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Opinion

Influenza immune escape under heterogeneous host immune histories

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In a pattern called immune imprinting, individuals gain the strongest immune protection against the influenza strains encountered earliest in life. In many recent examples, differences in early infection history can explain birth year-associated differences in susceptibility (cohort effects). Susceptibility shapes strain fitness, but without a clear conceptual model linking host susceptibility to the identity and order of past infections general conclusions on the evolutionary and epidemic implications of cohort effects are not possible. Failure to differentiate between cohort effects caused by differences in the set, rather than the order (path), of past infections is a current source of confusion. We review and refine hypotheses for path-dependent cohort effects, which include imprinting. We highlight strategies to measure their underlying causes and emergent consequences.

Host susceptibility shapes strain fitness

For influenza and other pathogens that evolve to escape adaptive host immunity, a combination of viral factors (including sequence and structure) and host factors (including adaptive immune repertoire), determine a strain's fitness, defined as its ability to exploit susceptible hosts. However, antigenic changes do not escape immunity equally in hosts with different immune histories [1–3]. **Episodic antigenic innovations** (see Glossary) allow influenza viruses of a given subtype (e.g., H1N1 or H3N2) to escape some population immunity and cause epidemics [3–5]. Many studies of immune escape by influenza focus primarily on viral factors, asking, for example, what constrains antigenic diversity and evolution (e.g., [4–7]), which sites on viral proteins are under positive selection (e.g., [8–10]), or which variants are likely to rise in frequency (e.g., [11–13]), without explicitly considering who in the host population has become most vulnerable to reinfection as a result of these antigenic changes.

A variant's potential to escape host immunity depends both on the variant's phenotypic dissimilarity from past strains (usually measured by **antigenic distance** [14–18]) and on the host's adaptive immune background. Models that account for path dependence in development of a host's immune repertoire, in which the order (path) and identity (set) of past exposures both affect susceptibility [14,19–24], can reproduce complex patterns observed in data. Such patterns include history-dependent immune escape, wherein the level of cross-protection that hosts gain against a given strain, y, from a recent infection or immunization with a closely related strain, x, depends on their immune history [1–3,14,22–35], despite the fact that there is a fixed degree of dissimilarity (a fixed antigenic distance) between strains x and y (Figure 1D, Key figure and Box 1). Still, approaches to modeling path-dependence vary [14,19–24], and some common modeling approaches do not account for path dependence at all. For example, some models assume that susceptibility depends only on the identity of strains (or antigens or epitopes) encountered in the past, not on the order of those encounters (Figure 1E) [4–7,18,36]. Models are a key tool used to study the relationship between influenza's epidemic and evolutionary dynamics, and to link observations made at the

Highlights

Influenza evolves to escape immunity, but antigenic substitutions rarely escape immunity equally in all hosts. Birth cohorts, each composed of hosts with similar infection histories, often differ in susceptibility to new influenza strains.

Descriptive studies, which identify cohort-associated differences in susceptibility and sometimes relate them to differences in initial infection, are common. However, the strength and persistence of observed effects varies, and the mechanisms underlying these patterns are ill-defined.

Understanding the epidemic and evolutionary impacts of cohort effects is not possible without a clear conceptual model for how these differences arise.

We argue that focusing on specific, measurable causes and consequences of cohort effects is the best way to cut through semantic confusion, draw appropriate connections between observed epidemiological examples, and understand their evolutionary implications.

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Key figure

Hypythesized mechanisms for preferential immune memory against strains encountered in childhood

