Potent protection against H5N1 and H7N9 influenza via childhood hemagglutinin imprinting

Katelyn M. Gostic, Monique Ambrose, Michael Worobey, James O. Lloyd-Smith

Two zoonotic influenza A viruses (IAV) of global concern, H5N1 and H7N9, exhibit unexplained differences in age distribution of human cases. Using data from all known human cases of these viruses, we show that an individual’s first IAV infection confers lifelong protection against severe disease from novel hemagglutinin (HA) subtypes in the same phylogenetic group. Statistical modeling shows that protective HA imprinting is the crucial explanatory factor, and it provides 75% protection against severe infection and 80% protection against death for both H5N1 and H7N9. Our results enable us to predict age distributions of severe disease for future pandemics and demonstrate that a novel strain’s pandemic potential increases yearly when a group-mismatched HA subtype dominates seasonal influenza circulation. These findings open new frontiers for rational pandemic risk assessment.

The key antigenic determinants for IAV susceptibility are the virus’s two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), where different numbered subtypes canonically indicate no cross-immunity. However, recent experiments have revealed that broadly protective immune responses can provide cross-immunity between different HA subtypes, particularly subtypes in the same phylogenetic group (8–14). HA group 1 contains human seasonal subtypes H1, H2, and H5, whereas group 2 contains seasonal H3 and avian H7. Combining these insights into heterosexual immunity with the concept of “original antigenic sin” (15) or “antigenic seniority” (16), we hypothesized that individuals imprint on the HA group of their first IAV exposure and thereby experience a reduced risk of severe disease from novel IAVs within that same phylogenetic group. This hypothesis predicts that the 1968 pandemic, which marked the transition from an era of group 1 HA circulation (1918–1968) to a group 2–dominated one (1968 to the present) (Fig. 1B), caused a major shift in population susceptibility that would explain why H5N1 cases are generally detected in younger people than are H7N9 cases (2, 17–19). Our analysis of human cases of H5N1 and H7N9 revealed strong evidence that childhood HA imprinting indeed provides profound, lifelong protection against severe infection and death from these viruses. These findings allowed us to develop new approaches for IAV pandemic risk assessment, preparedness, and response but also raise possible challenges for future vaccination strategies.

Reconstructing IAV exposure history by birth year

To investigate whether an individual’s initial childhood exposure to IAV influences later susceptibility to H5 and H7 viruses, we estimated the fraction of each birth-year cohort from 1918 to 2015 with first exposure to H1, H2, or H3—or the fraction still naïve—for each country in our study (China, Egypt, Cambodia, Indonesia, Thailand, and Vietnam). We estimated the annual probability of IAV infection in children using published age-seroprevalence data (20, 21) and then rescaled this baseline attack rate to account for year-to-year variability in IAV circulation intensity (supplementary text).

One resulting country-specific reconstruction of HA history is depicted in Fig. 1C. Although H3N2 has dominated since 1968, a non-negligible fraction of many birth-year cohorts from the 1970s onward was exposed first to H1N1 viruses, with notable peaks near the reemergence of H1N1 in 1977 and the 2009 pandemic.

H5N1 and H7N9 cases track HA imprinting patterns

Next, we compiled data on all known human cases of H5N1 and H7N9 with reported patient age (Fig. 2, A and B). These data encompass mostly clinically severe and fatal cases; total incidence remains unknown. Thus, our analysis focused on the determinants of severe cases. The possible existence of many undetected mild cases, as hypothesized for H7N9 (1), is consistent with HA imprinting because broadly protective immune responses are expected to provide partial protection (8, 14), i.e., to reduce severity without preventing infection altogether (4, 12, 22–25).

