Photo: Purdue University/Tom Campbell

Swine flu spurs vaccine makers

Turbo-Serum Under Way

Being hooked on a 78-year-old-technology, vaccine producers hope to speed up flu vaccine development by using novel production methods. In the case of the currently raging swine flu, however, they will probably be too late.

Imagine there's a flu epidemic and chickens are on strike. A bad joke? No, a serious nightmare, because so far there's no flu vaccine that doesn't make use of hens' eggs. Since the 1950s, inactivated flu viruses – and that is what flu vaccines are composed of – have been routinely grown by vaccine makers in fertilized hens' eggs (a technique that was originally discovered 78 years ago). Admittedly, an antique but established technology, its medical worth has been proven over decades. The trivalent influenza vaccine that is produced in advance each year, depends on the use of hen's eggs (see box on page 47).

Annual flu campaign: well-proven...

It's a tricky but not impossible task for the epidemiologists responsible to predict which flu strains are likely to emerge worldwide at a given point of time (and will therefore be chosen as vaccine targets). If the annual forecast fails, however, the death toll from this viral disease would be significantly higher than in "normal" flu seasons. Fortunately, this nightmare scenario hasn't materialised in recent years. In this respect, the WHO Global Influenza Surveillance Network has performed well.

After designing a vaccine with, casually speaking, the lowest common denominator (against H1N1, H3N2 and type B in recent years; see box on page 47), pharmaceutical companies are instructed by the WHO to produce it in advance as soon as possible. However, "as soon as possible" means 3 to 6 months at the best: each winter's vaccine was arranged in the preceding summer.

The main problem, however, are the eggs. As of November 2007, both the conventional injection vaccine and a novel nasal spray vaccine of live attenuated influenza virus ("FluMist"), have been manufactured using reams of chicken eggs.

...but unfeasible for pandemics

The old-fashioned way of producing vaccines in eggs has several drawbacks. Furthermore, it's too dependent on a steady supply of eggs to be reliable in the face of a pandemic emergency. In order to produce 300 million doses of vaccine (a normal annual global amount), egg-based production requires some 900 million eggs. In the case of an avian or swine flu pandemic, egg-producing flocks wouldn't suffice, jeopardizing vaccine production capabilities. Eggs are perishable, and chickens can't lay them on stock.

(photo above) At Purdue University, West Lafayette, USA, virologist Suresh Mittal is working on flu vaccines that are not based on eggs and can easily be modified to changes in the virus.

An additional limitation to the traditional method of producing flu vaccine is intolerance, since flu vaccines are contraindicated for those with severe allergies to egg proteins and people with a history of Guillain-Barré syndrome. Some virus variants (especially the avian flu subtypes H5 and H7) are too pathogenic to be successfully grown in hens' eggs. They would quickly kill them.

Most notably, however, as its major disadvantage, is that producing vaccines in chicken eggs is too time-consuming. It's convenient for predictable infectious illnesses like the "normal" annual flu, when there is advance data and enough time for the manufacturing process. If a new pandemic influenza strain, whose exact genetic composition isn't yet known, is spreading all over the world like wildfire, however, the "egg way" will take too long.

How can the international community respond effectively to a future pandemic, and how can vaccine producers take steps to speed up the vaccine manufacturing process? The chance to profit from infectious diseases such as flu is huge, in the light of a Datamonitor study from 2007, predicting that the worldwide influenza vaccine market will more than double in size to over €5 billion by 2016.

Cell culture to replace egg technology

To develop a vaccine that would prevent infections from swine flu (a new type of influenza A subtype H1N1), would take the usual 3 to 6 months. In the face of the flu plague's rapid worldwide spread, taking weeks not months, researchers are looking urgently for new ways to speed up the vaccine's manufacturing process.

A relatively new approach is cell-based vaccine production. Currently, this technique is used to produce the polio vaccine, for example. Virus strains are injected into laboratory-grown mammalian cells where they multiply (kidney cells, taken from green monkeys, are often used for this purpose). After having removed, harvested and purified the cells' outer walls, a vaccine can be produced in this way in a matter of weeks.

