Lecture 17. Viruses that Infect Lymphocytes: EBV and HIV

Long term Host - Pathogen Relationships

Host immune defense consistently fail to clear a few viral infections

Examine mechanisms pathogens use to evade immune defense

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Contrasting host-pathogen relationships of two prototype infections

Epstein-Barr Virus (EBV)

- Large DNA virus, e.g. herpesviruses
- Coevolved with host species over millions of years
- Genetically stable
- Persist in host in latent pattern of viral gene expression in response to T cell surveillance

Human immunodeficiency virus (HIV-1)

- RNA retrovirus
- Recently introduced into humans
- Error-prone viral replication mechanisms resulting in “swarms” of distinct strains “quasispecies”
- Overwhelm host by escaping from immune surveillance

2

Host-pathogen relationships

Some mechanisms of avoiding immune surveillance

1. Avoid recognition by cytotoxic T cells

- Evolution of viral strains that avoid presentation by MHC by mutating class I molecule peptide anchor amino acids or amino acids recognized by T cells in immunodominant peptides
- Blocking of antigen processing and presentation

3. Suppression of viral gene expression by the virus

Change from productive to latent mode by selective pressure of immune response

2. Modification of the immune response

e.g. release of anti-inflammatory cytokines, IL-10

3

Syndromes resulting from EBV infection

Age of host influences character of primary infection

- Primary infection with EBV in childhood usually subclinical
- 25-70 % of newly infected adolescents and adults develop infectious mononucleosis:
  - Fever
  - Lymphadenopathy
  - Pharyngitis
  - Transient heterophil antibodies
  - Activated CD8 cytotoxic anti EBV T cells (“atypical lymphocytosis”)

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Syndromes resulting from EBV infection

EBV-driven neoplasms

- Nasopharyngeal carcinoma, Gastric cancer subset
- B cell lymphomas:
  - Burkitt's Lymphoma
  - Immunoblastic lymphoma in immunosuppressed host
  - Subset of Hodgkin’s disease

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EBV is a B cell lymphotropic herpesvirus

Stages of EBV infection

Binding EBV surface glycoprotein to CD21 (CR2)

- CD21expressed on B cells as BCR co-receptor complex with CD19
- CD21 also expressed on some epithelial cells, accounting for tropism

Triggers T-independent polyclonal B cell activation Ig synthesis and B cell proliferation

Results in T-independent release of heterophil and other antibodies (Rheumatoid factor, cold agglutinins, ANA)

EBV enters the cell by receptor mediated endocytosis

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Initially EBV replicates as a productive lytic infection

- IgM antibodies to Viral Capsid Antigens (VCA) and Early Antigens (EA) are found at clinical presentation, indicating lytic replication and persists for 1-2 months.
- IgG anti VCA appears at time of clinical presentation and persists lifelong-"standard EBV titre"
- Antibodies to EA Peak at 3-4 weeks; marker of more severe disease
- Lytically infected cells are largely eliminated by EBV-specific cytotoxic cells, NK cells, interferon-mediated mechanisms and ADCC

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By 3-6 weeks EBV enters latent stage in majority of remaining infected B cells

- Antibodies to latent EB Nuclear Antigens (EBNA's) appear 3-6 weeks after initial infection; last lifelong
- Virus evades cytotoxic response in latent form
- CD8 T cells play a crucial role in enforcing the maintenance of latency and thwart proliferation of EBV infected B cells by killing the B cell

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- EBV is maintained in its latent infective cycle as a multicopy circular 172Kd ds plasmid minichromosome with replication linked to B cell proliferation

9 Latent proteins:
Six nuclear antigens:
EBNA1, 2, 3A, 3B, 3C, and EBNA leader protein (EBNA-LP)
Three latent membrane proteins:
LMP1, LMP2A, and LMP2B

- EBNA1 binds to the EBV *ori*, initiating replication and also acting as a transcriptional enhancer

EBNA1 contains a gly-ala repeat region that inhibits the ATP motor of the proteasome, impeding further insertion of EBNA1 into the proteasome, thus halting its degradation, a strategy for avoiding surveillance

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B cell lymphoblastoid cell line (BLCL)