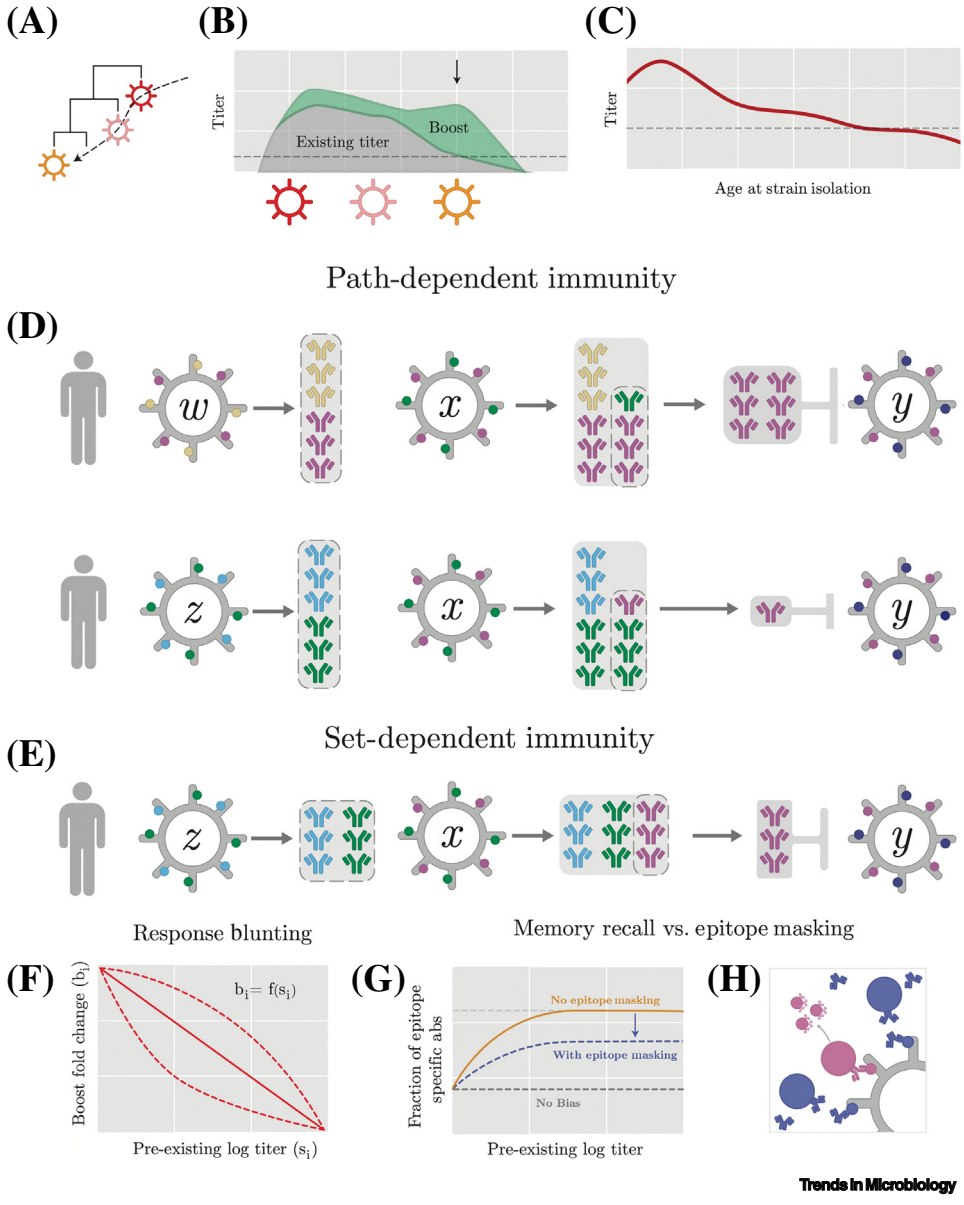

Figure 1. (A) Influenza viruses evolve antigenically over time. (B) A hypothetical example of an **antibody landscape** that reconciles two observed patterns: on average, infection causes the largest boost to the current strain (arrow) [32], but absolute titers remain highest to childhood strains due to **back-boosting**, wherein titers rise simultaneously to current and past strains, which have some antigenic homology [32,45,48,62]. The broken line represents a hypothetical 50% protective titer. (C) Repeated recall of cross-reactive memory leads to a pattern in which titers are, on average, highest to the strains that circulated in childhood [45,62]. (D) Original antigenic sin (OAS) can cause path-dependent immune repertoire development. When hosts with different immune histories are subsequently infected with strain x, existing

(Figure legend continued at the bottom of the next page.)

Glossary

Antibody landscape: a strategy for analysis and visualization of titers to many strains in which the x-axis shows the scaled antigenic distance between strains and the y-axis shows smoothed titers to each strain [32] (similar to Figure 1B).

Antigenic distance: a measure of dissimilarity between influenza strains. Most antigenic distance measures are derived from serological assays and sometimes incorporate sequence data.

Back-boosting: a pattern wherein infections recall and boost cross-reactive memory of antigens seen in the past. This results in an increase in titer that is temporally centered at the current strain, but that also raises titers to antigenically homologous past (depicted in Figure 1B).

Birth cohort: a group of individuals, born at a similar point in time, that are exposed to similar influenza viruses and may follow similar trajectories of immune repertoire development.

Cohort effects: birth year-specific differences in influenza susceptibility.

Episodic antigenic innovation: a pattern of epochal evolution in which genetic changes accumulate continuously, but phenotypic innovations are more sudden. Episodic innovations can be visualized as cluster transitions in antigenic maps, where antigenically similar strains cluster together, and occasional phenotypic innovations cause new clusters to appear.

Epitope bias: a phenomenon in which subsequent immune responses are dominated by recalled memory and increasingly focus on influenza virus epitopes conserved between current and past strains.

Epitope masking: the hypothesis that pre-existing antibodies to a given epitope can interfere with titer boosting to that epitope. This can occur if antibodies bound to a particular site on the antigen occlude B cell access, thereby inhibiting stimulation and replication of cognate clones (depicted in Figure 1G,H).

