Host Factors Impact Vaccine Efficacy: Implications for Seasonal and Universal Influenza Vaccine Programs

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ABSTRACT Influenza is a global public health problem. Current seasonal influenza vaccines have highly variable efficacy, and thus attempts to develop broadly protective universal influenza vaccines with durable protection are under way. While much attention is given to the virus-related factors contributing to inconsistent vaccine responses, host-associated factors are often neglected. Growing evidences suggest that host factors including age, biological sex, pregnancy, and immune history play important roles as modifiers of influenza virus vaccine efficacy. We hypothesize that host genetics, the hormonal milieu, and gut microbiota contribute to host-related differences in influenza virus vaccine efficacy. This review highlights the current insights and future perspectives into host-specific factors that impact influenza vaccine-induced immunity and protection. Consideration of the host factors that affect influenza vaccine-induced immunity might improve influenza vaccines by providing empirical evidence for optimizing or even personalizing vaccine type, dose, and use of adjuvants for current seasonal and future universal influenza vaccines.

KEYWORDS aging, microbiota, obesity, pandemic, pregnancy, seasonal influenza, sex difference

Each year, seasonal influenza epidemics cause 3 to 5 million severe cases of respiratory illnesses and 290,000 to 650,000 deaths worldwide (1). Such epidemics generally occur during the winter months when lower temperature and humidity favor virus dissemination. Although all people can acquire influenza viruses, children, the elderly, pregnant women, and individuals with either chronic medical conditions or immunosuppression are at higher risk (2). The influenza A virus (IAV) H1N1 subtype dominated in the early 20th century, the H2N2 subtype of IAV was predominant in the late 1950s and 1960s, and H1N1 and H3N2 subtypes of IAV and different lineages of influenza B viruses have been responsible for seasonal epidemics in last several decades (2, 3). Influenza A and B viruses frequently acquire point mutations on their surface glycoproteins (antigenic drift), resulting in variable levels of morbidity and mortality each season (2). In the United States, seasonal influenza epidemics since 2010 and 2011 have resulted in between 9.3 and 49 million cases, between 140,000 and 960,000 hospitalizations, and between 12,000 and 79,000 deaths annually (4). In the 2017–2018 season, influenza-related hospitalizations in the United States reached almost a million, with nearly 79,000 deaths, of which older adults (≥65 year of age) were affected most severely (4). In addition to annual epidemics, influenza pandemics occur sporadically at unpredictable frequencies, with four such pandemics occurring in last century (i.e., 1918, 1957, 1968, and 2009), which resulted in millions of deaths worldwide (2). Pandemic influenza viruses are generated by reassortment of genetic segments (antigenic shift) between two or more influenza viruses in swine or avian hosts. The resulting virus is antigenically different than seasonal influenza strains and causes severe illness and high mortality in the population, which lacks sufficient preexisting
immunity (2). Sporadic human infections have also been recorded in last few decades with novel avian origin IAVs (e.g., H5N1 and H7N9) and swine origin variant viruses (H3N2), which may undergo sufficient mutations and result in pandemics if sustained human to human transmission occurs (5, 6).

Vaccination is the most effective means to prevent influenza infection. Influenza vaccines have been in use since the 1940s in the United States (2). Most commonly used influenza vaccines are inactivated influenza virus (IIV) vaccines administered through the intramuscular route with the exception of intranasal live-attenuated influenza vaccines (LAIVs) recommended for use in young children (2). Current seasonal influenza vaccines contain two IAVs (H1N1 and H3N2) and one (in the trivalent influenza vaccine [TIV]) or two (in quadrivalent influenza vaccine [QIV]) influenza B viruses to increase the breadth of immunity (7). The immunity conferred by these vaccines is specific to the vaccine antigen and wanes over time (8); thus, annual vaccination is recommended. Currently, the strains to be used in the annual influenza vaccines are decided based on the surveillance report of World Health Organization (WHO) laboratories established throughout the world (7). To prepare seasonal influenza vaccines, the hemagglutinin (HA) and neuraminidase (NA) gene segments of the selected strains are reassorted into the replication efficient A/Puerto Rico/08/1934 (H1N1) IAV, and the virus is grown in embryonated chicken eggs, followed by chemical inactivation (2). LAIV, which is delivered as a nasal spray, was launched in the United States in 2003 targeting the pediatric population (9). In 2016, the use of LAIV was suspended in the United States owing to poor effectiveness particularly against A/H1N1pdm09 virus (9); other countries, however, including the United Kingdom and Finland continued its use and even the Advisory Committee on Immunization Practices (ACIP) in the United States recommended reintroduction of LAIV vaccine for the 2018/19 influenza season (9, 10).

