

A One-Size-Fits-All Flu Vaccine?

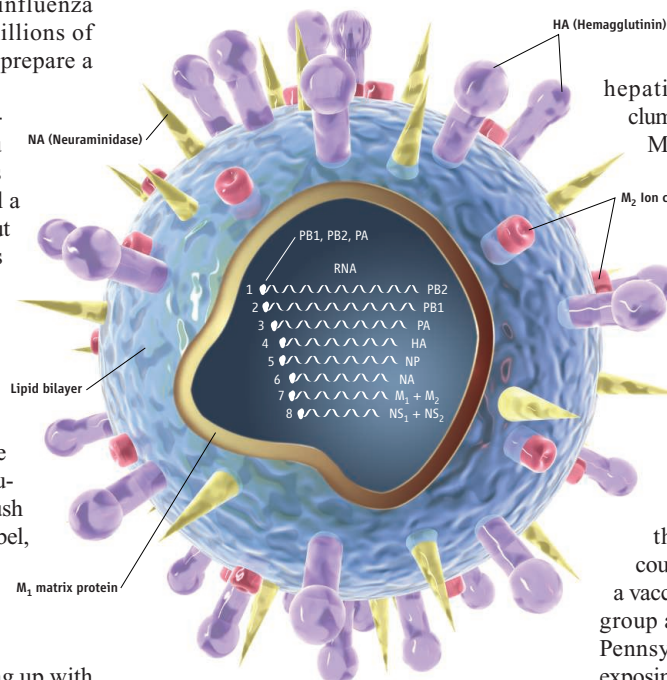
The threat of avian influenza has revived efforts to develop “universal” flu vaccines that protect against all human influenza strains. Although that goal remains elusive, vaccines that protect against seasonal flu variants could be closer

Modern medicine’s main weapon against the influenza virus is woefully unsophisticated. Each year, companies have to make a new batch of flu vaccine because unlike, say, polio or chickenpox, flu strains change every year. The vaccine is grown in eggs, a process that takes up to 9 months, and people have to be vaccinated annually, which many don’t bother to do. More troubling, if a pandemic strain of influenza came along, the virus could kill millions of people in the time it would take to prepare a matching vaccine.

What scientists dream of is a vaccine that can protect against any flu strain for years or even a lifetime. This so-called universal flu vaccine is still a long way off, if it’s even possible. But many labs are dusting off past projects on broad flu vaccines, spurred by new funding and fears that H5N1, the deadly avian influenza that has swept across half the world, could acquire the ability to be transmitted from human to human. Until now, “flu has never been before high enough on the radar screen” for companies in particular to follow through with a strong push for a universal vaccine, says Gary Nabel, director of the Vaccine Research Center at the U.S. National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Maryland.

Doing so, however, means coming up with an alternative way to stimulate immunity to the virus. The tried-and-true technique for seasonal flu uses a killed virus vaccine that works mainly by triggering antibodies to hemagglutinin (HA), the glycoprotein on the virus’s surface that it uses to bind to human cells. Hemagglutinin and neuraminidase (NA), another surface glycoprotein that helps newly made viruses exit cells, give strains their names (H5N1, for example). The sequences of HA and NA mutate easily, which is why each season’s flu strain—although it may be the same in subtype, such as H3N2—“drifts” slightly from the previous year’s, and the annual vaccine must be tailor-made.

To make a universal vaccine for influenza A, which includes the main seasonal flu strains and bird flu, as well as past pandemic strains, some scientists are hoping to use “conserved” flu proteins that don’t mutate much year to year. (Influenza B, the other type, occurs only in humans and causes milder symptoms.) Some of the conserved protein vaccines in the works



Weak spots. A universal flu vaccine would target “conserved” proteins, such as M2 or NP, an inner protein.

stimulate production of antibodies as do conventional flu vaccines, whereas others rouse certain immune system cells to battle the virus.

Other scientists are pursuing a slightly less ambitious goal: They are working on vaccines that match a particular HA, such as the H5 in H5N1, but that also protect against “drift” strains that typically emerge from year to year.

