The HIV Epidemic

- Over 50 million HIV/AIDS cases have occurred worldwide.
- Over 40 million people are currently living with HIV/AIDS.
- 95% of all cases are in developing countries.
- Highest prevalence in Africa.
- In some African countries 30% of adult population is infected with HIV and the average life span has declined by ~20 years.
- It is currently estimated that there are over 11 million orphans worldwide due to the HIV/AIDS epidemic.
- In the US over 700,000 people have been infected and over 400,000 people are currently infected.
Opportunistic Diseases in AIDS

Table 2  The main opportunistic diseases associated with HIV infection, seen in AIDS

<table>
<thead>
<tr>
<th>Viral infections</th>
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<tbody>
<tr>
<td>CMV*</td>
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<tr>
<td>Herpes simplex*</td>
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<tr>
<td>JC virus* (causing progressive multifocal leukoencephalopathy (PML))</td>
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<tr>
<td>Molluscum contagiosum*</td>
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<tr>
<td>Herpes zoster</td>
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<tr>
<td>Measles</td>
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<tr>
<td>HPV</td>
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<tr>
<td>HHV8</td>
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<td>Epstein-Barr virus (EBV)</td>
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<th>Bacterial infections</th>
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<tr>
<td>Recurrent bacterial pneumonia* (commonly <em>Strep pneumoniae</em>)</td>
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<tr>
<td><em>Mycobacterium tuberculosis</em></td>
</tr>
<tr>
<td>Non-tuberculosis mycobacteriosis* (particularly <em>Mavium-introcellulare complex</em>)</td>
</tr>
<tr>
<td>Systemic non-typhoid Salmonella infections* (notably <em>S.enteritidis and S.typhimurium</em>)</td>
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<tr>
<td>Pseudomonas spp. septicaemia and ‘vasculitis’</td>
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<tr>
<td><em>Bartonella</em> spp. (causing bacillary angiomatosis)</td>
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<tr>
<td><em>Rhodococcus equi</em></td>
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<tr>
<td><em>Nocardia</em> spp.</td>
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<th>Fungal infections</th>
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<tr>
<td><em>Candida</em> severe infection</td>
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<tr>
<td><em>Pneumocystis carinii</em></td>
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<tr>
<td><em>Cryptococcus neoformaris</em></td>
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<tr>
<td><em>Histoplasma capsulatum</em></td>
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<tr>
<td><em>Coccidioides immitis</em></td>
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<tr>
<td><em>Aspergillus</em> spp.</td>
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<tr>
<td><em>Penicillium mameffei</em></td>
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Opportunistic Diseases in AIDS (cont.)

Protozoal infections
- Toxoplasma gondii
- Cryptosporidium parvum
- Isospora belli
- Leishmania spp.
- microsporidia spp. (commonly Encephalitozoon intestinalis, Enterocytozoon bieneusi)
- Acanthamoeba spp.
- Trypanosoma cruzi

Tumours
- Kaposi's sarcoma
- Primary cerebral lymphoma
- High-grade non-Hodgkin lymphoma
- Carcinoma (invasive) of the cervix
- Carcinoma of the conjunctiva
- Carcinoma of the anus
- T-cell lymphoma
- Hodgkin's disease
- Lymphoproliferative disease, pre-lymphomatous

Other conditions
- HIV-wasting syndrome (fever, weight loss, diarrhoea)
- HIV-associated dementia
- Various dermatitis patterns (e.g. pruritic rash, eosinophilic folliculitis)
- Skeletal myopathy
- Peripheral and autonomic neuropathy
- Cardiomyopathy
- Pulmonary hypertension
- Vasculitis
- HIV-associated nephropathy (HIVAN)
- Haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP)
- Oral and oesophageal ulcers
- Dyshaemopoiesis and marrow serous atrophy

Those with an asterisk were in the 1987 & 1992 CDC lists for the case definition of AIDS.
A Crash Course in Immunology
To activate a T cell you need:
Foreign Antigen
Antigen Presenting Cell (APC)
HIV

Immature

- Gag Precursor
- Vpr
- Gag-Pol Precursor
- Cyclophilin

Mature

- Cyclophilin
- CA
- MA
- NC
- p6
- RT
- PR
- Vpr
- IN

Peptides processed between CA and NC, between NC and p6, and distal to PR.
HIV Genomic Structure

LTR gag pol vpr tat rev nef env vif vpu LTR

G. Aldrovandi
Viral RNA

RT & other virion proteins

gp120

p24

Infection

Fusion & Entry

Binding

CCR5

Reverse transcription

Nuclear localization & entry

Integration

CD4

CXCR4
Schematic View of the Course of HIV-1 and Disease

- Seroconversion
- Infection
- Death
- Symptomless
- Symptomatic
- AIDS

Cells per μL:
- CD4 T cells

4-8 weeks: Antibodies to HIV env, HIV-specific CTL, Plasma RNA copies
~10-12 years: Antibodies to HIV p24
2-3 years: Plasma HIV RNA copies/mL
The “dogma” in the AIDS field is that CD4 cells are lost.