Aside from accelerated manufacturing, cell-based vaccine production offers additional advantages: the infected cells can be frozen and stored in advance of a pandemic or developed rapidly in response. In addition, cell-based vaccine production dramatically reduces the risk for contamination and allegedly is more reliable and flexible. Allergic side effects aren't a problem, either.

Producing flu vaccines the classical way with eggs is time consuming and limited to a defined capacity. Alternatives, such as cell-culture based technologies, are approved but not sufficiently available at the moment.

At least 20 firms [and, in addition, several academic groups] are working on pandemic vaccines, WHO director Marie-Paule Kieny told *Reuters* in February. Some of these firms are also working on novel vaccine production techniques. The US Department of Health and Human Services (HHS), for example, paid €73 million to Sanofi-Pasteur in 2005 to develop a cell-based influenza vaccine technology and conduct clinical trials within five years, with the goal of obtaining an FDA license for this vaccine. The company is to establish a cell-based influenza vaccine manufacturing facility, capable of producing at least 300 million doses of a pandemic influenza vaccine within one year. In 2006, the HHS added fuel to the fire when it awarded five contracts totaling more than €750 million to accelerate alternative technologies for flu vaccine production within the U.S. (mostly based on cell-based production technologies).

Europeans at the cutting edge

While most US companies are still fiddling about, European companies are surging ahead. In April 2007, the Marburg, Germany-based Novartis Behring approved Optaflu, the first influenza vaccine to be produced in a mammalian cell line. According to data from a clinical phase III study in 2006, "the cell culture-derived influenza vaccine was comparable to conventional egg-based vaccines in efficacy and tolerability". Meanwhile, Novartis has established a factory in Marburg that produces this vaccine in large quantities and delivered the first batches in the 2006/2007 season. At present, Novartis is building another factory in North Carolina, US, which might, how-

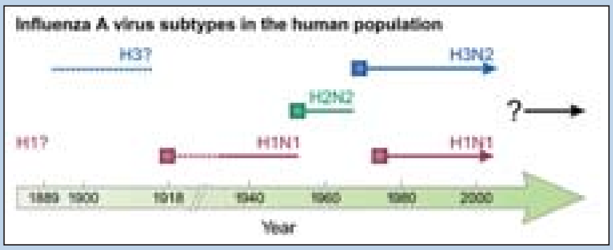
The seasonal flu vaccine

Guessing the Serotype

Each year, flu causes an estimated 50,000-60,000 deaths in the European Union alone (most people die not from the virus, but from secondary bacterial infections and complications). Among the three influenza types A, B, and C, the most virulent human pathogens are the highly variable type A viruses, causing the most severe disease. They can be subdivided into different serotypes based on the antibody response to these viruses, such as "H3N2", which caused Hong Kong Flu in 1968; "H5N1", which causes avian flu; or "H1N1", which caused Spanish flu in 1918, and swine flu in 2009 (see right picture).

Due to frequent changes in their genetic properties, each year different virus strains become dominant, hence, each year a new and effective vaccine formulation has to be developed.

The World Health Organization (WHO) monitors all regions of the world regarding currently circulating viruses and coordinates the timely development of an annual vaccine (one for the northern and one for the southern hemisphere, respectively). This annual flu campaign results in an (annually variable) trivalent vaccine, consisting of hemagglutinin surface glycoprotein components (see virus figure on page 48). In the recent ten years or so, the seasonal flu vaccine was effective against H1N1, H3N2, and type-B strains, thought most likely to cause significant human suffering in the coming flu season, too. -WK-



The various strains of influenza that have infected the human population in the 20th century (data taken from Peter Palese, Influenza: old and new threats, *Nature Medicine*, 2004).

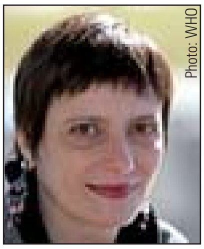


Photo: WHO

Marie-Paule Kieny, director of the WHO initiative for vaccine research since 2001.

ever, not be ready for use before 2010.

Baxter is saving the day for the US pharmaceutical industry. The company from Deerfield, Illinois, has a strong focus on vaccine development and production. For years, the US company has been developing both seasonal and pandemic influenza vaccines based on the company's "vero-cell" technology (a mammalian cell line, derived from green monkey cells).