It’s not yet clear whether any of these broad vaccines will ever work as well as a traditional, HA-matched vaccine. But they could help when

the annual vaccine doesn’t match the circulating strain exactly, and in a pandemic, they could reduce deaths until a matched vaccine is ready. “Anything that would dampen a pandemic would be useful,” says virologist Robert Couch of Baylor College of Medicine in Houston, Texas.

One for all

One of the most hotly pursued strategies for a universal vaccine against influenza A is based on a flu protein called M2. This protein forms an ion channel crossing the membrane of a virus particle or infected cell, barely jutting out from the surface. It’s an appealing target because the 23 amino acids that make up the ectodomain, or protruding part, of M2 (known as M2e) scarcely vary from one human flu strain to the next, even back to the 1918 Spanish flu.

Scientists first showed in the late 1980s that antibodies to M2 can slow flu infection in mice. In 1999, biochemist Walter Fiers’s team at Ghent University in Belgium reported in *Nature Medicine* that it had reduced flu deaths in mice with a vaccine made of M2e fused to another protein, the core of the hepatitis B virus (HepB). (These proteins clumped into viruslike particles bristling with M2e that stimulated more antibodies to M2e than did the protein by itself.) In its latest paper in *Vaccine* in January, Fiers’s lab, now collaborating with the vaccine company Acambis in Cambridge, Massachusetts, has improved the candidate vaccine by attaching three copies of M2e to the HepB core, delivering it nasally—which boosts immune responses compared to injection—and adding an adjuvant, an ingredient that also increases the body’s immune response.

Although M2e is typically conserved, there’s a small chance that the protein could still evolve, enabling the virus to evade a vaccine. To assess that risk, Walter Gerhard’s group at the Wistar Institute in Philadelphia, Pennsylvania, pushed the virus to mutate by exposing mice with weak immune systems to an H1N1 seasonal flu strain while giving them antibodies specific to M2e. As they reported last June in the *Journal of Virology*, M2e mutants appeared in some mice after 3 weeks, but there were only two types—fewer than might have been expected. “To us, that was reassuring,” Gerhard says, because it should be possible to make an M2e vaccine to match the few anticipated variants.

Another major caveat is that although M2 vaccines may prevent deaths from flu, they may not keep people from getting sick, the way conventional vaccines normally do, notes Couch. That’s because M2 antibodies seem to work by binding to infected cells and promoting their clearance, instead of blocking the virus (which

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sports few M2 surface proteins) from infecting new cells, as traditional vaccines are thought to do. Fiers's mice, for instance, still get sick and lose some weight, although they do survive. Fiers argues that, given the limitations of current seasonal flu vaccines—a regular flu vaccine matches the circulating strain only 80% to 90% of the time and often doesn't work at all in the elderly, whose immune systems aren't good at making new antibodies—M2 vaccines are a possible replacement. Others, including Gerhard, see M2 vaccines as a backup to regular vaccines, perhaps as an added component in annual flu shots.

Some experts caution that it's too early to say that M2 vaccines will work in people, as opposed to mice. Retired New York Medical College virologist Edwin Kilbourne, for instance, questions whether Fiers challenged mice with sufficiently high doses of virus.

Despite the skepticism, several companies hope to commercialize M2 flu vaccines, including Switzerland-based Cytos Biotechnology; Acambis expects to submit a clinical trial application to the U.S. Food and Drug Administration (FDA) this year. Acambis's Ashley Birkett agrees that “we need to see how it performs in the clinic.” Another contender is Merck, which has done animal tests on an M2 vaccine combined with an influenza B vaccine made from a conserved stretch of the virus's HA, says Merck researcher Antonello Pessi.

Looking inside

Another approach to a universal flu vaccine uses conserved internal proteins such as nucleoprotein (NP) to elicit a different kind of immunity, one based on a type of T cell called a cytotoxic T lymphocyte (CTL) rather than on antibodies. CTLs recognize and kill infected cells expressing viral antigens, fragments of proteins such as NP.

