Lecture 20

Evolution and vaccines
Today:

1. A bit more about drug therapy
2. Different sorts of vaccines
3. Could vaccines increase virulence?
4. Or decrease it?
5. Why HIV is hard to vaccinate against
Antiretroviral therapy and HIV/AIDS
There are 3 phases of HIV disease:
There are 3 stages of HIV disease:

- The **acute phase** lasts for about 12 weeks after initial infection and is characterized by a sharp drop in CD4 T-cells, a spike in viral load, and the first immune responses.

- The **chronic phase** can last many years and shows very low viral loads and reduced (but not catastrophic) T-cell counts. The immune system manages to suppress the infection fairly efficiently during this phase.
There are 3 stages of HIV disease:

- The **AIDS phase** is defined by a drop in CD4 T-cells to a dangerously low level.

- At this point, the immune system is overwhelmed and the viral load shoots up and the patient suffers from wasting, neurological impairment, malignancies, and opportunistic infections that would normally not be a problem.

- This phase is fatal in the absence of effective drug therapy.
Antiretroviral therapy

- With its high mutation rate, small genome, and large population size, HIV is highly likely to generate resistance mutations (as we’ve seen with AZT).

- What was needed was a way to increase the number of mutations that must arise in a virion’s genome to render it resistant to the drug.

- The key breakthrough was to use combination therapy, cocktails of multiple drugs acting together which are not only very effective, but which delay evolution of resistance.

- Such cocktails have been given the nickname Highly Active Anti-Retroviral Therapy (HAART).

Figure 3: Trends in annual rates of leading causes of death among adults aged 25–44 years in USA over the period 1982–1998. The data from 1998 are preliminary. (Source: Centers for Disease Control and Prevention.)
Antiretroviral therapy

• Currently, combination therapy involves some combination of reverse transcriptase inhibitors and protease inhibitors

Figure 3 Mechanism of action of nucleoside and non-nucleoside reverse-transcriptase inhibitors. To enable HIV to be integrated into the host DNA and so use the cell’s genetic machinery to make new virus, the single-stranded viral RNA must first be converted to double-stranded DNA by the viral enzyme reverse transcriptase, while the enzyme RNase-H hydrolyses the RNA after it has been copied. Nucleoside and non-nucleoside reverse-transcriptase inhibitors are two classes of antiretroviral drugs that suppress HIV replication by attacking reverse transcriptase.

a. Nucleoside reverse-transcriptase inhibitors are similar in structure to the building blocks that make up DNA. By incorporating themselves into the DNA nucleoside chain being produced by reverse transcriptase, they stop attachment of further nucleosides and so prevent ongoing viral DNA synthesis. b. Non-nucleoside reverse transcriptase inhibitors attach to the reverse transcriptase and affect the activity of the enzyme by restricting its mobility and making it unable to function. (Adapted from ref. 108 with permission.)
Antiretroviral therapy

• Currently, combination therapy involves some combination of reverse transcriptase inhibitors and protease inhibitors.

Figure 4 Mechanism of action of protease inhibitors. After transcription in the nucleus, viral mRNA enters the cytoplasm and uses the host’s cellular machinery to manufacture virus proteins. The viral components then gather at the cell membrane and immature viruses bud off the cell. Core proteins are produced as part of long polypeptides, which must be cut into smaller fragments by the enzyme protease in order to form mature, functional proteins. Protease inhibitors bind to the site where protein cutting occurs, and so prevent the enzyme from releasing the individual core proteins. In this way the new viral particles are unable to mature or become infectious. (Adapted from ref. 108 with permission.)
Antiretroviral therapy

- The goal of drug therapy is basically to extend the chronic phase indefinitely
- HAART can do just this. It drives the number if copies of viral RNA below the level of detection
- It also raises the CD4 lymphocyte numbers back to levels above the threshold for AIDS (200 per mL of blood)
- This can restore immune function, leading to clearing of opportunistic infection and dramatic health turnarounds
Antiretroviral therapy

• The impact on the natural history of AIDS in US/Western Europe has been huge

• Opportunistic disease has been reversed and prevented

• Healthcare costs have diminished

• Many ill and disabled patients have returned to normal and functional lifestyles (some with large VISA bills)

