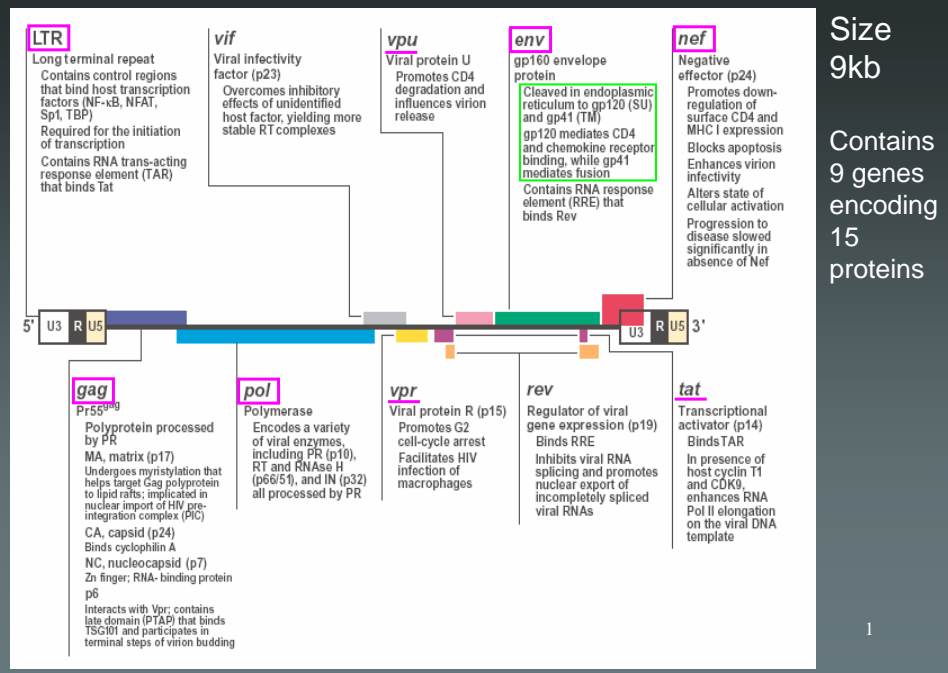
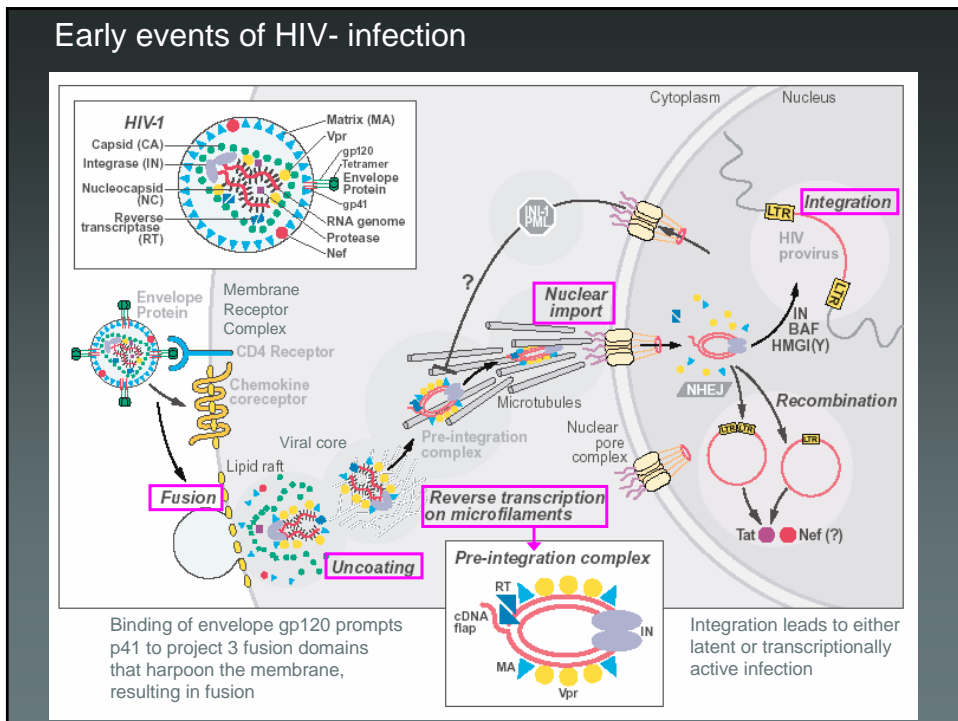


## Organization of HIV-1 Provirus



## Early events of HIV-1 infection



## Host Response to HIV-1 infection

**First Phase:** CD8 T cell response of immune system controls initial destruction of memory/effector CD4 T cells, but does not eliminate infectious virus primarily located in monocytes and memory CD4 T cells

Antibodies to HIV-1 are formed but these neither clear the infection nor are protective

