PERSPECTIVES: VIROLOGY

The Origin and Control of Pandemic Influenza

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A worldwide epidemic (pandemic) of type A influenza could occur at any time. Such an event will be caused by a "new" virus against which the human population has no immunity, and past experience indicates that this new virus will probably arise in China. With today's crowded conditions and rapid transportation, the epidemic is expected to reach every corner of the globe. Millions of people will become ill, and many will die.

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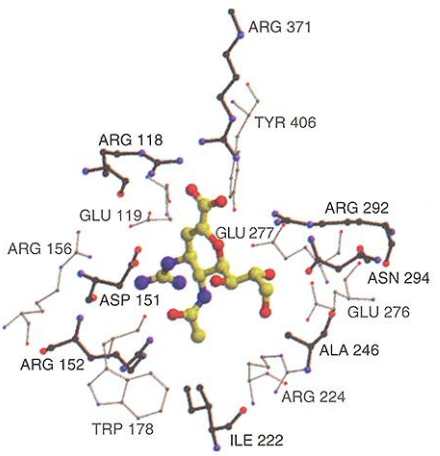
Why was the flu virus that caused the "Spanish influenza" pandemic of 1918—in which more than 20 million people died—so virulent? Fragments of RNA from the 1918 strain have been obtained from lung samples of flu victims preserved in pathology museums or frozen in the Alaskan permafrost. These RNA pieces have yielded the complete sequences of the genes for three crucial flu virus proteins: hemagglutinin, neuraminidase, and nonstructural protein. But, so far there are no obvious features in these sequences that hint why the 1918 virus was so virulent. The phylogenetic reanalysis of hemagglutinin gene sequences from humans, birds, and pigs by Gibbs *et al.* (see page 1842 for the report, and page 1773 for the Perspective) suggests that the 1918 virus hemagglutinin gene was a recombinant, and that the recombination event occurred at about the same time as the Spanish flu pandemic started (*1*). Whether this recombination in the hemagglutinin gene was the trigger that caused the virus to be so virulent is not yet known.

One mechanism by which influenza viruses with "new" antigens can enter the human population is through genetic reassortment between established human viruses and animal or bird viruses with different hemagglutinin and neuraminidase antigens. The 1957 (Asian) and 1968 (Hong Kong) strains arose in this way. It is clear, however, that new human pandemic viruses may arise in other ways. The avian influenza virus (subtype H5N1) that infected 18 Hong Kong residents in 1997, killing 6, was not a reassortant—all of its genes were of avian virus origin. This highly lethal chicken virus had spread from chickens to people but had not learned to spread from person to person, and the epidemic was stopped by killing all the chickens in Hong Kong. Now, in 2001, another H5N1 virus has appeared in the live chicken markets in Hong Kong. So far this virus does not appear to have infected anyone, and it differs from the 1997 H5N1 virus in its internal genes. Nevertheless, all of the chickens in Hong Kong have again been slaughtered as a precaution.



Beating the flu. Crystallographic structure of influenza virus neuraminidase (N9 subtype) showing the rationally designed anti-flu drug, Relenza (4-guanidino-Neu5Ac2en), bound to the active site of the enzyme. The drug is represented as an atom-colored ball-and-stick model (yellow, carbon; blue, nitrogen; red, oxygen). The neuraminidase catalytic site (conserved among all influenza A viruses) is shown with the closer carbon atoms in black and those farther away in gray. (Drawn with MolScript and rendered in Raster3D).

With reverse genetics, Hatta and colleagues (see page 1840 for the report, and page 1773 for the Perspective) have reconstructed some of the H5N1 viruses that killed the six people in Hong Kong in 1997 in an attempt to find out why this avian virus was so virulent for humans (*2*). They were able to divide the H5N1 viruses into two groups with high (HK483) or low (HK486) pathogenicity in mice. Reassortment experiments showed that it was the PB2 gene encoding one of the internal polymerase proteins, together with the high cleavability of the hemagglutinin, that seemed to be responsible for the difference in virulence (for mice) between the two groups of viruses.