• But all this comes at a cost:

• Expense, inconvenience, toxicity, resistance. At the moment, drug therapy is still mostly for the economically privileged
Some numbers to explain why it is easy for HIV to evolve resistance

- About 10 billion virions are generated daily
- Approximately one mutation is generated for each new genome
- Drug therapy generates strong selection for resistance
- Drug resistant virus is readily archived in latently infected cells to confound treatment modifications for the remainder of the patient’s life
- Resistant viruses can also be passed on to newly infected patients, up to 10% of new infection in some cohorts
<table>
<thead>
<tr>
<th>Feature</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safe</td>
<td>Vaccine must not itself cause illness or death</td>
</tr>
<tr>
<td>Protective</td>
<td>Vaccine must protect against illness resulting from exposure to live pathogen</td>
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<tr>
<td>Gives sustained protection</td>
<td>Protection against illness must last for several years</td>
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<tr>
<td>Features of effective vaccines</td>
<td></td>
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<td>-------------------------------</td>
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<tr>
<td><strong>Induces neutralizing antibody</strong></td>
<td>Some pathogens (such as poliovirus) infect cells that cannot be replaced (eg, neurons). Neutralizing antibody is essential to prevent infection of such cells</td>
</tr>
<tr>
<td><strong>Induces protective T cells</strong></td>
<td>Some pathogens, particularly intracellular, are more effectively dealt with by cell-mediated responses</td>
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</tbody>
</table>
| **Practical considerations** | Low cost per dose  
Biological stability  
Ease of administration  
Few side-effects |

Figure 14-23 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)
<table>
<thead>
<tr>
<th>Disease</th>
<th>Estimated annual mortality</th>
<th>Estimated annual incidence</th>
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<tbody>
<tr>
<td>Malaria*</td>
<td>1,124,000</td>
<td>300–500 million</td>
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<tr>
<td>Schistosomiasis</td>
<td>15,000</td>
<td>no numbers available</td>
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<tr>
<td>Worm infestation</td>
<td>12,000</td>
<td>no numbers available</td>
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<td>Tuberculosis</td>
<td>1,644,000</td>
<td>~8 million</td>
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<td>Diarrheal disease</td>
<td>2,001,000</td>
<td>~4,100 million</td>
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<td>Respiratory disease</td>
<td>3,947,000</td>
<td>~362 million</td>
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<tr>
<td>HIV/AIDS</td>
<td>2,866,000</td>
<td>~2 million</td>
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<tr>
<td>Measles†</td>
<td>745,000</td>
<td>~44 million</td>
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### Current immunization schedule for children (USA)

<table>
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<tr>
<th>Vaccine given</th>
<th>1 month</th>
<th>2 months</th>
<th>4 months</th>
<th>6 months</th>
<th>12 months</th>
<th>15 months</th>
<th>18 months</th>
<th>4–6 years</th>
<th>11–12 years</th>
<th>14–16 years</th>
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<tr>
<td>Diphtheria–tetanus–pertussis (DTP/DTaP)</td>
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<tr>
<td>Inactivated polio vaccine</td>
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<td>Measles/mumps/rubella (MMR)</td>
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<td>Pneumococcal conjugate</td>
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<tr>
<td><em>Haemophilus B</em> conjugate (HiBC)</td>
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<td>Hepatitis B</td>
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<tr>
<td>Varicella</td>
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</table>
• Vaccines must also be *perceived* to be safe. *Bordetella pertussis* causes whooping cough, which in small infants results in significant hospitalization (32% of cases), pneumonia (10% of cases) and death (0.2% of cases).

• The whole cell vaccine against *Bordetella pertussis* was developed in the 1930's and childhood vaccination in the US reduced the annual rate of infection from 200/100,000 in the 1940's to less than 2/100,000.

• Whole cell vaccine, given with tetanus and diphtheria toxoids, was associated with inflammation at the injection site. In a few children, high temperature and persistent crying occurred; very rarely, seizures or a transient unresponsive state were seen.