BLCL can be derived from nearly everyone, *in vitro*

Express all latency genes, a pattern designated latency III,

BLCL phenotype high expression of:
B cell activation markers CD23, CD30, CD39, and CD70
Cellular adhesion molecules LFA1 (CD11a/18), LFA3 (CD58), and ICAM1; (CD54)

Because of the adhesion molecules these BLCLs grow in large clumps in tissue culture

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Immunoblastic lymphomas

Develop in transplant recipients or other patients receiving T cell immunsuppressive therapies

- Resembles the BLCL phenotype
- Express all latency genes
- Start as multi-/polyclonal proliferations
- Withdrawal of immunosuppression results in regression
- Secondary transformation events may occur: monoclonal lymphoma

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Burkitt's lymphomas

Chronic immune system drive, e.g. by malaria implicated as cofactor, but no overt immune deficiency

- Exhibit a different gene expression pattern "latency I"
- Only abundant EBNA1 transcription is found
- Lymphoma cells display a distinct phenotype: CD10+ (CALLA) and CD77+ (BLA), but lack expression of activation and adhesion molecules
- In culture Burkitt tumor B cell lines grow as dispersed single cells

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The achilles' heel of the immune system

The T cell immune response to viruses often uses a very small number of different CD8 T cell clones directed to one or a few "immunodominant" peptides encoded by the viral genome, that are often presented by just one allelic type of an individual's HLA molecules

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EBV infection results in nasopharyngeal carcinomas in Papua New Guinea and southern China

HLA-A11distribution African 1.5%, Caucasian 6.9%, Asian 16.3%

HLA-A11-positive Caucasians nearly always respond to two immunodominant HLA-A*1101 epitopes of the nuclear antigen EBNA3B (EBNA4):

IVTDFSVIK 416 to 424

AVFDRKSDAK 399 to 408

These sequence motifs were often mutated in EBV strains in lowland Papua New Guinea and southern China, areas where more than 50% of individuals carry the HLA-A*1101 allele

All mutated strains have a point A -> C mutation, which produces a Lys -- Thr (K -> T) change in residue 424 of EBNA4 at position 9 of the CTL epitope (Frequency dependent selection)

Loss of recognition of immunodominant epitope and ability to recognize EBV is a mechanism of escaping the CTL response implicated in neoplastic transformation

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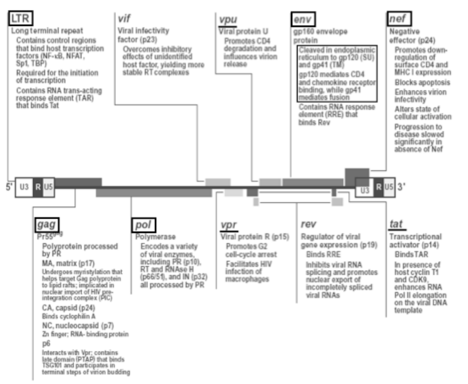
Organization of HIV-1Provirus



Size 9kb

Contains 9 genes encoding 15 proteins

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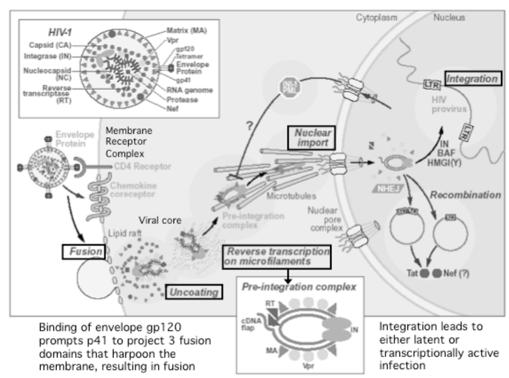
Early events of HIV- infection



Binding of envelope gp120 prompts p41 to project 3 fusion domains that harpoon the membrane, resulting in fusion

Integration leads to either latent or transcriptionally active 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 naïve CD4 T cells

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

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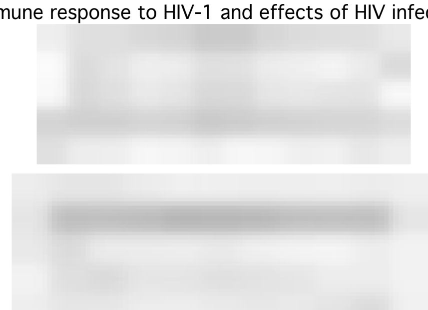
Immune response to HIV-1 and effects of HIV infection



CD4 T cells #/μl

CLINICAL

Flu-like Illness

Asymptomatic phase

Symptomatic phase AIDS

Chronic lymphadenopathy

"Set Point"

Mucous membrane Infections, etc.

