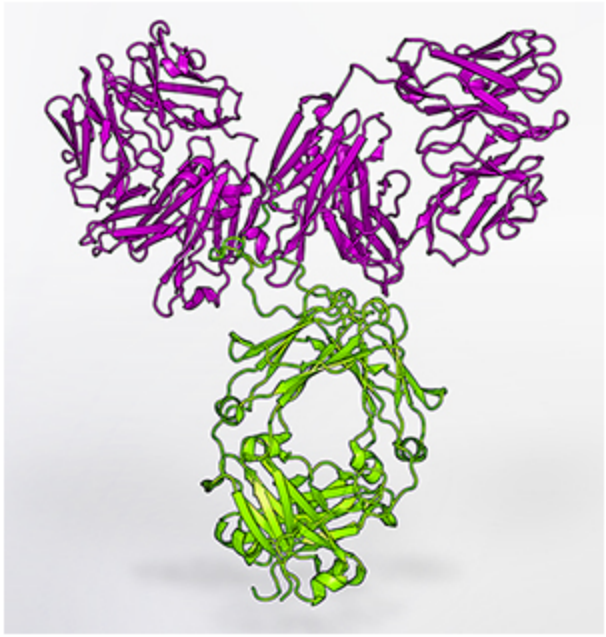


NIH Launches Large Clinical Trials of Antibody-Based HIV Prevention

Studies on Three Continents Could Have Broad Implications for HIV Prevention Research

Enrollment has begun in the first of two multinational clinical trials of an intravenously delivered investigational antibody for preventing HIV infection. Known as the AMP Studies, for antibody-mediated prevention, the trials will test whether giving people an investigational anti-HIV antibody called VRC01 as an intravenous infusion every 8 weeks is safe, tolerable and effective at preventing HIV infection. With a projected enrollment of 4,200 adults, the trials also are designed to answer fundamental scientific questions for the fields of HIV prevention and vaccine research.

The National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH), is sponsoring and funding the AMP Studies.



Model of the VRC01 antibody
Credit: NIAID
View larger image.

The NIAID Vaccine Research Center (VRC) discovered the VRC01 antibody in the blood of an HIV-infected person in 2010 and subsequently manufactured the antibody for these trials. Laboratory studies have shown that VRC01 stops up to 90 percent of HIV strains worldwide from infecting human cells, and thus it is considered to be a broadly neutralizing antibody.

"The AMP Studies could have a major impact on the future of HIV prevention and may be especially informative to HIV vaccine research," said NIAID Director Anthony S. Fauci, M.D. "Many scientists believe that if a vaccine were developed that elicited broadly neutralizing antibodies in healthy people, it would protect them from HIV infection. The AMP Studies will test this hypothe by directly giving people the VRC01 antibody."

In addition, the studies could clarify what level of broadly neutralizing antibodies a vaccine or other long-acting HIV prevention method needs to achieve and maintain to provide sustained protection from the virus.

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Points for Discussion

- HIV is a unique pathogen in that it targets the immune system directly—playing "offense"—killing or dysregulating the cells that specifically target it and "defense", employing more conventional immune escape mechanisms
- Despite this, the immune response, both antibody and CTLs, provide an important level of control over the virus for an extended period of time, keeping the reservoir relatively stable
- Vaccines could in principle employ similar strategies, but drugs are still the most effective treatment tool