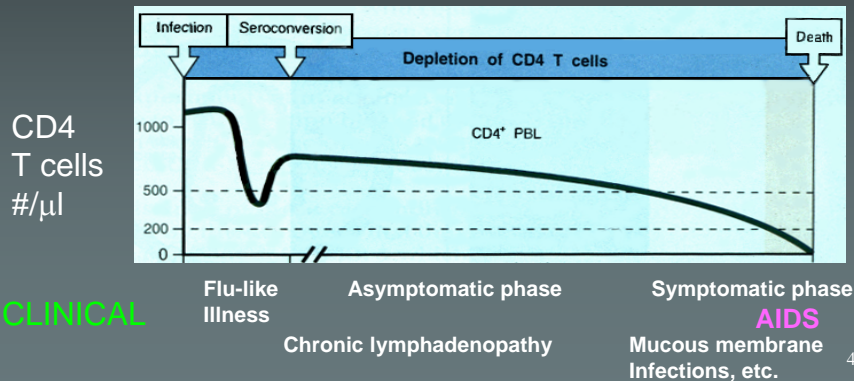
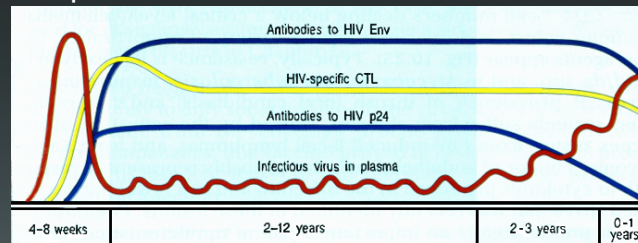
- Acute illness- “flu-like”
- Clinical asymptomatic phase- 2-12 or more years

**Second Phase:** HIV-1 escapes the CD8 T cell response and mutations in the viral envelope now favor infection and destruction of naive CD4 T cells

**Acquired immune deficiency (AIDS) appears upon depletion of critical CD4 T cell subsets**

3

## Immune response to HIV-1 and effects of HIV infection



4

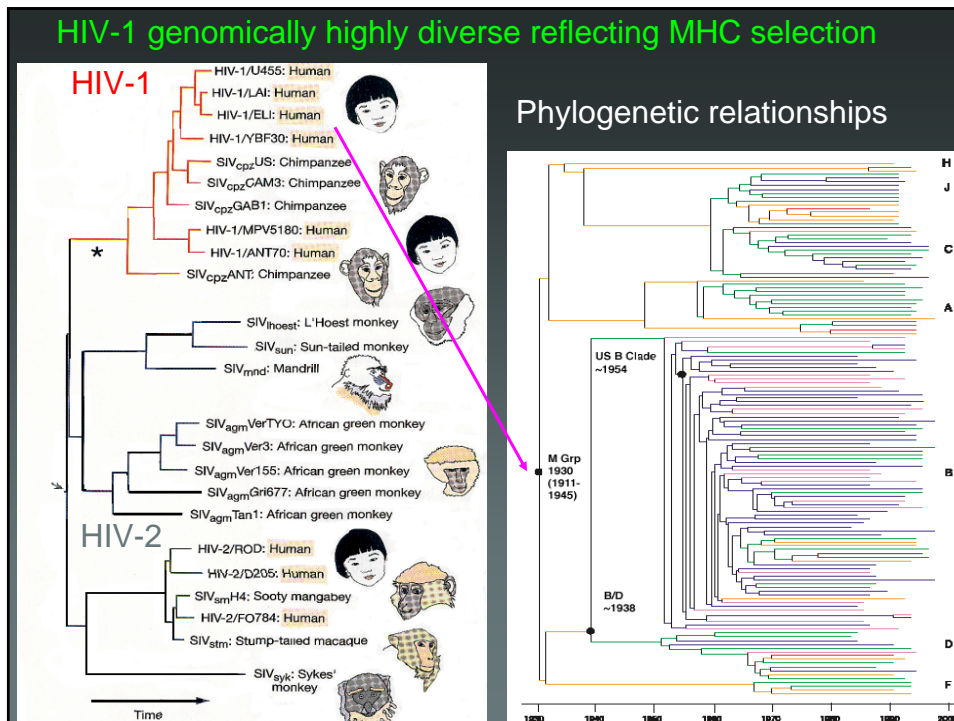
# Host - Parasite Relationships of HIV

Reverse transcriptase has no proofreading function and creates a vast number of mutations

HIV must adapt and evolve in an environment determined by attributes of the host's immune system

- MHC alleles
- TCR repertoire
- Polymorphism of viral entry receptors
- Chemokine and cytokine milieu (e.g. parasitic infections)
- Other genes regulating immune response
- Prior immune history
- Age

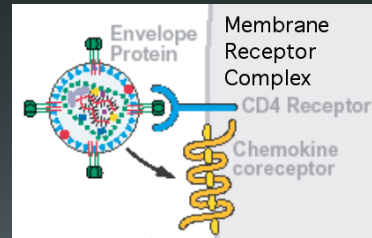
Outcome of infection depends on biology of host, especially whether immune response targets critical HIV structures and HIV-1 mutational capacity, etc.



