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Review Article

Advances in the development of universal influenza vaccines

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Despite the widespread availability and use of influenza vaccines, influenza still poses a considerable threat to public health. Vaccines against seasonal influenza do not offer protection against pandemic viruses, and vaccine efficacy against seasonal viruses is reduced in seasons when the vaccine composition is not a good match for the predominant circulating viruses. Vaccine efficacy is also reduced in older adults, who are one of the main target groups for vaccination. The continual threat of pandemic influenza, with the known potential for rapid spread around the world and high mortality rates, has prompted researchers to develop a number of novel approaches to providing immunity to this virus, focusing on target antigens which are highly conserved between different influenza A virus subtypes. Several of these have now been taken into clinical development, and this review discusses the progress that has been made, as well as considering the requirements for licensing these new vaccines and how they might be used in the future.

Keywords Clinical, influenza, vaccine.

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Introduction

The word 'universal' has two meanings in the context of influenza vaccines; vaccines that protect against all influenza viruses, and the vaccination of the entire population against influenza. This review will cover primarily the former, but will also discuss how these new vaccines could be used in vaccination policies of the future.

Currently licensed seasonal influenza vaccines, whether inactivated or live attenuated, split or whole virion, adjuvanted or not, induce antibodies against the highly polymorphic head of the viral haemagglutinin (HA). As the proportion of the human population with effective antibodies to the HA of the circulating virus increases following infection and recovery, or vaccination, variants of the virus capable of escaping this immunity by virtue of mutated HA sequences that either change the protein sequence or shield it by glycosylation are selected, resulting in continual antigenic drift of the circulating viruses. The immunodominant antibody responses induced by vaccination are in most cases highly specific for the HA molecules that were included in the vaccine, and when there is a significant mismatch between the vaccine and circulating virus, the vaccine efficacy is markedly reduced.[1] Periodic Influenza A pandemics occur when a virus of a new subtype infects humans and is transmissible resulting in rapid spread of the new virus to multiple geographic locations. The absence of antibodies specific for the pandemic HA results in an increased number of susceptible individuals, and high numbers of human infections occur until after a few years the majority of the population has been exposed and immune selection pressure again results in antigenic drift of the pandemic virus, which then becomes the current seasonal influenza virus. Current vaccine formulations require the precise HA sequence of the circulating virus to be known to produce the vaccine, resulting in a lag of several months before large numbers of doses of a new vaccine can be produced once a new pandemic virus has been identified. The realization from 2004 onwards that H5N1 viruses were repeatedly causing infections in humans, despite the fact that human-to-human transmission had only been observed in rare cases involving extremely high exposure, highlighted our susceptibility to influenza A pandemics, and resulted in new approaches to influenza vaccine development being undertaken. Three main strategies employing conserved regions of the influenza virus as antigens have emerged as potential solutions, and these will be reviewed below.

Animal models for testing candidate influenza vaccines

Although this review will focus on data from clinical trials, all of the vaccines discussed will have been tested in pre-clinical animal models prior to initiating clinical studies. The species used are most commonly mice, ferrets and macaques, as reviewed by Bodewes *et al.*[2] In mice, the pathogenesis of the infection does not resemble that of humans, and viruses for challenge experiments have to be adapted to infect mice. There are limited T cell reagents available for use in ferrets, which also appear to be highly susceptible to influenza A virus infection, perhaps more so than humans, and supply of sufficient quantities of animals can be a limiting factor in planning experiments. The immune system of a macaque is very similar to that of a human, and experimental data obtained in this model is a better predictor of the outcome in humans, but the cost of conducting experiments in an ethically approved manner, particularly when high-level containment is required for virus challenge, prevents the model from being used extensively. Pigs have also been used to test influenza vaccines[3] and have the advantage that there is no shortage of supply, reagents are available for T cell analysis, and they can be infected with viruses of many different subtypes as both α-2,3- and α-2,6-galactose sialic acid linkages are present on cells lining the pig trachea,[4] which provides an opportunity to study heterosubtypic protection induced by vaccination. A recent comparison of pandemic H1N1 vaccines in pigs[5] produced data that were in close agreement with a similar study in humans,[6] providing further support for the greater use of this model in future. However all of these models have the disadvantage that they cannot mimic the complex immune memory to influenza A virus found in humans after a lifetime of repeated exposures, and the results of experimental studies must be interpreted with this in mind.

