Danger from the Wild: HIV, Can We Conquer It?

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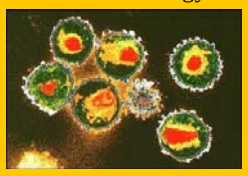
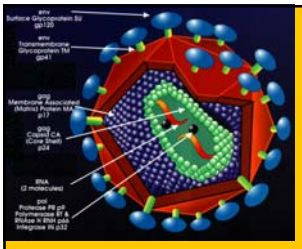




Part II

Why gene therapy might be a reasonable tool for attacking HIV

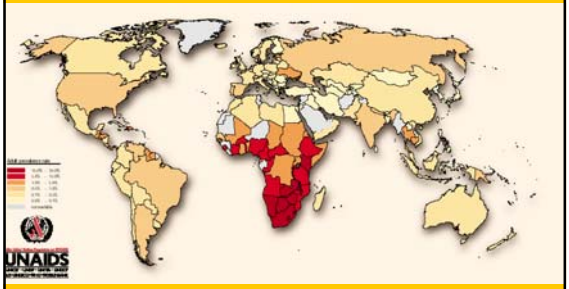
Application of molecular methodologies to infectious disease

- The outstanding vaccine candidates-- HIV, malaria, tuberculosis and others-- are unsolved because they are difficult: need new and better approaches
- Standard vaccine approaches for these agents, ones that induce antibodies, are not protective because the organisms elude antibodies
- We need new methodologies

How do they elude antibodies?

- HIV hides its jewels and varies its structure by mutation
- Malaria and others vary their structure by using many genes to change their coat structure serially
- Tuberculosis become latent and takes advantage of immune deficiencies
- And there are other tricks like direct immunoinhibition
- Polio, measles, etc lulled us into compliance

HIV: a global health problem



People living with HIV: 40 millions at the end of 2004
New HIV infections: 5 millions in 2004
Deaths due to AIDS: 3.1 millions in 2004

Data source: Joint United Nations Programme on HIV/AIDS (http://www.unaids.org/)

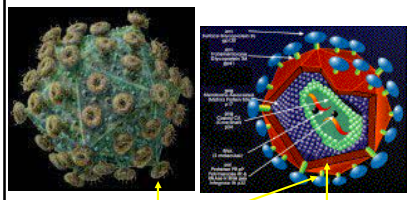
What Response is Possible?

- **Drugs-- very successful in the developed world and now being used in the less developed world**
- **Education-- quite effective in Uganda and Thailand but continuing infection in the US is a warning about the need for constant vigilance**

What Response is Possible?

- **A vaccine- the right response to any viral infection because it provides protection**
- **But HIV is not controlled by the immune system, making a vaccine problematic**

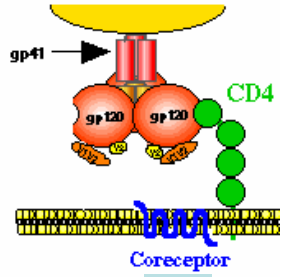
Why is HIV so resistant to antibodies?

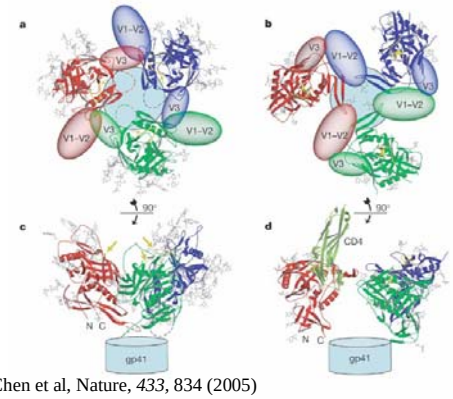


gp120 gp41

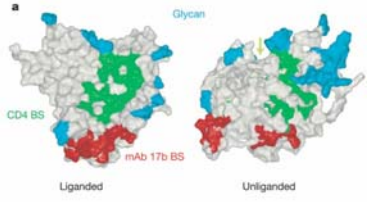
These are the proteins to attack with an antibody

How does HIV-1 enter its target cells?





Chen et al, Nature, *433*, 834 (2005)



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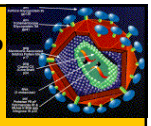
What has been the Response?



- Response of vaccine community has been to either
- Try to induce antibodies
  - But stimulating a broadly reactive antibody response has failed
- Move to T cells which partially control some viral infections but require stimulating with intracellular peptides
  - So have used DNA and viral vaccines
  - This is a new technology and we are learning our way; some hopeful results are being reported

Our Approach

- Because of the failure of standard approaches and uncertainty about progress, we have entertained a heroic notion: to use gene therapy to attack HIV
- We have taken two approaches, one using RNAi and a second, to be discussed in the third segment of this lecture, involving engineering the immune system

Both Involve Gene Therapy of Blood Stem Cells

- Gene therapy is an old idea still in search of an application beyond rare inherited immune deficiencies- we use HIV itself to bring therapeutically useful genes to the body
- The relevant stem cells are located in the bone marrow or circulating blood

