Structure of HIV

- Virion contains a membrane envelope with a single viral protein= **Env** protein
  - Important in receptor recognition
- Capsid made up of Gag protein
  - (group-specific antigen)
  - Icosahedral
- Interior of capsid- contains 3 enzymes
  - **Pol**: reverse transcriptase
  - **Prot**: protease
  - **Int**: integrase
    - Required for early stages of retrovirus infection
    - Maturational cleavage of these proteins occurs only after encapsidation and release of virion from infected cell
♦ Contains 2 identical +RNA strands
  – 7,000 to 10,000 bases long
  – RNA is capped and polyadenylated
  – 2 identical strand function unknown
    • Speculated that it may buffer against a too rapid mutation rate due to inaccuracy of RT

♦ Order of genes in Retrovirus


Cap and poly-A added by cellular genes

♦ R sequence
  – Present at both ends: 20-250 bases long
  – Important transcriptional signals that are only used in provirus

♦ U₅ sequence (untranslated)
  – Does not encode protein: 75-200 bases long
  – Has cis-acting regulatory signals

♦ PB (Primer binding site)
  – Specific tRNA primer for initiation of transcription binds
♦ **Gag:prot:pol:int:env**

- Gag: encodes coat proteins
- When gag:prot:pol in same reading frame they are expressed as one precursor protein that must be cleaved by protease
- Gag expressed in much higher amounts than gag:prot:pol

♦ There is a variable length sequence following env

- Contains polypurine tract (PP) important in generating DNA from virion RNA
- Untranslated sequence ($U_3$)
- 2nd copy of the R sequence

♦ **Two different types of HIV**

- **M-trophic**= most important in initial infection
  - Macrophage is initial target
  - Individuals who are homozygous for mutation in CCR5 are extremely resistant (but NOT immune) to HIV infection
  - Individuals who are heterozygous for mutation in CCR5 are less susceptible to infection than normal people
  - HIV integrates into macrophage genome (non-dividing, terminally differentiated cell, therefore HIV cannot replicate within macrophage)
  - Macrophage acts as “trojan horse” in bringing HIV to lymphatic cells
- **T-trophic** forms made by mutation in macrophage
CD4+ with CXCR4 co-receptor (chemokine receptor) become infected with HIV in lymph nodes and other lymphatic tissues

- T cells replicate within these tissues to try to respond to HIV infection
- HIV is continually being replicated in these T cells, and as cells are killed there is massive stem cell proliferation to replace the lost cells

- Increase in level of opportunistic infections begin as T cell levels drop to below 200/cc

**FIGURE 2-1.** Viral Replication in Human Lymphocytes. Scanning electron micrograph of HIV-infected human T4 lymphocyte. A, A single cell infected with HIV showing virus particles and microwells on the cell surface (magnified 7,000 times). B, Enlargement of a portion of the mountain-like cell surface in A, showing multiple virus particles building out of the cell surface (magnified 20,000 times). As each HIV exits the cell, it leaves a hole in the cell membrane. (Photograph courtesy of K. Nagashima, Program Resources, Inc., NCI-Frederick Cancer Research Facility)
HIV 1998

- In 1996 deaths from AIDS declined for the first time in > 10 years
  - Due to powerful therapies that retard the activity of HIV
    - Anti-RT drugs, protease inhibitors= cocktails
  - Trend in industrial countries not representative of the world as a whole
    - Expanding rapidly (thousands of separate epidemics)
  - Since early 1980s 40 million have contracted AIDS and ~12 million have died

- In 1997
  - Nearly 6 million people (16,000/day) acquired HIV
  - 2.3 million have died from HIV disease, including 460,000 children
  - More than 90% of HIV infected people live in developing countries, but > than 90% of the money spent for care and prevention is spent in industrial countries.
  - HIV therapies cost ~ $10,000/year per person (not available in developing countries)
The region below the Sahara in Africa has > 2/3 of the globe’s HIV infected population and ~ 90% of all infected children

- In areas of Botswana, Swaziland and several provinces of South Africa, one in four adults is infected
- Life expectancy is falling in Africa
- Unprotected heterosexual sex accounts for most of HIVs spread, but also due to contaminated blood supply. 25% of blood is NOT screened for HIV, and this is administered to women and children

India has 3-5 million HIV infected people
HIV is spreading into Thailand, Burma and Vietnam and China