Anti-M2e antibodies

The M2 protein forms a proton-selective ion channel which plays an important role in virus morphogenesis and assembly. It consists of only 96 amino acid residues, of which 23 are present on the surface of the virion, and only five of these exhibit any significant degree of polymorphism.[7] Although present in low abundance on the surface of the virion, M2 is also found on the surface of virus-infected cells, with approximately twice as many M2 molecules than HA molecules reported. Anti-M2e antibodies are not found following influenza infection, but can be induced by vaccination. These antibodies are not virus neutralizing, and most likely act via antigen-dependent cell cytotoxicity or complement-dependent cytotoxicity.[8] Various strategies have been employed to increase the immunogenicity of M2e vaccines in animal models and a number of studies have reported partial protection against lethal challenge and decreased viral shedding following induction of anti-M2e antibodies in mice.[9–11] In pigs, contrasting results have reported either exacerbation of disease following influenza challenge,[12] or partial protection with reduction in macroscopic lung lesions, but no reduction in virus shedding.[13]

Clinical trials have been undertaken by Sanofi Pasteur using the vaccine ACAM-FLU-A, which was found to be well tolerated, with no serious side effects. Antibody responses to M2e were induced in the majority of subjects. VaxInnate has also completed a Phase I trial of M2e fused to flagellin, again demonstrating immunogenicity with IgG specific for M2e detected in 96% of subjects after the second dose and acceptable vaccine safety.[14] Other M2e based vaccines are also in development, but as yet no clinical efficacy studies have been reported.[15] Thus, it has been demonstrated that although antibodies specific for M2e are not part of the response to influenza A virus infection in humans, it is possible to induce them by vaccination. It is still not known whether these antibodies will recognize all influenza A subtypes, if they can contribute to protection against disease following infection, what the mechanism of that protection might be and what antibody titre would be required to achieve a useful level of protection.

T cell responses to conserved antigens

In contrast to HA, the internal antigens of influenza viruses are very highly conserved across all subtypes and strains of influenza A. Nucleoprotein (NP) and matrix protein 1 (M1) are abundantly expressed in virus-infected cells,[16] and effector T cells capable of recognizing peptides derived from these antigens that are presented by major histocompatibility complex (MHC) molecules on the surface of virus-infected cells can kill the virus-infected cells, preventing further spread of the infection within the host. Several large epidemiological studies have provided evidence that recent infection with seasonal influenza reduces the risk of disease caused by pandemic virus. Analysis of the Cleveland family study found that infection with H1N1 prior to 1957 reduced the risk of infection with H2N2 at the start of the 1957 pandemic.[17] A new analysis of susceptibility to infection in the 1918 pandemic concludes that recent infection with a seasonal influenza virus provided heterosubtypic immunity to the pandemic virus,[18] and in the 2009 pandemic, recent seasonal influenza virus infection was associated with protection from infection with pandemic virus whereas recent vaccination against seasonal influenza increased susceptibility to pandemic virus.[19]

This heterosubtypic protection is mediated through T cells (either $CD4^+$ or $CD8^+$, or both) specific for antigens that are conserved between seasonal and pandemic viruses, rather than by antibodies to external antigens which differ between viral subtypes. A study in which volunteers were inoculated with live influenza virus demonstrated that those with cytotoxic T cell responses detected by lysis assays cleared influenza virus effectively and exhibited reduced virus shedding, even in the absence of antibodies specific for the HA of the influenza challenge virus.[20] Antibodies are undoubtedly the primary protective mechanism when the antibody specificity is a perfect match for the HA of the infecting virus, but vaccine failures occur approximately 1 out of 20 years, when the vaccine does not match the circulating viruses, demonstrating that it is not sufficient to have antibodies recognizing a particular influenza subtype (H1 or H3 for example), but that the antibodies must be specific for the precise variant of the subtype.[21] When these are not present and influenza virus is encountered, a strong T cell response can act rapidly to prevent spread of the infection, resulting in some cases in a completely asymptomatic infection with no viral shedding.