However CD8 T cells can also be depleted.
CD8+CD4dim Cells in the Peripheral Blood

- **Isotype Staining Control**
  - 0.0% for IgG
  - 0.0% for CD4
  - 25% for CD8

- **Normal Individual**
  - 67% for IgG
  - 4.5% for CD4
  - 1.0% for CD8
  - 27% for CD8

- **Bar Graphs**
  - % CD8+CD4dim of Total CD3+
  - % CD8+CD4dim of CD3+, CD8+
  - CD4/CD8 Ratio
Stimulation of CD8 cells by APC induces CD4 expression.

- **TOTAL CD8+**: 28%
- **CD45RO+ CD8+**: 7%
- **CD45RA+ CD8+**: 38%
HIV Replication in CD8+ T cells

Costimulated CD8+ T cells

Unsorted PBL

Day 2  Day 3  Day 4  Day 5  Day 6  Day 7

p24 ng/ml

Ada-M  Bal  89.6  HTLV-IIIB

Day 2  Day 3  Day 4  Day 5  Day 6  Day 7

p24 ng/ml

Ada-M  Bal  89.6  HTLV-IIIB
The HIV Long Terminal Repeat (LTR)

Fig. 1. HIV-1 long terminal repeat (LTR), indicating potential sites of interaction for DNA- and RNA-binding proteins. These include binding sites for chicken ovalbumin upstream promoter (COUP), activator protein (AP)-1, nuclear factor of activated T cells (NF-AT), upstream stimulatory factor (USF), T-cell factor-1α (TCF-1α), nuclear factor (NF)-κB, SP1, TATA, initiator, untranslated binding protein (UBP)-1 or leader binding protein (LBP)-1, UBP-2, and CTF/NF-1.
Central lymphoid organs

Peripheral lymphoid tissue

- Tonsils and adenoids
- Waldeyer's ring
- Lymph nodes
- Thymus
- Bone marrow
- Lymph nodes
- Spleen
- Peyer's patches
- Lymph nodes
- Lymphatic vessels
HIV Infection in the Lymph Node
Early disease  Intermediate disease  Late disease

Lymph node hyperplasia  Lymph node involution  Dissolution of FDC network

Trapping of HIV  Induce and maintain vigorous HIV-specific immune response  Spillover of virus into circulation

Induce and maintain vigorous HIV-specific immune response  Trafficking of lymphocytes  VIREMIA

Spillover of virus into circulation  Loss of HIV specific immune responses  Equilibration of viral load in PB and LN

Loss of the ability to respond to other pathogens  Compounding of Immunosuppression
HIV-1 Induced Depletion of CD4+ Cells

Mock

HIV-1 Infected Implant #80-4:

Day 22 P.I.

Day 30 P.I.
There is poor peripheral human cell reconstitution in SCID-hu mice.

How can we improve in vivo modeling to model pathogenesis and assess efficacy of drugs?
BLT (bone marrow-liver-thymus) mouse

-Better peripheral reconstitution than Thy/Liv alone (Spleen, Lymph node, Peripheral blood etc.)

-Can we establish efficacy for stem cell-based TCR approaches?
Multilineage Hematopoiesis in BLT mice

%CD45+
mean=53% ± 29%
range 19%-80%
n=12
Humanized Mouse Model of HIV Infection: The Modified BLT Model

1. Sort CD34+
2. Transduce with Anti-HIV TCR or Control TCR
3. Combine with fetal thymus tissue and liver stroma, implant under kidney capsule
4. Thaw and Transduce with Anti-HIV TCR or Control TCR
2a. Viably freeze fraction
5. Tail Vein Inject
6. Analyze TCR Expression/Function

Infect with HIV-1\textsubscript{NL4-3HSA-HA}

HLA-A\textsuperscript{*}0201+ Tissue

Fetal Liver

NSG

3 weeks

6-12 weeks

Suppression of HIV Replication by HIV-specific TCR

%CD45+HIV(HSA-HA)+ Cells

Week 2

Week 6

SL9-TCR  Control  Uninfected  SL9-TCR  Control  Uninfected
Control of Plasma Viral RNA

A.

![Graph showing copies of vRNA/ml over weeks 2 and 6 for SL9-TCR and Control TCR groups with p-values 0.05 and 0.02.]