Influenza vaccination is recommended in the United States for any individuals over 6 months of age who do not have any contraindications (9). Pregnant women, health care workers, children, the elderly, and immunocompromised individuals are prioritized for vaccination because the risk of severe disease is higher among them. Seasonal influenza vaccines, in general, reduce the risk of influenza infection and severe outcomes in individuals of any age compared with unvaccinated individuals (11). The US Centers for Disease Control and Prevention (CDC) estimated that vaccination averted around 5.29 million influenza-related illnesses, 2.64 million medical visits, and 84,700 hospitalizations in the United States during 2016–2017 influenza season (11). The protective benefit of seasonal influenza vaccines is mainly based on the ability to produce antibodies against HA, the major surface glycoprotein, of the virus. Such hemagglutination inhibition (HAI) antibodies bind to the head region of HA antigen and prevent the virus from binding to the receptor on respiratory epithelial cells (12). The HAI titer is the most commonly used correlate of protection for seasonal influenza vaccines where a titer of ≥40 is considered protective (13, 14). The seasonal influenza vaccines, however, provide only strain-specific protection and limited or no protection against novel strains with pandemic potential; the requirement of annual reformulation of vaccines results in vaccine strain selection being months behind in the arm race with the constantly evolving influenza viruses (5). Egg adaptation can result in additional mutations in the influenza vaccine strains which impair the functional ability of antibodies produced against the vaccine virus to neutralize circulating viruses further reducing influenza vaccine efficacy (15). In general, the effectiveness of seasonal influenza vaccines is highly variable from season to season and ranges between 10 and 60% (5).

As a result of variability in seasonal influenza vaccine efficacy, there is growing interest in the development of universal influenza vaccines that can provide broader and longer duration immunity. As per the strategic plan of National Institute of Allergy and Infectious Diseases (NIAID) to improve current influenza vaccines, the universal influenza vaccines should have at least 75% effectiveness, protect against both group 1 and 2 of IAVs, confer multiseason protection, and be effective in all age groups (5). Different strategies are being considered to develop universal influenza vaccines,
including chimeric or headless HA-based approaches that induce broadly protective HA stalk-specific antibodies (16), matrix protein 2 (M2) targeting vaccines that elicit antibodies against the highly conserved M2 protein with cross-protective properties (17), and T cell-stimulating vaccines based on conserved internal proteins, such as the nucleoprotein (NP) and matrix protein 1 (M1) (18). Although considerable attention is being paid to the rational design of improved influenza vaccines, including universal influenza vaccines, continued attention should be paid to the host-related factors, including age, sex, pregnancy, immune history, and obesity (Fig. 1), that can alter vaccine efficacy. Furthermore, little consideration has been given to the targetable mechanisms, including the composition of the gut microbiota, genetics, and even hormones that can contribute to how age, sex, pregnancy, and even obesity can impact influenza vaccine-induced immunity and protection.

**EFFECTS OF AGE ON INFLUENZA VACCINE OUTCOME**

Influenza vaccination in children above 6 months of age has been recommended since the 2002–2003 influenza season in the United States. Immunogenicity studies show that full-dose vaccination (i.e., two-dose vaccine at 4-week interval, if not vaccinated before) induces a protective level of serum antibodies (HAI ≥ 40) in children (19). One study using TIV in vaccine-naïve and seronegative 5- to 8-year-old children showed that 85, 68, and 48% of the children develop protective antibody response (HAI ≥ 40) against H1N1, H3N2, and influenza B virus strains, respectively, after receiving two doses of vaccine (20). In the case of LAIV, mucosal antibody and cell-mediated immune responses are associated with protective efficacy in children, but specific correlates of protection for LAIV vaccines are not well established (21). Randomized controlled trials have shown that the efficacy of IVs in children ranges from 43 to 91%, whereas for LAIV, the efficacy is 64 to 93% (22).

