Dynamic Perspectives on the Search for a Universal Influenza Vaccine

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A universal influenza vaccine (UIV) could considerably alleviate the public health burden of both seasonal and pandemic influenza. Although significant progress has been achieved in clarifying basic immunology and virology relating to UIV, several important questions relating to the dynamics of infection, immunity, and pathogen evolution remain unsolved. In this study, we review these gaps, which span integrative levels, from cellular to global and timescales from molecular events to decades. We argue that they can be best addressed by a tight integration of empirical (laboratory, epidemiological) research and theory and suggest fruitful areas for this synthesis. In particular, quantifying natural and vaccinal limitations on viral transmission are central to this effort.

Keywords. influenza vaccination; shedding-transmission relationship; viral eco-evolutionary dynamics.

Influenza A viruses (IAVs) and influenza B viruses (IBVs) are segmented ribonucleic acid (RNA) viruses that exert significant morbidity and mortality on human populations. Although IAVs infect multiple animal species, IBVs principally infect seals and humans. Governing the epidemiology and evolution of these viruses are dynamical processes at multiple scales, from molecular interactions to global transmission. Many control efforts have focused on vaccines, although current formulations are of only medium efficacy and vulnerable to the evolutionary dynamics of the virus. Currently, there is a major focus on universal influenza vaccine (UIV) to address these issues, and unraveling the biological dynamics underpinning this goal is vital.

Across scales, however, there remain significant gaps in empirical work and theory; these shortcomings center on global availability of observational and experimental data quantifying dynamic processes. The coevolution of data collection, processing, and availability with new theory will lead to insights into universal vaccination. In particular, understanding the potential impacts of a UIV on evolution and transmission dynamics *a priori* could aid in optimizing strategies for UIV development. In the rest of this section, we provide a brief overview of the processes governing influenza transmission and evolution, in addition to the techniques developed to quantify these processes. At the finest scale, host-pathogen interactions reflect

molecular interactions between target cells, viral particles, and the immune response. In particular, key processes of infection, immunity, and vaccinology center on the viral antigens, namely, the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). As a consequence of immune selection and variation, driven largely by antibody pressure and allowed by the error-prone RNA polymerase [1], these antigens significantly evolve on a rapid (often annual) timescale via “antigenic drift” [2]. Experimental and computational techniques have mapped influenza strains in antigenic space [3–5], clearly illustrating drift. As a result of rapid evolution, seasonal epidemics of influenza exert a significant burden of disease [6]. As such, surveillance programs (particularly the Global Influenza Surveillance Network of the World Health Organization [WHO]) have facilitated tracking drift over decades, and software has been developed to analyze real-time data [7]. Influenza A viruses are separated into subtypes based on their HA and NA, and these each comprise 2 phylogenetic clades; groups 1 and 2 [8]. A more drastic evolution of IAVs can occur through reassortment of antigens to which the human immune system has little or no previous immunity (eg, from avian and porcine origins) [2]. This antigenic shift caused the major pandemics of influenza (eg, 1918, 1957, 1968, 2009) [9], with significant impacts on human health.

Due to the rapid evolution of influenza viruses, developing a predictive science of control necessitates a comprehensive understanding of the processes governing antigenic drift. Influenza A viruses are single-stranded RNA viruses that have 8 segments. Two segments code for the major immunogenic antigens (ie, HA and NA), whereas other segments code for other proteins such as RNA polymerase subunits or structural proteins (eg, nucleoprotein [NP]). The HA protein facilitates viral

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entry and represents the major target of the humoral immune response, thereby a major component of current vaccines and candidate for vaccines. This protein is formed of the “head” (HA1) and the “stem” (HA2) subunits. The HA head is immunodominant, and antibodies targeting HA bind to exposed regions on its surface. These epitopes (or antigenic sites) have been determined experimentally (see [10]) or predicted computationally (see [11]). Due to strong selective pressures from host immune systems, seasonal viruses vary highly at these sites. Thus, seasonal vaccines targeting HA head epitopes need to be updated yearly. In contrast, other regions of HA (and other proteins) are more conserved. These regions, along with NA and other structural proteins that are conserved, are also considered for vaccine design [12]. Current seasonal vaccines are either inactivated (eliciting an antibody response to the HA head) or live attenuated (eliciting a broader response that includes T cells). The former approach is older and has clear correlates of protection, whereas the latter elicits a broader response but lacks proper correlates of protection (see [13] for a detailed comparison).