## Cellular Specificity, “Tropism” of HIV strains

### Based on envelope structure

- The viral envelope contains sequences that interact with a membrane viral receptor complex composed of CD4 and one of several chemokine receptors



- The sequence of a given viral envelope is specific for **one** of the chemokine receptor types
- The main two chemokine receptors are **CCR5** and **CXCR4** that are **distributed on different cell lineages**
- Strains that bind to **CCR5** are termed “**R5**” tropic and those that bind **CXCR4** are termed “**R4**” tropic

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## Chemokine Receptors:

### CCR5

- **Ligands:** **RANTES, MIP-1 $\alpha$ , MIP-1 $\beta$**  are produced in large quantities by activated CD8 and CD4 T cells in the immune response to HIV and **compete with R5 HIV binding to membrane receptor complex, blocking progress of the infection**
- **Distribution:** **CCR5 found on monocytes, DC and effector, memory or activated T cells**, not naïve CD4 T cells
- **Biology:** CCR5 responsible for migration of memory and effector T cells, monocytes and dendritic cells to sites of inflammation
- **Several CCR5 polymorphisms:** e.g.  **$\Delta 32$  mutant** allele render CCR5 unexpressed and incapable of binding HIV R5 strains. Homozygote frequency 1%, heterozygote ~10% in N.Euro. Caucasoids, but X4 strains are still infective

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## Chemokine Receptors: Coreceptors for HIV entry

### CXCR4

- **Ligand:** **Stromal derived growth factor 1 (SDF-1)** produced by stromal cells. Competes with HIV binding, but not produced in inflammation or by T cells
- **Receptor:** **expressed on** monocytes, **naïve T-cells**, B-cells, etc. **X4 virus preferentially infects naïve/activated T cells**
- **Biology:** **SDF-1 responsible for migration/homing of naïve T cells to lymph node**

(Because T-cell lines only express CXCR4 coreceptors and respond to HIV infection by forming syncytia, earlier X4 strains were termed “syncytia inducing, or T-tropic”)

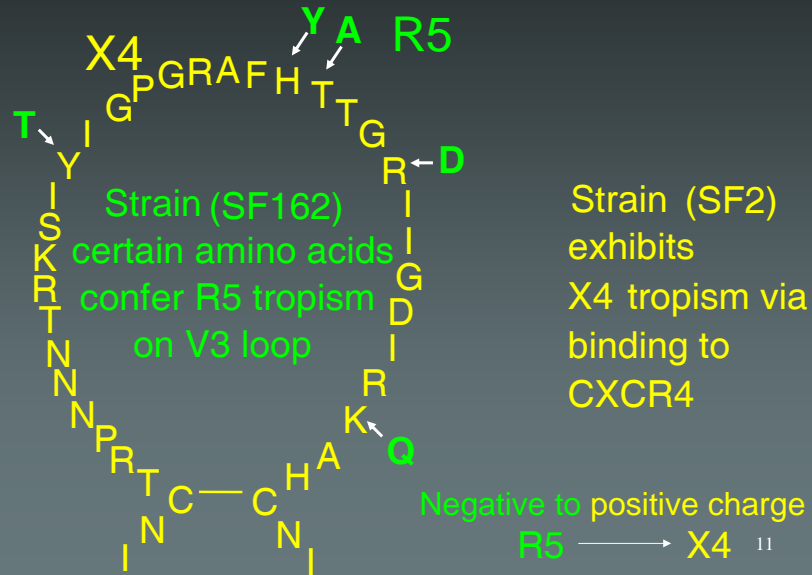
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## HIV strain early in infection

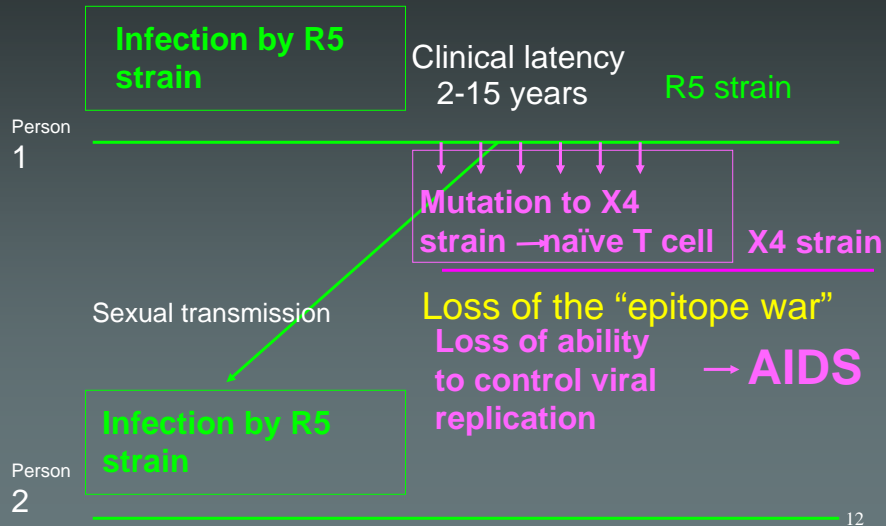
- *R5 is almost always the sexually transmissible form of the virus*
- **Primary isolates from newly infected individuals are usually R5**
- **R5 strains mainly replicate in monocytes.** Activated and memory T cells are infected, but at lower efficiency (old term = MT-tropic or monocyctotropic)
- Therefore much of the viral load in earlier phase of HIV infection is in the monocytes and macrophages and **the number of CD4 T cells though decreased, remains stable**