A more recent study of influenza challenge in healthy subjects who were all seronegative (defined as having an HI titre <10) for the challenge virus (either H3N2 or H1N1) at the time of infection found that pre-existing $CD4^+$ T cells rather than $CD8^+$ T cells specific for two internal antigens, NP and matrix protein 1 (M1) correlated with disease protection.[22] T cell responses were measured in interferon-γ ELISpot assays using pools of 18-mer peptides spanning the antigens of interest, in which either $CD4^+$ or $CD8^+$ cells were depleted prior to setting up the assay. The strength of the $CD4^+$ T cell response to NP and M1 showed a significant negative correlation with both the total symptom scores and the duration of illness for the nine volunteers infected with H1N1, and additionally with virus shedding for the 14 volunteers infected with H3N2, whereas the correlation was weaker when total T cell responses to NP and M1 were considered. However, the numbers in the study were small, and for the H3N2 group $CD4^+$ and $CD8^+$ T cell responses against these two antigens were very similar in magnitude (56% $CD4^+$ versus 44% $CD8^+$). Whereas $CD8^+$ T cells are believed to act directly on virus-infected cells, in other virus infections the role of $CD4^+$ T cells is thought to be in priming or maintaining the $CD8^+$ T cell response[23] or in recruitment of CD8+ T cells to the site of infection.[24] However, $CD4^+$ T cells may also act directly as antiviral cytotoxic cells, and Wilkinson *et al.* demonstrated that $CD4^+$ T cells taken from the volunteers in the human challenge study were cytotoxic and employed the perforin-granzyme pathway. If cytotoxic $CD4^+$ T cells are to have a protective effect following human influenza virus infection, it will require the expression of MHC class II on the respiratory epithelium, and substantial expression was demonstrated on explanted human lung tissue and cultured primary human bronchial epithelial cells,[22] supporting the hypothesis that cytotoxic $CD4^+$ T cells recognizing conserved influenza antigens may act directly to contain the spread of influenza A virus in the human respiratory tract. Earlier studies that measured cytotoxic T cell responses to influenza A virus in lysis assays[20,25,26] would therefore have detected both $CD4^+$ and $CD8^+$ responses.

The high degree of conservation of internal antigens such as NP and M1 across all influenza A virus subtypes allows T cells that were primed by infection with one viral subtype to recognize and kill cells infected with virus of a different subtype, resulting in the heterosubtypic immunity that is not conferred by antibodies to the polymorphic regions of HA. Lee *et al.*[27] demonstrated that blood donors in the UK had memory T cells that were capable of recognizing NP and M1, and to a lesser extent other internal antigens from H5N1 influenza virus. However the half-life of the T cell response has been calculated to be only 2–3 years.[26] The median T cell response to influenza in the population correlates with the number of influenza infections at that time, and decreases in influenza seasons when the number of influenza cases is low.

This short-lived period of effective T cell-mediated protection against influenza disease can affect the progression of an influenza pandemic in ways which have only recently begun to be understood. At the start of a new pandemic with a novel influenza subtype, some individuals have immunity which is capable of preventing symptoms of influenza infection from developing following infection with the new virus, although they may experience subclinical infections. New analysis of the progress of the 1918 pandemic[18] highlights the fact that many of those living in urban environments were apparently unaffected, despite having had a higher likelihood of exposure than those in isolated communities. The most likely mechanism for this is that they had recently been exposed to the former seasonal influenza virus and had sufficient T cell immunity to prevent disease occurring after exposure to the pandemic virus. The fact that influenza pandemics occur in waves, rather than infecting the whole population at the first exposure may be explained by the short-lived nature of the heterosubtypic immunity, with waning immunity in some of those who escaped illness in the first wave resulting in susceptibility to the second wave despite little antigenic drift occurring. Furthermore, adults had higher rates of pre-existing immunity than the young, and this immunity was better maintained. This may be a consequence of repeated exposures to influenza virus throughout life gradually modifying the T cell memory to this acute viral infection, with each subsequent encounter.

