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

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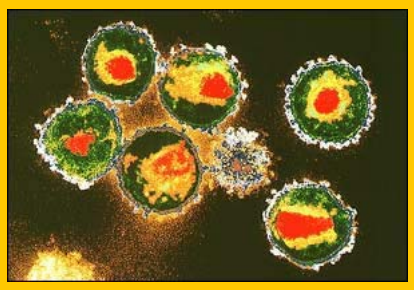
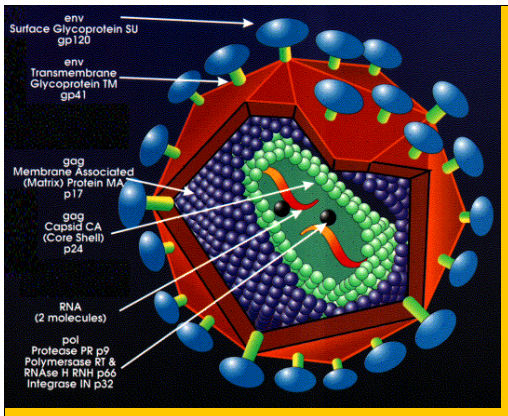




Part II

Why gene therapy might be a reasonable tool for attacking HIV

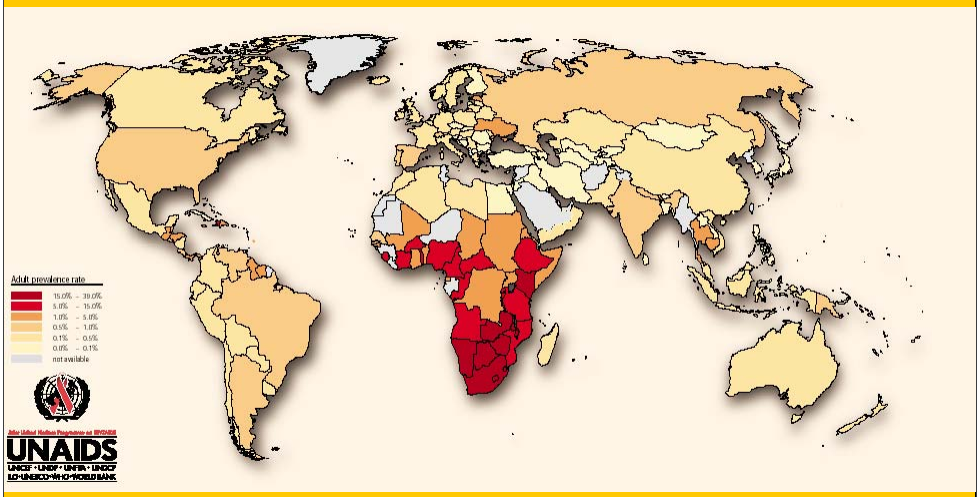
Application of molecular methodologies to infectious disease

- 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/)

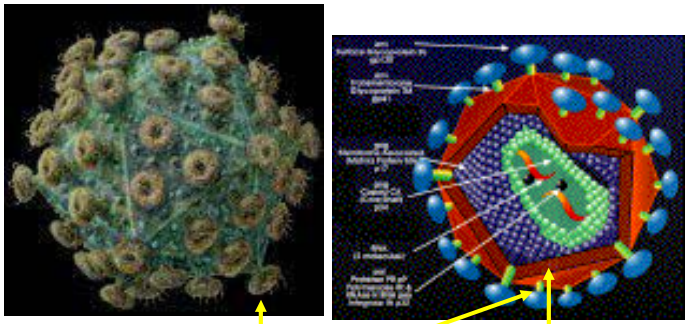
What Response is Possible?

- **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**

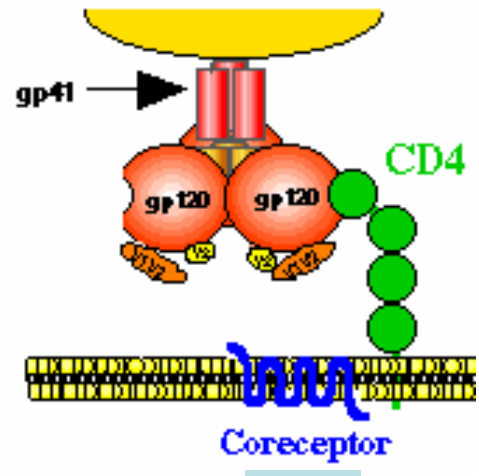
Why is HIV so resistant to antibodies?



