Lecture 20

Evolution and vaccines

then

Influenza virus evolution
Return of Chloroquine Antimalarial Efficacy in Malawi


BACKGROUND

In 1993, Malawi became the first country in Africa to replace chloroquine with the combination of sulfadoxine and pyrimethamine for the treatment of malaria. At that time, the clinical efficacy of chloroquine was less than 50%. The molecular marker of chloroquine-resistant falciparum malaria subsequently declined in prevalence and was undetectable by 2001, suggesting that chloroquine might once again be effective in Malawi.
METHODS
We conducted a randomized clinical trial involving 210 children with uncomplicated *Plasmodium falciparum* malaria in Blantyre, Malawi. The children were treated with either chloroquine or sulfadoxine–pyrimethamine and followed for 28 days to assess the antimalarial efficacy of the drug.

RESULTS
In analyses conducted according to the study protocol, treatment failure occurred in 1 of 80 participants assigned to chloroquine, as compared with 71 of 87 participants assigned to sulfadoxine–pyrimethamine. The cumulative efficacy of chloroquine was 99% (95% confidence interval [CI], 93 to 100), and the efficacy of sulfadoxine–pyrimethamine was 21% (95% CI, 13 to 30). Among children treated with chloroquine, the mean time to parasite clearance was 2.6 days (95% CI, 2.5 to 2.8) and the mean time to the resolution of fever was 10.3 hours (95% CI, 8.1 to 12.6). No unexpected adverse events related to the study drugs occurred.

CONCLUSIONS
Chloroquine is again an efficacious treatment for malaria, 12 years after it was withdrawn from use in Malawi. (ClinicalTrials.gov number, NCT00125489.)
First in the United States to identify Asian flu virus
Science

The Vaccine Hunter
Wednesday 21 June 2006 21:00-21:30 (Radio 4 FM)

Maurice R Hilleman saved more lives than any other scientist in the 20th Century. Given his extraordinary achievements, he is surprisingly little-known outside his field. He is the only scientist ever to make a flu vaccine in advance of a pandemic, a task scientists are now struggling to achieve in the face of bird flu. But, one of his creations, the combined MMR shot, remains controversial in the UK to this day.

Jeryl Lynn Hilleman presents the incredible story of her father, Dr Maurice Hilleman.

Audio is available for more than seven days

The Vaccine Hunter

Listen again to The Vaccine Hunter
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.
Figure 14-25 part 1 of 2 Immunobiology, 6/e. (©Garland Science 2005)
Figure 14-25 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Mutate virulence gene → Resulting virus is viable, immunogenic but avirulent. It can be used as a vaccine

Delete virulence gene
• **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
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\textsuperscript{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\textsuperscript{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
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).
Virulence-antigen vaccines

- In a study in Romania a steady increase in the number of vaccinated individuals was associated with a steady fall in observed frequency of toxigenic (versus mild) bacteria

- The majority were toxigenic at the beginning of the study, small minority were 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

*HOW?*
Virulence-antigen vaccines

- 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.

- Vaccine introduced in 1995, given to all children between 6 months and 14 years of age

- Four years later: large reduction in hospitalizations for whooping cough...

- ...for non-vaccinated (e.g. < 6 months old) as well as vaccinated children (Soubeyrand and Plotkin 2002)

*What was the response of Gandon et al.?*
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
vaccines
Evolutionary and immunological implications of contemporary HIV-1 variation

Bette Korber*,†, Brian Gaschen*, Karina Yusim*,†, Rama Thakallapally*, Can Kesmir*,† and Vincent Detours*,†

b) 1996 Influenza Sequences
   Hemagglutinin (H3)
   n=96

   0.10

c) HIV-1 Single Individual (v2-C5)
   Subtype B
   Asymptomatic phase
   Year 6 post sero-conversion
   n=9

   ...

e) Amsterdam (V2-C5)
   n=23

f) Democratic Republic of the Congo
   1997, n=193
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.
• The use of a consensus or reconstructed ancestral sequence effectively cuts diversity in half.

• If you could create one of these that was immunogenic, it should have broader range and last longer than field isolate.

• Currently, computer-reconstructed consensus strain is in trials in monkeys.

• We’re working to generate real ancestral sequences…
vaccines

[Image of a person in a laboratory setting, wearing protective gear and handling small samples.]

[Image of a person working at a table with various laboratory equipment.]
Results
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 variable proteins.

<table>
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<tr>
<th>Vaccine source</th>
<th>Differences (%)</th>
<th>Advantages and characteristics</th>
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| 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 |
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?
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."