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Immunobiology of Influenza Vaccines

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Vaccination is the primary strategy for prevention and control of influenza. The surface hemagglutinin (HA) protein of the influenza virus contains two structural elements (head and stalk) that differ in their potential utility as vaccine targets. The head of the HA protein is the primary target of antibodies that confer protective immunity to influenza viruses. The underlying health status, age, and gene polymorphisms of vaccine recipients and, just as importantly, the extent of the antigenic match between the viruses in the vaccine and those that are circulating modulate influenza vaccine protection. Vaccine adjuvants and live attenuated influenza vaccine improve the breadth of immunity to seasonal and pandemic virus strains. Eliciting antibodies against the conserved HA stem region that cross-react with HAs within influenza virus types or subtypes would allow for the development of a universal influenza vaccine. The highly complex network of interactions generated after influenza infection and vaccination can be studied with the use of systems biology tools, such as DNA microarray chips. The use of systems vaccinology has allowed for the generation of gene expression signatures that represent key transcriptional differences between asymptomatic and symptomatic host responses to influenza infection. Additionally, the use of systems vaccinology tools have resulted in the identification of novel surrogate gene markers that are predictors of the magnitude of host responses to vaccines, which is critical to both vaccine development and public health. Identifying associations between variations in vaccine immune responses and gene polymorphisms is critical in the development of universal influenza vaccines.

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Abbreviations: APC = antigen presenting cell; CaMK-IV = calcium/calmodulin-dependent protein kinase type IV; HA = hemagglutinin; HAI = hemagglutination inhibition; LAIV = live attenuated influenza vaccine; M2 = matrix 2 ion channel protein; MVS = master virus strain; NA = neuraminidase; NP = nucleoprotein; Th = T helper; TIV = trivalent influenza vaccine; TLR = Toll-like receptor

Critical advances in the understanding of the immunobiologic mechanisms leading to the protection conferred by influenza vaccines have been made over the past decade. In this review, we discuss the most relevant of these advances, with special emphasis on the use vaccinology tools for improved vaccine production and enhanced immunogenicity and on systems vaccinology for the early identification of vaccine responders. We also focus on heterotypic immunity to influenza and the immunologic basis for the development of a universal influenza vaccine. These challenges in influenza vaccine development and their corresponding opportunities are summarized in Table 1.

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Influenza Virus and Immune Mediators of Protection

The isolation of the influenza A virus in 1933[22] led to the first use of influenza vaccines in the 1930s and 1940s.[23-25] Influenza viruses contain eight single-stranded RNA segments encoding 11 proteins and are classified into three distinct types on the basis of major antigenic differences: influenza A, influenza B, and influenza C, with types A and B causing annual human epidemics.[26] The trimeric hemagglutinin (HA) glycoprotein is a major determinant of virulence and

