12/01/05 was World AIDS DAY
http://www.data.org/

AIDS is one of the greatest pandemics in medical history

Over 20 million people have died from AIDS (Acquired Immune Deficiency Syndrome)

Not only is this a human tragedy on unimaginable dimensions, it is also a threat to world security because of the potential for political destabilization
Excellent web sites:
http://www.unaids.org/

http://hivinsite.ucsf.edu/InSite.jsp

![Global Summary of the AIDS Epidemic December 2005](image)

<table>
<thead>
<tr>
<th>Number of people living with HIV in 2005</th>
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<tbody>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Adults</td>
</tr>
<tr>
<td>Women</td>
</tr>
<tr>
<td>Children under 15 years</td>
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</tbody>
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<table>
<thead>
<tr>
<th>People newly infected with HIV in 2005</th>
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<tbody>
<tr>
<td>Total</td>
</tr>
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<td>Adults</td>
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<table>
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<tr>
<th>AIDS deaths in 2005</th>
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</tr>
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<td>Adults</td>
</tr>
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</table>

The ranges around the estimates in this table define the boundaries within which the actual numbers lie, based on the best available information.

see also NYT  11/26/03
http://fire.biol.wwu.edu/trent/trent/hivstatistics.pdf
HIV = Human Immunodeficiency Virus
AIDS = Acquired Immunodeficiency Syndrome

- Infection with HIV is characterized by a relentless decline in both the numbers and function of lymphocytes called T-helper cells

- T helper cells play a central role in coordinating immune responses

- HIV infection may produce no symptoms for years, but typically within 10-15 years the weakened immune system loses control over viral replication and AIDS develops
Over the past couple of decades it has become apparent
- that some individuals escape HIV infection despite being at high risk for it
- other people are infected with the virus but progress to AIDS at an unusually slow rate

In other words, there is considerable heterogeneity in the clinical course of HIV infection.

Why do some individuals escape HIV infection despite being at high risk for it?

Why do some people who contract the virus progress to AIDS at an unusually slow rate?
What are the possible explanations for these observations?
What are the possible explanations for these observations?

• Genetic variants of the virus

• Variability among individuals with respect to other infections

• Variations in life-style (environment)

• Genetic variability among individuals: are there naturally occurring polymorphisms that confer some level of resistance to HIV infection?

• variation with respect to other infections
Genetic Variants of the Virus:

- Virologist estimate that an infected individual may produce as many as $10^9$ virus particles per day
- As with other viral RNA replicases, reverse transcriptases lack proof-reading abilities due to a lack of 3’-5’ exonuclease activity
- In the case of HIV, copying errors occur at a rate of $1 \times 10^{-5}$ to $1 \times 10^{-4}$
- Couple this mutation rate with the persistent nature of the infection and the large number of particles produced; tremendous opportunity for the production of viral genetic diversity
- Explains the rapid evolution of multi-drug resistance
Genetic variability among individuals: Are there naturally occurring genetic polymorphisms that confer some level of resistance to HIV infection?

How could these polymorphisms exert their effects?
These polymorphisms could affect:

- susceptibility to infection to HIV and other pathogens
- rate of progression of the disease
- final disease outcome
- immune response
- response to drug therapy
Recent findings reveal that some people who are partly or fully resistant to HIV-1 infections owe their good fortune to their genes.

HIV-1 is the virus responsible for most of the AIDS worldwide.

[HIV-2 restricted to certain regions of Africa]

In the mid-1980’s a group of investigators started a systematic search for genetic polymorphisms that could influence the course of HIV infection.

At this time most investigators were focussing on the genetic variability of the virus itself (that might influence virulence).
Systematic search for genetic polymorphisms that could influence the course of HIV infection:

Went on a “genetic fishing expedition” to try to find “resistance alleles”

Cohort or group of several hundred individuals at high risk for HIV infection:
  - IVD: intravenous drug users
  - MSM (homosexual men)
  - hemophiliacs who received tainted blood

Blood samples collected from each -- cell cultures made to supply continuing source of DNA

*When the study started, only 1000 human genes had been cloned*

But this was still too many genes to start screening randomly for polymorphisms
So, the scientists made some judicious choices of which genes to focus on:
• chose 50 genes that they whose proteins could potentially influence the HIV life cycle
• systematically examined these genes for allelic variations that correlated with resistance to HIV infection or slow progression of the disease