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## Mutation of R5 to X4: V3 Envelope Loop sequence and strain tropism change



Evolution of tropism in an individual from R5 to X4 is the precursor to developing immune deficiency, but R5 strains are preferentially sexually transmitted



HIV infection is controlled by the immune system, but only for a period of time

- What is the nature of the immune response to HIV and what mechanisms does HIV use to circumvent it?

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## Acute HIV-1 Infection "Flu-Like"

### Clinical

- Headache, retro-orbital pain, myalgias, pharyngitis, fever, Nonpruritic maculopapular rash in first 1-3 weeks  
Adenopathy and malaise may last for several months
- Transient thrombocytopenia and CD4 T-cell lymphopenia

### Viral

- Rapid appearance of **marked viremia with an R5 strain infecting monocytes and memory CD4 T cells**
- This results in acute **CD4 T-cell lymphopenia**
- **Integration in memory CD4 T cells provides a long-lived reservoir where HIV can remain latent**
- Structurally the **initial** virus strain has **no, or very limited diversity**

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## Acute Infection

### Development of anti HIV Immune Response

- With onset of a **CD8 T-cell immune response** viremia falls from  $\sim 5 \times 10^6$  /ml to  $< 10^4$  /ml
- The CD4 T-cell count rises from  $\sim 400$  to  $> 800/\mu\text{l}$
- Degree of viral suppression and return of CD4 T cell levels (**set point !**) varies and correlates with the length of the asymptomatic period
- HIV species begin to **diversify**, viral variants appear reflecting successful attempts to escape the suppression of the CD8 T cell response
- The virus mainly persists in monocytes / macrophages

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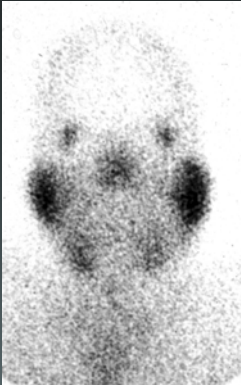
### CD8 T-cell Response to HIV-1

- Establishes asymptomatic phase of infection
- The CD8 T-cell responds to **HIV-peptides** by activation, clonal expansion, and differentiation to effector status
- Specific **lysis of HIV- infected target cells** (macrophages and CD4 T cells) via perforin pathway and/ or apoptosis via upregulation of *fas* ligand
- **Strong inhibition of viral infectivity by release of chemokines** (MIP-1 $\alpha/\beta$ , RANTES) that **bind to CCR5 and compete with coreceptor dependent entry of R5 HIV-1**
- Release of **IFN- $\gamma$  and secondarily TNF- $\alpha$** , decrease LTR-driven transcription

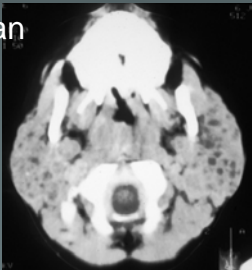
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Excessive anti HIV CD8 T cell response may result in diffuse infiltrative lymphocytosis syndrome (DILS) simulating Sjogren's syndrome



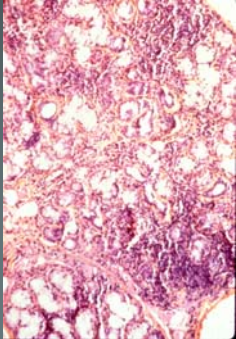
Nuclide scan



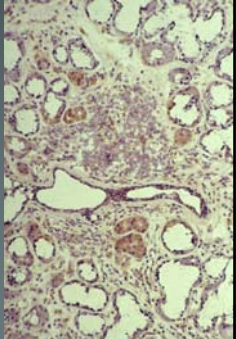
CT scan

CD8 T cells >2000/ $\mu$ l

Salivary gland biopsy





H & E



HLA-DR stain

DILS is usually associated with long term non progression and a favorable outlook

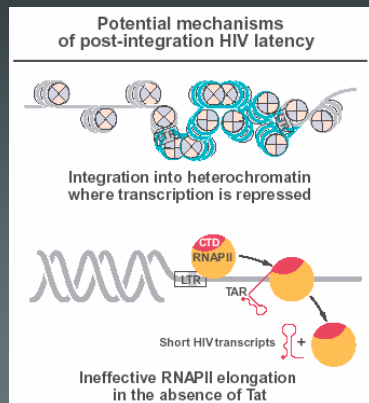
However, it is also associated with a type of B cell lymphoma that occurs early in the course of HIV infection, reflecting chronic B cell stimulation

