society for general Microbiology

ROYAL SOCIETY & ACADEMY OF MEDICAL SCIENCES CONSULTATION ON PANDEMIC INFLUENZA

EVIDENCE SUBMITTED BY THE SOCIETY FOR GENERAL MICROBIOLOGY (SGM)

Introduction

Influenza viruses (IVs), members of three genera *Influenzavirus* (A, B, C) of the *Orthomyxoviridae* family, infect humans and many animal (avian and mammalian) species. Type A IVs are further differentiated into 16 haemagglutinin (HA) subtypes (H1-H16) and 9 neuraminidase (NA) subtypes (N1-N9) (Russell *et al*, 2004). Until recently, viruses of only 3 HA (H1-H3) and 2 NA (N1, N2) subtypes have been isolated from human (with a very few exemptions). Since 1997 H5N1 IVs, normally circulating and replicating in birds, have been isolated from human patients with clinical influenza. To the beginning of 2006, about 50 % of approximately 170 patients with proven human influenza have died. So far all patients with H5N1 influenza seem to have been infected by intense contact with infected domestic (and sometimes feral) birds. Until now, human-to-human transmission has not been reliably documented, although it may exist. Genetically, the human H5N1 IV isolates are of avian origin in all eight genes (RNA segments), i.e. they are not reassortants like the human IVs of type A that caused the major pandemics of 1957 and 1968 (Subbarao *et al*, 2006). The 1918 pandemic H1N1 influenza A virus is possibly derived from an avian source (Tumpey *et al*, 2005; Taubenberger and Palese, 2006). The exact origin of the 1977 H1N1 viruses causing a moderate pandemic remains to be elucidated.

Avian IVs use sialic acid (SA) bound in α2,3-linkage to galactose (Gal) of cell surface glycoproteins as their receptor (SAα2,3Gal), whereas human IVs use SAα2,6Gal as their receptor. However, it has recently been shown that cells of the lower respiratory tract of human (in part alveolar epithelium) contain SA α2,3Gal receptors that can act as receptors for avian IVs (Matrosovich *et al*, 2004; Shinya *et al*, 2006). An interesting and worrying receptor switch, by change of a single amino acid of the HA of the 1918 H1N1 virus, has been described by Glaser *et al* (2005).

Avian IVs circulate widely in domestic, and probably also feral birds, as ‘low pathogenicity avian influenza’ (LPAI) viruses but can turn into ‘highly pathogenic avian influenza’ (HPAI) viruses within short periods of time by several mechanisms (Perdue *et al*, 1997; Suarez *et al*, 2004; Kawaoka Y, personal communication). It is feared that the H5N1 virus, which has recently spread from SE Asia to Africa and many parts of Europe, may acquire point mutations (in several genes, particularly the HA gene) or acquire genes from other IVs circulating in humans (via reassortment) which may make it capable of human-to-human transmission thus achieving extensive spread within a short time. Therefore ‘pandemic preparedness is fully merited’ (Webster, 2006).

Comments

These comments overlap in part with evidence, comments and suggestions already provided by the submission of Fellows of the Academy of Medical Sciences to the House of Lords Science and Technology Select Committee in reply to their enquiry into 'pandemic influenza' in September 2005. Many chapters of the recent book on *Influenza virology* by Y Kawaoka (2006) have been cited as reviews.

I.1.a. Implications of scientific understanding for treatment with drugs

Deeper understanding of structural components of viral particles (RNP, HA, NA, M2; see reviews in Kawaoka (2006); Russell *et al*, 2004: Gamblin *et al*, 2004) and of viral components on the plasma membrane of IV-infected cells (M2), as well as profound insights into the mechanisms of viral entry, import of viral RNA into the cell nucleus and export of RNP thereof, viral RNA transcription and replication (Elton *et al*, 2006), virus assembly (Muramoto *et al*, 2006; Noda *et al*, 2006) and virus release has given rise to the development of IV-specific antiviral drugs (M2 channel inhibitors, NA inhibitors; Lamb and Pinto, 2006; Gubareva and Hayden, 2006). Such knowledge may in the future lead to clinically applicable antivirals that act by blocking viral fusion with the cellular membrane, nuclear import or export, or viral assembly. It is very clear that future rational drug design will be based on the thorough understanding of viral replication mechanisms.