Epidemiologists have found that:
- Groups whose human rights are least respected are most affected
- As epidemics “mature” the epidemic shifts from the primary population to those who were socially marginalized or discriminated against before the epidemic began (gender, race, economic status, culture, religion, …)
In 1988 whites accounted for 60% of infections and blacks and hispanics 39%
– By 1996 38% of new cases diagnosed in whites and 61% in blacks and Hispanics
– Between 1995-96 AIDS declined 13% in whites but not at all in blacks and Hispanics

Future
– AIDS will become more concentrated and expand faster in developing countries
– HIV will enter areas where it has not been seen before
– Will slow in industrial nations for some populations but increase in marginalized groups
– Cost of care will rise dramatically
– Highest priority must be given to finding a vaccine and making it available to those who need it most & also to educate those people who need it most
Improving HIV Therapy

♦ Until a few years ago, HIV infection was invariably a progressive, lethal disease that robbed its victims of dignity
  – Most medical interventions focused on treatments for pneumonias and other opportunistic infections, rather than controlling HIV itself
  – Since 1995 advances have led to a shift in prospects for most patients who get treatment

♦ Between 1996 and 1997 deaths from AIDS in the US declined by 44%
  – During same time hospitalizations due to AIDS related complications (opportunistic infections) also dropped
  – Due to intensive cocktail therapies
    • Can this be maintained? No long term data yet as to how long AIDS symptoms can be delayed with these therapies
    • Treatment is COSTLY
    • Some people do not respond well, while others do very well??
  ♦ Ultimate goal is CURE, not maintenance
  ♦ Management of AIDS is “real”, but a CURE is probably not possible (?)
How HIV Harms

♦ Spread
  – Sexual contact
  – Direct exposure to contaminated blood
  – Mother to fetus (vertical transmission)- through placenta and through milk
  – Contaminated needles
  – NOT TRANSMITTED BY:
    • SALIVA, KISSING, UTENSILS, HUGGING, SNEEZING, MOSQUITO, SWEAT

♦ HIV infects primarily CD4+ cells (Helper T lymphocytes), but can invade other cells (macrophages, dendritic cells)
  – Replicates and is transmitted to other cells
♦ Severity of infectious symptoms and expected life span after infection depends on infectious viral load

♦ At start of infection there is a high level of viral replication as measured by viral levels in the blood and by a drop of CD4+ cells
CD4+ cells normally are about 800 cells per cubic millimeter of blood. True AIDS occurs when these cells drop below 200.

About 3 weeks into infection (acute phase) many people display symptoms like mononucleosis:

- Fever, enlarged lymph nodes, rash, muscle aches and pains, and headaches.
- These symptoms resolve in ~3 weeks as immune response begins to control infectious process (killing of virally infected CD4+ cells by CD8+ cytotoxic cells).
- Antibody molecules produced
By six months rate of viral replication is at a lower, but steady state

- Seroconversion (antibody levels detectable and can be measured by ELISA)- at 6 months
- Level of virus replication is patient dependent and will determine the subsequent rate of disease progression
  - Generally, 8-10 years pass before major HIV-related symptoms appear
  - Chronic-prolonged phase of infection
- Over time, CD4 levels gradually fall. When less than 200 cells/cc patient has “AIDS”

HIV is able to infect cells other than CD4+

- In addition to the CD4 antigen there are other co-receptors to which the HIV binds
  - CCR5- found on macrophages
  - CXCR4- found on T cells
- If these co-receptors are mutated on the cells then HIV is unable to adsorb (attach) and the individual is resistant to infection

HIV replicates only when the infected cell replicates

- When specific immune response begins, specific helper cells die
♦ HIV mutates at high rate, and immune response cannot keep up with antigenic changes after 10-12 years

♦ As CD4 levels drop below 100, HIV levels in blood increase
  - Bacteria that are normally contained begin to proliferate and cause opportunistic infections
    • Pneumocystis carinii and toxoplasmosis
  - Once these symptoms begin to appear, the patient usually has 1-2 years before the disease is lethal

♦ Once HIV replication begins, the RT begins to replicate both the viral genome and the host cell genome (host cell activation and replication)
  - Protease enzyme cuts new viral proteins into forms that are packaged with the viral RNA (two identical copies of RNA)
  - Viruses bud from cell, pick up host cell membrane, and infect other cells
  - When these cells become specifically activated the virus replicates and cycle continues with many mutations
  - Most mutations probably make non-productive virus, while others give resistance and change antigenicity of virus
Anti-Retroviral Drugs
– Block viral replication in two ways:
  • Inhibition of reverse transcriptase (prevent integration into host genome by preventing RNA → DNA transcription)
    – Nucleoside analogues: resemble natural nucleotides but prevent completion on growing strand
      • AZT (1987- zidovudine)
  • Inhibition of HIV proteases: block catalytic site of HIV protease preventing it from cleaving newly made HIV proteins