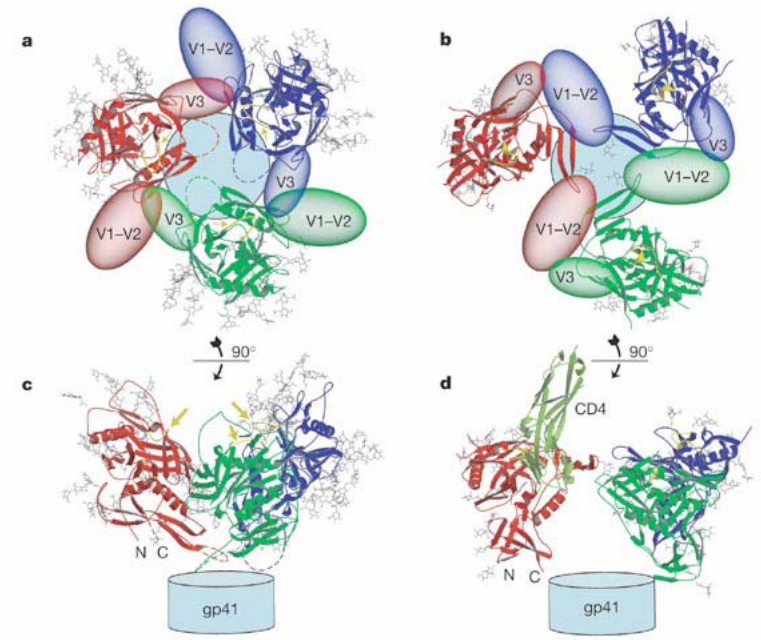
gp120 gp41

These are the proteins to attack with an antibody

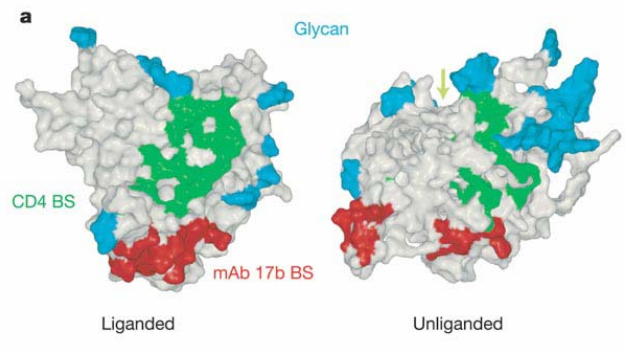
How does HIV-1 enter its target cells?



CCR-5



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



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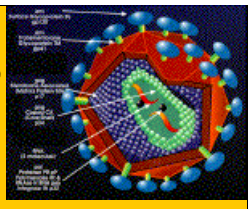
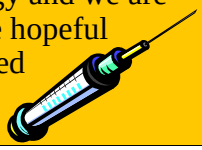
What has been the Response?



- 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

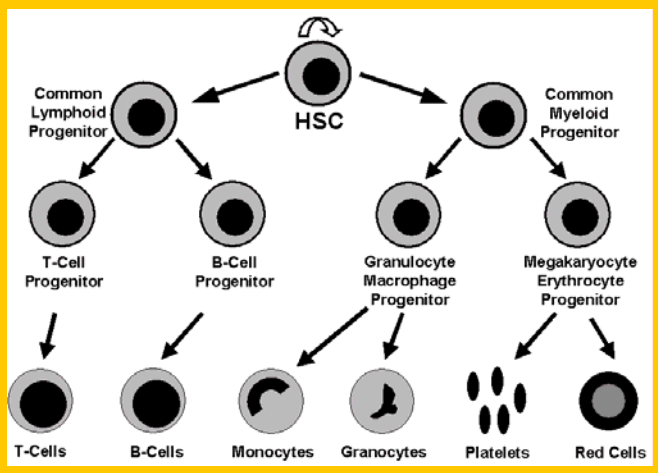
Tulips
[Tulipmania]