Researchers at Merck and Vical Inc., a biotech company in San Diego, California, reported 13 years ago that a vaccine based on NP partially protected mice from seasonal influenza A, although some animals still died. Instead of immunizing the animals with NP itself, the researchers used DNA encoding the protein as the vaccine, a strategy that often generates a more powerful cellular immune response. Last year, FDA researcher Suzanne Epstein and others showed that mice survived seasonal flu and could be partially protected against dying from H5N1 by an NP-based DNA vaccine boosted by ferrying the DNA into cells inside an adenovirus disabled so it can't replicate.

Like M2 vaccines, vaccines based on internal viral proteins won't prevent infection altogether because CTLs target already infected cells. Still, says Epstein, they could offer some protection until a pandemic vaccine is produced. Her group is now looking at a DNA vaccine that combines NP and M2, a strategy also being pursued by Vical with NIAID support. Others are considering the inner proteins not for a stand-alone vaccine but as adjuvants that could broaden the immune response to HA-based vaccines.

One overarching question is whether long-lasting immune protection against different flu subtypes—whether through CTLs, M2, or

some other mechanism—is possible in humans. The epidemiological data have been scanty. But Epstein recently found suggestive evidence by analyzing old records from a study of 60 Cleveland, Ohio, families who experienced the 1957 H2N2 flu pandemic.

Epstein reports in the January 2006 *Journal of Infectious Diseases* that adults (but not children) who had lab-verified H1N1 flu in the years before that pandemic were one-third as likely to get sick with the 1957 H2N2 flu.

Narrowing in

With the two main approaches to making a truly universal flu vaccine still a question mark, some investigators are working on a seemingly more approachable goal: making HA-specific vaccines that protect against drift strains within the same HA family.

One way to achieve this broad immunity is with a live attenuated vaccine, which consists of a virus that can still infect cells and thus should induce

CTL responses. A live attenuated nasal vaccine made each year for annual flu, FluMist, has been on the market since 2003 in the United States. Manufacturer MedImmune says that it protects against mismatched strains. MedImmune and NIAID will soon begin clinical testing of FluMist versions for potential pandemic strains such as H5N1 and H9N2. The downside of live vaccines, however, is that there is a small risk that the virus could revert to a dangerous form, perhaps even creating a new pandemic strain.

A safer way to achieve protection against drift pandemic strains may be with DNA encoding the HA surface protein delivered by means of a viral vector. Compared to the traditional killed virus vaccine, this should stimulate broadly protective CTL responses to conserved parts of the HA protein that are shared by related strains. Separate teams at the University of Pittsburgh in Pennsylvania and Purdue University in West Lafayette, Indiana, both collaborating with the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, reported in *The Lancet* and the *Journal of Virology* in February that an adenovirus-delivered vaccine based on H5 DNA protected against both the 1997 Hong Kong strain of H5N1 and the 2004 Vietnam strain. One advantage compared to conventional egg-grown vaccines: The manufacturing of the vaccine is done with cells, and “you can make millions of doses in a month's time,” says



Broad thinker. Belgian biochemist Walter Fiers hopes to take advantage of the similarities among flu viruses.

Some Proposed Universal or Broad Flu Vaccines					
	Vaccine Type	Antibodies	Cytotoxic T lymphocytes	Who	Clinical trials
All HA Strains	M2e			Acambis, Merck, Cytos Biotech., Wistar Inst., Wash. U./Vaccine Res. Inst.	Acambis 2007? Merck 2006?† Cytos 2007?
	Conserved HA (influenza B)			Merck	Merck 2006?†
	DNA vaccine with NP, sometimes M2 gene†			FDA, Vical/St. Jude	
HA Drift Strains	DNA vaccine with HA gene			PowderMed	Seasonal flu 2005; H5N1 2006
	Adenovirus-based with H5 HA gene			U. Pittsburgh/CDC, Purdue/CDC	2007?
	Live attenuated			MedImmune/NIAID	FluMist (seasonal flu) on market; pandemic vaccine trials 2006

* Merck is testing a new flu vaccine but has not disclosed which one. † FDA vaccine included adenoviral boost.

Influenza

Suryaprakash Sambhara of CDC, senior author on the *Lancet* study.

