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Inactivated influenza virus vaccines: the future of TIV and QIV

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Abstract

Influenza viruses continue to be a major public health concern, despite the availability of vaccines. Currently licensed influenza vaccines aim at the induction of antibodies that target hemagglutinin, the major antigenic determinant on the surface of influenza virions that is responsible for attachment of the virus to the host cell that is to be infected. Currently licensed influenza vaccines come as inactivated or live attenuated influenza vaccines and are tri- or quadrivalent as they contain antigens of two influenza A and one or two influenza B strains that circulate in the human population, respectively.

In this review we briefly compare trivalent and quadrivalent inactivated influenza vaccines (TIV and QIV) with live attenuated influenza vaccines (LAIV). The use of the latter vaccine type in children age 2 to 8 has been disrecommended recently by the American Centers for Disease Control and Prevention due to inferior vaccine effectiveness in this age group in recent seasons. This recommendation will favor the use of TIV and QIV over LAIV in the near future. However, there is much evidence from studies in humans that illustrate the benefit of LAIV and we discuss some of the mechanisms that contribute to broader protection against influenza viruses of different subtypes induced by natural infection and LAIV. The future challenge will be to apply these insights to allow induction of broader and long-lasting protection provided by TIV and QIV vaccines, e.g. by the use of adjuvants or combining LAIV with TIV and QIV. Other immune factors than serum hemagglutination inhibiting antibodies have shown to correlate with protection provided by TIV and QIV, which illustrates the need for other correlates of protection than hemagglutination inhibition by serum antibodies and justifies more focus on influenza antigens in the TIV and QIV other than hemagglutinin.

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Keywords

Influenza; Vaccine; TIV; QIV; Live attenuated influenza vaccine; heterosubtypic immunity; correlate of protection

Manuscript

Influenza continues to be a major health problem with up to 500.000 deaths and many more hospitalizations every year and worldwide (<http://www.who.int/mediacentre/factsheets/fs211/en/>). It is striking that the disease burden caused by circulating influenza viruses is so high, despite being a vaccine-preventable disease. Young children, the elderly, pregnant women and immunocompromised individuals belong to the most critical target groups for influenza vaccination, since they are at higher risk for influenza-related comorbidities. Also frontline health care providers are highly advised to be vaccinated since they are highly exposed to influenza virus during influenza epidemics and pandemics, and can contribute to influenza spread among hospitalized patients when infected [1]. Human influenza vaccines typically contain two influenza A and one or two influenza B strains, hence they are called trivalent or quadrivalent. Influenza A viruses that circulate in humans typically belong to the subtypes H1N1 and H3N2, where H stands for hemagglutinin (HA), N stands for neuraminidase (NA) and the numbers refer to the subtype of these proteins. Influenza B strains typically belong either to the Victoria or the Yamagata lineage. Because it has been proven hard to predict which B strain will be dominant in the next influenza season, quadrivalent vaccines containing antigens of both B strains have been introduced recently [2]. Influenza vaccines were estimated at a \$2.9 billion market value in 2011 with many major vaccine manufacturers producing them (http://who.int/influenza_vaccines_plan/resources/session_10_kaddar.pdf).

When it comes to licensed vaccines for use in humans, we have the choice between live attenuated, inactivated, and recombinant variants of the influenza vaccine. Live attenuated influenza virus (LAIV) are temperature-sensitive vaccine strains that grow at lower temperatures which restricts their replication to the upper respiratory tract when administered intranasally [3]. For inactivated influenza virus vaccines (IIV), vaccine virus propagation is often followed by chemical inactivation, e.g. by exposure to beta-propiolactone, which will result in whole inactivated virus (WIV) vaccines. Dissociation of virions with detergents and enrichment for the viral HA can reduce adverse effects sometimes associated with WIV vaccination. However, split and subvirion vaccines can be less immunogenic compared to WIV [4]. Trivalent and quadrivalent IIV are referred to as TIV and QIV, respectively and are administered intramuscularly or intradermally.

Since influenza vaccines are available, why are we doomed to the yearly flu shot?

The globular head of the influenza HA contains the binding site with which influenza viruses bind to sialylated proteins that function as host cell receptors. Therefore, antibodies targeting the head of HA can neutralize the virus by preventing viral attachment and entry and virus antibodies in serum are measured by virus neutralization assays in vitro as correlates of TIV and QIV vaccine protection. A serum hemagglutination inhibition (HI)