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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.

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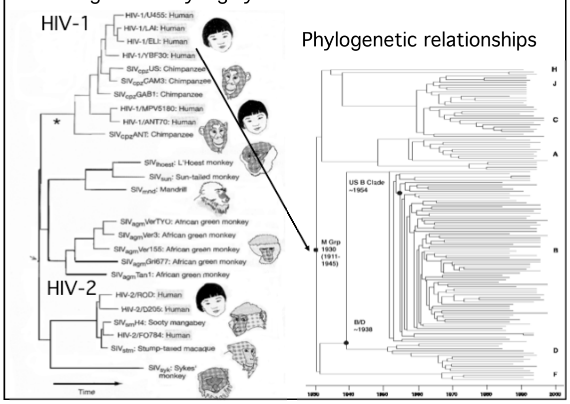
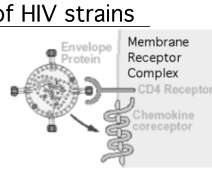
HIV-1 genomically highly diverse



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-1α, MIP-1β are inflammatory cytokines made 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. Δ32 mutant allele render CCR5 unexpressed and incapable of binding HIV R5 strains.
- Δ32 Homozygote frequency 1%, heterozygote ~10% in N.European 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/CXCR4 responsible for migration/homing of naïve T cells to lymph node

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HIV strain tropism 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
- 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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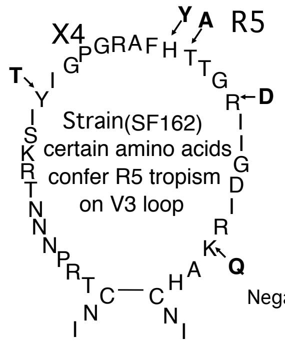
Mutation of R5 to X4: a few changes in envelope V3 Loop sequence changes strain tropism



Strain (SF2) exhibits X4 tropism via binding to CXCR4

Negative to positive charge R5 → X4

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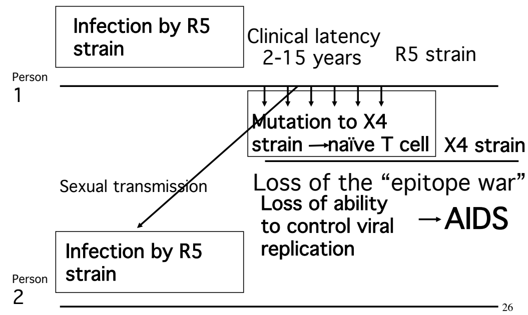
Evolution of tropism in an individual from R5 to X4 is the precursor to developing immune deficiency, but R5 strains are preferentially sexually transmitted



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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 ~5x10⁶ /ml to <10⁴ /ml
- The CD4 T-cell count rises from ~400 to >800/μl
- 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

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CD8 T-cell Response to HIV-1

- Establishes asymptomatic phase of infection
- Specific CD8 CTL 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-1α/β, RANTES) that bind to CCR5 and compete with coreceptor dependent entry of R5 HIV-1
- Release of IFN-γ and secondarily TNF-α, decrease LTR-driven transcription

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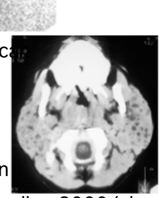
Excessive anti HIV CD8 T cell response may result in diffuse infiltrative lymphocytosis syndrome (DILS) simulating Sjogren's syndrome