The aim of boosting heterosubtypic T cell responses to conserved influenza antigens by vaccination underlies the development of a number of novel universal influenza vaccines. The first of these to enter clinical development was Modified Vaccinia virus Ankara (MVA)-NP + M1, using the replication-deficient poxvirus vector MVA to express the NP and M1 of influenza A. MVA as a vaccine vector has been tested in many clinical trials of novel vaccines against malaria, tuberculosis and HIV.[28] It has been found to have an excellent safety profile in all sections of the population from children[29] to the elderly (Richard D. Antrobus, Patrick J. Lillie, Tamara K. Berthoud, Alexandra J. Spencer, James E. McLaren, Kristin Ladell, Teresa Lambe, Anita Milicic, David A. Price, Adrian V. S. Hill and Sarah C. Gilbert; unpublished data.), and is highly effective at boosting T cell responses however they were first acquired. In a first Phase I study demonstrating safety and immunogenicity in healthy young adults,[30] the T cell response to NP and M1 was found to be predominantly $CD8^+$ prior to vaccination and the CD4:CD8 ratio was not altered by vaccination. A subsequent Phase IIa influenza challenge study then provided the first demonstration of efficacy of a vaccine designed to boost T cell responses to influenza, with a significant reduction in duration of viral shedding in the vaccinated group and also a reduction in the numbers of subjects experiencing symptoms of influenza virus infection.[31] The NP and M1 sequences in MVA-NP + M1 are derived from an H3N2 virus, and the challenge was performed with a virus of the same subtype. A further study has examined the safety and immunogenicity of the vaccine in older adults, demonstrating remarkable immunogenicity even in those aged over 70 years (Antrobus, submitted). Indeed, the large increases in the number of T cells recognizing NP and M1 following a single vaccination with MVA-NP + M1 are a notable feature of these clinical studies, with a >10-fold increase (mean of all subjects) in T cell response to the influenza antigens at the highest dose tested.[30]

Other T cell boosting vaccines are also in development, with BiondVax, SEEK, Immune Targeting Systems and Bionor Pharma all employing peptide or protein-based vaccinations to increase T cell responses to influenza. Vical has tested a trivalent DNA vaccine formulation, in which the three plasmids express H5 HA, NP and M2. T cell ($CD4^+$ and $CD8^+$ combined) responses to NP were assessed by interferon-γ ELISpot assay, and a threefold increase following vaccination was recorded in between 20% and 60% of subjects.[32] BiondVax is developing Multimeric-001, a protein consisting of conserved regions of the virus (including five linear epitopes from HA, three from NP and one from M1 of influenza A and B) which is produced in *Escherichia coli* and administered with Montanide ISA 51VG adjuvant. Clinical trials have been completed in younger and older adults with good safety. IgG titres against the vaccine were increased by up to 50-fold, and cellular responses were assessed by proliferation of peripheral blood mononuclear cells (PBMCs) from donors with up to 90% of subjects demonstrating a twofold increase in proliferation following vaccination.[33] SEEK have produced a synthetic multiepitope vaccine FLU-V which is administered with Montanide ISA 51 adjuvant,[34] and has been tested in Phase I and Phase IIa clinical trials. The vaccine consists of an equimolar mixture of four peptides encoding regions from NP, M1 and M2. Immune response was assessed by measuring interferon-γ in the supernatant of PBMCs from vaccinees following incubation with the four peptides, with all vaccinees in the high-dose group demonstrating a twofold increase over the pre-vaccination response.[35] Immune Targeting Systems have produced a synthetic nanoparticle vaccine FP01 consisting of six peptides each conjugated to a fluorocarbon molecule which is now in clinical testing. Bionor Pharma also has a peptide-based influenza vaccine based on conserved regions of influenza antigens in development.[15]

There is still much work to be done in defining the phenotype of protective T cells and determining the duration of immunity induced by vaccination, as naturally acquired T cell-mediated immunity to influenza is short lived. However, the known protective effect of T cell responses acquired by influenza virus infection and the potential to protect against all influenza A viruses with a single vaccine makes this an extremely important area of vaccine development.