To understand biological dynamics, considerable insight can be gleaned from appropriate abstraction. Mathematical models in virology, immunology, and epidemiology have made significant contributions to understanding disease dynamics and evaluating disease control strategies [4, 14–16]. The first complexity that arises for influenza is a result of the large number of interactions for multistrain pathogens. To resolve this issue, modeling from the perspective of the pathogen instead of the host (ie, status-based modeling) [17] is an appropriate mathematical approach. Moreover, for rapidly evolving pathogens, evolutionary processes cannot be decoupled from transmission dynamics. Cross-scale approaches are crucial for understanding key levels of viral and immune selection (cellular, whole organism, transmission, herd immunity, global dispersal). “Phylogenetics” [4, 18] integrates these processes. Explicit fitness models of HA have also been formulated [19, 20], with remarkable predictive ability for yearly predictions of emerging strains based on existing data.

Figure 1 illustrates these processes at different integrative scales. Fully understanding influenza ecology, evolution, and immunological control requires synthesizing dynamics across these levels, particularly in the context of vaccine design and the search for a universal vaccine. From the molecular processes to phylogenies on a global scale, by way of viral transmission to local epidemics and regional heterogeneity, many experimental and modeling studies have highlighted how these factors influence long-term disease dynamics outcomes (see Refs. [21 and 22]).

Influenza vaccinology has had a major impact on the reduction of disease and transmission. Iterations of current influenza vaccines are reviewed elsewhere [12]; the ultimate goal is a universal vaccine with broad heterosubtypic immunity. Influenza virology and vaccinology history has been guided by often highly empirical approaches; however, there is growing interest

in “rational vaccinology,” based on a solid informatic and theoretical basis. In this paper, we review recent developments at the interface of theory and experiment, while emphasizing the remaining issues to be addressed. We first briefly summarize the current state of UIV, then we set this in context of theoretical and empirical demands to understand cross-scale influenza dynamics and evolution. Finally, we address perspectives for success in the pursuit of UIV, based on dynamic processes with empirical work.

CURRENT PROGRESS OF VACCINES AGAINST INFLUENZA VIRUSES

Understanding the evolutionary and transmission dynamics of influenza viruses provides an important informatic basis for informing prevention strategies, and recent experimental approaches, sometime in conjunction with theory have led to exciting developments. Diverse approaches toward vaccination are currently in use, under trials, or have been proposed, and many of these involve either HA or NA (Figure 2). Targeting different regions (eg, conserved or variable epitopes) on various influenza proteins (eg, HA, NA) could result in differing selection pressures on influenza viruses, and thus may alter their evolutionary dynamics, in addition to affecting host susceptibility and transmission. Understanding this interplay is important for UIV success, to ensure reduced susceptibility and prolonged immunity.

ADVANCES IN HEMAGGLUTININ MODELING AND PREDICTION OF IMMUNITY

In terms of HA, key observations for UIV developments have been gleaned from significant progress in understanding molecular mechanisms underlying HA evolution and immune response. Perhaps the most important foundation to this progress is surveillance. To monitor strains present in the population, the HA inhibition assay is used; based on these surveillance data, the WHO selects strains for vaccine development. Currently, seasonal vaccines target variable regions on the HA head (Figure 2), but this approach is limited and requires frequent updating of the vaccine formulation. Moreover, from a molecular biophysical perspective, there are many other potential complexities with achieving herd immunity, one example being antibody interference [31, 34, 35].

HEMAGGLUTININ STEM APPROACH: ISSUES AND POTENTIAL SOLUTIONS

Whereas new antigenic variants of the HA head subunit emerge often, HA stem regions exhibit considerably stronger conservation [36]. This observation has led to the scrutiny of HA stem regions for UIV, particularly because transmission and replication of influenza viruses are impeded by antibodies to HA stem [37]. In addition, vaccines eliciting stem antibodies have potential for heterosubtypic protection within HA groups [38], and vaccines targeting group 2

Figure 1. Processes driving influenza evolution and transmission dynamics at molecular (1), within host (2), population (3), and phylogeny (4) scales. New strains emerge due to mutation, and they are subject to selection pressures within hosts. As a result of a primary infection, imprinting provides broad heterosubtypic cross-protection. Host heterogeneity and local epidemics encompass population-level dynamics. These previous processes in conjunction with regional heterogeneity result in distinct lineages that are represented in phylogenies. This figure is adapted from Refs. [4, 23–26], and imprinting is presented in Ref. [27].