Nuclide scan

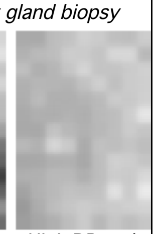


CT scan

CD8 T cells >2000/μl

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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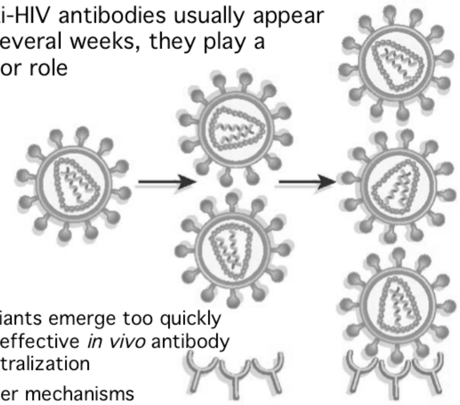
Anti-HIV antibodies usually appear in several weeks, they play a minor role



Variants emerge too quickly for effective *in vivo* antibody neutralization

Other mechanisms

Immune Responses in asymptomatic phase

Depends on a relatively few CD8 T cell clones

- Maintenance of a few CD8 T-cell expanded memory/ effector CTL clones, each comprising 1-5% of CD8 T cell repertoire
- Clones each recognize different **immunodominant** HIV peptides, great individual variation in number and particular peptide recognized
- More clones = generally good outlook for long asymptomatic period (>12yrs), fewer clones =rapid progression of HIV infection (<2yrs)

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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 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**XXXXXX[**KRYL**]		X**P**XXXXXX**Y**	X**P**XXXXXX**L**
Peptides able to bind each allelic molecule				
	I**R**GKVQKE**Y**	K**R**RVVQRE**K**		D**P**NPQEVV**L**
	I**R**PVVSTQ**L**	A**R**ILAVER**Y**		K**P**CVKLTP**L**
	T**R**PNNNTR**K**	E**R**DRDRSI**R**		R**P**VVSTQL**L**
	I**R**IQRGPG**R**	L**R**SLCLFS**Y**		S**P**LSFQTH**L**
	S**R**AKWNNT**L**	T**R**IVELLG**R**		I**P**RRIRQG**L**
	L**R**EQFGNN**K**	C**R**AIRHIP**R**		
	F**R**PGGGDM**R**	I**R**QGLERI**L**		
	W**R**SELYKY**K**			
# of peptides	**15**		**0**	**6**

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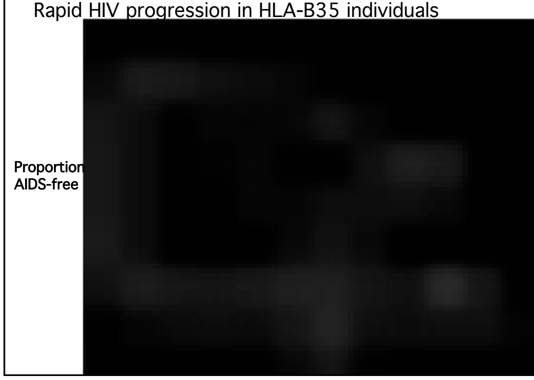
Role of MHC in Recognition of HIV peptides

Rapid HIV progression in HLA-B35 individuals



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

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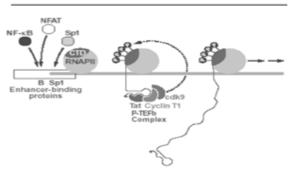
Another reason for CD4 T cell loss

CD4 T cell activation initiates HIV replication

T cell activation causes, among other effects, a marked increase in cyclin T1, NFAT and NFκB

Transcriptional activation of HIV-1 gene expression



Tat and cyclin T1 binding to TAR activates Cdk9, leading to phosphorylation of the C-terminal domain (CTD) of RNAPII and effective elongation

This links viral expression to T cell activation, resulting in viral pathogenic effect

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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

Mycobacterium tuberculosis reactivation, Cryptosporidium

Activation of latent herpes zoster

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

Pneumocystis carinii

Progressive cytomegalovirus infections, M. avium complex

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HIV virus vaccines have failed, Why?

- Immunization with rENV produce anti HIV antibodies
- But antibodies induced by immunization fail to protect as shown in multiple trials
- A live attenuated virus has not yet proved achievable
- But recombinant viral vectors vaccines with portions of the HIV genome have been developed and produce CD8 immunity

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HIV virus vaccines have failed, Why?