Immunodominance: the phenomenon whereby the immune system does not target all epitopes equally. Immunodominant epitopes are the focus of an immune response. Immunodominance hierarchies can depend on both viral factors (structural accessibility and binding affinity of

Box 1. Examples of imprinting and other cohort effects

A strain's fitness

- In the 2013–2014 influenza season, H1N1 influenza acquired a K166Q substitution. This substitution abrogated immunity and caused a disproportionate number of cases in middle-aged adults born between 1965 and 1979 [2,52].
- Some middle-aged adults (born in the 1960s and 70s) are perpetually susceptible to the 3c2.A clade of H3N2 influenza. Repeated recall of antibodies that can bind but not neutralize these viruses interferes with the development of *de novo* responses capable of preventing infection [1].

Vaccine effectiveness

- In the 2018–2019 influenza season, vaccine effectiveness against the dominant H3N2 strains that circulated in Europe and Canada varied dramatically by birth cohort [29]. Measured vaccine effectiveness was much lower in 35- to 54-year-olds than in any other cohort. Although the exact mechanism has not been resolved, cohort-associated differences in immune history are the main hypothesis [29,31].

Susceptibility to pandemic influenza viruses

- The 1918 H1N1 pandemic was characterized by an unusual 'W-shaped' mortality pattern, in which deaths occurred disproportionately in young adults (who formed the 'W's' middle peak), and at the extremes of age [53]. One hypothesis for these unusual mortality patterns centers on differences in imprinting: childhood exposures to a mismatched strain may have left young adults (born between 1889 and 1900) susceptible to severe disease in 1918 [40,43].
- The 2009 H1N1 pandemic strain caused a disproportionate number of cases in younger cohorts (born after 1980) but spared older cohorts who had cross-protective immunity from exposures to historical H1N1 strains [3,35,54,55]. Primary infection with the 1957 pandemic strain has also been associated with elevated risk in the 2009 pandemic [41].

Susceptibility to avian influenza viruses

- Avian influenza subtypes H5N1 (group 1) and H7N9 (group 2) have spilled over repeatedly from poultry into humans. Most H5N1 cases occur in children and young adults, whereas H7N9 cases occur in older adults. These patterns can be explained by differences in childhood imprinting: older cohorts (imprinted to group 1 flu viruses) show the strongest protection against H5N1, whereas younger cohorts (imprinted to group 2 viruses) show the strongest protection against H7N9 [37].

Susceptibility to seasonal influenza subtypes

- Two influenza A virus subtypes, H1N1 and H3N2, currently circulate seasonally. Although the extent of each subtype's circulation and exact case–age distributions vary from year to year, across the past two decades H3N2 has generally caused a greater proportion of cases in high-risk older adults than H1N1 [38,39,56]. This pattern is consistent with the hypothesis of imprinting to specific hemagglutinin subtypes.
- The two lineages of influenza type B have distinct age distributions of cases that are consistent with a combination of set- and path-dependent cohort effects [44]. Certain cohorts have lower susceptibility to B/Yamagata than to B/Victoria both because most people in those cohorts were imprinted with B/Yamagata and because many people have not been infected with B/Victoria at all.

molecular, within-host and population level. A more systematic understanding of the within-host causes and population-level consequences of path dependence in development of the immune repertoire will facilitate a better understanding of when it is important to account for these patterns, and how to model them.

immune memory reduces the total size (response blunting) and shapes the epitope specificity (epitope bias) of the subsequent response (the broken outline separates the new response from the standing repertoire). The following year, strain y has one epitope in common with strain x, but the strength of cross-immunity hosts gain against strain y from a past infection with strain x depends on immune history (adapted from [49]). (E) Set-dependent immunity implies that differences in the development of the immune repertoire depend on the identity of the antigens experienced in the past, but not on the order in which they were experienced. Here, immunity accumulates additively, so that all hosts gain the same cross-protection against y from a past infection with x, regardless of their immune history. (F) Measuring response blunting: current evidence shows a linear decrease in log boost with log titer to specific influenza epitopes [23,24,77] or strains [24] (unbroken line) [77], but evidence for this quantitative relationship is limited and it could differ in other contexts (broken lines). (G) Measuring epitope bias involves tracking how the fraction of antibodies or antibody-secreting cells specific to a given epitope changes as a function of pre-existing titer to that epitope. The exact functional relationship and the degree of epitope masking is predicted to depend on factors including antigen dose and the degree of steric interference between epitopes [22–24]. (H) Illustration of epitope masking, in which pre-existing antibodies to the blue epitope occlude binding of cognate B cells, whereas B cells to the pink, novel epitope are more able to bind and replicate.