A meta-analysis of vaccine efficacy studies in 10 different randomized trials in adults (18 to 65 years) showed an average pooled efficacy of 59% for TIV (23), with QIV resulting in seroprotection (HAI ≥ 40) and seroconversion (i.e., 4-fold increase in HAI titer postvaccination) rates that are comparable or greater than TIV in young adults (24).
In general, the clinical vaccine efficacy estimates in elderly (≥65 years of age) are lower than in young adults (<65 years of age) (25). A review of vaccine antibody response studies from 1986 to 2002 showed that the seroconversion rates were higher in young adults compared to aged adults against H1N1 (60% versus 42%), H3N2 (62% versus 51%), and influenza B (58 versus 35%) viruses (25). Likewise, seroprotection rates were also greater in young adults than in aged adults against H1N1 (83% versus 69%), H3N2 (84% versus 74%), and influenza B (78% versus 67%) viruses (25). Vaccine effectiveness (based on observational studies) is similar among children, young adults, and aged adults against A/H1N1 and influenza B viruses; whereas vaccine effectiveness is highest in children, lower in young adults, and lowest in aged adults against A/H3N2 (26).

Influenza vaccine effectiveness in the UK during 2016/17 season also showed a declining trend of vaccine effectiveness in aged adults against all influenza virus antigens compared to younger adults (27). In general, influenza vaccine-induced immunity declines with age, which is likely affected by host factors, such as age-associated immunosenescence and immune history, as well as virus factors, including antigenic drift.

Regarding vaccine safety, influenza vaccines in general are safe and well tolerated by different age groups with rare serious or clinically important adverse events (28). Mild and transient local and systemic reactions to influenza vaccines are reported in all age groups, with comparatively higher adverse reactions being observed in younger subjects (29, 30). The adverse reactions to influenza vaccines may vary depending upon the nature of the vaccine (e.g., IIV or LAIV), vaccine composition (selection of antigens and adjuvants), route of administration (intramuscular, intranasal, or others), and the dose of vaccines. Hence, close monitoring of the safety profile is necessary across different age groups while testing novel vaccine platforms in clinical studies.

The reduced efficacy of influenza vaccine in aged humans has been associated with age-dependent changes in innate and adaptive immune function due to immunosenescence. Lower expression of CD80 costimulatory molecules in activated monocytes (31), a reduced number of plasmacytoid dendritic cells and a generalized decrease in Toll-like receptor 7 (TLR7)- and TLR8-induced cytokines (alpha interferon [IFN-α] and interleukin-12p40 [IL-12p40]) in dendritic cells (32), and impaired inflammatory responses in aged adults due to elevated levels of the anti-inflammatory cytokine IL-10 (33) are associated with lower seroconversion and seroprotection rates of vaccines in older adults compared to young adults. Systems biology approaches have found the association of higher baseline innate inflammatory responses in aged adults with diminished antibody responses postimmunization with TIV over five consecutive influenza seasons (34). Reduced influenza-specific activation-induced cytidine deaminase (AID) responses and the percentage of switch memory B cells (35, 36), reduced affinity maturation (37), lower number of antibody-secreting cells or plasmablasts (38), impaired memory B cell to plasma cell differentiation (39), diminished antibody diversity (40), and reduced plasticity of B cell receptor repertoires (41) are likely to be the B-cell-associated defects associated with inefficient antibody responses after influenza vaccination in aged adults. Shortening of telomere length in B cells is also linked to lower antibody production and inefficient CD8+ T cell proliferation in aged adults (42).

The loss of CD28 molecules that are pivotal in T cell activation and germinal center development and accumulation of CD8+ CD28- T cells (43, 44), a decline in influenza-specific memory T cells (45), a reduced frequency and function of circulatory T follicular helper cells (46), and age-dependent decline in granzyme-, perforin-, and IFN-γ-mediated cytolytic activity of CD8+ T cells (47, 48) represent some of the T cell events in aged adults that may contribute to lower vaccine-induced immunity and protection. The expression of the proliferative senescence marker, KLRG1, and the inhibitory receptor CD57 on CD8+ T cells prevaccination can also predict lower antibody production in aged adults (49). Infection with cytomegalovirus, which is more prevalent in older subjects, also affects influenza vaccine-induced immunity in aged individuals (50). A recent study has shown that plasmablasts from older individuals have reduced somatic hypermutation of immunoglobulin variable genes, resulting in limited adapt-
ability of antibody responses to drifted epitopes of influenza virus and highlighting the fact that a better vaccine for older individuals should focus on enhancing the antibody affinity maturation process (51). Studies in aged humans also show that high-dose vaccine and adjuvant supplementation improves the performance of inactivated vaccines compared to the standard-dose vaccination, with these strategies now incorporated in seasonal influenza vaccine campaigns for older individuals (52, 53).