The spillover of novel influenza A viruses (IAV) is a persistent threat to global health. H5N1 and H7N9 are particularly concerning avian-origin IAVs, each having caused hundreds of severe or fatal human cases (20). Despite commonalities in their reservoir hosts and epidemiology, these viruses show puzzling differences in age distribution of observed human cases (1, 2). Existing explanations—including possible protection against H5N1 among older birth-year cohorts exposed to the neuraminidase of H1N1 as children (3, 4) or age biases in exposure to infected poultry (5–7)—cannot fully explain these opposing patterns of severe disease and mortality. Another idea is that severity of H5N1 and H7N9 differs by age and so leads to case ascertainment biases (7), but no explanatory mechanism has been proposed.

No data available from 1918 to 1921. One resulting country-specific reconstruction of HA history is depicted in Fig. 1C. Although H3N2 has dominated since 1968, a non-negligible fraction of many birth-year cohorts from the 1970s onward was exposed first to H1N1 viruses, with notable peaks near the reemergence of H1N1 in 1977 and the 2009 pandemic.
**HA imprinting explains age distributions**

To formally assess the HA imprinting hypothesis alongside previous explanations (1, 3–7) for observed H5N1 and H7N9 age distributions, we developed a set of multinomial models. These models related the probability that a case occurred in a given birth cohort to country- and year-specific demography; to risk factors including age-based risk of exposure to poultry, age-based risk of severe disease or case ascertainment; and to reconstructed patterns of first exposure (and, hence, potential immunological imprinting) to HA or NA subtypes (table S1). Model comparison showed that HA imprinting was the dominant explanatory factor for observed incidence and mortality patterns for both H5N1 and H7N9 (Table 1). It was the only tested factor included in all plausible models for both viruses (i.e., all models with Akaike weights greater than 4e−5).

The best models also included age-based risk of severe disease, echoing patterns known from seasonal influenza epidemiology. Age-based poultry exposure risk [estimated based on contact data from China (6, 7)] was included for H7N9 but not H5N1, which may reflect that age-specific poultry exposures vary across the multiple countries affected by H5N1 or that humans interact differently with ill (H5N1-infected) versus asymptomatic (H7N9-infected) poultry. In models including HA imprinting, NA imprinting never showed any significant effect (table S2). Remarkably, despite differences between the viruses and age cohorts involved, the estimated protective effects of HA imprinting were nearly identical for H7N9 and H5N1. In all models, protective HA imprinting reduced the risk of severe infection with H5N1 or H7N9 by ~75% and the risk of death by ~80% (Table 1, figs. S5 to S7, and table S2).

**Antigenic seniority across influenza subtypes**

Most individuals born before the emergence of H3N2 in 1968 and exposed first to group 1 HA antigens (Fig. 1) have also been exposed to H3N2 after 1968—probably multiple times. Yet these seasonal group 2 exposures later in life evidently fail to override group 1 HA imprinting from childhood (Fig. 2). The birth year–specific protection seen for human H5N1 and H7N9 thus clearly indicates that clinically relevant antigenic seniority—preferential recall of immunological reactivities to antigens encountered earlier in life upon later exposure to cross-reactive antigens (16)—can act across HA subtypes of the same HA group, not only within subtypes as often assumed.

Although the precise mechanism underlying antigenic seniority in this context remains to be determined, antibodies directed against conserved HA epitopes provide a plausible explanation for protection at the level of HA groups. For example, research following the 2009 H1N1 pandemic drew attention to the fact that primary exposure to a
novel IAV can preferentially boost broadly protective antibodies that bind conserved HA head or stem epitopes shared by different HA subtypes (8–14), even though immune memory against more variable epitopes on the novel HA head may be absent. This absence may in fact enable robust expression of otherwise-subdominant, broadly protective responses to conserved epitopes such as those on the HA stem (8). In particular, primary exposure to H5N1 or H7N9 can activate HA stem–specific reactivities induced by previous infection by H1 or H3, respectively (12, 13, 26).