immune cells to specific epitopes) and host factors (immune history and precursor frequency of immune cells with specificity to each epitope).
Imprinting effects: cohort effects caused by differences in immune protection against influenza strains similar to those that caused an individual's earliest childhood infections.
Original antigenic sin (OAS): preferential recall of antibody responses originally raised against the primary strain.
Path-dependent: wherein differences in past infection order affect the specificity of immune memory that different cohorts gain upon infection with the same strains.
Response blunting: a phenomenon wherein subsequent immune responses are (on average) smaller in magnitude than the primary immune response.
Serological studies: studies measuring serum antibodies to a given influenza strain. Assays measure antibodies to different antigens or epitopes of influenza. Hemagglutinin inhibition (HI) assays, ELISA, neutralization assays, and neuraminidase inhibition are the most common serological assays for studying influenza responses.
Set-dependent: differences in susceptibility due to older cohorts, but not younger cohorts, having experienced cross-protective antigens from the past.

At the population level, birth year-associated differences in immune specificity and susceptibility to new strains (Box 1) demonstrate the epidemiological impacts of path dependence. Individual immune histories are rarely known, but **birth cohorts** are subsets of the host population born at similar times that are assumed to have similar infection histories. Susceptibility to a given antigenic phenotype can differ between cohorts [1–3,35,37–43], a pattern known as a **cohort effect**. **Imprinting effects** are a special kind of cohort effect in which hosts gain stronger immune protection against antigenic phenotypes that circulated in childhood than against phenotypes encountered later in life [37–39,44]. **Original antigenic sin** (**OAS**) is a within-host process in which repeated recall and boosting of cross-reactive antibodies reinforces strong immune memory of earlier strains [45–51]. Although the existence of these patterns shows that the order, not just the identity, of past infections can leave birth cohorts vulnerable to different antigenic substitutions, and can cause a strain's fitness to vary across birth cohorts, the observed strength and persistence of these effects varies, and their broader importance to population-level evolutionary and epidemic dynamics is not clear.

We argue that the current lack of a clear conceptual model for how cohort effects arise is a key obstacle to synthesis. Descriptive studies of cohort effects, imprinting effects, and OAS are common. Further development of specific and quantitative hypotheses for these patterns will make it easier to compare and reconcile effects observed in different contexts, and to agree on definitions for the patterns we observe. To help facilitate these comparisons, we review and highlight gaps in current conceptual frameworks for how birth year-associated differences in susceptibility arise, focusing on measurable patterns that provide a basis for comparison across studies.

Cohort-specific differences in susceptibility

Individuals born in a particular year are, on average, exposed to a similar series of influenza viruses from childhood to adulthood. Therefore, cohort effects are evidence that infection history, including the set of all strains seen in the past, and the order in which cohorts of different ages are exposed to the same strains, can shape host susceptibility. In turn, strain fitness will vary with host susceptibility if susceptible cohorts are more likely to become infected, or more likely to shed high viral loads and contribute to transmission.

In many examples, cohort effects shape population susceptibility to avian, seasonal, or pandemic influenza viruses (Box 1). However, the main similarity between these examples is the emergent pattern – differences in the specificity of adaptive immunity associated with birth year – not necessarily the underlying mechanism.

Cohort effects can occur due to set-dependent or path-dependent differences in immune history

Failure to differentiate between distinct mechanisms that give rise to cohort effects is one main source of confusion about their broader biological significance. We argue that there are two main reasons cohorts can differ in susceptibility: **set-dependent** differences in immune history, in which older cohorts, but not younger cohorts, were born early enough to have 'seen' a cross-protective antigen from the past; and **path-dependent** differences in infection history, in which differences in lifelong infection order affect the specificity of immune memory that different cohorts gain on infection with the same cross-protective strain (Figures 1 and 2).

The concept of set dependence is fundamental to the concept of adaptive immunity: hosts previously infected with a given antigen gain protection, while hosts not previously infected lack protection. It is also fundamental to the concept of immune escape: novel antigens have a fitness