Table 1—*Challenges and Strategies in Influenza Vaccine Development*

Challenges	Strategies
Antigenic drift/shift of influenza virus	Enhanced antigenic match by development of universal influenza vaccine (protects against most varieties of influenza strains and subtypes): 1. Candidate vaccine would elicit antibodies against the conserved HA stem region that cross-react with H1 and H3 HA[1] 2. Candidate vaccine would use the extracellular domains of M2 and NP as protective antigens[2] 3. Development of candidate universal influenza vaccines based on gene signatures linked to development of broadly protective antibodies[3] Enhancement of cell-mediated responses by LAIV[4]
Individual variations in immune response to influenza	Use of a patient-centered, individualized approach: 1. Increase current knowledge of host genetic polymorphisms in the immune response to influenza vaccine[5] 2. Use of systems vaccinology tools to further understand the impact of individual genetic variations in the development of protective immunity[3]
Suboptimal immunogenicity in at-risk populations: elderly people (immunosenescence), people with comorbidities (severe asthma)	Development of adjuvanted influenza vaccines[6] Robust priming of the cellular arm of the immune system with virosomes[7] Production of high-dose influenza vaccine formulations[8] Early identification of nonresponders by use of vaccine chips[3]
Need for vaccines that induce a rapid rise of protective antibodies in the setting of an influenza pandemic	Prepandemic priming by an MF59-adjuvanted influenza vaccine followed by subsequent boosting of long-lived human memory B cells in the pandemic setting[9]
Need for vaccines that can be produced rapidly in response to an incipient pandemic	Surveillance aimed at identifying precursor pandemic viruses with novel NA[10]
Limited availability of subunit HA and NA proteins for vaccine production	Antigen-sparing vaccine regimes by development of adjuvant-based vaccines[11-15]
Prolonged vaccine manufacturing timetable	Shortening of vaccine production time frame by: 1. Use of plasmid-based reverse genetics technology for generation of vaccine reference strains[16,17] 2. Development of a nonreplicative adenovirus-vectored influenza vaccine that can be produced without the prerequisite of growing influenza virus in eggs or cells[18]
Unbalanced response to HA and NA in current influenza vaccines	Development of influenza vaccines with higher amounts of NA antigen[19]
Low immunogenicity of pandemic influenza strains in immunologically naive individuals	Development of new adjuvanted vaccines[9]
Prolonged time needed to evaluate new influenza vaccines in clinical trials, especially in the context of a pandemic	Identification of early molecular signatures that can be used to predict later immune responses[20,21] Application of knowledge gained from early innate immune signatures of suboptimal vaccines to develop better ones[3]

HA = hemagglutinin; LAIV = live attenuated influenza vaccine; M2 = matrix 2 ion channel protein; NA = neuraminidase; NP = nucleoprotein.

is responsible for attachment of the virus to specific sialic acid-containing proteins on the host cell surface and, ultimately, fusion between the viral and endosome membranes and release of viral nucleic acids into the cytoplasm. The HA protein contains two structural elements: the head (the primary target of antibodies that confer protective immunity to influenza viruses[27]) and the stalk. Both elements differ in their potential utility as vaccine targets (discussed in " Steps Toward Development of a Universal Influenza Vaccine" section). Proteolytic cleavage of the HA is necessary to generate infectious virus, but the role of HA cleavability in pathogenesis in humans currently is unknown. However, influenza viruses are known to use the nonstructural protein 1 as a mechanism to circumvent the host type I interferon response (discussed in "Using Transcriptional Profiling to Identify Influenza-Specific Virulence Factors and Responses to Human Vaccine Adjuvants" section). Neuraminidase (NA) is a glycoprotein that removes sialic acid from viral proteins and prevents aggregation of the virus by the HA protein binding to other proteins. Antibodies to NA aggregate viruses on the cell surface, effectively reducing the amount of virus released from infected cells.[19] Although immune response to influenza surface glycoproteins is mainly humoral, $CD4^+$ and $CD8^+$ T cells also play important roles in immunity to influenza.

In contrast to the strain-specific response of antibodies, cell-mediated immunity tends to be more cross-reactive among viral subtypes, recognizing more conserved epitopes on the surface proteins and internal viral proteins.[28] The discovery of epitopes on relatively well-conserved influenza proteins, such as nucleoprotein (NP) M, and the stem of the HA trimers serves as the basis for the development of a universal influenza vaccine capable of generating responses that are cross-reactive between subtypes (discussed in "Steps Toward Development of a Universal Influenza Vaccine" section).

Because of their error-prone polymerases, influenza viruses undergo genetic changes that lead to gradual antigenic changes in both HA and NA, a process known as antigenic drift that leads to the emergence of new variant strains.[29] Antigenic shift occurs when the currently circulating influenza A virus disappears and is replaced by a new subtype with novel glycoproteins to which antibodies against the previously circulating subtype do not cross-react.[30] However, exposure to a 1918-like H1N1 virus (from previous infection or vaccination) contributed to the induction of cross-reactive antibody response to the 2009 influenza A(H1N1) virus.[31]