Both universities are seeking funding for clinical tests from NIAID and companies. Sambhara plans to give the Purdue team an adenovirus-based vaccine containing genes for NP and M, which codes for M2 and an inner protein, as well as H5HA; and Andrea Gambotto of Pittsburgh hopes his vaccine, which also worked in chickens, may be picked up as a bird vaccine. A company called PowderMed avoids using adenoviruses to

deliver the DNA, which have some drawbacks, instead using gold-coated particles and high-speed injection to get HA-based DNA vaccines into a person's skin cells.

Although the variety of approaches to broader flu vaccines can be dizzying, "having all of those efforts moving forward gives us more weapons in the arsenal and makes us more likely to find the best platform," NIAID's Nabel says. Even if one approach rises to the top, there are many obstacles ahead, such as

persuading regulatory agencies—who now approve flu vaccines based only on HA antibody responses—to use CTL responses as a measure of efficacy instead, notes virologist Albert Osterhaus of Erasmus University in Rotterdam, the Netherlands. Still, if universal—or at least broader—flu vaccines can make it to the market, they could save lives during regular flu season and stave off disaster when the next pandemic strikes.

—JOCELYN KAISER



NEWS

Oseltamivir Becomes Plentiful—But Still Not Cheap

The shortage of oseltamivir may soon be a thing of the past. But whether the drug will become cheap enough for developing countries and how well it will work against a pandemic remain to be seen

For more than 6 months, German physician Tido von Schoen-Angerer has "desperately" tried to order oseltamivir from Roche, the Swiss company that produces the anti-influenza drug. "But I've given up," he says. Von Schoen-Angerer, research and development director of the Campaign for Access to Essential Medicines at Médecins sans Frontières in Berlin, wanted 100,000 treatment courses to protect MSF personnel and to treat patients, should the organization ever find itself at the cradle of a pandemic. But Roche kept saying it simply didn't have enough of the drug, Von Schoen-Angerer says. He eventually picked up 500 treatments from a Dutch wholesaler last month, at a price he says was too high.

As global demand for oseltamivir, better known by Roche's brand name Tamiflu, reached the stratosphere, many clients found themselves at the end of a long queue. But their frustrating wait should soon be over. Last month,

Roche announced a series of deals with other companies to dramatically ramp up production of oseltamivir; in 2007, it says it will be capable of producing 400 million treatment courses (each consisting of 10 capsules) yearly. That's up from just 6 million 3 years ago and much more than the expected demand. Supply will be boosted further because a handful of generic drug makers have started producing their own versions of oseltamivir—some with a sublicense from Roche, others without.

Although production may finally meet worldwide demand, several questions remain. It's unclear whether oseltamivir's price will drop enough for poor countries to stockpile the drug; many fear that they will be left behind (*Science*, 18 November 2005, p. 1103). That's why it's essential to find simpler ways to produce the drug, some say. One such method may be a revolu-



Drug of choice. More than 65 countries are stockpiling oseltamivir, better known as Tamiflu.

tionary, easy, and cheap synthetic pathway that Harvard University chemist and Nobel laureate Elias Corey and his team are now reporting.

Also unanswered is the question of how well oseltamivir will work against a pandemic virus. The drug is effective against seasonal flu strains, but there aren't solid data about its efficacy against H5N1, the avian influenza strain that some suspect is a prime candidate to evolve into the next flu pandemic. "We think it's effective" against H5N1, says Nikki Shindo, a medical officer at the World Health Organization (WHO) in Geneva, Switzerland, "but that's a feeling. We would like to have more evidence." Some studies on the drawing board aim to get just that.

Change of heart

Until less than a year ago, Roche routinely dismissed suggestions that it sublicense oseltamivir, saying it needed tight control over the complex production process, which includes a risky step involving an explosive intermediate compound called an azide. It also refused to say how much oseltamivir it produced or how much it was charging governments for it.

But the company did an about-face last fall. In the past 6 months, it has signed deals with more than 15 companies, each of which will help carry out a step in Tamiflu's production process. In addition, Roche has sublicensed Shanghai Pharmaceuticals and HEC, both in China, and Hetero in India, to make oseltamivir from beginning to end. Those companies will produce generic versions for local use; they won't be named Tamiflu, and Roche will not control quality, production volume, or pricing policy, says David Reddy, Roche's pandemic task force leader.