titer of 40 or a four-fold raise in HI titer upon vaccination has been referred to as the golden correlate of protection for influenza vaccines for many years now, since this level of virus-neutralizing antibodies has been associated with reduced likelihood of morbidity upon infection [5]. By using this criterion to estimate to what extent the vaccine will protect in the field, we already exclude the contribution of immune factors that can contribute to protection other than HA neutralizing antibodies. Conventional inactivated vaccines aim at the induction of neutralizing antibodies targeting the head of the influenza HA. The poor fidelity of the influenza replication machinery can however result in antigenic drift. The segmented nature of the influenza genome also allows the exchange of whole gene segments between influenza viruses that simultaneously infect the same cell, which can result in new influenza viruses with a new antigenically unrelated hemagglutinin. This process is called antigenic shift. Antigenic drift and shift allow the influenza virus to escape neutralizing antibodies that target the viral HA by changing its antigenic makeup. Antigenic drift is the reason why we have yearly epidemics and why conventional influenza vaccines need yearly updates. Antigenic drift can result in poor vaccine effectiveness (VE) since it may be the reason why vaccine strains do not sufficiently match the circulating ones. Antigenic drift occurs gradually during the influenza season and geographical differences in antigenic drift have been reported [6]. Therefore continuous monitoring of antigenic drift at different locations is important to estimate VE for used vaccines and to monitor antigenic variation of circulating influenza viruses in the field. The Centers for Disease Control and Prevention (CDC) monitor effectiveness of seasonal influenza vaccination in collaboration with the U.S. Flu Vaccine Effectiveness Network in order to measure the benefits of influenza vaccination for public health (<https://www.cdc.gov/flu/professionals/vaccination/effectiveness-studies.htm>). Effectiveness of influenza vaccines in previous seasons has been especially poor for the H3N2 component, with VE for H3N2 as low as 10% (95% CI –25 to 35) in 2013–2014 and 7% (95% CI –32 to 34) for 2014–2015 [6]. Vaccine effectiveness also differs between vaccine types, i.e. LAIV or IIV, TIV or QIV, as well as between age groups and vaccinees with different immune status [6,7]. Recently, the American Centers for Disease Control stopped recommending the use of LAIV in children ages 2–8 years, based on inferior VE as compared to IIV obtained from observational studies performed by the US FLU VE network (<https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2016-06/influenza-05-flannery.pdf>). This is quite surprising since LAIV used to be the preferred vaccine type for this age group due to its superior VE in previous seasons [7]. This recommendation will most likely contribute to the dominance of inactivated vaccines such as TIV and QIV at the cost of LAIV, at least in the US.

The fact that influenza viruses can easily escape host immunity and the sometimes poor VE of conventional influenza vaccines in recent seasons illustrate the need for better influenza vaccines that can provide broader and longer protection. The quest for so called “universal and multi-season vaccines” has been ongoing for many decades now and different approaches have been followed. More conserved epitopes like the ectodomain of matrix 2 (M2e) protein, the stem region of HA or conserved regions of the nucleoprotein (NP) and neuraminidase (NA) are promising vaccine candidates [8–11]. Host immune responses targeting these epitopes have shown to be able to provide hetero(sub)typic immunity against different influenza viruses [12–15]. It is intriguing that for most of the mentioned

approaches, protection correlates with the induction of non-neutralizing antibody or T cell responses, the former often in a Fc receptor-mediated way, but not with virus neutralization in vitro which is still the recommended correlate of protection for conventional influenza vaccines like TIV and QIV [12,16–21]. In order to identify vaccination strategies that afford broader protection, much can be learned from the host immune responses induced by influenza infection. Influenza vaccines used until 2009 did not protect from the H1N1 pandemic virus that emerged that year. However, T cells induced by infection with influenza viruses that circulated before the 2009 pandemic H1N1 virus may have contributed to protection against the pandemic H1N1 virus in the absence of vaccine-induced neutralizing antibodies [22–24]. Recent infection with a seasonal virus also correlated with protection from subsequent infection with 2009 pandemic H1N1 virus [25]. Because of these reasons one would expect that vaccination with LAIV would result in a broader immune response as compared to TIV and/or QIV. LAIV administration can indeed induce T cell responses [26,27] and a cross-reactive plasmablast response to heterovariant influenza strains that is greater than with IIV [28]. LAIV is also better for induction of influenza-specific IgA in the upper respiratory tract [27], which is considered one of the first lines of defence against influenza virus. However, vaccine-specific HI titers against homologous virus are sometimes lower after LAIV administration compared to IIV, and also induction of cross-reactive ADCC-antibodies in sera are not as good as with IIV, whereas natural and experimental infection do perform similarly [16,17]. Until recently, universal vaccines for influenza were typically being explored as an addition to the conventional seasonal vaccines. The idea of combining a universal vaccine component with a seasonal vaccine, e.g. TIV with a M2e-expressing virus like particle, has been tested in preclinical settings [29,30]. The concept behind this approach is that even partial protection provided by a universal influenza virus component still can reduce disease burden in case of failure of the seasonal vaccine. However, IIV also may have good assets for induction of hetero(sub)typic immunity as well. Whole inactivated virus has been used for the induction of heterosubtypic immunity (HSI) in animal models, which correlated with the induction of cross-reactive T cells targeting nucleoprotein (NP) and was dependent of the method of inactivation used [31]. In humans, antibody responses targeting the NP induced by seasonal vaccination cross-react with H7N9, which suggests they can contribute to HSI [16]. Antibody responses against NA can be induced by seasonal IIV and have shown to contribute to protection from influenza in humans [32,33]. Therefore, IIV that still contain viral antigens other than HA alone like NP or NA should have the potential to induce broader immune responses that correlate with immune factors other than HI antibody titers. In current TIV and QIV, only the amount of HA is standardized. In order to estimate the contribution of other vaccine components to the induction of (heterosubtypic) immunity, the non HA viral antigens should also be present at reasonable amounts in the vaccine. Although seasonal TIV used before 2009 did not protect against the new pandemic H1N1 virus, they may have primed for cross-reactive antibody responses that target the stem of HA [34] which were potentially boosted upon infection or vaccination with the pandemic 2009 H1N1 virus. Thus, exposure to a virus that did not circulate in the population before may allow memory B cells specific for the conserved stem of HA to be boosted whereas memory B cells specific for the more variable epitopes in the head of HA will not be boosted by the new virus [35,36]. Therefore, TIV and QIV may contribute to the priming of broadly reactive antibody responses that are boosted by