Early beliefs of HIV disease
– Suggested that only a few CD4+ cells were actually infected and that HIV replicated weakly for a long period of time
  • This view implied that most of the lost T cells were killed by a mechanism other than by HIV replication (if this were true then HIV directed drugs might not be able to prevent this T cell killing)
– It is now believed that HIV replicates fast from the start
  • HIV levels remain stable for several years because body responds by making very high levels of CD4+ T cells (replaces those that are killed)
The strength of the initial immune response has a significant effect on progression to AIDS

- Those who respond with strong CD8+ activity get greater suppression of viral replication early in infection and progress more slowly towards AIDS than those who mount a weak response
- A strong initial response helps to later manufacture the subset of CD4+ cells that specifically react to HIV
  - Once these specific T cells are lost they may not come back with treatment even though other CD4+ T cells are made to increase T cell number to greater than 200

At any stage, viral levels correlate with prognosis

- Patients whose viral levels fall into the undetectable range and stay there are most likely to avoid progression to AIDS

Thus, the amount of virus in the system plays a major role in determining a patient’s eventual outcome

- Therapy aims to shut down viral replication
- For those patient’s whose immune systems are suppressed, this is best way to keep viral levels down
- All patients must stay on medications!!
Optimal Therapy (HAART= highly active antiretroviral therapy)

- Consists of triple therapy:
  - Two nucleoside analogues and a protease inhibitors
    - Dosage (# of pills/day, empty vs full stomach, side effects, etc.) may be too much for some patients and they stop therapy
    - Least number of pills is 8/day and most is ~24/day
  - Cost ~ $10,000-$12,000/year
### Anti-HIV Drugs Now on the Market

<table>
<thead>
<tr>
<th>Generic Name (Other Names)</th>
<th>Typical Dosage</th>
<th>Some Potential Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nucleoside Analogues</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Didanosine (Videx, ddI)</td>
<td>2 pills, 2 times a day on empty stomach</td>
<td>Nausea, diarrhea, pancreatitis, peripheral neuropathy</td>
</tr>
<tr>
<td>Lamivudine (Epivir, 3TC)</td>
<td>1 pill, 2 times a day</td>
<td>Usually none</td>
</tr>
<tr>
<td>Stavudine (Zerit, d4T)</td>
<td>1 pill, 2 times a day</td>
<td>Peripheral neuropathy</td>
</tr>
<tr>
<td>Zalcitabine (Hivid, ddC)</td>
<td>1 pill, 3 times a day</td>
<td>Peripheral neuropathy, mouth inflammation, pancreatic inflammation</td>
</tr>
<tr>
<td>Zidovudine (Permin A-ZT)</td>
<td>1 pill, 2 times a day</td>
<td>Nausea, headache, anorexia, neutropenia (reduced levels of neutrophils, white blood cells, weakness, insomnia)</td>
</tr>
<tr>
<td>Pill containing lamivudine and stavudine (Combivir)</td>
<td>1 pill, 2 times a day</td>
<td>Same as for didanosine</td>
</tr>
</tbody>
</table>

**Nucleotide Analogues**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Typical Dosage</th>
<th>Some Potential Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Didanosine (ddI)</td>
<td>4 pills, 3 times a day (mixed into water), not within an hour of entecic acid or didanosine</td>
<td>Rash, headache, hypertension</td>
</tr>
<tr>
<td>Nevirapine (Viramune)</td>
<td>1 pill, 3 times a day</td>
<td>Rash, hepatitis</td>
</tr>
</tbody>
</table>