What can be done if a new influenza virus suddenly appears and spreads with alarming speed around the world? Slaughter and quarantine of people is not an option and vaccines would take some time to develop. So, antiviral neuraminidase inhibitors might provide the first line of defense against a new flu virus. Neuraminidase—one of the glycoprotein "spikes" on the surface of the influenza virus—is an enzyme that cleaves sialic acid residues from receptors for the virus, enabling the virus to spread throughout the body. Inhibition of this enzyme stops this spread and effectively curtails the infection.

Two inhibitors specific for influenza virus neuraminidase are currently being used to control influenza infections, and two others are under development. Relenza (4-guanidino-Neu5Ac2en) was invented in Australia and is marketed by Glaxo-SmithKline (see the figure). The other, Tamiflu [4-acetamido-5-amino-3-(1-ethylpropoxyl)-1-cyclohexene-1-carboxylic acid ethyl ester], was invented by Gilead Sciences and is marketed by Hoffman-LaRoche. Passage of influenza virus in the presence of these inhibitors, either in vitro or in clinical trials, has led to the selection of drug-resistant viral mutants (*3*). These are of two kinds: those with sequence changes in the hemagglutinin, and those with sequence changes affecting the catalytic site on the neuraminidase. Drug resistance, however, may not be a problem if and when the drugs are used widely in the community to control influenza (*3*). Although these compounds are able to stop virus replication, they cannot repair the damage already done by the virus; hence, the drugs must be given very soon after the initial infection in order to be effective. Relenza is a powder inhaled into the lungs; Tamiflu is a pill. These drugs are effective only against the influenza virus, and not against other viruses or bacteria that cause clinical symptoms similar to those of the flu.

Clearly, rapid, sensitive, simple and cheap diagnostic tests for influenza are needed for the neuraminidase inhibitors to be used effectively in the community. Furthermore, because general practitioners are likely to be swamped with flu patients in the event of an epidemic, it is desirable that these tests be available for use in the local pharmacy or even at home. One such diagnostic test under development by ZymeTx Inc. (Oklahoma, USA) uses a substrate that is specific for influenza neuraminidase and is not cleaved by the neuraminidases of other viruses or bacteria that are likely to be present in the respiratory secretion samples

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(*3*). This test, which uses a chemiluminescent reporter group and sensitive Polaroid film, is highly accurate and suitable for use in the local pharmacy. Another diagnostic test under development by Biota Holdings (Melbourne, Australia) and BioStar (Colorado, USA) uses a silicon chip biosensor and optical immunoassay technology (*3*). The chip has antibodies to flu A and B nucleoproteins attached to its surface, and the refractive index changes if these antigens are present in the test sample, yielding a purple color easy to see by eye. Both of these tests give a result in no more than 20 min.

It is doubtful whether vaccination would be useful in controlling an influenza pandemic, at least in the early stages. Such an exercise was, in fact, attempted in January 1976, when a swine flu outbreak occurred among army recruits at Fort Dix, New Jersey (*4*). It was thought that the 1918 "Spanish influenza" virus might have returned, prompting President Ford to authorize the expenditure of $350 million to "vaccinate every man, woman, and child in the U.S.A." This mass vaccination program experienced a number of problems—low antibody titer, vaccine side effects, and litigation tangles—that could happen again if such an exercise were ever to be repeated. (The expected pandemic never materialized.)

The influenza vaccines currently in use are inactivated subunit vaccines containing hemagglutinin and neuraminidase obtained from various strains of cultured flu virus. They are reasonably effective against the strain used to make the vaccine and are cost-effective. However, it would be difficult to make, test, and safety-test enough vaccine in time to protect many people against a new virus. Vaccines currently being developed may show more promise. These include vaccines prepared by reverse genetics, DNA vaccines, vaccines against the conserved regions of the M2 ion channel of the flu virus, and even vaccine "cocktails" containing all known hemagglutinin subtypes.