exposure to a hetero(sub)typic virus or vaccine. The feasibility of this approach to induce HA stem-reactive antibodies that provide heterosubtypic immunity is now investigated by consecutive vaccination using split IIV with different exotic HA heads but conserved HA stalks [37]. However current IIV manufacturing processes purify and enrich for the HA protein to an extent that they remove intrinsic adjuvanticity and conserved antigens present in WIV, e.g. they remove part of the internal proteins and viral RNA. Purification of IIV helps avoiding adverse vaccine effects like fever sometimes associated with whole-inactivated virus influenza vaccines. Can we optimize downstream processing of IIV such that we keep the intrinsic adjuvanticity of the virion and the more conserved viral antigens while minimizing adverse effects?

Another heavily explored vaccination strategy that aims for broader and more potent immune responses induced by conventional influenza vaccines is the use of adjuvants. Adjuvants can promote B and T cell activation upon IIV vaccination, which in turn can correlate with more qualitative and quantitative antibody responses [38–42]. Both CD4+ and CD8+ T cells can clear infected cells and these correlate with protection from symptomatic influenza in humans [23,24,43]. CD4+ T cells are also important providers of bystander help to the antibody-producing B cells [44,45]. It has recently been shown that consecutive vaccination with IIV can result in the maintenance of memory CD4+ T cells in humans [46]. Bystander help from T cells can not only lead to higher antibody titers and antibody avidities, but it is also needed for efficient isotype class switching. This is indeed observed for oil in water adjuvants that were tested with IIV in humans [40]. Antibody isotype class switching can be crucial for (universal) influenza vaccine types that rely on antibodies that require Fc receptor mediated mechanisms in order to provide protection, since different antibody subtypes can engage different Fc receptors on effector cells with different efficiencies [19–21]. Enhancing antibody responses with the use of adjuvants allows antigen dose sparing, which means less vaccine needs to be used in to induce a protective immune response.

In the end, the classical dogma of vaccination still holds: training the immune system against influenza virus infection by mimicking natural infection with an adjuvanted inactivated virus vaccine, or with live attenuated influenza virus, or with both. TIV and QIV seem to outperform LAIV during recent seasons when VE is considered. It is important to look into the reasons why LAIV came with poor VE in recent seasons, since LAIV mimics best natural infection resulting in the induction of broader and long-lasting immune responses. Eventually, a vaccination regime in which LAIV are alternated with IIV may provide the vaccinee with benefits of both vaccine types in the long run. In order to broaden protection provided by TIV and QIV to other influenza subtypes, more attention should be paid to the inclusion and to the quality of other viral components like NA, NP and M1 in the vaccine. Another reason to maintain NP and eventually viral RNA levels high in TIV and QIV is the recent finding that the ribonucleoprotein can interact directly with the cytosolic innate sensor molecule DAI/ZBP1. This interaction can result in inflammation by the induction of necroptosis [47,48]. Therefore, vaccine-derived NP and viral RNA can potentially even provide an adjuvant effect for TIV and QIV vaccination. A better understanding of how the individual aspects of the immune response induced by TIV and QIV vaccination contribute to vaccine effectiveness will allow further optimization of these vaccines. It is clear that as

for most universal vaccine approaches as well as for LAIV, protection provided by TIV and QIV may correlate with other immune factors different from virus-neutralizing antibodies in sera. This urges us to rethink the correlates of protection for influenza vaccines beyond inhibition of hemagglutination of red blood cells by serum antibodies.

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Highlights

- Currently licensed influenza vaccines sometimes come with poor vaccine effectiveness and their protection is limited in time
- Training the immune response against influenza antigens other than the hemagglutinin head can contribute to broader protection against influenza viruses
- A better understanding of the reasons why vaccine effectiveness was low for live attenuated influenza virus and H3N2 vaccines during recent seasons will allow further optimization of influenza vaccines and future vaccination strategies for influenza