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## Reasons for failure of CD8 T cells to totally eliminate HIV-1

### No expression of viral peptides



(also inaccessible to Rx)

### Thwarted immunosurveillance

Nef and vpu diminish MHC class I expression, thus avoiding infection surveillance, especially when in monocytes

Nef is particularly clever since it decreases HLA-A and HLA-B, but not HLA-C and HLA-E, thus avoiding most NK cell surveillance

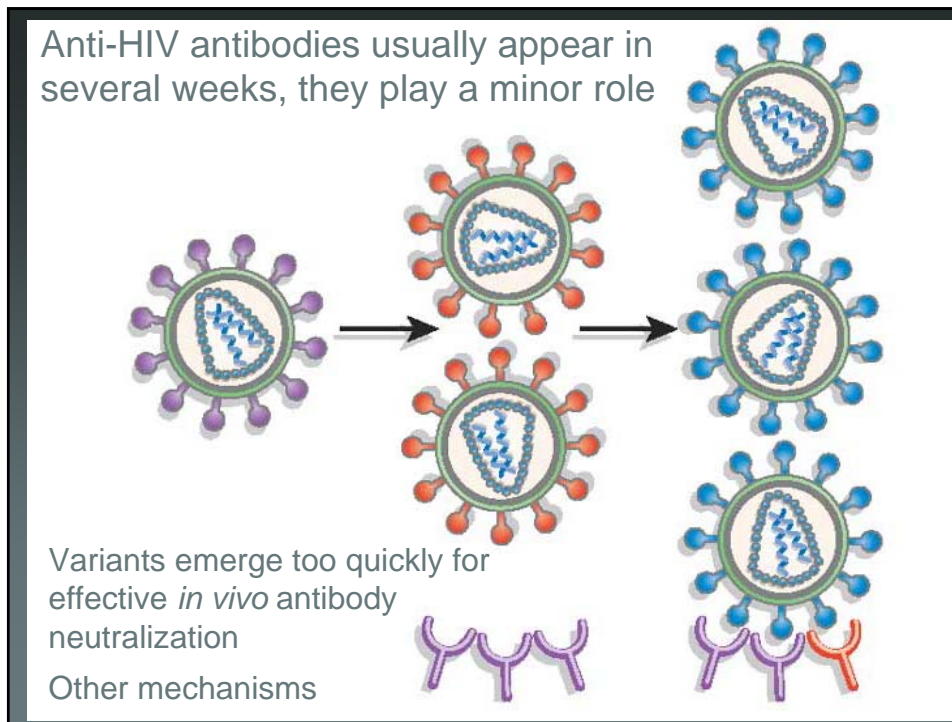
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### Thwarted immunosurveillance

#### Dendritic cells used as a "Trojan Horse"

- Immature DCs, typically located in the submucosa express a C-type lectin DC-SIGN
- HIV-1 envelope binds to DC-SIGN with high affinity
- The virions are internalized and remain in acidic endosomal compartments while the DC matures
- Intact infectious virions are reexpressed on the surface when the DC enters the lymph node

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## Immune Responses in asymptomatic phase

Depends on a relatively few CD8 T cell clones

- Maintenance of <5-20 CD8 T-cell expanded memory/ effector CTL clones, each comprising 1-5% of CD8 T cell repertoire
- Clones each recognize different HIV peptides, great individual variation in number and particular peptide recognized
- Many clones = generally good outlook for long asymptomatic period (>12yrs), few clones = rapid progression of HIV infection (<2yrs)
- The number of clones and survival duration correlates with the viral "set point" established in the acute infection

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## Long term non progressors

- A subset of infected individuals that remain asymptomatic for >12 years
- Particular HLA types, e.g. HLA-B27, B57, etc.
- Low levels of plasma virions, CD4 counts >500/ul
- High CD8 T-cell counts, may be > 3,000/ul
- High chemokine release (RANTES, MIP)
- CTL response is against critical conserved region of HIV gag, env, pol that cannot readily be mutated without loss of viral function-This appears to be the key factor !

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The particular peptide recognized by CD8 T cells is critical to whether the infection will be controlled

**If the recognized peptide encodes a region that is essential for HIV function, any mutation in that site will be lethal for the virus**

