Influenza: achievements and challenges
An historical snapshot

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I have worked with influenza over a period of 40 years and, with each passing year, I have become more aware of its impact and more intrigued by the fascinating biology and natural history of the causative viruses.

Influenza wasn't my first experience in virology, having worked initially in a poliomyelitis vaccine R&D programme following the introduction of Salk vaccine into Australia by Val Bazeley. I had something of a jaundiced view when my next project was to assist in applying the virus dissociation methods of Laver & Webster [1] to the production of an improved, less reactogenic influenza vaccine. Although William Farr had alerted the medical profession as early as 1847 to the excess mortality associated with influenza outbreaks [2], like many others, I regarded influenza as little more than a winter season inconvenience.

Nevertheless, through the lectures of David White at Melbourne University and reading the works of Burnet, I appreciated that the influenza viruses had fascinating biological properties and that, for unknown reasons, there had been a dramatic and lethal pandemic in 1918-19. However, during my school years, I had lived through the next recorded pandemic of Asian influenza in 1957 with no real memory of its effects, whereas I had very clear memories of the impact of poliomyelitis, including the death of a fellow pupil and crippling of others.

Historical texts indicate that humans have suffered typical influenza-like illness through the centuries and that, on three to four occasions each century, severe, widespread, probably pandemic outbreaks have occurred [3]. But, despite William Farr's early findings, it is only relatively recently that we have begun to appreciate the very significant inter-pandemic impact of the disease seen as excess mortality, hospitalisation and economic costs [4]. In fact, it has been suggested that influenza is the singular cause of winter season excess mortality in developed countries with temperate climates [5]. Most recently, it has been shown that the impact in tropical regions is similar to that in cooler climates [6], something not previously appreciated due to its less seasonal nature, masking by other febrile illnesses and inadequate diagnosis.

Study of the causative viruses commenced with successful transmission to ferrets [7]. However, progress in understanding the natural history of influenza and attempts at its control grew from Burnet's successful isolation of the virus by amniotic inoculation and adaption to growth in the allantois of the embryonated chicken egg; this provided a means of preparing virus in quantity in the laboratory [8]. The observation by Hirst that egg-grown virus agglutinated chicken erythrocytes paved the way for the beginnings of antigenic characterisation by the haemagglutination-inhibition test still used today [9].

Although Burnet was pursuing development of a live attenuated vaccine in Australia, successful trials of vaccines containing inactivated egg-grown virus in the US armed services saw their acceptance for use by the Allied forces towards the end of World War II, prompted largely by the experience with the Spanish influenza pandemic in the closing stages of World War I [10].

However, in 1946-7, a combination of laboratory observations and vaccine failures heralded the circulation of an influenza A strain whose surface antigens differed significantly from its predecessors [11]. There was concern amongst virologists that the processes involved in the evolution of antigenically different viruses may also give rise to a strain with increased pathogenicity and pandemic potential similar to the Spanish influenza [11].

Consequently, one of the initial acts of the newly established World Health Organization was to establish an international surveillance programme to study the antigenic changes in the viruses and to provide updated strains for vaccine production [12]; this has evolved as the WHO Global Influenza Programme [13]. Australia joined this programme in 1951 with the designation of a Regional Influenza Centre at what was then the Commonwealth Serum Laboratories, a designation upgraded to Collaborating Centre in 1992 (see article by Catton in this issue) and, over the years, three Australian National Influenza Centres were designated in Melbourne, Perth and Sydney.

Early studies, particularly those of Burnet, established that there were gradual changes in the surface antigens of influenza viruses, referred to by Burnet as 'antigenic drift', and also that genetic hybridisation of influenza viruses was possible [11, 14]. Burnet's team at the Walter and Eliza Hall Institute also discovered

and characterised the enzymic activity, neuraminidase (NA), on the surface of the virus [15]. Some 3 decades later, Australian scientists succeeded in crystallising neuraminidase from an influenza virus, determining its structure by x-ray crystallography [16] and then designing [17] trialling and registering the first of a new class of drugs, the NA inhibitors, which are playing a prominent role in the pandemic preparedness plans of governments.

While the viral NA is antigenic and has an important role in viral replication, it is the minor of the two exposed surface antigens, and the viral haemagglutinin (HA) is the primary target of protective immunity [18]. Although both antigens had been found to vary, the appearance of an influenza A virus, with dramatically different HA and NA as the cause of the 1957 pandemic, subsequently referred to as an 'antigenic shift' [19], together with the progressive isolation of influenza A viruses from birds and animals, brought suspicions that the latter may be the source of new pandemic viruses [20]. This view was strengthened when the HA of the 1968-69 pandemic virus was found to be antigenically related to avian and equine influenza viruses isolated some years earlier [21]. However, the NA antigen of the Hong Kong pandemic virus appeared antigenically equivalent to that of the previously circulating human viruses which, along with other data, suggested that the pandemic virus may have been derived by genetic reassortment [22].

As the molecular structure of the virus and its genome was gradually resolved, the mechanisms of antigenic drift and antigenic shift became clear – the former due to the high mutation rate in the single stranded RNA genome and the latter, in the case of the 1957 and 1968 pandemic strains, by genetic reassortment between human and avian viruses possibly because of the segmented nature of the genome [23]. What remains to be resolved is the host species in which this occurs and what drives the change in receptor specificity from avian to human in the HA which is responsible for cell attachment. Probably the most remarkable laboratory achievement with influenza is deciphering the genome of the 1918 pandemic virus from small RNA fragments in human tissues and then reconstruction of the virus itself [24]. Surprisingly, from this has come an indication that this virus may not be a reassortant but an avian strain that simply adapted to humans by mutation [24], something that has implications with respect to the current epizootic of influenza A(H5N1) in poultry.