**Protease Inhibitors**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Typical Dosage</th>
<th>Some Potential Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indinavir (Crixivan)</td>
<td>2 pills, 3 times a day on empty stomach or with a low-fat snack, and not within 2 hours of didanosine</td>
<td>Kidney stones, nausea, headache, blurred vision, depression, mild rash, fever, fever itch, abnormal distribution of fat, elevated triglyceride and cholesterol levels, glucose intolerance</td>
</tr>
<tr>
<td>Nelfinavir (Viracept)</td>
<td>3 pills, 3 times a day with food</td>
<td>Nausea, abdominal distribution of fat, elevated triglyceride and cholesterol levels, glucose intolerance</td>
</tr>
<tr>
<td>Ritonavir (Norvir)</td>
<td>6 pills, 3 times a day (or 4 pills, 2 times a day if taken with didanosine) with food, within 2 hours of didanosine</td>
<td>Nausea, vomiting, diarrhea, abdominal pain, headache, itching, fever, fever itch, rash, abnormal distribution of fat, elevated triglyceride and cholesterol levels, glucose intolerance</td>
</tr>
<tr>
<td>Saquinavir (Invirase, a hard gel capsule, Fortovase, a soft gel capsule)</td>
<td>6 pills, 3 times a day (or 2 pills, 2 times a day if taken with didanosine) with a large meal</td>
<td>Nausea, diarrhea, headache, abnormal distribution of fat, elevated triglyceride and cholesterol levels, glucose intolerance</td>
</tr>
</tbody>
</table>

**Drug Choices**

Today, there is a great variety of drugs available. These drugs are designed to be as effective as possible, but they must be used carefully. Antiretroviral drugs can cause serious side effects, including nausea, vomiting, diarrhea, headache, and abnormal distribution of fat. These side effects can be reduced by taking the drugs with food or by taking them with other medications.

### Typical Protocols for Postexposure Prevention

**Preferred two-drug regimen:**

- Zidovudine (Retrovir, AZT)
- Lamivudine (Epivir, 3TC)

**Alternative two-drug regimen:**

- Stavudine (Zerit, d4T)
- Didanosine (Videx, ddI)

If the patient has advanced HIV disease, has a high viral load, or has previously been treated with any drug in the selected two-drug regimen, consider adding:

- Nevirapine (Viracept)
- Indinavir (Crixivan)

**DRUG REGIMENS may, if instituted quickly, reduce the chance of HIV infection after a risky exposure. Therapy is usually maintained for four weeks.**
Drug Resistance

♦ Mediated by mutations
  – Nucleoside analogue resistance may be caused by a single mutation to reactive protein
  – Protease resistance to drugs usually requires at least two mutations in a single gene

♦ HIV makes ~ 10 billion replicates/day
  – Done without accuracy: genome of each new particle probably differs from “parent” genome in at least one spot
  – Thus, every mutation able to contribute to drug resistance is likely to be made in some of the particles
Thus, if patient has never been treated, any compound that is given will encounter some HIV variant that is already resistant.

If antiretroviral drug taken, drug resistance can be attained by only <5 mutations in a genome (will block not variants, but resistant forms will proliferate, and some of the semi-resistant variables will continue to divide and allowed to generate other mutations towards resistance.

- Antiretroviral drugs will select for variables (mutants)
- Use of polytherapy vs monotherapy

If virus is detected in blood after 4-6 months of therapy then probably a variant that is drug resistant. Must alter therapy depending upon the resistance type found.

Viral levels assessed by viral-load assays which count copies of HIV RNA in a milliliter of plasma. The number of viral particles is ½ the RNA count

- Current test sensitive to RNA concentrations of 500 or more copies/ml
- Within 1st 8 weeks of therapy viral loads should drop about 10X; by 6 months undetectable
- Triple therapy successful in 75-85% of patients
♦ Viral load tests measure viral RNA/ml—Number of Viral particles is \( \frac{1}{2} \) the number of RNAs found (why??)
  – Each viral particle contains two RNA strands

♦ In 1996 researchers examined ~1,600 samples of blood from untreated patients and compared viral load with prognosis of survival
  – Found HIV replicates at high rate from start
  – Found viral load directly correlated with survival
    • 70% with >30,000 copies/ml died within 6 years
    • If <500 <1% died in 6 years (average > 10 years)
New challenge now is to identify cells that contain provirus (resting T cells in which virus does not replicate)

- Current drugs do not do this
- To develop these types of drugs, researchers must develop new viral load assays that measure infected cells
  - This will allow for measurements of success of new treatments to eliminate proviral cells
  - these cells would have no markers specific for virus (how would you find these and identify them?)