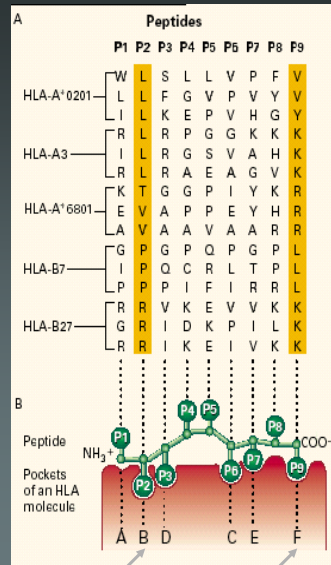
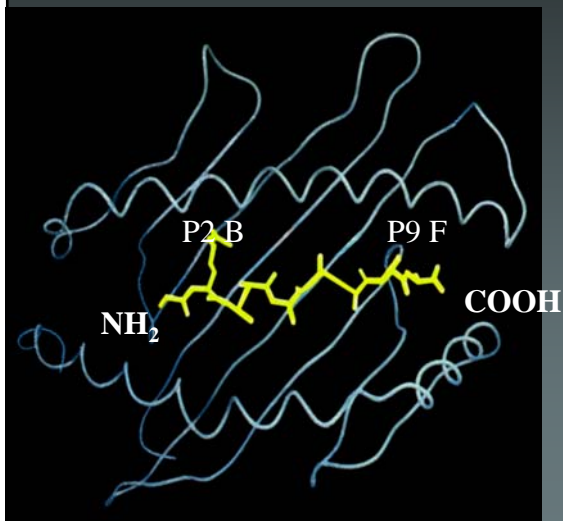
**For this to occur two conditions must be met:**

**1. The correct peptide must be presented.** The individual's class I MHC alleles are the major determinant of which peptide is recognized. They determine the particular peptides that are bound and presented

**2. The peptide must be recognized by a T cell clone.** Not all bound peptides are equivalently recognized by T cell clones in the repertoire. Only a few bound peptides are "immunodominant", and readily recognized

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## The T cell ligand: combination of peptide and class I MHC



The environment formed by peptide binding properties of MHC molecules influences evolution of the HIV infection. HLA alleles influence the number of peptides in a protein that can be recognized (Example HIV envelope protein)

Allele: **HLA-B\*27052**

**HLA-B\*3501**

**HLA-B\*0702**

Motif X**R**XXXXXXXX**[KRYL]**

X**P**XXXXXXXX**Y**

X**P**XXXXXXXX**L**

Peptides able to bind each allelic molecule

<b>I</b> RGKVQ <b>K</b> <b>Y</b>	<b>K</b> R <b>R</b> VVQ <b>R</b> <b>E</b> <b>K</b>	<b>D</b> P <b>N</b> PQ <b>E</b> V <b>V</b> <b>L</b>
<b>I</b> R <b>P</b> VV <b>S</b> T <b>Q</b> <b>L</b>	<b>A</b> R <b>L</b> A <b>V</b> E <b>R</b> <b>Y</b>	<b>K</b> P <b>C</b> V <b>K</b> L <b>T</b> P <b>L</b>
<b>T</b> R <b>P</b> N <b>N</b> N <b>T</b> R <b>K</b>	<b>E</b> R <b>D</b> R <b>D</b> R <b>S</b> <b>I</b> R	<b>R</b> P <b>V</b> V <b>S</b> T <b>Q</b> <b>L</b> <b>L</b>
<b>I</b> R <b>I</b> Q <b>R</b> G <b>P</b> G <b>R</b>	<b>L</b> R <b>S</b> L <b>C</b> L <b>F</b> S <b>Y</b>	<b>S</b> P <b>L</b> S <b>F</b> Q <b>T</b> H <b>L</b>
<b>S</b> R <b>A</b> K <b>W</b> N <b>N</b> T <b>L</b>	<b>T</b> R <b>I</b> V <b>E</b> L <b>L</b> G <b>R</b>	<b>I</b> P <b>R</b> R <b>I</b> R <b>Q</b> G <b>L</b>
<b>L</b> R <b>E</b> Q <b>F</b> G <b>N</b> N <b>K</b>	<b>C</b> R <b>A</b> I <b>R</b> H <b>I</b> P <b>R</b>	
<b>F</b> R <b>P</b> G <b>G</b> G <b>D</b> M <b>R</b>	<b>I</b> R <b>Q</b> G <b>L</b> E <b>R</b> I <b>L</b>	
<b>W</b> R <b>S</b> E <b>L</b> Y <b>K</b> <b>K</b>		

# of peptides

**15**

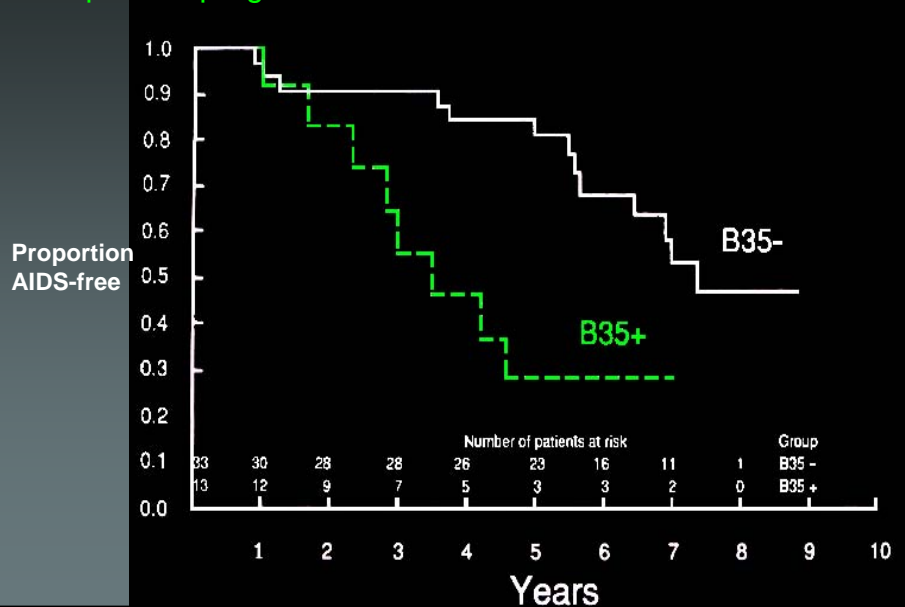
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## Role of MHC in Recognition of HIV peptides