Phylogenetic analyses of influenza gene sequences showed that strains of the 20th century pandemics originated by multiple reassortments of viral genes between human and animal viruses. Although a new subtype has always a novel HA, these analyses showed that novel NA and internal genes were introduced into the prevailing human virus strains before acquisition of the novel pandemic HA. Surveillance measures aimed at identifying these precursor viruses could alert public health officials in advance to the emergence of future pandemics.[10]

Vaccination is the primary strategy for prevention and control of influenza,[32,33] but the influenza vaccine has to be reformulated almost every year to take into account the ever-changing virus. Protection after natural infection is primarily mediated by HA-specific antibodies in serum and mucosa, whereas antibody directed against the NA, conserved influenza proteins, and T-cell responses reduces disease severity through enhancing viral clearance.[26,30,34] Based on serologic studies of influenza using human serum, a hemagglutination inhibition (HAI) titer of 1:32 to 1:40 represents the titer range at which approximately 50% of individuals will be protected from infection.[35] There is no HAI titer that can guarantee protection from infection,[36-39] although elevated levels of serum anti-HA antibody generally are correlated with resistance to influenza infection, and lower antibody levels are associated with increased risk of illness among persons exposed to influenza.[40-43] The identification of additional early adaptive and innate immune mediators is of particular importance in the development of pandemic influenza vaccines (discussed in "Innate Immune Signatures Predictive of Influenza Vaccine Immunogenicity" section).

Development of Cross-Protective Influenza Vaccines

In addition to recipient's age and health status, the protection conferred by vaccines depends on the extent of antigenic match between the viruses in the vaccine and those that are circulating during a given influenza season.[44]

Traditional Antigens in Influenza Vaccine Production

Each dose of seasonal influenza vaccine is formulated to contain the viruses (or their HA proteins) representing the influenza A(H3N2), A(H1N1), and influenza B strains considered to be the most likely to circulate in the upcoming influenza season. Vaccine production in the United States begins with the generation of vaccine reference strains, that is, hybrid viruses with the HA and NA genes from the drifted variant combined with other genes from a laboratory strain adapted to grow well in eggs.[26] Plasmid-based reverse genetics technology is now being used to reliably generate reference strains in a shorter time frame.[16,17] The virions harvested from eggs are chemically inactivated, the viral envelope is disrupted with detergents, and the HA and NA proteins are then purified. HA is the main immunogen in inactivated influenza vaccines, and levels of HA are used to standardize vaccine doses. The quantity of NA is not standardized and may vary greatly for different manufacturers and production lots. The levels of NA antibody also appear to be critical for protection, and there is concern that human influenza vaccines do not include enough NA protein to induce a strong protective antibody response.[19]

The master virus strains (MVSs) for each of the three influenza virus components in the live attenuated influenza vaccine (LAIV) are created through genetic reassortment. In this process, the stable, attenuated, cold-adapted master donor virus is mated in tissue culture with the anticipated epidemic wild-type strain, yielding an MVS that contains two genetic segments that encode for HA and NA derived from the antigenically relevant influenza viruses recommended for inclusion in the annual vaccine formulation by the Centers for Disease Control and Prevention and US Food and Drug Administration. The remaining genetic backbone of the MVS is derived from the master donor virus containing mutations that confer the cold-adapted, temperature-sensitive, and attenuated phenotypes.[45] LAIV stimulates a strong cross-reactive antibody response in children and provides a high

level of protection against antigenic drift strains in an influenza season in which there is a suboptimal match between the vaccine strain and the epidemic strain. Additionally, LAIV induces nasal IgA antibody, which correlates with protection.[4]

Alternative Antigens in Influenza Vaccine Production

Vaccine candidates can be developed quickly after the HA gene has been sequenced, thereby eliminating the need to use pathogenic viruses or to adapt viruses to grow in eggs or cell culture.[26] HA genes have been cloned into vector viruses, resulting in recombinant vaccines expressing the HA protein that induce protective cellular and antibody responses against the vaccine virus and antigenically drifted strains in animal studies.[46-50] Intranasal and epicutaneous administration of an adenovirus-vectored influenza vaccine has been shown to be an effective means of eliciting protective HA antibodies in early safety and immunogenicity clinical trials.[18]