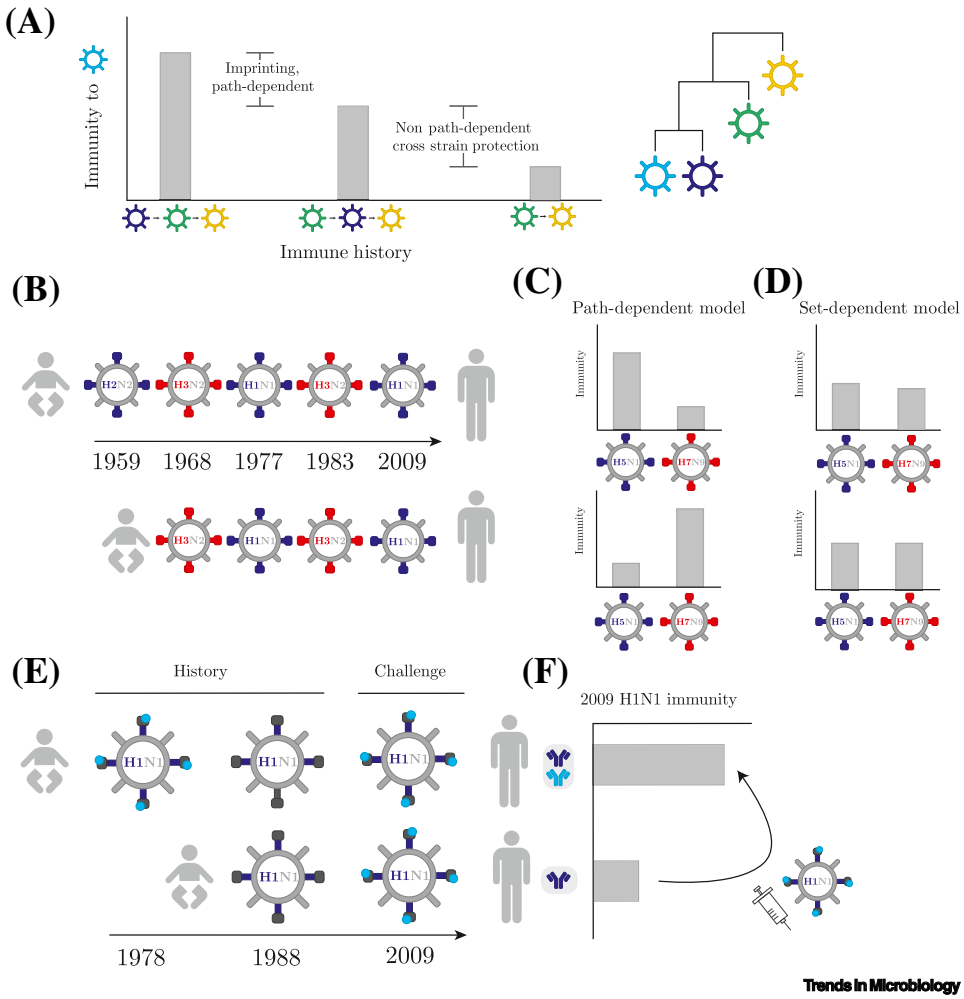

Figure 2. Set-dependent versus path-dependent cohort effects. (A) Cohorts can differ in susceptibility due to differences in the order or identity of past infections. (B–D) An example of imprinting. (B) Adult cohorts have lived through decades of cocirculation of influenza A strains whose hemagglutinin antigens fall on two distant branches of the phylogenetic tree. (Seasonal strains from hemagglutinin group 1, H1N1 and H2N2, are shown in blue, and strains from group 2, H3N2, are shown in red.) If immunity accumulated in a strictly set-dependent manner, adult cohorts would show equally strong protection against both groups, given repeated infections and immunizations (D), but instead cohorts show stronger protection against the group that circulated in childhood, consistent with imprinting (C) (Box 1). (E,F) An example of non-imprinting cohort effects. (E) Immune memory of a cross-reactive epitope present in past H1N1 strains (light blue dot), which circulated most recently in the late 1970s, provided cross-protection against the 2009 H1N1 pandemic strain. However, this epitope was shielded by a glycan and inaccessible from 1983 to 2009 [2,58]. (F) Younger cohorts born after this cross-reactive epitope became inaccessible were immunologically naive to the 2009 pandemic strain, whereas older cohorts had some cross-protective immunity. However, this set-dependent cohort effect did not persist. Younger cohorts quickly built new immune memory on infection or vaccination with the 2009 strain (arrow).

advantage. Set-dependent effects are already incorporated into most conceptual or quantitative models of influenza fitness and evolution.

Set-dependent cohort effects primarily occur when past antigens re-emerge in circulation. Antigenic reversion to an ancestral state [57], loss of an epitope-shielding glycan [2,58], and emergence of a reassortant pandemic strain [54,55,59] could all cause set-dependent cohort effects. During pandemics, set-dependent cohort effects often give rise to a pattern called 'senior

sparing', in which pre-existing immunity in older and more immunologically experienced cohorts often shifts the mortality impacts of pandemic strains toward younger cohorts, relative to that of seasonal strains [59,60].

