A universal influenza vaccine – are we almost there?

Alan W Hampson

Honorary Senior Research Fellow
Monash University, VIC
Chairman, Influenza Specialist Group

Seasonal influenza has a significant health care and economic impact and pandemic influenza is believed to have the potential to result in a global catastrophe[1]. Regardless of developments with antivirals, vaccination remains the most effective option for limiting the impact of both seasonal and pandemic influenza[2-4].

Current inactivated virus vaccines, while useful against seasonal influenza, have serious deficiencies. They induce immunity that is largely dependent on circulating antibody directed to the exposed highly variable head of the major surface protein the haemagglutinin (HA) and, to a lesser extent a second surface protein the neuraminidase (NA)[5]. Ongoing antigenic changes in these molecules ('antigenic drift'[6]) require vaccine reformulation and administration on essentially an annual basis with the potential for vaccine mismatch[7]. These vaccines have reduced effectiveness in older adults, a major risk group, possibly due to immunosenescence[8]. Vaccine production has a lead time of 4–6 months[9] and global production capacity is limited[4], which restricts the ability to respond to antigenic drift changes and to the emergence of a pandemic[10,11].

Living vaccines, adjuvants such as the squalene-based oil in water formulations MF59[5] and AS03$_A$[12] and new delivery systems including intradermal injectors[13], all of which are currently licensed, broaden and strengthen the immune response and may reduce the effect of antigenic drift, and of immunosenescence[5,10]. In addition, new vaccine production approaches, which do not rely on embryonated chicken eggs, the vaccine substrate used for over 60 years, may help to reduce response times[14,15]. Nevertheless, there is great appeal in the concept of a 'universal' influenza vaccine that would protect against all influenza viruses particularly all influenza A subtypes and strains. Research has been stimulated recently by the perceived threat of an H5N1 pandemic, plus some encouraging findings and these have prompted enthusiastic media reports that "The vaccine that will protect you against EVERY strain is just years away"[16]. Is this a true reflection of current progress?

The best correlate of protection against influenza in humans is circulating antibody against the HA antigen measured by haemagglutination-inhibition[17,18] which is used by regulators as a measure of effectiveness for inactivated virus vaccines[19]. However, immunity acquired by infection and by vaccination with living attenuated virus remains incompletely defined, and clearly includes other components including mucosal IgA and cell-mediated immunity (CMI)[20-22]. Most *in vivo* studies on influenza immunity, immunogens and delivery systems are performed in laboratory mice, which are not a natural host for influenza. The clinical pathology differs from that in humans[23], death is often the endpoint and mice readily demonstrate a level of heterotypic immunity following infection with influenza or immunisation with attenuated and inactivated vaccines which is not observed in humans[5]. Hence, results obtained in mice should be viewed with caution[24].

There is only meagre evidence that humans naturally mount a heterotypic response to infection with influenza. Retrospective analysis of data from the 1957 pandemic[25] provided suggestive evidence for heterotypic immunity as did analysis of school outbreaks of H1N1 and H3N2 influenza[26]. The replacement of the previously circulating subtype by the 1957 and 1968 pandemic viruses is also suggestive of, at least, a short-lived heterotypic immunity[27].

The ferret is naturally susceptible to influenza, largely reproduces the characteristics of human disease and is considered a more appropriate model. However, it is not widely used because of difficulties in availability, caging and handling and the absence of immunological reagents to analyse the response to vaccination or infection. Some heterotypic, antibody-independent protection has also been reported in the ferret[28,29], but it may be that this reproduces the weak heterotypic protection in immunologically primed humans[5,29]. Other animals where human-like infection occurs, pigs and non-human primates, are generally impractical for experimentation.