### Rapid HIV progression in HLA-B35 individuals



## Basis of outcome with HLA type

### HLA-B35 RAPID PROGRESSION

xPxxxxxxY peptides recognized, if any, are in non critical parts of HIV genome permitting mutations in MHC anchor residues. Peptides weak stimulators  
Rapid viral replication and evolution not restrained

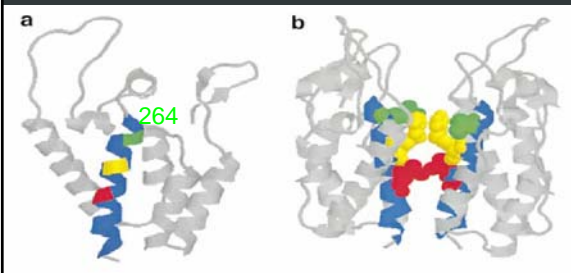
### HLA-B27 SLOW PROGRESSION

xRxxxxxx[KRYL] peptides recognized are often in critical parts of HIV genome and mutations not permitted in MHC anchor or TCR recognition residues  
Viral replication and evolution greatly slowed

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## An example of HIV-1 escape from a CD8 T cell clone

HLA-B27 hemophiliac, infected ~1983 by blood products



*CTL clone to gag  
p24<sub>263-272</sub>  
controlled HIV-1  
replication for >10  
years*

	Virions/ml	CD4/ $\mu$ l	Gag p24				Tropism	CTL					
1984	1,800	510	K	<sup>264</sup> R	W	I	L	G	L	N	K	R5	+++
1993	780	400	-	-	-	M	-	-	-	-	-	X4	+++
1995	21,400	60	-	-	-	M	-	-	-	-	-	X4	+++
			K	-	-	M	-	-	-	-	-	X4	0
1996	530,970	10	K	-	-	M	-	-	-	-	-	X4	0

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Kelleher, JEM 2001

## Immune responses in asymptomatic phase

### Shifting immunodominance in "epitope war"

- Usually recurrent pattern of HIV escape from immunodominant CTL effect by mutation followed by regain of CD8 CTL control via next HIV peptide that can be presented by MHC class I and recognized by TCR in **hierarchy of HIV peptide immunodominance**
- During the progression of the infection in a person a huge number (swarm) of mutant forms arise (quasispecies)

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## Viral Response near end of asymptomatic period

- Rate of cellular infection and potential mutations increases
- Definitive **viral escape** occurs when virus is no longer presented by MHC to available CD8 T cell clones
- Continual generation of env mutations
- **Selection against R5 variants by CD8 T-cell CCR5 chemokines that blocks infection is finally bypassed**
- Change in cellular tropism by env mutations leads to **X4 phenotype** (CXCR4, T-tropic)
- **Enhanced T-tropism** of X4 leads to more significant impairment of CD4 T-cell compartment

**Loss of the “epitope war”**

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## Reasons for CD4 T cell loss in HIV-1 Infection

During asymptomatic phase and transition to AIDS

Accelerated loss in number of CD4 T cells

- Activation of large numbers of mature and naïve CD4 T cells by cytokines, etc. during antiviral response (Bystander activation, homeostatic regulation) leads to loss of repertoire by physiologic apoptosis
- Thymic derangement results in failure to generate new naïve CD4 T cells to repopulate repertoire
- CD8 T cell killing of infected CD4 T cells
- ADCC by NK cells, etc. to infected CD4 T cells

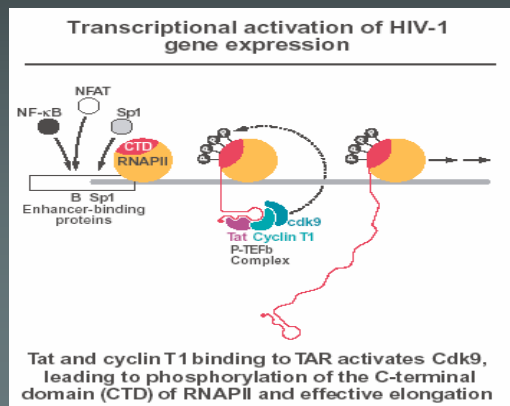
32



## Another reason for CD4 T cell loss

CD4 T cell activation initiates HIV replication  
HIV replication initiates CD4 T cell activation