• Anecdotal reports that irreversible brain damage might be a rare consequence of pertussis vaccination, coupled with two deaths in Japan, lead to a decline in vaccination rates in the late 1970's and a rise in whooping cough and death due to pertussis infection, especially in Japan and in Great Britain.
• As a result of those 2 deaths in Japan that were feared to been due to the vaccine, the vaccine was temporarily suspended, the given only to older children

• A few years later there was a big outbreak (13000 cases) and 41 kids died.

• Careful studies did not confirm that pertussis vaccination was a primary cause of brain injury, but in response to public concerns an **acellular vaccine** was developed containing purified antigens that induce protective immunity

• This vaccine is as effective as the whole cell vaccine and does not induce the common side-effects of the original vaccine.

• Recent anecdotal reports of association between childhood vaccination (particularly with MMR) and autism have raised concerns in parents; worldwide studies have found no association between the incidence of vaccination and autism.
The original Salk polio vaccine is an example of an **inactivated** (killed) **vaccine**. It is made by growing virulent polio virus in tissue culture, then treating the virus with formaldehyde so that it cannot reproduce in the person who receives the vaccine.

Neutralizing antibody produced to polio virus is very efficient at blocking the ability of the virus to infect host cells and offers good protection from infection.

- risk of infection is very low

- use of the whole virus stimulates immunity to antigens in their natural conformation on the virus surface (essential for neutralizing antibodies).
• The Sabin oral polio vaccine and the measles, mumps, and rubella (MMR) vaccine are examples of **attenuated** (weakened) vaccines.

• Attenuated vaccines are generally more potent than killed ones. 

  *Why?*

• To make an attenuated vaccine, the pathogen is grown in animals or tissue culture under conditions that make it less virulent.
The pathogenic virus is isolated from a patient and grown in human cultured cells.

The cultured virus is used to infect monkey cells.
The virus acquires many mutations that allow it to grow well in monkey cells.

The virus no longer grows well in human cells (it is attenuated) and can be used as a vaccine.
• attenuated vaccines can stimulate generation of memory cellular as well as humoral immune responses;

WHY?

• the ability of the virus to multiply in the host means that less virus must be injected to induce protection; and use of the whole virus stimulates response to antigens in their natural conformation

• Additional advantages of the Sabin vaccine are that it can be administered orally, which is less expensive than giving injection, and that it can spread between family members

• Disadvantages of attenuated vaccines are that the virus may very rarely revert to its virulent form and cause disease. Because the incidence of vaccine-acquired polio is much higher than that of naturally acquired polio in the US, vaccination recommendations changed recently so that infants will receive killed polio vaccine prior to receiving the oral vaccine. The oral vaccine is being used in the WHO polio eradication campaign.
• **Subunit vaccines** contain purified antigens rather than whole organisms; an example is the *Bordetella pertussis* antigens included in the acellular vaccine.

• Subunit vaccines are not infectious, so they can safely be given to immunosuppressed people; and they are less likely to induce unfavorable immune reactions that may cause side effects.

• The disadvantages of subunit vaccines are that the antigens may not retain their native conformation, so that antibodies produced against the subunit may not recognize the same protein on the pathogen surface.

• And isolated protein does not stimulate the immune system as well as a whole organism vaccine.

• Other protein vaccines that induce good protective immunity are the diphtheria and tetanus toxoid components of DPT (we’ll talk about diphtheria in a bit).

• These are toxins that have been treated to eliminate their toxicity; they are still able to induce antibodies that can neutralize the native toxins.
• A new approach to developing vaccines to parasites is to isolate parasite peptides from host cell MHC and use those peptides (synthesized in bulk in the lab) to induce immunity.

• These **peptide vaccines** target particular peptides to which a protective response can be developed. Peptides have no native structure and do not bind the pattern recognition molecules on phagocytes that promote pathogen uptake.

• Peptide immunogenicity can be increased by giving them in lipid micelles which transport the peptides directly into the cytoplasm of dendritic cells for presentation on Class I MHC.