Baxter is licensed to produce cell culture-based vaccines at its commercial scale manufacturing facility in Bohumil near Prague, Czech Republic. Another Baxter vaccine, Celvapan, the first cell culture-based H5N1 (avian flu) pandemic vaccine, recently smashed through the last approval hurdle from the European Medicines Agency (EMEA) and thus can be used if the WHO officially declares an avian flu pandemic.

Making swine flu vaccines is like ...

What about the 2009 swine flu outbreak? Baxter officials recently declared that the company's technology allows it to develop vaccines in half the time it usually takes (13 weeks instead of 26). The firm requested (and received) the new H1N1 Mexico strain from the WHO, in order to develop a vaccine. A Baxter official recently told an Austrian newspaper that, after a prefabrication delay of 1-2 weeks, "In 8 to 12 additional weeks we should have the first batches". Baxter claims to have the production capacity for 60 million doses per month.

Other vaccine makers made similar statements, affirming that the cell-based approach will not be perfected in time to provide the vaccine supplies that could be needed to fight the swine flu pandemic that started in April 2009. According to a *New York Times* article, an effective swine flu vaccine is "months away". US federal officials' statements are likewise. They say it would take until late November at the earliest to make enough vaccine to protect all Americans (306 million, each of them needing two shots) from a possible epidemic of swine flu

The sinister enemy, influenza A/H1N1, under the electron microskope.

Diagnostic assays, synthetic genes, antiviral drugs and more

Profiting from Swine Flu

Somebody always cashes in on disasters. As *Reuters* reported, the venture capital firm Kleiner Perkins Caufield & Byers stands to benefit from the swine flu outbreak in Mexico because two of its portfolio companies (BioCryst and Novavax) have technologies that could be used to fight the epidemic. As a consequence, the stock of both companies surged when investors scrambled for shares.

Several companies offer diagnostic virus tests. Berlin-based biotech firm TIB Molbiol, for example, in May introduced a test that is optimised for Roche's Lightcycler systems, selectively identifying the new influenza A/H1N1 variant. Qiagen, as a provider of sample and assay technologies, also provides molecular diagnostics, such as PCR enzymes for virus detection (see photo) and kits to isolate viral RNA.

Geneart, a synthetic biology specialist from Regensburg, Germany, is involved in vaccine development. The company recently produced several synthetic genes for an undisclosed pharmaceutical customer and for the Robert Koch Institute (Berlin). These were mainly variations of the viral coat proteins of the swine flu strain (H1N1), designated for the development of a vaccine against swine flu. Geneart's CEO, Ralf Wagner, told *Lab Times* that his company has developed "an emergency plan for such scenarios to deliver in the shortest possible time".

A molecular diagnostics test to detect a variant of the influenza A virus.

Roche and GlaxoSmithKline are also likely to benefit from current global pandemic fears. The pharmaceutical companies produce Tamiflu and Relenza, respectively, the only antiviral drugs that appear to be effective against the new kind of influenza, thus prompting governments and companies to stockpile them.

Due to escalating hoarding of antiviral drugs all over the world, resulting in shortages in several countries, the drugmakers have started to allocate their stock. Roche, for example, doesn't supply French pharmacies any longer because of a run on Tamiflu. Presently, only hospitals in France are given the drug. After a tenfold increase of daily prescriptions for antiflu drugs, Roche and Glaxo quickly boosted their production.

Tamiflu (active ingredient: the small molecule Oseltamivir) is a chemically synthesised and orally active neuraminidase inhibitor, preventing new viral particles from being released by infected cells. According to the CDC, however, Tamiflu may be susceptible to resistance and unable to combat the influenza A virus, the most common seasonal flu virus in recent years – a fact that personal stockpilers mostly ignore.

Relenza (Zanamivir), discovered in 1989, is the first neuraminidase inhibitor to be commercially developed (originally by the Australian biotech company Biota). The Glaxo drug is unpopular because it must be breathed in with an inhaler. According to a paper, however, published in 2005 in *The Lancet*, Relenza is at least as effective as Tamiflu, has fewer side effects and less tendency to make the virus resistant. -WK-

(and years to produce enough swine flu vaccine to satisfy global demand).