Studies in mice also reveal that immunization of aged mice (>16 months) with either monovalent or trivalent IVVs induces lower antibody responses and protection compared to young mice (2 to 3 months) (54–56). Such differences are in part associated with age-dependent changes in the hormonal milieu, cytokine profile, and antigen-presenting cell responsiveness after immunization (54–56). Influenza nucleoprotein-based vaccine studies also indicate that aged mice have alterations in CD4⁺ T-helper and CD8⁺ cytotoxic T cell frequencies compared to young mice (57, 58). Aged mice also require multiple doses of vaccines, higher quantities of antigen, or the addition of adjuvant to elicit improved immune responses and protective efficacy of influenza vaccines compared to young mice (59, 60).

In mice, IAV NP and M2-based universal influenza vaccine candidates tested across different age groups indicate that antigen-specific antibody and T cell responses decline with age, leading to a reduced protective efficacy in older mice (61). Studies in humans and mice highlight the necessity of including age in preclinical and clinical studies of seasonal and universal influenza vaccines.

SEX DIFFERENCES IN INFLUENZA VACCINE EFFICACY

Biological sex (i.e., being male or female based on sex chromosome complement, gonadal tissues, and sex steroid concentrations) and gender (i.e., sociocultural influences that affect roles, behaviors, and activities that are deemed appropriate for men or women) are associated with the influenza vaccine acceptance rate, the reporting of adverse reactions, and the development of immune responses postvaccination (62). The influenza vaccine acceptance rate is reportedly lower, whereas passive reporting of local and systemic adverse reaction is higher, in females than males (62). In young adults (18 to 49 years), immunization with seasonal TIV vaccine resulted in at least two times greater HAI antibodies in females than males (63). Administration of a half-dose TIV in females induced antibody responses to the H1N1, H3N2, and influenza B antigens that are almost equivalent to the full-dose vaccine response in males (63). Higher neutralizing antibodies are observed regardless of age in females after either monovalent vaccination or TIV, with the differences being reported against both influenza A and B viruses (56, 63, 64). Among aged individuals (>65 years), both the seroconversion and the seroprotection rates are higher in females compared to males after immunization with TIV (65). A greater HAI antibody response in aged adult females is also observed against H1N1 and H3N2 viruses after immunization with high-dose seasonal TIV (66). Vaccination with a monovalent, unadjuvanted H1N1 pandemic 2009 vaccine resulted in females having significantly higher seroprotection and seroconversion than males in Taiwan (67). Likewise, aged females have significantly higher postvaccination HAI titers than similarly aged males after a single dose of unadjuvanted 2009 H1N1 pandemic vaccination in some (68) but not all (56) studies in the United States. The impact of biological sex on immune responses or adverse reactions after the receipt of either QIV or LAIV has not been reported in humans.

Limited human studies have considered the effects of biological sex on vaccine effectiveness studies but have consistently shown that influenza-vaccinated females have a lower risk of hospitalizations and deaths compared to vaccinated males (69–71). A recent study evaluated vaccine effectiveness across seven seasons in Canada and showed that overall vaccine effectiveness was higher in females than in males; the difference was more pronounced in response to A/H3N2 and influenza B virus than A/H1N1 (72), with the observed sex difference being more pronounced in older (>50 years of age) than in younger (<20 years of age) individuals (72). These findings
highlight the need to include sex as a potential modifier of influenza vaccine outcome in future vaccine effectiveness studies.

Studies in mice further illustrate sex differences in immunity and protection with influenza vaccines. After primary inoculation with a sublethal dose of H1N1 or H3N2 influenza virus, adult female C57BL/6 mice generated higher neutralizing and total anti-influenza virus antibodies (73). After secondary challenge, males and females show similar levels of protection against homologous virus, but females have better protection against heterosubtypic viruses, indicating that females developed better cross-protective immunity than males (73). After vaccination with either whole inactivated IAV, TIV, or QIV, adult female mice generate greater quantity and quality of influenza-specific antibody responses than do males (74–76). Antibody derived from vaccinated females also is better at protecting both naive males and females than antibody from that from males, and this protection is associated with increased antibody specificity and avidity to the H1N1 virus (76). The TLR7 gene (Tlr7) is encoded on the X chromosome, is also expressed in B cells, and plays a role in isotype switching (77). The expression of Tlr7 is greater in B cells from vaccinated females than in B cells from males and is associated with reduced DNA methylation in the Tlr7 promoter region, a higher neutralizing antibody, class switch recombination, and antibody avidity in females (76). Deletion of Tlr7 reduced sex differences in vaccine-induced antibody responses and protection after challenge and had a greater impact on responses in females than males. Taken together, these data illustrate that greater TLR7 activation in B cells and antibody production in females improves the efficacy of IIVs against influenza.