*Why bother with this?*

• One limitation of the peptide approach is that it is tightly linked to particular HLA (MHC) alleles, so some peptides may not be universally effective at inducing protective immunity.
Particular HLA molecule found to have most affinity for nonapeptides with proline as second residue

Assembly of HLA protein in the presence of each of the candidate peptides is assayed

Candidate nonapeptides with proline as second residue are identified

Proliferation assay conducted with lymphocytes from infected patients

Peptide identified as having potential for vaccine development

Figure 14-26 Immunobiology, 6/e. (© Garland Science 2005)
Recombinant vaccines are those in which genes for desired antigens are inserted into a vector, usually a virus, that has a very low virulence.

The vector expressing the antigen may be used as the vaccine, or the antigen may be purified and injected as a subunit vaccine.

The only recombinant vaccine currently in use in humans is the Hepatitis B Virus (HBV) vaccine, which is a recombinant subunit vaccine.

Hepatitis B surface antigen is produced from a gene transfected into yeast cells and purified for injection as a subunit vaccine.

This is much safer than using attenuated HBV, which could cause lethal hepatitis or liver cancer if it reverted to its virulent phenotype.

Recombinant DNA techniques can also be used to make safer attenuated pathogen vaccines.
Isolate pathogenic virus

Isolate virulence gene

- Receptor-binding protein
- Virulence
- Core proteins
Mutate virulence gene

Delete virulence gene

Resulting virus is viable, immunogenic but avirulent. It can be used as a vaccine
• **DNA vaccines** are the newest vaccines and are still experimental

• Like recombinant vaccines, genes for the desired antigens are located and cloned

• In the case of DNA vaccines, however, the DNA is coated onto minute metal projectiles then injected into the muscle of the animal being vaccinated, usually with a "gene gun" that uses compressed gas to blow the DNA into the muscle cells.

• Some muscle cells (mysteriously) express the pathogen DNA (they make transcribe and translate it so you get the protein) and thereby stimulate the immune system

• Both humoral and cellular immunity have been induced by DNA vaccines.
Clone gene for influenza hemagglutinin in a plasmid

Inject cloned gene into muscle tissue
Figure 14-28 part 2 of 2

Infect mice with influenza virus → Measure virus titer

Virus titer

uninjected mice (control)
mice injected with DNA

Time

Immunobiology, 6/e. (© Garland Science 2005)
Imperfect vaccines and the evolution of pathogen virulence

Sylvain Gandon*, †, Margaret J. Mackinnon*, †, Sean Nee*, & Andrew F. Read*
Vaccines rarely provide full protection from disease. Nevertheless, partially effective (imperfect) vaccines may be used to protect both individuals and whole populations\(^1\)\(^-\)\(^3\). We studied the potential impact of different types of imperfect vaccines on the evolution of pathogen virulence (induced host mortality) and the consequences for public health. Here we show that vaccines designed to reduce pathogen growth rate and/or toxicity diminish selection against virulent pathogens. The subsequent evolution leads to higher levels of intrinsic virulence and hence to more severe disease in unvaccinated individuals. This evolution can erode any population-wide benefits such that overall mortality rates are unaffected, or even increase, with the level of vaccination coverage. In contrast, infection-blocking vaccines induce no such effects, and can even select for lower virulence. These findings have policy implications for the development and use of vaccines that are not expected to provide full immunity, such as candidate vaccines for malaria\(^4\).
Could vaccines breed viciousness?

- Gandon et al. used mathematical modeling to show that vaccines designed to reduce pathogen growth rates, or neutralize toxins, can diminish selection against virulent pathogens (host mortality).

- The idea is that immunity (say to the toxin) reduces the risk of host death and shifts the optimal virulence higher. If hosts don’t suffer from the toxin, the pathogen can evolve to higher levels of virulence if that helps transmission (increases $R_0$).

- Post-vaccination, pathogens evolve to higher levels of intrinsic virulence in unvaccinated individuals.

- Can erode population-wide benefits and even increase overall mortality rates.

- Infection-blocking vaccines don’t have this problem.

Why?
Virulence-antigen vaccines

• In principle, vaccines can also be used as evolutionary tools to favor evolution towards benignness, and Gandon et al.’s results do not apply generally.