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



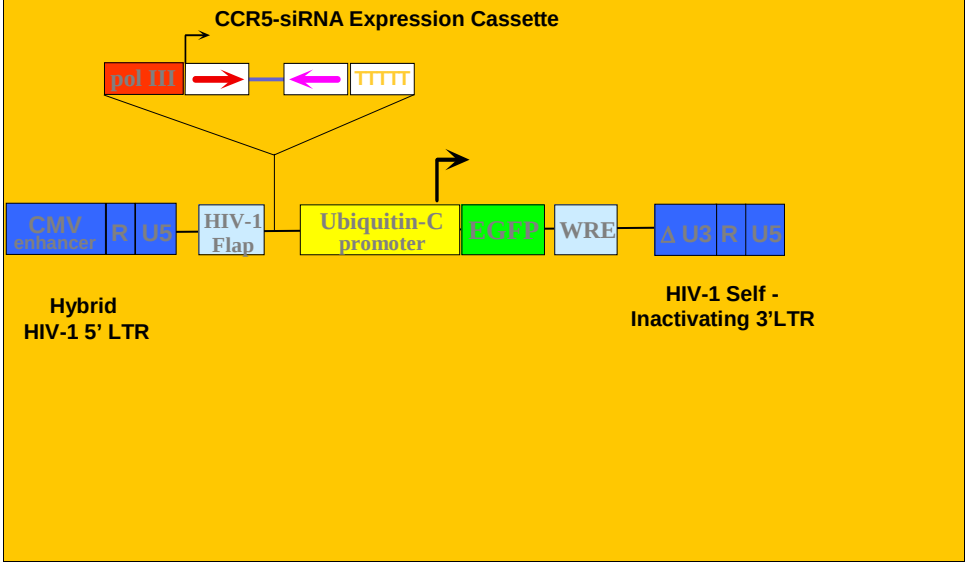
Gene Therapy Target: the CCR5 Co-receptor

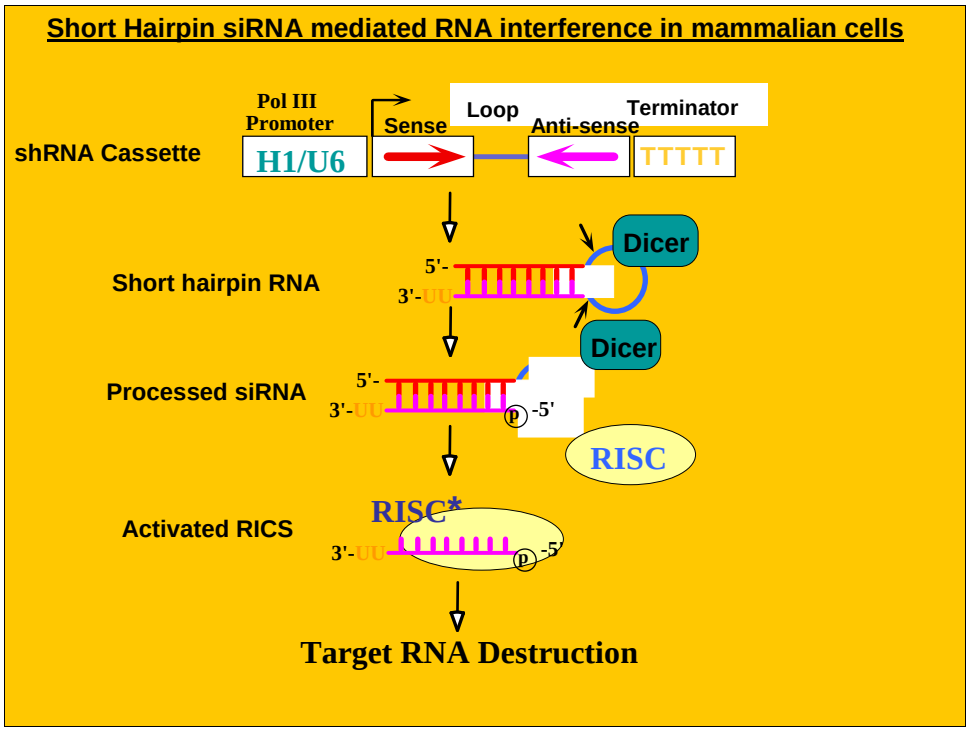
- Why is it a possible target?
  - There is a natural population of people with two mutant copies of the gene
    - Leads to loss of surface expression of CCR5
    - People are resistant to HIV infection
    - Have no defect in immune functions
  - People with one mutant gene have less than 50% of CCR5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