HA stem regions may generate broader responses than those for group 1 [39]. However, the immunodominance of the HA head can lower the effectiveness of stem vaccines, but the replacement of heads with zoonotic variants in a chosen vaccine strain (resulting in chimeric HA vaccines) avoids a strong immune response to the head, preventing interference [40]. Although the HA stem has many characteristics that are promising for future vaccines, further

work remains to resolve the role of stem epitopes (and antibodies) in “imprinting”, that is, the heterosubtypic heightened immune response within phylogenetic groups of IAVs [27]. Further work remains to resolve the role of stem epitopes in imprinting, that is, the heterosubtypic heightened immune response within phylogenetic groups of IAVs, in addition to delivery of these antigens to maximize the immune response that could overcome imprinting.

Figure 2. Current and emerging vaccination strategies to control influenza viruses. For HA, these include strain-specific, seasonal vaccines targeting regions on the head (1), others targeting conserved stem epitopes for broader cross-immunity (3), or both stem and head conserved epitopes. Other approaches involve targeting the NA conserved epitope (4) or epitopes that elicit broad responses (5). This figure is adapted from Refs. [28–33].

A significant current issue with targeting the stem of HA for vaccine development is that previous exposure to any influenza strains leads to an immune response toward epitopes on the head of HA. This response leads to an inhibition of vaccine-induced immunity toward the stem, because serum concentration of antibodies to stem regions are low [41]. To circumvent this problem, it may be necessary to select conserved epitopes on both the head (eg, see [36] for a new such epitope) and the stem [32], but further investigation into selection pressure on escape mutations is required for the HA stem. Moreover, although there are data (for H1) pertaining to single mutation effects in vitro [42], more of these for other subtypes,

in conjunction with studying epistatic interactions between HA residues and in vivo experiments, could aid in careful selection of HA stem epitopes for higher vaccine effectiveness. These developments could eventually lead to omission of HA head epitopes in vaccines.

OTHER APPROACHES TO CROSS-PROTECTION IMMUNIZATION

Although a large fraction of the existing literature has focused on vaccine design based on HA regions, there are other approaches that are under study. In particular, there has been significant recent interest in vaccines eliciting NA antibodies

[43], because these could improve existing vaccines rapidly [44]. For instance, certain NA epitopes elicit broad immune response [28], and their inclusion in seasonal vaccines could drastically change protection [43, 44]. For example, there is a unique conserved NA epitope in IAVs and IBVs that was found to be key for proper NA function [33]; however, antibodies to this epitope seem to provide low cross-protection, and targeting this conserved region could improve vaccine effectiveness.

In addition to NA, internal antigens could also be targets of vaccines, particularly because these tend to have higher

conservation due to weaker selection pressures. There have also been developments toward an influenza vaccine targeting the matrix protein M2 ectodomain (M2e), a highly conserved internal protein [45–47]. Among others, T-cell epitopes generate broader cross-protection [48] than HA regions, thereby generating significant attention in the pursuit of UIV [49, 50]. Other conserved internal proteins that have been used for vaccine design are the NP and the matrix protein M1 [51–54], with these approaches aiming to elicit T-cell immunity.

Box 1. Shedding, transmission, and the basic reproduction number

Understanding the dynamics of viral shedding from a host is central to quantify transmission, hence evolution and the impact of control. Host shedding is depicted schematically as the curve a in panel I [55]. Immunity from vaccination or previous infection alters the shedding after the initial incubation (ending at t_0) in 2 ways. Either immunity reduces the maximum shedding (curve b) or decreases the total infectious period (curve c). The explicit relationship between viral shedding and transmission remains elusive (see panel II for a possible illustration), yet it underlies scaling from viral kinetics to population-levels dynamics. Simple models interpreting data showed that the best fit for H3N2 is $\text{Transmission} \propto \text{Shedding}^{0.156}$ [56], but these models do not consider defective interacting particles [57, 58] that may reduce transmission at high viral shedding.

The basic reproduction number R_0 is defined as the number of infectious individuals that 1 individual would infect in a fully naive population [15]. Partitioning the components of R_0 gives

$$R_0 = \text{infection rate per day} \times \text{infectious period}.$$

As the infection rate varies over time, due to the change in viral shedding,

$$R_0 \propto \int_{t_0}^t \text{Shedding } d\tau \quad [59],$$

with this area under the shedding curve a shown in panel I. For influenza, $R_0 \approx 2$ [59]. With no heterogeneity, the aim for herd immunity threshold, ie, the proportion p of individuals needed to be vaccinated to avert an epidemic [14], is