But the most promising first line of defense does seem to be antiviral drugs, and of those currently available, the neuraminidase inhibitors, although expensive, appear to be the best. Use of these drugs in the face of an exploding influenza epidemic would, however, be beset with immense social, political, economic, and logistical problems. For the drugs to be of any use, huge quantities would need to be immediately available, and means for their rapid distribution would need to be in place beforehand. Supplies of these drugs at the moment are woefully inadequate. In addition, the companies producing the drugs now seem to have a diminished interest in influenza—possibly because the last flu season was the lightest for many years, and little demand for neuraminidase inhibitors meant few sales and meager profits for the pharmaceutical companies involved. The concept that it is hard to sell umbrellas in a drought seems to have escaped them!

Imagine the following: Somewhere in China, an influenza virus, subtype H9N2, suddenly acquires the ability to infect humans. The virus is highly infectious and highly transmissible, although the disease it causes is fairly mild. Because of this, perhaps, the identity of the new strain is determined only after it has infected a large number of people in China. Some of them carry the new virus into Hong Kong, others into Taiwan, and still others into a number of other countries. There is an explosive pandemic, much social and economic disruption, and a good deal of misery among the victims. Rapidly growing strains of the new virus are created, and vaccine production starts but progresses slowly. The neuraminidase inhibitors Relenza and Tamiflu are eagerly sought but are in very short supply. Who should get these new drugs? Health care workers and those in essential services, obviously, but who will identify them? Even then, there will not be nearly enough of the drugs for all those who need them in the developed world, let alone the rest of the world's population.

The answer seems to be: Stockpile these drugs now, in huge quantities. Their shelf life has not yet been determined, but they are simple chemical compounds and there is no reason to suppose they are not stable. In any case, it would be possible to replace the stockpile every 5 years or so. The cost of making the drugs, as opposed to the prices the pharmaceutical companies charge consumers, would not be exorbitant. Such an expenditure by governments would be a worthwhile investment in their defense against this debilitating and often deadly illness.

References and Notes

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2. M. Hatta, P. Gao, P. Halfmann, Y. Kawaoka, *Science* **293**, 1840 (2001).
3. Proceedings of the Royal Society meeting on "The origin and control of pandemic influenza" held at the Royal Society in London, UK, 25 to 26 April 2001.
4. W. R. Dowdle, *J. Infect. Dis.* **176**, S69 (1997).
5. We thank J. Murray for help with the figure.

PERSPECTIVES: CLIMATE CHANGE

Devil in the Detail

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The Intergovernmental Panel on Climate Change (IPCC) this year confirmed a global mean warming of 0.6 ± 0.2°C during the 20th century and cited anthropogenic increases in greenhouse gases as the likely cause (*1*). However, this mean value conceals the complexity of observed climate change. If the recent past is a guide to the future, regional climate changes will have more profound effects than the mean global warming suggests.

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Global maps of observed climate change reveal a complicated pattern. Trends in mean annual air temperature for 1950–98 indicate three areas of particularly rapid regional warming, all at high latitudes (*2*): northwestern North America and the Beaufort Sea, an area around the Siberian Plateau, and the Antarctic Peninsula and Bellingshausen Sea. The last area provides a valuable case study, remote from the complications of urban warming and sulfate aerosols.

The mean temperature trend for all Antarctic stations for 1959–96 is +1.2°C per century (*3*), well above the global mean. Regional responses have, however, varied widely. Annual air temperatures have cooled at Amundsen-Scott base at the South Pole since 1958 (*4*) but have warmed on the Antarctic Peninsula (see the first figure) since reliable records began in the 1950s (*3*, *5*). The longest records from the peninsula (*4*) show a warming in the northwest Antarctic Peninsula that is considerably larger than the mean Antarctic trend. The shorter records (*4*, *6*) suggest that the warming extends further south and east. Antarctic Peninsula records are too short to show when the rapid regional warming began. However, warming at Orcadas began in the 1930s, and annual temperatures at Orcadas correlate well with the Faraday record. Warming in the Antarctic Peninsula may thus have begun at a similar time.

The importance of this recent rapid regional warming is highlighted by its impact on the local environment, such as expanding ranges of flowering plants (*7*), retreat-