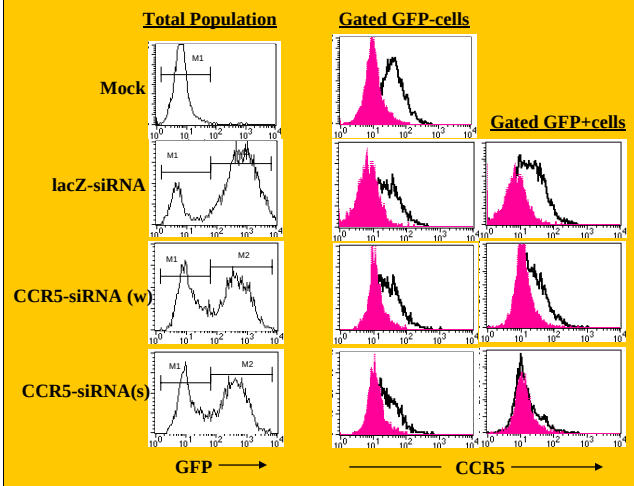


Dicer: a high conserved member of RNasIII family of ribonuclease involved in the procession of ds and hairpin RNA molecules.

RISC: RNA induced silencing complex, which contains multiple protein subunits including Ago2, eIF2C1 and eIF2C2.

RISC*:the antisense strand of siRNA incorporated RISC which is active in catalyzing target RNA cleavage using the loaded siRNA antisense strand as the guide. Activation of RISC involves an ATP dependent step of ds siRNA unwinding and conformation change.

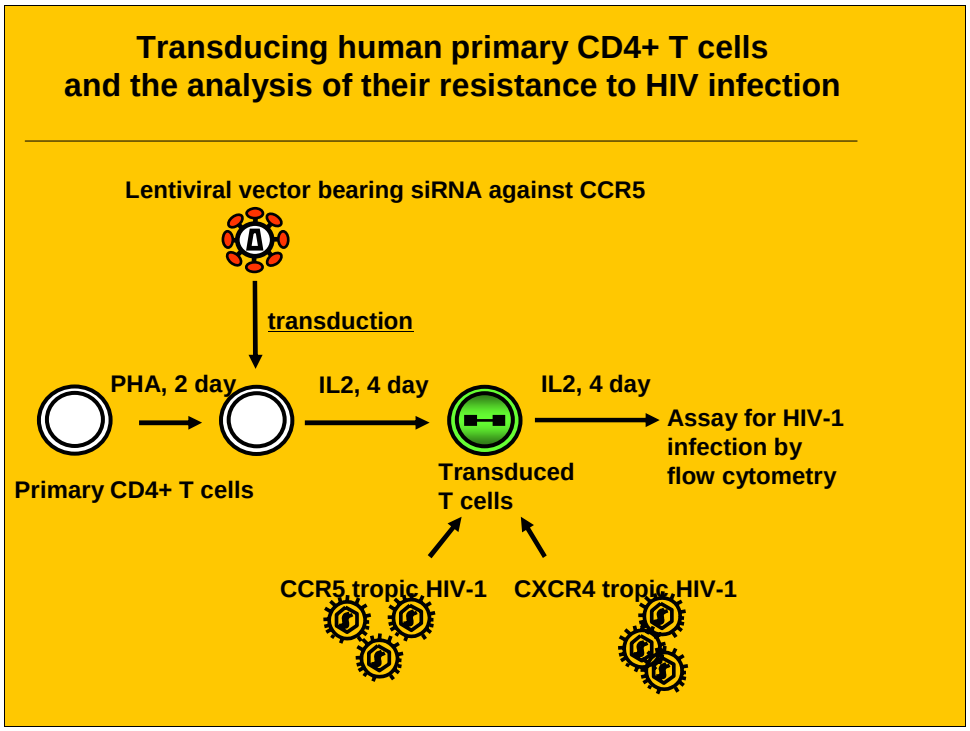
Selection of siRNA constructs that can potently inhibit CCR5 expression in Magi-CCR5 cells