Indeed, others have suggested that heterosubtypic antibodies might attenuate disease from other IAV strains and may be imprinted to some degree by childhood exposure, although their serological assays provided no ability to detect or predict actual patterns of protection relevant to H5N1 and H7N9 in human populations (27).

Given the immunodominant nature of HA head reactivities (13, 14, 26, 28), conserved HA head epitopes shared within, but not between, HA groups (29) may play a role in these patterns of protection. Cross-reactive HA-specific CD4+ or CD8+ T cell responses should also be investigated, because they, too, are likely to be disproportionally shared within HA groups (given the sequence similarities within each clade) and might be especially capable of facilitating the sort of long-term immunity indicated by the data. Nevertheless, current data, including the high degree of sequence conservation of stem domains within each HA group (Fig. 1A and fig. S1), seem most consistent with a stem-directed mechanism for the antigenic seniority acting at the HA-group level (23). Divergence in HA stem amino acid sequences within each phylogenetic group is comparable to divergence in HA head sequences within a single HA subtype [i.e., the scale at which antigenic seniority is already known to act (16)] (fig. S1), but stem divergences between the two HA groups are markedly higher. Notably, H3 and H7 are as divergent as any pair of group 2 HAs; because H3 childhood exposure provides protection against H7, it may thus protect as well or better against the other group 2 HAs (H4, H10, H14, and H15) but perhaps not at all against more divergent group 1 HAs (fig. S1C). Similarly, the joint consideration of protein sequence conservation patterns (Fig. 1A and fig. S1) along with immunological and epidemiological data suggests that H1 or H2 childhood exposure may protect generally against zoonotic group 1 HAs but not group 2 HAs.

The putative generality in HA imprinting protection patterns for novel HA subtypes other than H5N1 or H7N9 is tentatively supported by the preponderance of HA group–mismatched childhood exposures among the small number of clinically significant human cases detected to date. By pooling data from 28 human cases of H5N6, H6N1, H7N7, H9N2, H10N7, and H10N8, we found that age patterns are consistent with HA imprinting (P = 0.019; see supplementary text), but case numbers are insufficient to investigate particular subtypes. Immunological experiments [e.g., using chimeric HA proteins (28)] are needed to systematically map HA cross-protection patterns across all HA subtypes, both within and between HA groups.

### Table 1. Estimated protection against severe infection from HA imprinting

<table>
<thead>
<tr>
<th>Factors in model</th>
<th>HA imprinting protection (95% CI)</th>
<th>△AIC</th>
<th>Akaike weight</th>
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<td>H5N1</td>
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<tr>
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<td>H7N9</td>
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</table>

### Fig. 3. Projected effects of HA imprinting on future pandemics.

(A) Attack rate of severe cases, by age group, for hypothetical H5 (blue) and H7 (red) IAV pandemics in 2015 (R0 = 2.5, relative infectiousness of imprinting-protected individuals (ω) = 0.5), if one assumes UK demography and age-structured mixing (see supplementary text). Lines show the average of 100 simulated outcomes, and shaded regions show the central 95%. Three vaccination scenarios were explored: vaccination of IAV-naïve children could cause dual imprinting to both HA groups (dashed lines), prevent imprinting to both groups (dotted lines), or have no effect on imprinting (solid lines). (B) Projected change in R0, for hypothetical H5 (blue) or H7 (red) IAV with R0 = 1.2 and ω = 0.5, if group 1 IAVs make up 100% or 75% of seasonal circulation after 2015.
higher and more evenly distributed across age groups than the severe attack rates shown here.

Over any prolonged period when IAV circulation is dominated by one HA group, imprinting generates growing herd immunity against zoonotic IAV strains from that group. Conversely, zoonotic strains from the mismatched HA group benefit from the rising proportion of humans without protection. So long as mild cases arising in people with group-matched imprinting contribute any less to transmission than unprotected cases, or if some fraction of infection events is prevented by imprinting-derived immunity, imprinting will alter the transmissibility of zoonotic IAV strains in the human population. This is summarized by the effective reproductive number, \( R_{eff} \), which quantifies transmission in a partially immune population (Fig. 3B). Crucially, a zoonotic strain that is initially subcritical (i.e., with \( R_{eff} < 1 \) and therefore unable to spread sustainably) could—solely because of susceptibility changes in the human population—emerge as supercritical and hence as a pandemic threat, if the mismatched HA group dominates IAV circulation for a sufficient period (Fig. 3B).