Heterosubtypic anti-HA antibodies

Although the humoral response to influenza HA is generally highly subtype specific, in recent years human antibodies that recognize a large number of subtypes have been identified. In 2009, Ekiert *et al.*[36] reported the isolation of antibody CR6261, which recognizes a highly conserved region in the stem region of HA, and can neutralize influenza virus by preventing membrane fusion. This is contrast to the majority of anti-HA antibodies which bind to hypervariable regions around the receptor binding site and prevent binding of the virus to host cells. CR6261 is able to bind to most group 1 HAs, including H1, H2 and H5. This was followed by the isolation of CR8020 which is capable of neutralizing most group 2 HAs including H3 and H7.[37] Corti *et al.*[38] reported the isolation of an antibody capable of binding all group 1 and group 2 HAs. These antibodies, used singly or as a cocktail of monoclonal antibodies, could be used to provide passive immunity in cases of severe influenza, providing a new therapeutic opportunity. Although isolated from human blood samples, these broadly neutralizing anti-stem antibodies appear to constitute a very minor component of the human immune response to influenza. However following the 2009

influenza pandemic, it was demonstrated that the anti-HA response was dominated by broadly neutralizing antibodies, raising the possibility that with the right immunogen design, this type of antibody could be induced to protective levels by vaccination.[39] There was evidence of extensive affinity maturation suggesting that these antibodies were produced after multiple exposures to antigen, and that it may be necessary to employ a complex multi-stage vaccination protocol to achieve broadly neutralizing antibodies.

The structure of HA and binding sites of broadly cross-neutralizing antibodies has been reviewed by Nabel and Fauci.[40] In pre-clinical studies, vaccination with plasmid DNA encoding HA followed by boosting with homologous inactivated influenza vaccine resulted in broadly neutralizing antibodies, including stem-specific antibodies that were protective against infection in mice and ferrets.[41] Broadly neutralizing antibodies were also induced in non-human primates using the same regime.[41] Steel *et al.*[42], have designed a novel vaccine based on the stem of HA without the globular head, which can be produced as protein in HEK293 cells. Mice vaccinated with this construct produced broadly neutralizing antibodies and were protected against lethal influenza virus challenge. A recombinant protein consisting chiefly of the HA2 portion of HA produced in *E. coli* and refolded is highly immunogenic in mice. Antibodies induced by vaccination were protective against homologous challenge, and exhibited cross-strain protection within the H3 subtype, but were not protective against H1 challenge.[43]

The approach of DNA priming and inactivated influenza vaccine boosting using H5 monovalent inactivated vaccine (MIV) has now been tested in clinical trials.[44] The regime resulted in increased humoral responses to H5 HA compared with two doses of MIV alone. Anti-stem antibodies were induced, which in some cases were capable of neutralizing a distinct H5 virus and an H9 virus. This provides evidence that broadly neutralizing anti-stem antibodies can be induced in humans by vaccination, and that the induction of increased helper T cell responses following the DNA vaccination may underlie the increased breadth of humoral responses. However, it is by no means certain that a neutralizing antibody response of a sufficiently broad specificity and titre can be induced in all humans by vaccination. It may be more realistic to aim to induce broadly neutralizing antibodies rather than universally neutralizing antibodies. A human monoclonal antibody recognizing a conserved epitope on the globular head of the majority of H1N1 viruses has been identified.[45] The use of an adjuvant with trivalent inactivated vaccine (TIV) or viral-vectored delivery of HA[41,46,47] also results in greater cross-reactivity than immunization with inactivated virus or recombinant protein alone, and these approaches have the potential to improve protective immunity against drifted variants of the same subtype at least, with the possibility for some cross-subtype neutralization.

What do we expect from a universal vaccine?

Having reviewed the different approaches that are being followed with the aim of developing a universal influenza vaccine, it is useful to consider what we expect a universal influenza vaccine to achieve. Will it be a 'one shot for life' vaccine given in infancy? Will it be a vaccine to be stockpiled in case of a pandemic rather than used to prevent seasonal influenza infections? Or a vaccination given to the whole population every year with efficacy against seasonal influenza at least as high as the currently licensed vaccines, but the same level of efficacy against drifted seasonal variants and pandemic viruses. If the latter, and the vaccine was used worldwide, the resulting immunity could prevent any new pandemic from occurring as the number of 'susceptibles' in the population would be very low. This could achieve containment of disease caused by Influenza A, although the continued presence of large reservoirs of the virus in avian species will require the rate of vaccination to be maintained continually.[48]