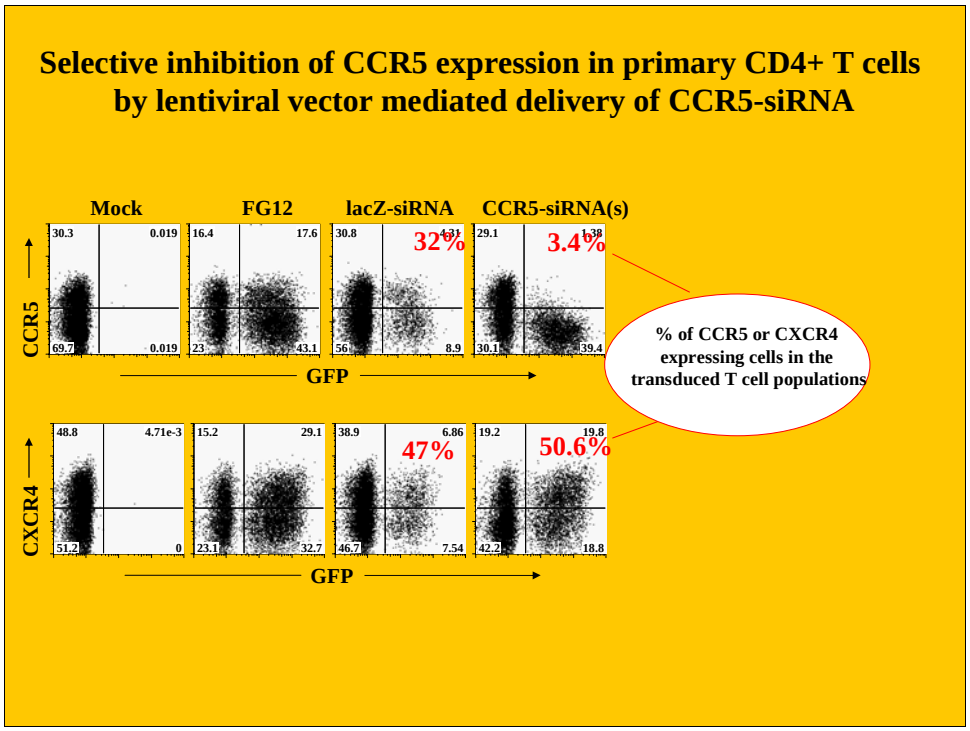


CCR5-siRNA (w): a weak anti-CCR siRNA.

CCR5-siRNA (S): a strong anti-CCR siRNA.

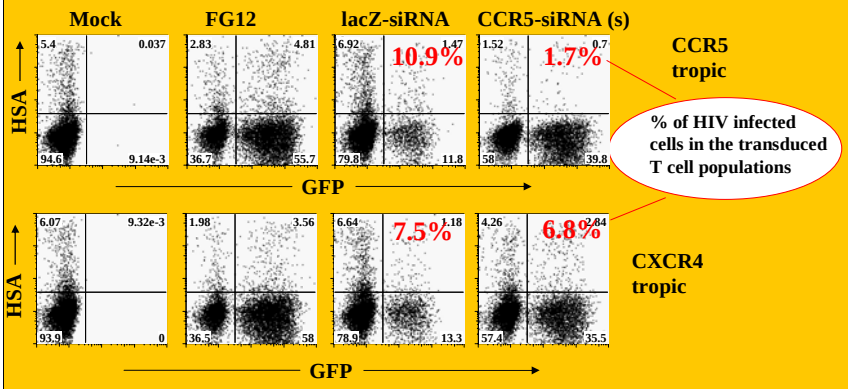
The transduced cells were stained with human CCR5 antibody for the surface expression of CCR5 and analyzed by two-color FACS. On the left, the transduced (GFP+) and untrsnduced (GFP-) were identified based on the GFP signal. On the right, the CCR5 specific staining is represented by the open curve and the isotype control staining by the pink closed curve.


Transducing human primary CD4+ T cells
and the analysis of their resistance to HIV infection
Lentiviral vector bearing siRNA against CCR5
transduction
PHA, 2 day
IL2, 4 day
IL2, 4 day
Assay for HIV-1 infection by flow cytometry
Primary CD4+ T cells
Transduced T cells
CCR5 tropic HIV-1
CXCR4 tropic HIV-1



CCR5-siRNA (s): the strong antiCCR5 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**