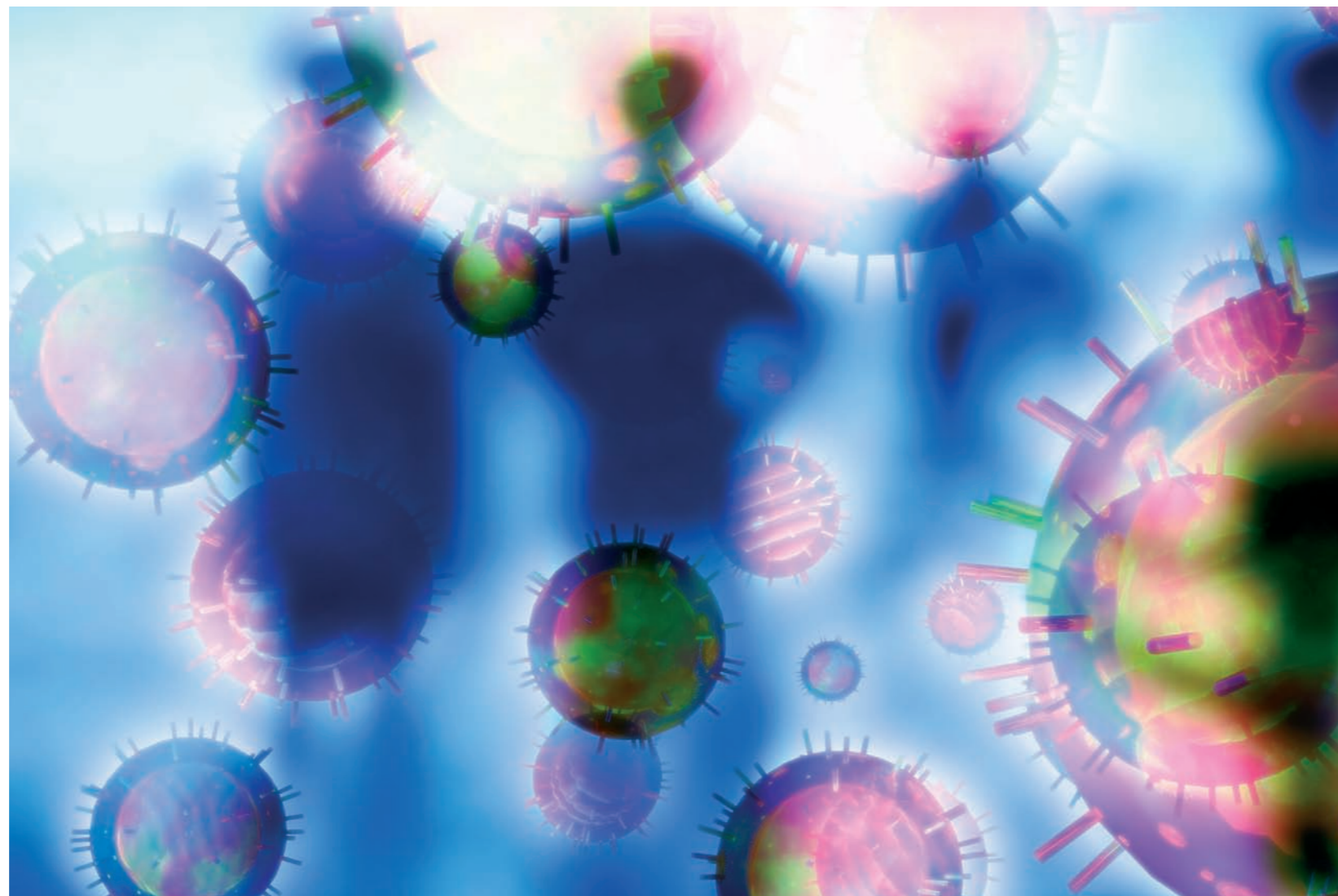




▲ Coloured transmission electron micrograph of influenza A virus strain H5N1. Dr Gopal Murti / Science Photo Library

► Computer artwork of influenza virus particles with people. Pasieka / Science Photo Library



There are worldwide anxieties about the spread of bird 'flu and the possible mutation of the virus into the cause of a human pandemic. **Wendy Barclay** describes the latest developments in vaccines to combat this huge threat to health

Influenza vaccines

Influenza A virus is a notorious human pathogen which claims around 10,000 lives every year in the UK, especially in the elderly and very young. Periodically, a new influenza subtype emerges in the human population which causes a pandemic. The 1918 Spanish 'flu pandemic was the most severe recorded to date, claiming up to 40 million deaths worldwide, but the two other well documented pandemics of the 20th century, Asian influenza in 1957 and Hong Kong influenza in 1968, are also remembered with apprehension. Currently, we are in the grip of an outbreak of avian

influenza on a historically unprecedented scale. This particular H5N1 strain of the virus is deadly for birds and also for those humans it manages to infect. Luckily, the numbers of people infected so far have been small. Only 132 people have had laboratory-confirmed infection, although 68 of them have died. However, given the plasticity of the influenza genome, it is highly likely that the H5N1 virus could mutate to acquire the ability to be readily transmitted from human to human.