Unfortunately, the high intrinsic point mutation rate of the viral genome, exerted by the RNA-dependent RNA polymerase will allow the virus to escape from treatment with particular drugs relatively quickly by the development of drug-resistant (*dr*) mutants which will advantageously replicate in the presence of the drug (both *in vitro* and *in vivo*). This has been observed for the M2 channel blockers (e.g. Bright *et al*, 2005) and also recently for the NA inhibitor oseltamivir (Le *et al*, 2005). It appears that in many cases *dr* viruses are readily transmissible and pathogenic. Thus, for effective and widely applied treatment, combination therapies should be envisaged with drugs interfering at different steps of the viral replication cycle.

The main application of antivirals has so far been prophylaxis (Gubareva and Hayden, 2006). For NA inhibitor-resistant IVs it is of importance that *dr* mutants with NA mutations are often associated with reduced infectivity, replication capacity and pathogenicity and so may spread less widely and less easily than wild type (*wt*) viruses. This was also confirmed by mathematical modelling (Ferguson *et al*, 2003; and see below).

I.1.b. Implications of scientific understanding for prevention with vaccines

In the case of vaccines, basic research will be more important in the medium-to-long term. It will enable understanding of issues such as the lower immune response and therefore lower efficacy of avian influenza virus vaccines, and how to increase the efficacy of so that less virus is needed per patient to increase the number of doses available during production.

Current worldwide vaccination against influenza is based on an established annual scheme in which each February a WHO sponsored influenza expert committee selects strains that will be included into the vaccine of the upcoming season, based on the analysis of the antigenicity of strains of the previous season. This allows the production of relevant vaccine viruses (as 'high yielding reassortants') in embryonated eggs and the production of inactivated, purified and adjuvanted subunit vaccines for use in autumn.

Live attenuated vaccines have been developed and recently licensed in the US, using master strains with cold adapted (*ca*) and temperature sensitive (*ts*) phenotypes based on multiple mutations and annually updated as 6:2 reassortants.

Both these procedures follow the antigenic drift of cocirculating IVs and would be ineffective against a virus with a novel HA previously not experienced by the human population.

New vaccine strategies are based on (Katz *et al*, 2006):

- optimisation of dose, delivery, and combinations of current vaccines;
- cell culture grown virus (replacing egg grown virus);
- baculovirus recombinant expressed virus specific proteins (mainly HA but also others, e.g. M2) in combination with established or novel adjuvants;
- the use of modern reverse genetics technology.

The latter is most promising, as the 'plasmid only approach' in a number of different versions (Fodor *et al*, 1999; Neumann *et al*, 1999; Hoffmann *et al*, 2000; Hoffmann *et al* 2002a, 2002b; Neumann *et al*, 2005) is now worked out so well that targeted and multiple mutations in any gene can be engineered relatively easily and quickly into infectious virus (e.g. Jin *et al*, 2004) and investigated for desired phenotypes (high yields for subunit production, cumulative attenuation for live attenuated vaccine candidates and safe production, etc).

HPAI viruses have the capacity to transmit from their natural hosts to humans and to cause severe disease. This has been seen not only with H5N1, but also with H7N7 and H9N2 viruses. In humans such viruses may reassort with human IVs of type A and form reassortants carrying the avian HA in the genetic background of the man-adapted virus and thus be able to transmit from human-to-human. Therefore, it is tempting to consider developing vaccine candidate strains prospectively which carry H5, H7, H9 etc HAs and/or different non-human NAs (e.g. N7). There is, however, the problem that the HA of a prospectively created vaccine candidate may not be sufficiently close in antigenicity to that of a strain which may finally succeed in becoming pandemic. Also there are production problems of such strains, leading to the use of apathogenic 'surrogate' AI virus strains, and the antigenicity and immunogenicity of such strains in an unprimed human population may be poor.

In summary, however, it is anticipated that a combination of 'suitable' IV strains and their modification by 'clever' reverse genetic manipulations will overcome some of those problems. Such developments have to go along with new testing and licensing guidelines (see below).