Adjuvants have been used to augment the immune response to vaccine antigens since the 1920s. Both their benefits and their side effects are associated with activation of certain components of the innate immune system.[51] Adjuvants permit antigen-sparing regimes, provide flexibility in the time interval between vaccinations, improve antibody avidity and breadth of immunity to drifted virus strains, and increase vaccine response in elderly people.[6,11-15]

The squalene-containing oil-in-water emulsions currently used as adjuvants for licensed inactivated influenza vaccines outside of the United States include MF59 and AS03.[52] In AS03, squalene is combined with α-tocopherol, increasing the total oil content in AS03 compared with MF59. MF59 works in a Nalp3-independent fashion.[53,54] Its effects are exerted at the injection site where it induces a strong influx of granulocytes (neutrophils, eosinophils) and several antigen-presenting cell (APC) types (monocytes, macrophages, and dendritic cells) that take up antigen and adjuvant and transport them to draining lymph nodes.[55] Distant priming with MF59-adjuvanted influenza vaccines results in a pool of cross-reactive memory B cells that can be boosted rapidly years afterward by a mismatched MF59-adjuvanted vaccine to generate high titers of cross-reactive neutralizing antibodies.[9] AS03 enhances the antigen-specific adaptive immune response by promotion of monocytes, not dendritic cells, as the principal APCs and by its effects on granulocyte function and cytokine production.[56]

Virosomes consist of phospholipids that spontaneously form virus-like vesicles to which the influenza virus surface glycoprotein HA and NA are anchored.[57] Virosomes interact with B lymphocytes and are taken up by APCs, inducing a stronger T helper (Th) 1 response than that induced by nonadjuvanted influenza vaccines.[7]

Steps Toward Development of a Universal Influenza Vaccine

A universal influenza vaccine has the capacity to protect against most varieties of influenza strains and subtypes. Many strategies for developing a universal influenza vaccine are based on raising an immune response against influenza proteins that are highly conserved across all strains.[58,59] Heterotypic immunity (cross-protection) refers to the protection of some individuals to subtypes of influenza virus to which they have not previously been exposed through natural infection or immunization. A possible explanation for cross-protective immunity has arisen from an understanding of the molecular targets of broadly neutralizing monoclonal antibodies directed against various portions of the HA protein.

A specific region of the HA stem is highly conserved among many viral strains, and it has been possible to stimulate the production of stem-targeting antibodies with cross-neutralizing properties in several host species, including nonhuman primates.[60] Immune responses to the 2009 pandemic H1N1 influenza infection include broadly cross-reactive antibodies against the HA stalk and head domain epitopes of multiple influenza strains.[61] The majority of HA-specific antibodies from healthy recipients of the pandemic H1N1 influenza vaccine also showed broad cross-reactivity to the HA head, and three broad cross-reactive antibodies were shown to bind the HA stem.[62]

Human heterotypic (cross-protective) antibodies were generated after administration of seasonal influenza vaccination, resulting in production of serum IgG that cross-reacted with the H5 HA. By immortalizing memory B cells from these individuals, the researchers isolated a panel of 20 heterotypic neutralizing monoclonal antibodies that neutralized viruses belonging to several HA subtypes (H1, H2, H5, H6, and H9), including the pandemic A/California/07/09 H1N1 isolate.[63] By interrogating $>100{,}000$ plasma cells from eight human donors infected with or immunized against multiple strains of influenza A, scientists isolated a neutralizing monoclonal antibody (F16) that recognized the HA proteins present on all 16 influenza subtypes and neutralized both H1 and H3 influenza A viruses by binding to a conserved epitope in the fusion subdomain (the stem of the HA trimers). Passive transfer of this antibody conferred protection from infection and death in animals, suggesting that eliciting antibodies against the HA stem region that cross-react with H1 and H3 HA (or across subtypes) is a realistic approach to developing universal influenza vaccines.[1]