Despite achievements to date, there are still important gaps in our knowledge and the progress towards control of influenza has been slow. For example, we have no firm basis for predicting the pandemic potential of viruses such as H5N1 and, although there are indications that an excessive cytokine response may be involved, we still do not adequately understand the reasons for the severity of human infections by the 1918 virus and the current avian H5N1 virus [25]. Inactivated vaccines, although improved in purity and reduced in reactogenicity, are, if anything, less protective than the earliest vaccines and production still depends on embryonated eggs [26].

Of current concern is the unexpectedly poor immunogenicity in humans of A(H5N1) vaccines [27]. The use of cell culture production systems and of live attenuated vaccines have been shown feasible and have even achieved limited registration, but factors such as cost, regulatory hurdles and stability appear to have impeded replacement of the current inactivated egg-grown product. The dream of a 'universal' vaccine, effective against all influenza strains, remains just that [27]. In all probability, current vaccines, with their limitations and limited global production capacity, would eventually have only limited impact on a pandemic in the near future, as will available supplies of antivirals.

References

1. Webster RG & Laver WG. Influenza virus subunit vaccines: immunogenicity and lack of toxicity for rabbits of ether- and detergent-disrupted virus. *J Immunol* 1966; 96:596-605.
2. Farr W. Deaths. In: *Vital Statistics: A Memorial Volume of Selections from the Reports and Writings of William Farr.* The Scarecrow Press Inc. Metuchen, NJ 1975, p.330-3.
3. Potter CW. A history of influenza. *J Appl Microbiol* 2001; 91:572-9.
4. Sullivan KM. Health impact of influenza in the United States. In: Hannoun C & Crampton R (Eds). *PharmacoEconomics Supplement 3.* Adis International Ltd Auckland 1996, p.26-33.
5. Reichert TA & Sharma A. The seasonality of human mortality: the role of influenza. In: Osterhaus ADME, Cox N & Hampson AW (Eds). *Options for the Control of Influenza IV.* Elsevier Science BV Amsterdam 2001, p.95-101.
6. Chow A, Ma S, Ling AE & Chew SK. Influenza-associated deaths in tropical Singapore. *Emerg Infect Dis* 2006; 12:114-21.
7. Smith W & Andrewes CH. A virus obtained from influenza patients. *Lancet* 1933; ii:66-8.
8. Burnet FM. Growth of influenza virus in the allantoic cavity of the chick embryo. *Aust J Exp Biol Med Sci* 1941; 19:291-5.
9. Hirst GK. The quantitative determination of influenza virus and antibodies by means of red cell agglutination. *J Exp Med* 1942; 75:49-64.
10. Dowdle WR. Influenza pandemic periodicity, virus recycling and the art of risk assessment. *Emerg Infect Dis* 2006; 12:34-9.
11. Burnet FM. The influence of host immunity on the virus: immunological drift. In: *Principles of Animal Virology.* New York: Academic Press Inc. 1955, p.380-403.
12. Payne AM. The influenza programme of WHO. *Bull World Hlth Organ* 1953; 8:755-74.
13. Stohr K. Overview of the WHO Global Influenza Programme. *Dev Biol (Basel)* 2003; 115:3-8.
14. Burnet FM & Lind PE. Recombination of characters between two influenza strains. *Aust J Sci 1949;* 12:109-10.
15. Gottschalk A. Neuraminidase: its substrate and mode of action. *Adv Enzymol* 1958; 20:135-46.
16. Varghese JN, Laver WG & Colman PM. Structure of the influenza virus glycoprotein antigen neuraminidase at 2.9Å resolution. *Nature* 1983; 303:35-40.
17. von Itzstein M, Wu WY, Kok GB, Pegg MS, Dyason JC, Jin B, Van Phan T, Smythe ML, White HF & Oliver SW. Rational design of potent sialidase-based inhibitors of influenza virus replication. *Nature* 1993; 363:418-23.
18. Couch RB. An overview of serum antibody responses to influenza virus antigens. *Dev Biol (Basel)* 2003; 115:25-30.
19. Dowdle WR & Schild GC. Influenza: its antigenic variation and ecology. *Bull Pan Am Health Organ* 1976; 10:193-5.
20. Andrewes CH & Worthington G. Some new or little-known respiratory viruses. *Bull World Health Organ* 1959; 20:435-43.
21. Laver WG & Webster RG. Studies on the origin of pandemic influenza. 3. Evidence implicating duck and equine influenza viruses as possible progenitors of the Hong Kong strain of human influenza. *Virology* 1973; 51:383-91.
22. Webster RG. On the origin of pandemic influenza viruses. *Curr Top Microbiol Immunol* 1972; 59:75-105.
23. Zambon MC. Epidemiology and pathogenesis of influenza. *J Antimicrob Chemother* 1999; 44 Suppl B:3-9.
24. Tumpey TM *et al.* Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science* 2005; 310:77-80.
25. Peiris JSM, Yu WC, Leung CW, Cheung CY, Ng WF, Nicholls JM, Ng TK, Chan KH, Lai ST & Lim WL. Re-emergence of fatal human influenza A subtype H5N1 disease. *Lancet* 2004; 363:617-9.
26. Hilleman M. Realities and enigmas of human viral influenza: pathogenesis, epidemiology and control. *Vaccine* 2002; 20:3068
27. Hampson AW. Ferrets and the challenges of H5N1 vaccine formulation. *J Infect Dis* 2006; 194:143-5.