I.2. Implications of scientific understanding for clinical care: diagnosis; disease understanding; infection control; transmission

a) Diagnosis

The application of reverse transcription (RT)-polymerase chain reaction (PCR) technology with appropriate primers will not only complement more traditional diagnostic techniques, but also replace them in various situations (as the new 'gold standard'). On the other hand , the molecular diagnostic techniques will have to be confirmed and weighted by virus isolation in a number of situations as the presence of a piece of RT-PCR amplified cDNA does not necessarily allow the conclusion that a virus from which it is derived is the real cause of cases of disease.

b) Disease understanding

Disease understanding has been promoted in a major way by the detailed analysis of virus-host cell receptor interactions (Matrosovich *et al*, 2006), recognition of certain animals (pigs) as 'mixing vessels' (C Scholtissek; Subbarao *et al*, 2006) of co-infection/reassortant formation of IVs, but also by the detailed analysis of humoral and cellular immune responses and the mechanisms of interferon (IFN) and, more recently, differential cytokine production. In IVs and other viruses, IFN resistance genes have recently been described (Garcia-Sastre *et al*, 1998; Haller *et al*, 2006). Finally, bacterial proteases have been recognized as activators of HA cleavage (in virus/bacterium co-infected respiratory tract tissues), thus enhancing viral infectivity and possibly also pathogenicity.

c) Infection control

Infection control of nosocomial influenza outbreaks in hospitals, outbreaks in old peoples' homes, etc., is improved by the application of IV specific antivirals to potential uninfected contacts and patients in early stages of infection/disease (leading to moderate attenuation and time reduction of symptoms). Prophylactic use of NA inhibitors in the prephase of an epidemic pandemic is a rational approach in principle, but has problems in supply, logistics, identification of appropriate recipients and possibly also in efficiency (Gubareva and Hayden, 2006).

d) Transmission

Much of the research into transmission has been overlooked recently. Here, basic research can have a real impact in the short term, by identifying how the virus needs to adapt for efficient transmission from either poultry-to-human, or indeed human-to-human. Understanding the barriers that prevent this from occurring and understanding the way the virus is mutating to overcome these barriers is of utmost importance, so that strains capable of rapid and efficient transmission can be identified and appropriate measures taken.

In the short and long terms, rapid diagnosis is essential for effective patient treatment — for example treatment with drugs or appropriate quarantine, or prophylactic treatment of contacts. This become especially important when considering that there will probably never be enough anti-influenza drugs available to treat a whole population, so appropriate use of the available drugs is critical.

In the case of the recent HPAI virus H5N1 outbreaks in domestic birds in SE Asia, H7N7 in the Netherlands, and H7N3 in Canada, early diagnosis and vigorous interruption of transmission chains by exposure prophylaxis and depopulation of diseased/infected animals and close contacts have been effective in controlling and conquering the outbreaks in animals and also, as a consequence, suppressing transmission from animals to humans. The execution of such measures is in all cases very painful and has to be accompanied by establishment of a number of public health instruments (see below).

I.3. Implications of scientific understanding for strategies for preparedness for an outbreak: surveillance and modelling

Basic research can help in pandemic preparedness and outbreak control planning. Understanding transmission and the virus life cycle will help to plan exclusion or quarantine areas (in a worst case scenario). Also, knowing which mutations could rapidly lead to a more virulent or transmissible strain of virus is going to be critical for surveillance.

The close collaboration of clinician virologists, veterinary virologists and basic virologists in surveillance has lead to one of the major achievements in influenza virology. It has allowed tracking of the evolution of these viruses, their frequent genetic interaction by reassortment in animal and human hosts, and the establishment of an early warning system that has become exemplary for the surveillance of other viral zoonotic diseases.

New guidelines of the EC are at present being considered for the surveillance of AI. Such surveillance should not be restricted to the diagnosis and management of HPAI viruses but also comprise a routine 3-monthly surveillance of flocks of domestic birds above a certain size for the presence of LPAI viruses. This approach should be supported. However, it has some difficulties in detail: e.g., what are the consequences of finding LPAI viruses in a domestic flock of birds, when and to what extent should such flocks be depopulated, to what extent can producers be compensated for loss of income, should birds be vaccinated, etc. In the surveillance of domestic birds, possible contacts with potentially infected feral birds should not be underestimated, e.g. common use of water troughs into which infectious virus could be secreted in large quantities, etc.