Set-dependent cohort effects reflect a simple difference in adaptive immunity between cohorts that were or were not yet born at the time cross-protective antigens last circulated. However, they often involve protection from strains that older cohorts encountered at relatively young ages, and therefore are easily conflated with path-dependent patterns, such as OAS and imprinting [60]. Given influenza's perpetual evolution, it is unlikely that two humans would ever be naturally infected with an identical set of strains in different orders (as in Figure 2A). Therefore, animal model experiments may be needed to fully disentangle confounded set- and path-dependent differences in susceptibility. However, it should usually be possible to identify set- or path-dependent effects as the dominant driver of a cohort effect by (i) identifying key antigenic sites that facilitate pre-existing immunity against the strain of interest, and (ii) asking whether all cohorts have had (roughly) equal opportunities for exposure to cross-protective antigens in the past (Figures 1 and 2).

Like senior-sparing effects, imprinting effects also involve strong immune memory of childhood strains, but arise due to path-dependent, not set-dependent, differences in immune history. In the following text, we outline hypotheses for path-dependent cohort effects, which are more complex and less understood than set-dependent effects (see Outstanding questions).

Repeated recall reinforces memory of the strains that circulated in childhood

OAS is the leading hypothesis for imprinting [25,37,50,61]. However, the term OAS is used inconsistently and its definition has been repeatedly clarified or reinterpreted [49–51,62,63]. Here, we briefly define OAS and its relationship to imprinting, although we further argue that focusing on specific and measurable mechanisms underlying OAS is the best way to cut through semantic confusion.

Influenza viruses evolve incrementally, and subsequent strains contain some antigenic structures that are identical or similar to those of the primary strain. OAS is an immunological process in which repeated recall and boosting of cross-reactive memory responses reinforce strong immunity against the antigenic phenotypes encountered earliest [47,48,62,64]. In cross-sectional data, this leads to a characteristic pattern in which individuals' antihemagglutinin antibody titers are higher, on average, against the influenza strains encountered in childhood than against strains encountered later in life [26,27,45,46,65] (Figure 1B). Hierarchical memory of the strains encountered earliest is also known as antigenic seniority [62]. Here, we refer to OAS primarily as the underlying within-host process (recall and reinforcement of cross-reactive memory) not to the emergent serological population-level pattern (titers are highest to strains from childhood), which is consistent with the conventions of some (e.g., [28,49,50,66–68]) but not all existing studies (e.g., [18,20,25,62,63]).

Imprinting effects: a potential epidemiological consequence of OAS

The hypothesis that OAS gives rise to population-level imprinting effects extends beyond established serological evidence in two key ways. First, imprinting effects are an epidemiological pattern involving birth year-associated differences in protection against a current strain or subtype, not just a serological pattern involving differences in titer (Box 2). Second, immunological biases toward the earliest encountered strains almost certainly involve many dynamically intertwined arms of immune memory, including various interacting B and T cell subsets [69,70], not just the antibodies measured by common serological assays. With these caveats in mind, imprinting effects may be an emergent, epidemiological consequence of OAS.

Box 2. Limitations of serological data

The relationship between OAS's emergent serological and epidemiological (imprinting) patterns is surprisingly complicated and is still being resolved. Titers are a correlate of influenza protection [71,74]. However, OAS does not prevent adults from mounting high titers against current strains [32,45,48,62]. (As illustrated in Figure 1(B,C) in main text, titers to childhood strains can be marginally higher, on average, than titers to strains from adulthood, but both can simultaneously be above the threshold associated with protection.)

Rather than completely preventing adults from developing antibodies to new strains, OAS is more likely to affect protection by modulating the quality and specificity of a titer's component antibodies. For example, OAS can cause hosts to deploy antibodies that bind [25,28,47,67,75] or neutralize [1,28] past strains more effectively than the current strain. If recalled antibodies neutralize the current strain poorly, then hosts showing high titers may still be susceptible to infection [1]. Overall, evidence shows that OAS can, but does not always, interfere with the quality of protection against strains encountered later in life [49]. Measuring differences in neutralization, or differences in protection from severe and detectable disease, not just differences in titer, is therefore essential when studying imprinting.

Cross-protective breadth

In common serological assays, antibodies cross-react poorly to influenza antigens of different subtypes. Thus, OAS has traditionally been described at the within-subtype level. However, there is growing interest in antibody, B, and T cell responses that target conserved influenza epitopes, which can provide broader cross-subtype protection, and provide a potential basis for universal influenza vaccination [71,72]. The proliferation of serological techniques to measure antibodies to conserved epitopes has revealed that, contrary to the classical within-subtype paradigm, OAS can also shape the quality and specificity of broadly protective, cross-subtype immunity [25,73]. Similarly, the breadth of cross-protection from imprinting and other cohort effects is context-dependent and depends on which epitopes are the dominant immune targets and how conserved those epitopes are. For example, epitopes that are conserved between influenza subtypes that have and have not previously circulated in humans are, by definition, the only recognizable immune targets present on novel, avian subtypes, such as H5N1 and H7N9. Imprinting protection against zoonotic H5N1 and H7N9 infections involves memory of these conserved epitopes and provides broad cross-subtype protection [25,37,73]. On the other hand, memory responses against familiar seasonal strains are more likely to target variable, immunodominant epitopes, and in this context imprinting acts narrowly within a subtype [38,39]. The fact that immune breadth can shift in contexts where variable or conserved epitopes are the dominant immune targets emphasizes the importance of specific antigenic hypotheses when characterizing or comparing cohort effects.