Although we tend to categorize people as 'susceptible' or 'immune' to influenza, in reality there are more possible outcomes of exposure to influenza virus than either no illness or severe illness/death. The possible outcomes and the immune mechanisms that are thought to be responsible for them are shown in Table 1. It is also necessary to consider how influenza vaccines are tested for efficacy. In Phase IIa, or controlled challenge studies, healthy individuals aged 18–45 years with low haemagglutinin inhibition (HI) titres to the challenge virus receive intranasal inoculation of the challenge virus while housed in a quarantine unit. Twice daily symptom questionnaires and daily nasal washes for virus quantification maximize the chances of detecting 'laboratory-confirmed influenza', which in this population is generally a very mild illness. Due to the unpredictable and sometimes low rate of infection of unvaccinated subjects, a control group of the same size as the vaccinated group must be included and it may be necessary to repeat the study in multiple cohorts of volunteers to achieve a statistically significant estimate of vaccine efficacy. It is essential to have the control group challenged with the vaccinated group rather than using data from a historical set of control subjects, as the reasons for the rate of infection in the control group are still not well understood and may be affected by the strain and prevalence of the seasonal viruses circulating in the months prior to the challenge.

Phase IIb or field efficacy studies require several 100 or 1000 people to be recruited at the beginning of the influenza season, with half of them receiving the vaccine under

Table 1. Possible outcomes of human interactions with influenza A

Outcome	Virus shedding and likelihood of onwards transmission	Immune mechanism responsible for protection	Scored as lab-confirmed flu? (symptoms and virus shedding)
1 No exposure	None	Can only be achieved by non-pharmaceutical interventions such as masks, mobility restriction	No
2 No infection	None	High-titre neutralizing antibodies (NAb) to the circulating virus	No
3 Asymptomatic infection	None or very low	Lower NAb titre, or protective T cell response, possibly anti-M2e antibodies	No
4 Mild illness: 'a cold' or 'man flu'	Moderate to high	Insufficient pre-existing immunity to prevent disease, but rapid increase in NAb and T cells to prevent spread of infection resulting from expansion of immune memory	Yes in quarantined challenge study, possibly in field study
5 Severe illness: 'the flu'	High	Insufficient pre-existing immunity to prevent disease, lack of appropriate immune memory to rapidly control spread of infection	Yes
6 Serious illness requiring hospitalization	High	Insufficient pre-existing immunity to prevent disease, lack of appropriate immune memory to rapidly control spread of infection, immunodeficiency from any cause, secondary bacterial infection	Yes
7 Death	High	Insufficient pre-existing immunity to prevent disease, lack of appropriate immune memory to rapidly control spread of infection, immunodeficiency from any cause, secondary bacterial infection	Yes

test, and the other half receiving placebo, or TIV as a comparator, as has been done for some studies of live attenuated influenza vaccine.[49] Follow-up consists of weekly monitoring telephone calls or web-based questionnaires to capture information on influenza-like symptoms, plus use of nasal swabs to sample virus when symptoms are present. This requires participants to remember to report all possible influenza symptoms for several months, take swabs correctly when indicated and provide them for virus detection. Thus, Phase IIa studies will capture all instances of mild disease, but provide no information about more severe illness and can only be used in a the age range least likely to suffer severe disease. Phase IIb studies will miss some cases of mild disease but can include a much wider age range, and if sufficiently large may be able to indicate vaccine efficacy against severe disease.

Phase IIa studies can only determine efficacy at a given time point following vaccination, which is usually only a few weeks. Phase IIb studies collect information for a whole influenza season, and may be extended to a second season. Virus isolation allows an assessment of efficacy against both strains that are antigenically similar to the vaccine and drifted variants to be assessed.[49]

Either of these approaches may be used to test the efficacy of novel influenza vaccines, but only against seasonal influenza. Efficacy testing against virus subtypes other than H1N1 and H3N2 can only be conducted in animal models, or using functional *in vitro* tests for neutralizing antibodies and cytotoxic T cells to predict vaccine efficacy against pandemic viruses. As novel 'universal' influenza vaccines can only be fully tested for efficacy against mild or possibly severe seasonal influenza in humans, if the efficacy is sufficient to recommend their use, they could then be used in place of the current seasonal vaccines.