• Vaccines can exert selective forces: influenza, measles, hepatitis B

• The virulence-antigen strategy describes how to use to our advantage:

• Target just the most virulent forms of a pathogen by making the virulence gene the target

• Such vaccines should disproportionately suppress severe forms, but leave behind mild forms that can act as natural “vaccine”
Virulence-antigen vaccines

• The diptheria vaccine works in just this way

• Active component is derived from diptheria toxin

• When iron levels are low, *Corynebacterium diptheriae* produces the toxin, killing nearby cells and freeing up iron

• Toxin is impotent in immunized person, just a waste of energy (about 5% of the protein budget to make a product that doesn’t work)

• Toxigenic variants should be at a disadvantage

• Accordingly, diptheria, but not *C. diptheriae*, has disappeared in areas using the vaccine

• Mild forms persist even after immunization stops
Virulence-antigen vaccines

• In a study in Romania a steady increase in the number of vaccinated individuals was associated with a steady fall in toxigenicity

• The majority were toxigenic at the beginning of the study, but non-toxigenic at the end.

• In terms of reductions in morbidity and mortality per unit investment, the diphtheria vaccination program is second only to smallpox

• This is in large part because the mild form favored by evolution acts as a natural attenuated vaccine

• Similar story with a pertussis toxoid vaccine in Sweden: large reduction 4 years after initiation of vaccination program not only in vaccinated, but also unvaccinated children.
Figure 10.6. Evolution of *C. diphtheriae* toward benignness, as evidenced by a decrease in the percentage of isolates that were toxigenic, during an extensive vaccination effort in Romania (data from Pappenheimer 1982).
HIV/AIDS vaccines
HIV vaccines in a nutshell

• A safe, effective, broadly cross-reactive, long-lasting protective vaccine is the holy grail of HIV/AIDS research at the moment

• Despite initial optimism, the question now is not when but if such a vaccine will ever be developed

• A highly promising candidate vaccine is not at hand

• New thinking will have to be applied to this problem
Why HIV is a hard target

• Spread both sexually and blood so need both mucosal immune responses and systemic

• Probably transmitted both as cell-free virus and cell associated and therefore probably need both neutralizing antibody AND T-cell mediated immune response

• Worst of all, our own immune systems can’t stop the replication of the virus

• Here, the virus’s evolution is the central issue

• maybe it will never be possible to generate immune protection against the virus

• Ignoring this, for the moment, you then still would need to contend with the tremendous genetic diversity of the virus
A maximum likelihood phylogenetic tree of HIV-1 group M (top) plus schematic representations of the M group subtypes (below). The phylogeny is courtesy of Andrew Rambaut and is based on partial env gene (V2-V5) sequences collected both within the Democratic Republic of the Congo (black branches) and outside the DRC (colored branches) (http://www.hiv.lanl.gov). The branches radiating from the center are drawn to scale. The ancestor of each subtype/CRF is marked with a circle. Several DRC strains fall basal to these points, and the much more extensive diversity of group M lineages encountered in the DRC indicates that this region has experienced a long, continuous epidemic.
Vaccine design for HIV

- The most attractive long term solution is a vaccine that prevents infection or reduces transmission.
- Limiting transmission may be the only realistic goal.
- Aim is to reduce peak viral loads from 30000 copies per mL to 1000 copies per mL.
- CTL vaccines may be the key.
- Naked DNA or whole proteins.
- Need to know which CTL responses contribute to reducing viral replication, then target them.
- Perhaps target conserved genomic regions?
- Maybe force escape mutation in the acute phase that has a high fitness cost later?
Diversity Considerations in HIV-1 Vaccine Selection

Brian Gaschen,¹ Jesse Taylor,¹ Karina Yusim,¹ Brian Foley,¹ Feng Gao,² Dorothy Lang,¹ Vladimir Novitsky,³ Barton Haynes,² Beatrice H. Hahn,⁴ Tanmoy Bhattacharya,¹ Bette Korber¹,5*

Science, Vol 296, Issue 5577, 2354-2360, 28 June 2002
[DOI: 10.1126/science.1070441]

Globally, human immunodeficiency virus–type 1 (HIV-1) is extraordinarily variable, and this diversity poses a major obstacle to AIDS vaccine development. Currently, candidate vaccines are derived from isolates, with the hope that they will be sufficiently cross-reactive to protect against circulating viruses. This may be overly optimistic, however, given that HIV-1 envelope proteins can differ in more than 30% of their amino acids. To contend with the diversity, country-specific vaccines are being considered, but evolutionary relationships may be more useful than regional considerations. Consensus or ancestor sequences could be used in vaccine design to minimize the genetic differences between vaccine strains and contemporary isolates, effectively reducing the extent of diversity by half.
Table 1. A summary of classes of potential vaccine strains for use in subtype C epidemic regions. Differences show typical values of the percentage of amino acid changes observed when comparing the potential vaccine strain sequences to the sets of available C clade protein sequences. The lower bound represents conserved proteins, the upper bound variable proteins.