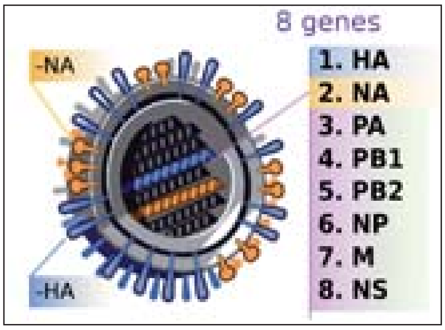
A study, done by the WHO and the International Federation of Pharmaceutical Manufacturers and Associations, estimates that it will take four years to produce enough vaccine to protect global population against the 2007 bird flu strain. In the case of a swine flu pandemic (and a vaccine to prevent it), it would take similarly long. By 2014 at the earliest, the study estimates, production capacity will increase to 5-15 billion doses, taking 1-2 years to meet global demand.

... robbing Peter to pay Paul

These limited production capacities, whether with the old-fashioned egg technology or novel cell-culture techniques, beg a fundamental question. Producing large quantities of swine flu vaccine would necessarily interfere with the production of the seasonal flu vaccine for next winter (which is ineffective against the new strain). Thus, in the case of a pandemic, the WHO would have to arrange a compromise with flu vaccine makers like Baxter and Novartis. So far nothing has changed, according to Marie-Paule Kieny, WHO director of the initiative for vaccine research, who recently announced that production of the unchanged seasonal vaccine should continue for now.

On May 1st, however, a senior World Health Organisation (WHO) official said that some drug makers will shift within a few weeks to producing pandemic flu vaccines against the new swine flu strain, according to a *Reuters* report. Officials at Sanofi-Aventis, which is manufacturing the conventional seasonal flu vaccine and has not been asked to produce a vaccine against the new H1N1 strain, also confirmed that it would be difficult to manufacture without diverting facilities from current seasonal flu production.

Swiss Novartis announced at the end of April to use data on the new adjuvant MF59 to design a new vaccine specifically for swine flu. Andrin Oswald, chief executive of Novartis Vaccines and Diagnostics, said to *Reuters*, "We will use these new insights, as well as our [knowledge] in cell based vaccine manufacturing, to develop a vaccine against the current outbreak."



The influenza A virus contains 8 segments of negative-sense ssRNA, with a total genome length of 13,588 nucleotides, coding for 11 proteins. Its classification is based on the viral surface proteins hemagglutinin (HA) and neuraminidase (NA), resulting in names such as "swine influenza A (H1N1)" (as pictured).

Small biotechs venture out

In the light of limited production capacities, small biotech firms try to step into the breach. Take Protein Sciences from Meriden, Connecticut. While awaiting federal approval for its first seasonal flu vaccine, FluBlok, the US company's chief executive, Dan Adams, said his firm could start making a swine flu vaccine in about six weeks. "We can switch production to make it", Adams said to a local newspaper.

Nice wording, but who is hindering him to start swine flu vaccine production? According to Adams' words, Protein Sciences isn't ready to take the risks involved in producing the vaccine without government funding. "We would need to disrupt manufacturing our seasonal flu vaccine, and doing so could cost us revenues," Adams pointed out, adding that, "without a large-scale manufacturing facility, Protein Sciences can only make limited amounts of the vaccine".

Another problem is the probable unfair distribution of the vaccine. Most of the vaccine batches will go to countries that produce the vaccine, because countries will restrict exports in a pandemic, experts predict (see box on page 48). In other words: countries like Mexico, where the epidemic hits hardest, wouldn't get enough vaccine.

Incalculable risk of an antigen shift

The most crucial question, however, is this: will the completed vaccine really protect people against illness? Virologists fear one thing above all: an unexpected antigenic shift, when different strains of a virus combine to form a new subtype (from a H1 to a H5 subtype, for example). Such a rare but not impossible event could foil all their efforts, resulting in a global pandemic, with an ineffective vaccine as the only counteragent. Winfried Koeppelle