The One Natural Useful Thing Viruses Do

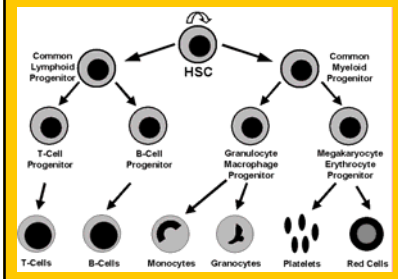
Tulips
[Tulipmania]

Carnation

The useful thing scientists can use viruses to do: gene therapy

- Retroviruses integrate their genetic material (as a DNA copy) into the genetic material of the cells they infect
- But they do not kill the cells
- So they are natural carriers of genes into cells
- By carrying "good" genes into cells they become vectors of gene therapy

Multipotent Hematopoietic Stem Cells



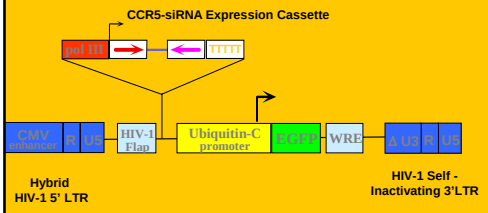
Gene Therapy Target: the CCR5 Co-receptor

- Why is it a possible target?
  - There is a natural population of people with two mutant copies of the gene
    - Leads to loss of surface expression of CCR5
    - People are resistant to HIV infection
    - Have no defect in immune functions
  - People with one mutant gene have less than 50% of CCR5 and develop AIDS more slowly

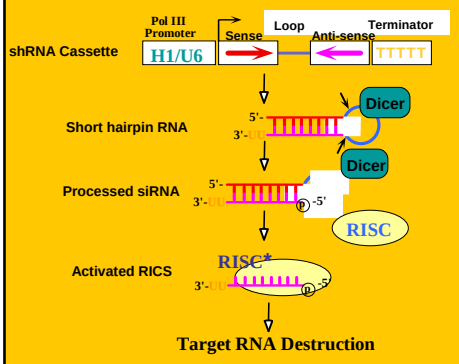
Gene Therapy Method

- To bring into helper T cells an interfering RNA (RNAi, siRNA or shRNA) that can block the translation of the mRNA for CCR5 and thus act like the mutation.

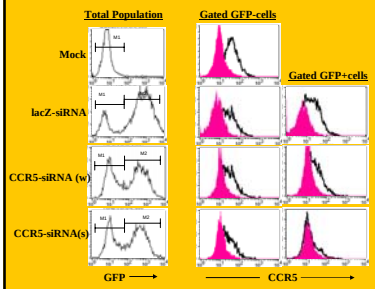
Turning HIV into a vector that will carry an RNAi against HIV into a cell



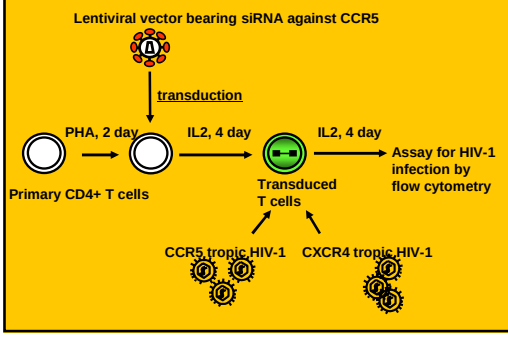
Short Hairpin siRNA mediated RNA interference in mammalian cells



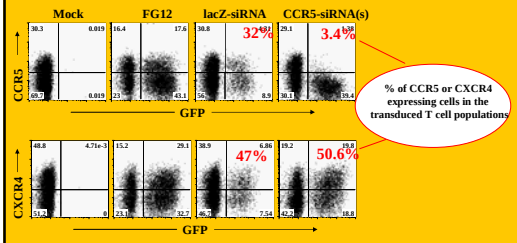
Selection of siRNA constructs that can potently inhibit CCR5 expression in Magi-CCR5 cells



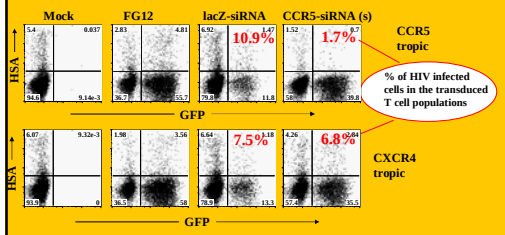
Transducing human primary CD4+ T cells and the analysis of their resistance to HIV infection



Selective inhibition of CCR5 expression in primary CD4+ T cells by lentiviral vector mediated delivery of CCR5-siRNA



CCR5-siRNA transduced human T cells are resistant to CCR5 tropic HIV-1 virus infection



Conclusions

- Lentiviral vector can deliver CCR5-siRNAs to primary PBL
  - 7-12 fold reduction of CCR5 surface levels
  - The inhibition was target specific (neither CD4 and CXCR4 were affected)
- Inhibition of CCR5-tropic HIV-1 infection of the transduced cells
  - 3-7 fold reduction of total infected cells
  - 3 fold reduction in p24 production
  - CXCR4-tropic HIV-1 infection was not affected

Next Steps

- To make this a real therapy, need to optimize the inhibition of HIV growth-- we now have very effective siRNA-delivering vectors
- Then need to organize a clinical trial-- we are in the process of forming a company that will do that