Of the 16 different subtypes of influenza virus that reside in the natural avian host, two are more feared than

the others. H5 and H7 influenza can be highly pathogenic in chickens and in the past were known as the 'fowl plague'. This trait is encoded by a motif in the gene for the spike protein, HA, that allows growth in a wide range of tissues other than lung and gut and thereby produces a rapid fatal pathogenesis. H5N1 viruses with this motif have spread across South Eastern Asia carried by water birds whose migratory routes could bring them to Europe and beyond next year. The UK has a pandemic plan which relies on antiviral drugs in the first wave, but an effective vaccine against H5N1 would be on every public health planner's wish list.

Annual vaccines

Annual influenza epidemics are controlled by a vaccination policy that relies on tremendous cooperation between WHO collaborative centres conducting surveillance worldwide and

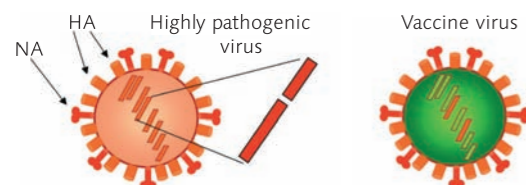
vaccine manufacturers. They track antigenic drift in seasonal influenza strains, and, based on this information, a new strain is chosen each year to update the vaccine. The vaccine itself is manufactured from a reassortant virus, a genetic recombinant in which the surface genes of the virus, HA and NA, are introduced into a genetic backbone that has been safely used to generate 'flu vaccine for many years (Fig. 1). This 'PR8' backbone strain also has the property of high growth in eggs, the current substrate used by manufacturers for vaccine production. After mass production of the reassortant virus, it is partially purified and chemically inactivated, and in some cases the HA and NA components are purified out to produce a 'split' or 'subunit' vaccine. Since this procedure is routine, it might seem a simple task to apply the same sequence of events to producing vaccine that could protect people against avian strains of influenza like H5N1.

Developing H5N1 vaccines

However, in 1997 when the H5N1 threat first loomed, it became apparent that production of vaccines against avian influenza would not be a simple task after all. First, since avian influenza viruses differ from human viruses in every gene segment, there may be incompatibilities for generating traditional reassortant viruses with the human PR8 strain. Moreover, the H5 and H7 avian strains of virus are considered so dangerous to both humans and birds that work with them needs to be performed under high containment, slowing the reassortment procedure and limiting where vaccine could be produced. One way around this problem, that only became feasible in 1999, is to use 'reverse genetics' to reliably create a safer, but antigenically identical virus as vaccine. To do this, pieces of DNA representing the entire genome of the desired virus are assembled. The internal virus genes are derived from the 'PR8' vaccine strain and the HA spike gene can be engineered to remove the pathogenic motif. Finally, the DNAs are combined in cells to recover the designer virus. This step must be performed under high containment, for example in facilities at the National Institute for Biological Standards and Control (Fig. 2). After suitable safety tests, the seed viruses can be distributed to vaccine manufacturers worldwide.

Alternative vaccine types

But this is only the first step and there are more problems to overcome. Earlier this year a reverse genetics (RG) H5 vaccine produced in the USA failed, even after two huge doses of 90 µg HA each, to induce sufficient serological response in volunteers. It would seem that since humans are immunologically naive to all avian influenza subtypes, their primary immune responses are much lower than those we expect each year following 'boosters' with just 15 µg HA from the updated human strains. The answer could lie in adjuvants. Indeed, studies in the UK have already shown that the dismal human immune response to avian H5 HA can be enhanced by using the MF-59 adjuvant.



▲ Fig. 1. To engineer a safe virus from the 'fowl plague' strains, the 4th RNA segment that encodes the HA spike protein is genetically engineered to remove a pathogenicity motif. Then the genes for spike proteins from the avian virus, HA and NA, are combined with genes for internal proteins from a safe but high-yielding human virus, PR8, to create a chimaeric virus safe for vaccine production. W. Barclay

▶ Fig. 2. Making safe H5 or H7 influenza vaccine. In containment level 4 laboratories at NIBSC, cells are transfected with DNA to make designer 'flu viruses suitable for vaccine production. W. Barclay

Alternatively, instead of the 'split' vaccine which is rather poorly immunogenic, we could turn to the use of cruder whole-virus preparations for immunization. A recent announcement from Hungary describes just such a product, although authorities elsewhere have been reluctant to use this type of vaccine which in the past was associated with side effects.