Measurable components of OAS

There is no standard way to measure or objectively compare OAS across disparate studies. However, OAS can be broken down into two measurable components, **epitope bias** and **response blunting**, which provide a potential basis for comparison, and synthesis.

The first measurable component of OAS is epitope bias, which refers to a shift across subsequent infections in the secondary **immunodominance** of epitopes that are conserved between current and past strains [22,28,35,47,67,76] (Figure 1D,G). Figure 1D depicts a simple but unrealistic case in which all epitopes are equally accessible, immunogenic, and mutable, while realistically, epitopes differ intrinsically in primary immunodominance due to differences in antibody binding affinity or structural accessibility. Evidence for OAS supports the hypothesis that the secondary immunodominance of conserved epitopes increases across a series of infections, in that new infections often boost responses specific to past antigens [28,47,67], but there is support for an opposing pattern, **epitope masking**, in which existing antibodies can occlude binding and replication of B cells cognate to the same epitopes (Figure 1H). Because B cells produce antibodies, this can interfere with the generation of new antibodies to familiar epitopes [22–24]. Epitope masking

is predicted to interfere with OAS most strongly in the context of vaccination, when antigen dose is low, and antigen does not replicate [22].

The ideal strategy to measure changes in secondary immunodominance would involve quantifying how the fraction of antibodies or antibody-secreting cells specific to a given epitope changes as a function of pre-existing titer (or of antibody-secreting cell precursor frequency) [22]. Further experiments and improved methods are needed to quantify these relationships (Figure 1G). Currently, absorption experiments [25,38,65] and measurement of antibody affinity to engineered antigens [28,75,76] can be used to infer where within an antigen antibodies are binding. The main limitation of these methods is that they usually provide coarse spatial resolution, for example, providing the ability to quantify binding to the hemagglutinin head or stalk domain, but less often to specific epitopes (but see [78,79]). Recent advances in microscopy have enabled finer-scale direct mapping of antibodies in polyclonal sera to specific epitopes [66–69], but these methods are not yet quantitative. Ongoing development of high-throughput microscopy methods and of methods to infer preferred epitopes from polyclonal mixtures of antibodies [18] will improve our ability to measure changes in secondary immunodominance going forward.

The second measurable component that could play a role in OAS is response blunting, in which subsequent immune responses are smaller in magnitude than the primary response [19,20,80,81]. Studies analyzing longitudinal titer data and some data on repeated vaccination support the idea that individuals with higher pre-existing titers experience weaker boosts [19–21,24,80,82], and have referred to these patterns as 'negative interference' [14] or 'antibody ceiling effects' [21,80]. Hypothesized mechanisms include that antibodies may neutralize and clear antigen, or antibodies may inhibit B cell activation through epitope masking, Fc receptor-mediated mechanisms, and other forms of occlusion [14,23,24]. As illustrated in Figure 1D, response blunting could act synergistically with epitope bias to limit the magnitude of *de novo* responses to new antigenic structures.

The magnitude of blunting in **serological studies** can be quantified (measured) by estimating the decrease in boost size per unit increase in pre-existing titer. In infection or vaccination experiments, this is equivalent to the regression coefficient of boost size (fold change in titer) on pre-existing log titer (Figure 1F) [22,24]. In longitudinal or observational studies, response blunting can be estimated as a parameter in dynamical or statistical models [19–21,80].

Path dependence in development of strain-specific immunity

OAS gives rise to path dependence in the development of the immune repertoire (Figure 1D,E). In turn, path dependence can give rise to cohort effects that are counterintuitive, and rarely included in quantitative or conceptual models of strain evolution and host immunity. For example, hosts with similar (and high) protective titers to a current strain (e.g., strain x in Figure 1D,E) can be vulnerable to different antigenic escape mutations in the presence of path dependence [49]. Likewise, in examples of imprinting, cohorts recently infected (or experimentally inoculated) with the same seasonal strains deployed antibody responses that differed in specificity and effectiveness against a given challenge [25,37–39] (Figure 2B). These observations suggest that subsets of the host population can drive selection of different antigenic phenotypes [34,79,83]. Theoretical studies have shown that host heterogeneity can shape dynamics of competition between strains and conditions under which strains can coexist [84,85], but these studies have not specifically considered path dependence. Integrating path dependence into mathematical models that link influenza's evolutionary and epidemic dynamics (e.g., [4–6,86]) could help to elucidate whether it primarily promotes strain competition (e.g., strains with high fitness in different cohorts competitively exclude one another) or coexistence (e.g., through cohort-specific niche partitioning).