<table>
<thead>
<tr>
<th>Vaccine source</th>
<th>Differences (%)</th>
<th>Advantages and characteristics</th>
</tr>
</thead>
</table>
| Isolates, subtype B            | 10–30          | Furthest along in vaccine testing  
Based on an actual virus, and strains can be selected on the basis of advantageous biological characteristics |
| Isolates, subtype C            | 5–15           | The closest natural form to C-subtype circulating strains  
Like subtype B isolates, based on actual viruses, and thus can be selected on the basis of biological features |
| Consensus, subtype C           | 3–8            | Central to C-subtype circulating strains  
Each amino acid is most commonly found at that position |
| Ancestral, subtype C           | 3–8            | Representative of the C subtype  
Maximum likelihood model of ancestor sequence |
| M-group consensus              | 5–15           | Representative of the HIV-1 epidemic  
Most likely to cross-react with all clades  
Consensus of the subtype consensus sequences |
Fig. 4. Amino acid percent differences between vaccine strain sequences and C-subtype sequences in CTL epitopes. This analysis was limited to protein subregions known to be immunogenic by requiring overlap with at least one well-characterized CTL epitope from the HIV immunology database (3). The consensus, ancestral, and vaccine sequences were compared with all subtype C sequences, and subtype C sequences broken down by country of origin for Botswana, India, and South Africa. The median difference between the query and the C-subtype sequence set is shown. Seventy-nine subtype C sequences were used for the p24 comparison, 79 for p17, and 97 for gp160. The range of differences is indicated only for the South African set, which tends to be typical, to simplify the figure. The comparisons are numbered along the x axis: 1, Botswanan C consensus; 2, Indian C consensus; 3, South African C consensus; 4, C clade consensus; 5, C ancestral sequence; 6, M-group consensus; 7, M-group ancestral sequence; 8, C.ZA.DU422; 9, C.ZA.ZA003; 10, C.ZA.ZA009; 11, C.ZA.ZA012; 12, C.ZM.ZM651; 13, C.BR.BR025; and 14, B.FR.HXB2R.
Circumcision cuts HIV risk: study

Wed Oct 26, 2005 7:05 PM BST

NEW YORK (Reuters Health) - It has long been noted that circumcised men appear to be less likely to become infected with HIV, but whether there's a benefit to actively circumcising adults for this purpose has been an open question.

Now, investigators in France and South Africa report that circumcising men does afford them some protection against HIV.

Dr. Bertran Auvert, from Hopital Ambroise-Pare in Boulogne, France, and colleagues conducted a clinical study to test this prevention strategy. They randomly assigned 1,546 uncircumcised, HIV-negative men ages 18 to 24 years residing in South Africa to be circumcised and 1,582 to a wait "control" group.
Those who underwent circumcision were instructed to abstain from sex for 6 weeks after the procedure.

During 21 months of follow-up, 20 cases of HIV infection occurred in the circumcision group and 49 in the control group, the team reports in the medical journal PLoS Medicine.

The researchers suggest several possible ways that circumcision may protect to some extent against HIV infection: "keratinization of the glans when not protected by the foreskin, short drying after sexual contact, reducing the life expectancy of HIV on the penis after sexual contact with an HIV-positive partner, reduction of the total surface of the skin of the penis, and reduction of target cells, which are numerous on the foreskin."

Auvert's group recommends male circumcision for reducing the risk of HIV infection in areas where the disease is rampant. However, they also caution men not to think circumcision gives them total protection. "If perceived as full protection, it could lead to reduction of protection of men who, for example, decrease their condom use or otherwise engage in riskier behavior."