Targets for a universal influenza vaccine are, by definition, highly conserved or invariant immunogenic sequences of the viral proteins. As suggested in one recent publication[30], to be effective these will require generation of 'unnatural immunity' – a better, or differently directed response than that created by infection. T-cell epitopes, principally within the internal proteins of the virus, have been considered as the principal targets for heterotypic cell-mediated responses[31-33] and many studies have demonstrated that such responses can protect mice against challenge. Limited experiments in ferrets have, however, shown somewhat mixed and less optimistic results, particularly when an H5N1 challenge is employed[34-36]. Recently the first clinical trial of a vaccine solely targeting T-cell epitopes was reported[37]. Two internal influenza antigens delivered in a modified vaccinia virus vector induced a largely $CD8^+$ response and offers proof of principle that the boosting of influenza T-cell immunity in humans is possible. Clearly, T-cell immunity is an important adjunct to specific B-cell immunity and the establishment of immune memory, which may be important in the elderly[22]. Nevertheless, there is a need to better understand the determinants and correlates of this immunity if it is to be developed as a vaccination strategy in its own right[31-33].

Surprisingly, development of antibody-mediated universal vaccination appears further advanced than T-cell immunity. One

target that has been the subject of extensive study is the highly conserved 24 amino acid ectodomain (M2e) of the third influenza A integral membrane protein. Several candidate vaccines have been developed based on a variety of constructs and some have entered clinical trials. In mice M2e vaccination ameliorates rather than prevents infection and M2e antibodies do not neutralise the virus but act via antibody-dependent complement or cell-mediated cytotoxicity[38-40]. Although pharmaceutical company data refer to successful completion of Phase I clinical trials[40-42], and to successful *in vivo* testing in ferrets[42], a search of the current literature fails to reveal relevant peer-reviewed publications to date.

While earlier monoclonal antibody studies indicated that there are neutralising epitopes shared widely by influenza A viruses[43,44], only recently have these emerged as serious vaccine candidates[45-47]. Antibodies to epitopes within the stem of the HA interfere with the critical membrane fusion process that occurs during infection. The 16 influenza A subtypes fall into two phylogenetically distinct groups and monoclonal antibodies raised in one of the studies[46] inhibit only Group 1 viruses, which contains 10 of the subtypes, including H1, H2 and H5, but not the H3 subtype that is frequently responsible for epidemic mortality. Another study involved polyclonal antibody raised to HA molecules engineered to lack the highly immunogenic globular head[47] and the results of the limited cross-protection studied suggested a similar restriction to Group 1 viruses. Differences between the HA stem region of Group 1 and Group 2 viruses include a potential glycosylation site that may mask the relevant epitope and it has been suggested that this may explain the reason that antibodies are HA group-specific[46]. Nevertheless, it has recently been reported that antibodies raised to an H3 (Group 2) stem peptide also demonstrated some protection against H1 (Group 1) viruses in mice[48]. A more broadly reactive antibody raised to the conserved N-terminal region of the HA fusion peptide, rather than the HA stem, provides partial neutralisation and partial inhibition of the fusion activity of all influenza A and B viruses tested[49].

However, as yet it seems that no target has been identified which could be claimed unequivocally to both cover all subtypes and provide solid protection against infection. For those candidate vaccines that do go forward in clinical trial there will be substantial hurdles. One will be how to ethically demonstrate at least equivalence to existing vaccines in risk groups such as the elderly, another will be whether effectiveness against a new subtype in a population lacking any priming immunity to related HA can actually be assessed until such an event occurs. In conclusion – no, we are not nearly there; there are indications that a universal vaccine may be feasible but much remains to be done. The most practical prospect in the immediate future may be to produce improved more broadly protective vaccines by combining proposed universal targets with strain-specific antigens and improved methods of delivery.

Biography

A virologist with over 40 years' experience working with influenza, Alan's career includes responsibility for developing influenza vaccine production processes and his role as Deputy Director and operational head of the WHO Collaborating Centre for Reference and Research on influenza, Melbourne, from its designation in 1992 until his retirement in September 2005. Since retirement he has maintained an active role as a consultant, to the government and WHO, as Chairman of the Australian Influenza Specialist Group (ISG) and as Editor in Chief of a new international journal *Influenza and Other Respiratory Viruses*. Alan was awarded an AOM and an Honorary Doctorate of Medicine for his public health contributions on influenza.

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