Other approaches have focused on using conserved epitopes of the viral proteins, including the extracellular domain of matrix 2 ion channel protein (M2) and NP as protective antigens. Based on H5N1 challenges in ferrets, immunization with DNA vaccines encoding for HA induces higher neutralizing antibody titer than NP and M2 DNA immunization, suggesting that NP and M2 may require combinatorial vaccination with HA to be suitable candidates for universal influenza vaccines.[2]

A thorough understanding of broadly protective antibody responses to conserved epitopes is critical to the development of a universal vaccine, but host responses are also highly important. Multiple associations between variations in humoral immunity to seasonal influenza vaccines and human leukocyte antigen, cytokine, and cytokine receptor gene polymorphisms have been identified. Identifying associations between variations in vaccine immune responses and gene polymorphisms is critical in the development of universal influenza vaccines capable of generating long-lasting protective immune responses against highly conserved influenza proteins because these genes are important transcriptional targets during vaccine immune responses.[5] Nevertheless, the current evaluation of the immunogenicity of influenza vaccines based exclusively on the end result of development of adaptive immune responses is, at the very least, incomplete and does not acknowledge the potential of innate responses that develop shortly after vaccination as immunologic markers of response (discussed in "Innate Immune Signatures Predictive of Influenza Vaccine Immunogenicity" section)

Systems Vaccinology

Our understanding of innate and adaptive immune responses in vivo has been greatly improved through the use of systems biology. This evolving interdisciplinary approach systematically describes the interactions between all the parts of a biologic system with a view to predicting the behavior of that system.[64] Systems biology, or systems vaccinology in the context of vaccine development, requires the use of highly sophisticated and powerful mathematical and computational modeling techniques to reveal, measure, and predict vaccine-induced immunity phenomena, including, but not limited to, the differences in gene expression through hundreds to thousands of probe sets (collection of probes) designed to interrogate a given sequence.

Generic and Influenza-Specific Transcriptional Profiling

The DNA microarray chip is a systems biology tool comprising a large ordered array of DNA probes of known sequence printed onto a solid support and fabricated on a very small scale. The probes are used to interrogate the composition of complex DNA (including cDNA generated from RNA transcripts) mixtures through hybridization.[65] Such DNA microarrays are used to generate a transcriptional profile (gene expression signature), which represents a snapshot of genes expressed at a particular point in time by a specific cell type or tissue.

A combined analysis of published transcriptional profiling data generated from 32 studies and 77 different host-pathogen interactions led to the identification of a cluster of 511 genes that comprise a common host response induced in many different cell types to different pathogens.[65] The group of genes that is most strongly and consistently upregulated consists of those encoding cytokines (called the inflammatory/chemotactic cytokine cluster). Of note, upregulation of the inflammatory/chemotactic cytokine gene cluster is relatively weak after influenza virus infection, which activates mammalian cells through the innate immune receptor for single-stranded RNA Toll-like receptor (TLR) 7[66] and possibly through TLR3. In contrast, the influenza virus causes a greater upregulation of interferon and interferon-induced genes, including several chemokine genes, favoring the development of a Th1-type immune response.

Using Transcriptional Profiling to Identify Influenza-Specific Virulence Factors and Responses to Human Vaccine Adjuvants

Transcriptional profiling has been used successfully to compare the influence of viral proteins from different strains and subtypes on the immune response. This approach has shown that repression of host gene expression can be localized to virus proteins that are known to cause severe disease. For example, the *NS1* gene of the influenza A virus plays a central role in inhibiting interferon-, cytokine-, and nuclear factor κB-dependent signaling pathways. Viruses containing the 1918 pandemic *NS1* blocked the expression of interferon-regulated genes more effectively than those containing *NS1* from more contemporary strains.[67] Transcriptome analyses have also shown that MF59 is a potent inducer of genes involved in leukocyte migration, specifically *JunB* and *Ptx3*.[68]