- Heterogeneity of HIV strains: need many immunodominant peptides directed to critical regions of viral genome for different MHC types because no cross protection (Think Zinkernagel-Doherty experiment)

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HIV virus vaccines have failed, Why?

However, the most telling reason is that we lack critical information about what is occurring during HIV infection

Vaccination produces CD8 T cell immunity

But:

Does not confer protection
May cause the infection to progress more rapidly

Two examples:

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vCP205 a recombinant live virus canarypox vector vaccine expressing gp41, Gag and Protease HIV genes induces CD8 T cell immunity

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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 HIV-negative male homosexual

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 KRWIIlGLNK in central and peripheral memory/effector CD8 T cells CD28+CCR7+CD45RO+ and CD28-CCR7-CD45RO-

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

Shortly thereafter, he developed flu-like symptoms and 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% 0f CD8 T cells to 9.8% of CD8 T cells, and this remained the dominant clonotype during acute infection

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By 32 months after diagnosis the predominant virion-encoded Gag peptide sequence mutated from KRWIIlGLNK to KGWIIlGLNK, thus thwarting binding and presentation of the peptide by HLA-B*2705

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*

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His CD4 T cell count continues to decline, presently 400 cells /μl 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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November 2007

Another failed trial

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ClinicalTrials.gov
A service of the U.S. National Institutes of Health

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Expanded Characterization of Immune Response to Merck Adenovirus 5 Gag/Pol/Nef Vaccine Given to HIV Uninfected Adults

This study has been suspended.
(Based on an interim data review, the DSMB concluded that the vaccine cannot be shown in this trial to prevent HIV infection or affect the course of the disease.)

Sponsors and Collaborators:	National Institute of Allergy and Infectious Diseases (NIAID) **HIV** Vaccine Trials Network
Information provided by:	National Institute of Allergy and Infectious Diseases (NIAID)
ClinicalTrials.gov Identifier:	NCT00486408

Purpose

The purpose of this study is to intensively characterize the immune response, particularly the T-cell response, to a three-dose regimen of an **adenovirus**-based **HIV**-1 vaccine in **HIV**-uninfected adults.

Condition	Intervention	Phase
HIV Infections	Biological: MRKAd5 **HIV**-1 gag/pol/nef	Phase I

MedlinePlus related topics: AIDS

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Merck AIDS Failure Hurts Global HIV Vaccine Research (Update1)

By John Lauerman

Nov. 15 (Bloomberg) -- The surprise failure of **Merck & Co.**'s AIDS vaccine may doom other test projects and force researchers to consider whether a protective shot against HIV is feasible.

Merck halted clinical trials of its vaccine in September, and last week researchers said that a cold virus used in the shot may have made test subjects more likely to catch HIV. The finding may cause the U.S. to cancel a study of a similar vaccine and raises concern about projects at **Crucell NV**.

The Merck setback means that 25 years into the AIDS epidemic doctors are still far from having a protective vaccine. An international team of scientists has been recruited to determine precisely what went wrong. Meanwhile, leading researchers are re-examining their vaccine strategies, said **David Ho**, who has studied HIV since the epidemic began.

``It's a giant step backwards,'' Ho, scientific director of the **Aaron Diamond AIDS Research Center** in New York, said in a telephone interview. ``It calls into question all the vaccines using related strategies that have been leading the field.''

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Merck vaccine candidate (V520) for CD8 immunity

adenovirus type 5 vector containing gag, pol and nef

- The STEP study enrolled 3,000 HIV-negative volunteers from diverse backgrounds between 18 and 45 years of age at high risk of HIV infection

- The vaccine did not prevent infection

  19 developed HIV /672 vaccinated

  11 developed HIV /691 placebo control

- And did not reduce the amount of virus in the blood of those who became infected

  40,000 copies/ml in vaccine group
  37,000 copies/ml in placebo group

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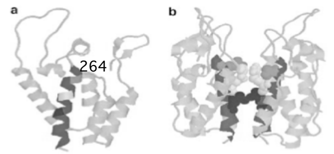
An example of HIV-1 escape from a CD8 T cell clone

HLA-B27 hemophiliac, infected ~1983 by blood products



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

	Virions/ml	CD4/μl	Gag p24 (264)	Tropism	CTL
1984	1,800	510	K R W I 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