T cell activation causes, among other effects, a marked increase in **cyclin T1**, **NFAT** and **NF $\kappa$ B**



This links viral expression to T cell activation

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## AIDS is the consequence of progressive CD4 loss

T cell immune function progressively deteriorates reflecting the central role of CD4 T cells

Stages:

- Loss of antigen-specific clonal responses (in vitro proliferation and skin test to various antigens, including those from immunizations)
- Loss of ability to generate new CD8 T cell responses
- Loss of Mixed Lymphocyte Culture responsiveness
- Loss of PHA responsiveness

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## AIDS is the consequence of progressive CD4 loss

Appearance of different infections as severity of immune deficiency increases

Candida (Thrush)

Salmonella - microbial persistence (Reactive arthritis?)

Mycobacterium tuberculosis reactivation, Cryptosporidium

Activation of latent herpes zoster

EBV reactivation and development of polyclonal lymphomas, Kaposi's sarcoma (HHV-8)

Pneumocystis carinii

Progressive cytomegalovirus infections, M. avium complex

## HIV virus vaccines have failed, Why?

- Immunization with rENV produce neutralizing antibodies
- But **neutralizing antibodies induced by immunization fail to protect as shown in multiple trials**
- A live attenuated virus has not yet proved achievable
- The second larger issue is heterogeneity of HIV strains, **need many immunodominant peptides** directed to **critical regions** of viral genome because no cross protection
- Some strains, mainly X4 tropic have **evolved to circumvent MHC presentation** by some common alleles. With high numbers of infected individuals there is increasing chance of infecting a person with the same HLA by a strain evolved to avoid immunosurveillance
- Recombinant live virus vaccines are under trial, there. a major issue is providing HIV **peptides able to bind divergent MHC class I** of a large proportion of the population

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But vCP205 a canarypox vector expressing gp41, Gag and Protease HIV genes addresses- in theory- all of these concerns

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### Case Report of a failure of a recombinant live vaccine

Betts et al. PNAS 2005, **102**:4512

Case # 202-T07, an HLA-B\*2705 male homosexual HIV-negative individual

vCP205 canarypox vector expressing gp41, Gag and Protease vaccination course given over 5 months

Immune response documented to two CD8 epitopes and one CD4 epitope including response to the HLA-B\*2705-restricted Gag peptide KRWIIIGLNK in central and peripheral memory/effector CD8 T cells  
CD28+CCR7+CD45RO+ and CD28-CCR7-CD45RO-

Approximately 18 months later 202-T07 had unprotected anal intercourse with an undisclosed HIV+ partner

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Shortly thereafter, he developed flu-like symptoms

He was then found to be positive for HIV antibodies, with a plasma viral load of 234,695 HIV-1 virions/ml

The acute infection induced a recall response to the B\*2705-restricted clone, expanding it from 0.05% of CD8 T cells to 9.8% of CD8 T cells, and this remained the dominant clonotype during acute infection

During the acute infection period there was no evidence of viral escape, but by 32 months after diagnosis the predominant virion-encoded Gag peptide sequence mutated from KRWIIIGLNK to KGWIIIGLNK, thus thwarting binding and presentation of the peptide by HLA-B\*2705

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Viral escape this early is extremely unusual, the average time to development of this escape mutation in unvaccinated individuals is >9 years

Moreover, the average survival until AIDS in an HLA-B\*2705 individual is >14 years

CD4 T cell count continues to decline, presently 400 cells / $\mu$ l at 32 months post infection, and viral titre remains high, despite optimal anti-retroviral therapy

*The authors raise the strong possibility that a vaccine developed according to the best notions of current immunological knowledge not only did not protect against HIV infection but accelerated development of the escape mutation in the vaccinated individual, thus hastening progression of the viral infection*

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## Summary

### Host-Viral Relationship in HIV-1 infection

HIV-1 infects cells by binding to a membrane receptor complex consisting of CD4 and a chemokine receptor, the nature of the latter determines tropism of the virus for different populations of CD4 T cells and monocytes

**First Phase:** “flu-like” infection by R5tropic virus triggers **CD8 T cell response** of immune system that controls viral replication but does not eliminate latent infectious virus primarily located in **monocytes and memory CD4 T cells**

Antibodies to HIV-1 are formed but these neither clear the infection nor are protective

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### **Clinical asymptomatic phase:** 2-12 or more years

Ability of immune system to control viral proliferation depends on host’s HLA class I alleles, etc. and relies on a few CD8 T cell clones

HIV-1 mutates because of the lack of proofreading by RT and variants **escape** the CD8 T cell response, further mutations in the viral envelope change to R4 tropism that favors infection and destruction of **CD4 T cells**

**Symptomatic phase:** acquired immune deficiency (AIDS) appears upon depletion of critical CD4 T cell subsets

Vaccination efforts have been unsuccessful, and the most sophisticated recombinant vaccines may hasten progression to AIDS

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