B.

**AMINO ACID ALIGNMENT**

- **INPUT VIRUS**: SLYNTVATL
- **Control TCR-CONTAINING MICE**: SLYNTVATL
- **HIV SL-9 TCR-CONTAINING MICE**: SLYNTVATL
Why Do Current Therapies Fail?

- Toxicity mandates cessation of therapy
- Drug resistance (mutation)
- Failure to clear all reservoirs
Mutation causes:

Drug resistance
Escape from immune response
Altered tropism
Increased replication potential
Effect of NVP on Viremia

Plasma viral RNA determinations (a), CD4+ lymphocyte counts (b), and percentages of mutant viral genomes in plasma and PBMCs (c) of subjects initiating treatment with NVP. Subjects were participants in a clinical protocol assessing the effects of NVP when added to existing treatment with ddI (subject 1625) or ddI plus zidovudine (subjects 1619, 1624, 1625). Treatment with NVP was initiated at week 0 using 200 mg per day and was increased to 400 mg per day after 2 weeks. ddI and zidovudine dosages were 400 mg per day and 300–600 mg per day, respectively. Viral RNA (●) was determined by QCR assay. CD4+ lymphocytes (●) were quantified by flow cytometry. Frequencies of viral genomes containing NVP-resistant-associated mutations in plasma (■) and PBMCs (□) were determined by automated DNA sequence analysis (Fig. 3, legend), with each data point representing the average of 3–6 independent PCR amplifications and sequence determinations.

Wei et. al., Nature 373, 1995
The Anti-Viral immune response/Escape By Virus

Antibodies to viral proteins are made
They are poorly neutralizing
Viral env protein mutates, and escapes from antibody responses
Relevant epitopes are physically hidden from immune response
  buried by other regions of env
  blocked by glycosylation
CD4 cells respond, but CD4 numbers are low
CD8 cells are present
  Up to 50% can be virus-specific as assessed by tetramer binding
Escape mutants have been demonstrated
Nef causes down-regulation of MHC I A and B (not C)
  This results in decreased CD8-mediated killing
CD8 cells can be infected (CD4-mediated)
APC function is perturbed
The physical demand to replace lost T cells may alter BM function
HIV infection perturbs myeloid and erythroid hematopoiesis
CD4/gp120 Interaction

Crystal clear. The fine structure of gp120 reveals new targets for therapy.
Is There Hope For A Vaccine?

Attenuated viruses can elicit protective immunity

However, these can cause disease in immunocompromised Individuals

Phase III trials of recombinant env have failed

DNA vaccines or viral vectors hold much promise

Phase III trials of viral vector plus recombinant env have Shown a small amount of efficacy.

A successful vaccine will most likely need to generate: Strong T cell-mediated responses IgA responses to protect mucosal surfaces
Effect of Cessation of Therapy On Viral Load

Viremia

Limit of Detection

Add HAART

Remove HAART

Years
HAART eliminates productively infected cells
HAART eliminates productively infected cells
HAART eliminates productively infected cells
But does not eliminate latently infected cells
HAART eliminates productively infected cells
But does not eliminate latently infected cells

We must eliminate latent virus
to “cure” HIV disease
Frequency of Latently Infected CD4+ T Cells as a Function of Time on HAART

\[ t_{1/2} = 44.2 \text{ months} \]
\[ 73.4 \text{ years} \]

R. Siliciano
Vaccines may be decades away
Vaccines may be decades away

Can we target viral reservoirs to deplete virus?
Targeting The Latent Reservoir
The Rationale

Low-level activation signals should stimulate expression of latent virus, without compromising the function of other cells. This might allow the immune system to kill the infected cell.

A specific anti-viral approach (immunotoxin) may also kill cells now producing virus, so that the latently infected cell is eliminated.

An immunotoxin is an antibody linked to a toxic molecule. The antibody binds to virus proteins on the cell, causing the toxin to kill the cell before new virus is made.
Prostratin or IL-7
Minimal activation plus immunoxin results in:

1. Elimination of 80% of the latent virus
2. No effect on normal, uninfected cells
Effect of Cessation of Therapy On Viral Load

Viremia

Add HAART
IL-7/Prostratin
Remove HAART

Limit of Detection

Years
What is ongoing now?

We have developed improved activators that induce virus to express from latently infected cells.

In normal mice, we can activate 50% or more of splenocytes with a single dose of these activators.

Studies are ongoing to test these systems, coupled with immunotoxins or anti-HIV CTL, in humanized mice to determine if we can eliminate the latent reservoir in vivo.

If successful, we may have a new form of therapy which could be used to provide a “functional cure” for HIV disease.