Found one gene called CCR5 that showed a statistically significant difference in genotypes among the infected and uninfected cohorts
• CCR-5 gene codes for a transmembrane protein that is involved in signal transduction events stimulated by chemokines

• Chemokines are a group of small polypeptides (70-80 amino acids) -- they induce cells to migrate to the size of inflammation

Investigators found that
• the CCR5 gene is polymorphic
• There is a major variant allele called ΔCCR5found found initially in the US population that is missing 32 nucleotides
• Results in a frameshift and truncation of the protein
The frequency of this allele has been investigated in a group of individuals who are either
1. infected with HIV or
2. uninfected with HIV but at high risk for HIV because of repeated high risk exposures (IVD, MSM, hemophiliacs) -- this group many represent resistant individuals

<table>
<thead>
<tr>
<th>HIGH RISK INDIVIDUALS</th>
<th>CCR5⁺/ CCR5⁺</th>
<th>CCR5⁺/ ΔCCR5</th>
<th>ΔCCR5/ ΔCCR5</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV Infected</td>
<td>85%</td>
<td>15%</td>
<td>0%</td>
</tr>
<tr>
<td>n=1343</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV Uninfected</td>
<td>83%</td>
<td>14%</td>
<td>3%</td>
</tr>
<tr>
<td>n=667</td>
<td></td>
<td></td>
<td></td>
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- In the general population of American Caucasians, the frequency of ΔCCR5/ΔCCR5 homozygotes is ~1.2%, so we would have expected about 16 HIV-infected individuals to be of this genotype
- That 0% of the HIV infected individuals were homozygous for the mutant allele was statistically significant.

More recent study on 17,214 Europeans (from 2000 GSA meeting: S. O’Brian)

**HIV infected:**
+/- = 3,823  +/-Δ =727  Δ/Δ = 1

**Control (NOT at high risk for HIV infection)**
+/- = 10,273  +/-Δ =2,221  Δ/Δ = 169
What is surprising about this life-cycle diagram?
HUH?
CCR5 receptor for HIV?
CD4 receptor for HIV?

The virus co-opts the receptor for its own purposes:
• A protein on the surface of the virus particle binds to the cell surface”receptor”
• the virus envelope fuses with the cell membrane and the entire virus particle enters the cell
Recently it has been shown that entry of the HIV virus into a host cell involves two different cell-surface receptors on target cells called CD4 and CCR5.

- CD4 is a cell surface receptor involved in T cell antigen recognition.
The following study was performed on a group of HIV-positive Danish homosexual men who showed different phenotypes with respect to the progression of the disease.

<table>
<thead>
<tr>
<th>Population</th>
<th>CCR5^+/CCR5^+</th>
<th>CCR5^+/ΔCCR5</th>
<th>ΔCCR5/ΔCCR5</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Danish population</td>
<td>81 %</td>
<td>18 %</td>
<td>1%</td>
</tr>
<tr>
<td>HIV Infected: long-term, slow progression</td>
<td>34%</td>
<td>66%</td>
<td>0%</td>
</tr>
<tr>
<td>HIV Infected: normal progression</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
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</table>
Look at this table again

What about the blue-highlighted individuals? They are homozygous for the wild-type allele

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Does the fact that 83% of uninfected high risk individuals are +/+ undercut the argument that the mutation confers resistance?

See problem 9 on final exam study sheet
Frequency of ΔCCR5 allele is high enough in Caucasian populations for it to be considered a polymorphism.

<table>
<thead>
<tr>
<th></th>
<th>mutant allele freq.</th>
<th>CCR5⁺/CCR5⁺</th>
<th>CCR5⁺/ΔCCR5</th>
<th>ΔCCR5/ΔCCR5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian Population</td>
<td>10%</td>
<td>81%</td>
<td>18%</td>
<td>1%</td>
</tr>
<tr>
<td>q</td>
<td>(1-q)²</td>
<td>2(1-q)q</td>
<td>q²</td>
<td></td>
</tr>
</tbody>
</table>

Are the genotype frequencies what Hardy would have predicted?

What assumptions did Hardy make about his idealized population?

Are ΔCCR5/ΔCCR5 individuals present at the expected frequency?
The discovery of the ΔCCR5 polymorphism prompted numerous studies on the distribution of this allele in various populations worldwide.