Global gene expression analysis of B cells from healthy human adults also indicates a differential expression of genes between male and females, particularly those that contain estrogen response elements in their promoter regions, indicating that hormone signaling may regulate gene expression in B cells (78). In mice, 17β-estradiol is positively associated with IAV neutralizing antibody production in females, indicating the role of estrogen in modulating influenza vaccine-induced immunity in females of reproductive age (56, 79). In humans, the lower neutralizing antibody response in males compared to females after TIV vaccine administration is associated with a higher level of serum testosterone and greater lipid metabolism (64).

To date, no animal studies of universal influenza vaccines have examined sex differences in vaccine-induced immunity or protection. To gain insight into the consideration of biological sex in universal influenza vaccine studies in animal models, we performed a literature search in PubMed using the keywords “universal influenza vaccine” for the year 2018. This search resulted in 42 influenza vaccine studies in different animal models, with 86% (36/42) of them using only female animals; 7% (3/42) using both sexes, but not disaggregating results based on sex; and the remainder (7% [3/42]) either using only male animals or not reporting the sex of the animals. To date, preclinical studies have failed to acknowledge the importance of biological sex in vaccine-induced immunity and protection.

EFFECTS OF IMMUNE HISTORY ON INFLUENZA VACCINE EFFICACY

Immune history is acquired over time through both virus exposures and vaccination, which affects the quality and quantity of antibody developed against influenza viruses later in life. Early life exposure to influenza viruses that occurs within the first decade of life presumably dominates the development of influenza-specific antibody responses later in life (80, 81). This phenomenon is known as “original antigenic sin” (OAS) and was put forward by Thomas Francis, Jr., in the 1960s (82). Currently, the concept of OAS is also referred as “immune imprinting” to address both the positive and the negative aspects of immune history on influenza virus vaccine efficacy (80). Immune imprinting facilitates the activation of memory B cells over de novo activation of naive B cells, thereby establishing a hierarchy of antibody responses where the highest response is generated against the strains from childhood, with subsequent strains inducing lower titers of antibody (80).
A cross-sectional study in China showed that neutralizing antibodies remained highest against the H3N2 viruses that circulated in the first decade of participants’ life, with lower neutralizing antibody responses observed against other H3N2 strains that circulated in subsequent years (83). Similarly, a longitudinal study over a 20-year period indicated that neutralizing antibodies against previously encountered influenza virus strains expand continuously over time (84). High-throughput studies of human plasmablasts induced by vaccination suggest that influenza vaccination induces preferential recall of memory B cells specific to influenza virus strains that circulated in previous years compared to the strains used for vaccination in more recent years (85, 86). Immune imprinting can also be replicated in the laboratory using sequential influenza virus infections of mice, rats, or ferrets (87–89). A study in mice, for example, showed that the effect of immune imprinting is more pronounced if the first exposure to IAV is through infection rather than vaccination (89).

The differences in the quality or cross-reactivity of antibody responses after influenza vaccination in different age cohorts is also partly explained by the differences in imprinting to viruses associated with birth year (90). Higher influenza virus susceptibility in older individuals may be caused by early life immune imprinting altering antibody responses against drifted influenza viruses later in life, despite the high immunization rate within this population (91). As a result of a mutation in the HA of the circulating H1N1 IAV during the 2013–2014 influenza season, the infection rate was unusually high in the middle-aged population, partly because these individuals developed higher antibody titers against the K166 HA of the H1N1 viruses that circulated during their birth years (1965 to 1979) than against the drift variant (K166Q) that was currently in circulation (92). A similar reduction in vaccine effectiveness in older and middle-aged people in the United States and Canada was observed during the H1N1-dominated 2015–2016 influenza season, which also could be associated with immune imprinting to sufficiently different H1N1 strains (93, 94). Mouse studies have shown that the adverse effect of immune imprinting can be avoided by including adjuvants in vaccine formulation or through repeated immunizations (95). Human studies suggest that an adjuvanted seasonal influenza vaccine is more effective at inducing antibody responses than a comparable unadjuvanted seasonal influenza vaccine, at least in an aged human population (53, 96).