We hypothesize that a key difference between path-dependent and set-dependent cohort effects is their persistence over time. Imprinted biases in the specificity of immune memory can persist for years or decades, even in cohorts repeatedly exposed to diverse flu phenotypes [1,37] (Figure 2B–D). In contrast, we hypothesize that set-dependent cohort effects are more likely than path-dependent cohort effects to be ephemeral, eroding within one or two influenza seasons as naive cohorts gain immunity through infection or vaccination. For example, infection or vaccination with the 2009 H1N1 pandemic strain allowed younger cohorts to build protective titers, eroding differences in immunity with older cohorts (Figure 2F) [35]. Similarly, set-dependent 'senior sparing' effects do not persist once a pandemic strain becomes established in seasonal circulation [59].

Cohort effects that cause large differences in susceptibility and persist across many epidemic seasons are likely to meaningfully influence influenza's evolutionary trajectory. Weak cohort effects are less likely to have a meaningful impact, especially if susceptibility varies more among individuals within a cohort than between cohorts. The relationship between epitope mutability and immunodominance, or mutability and neutralization potency (neutralizing antibodies block viral replication), is likely to shape the magnitude and persistence of path-dependent cohort effects. Imprinting effects that persist for decades most likely involve epitopes that are conserved and phenotypically stable across a host's lifetime [38], but the role of more conserved and persistent epitopes in defense against seasonal influenza is still being resolved. Responses to conserved influenza epitopes are often recalled and boosted across repeated infections [28,87], and they are a correlate of protection against seasonal influenza [68,71], but they are generally considered immunosubdominant to, and less potent than, responses to variable sites, which undergo rapid antigenic drift [28,35,51,68,88]. If there is an inverse relationship between influenza epitopes' conservation (persistence) and their immunodominance or neutralization potency (importance in immune protection), then there may also be an inverse relationship between the persistence and strength of cohort effects. (For example, for seasonal influenza, imprinted biases in memory of conserved epitopes are persistent, but have relatively weak effects, perhaps because conserved epitopes only play a small role in protection [38,39].) Testing this hypothesis would require routine evaluation of cohort effects' strength and persistence. Cross-sectional data collected across many influenza seasons provides insight into persistence (e.g., [1,38,39,56]). Strength can be measured by comparing a cohort's observed risk to their expected risk in a null model, or in long-term baseline data (e.g., [37–39,44]).

Concluding remarks

For influenza and other shape-shifting pathogens that evolve to escape host immunity, strain fitness depends on a combination of viral factors and host factors. Cohort-specific differences in susceptibility are a well-documented pattern with unknown effects on influenza strain fitness and evolution.

Differentiating between set-dependent and path-dependent examples of cohort effects is a needed first step toward organizing observed patterns and determining which observations are at odds with common assumptions. Set dependence is fundamental to the concept of adaptive immunity and to immune escape, so most studies of influenza virus evolution already account for set-dependent differences in host susceptibility. In contrast, the epidemic and evolutionary effects of path dependence are less studied.

Focusing on specific and measurable aspects of path dependence, such as response blunting and epitope bias, can help us draw connections between observed patterns. The strength and persistence of cohort effects are also measurable. Systematically studying how strength and persistence vary in different epidemic contexts can help us to understand the importance of cohort effects in shaping population susceptibility and strain fitness.

Outstanding questions

How does heterogeneity in host immune history and host imprinting shape influenza's epidemic and evolutionary dynamics?

To what extent do set-dependent or path-dependent differences in host immune history contribute to competition between, or coexistence of, multiple influenza variants?

How can we predict which subsets of the host population are most vulnerable to specific antigenic substitutions, or to specific forms of vaccine mismatch?

Can we quantify the relative contributions of epitope bias, response blunting, and other forms of interference between host immune memory and *de novo* responses?

Does the relative dominance of a pathogen's variable and conserved epitopes alter the dynamics of interference?

How can path-dependent models be used to accurately predict (i) how influenza strains are selected for at the population level, and (ii) which cohorts were responsible for the selection?

What level of model complexity is needed to understand the consequences of cohort effects?

Do a person's first few exposures to a pathogen have lifelong protective effects, or does the first exposure alone dominate?

Do initially large cohort-specific differences in susceptibility to a given influenza lineage generally decrease over time as the lineage continues to circulate?

Can we generalize insights from influenza to study how past infections shape future population immunity, and strain fitness, for other antigenically evolving pathogens?

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Declaration of interests

There are no interests to declare.

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