$$p = 1 - \frac{1}{R_0} \approx 0.5.$$

Although previous immunity for influenza and other partially immunizing pathogens can reduce shedding and thus affect transmission, past exposure can also reduce susceptibility. Human populations almost always have some immunity to a new influenza strain due to previous infection. As amino acid homology between 2 strains decreases, the infectious period and the probability of infection both increase (panel III) [60]. Transmission also increases with a decrease in homology (panel III), leading to larger epidemics and thus illustrating the importance of relating viral shedding to transmission for different levels of homology. For pathogens with previous immunity in the population, the effective reproduction number, defined as

$$R_{\text{eff}} = R_0 \times \text{proportion susceptible} \quad [15],$$

governs the dynamics of a new strain. If this quantity is greater than 1, then an outbreak occurs, whereas if it is less than 1, the strain dies out. Thus, measuring shedding under different vaccination scenarios, in conjunction with determining the relationship between transmission and shedding, is important to lower influenza burden. Moreover, determining the proportion susceptible is also crucial, yet measuring this requires knowledge of previous infections. Hosts will have invariably seen multiple infections across their lifetimes, and cross-immunity between strains is complicated by processes such as imprinting and antibody interference [27, 31].

FROM MOLECULAR TO GLOBAL: CROSS-SCALE DYNAMICS AND VACCINATION

In parallel with a mass of experimental work on influenza immunology, vaccinology, and epidemiology, there has been considerable theoretical research to unravel the dynamical processes at their roots. However, the experimental and theoretical approaches have not fully converged. Arguably, the central process is transmission. In this study, population biology helps us to summarize this complex process (Box 1). Essentially, transmission determines whether strains will successfully spread (and thus governs vaccine coverages needed to avert epidemics), and quantifying transmission requires understanding its relationship with viral shedding.

Table 1 summarizes recent progress in computational biology and modeling to quantify influenza transmission dynamics and its implications for epidemiology and control. However, a crucial step is shaping the empirical work to emphasize connections with new theory, and this is also summarized in Table 1. Essentially, the formulation and analyses of data-driven models will be ameliorated through new data collection methods that improve speed and accuracy of sampling and detection of infection, in conjunction with software for data analysis (eg, see [7]). In this section, we review theoretical progress on understanding influenza dynamics across scales, with specific focus on the interplays of immunity and vaccination with individual and population-level dynamics.

For previously infected individuals, cross-immunity to an incipient strain impacts successful transmission, but also may have dramatic repercussions on population-level dynamics and the global spread of influenza. Perhaps the first proposed within-host mechanism involved in cross-immunity is “original antigenic sin” [85], or “antigenic seniority” [86]. In other words, an individual’s immune response pattern and HA-specific B-cell repertoire is established by the initial subtype exposure, and thus individual cross-immunity is highest for strains within this subtype throughout the individual’s life. Through the examination of H5N1 and H7N9 incidence coupled with historical data, recent statistical findings have shown that antigenic seniority extends to heterosubtypic immunity within HA phylogenetic groups [27]. This imprinting (Table 1) explains the varying incidences in different age cohorts, and it raises significant concern for years with epidemics with low heterosubtypic immunity. There are currently ongoing studies in child cohorts to unravel the mechanisms governing imprinting, and including these in phylodynamics models could result in critical insight for emerging strains capable of pandemics. Moreover, other statistical analyses indicate that immunity to pandemics may be weaker if a previous pandemic was seen at a young age [83], highlighting the importance of considering population-level processes.

Biophysical factors of the HA protein have been implicated in, or correlated with, transmission and evolution. Charge [62, 63], glycosylation, and thermostability [61] are

Flowchart of processes at different scales that are presented in Table 1.

Table 1. A Summary of the Important Multiscale Dynamical Processes Governing Influenza Epidemiology and Evolution^a