“Trial” patients versus “field”/Clinic patients have different results

- Trial patients have 75-85% success
- Field patients exhibit ~50% success
  - Field patients more heterogenous
  - Field patients often start later in disease process
  - Field patients often do not follow protocol and/or stop when they feel sick
  - Field patients may have been on anti-retrovirals previously and contain resistant forms

Even successful therapy does not restore immune function totally

- Mix of CD4+ cells may be abnormal (may not recognize as many pathogens or may be less effective
♦ Resting T cells with provirus are not killed by current therapies
  – If become activated will replicate virus
  – Certain cells act as reservoirs of HIV
    • macrophage-short lived, brain neurons-long lived)

♦ How to activate immune system?
  – Immune cells must “see” antigen to destroy cell
    • Give substance that activates infected cells
      – Give HAART to prevent other cells from being infected
    • Remove stem cells, grow in vitro and add back
    • Remove stem cells, add a gene protective against HIV, grow in vitro to expand, and return to patient
    • IL-2 therapy (only cells with IL-2R)- T and B cells

♦ Other Strategies for preventing infection
  – Block integrase enzyme
  – Knock out Zn from protein that needs it in order to draw HIV RNA into new particles being made in cell
  – Use of antisense DNA to inactivate two (tat and rev) that are necessary for manufacture of other proteins
  – Block entry into cells
    • Must bind to CD4 and co-receptor proteins
    • CD4 blockage not very effective, but blockage of other co-receptors effective (SA, Sept, 1997)
  – Use altered (designer) virus that infects HIV infected cells and kills those cells
HIV Vaccines

♦ Natural immune response that vaccine elicits does not destroy HIV in cells.
  – Will serve to block HIV from infecting new cells (humoral arm of immune system)
    • No vaccine made yet to activate cellular arm
  – Will act as a protective mechanism for infection
  – Cannot immunize with vaccine against all variants of HIV
  – Danger of using both whole killed viral vaccine and live attenuated viral vaccines
♦ Best vaccine will activate both humoral and cellular branches of immune response

♦ HIV env protein is a gp160
  – Composed of gp 120 that interacts with CD4 protein on $T_H$ cells
  – Composed of gp 41, anchors gp120 to membrane of HIV
♦ Antibodies to both gp160 and gp120 elicit Ab formation and block HIV infection in test tubes
  – Only recognize strains from which they have been made, not other strains
    • Antigenic sites may be different between lab strains and patient strains
    • Difference in coiling may expose hidden antigenic sites that are not protective in patient strains
In vivo strains may have surface antigens that are highly coated with sugars that would serve to block the ability to recognize antigenic sites.

People who are infected with HIV but remain healthy make a very small amount of antibody which can neutralize viruses from many different patient isolates.

– If understand differences in antibodies made then might be able to develop vaccine for HIV.

Making a killed virus vaccine requires a rigorous inactivation procedure.

– Residual genetic material may be dangerous.
– Harsh treatment makes vaccine less effective.
– Inactivation may cause HIV to lose its gp120, and make it less effective.

Envelope proteins embedded in pseudovirions (empty lipid shells).

To make T cytotoxic response must associate Ag with cell membranes and self MHC.

– Viral gene inserted into host target cells by viral vector.
Most common viral vector being used currently is the “Canarypox” virus
- Canarypox is defective and will not multiply
- Genes for env and gp120 and other nonsurface HIV proteins inserted into canarypox (gag and protease genes)
- Have proved safe in human testing and have elicited a modest T_C immune response
  - Need to have viruses that cause production of larger amounts of HIV proteins
    - Multiple shots, boosters
Other researchers use of viral peptides
- Does not elicit good immune response
  - May be degraded too fast
Injection of naked HIV DNA
– Does get into cells and direct production of viral proteins
  • Does seem to cause production of Tc
  • Must evaluate safety and effectiveness

Combination vaccines
– To activate both arms of immune response
  • First may be exposed to canarypox virus carrying env to stimulate cellular response
  • Months later receive pure gp120 to elicit antibody Rx
– Need to improve delivery system and strength of response (use more HIV proteins)
Use of live attenuated vaccine

– Must delete genes critical for HIV replication while maintaining antigenicity of virus

– Recently a group of physicians volunteered to be given an attenuated virus so that the response process could be monitored
  • Volunteers feel that value of testing this approach outweighs the potential risks to their health!

– Attenuated SIV in monkeys
  • Some have proved very effective at inhibiting disease progress of virulent strains of SIV
  • Protected monkeys do NOT have high Ab’s or Tc cell activities so basis of immunity unknown
    – May be combination of Ab, Th and Tc
  • Some monkeys do progress to AIDS symptoms
♦ At present there is no proof that vaccines will provide long-term, full immunity to disease, and may even lead to disease

♦ Vaccines may give body a “head start” for protection and thus lower initial level of viral load

♦ Wide genetic variability of HIV will limit effectiveness of vaccine
  - (HIV from one patient or one part of the world would be different that from another)

♦ Vaccine use will not be available for at least 5 more years
  – Phase III trials necessary 1st