Mathematical modelling of viral transmission and disease development under various conditions has been very successful in determining how an epidemic may spread and what the likely outcome of intervening measures might be (Anderson and May, 1991). Application of mathematical modelling to

infections with monotypic viruses (e.g. measles virus infections, epidemiology and vaccination outcome) has been very successful. In the case of IV infections and disease with multiply drifted antigenic variants and multifaceted immune responses, mathematical modelling is much more complex and difficult. However, for the assessment of the impact of a monovalent/monotypic vaccine (e.g. H5 vaccine) these procedures will have their place.

II. What lessons can be learnt from other disease outbreaks and more general public emergencies, and the associated emergency planning responses?

A major lesson is probably that an emergency 'pandemic plan' is transparent and widely known so that confusion of competences, irritation and public ignorance, etc., can be avoided and that wide understanding and acceptance of anti-epidemic measures are achieved. Experiences from the planning for preparedness to deal with events of bioterrorism (e.g. in the city of New York) point in the same direction.

It is also very important to ensure a just distribution and application of vaccines and antivirals. Surely, in case of an influenza pandemic everybody would wish to be protected optimally by antiviral treatment and vaccination. A scarcity of both, antivirals and vaccines, has lead to the conclusion that more emphasis needs to be put on exposure prophylaxis.

III. How do wider ethical, social and regulatory issues, including those associated with the development of new technologies or treatments, influence current policymaking and future preparedness?

In the case of a pandemic, disturbances of daily life will go far beyond that of the health care system. Problems will arise in the economy, trading, travelling, provision of various services, maintenance of public order, etc. Details of such consequences have been described very clearly (in part drastically) in the July/August 2005 Issue of *Foreign Affairs* (Vol 84, Number 4).

The technology of reverse genetics allows rapid production of vaccine candidate strains. For this reason and in the light of great urgency of vaccine production, currently used guidelines for vaccine production and evaluation should be reconsidered (without decreasing the significance of safety issues).

IV. How is the scientific evidence (academic, public or commercial) being incorporated into policy making?

Various areas of scientific evidence on influenza (avian and pandemic) have already been incorporated into policies: planning of vaccines (current and prospective), storage of IV-specific antivirals; comprehensive and prospective structured surveillance of humans and animals for influenza viruses (molecular epidemiology and viral evolution), etc.

However, this process could be strengthened through more interaction of the influenza community — NIBSC, HPA, IAH and VLA could be encouraged to

seek more collaboration with academia through joint meetings and exchange of personnel.

The influenza pandemic plan needs to be updated constantly and to be transparent and justifiable.

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Sources
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About the SGM
The Society for General Microbiology, founded in 1945, is an independent professional scientific body dedicated to promoting the 'art and science' of microbiology. It has now established itself as one of the two major societies in the world in its field, with some 5,500 members in the UK and abroad.

Society membership is largely from universities, research institutions, health and veterinary services, government bodies and industry. The Society has a strong international following, with 25% of membership coming from outside the UK from some 60 countries.

The Society is a 'broad church'; its members are active in a wide range of aspects of microbiology, including medical and veterinary fields, environmental, agricultural and plant microbiology, food, water and industrial microbiology. Many members have specialized expertise in fields allied to microbiology, including biochemistry, molecular biology and genetics. The Society's membership includes distinguished, internationally-recognised experts in almost all fields of microbiology.

Among its activities the Society publishes four high quality, widely-read research journals (*Microbiology*, *Journal of Medical Microbiology*, *Journal of General Virology* and *International Journal of Systematic and Evolutionary Microbiology*). It also publishes a highly respected quarterly magazine, *Microbiology Today*, of considerable general educational value. Each year the Society holds two major scientific meetings attended by up to 1500 microbiologists and covering a wide range of aspects of microbiology and virology research.

The governing Council of the SGM has a strong commitment to improving awareness of the critically important role of microbiology in many aspects of human health, wealth and welfare. It has in this connection recently initiated a 'Microbiology Awareness Campaign' aimed at providing information to the

government, decision makers, education authorities, media and the public of the major contribution of microbiology to society.

An issue of major concern to the Society is the national shortage of experienced microbiologists, particularly in the field of clinical microbiology and in industry. To attempt to improve this situation long-term, the Society runs an active educational programme focused on encouraging the teaching of microbiology in university and college courses and in the school curriculum, including primary schools. Some 400 schools are corporate members of SGM.

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