Transcriptional Profiles Underlying Clinical Symptoms of Influenza Infection

Studies have analyzed in vivo changes in gene expression in peripheral blood leukocytes of subjects with influenza A infection.[69-71] Key transcriptional differences between asymptomatic and symptomatic host responses to influenza A infection have been observed. Specifically, a cluster of genes, including those encoding several pattern-recognition receptors

that regulate immune responses acting as viral RNA sensors (TLR7, RNA helicases, and interferon induced with helicase C domain 1), is strongly activated only in symptomatic subjects. This unique transcriptional signature manifests 36 h before peak symptoms and is predictive of disease severity.[71]

Innate Immune Signatures Predictive of Influenza Vaccine Immunogenicity

Systems biology approaches (using multiplex cytokine analysis, flow cytometry, and microarray transcriptional profiling) have been used to identify early molecular signatures that can be used to predict later immune responses and gain a better insight into the mechanisms that underlie immunogenicity.[20,21] Genes whose products are involved in viral sensing through TLR7 and TLR8, major histocompatibility complex class 1-mediated antigen presentation, interferon signaling, inflammation, and the nuclear factor κB and Janus kinase/signal transducers and activators of transcription signaling pathways all play a central role in the vaccine response to trivalent influenza vaccine (TIV) in adults within 24 h after immunization.[20] Analyses of these early patterns of gene expression revealed a 494-gene transcriptional signature that correlated with the magnitude of the antibody response. Expression of *CD74*, *HLA-E*, *E2F2*, and *PTEN* most strongly correlated with the magnitude of the antibody response. *STAT1* expression (associated with interferon signaling pathways) increases after vaccination, most prominently on day 1 and in the high-responder group. *E2F2* expression (transcriptionally represses cell cycle genes to maintain quiescence) is downregulated after vaccination, most prominently on day 3 and in the high-responder group. The difference between *STAT1* and *E2F2* expression was sufficient to predict early after vaccination whether an individual would ultimately be a high or low responder, as judged by antibody responses.[20]

Nakaya et al[21] used systems biology tools to compare the innate and adaptive immune responses to vaccination with TIV and LAIV. Among the genes induced by vaccination with TIV, these investigators found that genes that were preferentially expressed by antibody-secreting cells were enriched. This result may have reflected the rapid proliferation of plasmablasts after vaccination; however, microarray analysis of B cells sorted from vaccinated subjects favored the conclusion that the changes in expression observed represented real transcriptional changes in B cells. Of note, expression of *XBP-1*, a transcription factor essential for the differentiation of B cells (as well as its target genes), was strongly upregulated at day 7 after vaccination. This upregulation correlated with HAI responses. In contrast to results obtained for TIV, analysis of the transcriptional signature induced by LAIV showed considerable enrichment for genes highly expressed in T cells and monocytes. In particular, expression of interferon-related genes in monocytes on day 3 after vaccination was correlated to the HAI response, suggesting a link between the interferon response and the antibody response.

With the use of bioinformatics modeling, such as discriminant analysis through mixed-integer programming, 42 sets of genes were identified that could be used to predict a magnitude of the antibody response of fourfold or greater 4 weeks after TIV vaccination. Key genes in the predictive gene signatures were *TNFRSF17* (tumor necrosis factor receptor superfamily member 17, a B-cell maturation factor), a gene previously used to predict the magnitude of antibody responses to vaccination with the yellow fever vaccine YF-17D,[72] and *CD38*, which encodes a surface protein important in lymphocyte development. It remains to be seen whether the *TNFRSF17* is part of a large network of genes whose transcriptional signature represents a common predictor of antibody responses to other vaccines. Another gene, *CAMK4* (encoding the calcium/calmodulin-dependent protein kinase type IV [CaMK-IV]), was also identified in the TIV discriminant analysis through mixed-integer programming model.[21] The expression of *CAMK4* at day 3 postvaccination was inversely correlated with plasma HAI antibody titers at day 28. Vaccination of CaMK-IV-deficient mice with TIV induced enhanced antigen-specific antibody titers, demonstrating an unappreciated role for CaMK-IV in the regulation of antibody responses. These data suggest that novel surrogate gene markers may be useful in predicting the magnitude of host responses to influenza vaccines and in shortening the time needed to evaluate protective vaccine responses in clinical trials by focusing on predictive innate responses at strategic early time points (eg, days 0, 3, 7) rather than on humoral responses developing weeks after vaccination. To the best of our knowledge, there are no published data to date on transcriptional profiling signatures generated by IgA-secreting B cells in the nasal mucosa of recipients of TIV or LAIV.