DNA and aa sequence of a portion of the CCR5 gene:

DNA sequence reads 5’ to 3’

- Top = wild-type
- Bottom = ΔCCR5 allele (32 bp deletion is shaded)
PCR based allele assay:
CCR5 sequence was amplified from genomic DNA using primers that flank the deletion. Shown here is an ethidium bromide stained agarose gel. Marker lane: 100 and 200 bp size standards EU2 and EU3 are resistant to HIV infection.
Data from earlier studies:
Scientific American Sept. 1997

Frequency of ΔCCR5 allele in %

Geographic distribution in Europe & Asia: north to south allele-frequency gradient, with the highest allele frequencies in Northern Europe (14%) ranging to a low in Greece (4.4%)

Updated information on allele frequencies in various ethnic and racial groups:
- Mainland China 1/407 = het; f (allele) = 0.123%
- Asians - another study found no hets found among 606 individuals
- Pacific Islanders: f (het) = 1.8%
Frequencies of the ΔCCR5 allele in various European populations
Each % value with confidence intervals is located at the approximate geographic site from which the corresponding peoples originated
Genetic composition of a population is the product of three fundamental evolutionary influences:

Adaptation (selection)

Chance (stochastic nature of mutation, genetic drift)

History (the course of evolution is contingent on prior historical events)
What explains the high frequency of this mutant allele in the Caucasian population and the absence or low frequency of it in other populations?

**Bottom line:**
Data collected on this polymorphism are consistent with a single ancestral mutation event producing the ΔCCR5 allele that is present at high frequencies today -- *possibly due to natural selection*
Selection?

*Natural selection*: differential reproduction of genotypes

*Darwinian fitness* is the relative probability of survival and reproduction for a given genotype.

*Fitness is a consequence of the relationship between the phenotype of the organism and the environment in which the organism lives, so the same genotype may have different fitnesses in different environments.*

*(Recall discussion of \(str^+\) and \(str^R\) bacteria)*
Selection?

• some undefined selective advantage it may have conferred to its carriers in the distant past
• possibly a catastrophic epidemic gave individuals who harbored this mutation a selective advantage?

• the geographic distribution of ΔCCR5 allele frequencies is consistent with a strong, historic selection event driving the allele frequency upward in ancestral Caucasian populations

• perhaps a widespread, fatal epidemic involving a pathogen that, like HIV, required the CCR5 protein for its pathogenic effects

Original candidate for the selective sweep was Bubonic Plague (a bacterial infection)
650 years ago, the BLACK DEATH or Bubonic Plague stalked Europe (1346-1352)
Before it burned itself out, the epidemic had killed about one third of the European population
70-80% of those who contracted the plague in the 14th century died from it
Of those who survived the plague -- where they genetically blessed, were they immune to the disease or was it just luck??

AIDS resistance and the Bubonic Plague

Any direct evidence of a tie?
NO, this is pure speculation at this point
Evaluating plague and smallpox as historical selective pressures for the CCR5-Δ32 HIV-resistance allele

Abstract

The high frequency, recent origin, and geographic distribution of the CCR5-32 deletion allele together indicate that it has been intensely selected in Europe. Although the allele confers resistance against HIV-1, HIV has not existed in the human population long enough to account for this selective pressure. The prevailing hypothesis is that the selective rise of CCR5-32 to its current frequency can be attributed to bubonic plague. By using a population genetic framework that takes into account the temporal pattern and age-dependent nature of specific diseases, we find that smallpox is more consistent with this historical role.

(see smallpox link on 321 home page)

Others want to pin the blame on the VIKINGS....
The chemokine receptor CCR5 constitutes the major coreceptor for the macrophage-tropic strains of HIV-1. A mutant allele of the CCR5 gene named Delta32 was shown to provide to homozygotes a strong resistance against infection by HIV. The frequency of the Delta32 allele was collected in 7328 noninfected unrelated individuals from 31 different European populations, and in Cyprus, Turkey, Daghestan, and North-Africa. The Delta32 allele was found in all populations studied, with a mean frequency of about 8.0%. A north to south gradient correlating latitude with Delta32 allelic frequencies was found, with highest allele frequencies in Nordic countries. We hypothesized that the Delta32 allele was disseminated in Europe by the Vikings during the eighth to the tenth centuries, because the most elevated values of this variant are actually found in their actual populations, and because they raided during the corresponding period in most European countries.