Immune imprinting can provide a protective benefit during influenza virus infection later in life. One such example is the 2009 influenza pandemic, in which aged individuals with prior immune history with the pandemic 1918 H1N1 influenza virus were less susceptible to 2009 H1N1 than were younger age individuals (97). Likewise, the lower susceptibility observed in older than in younger individuals (i.e., people 18 to 49 years of age) during the 1918 influenza pandemic is hypothesized to be due to exposure to earlier strains of H1 viruses in childhood (98). Using statistical modeling, childhood imprinting with H1N1 and H3N2 virus can also provide protection against H5N1 and H7N9 influenza viruses, respectively, later in life (99). Immune imprinting boosts memory B cell responses that produces broadly neutralizing HA stalk-specific antibodies (80).

Despite reports of both positive and negative effects of immune imprinting on influenza vaccine efficacy, the extent to which immune imprinting is induced by different types of exposures, including different vaccine platforms, is not well characterized. Few studies have examined the role of host factors, including the combined effect of sex and age, in influencing the strength and magnitude of immune imprinting both in humans and nonhuman animals.

PREGNANCY AND INFLUENZA VACCINE EFFICACY

Pregnancy is associated with physiological and immunological alterations intended to maintain an optimal environment for the growing fetus. Hormones, including estradiol and progesterone, which are immunomodulatory, vary considerably during different stages of pregnancy (100). Cytokines that mediate inflammatory or anti-inflammatory responses and immune cells associated with innate and adaptive im-
immune systems also fluctuate during pregnancy (100, 101). Together, these pregnancy-
associated physiological and immunological changes contribute to immunological
shifts in pregnant compared to nonpregnant females.

In the case of influenza, pregnant females are at higher risk of influenza virus
infection compared to nonpregnant females (102). Considering the greater risk of
influenza infection and outcomes, the WHO has prioritized influenza vaccination for
pregnant women (103). Immunization during pregnancy serves the dual function of
protecting the health of mothers and infants. Because no influenza vaccines are
recommended before 6 months of age, neonatal protection from influenza relies on
passively transferred antibodies from the mother (103). A study in the United States
using TIV showed comparable seroconversion rates when females were vaccinated
during either the first or the third trimester of pregnancy (104). As in other populations,
a history of prior immunization and greater baseline antibody titers are associated with
lower seroconversion rates in pregnant women (104). IIVs result in comparable sero-
protection and seroconversion rates between pregnant and nonpregnant women (105,
106). The randomized controlled trials have shown highly variable efficacy of TIV during
pregnancy. One study in Nepal showed only 19% efficacy of TIV in preventing
influenza-like illnesses in pregnant women, while another study in South Africa ob-
served a 50.4% vaccine efficacy (107, 108). The efficacy of maternal influenza immuni-
zation in preventing laboratory-confirmed influenza infection in infants also varies from
30 to 63% in different clinical trials (107–109).

Multiple studies show that maternal immunization with IIVs is not associated with
increased risk of adverse events in maternal or fetal health (110, 111). A study in 2017
showed that women who received the inactivated pandemic H1N1 2009 vaccine during
the first trimester of pregnancy had a greater risk of miscarriage if they had received the
same vaccine in the previous year (112). Though this finding does not indicate a causal
relationship, it highlights the importance of continued active surveillance in pregnant
women and infants, which are all too often not included in clinical trials of drugs or
biologics, including vaccines (113).

Studies in mouse models of pregnancy have shown that disruption of cytokine and
hormonal pathways, as well as alterations of placental and respiratory pathophysiology,
is responsible for the adverse effects of influenza infection during pregnancy, which are
mediated by infection-induced suppression of circulating progesterone (114, 115).
Influenza vaccination during pregnancy in mice suggests greater protection of neo-
ates through maternal immunization (116, 117). To date, no influenza vaccine has
been designed to specifically target pregnant females. In order to develop safe,
immunogenic, and highly efficacious vaccines including universal influenza vaccines,
pregnancy-associated changes and their impact on vaccine-induced immunity should
be considered in experimental studies.