Systems Vaccinology and Influenza Vaccine Development

The individual variability in immune responses to influenza within a population is affected by age. Up to 50% of elderly recipients of influenza vaccines fail to respond to TIV with a fourfold increase in HAI titers,[35] and the presence of comorbidities, such as asthma, results in lower seroprotection rates in elderly subjects than in younger subjects to the same vaccine dose, particularly those with severe asthma.[8] Elderly

people exhibit suboptimal cytokine secretion, a chronic inflammatory state ("inflammaging") as well as diminished T-cell, B-cell, and APC function (generally referred to as immunosenescence). More specifically, frequencies of TIV-specific antibody-secreting cells circulating after vaccination are significantly lower in elderly subjects than in younger subjects, resulting in dysfunctional immunity and impaired memory formation.[73,74]

The identification of predictive signatures of vaccines would allow for the development of a vaccine chip with a few hundred genes that could interrogate a particular type of innate or adaptive immune response (eg, magnitude of effector $CD8^+$ T-cell response; frequency of Th cells; balance of Th1, Th2, and Th17 cells; high-affinity antibody titers). Such a chip would permit rapid identification (or prediction) of suboptimal responses in elderly people and other groups, such as infants and immunocompromised individuals.[3] Moreover, these chips could be useful for the identification of safe and effective adjuvants that could be used to boost antibody responses in such low responders.

Early identification of low responders is of critical importance in the context of pandemics or when conducting vaccine clinical trials under adaptive designs. In both situations, systems vaccinology would allow for rapid evaluation of strength, type, duration, and quality of protective immune responses stimulated by the vaccine and guide the refinement of vaccine formulations, delivery systems, and the overall development of vaccines with improved immunogenicity.[3,75]

Conclusions

Analyzing early changes in the transcriptome after influenza vaccination and how those changes correlate with or can be used to predict local antibody responses is critical to influenza vaccine development and public health. Future studies should focus on analyzing changes in the transcriptome of the nasal mucosa after vaccination with LAIV.

Systems vaccinology will continue to play an essential role in the discovery of correlates (most importantly, early signatures) of protection and predictors of antibody responses to other vaccines and common gene networks critically involved in regulating antibody responses to different vaccines. Systems approaches should provide a better understanding for how specific genetic variations contribute to protective immunity at both the individual and the population level. These techniques should also define the process of immunosenescence, which impedes the protection afforded by current seasonal influenza vaccines at the population level and could similarly impede the protection provided by a universal influenza vaccine. Systems vaccinology could further support the development of a universal influenza vaccine by revealing genetic signatures of plasmablasts and plasma cells that have produced broadly protective antibodies in subjects infected or vaccinated with seasonal and pandemic influenza vaccines.

The use of high-throughput screening methods has led to the recent identification of a human neutralizing monoclonal antibody that recognizes the HA glycoprotein from all 16 subtypes and neutralizes H1 and H3 HA of influenza A viruses. Such studies could further inform universal vaccine development through the identification of rare epitopes on viral proteins such as HA, M2, NP and others recognized by broadly protective antibodies. Finally, adjuvants permit antigen-sparing regimens, which are of critical importance for vaccine manufacturing in the context of a pandemic. Moreover, adjuvants rapidly stimulate the production of long-lived memory B-cell responses, leading to long-lasting protection.

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