Vaccines for all

It should not be forgotten that the number of cases of severe influenza disease and death in different age groups is affected more by naturally acquired immunity than either exposure to the virus, or vaccination, with the majority of deaths from seasonal influenza occurring in the very young or the elderly. Any form of immunosuppression, including pregnancy and obesity, increases the probability of severe illness,[50] and in the elderly, currently licensed vaccines are considered to have low efficacy, although robust evidence is lacking.[51] Repeated use of TIV in influenza-naïve individuals prevents the acquisition of heterosubtypic T cell immunity.[52] In animal models, the heterosubtypic immunity acquired following virus infection is partially protective against infections with influenza viruses of other subtypes, and acquisition of heterosubtypic immunity is prevented by use of TIV or whole inactivated virus vaccines.[53–55] The ideal influenza vaccine for infants or young children would

therefore be designed to prime broad immunity, either cytotoxic T cell or neutralizing antibody mediated (but preferably both), paving the way for further development of the immune memory at each subsequent encounter with influenza virus rather than providing sterilizing immunity. For older children and adults, this broad immunity would be boosted by periodic vaccination with a different vaccine which may contain both 'universal' and 'seasonal' components. For example, use of MVA-NP + M1 co-administered with TIV results in broadly cross-reactive T cell responses to NP and M1 as well as high-titre antibodies specific to the HA components of TIV (Figure 1, and Caitlin E. Mullarkey, Arjan van Laarhoven, Amy Boyd, Eric Lefevre, Teresa Lambe, Sarah C. Gilbert; unpublished data). Use of such a vaccination regime would accelerate the onset of highly effective, broad immunity. For the elderly, boosting immune memory has a greater chance of success than priming new immune responses, and this approach could increase the upper age limit at which vaccination ceases to become effective in the face of immunosenescence. At the extremes of age the chance of exposure to the virus will be reduced by effective, broad immunity in the rest of the population, and vaccination to induce neutralizing antibodies in late pregnancy could improve protection of infants prior to their first vaccination.[56]

Influenza A B C

All of the above refers to vaccination against influenza A, whereas we currently vaccinate against influenza B as well, but not influenza C which causes only very mild disease. The lack of a significant animal reservoir of influenza B[57] means that pandemics do not occur, and that with widespread use of an effective vaccine this virus could in theory be eradicated. Infections result in disease in children; once immunity has been acquired influenza B rarely causes disease in healthy adults but then affects the elderly. The same approaches to inducing universal or broad immunity to influenza A with a vaccine that has an improved level of efficacy in the elderly, who form the main reservoir for influenza B virus, could also be applied to influenza B, and vaccine formulations could continue to cover both virus types.

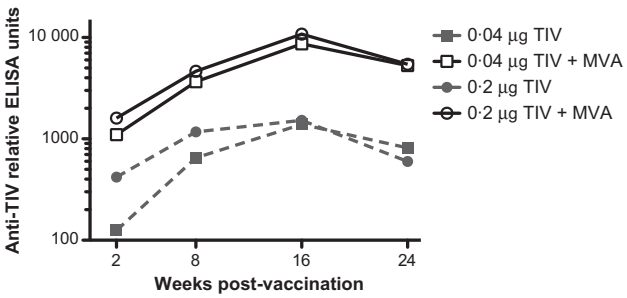

Figure 1. Balb/c mice were vaccinated with a low dose of trivalent inactivated vaccine (TIV, where 0·2 μg represents 1·3% of the human dose, and 0·04 μg is 0·27% of the human dose), either alone or co-administered with 10^6 pfu Modified Vaccinia virus Ankara-nucleoprotein + M1 (0·67% of the human dose). Serum anti-TIV responses were measured by ELISA at the time points indicated.

The path ahead

There has been little significant change in our approach to vaccination against influenza for many years, but there is now enormous scope for applying novel technologies to produce vaccines that will provide better protection against seasonal influenza in all age groups at the same time as very useful protection against pandemic influenza that is at least capable of reducing the number of deaths and reducing the severity of disease in those who do become infected. The diversity of approaches being pursued and uncertainty over what each could achieve has resulted in reluctance from large vaccine manufacturing companies to commit to any one of them until larger efficacy studies have been completed. These studies will therefore require public funding, which is unquestionably warranted when the return on investment in terms of improved public health and security against pandemic influenza is taken into account. Influenza A cannot be eradicated, and to gain control over this virus it may be necessary to vaccinate a high proportion of the population at intervals throughout life. However, universal vaccination with universal vaccines would put an end to the threat of global disaster that pandemic influenza can cause, and is a goal well worth pursuing.

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