Very good immune responses might be raised following vaccination with a live attenuated vaccine. Cold-adapted strains were recently licensed for use in children in the USA against annual epidemic flu. Trials with H5N1 versions of these viruses will be conducted shortly in volunteers kept in strict isolation. After all, the consequence of natural reassortment between these vaccine strains and a circulating human virus could be the generation of a new virus with pandemic potential of its own!

More problems

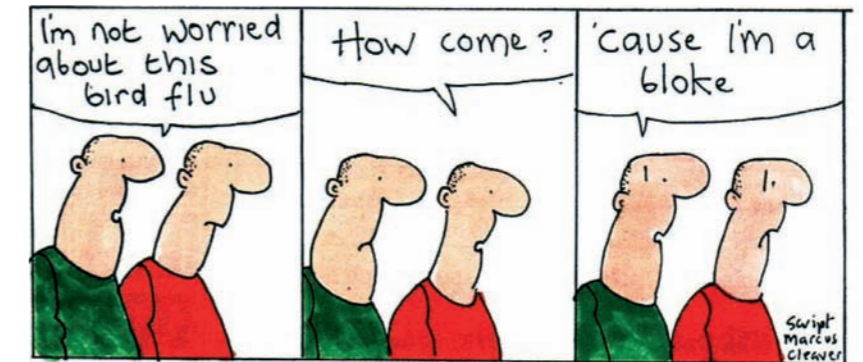
No matter which format of vaccine is chosen, they all have one drawback at present; current influenza vaccine production relies on growing the virus in large numbers of chicken eggs. In a pandemic situation the availability of eggs might be limited, due either to eggs not being ordered in advance or to an avian virus outbreak reducing the supply. The obvious solution is to switch to cell cultures for vaccine manufacture, but yields of virus from the licensed cell substrate, Vero cells, are low. Companies such as Sanofi Pasteur, the largest provider of 'flu vaccine in the world, are actively looking into which other cell lines might be used. One promising cell line (developed by Crucell Holland BV) called PERC.6, can be used to culture both human and avian influenza viruses and may be a good substrate for the future.

Some people have argued that we should not invest in production of an H5N1 vaccine yet since we do not know exactly which H5N1 virus will be the source of the pandemic. Indeed there are already at least five phylogenetically and antigenically distinct clades of the H5N1 viruses. In response



labs worldwide are producing a repertoire of different H5N1 RG vaccines representing each clade. Some of the variation in the H5N1 strains may itself be driven by vaccination, not of humans but of birds. In China we know that an RG vaccine has been produced and given to poultry in attempts to ring-vaccinate outbreaks. If suboptimal doses are used, the immunity produced protects birds from death, but not from infection. Virus replication in birds with some level of H5 antibody can then drive evolution of new antigenic variants, escalating the problems of producing human vaccine. In the light of the antigenic variation of current H5N1 strains, it will be important to know the level of cross protection to be expected from an imperfectly matched vaccine.

The dream of all influenza vaccinologists would be a universal vaccine that protected against related strains and even across the subtype barrier. There are conserved regions of the influenza virion which might be suitable targets. Indeed, some success has been achieved at least in animal models using a small peptide representing the ectodomain from the M2 protein, a minor constituent of the virus envelope, in a fusion with hepatitis B core. And it has been known now for some time that internal genes of influenza such as NP and matrix protein contain highly conserved T cell epitopes. However, whether a T cell vaccine for influenza



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would prevent infection or even enhance symptoms is not clear.

The future

In the next few years, it may be that the H5N1 or any other avian influenza subtype emerge as pandemic viruses by acquiring human transmissibility, or it may be that the threat does not mature for biological reasons we do not understand. However, it is certain that during that time we will learn more about influenza viruses that cross the species barrier from birds and how we can use vaccines to control them when they do.

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Wendy is part of a European consortium known as 'FLUPAN', funded by the EU, to rehearse the response to the pandemic threat posed by influenza; she also conducts basic research into the host-range restrictions that limit spread of avian influenza into other species.

Further reading

www.who.int/csr/disease/avian_influenza/en/

www.hpa.org.uk/infections/topics_az/avianinfluenza/menu.htm (includes the pandemic plan for the UK)

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From lethal virus to life-saving vaccine: developing inactivated vaccines for pandemic influenza. *Nat Rev Microbiol* 10, 842–847.

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Confronting the avian influenza threat: vaccine development for a potential pandemic. *Lancet Infect Dis* 8, 499–509.

When the H5N1 threat first loomed, it became apparent that production of vaccines against avian influenza would not be a simple task.