**OBESITY AS A COMORBIDITY IN INFLUENZA VACCINE EFFICACY**

Obesity is an independent risk factor for influenza-related illnesses and hospitaliza-
tions (118). Obesity also impacts the effectiveness of influenza vaccines. Obese adults
immunized with TIV, despite developing efficient antibody responses, are at two times
greater risk of developing influenza or influenza-like illnesses (119). The relatively lower
effectiveness of influenza vaccines in obese individuals is hypothesized to be mediated
by insufficient T cell function, since peripheral blood mononuclear cells from TIV-
vaccinated obese adults have decreased activation of cytotoxic T cells and reduced
expression of functional markers, including IFN-γ and granzyme B (120). Influenza
vaccination is still important in obese populations since vaccinated obese children are
three times less likely to acquire PCR-confirmed influenza and miss significantly fewer
school days compared to their unvaccinated obese counterparts (121). At least one
study using diet-induced obesity in adult male mice illustrates that the induction of
chronic inflammation might be responsible for reduced efficacy of inactivated mon-
ovalent influenza vaccine in obese mice (122). The impact of obesity on immune
responses and protection after influenza virus challenge could not be reversed by
increasing the dose of vaccine antigen or using an adjuvant in obese mice (123); therefore, further studies are needed to explore the underlying mechanisms for reduced protection against influenza virus infection in obese individuals which will guide the development of an effective influenza vaccine. The microbiome of lean and obese individuals differs considerably in terms of richness and diversity (124) and, given the role of the microbiome in host immunity (125), studies should dissect the interaction of the microbiome with obesity on influenza vaccine effectiveness.

HYPOTHESES FOR HOST-RELATED MECHANISMS AFFECTING INFLUENZA VACCINE EFFICACY

Microbiota. In murine models of influenza infection, the commensal microbiota plays an important role in the establishment of proper innate and adaptive immune responses. After antibiotic treatment or in germfree mice, the innate and adaptive immunity to influenza virus is severely compromised compared to specific pathogen-free mice, but the sexes and ages of these mice are inconsistently reported in these studies (126, 127). The intestinal microbiota can regulate the TLR7 pathway after influenza virus infection, at least in adult female mice (128). Several studies in mice have reported the beneficial prophylactic effect of oral and intranasal administration of probiotics, such as *Lactobacillus*, on influenza virus-specific innate and adaptive immune responses (129, 130). The immunological benefits of adding probiotics into HA- and M2e-based influenza vaccine formulations have also been shown in mice (131, 132). A randomized controlled study in adult humans in Italy showed that those who consumed probiotics before receipt of the seasonal TIV had significantly higher vaccine-specific antibody responses compared to placebo controls (133). Similarly, in the United States, healthy adults that consumed probiotics for 28 days postvaccination with TIV had significantly improved seroprotection against the H3N2 virus strain compared to placebo controls (134). The beneficial effects of probiotics on antibody responses to TIV are also observed in older adults who consumed probiotics continuously either before or after immunization (135, 136).

TLR5, which senses bacterial flagellin produced by the microbiota, is associated with improved efficacy of influenza vaccines. In healthy adults immunized with TIV, there is an association between early expression of TLR5 in blood with the subsequent induction of vaccine-specific antibody responses (137). Because TIV alone does not stimulate TLR5, such activation is likely mediated by other factors, including the host commensal microbiota. A follow-up study in mice that did not indicate either the sexes or the ages of the mice revealed that TLR5-mediated sensing of flagellin produced by intestinal microbiota is necessary for efficient antibody production after influenza vaccination (138). Antibody responses to an IIV also were severely impacted in germfree or antibiotic-treated wild-type mice, as well as in TLR5 knockout mice after immunization with TIV. The immunity was restored in germfree and antibiotic-treated mice after oral administration of a flagellated strain of *Escherichia coli* or after coinjection of flagellin and TIV (138). This study highlights the influence of microbiota on influenza vaccine responses. Additional studies are necessary to compare how intestinal and respiratory microbiota are associated with influenza virus infection and immunity. Furthermore, most studies evaluating the impact of the microbiota on influenza vaccine-induced immunity do not consider the sex, age, body mass, or reproductive status of the subject and may miss important associations that could explain some variability in the impact of the microbiota on vaccine efficacy.