Level	Process	Modeling References	Empirical Gaps	Next Steps
Molecular	1. Escape and protein stability	[61]	Data quantifying escape probability; relationship between protein stability and infection, shedding	Animal challenge studies; deep sequencing from shedding
	2. Charge and glycosylation	[62, 63]	Relationship between charge and infection, shedding	Animal challenge studies; deep sequencing from shedding
Tissue and organism	3. Local spatial dynamics	[64, 65]	Accurate parameter values for viral life cycle in lungs	Animal challenge studies examining infected lungs
	4. Modeling influenza in the respiratory tract	[66]	Data for human, bird, and swine infections	PET scan with labeled viruses
	5. Aggregate models	[67–69]	Human immune response data	Human challenge studies
	6. Quasispecies	[70, 71]	Data on transmission chains	Deep sequencing along heterogeneous transmission chains
	7. Imprinting	[27]	Understanding immune, viral kinetics and transmission	Better immune assays and read outs; B-cell transcriptomics for community dynamics of B cells
	8. Correlates of protection	[3]	Currently relies on HA1, but this does not correspond to protection	Development of neutralization assays
Transmission	9. Scaling with local population density	[72]	Current data are only for turkeys and H7N1	Measuring transmission rates for humans (and other animal models) for H3N2 (and other subtypes)
	10. Impact of immune escape	[60, 73]	Shedding and transmission relationship	More animal/human challenge studies (reliable transmission models)
Local epidemic dynamics	11. Estimation of R_0 , R_{eff}	[74, 75]	Shedding and transmission relationship; measures of immunity from previous infections	Reliable transmission models; better surveillance to track susceptibility
	12. Transmission and seasonality	[76, 77]	Shedding and transmission relationship	Deep sequencing along heterogeneous transmission chains
	13. Herd immunity, immune escape, and vaccination	[78–83]	Levels of immunity and cross-protection; effective vaccine design	Measures of family and population level transmission for indirect protection; UIV development
Regional dynamics and coupling	14. Gravity models	[84]	Age-structured movement data	Age-structured cohort studies that include spatial movement
Global dynamics	15. Global spread	[22, 25]	Heterogeneous global surveillance	Improved surveillance for HA and phenotypic data, properly curated for immune response
	16. Multiscale dynamics	[21]	Shedding and transmission relationship; heterogeneous global surveillance	Age-structured powered cohort studies; improved surveillance
	17. Phylodynamics	[4, 5, 19]	Heterogeneous global surveillance	Age-structured powered cohort studies; improved surveillance; deep sequencing from shedding

Abbreviations: PET, positron emission tomography; UIV, universal influenza vaccine.

^aReferences that address theoretical developments are included.

also associated with antigenicity and attraction for certain antibody sequences (Table 1). However, further studies are necessary to elucidate the biophysical factors of HA and NA that are central to influenza dynamics, and the role of these factors on processes across scales could be gleaned through extensive statistical analyses. On a similar note, although there have been studies modeling viral kinetics of influenza [67–69] (Table 1), tying these to population-level processes will enhance evolutionary dynamics models and could perhaps resolve the effect of processes such as hindrance or imprinting on global spread.

The impacts of vaccination in evolutionary and transmission dynamics of influenza viruses have also been studied from a theoretical perspective (Table 1). For previous seasons, statistical analyses have revealed that seasonal vaccination

in the United States reduced infections significantly [81] by direct (ie, actual vaccination) and indirect (ie, reduction in disease-transmitting contacts) protection. Because a universal vaccine would bestow broad immunity, and hopefully high efficacy and effectiveness (compared with current strain-specific vaccines [26]), these findings raise the alluring possibility of largely controlling seasonal outbreaks. Moreover, in terms of influenza antigenic evolution, Arinaminpathy et al [62] used phylodynamics frameworks to show that significant vaccine-induced cross-immunity could lead to the emergence of fewer distinct antigenic variants. A comparison of seasonal to universal vaccines confirmed this point and showed that strain-specific vaccination may actually have the opposite effect [80]. In fact, given a long enough period of cross-immunity for universal vaccination, seasonal epidemics, antigenic

evolution, and pandemics were predicted to be controlled best by universal vaccination [80].

FUTURE OUTLOOK TOWARD NEXUS OF EMPIRICAL WORK AND THEORY

Although there has been progress to bridge empirical work and modeling, many questions remain. Table 1 highlights the empirical gaps and next steps for existing modeling efforts; recurring themes are animal and human challenge studies for reliable transmission models, deep sequencing of shedding, age-structured cohorts, and improved surveillance. With these in hand, unraveling the central relationship between shedding and transmission (Box 1) should be possible, and more accurate insights into global dynamics will bolster predictions regarding emerging strains and vaccination. In particular, elucidating the interplay of molecular and within-host mechanisms with universal vaccination is at the junction of empirical and theoretical work, and progress in this area will guide universal vaccination strategies. For instance, cohort studies coupled to phylodynamics models that incorporate imprinting would yield focal understanding of future universal vaccination impacts on global spread. Another central need for improved surveillance is the development of appropriate correlates of protection, to obtain reliable data for model parameterization. Reliable data, coupled with statistical analyses to glean the role of molecular processes in population level processes and evolution, could enhance the formulation and analyses of cross-scale predictive models. Further synergy between empirical work and theory could continue to advance our understanding of the molecular, within host, and population-level dynamics governing influenza in the context of UIV development and administration.

Notes

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