Genetics. Host genetic factors play an important role in influenza pathogenesis and immunity to vaccines. Single nucleotide polymorphisms of genes associated with human leukocyte antigen (HLA) molecules, cytokines, and cytokine receptors influence the humoral immune responses to influenza virus vaccines (139). For example, polymorphisms in the IFN-inducible transmembrane protein 3 (IFITM3) gene are associated with increased risk of influenza virus infection, and recent meta-analyses indicate that the polymorphism in IFITM3 is associated with an increased risk of severe influenza in Asians and Caucasians but not in other racial or ethnic groups (140, 141). The IFITM
gene, which is induced by IFNs, mediates antiviral response and can also shape adaptive immunity by protecting the survival of resident memory cytotoxic T cell population (142). In adult humans, the association of host genetics and antibody responses to seasonal TIV revealed 20 genes that mediate antibody responses to TIV, including genes related to antigen processing and intracellular trafficking (143). Another study suggested an association between the presence of certain HLA class II alleles in older aged individuals with a higher seroprotective response after immunization with TIV (144). A study by Avnir et al. showed that a phenylalanine (F)-to-leucine (L) polymorphism in the immunoglobulin heavy-chain variable locus (IGHV1-69) can modulate B cell clonal expansion, somatic hypermutation, and neutralizing antibody responses after H5N1 influenza vaccination (145). Vaccinees bearing F alleles develop higher stem-directed broadly neutralizing antibody and microneutralization antibody responses versus individuals carrying L alleles (145). A better understanding of the relationship of genetic variations and influenza vaccine-induced immunity is necessary to predict effectiveness of seasonal or universal influenza vaccine responses, as well as how genetics and even epigenetics may explain the impact of host demographic variables on vaccine-induced immunity.

Hormones. Sex steroids, mainly estrogens and progesterone in females and testosterone in males, fluctuate over the life course and mediate a wide range of immune responses during infection or vaccination by interacting with their respective receptors expressed in cells of the innate and adaptive immune system (146). Estradiol can directly upregulate AID and induce somatic hypermutation and class switch recombination of immunoglobulins, which indicate its potential role in antibody production and function (147). In adult female mice, ovariectomized mice have significantly lower influenza-specific antibody responses than gonad-intact females after immunization with monovalent inactivated vaccine, which is restored after administration of estrogen to ovariectomized females (56, 79). The rapid decline of estradiol in women after menopause, associated with health disorders including osteoporosis and atherosclerosis, can be managed by hormonal replacement therapy (HRT). HRT that consisted of equine estradiol and medroxyprogesterone reduces baseline the concentration of proinflammatory cytokine and increases the numbers of circulating B cells in postmenopausal women (148, 149). Plasma estradiol levels in postmenopausal women, including those on HRT, are positively correlated with the fold increase in influenza-specific IgG antibody titers after immunization with TIV (150). In both humans and mice vaccinated with a monovalent 2009 H1N1 inactivated vaccine, serum estradiol levels are positively correlated with neutralizing antibody titers in both young and aged adult females (56).

Circulating testosterone concentrations decline gradually in men as they age, and one previous study has indicated that men with higher serum testosterone concentrations have lower antibody responses to TIV (64). In both humans and mice vaccinated with a monovalent 2009 H1N1 inactivated vaccine, higher serum testosterone concentrations are negatively associated with neutralizing antibody titers in young adult but not aged adult individuals (56). In mice, removal of the gonads, and hence the production of estrogens and progesterone in females and testosterone in males, eliminates sex differences in IIV-induced immunity, which can be reversed by the replacement of estradiol in females and testosterone in males (56). Further studies into how sex steroids mediate the effects of sex, age, and pregnancy are required, especially in studies of universal influenza vaccine platforms.

CONCLUSIONS

The data pertaining to seasonal IIVs suggest that the “one size fits all” approach of vaccine administration does not necessarily protect equally across distinct ages, sexes, reproductive periods, or comorbid conditions, with commensal microbial, genetic, and hormonal mechanisms contributing to this variability. As we move closer toward the goal of development of universal influenza vaccines that provides efficient protection across all age groups, host factors must be given greater consideration. Preclinical
clinical, and epidemiological studies must continue to disaggregate and explore the influence of host-specific factors on influenza vaccine-induced immunity and protection. Through greater consideration of the host factors reviewed, either alone or in combination, we may mitigate disparities in influenza vaccine efficacy and develop safer and more efficacious universal influenza vaccines.

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