Our work implies that we have never seen a true “virgin soil” influenza pandemic and that all prior estimates of \( R_0 \) for pandemic IAVs are systematic underestimates because they do not account for protection induced by HA imprinting. Conversely, we see that imprinting raises the threshold \( R_0 \) necessary for a novel subtype to invade. Note that the cocirculation of group 1 and 2 HAs since 1977 has balanced herd immunity in a way that increases the inherent transmissibility needed for a novel subtype from either HA group to invade. As a generality, \( R_{eff} \) for zoonotic influenza strains will change through time depending on seasonal influenza patterns and demographic background, and the magnitude of change will depend on the infectivity of imprinting-protected cases (Fig. S9).

Discussion

Our findings show that major patterns in zoonotic IAV epidemiology, previously attributed to patient age, are in fact driven by birth year: IAV strains circulating during an individual’s childhood confer long-term protection against novel HA subtypes from the same phylogenetic group. Hence, antigenic seniority extends across IAV subtypes, introducing previously unrecognized generational structure to influenza epidemiology. These immune imprinting effects have implications for public health and highlight that influenza virulence represents a joint phenotype between virus and host—even for strains not yet adapted to the human population.

These findings support the hypothesis that the unusually high mortality in young adults during the 1918 H1N1 (group 1) pandemic may have arisen primarily from mismatched H3 (group 2) imprinting in the cohort born between ~1880 and 1900 (39). This same cohort was strongly affected during the (group 1) 1957 pandemic (33), yet they suffered no excess mortality when they were even older, during the (group 2) 1968 pandemic (34). The possibility that mismatched HA imprinting currently contributes to the greater health impacts of seasonal H3N2 (relative to H1N1) in today’s older age classes is worth investigating. And a diagnostic assay to determine whether an individual was imprinted on a group 1 or group 2 HA may be useful for individualized clinical care and vaccine design strategies, both for pandemic and seasonal IAVs.

Our findings raise questions about whether seasonal influenza vaccination might boost broadly protective anti-HA responses (27) or alter imprinting from natural infection in IAV-naive children. By exposing IAV-naive children simultaneously to group 1 (H1N1) and group 2 (H3N2) antigens, vaccination might confer dual imprinting to both HA groups or prevent strong imprinting against either HA group—or it could have no effect beyond delaying the age of imprinting via the first natural infection. Our sensitivity analyses demonstrated that, given the low IAV vaccination coverage in H5N1- and H7N9-affected countries, none of these effects would change our study’s conclusions (fig. S7). However, to properly inform early childhood vaccine policy, future research must determine which, if any, of these effects occur.

HA group imprinting also might complicate “universal” vaccination approaches targeting conserved HA epitopes. Our findings indicate potent, long-lasting cross-protection between subtypes, putatively based on such responses. However, universal vaccination may have to outperform natural infection in its ability to induce broad immunity in the face of previous imprinting. The persistence of group 1 imprinting in older adults, despite decades of natural exposure to H3N2 after 1968 (Fig. 2), as well as the relative weakness of group 2 anti-HA stem reactivities in these older groups (2), suggests that HA exposures later in life do not readily alter broadly protective responses in individuals already imprinted to a particular HA group. To be effective, would bivalent (group 1 and group 2 HA stem) universal vaccines need to be delivered to infants before natural IAV infection? Or might universal vaccines even impair natural, long-term protection of the sort we have detected against H5N1 and H7N9 if received before an individual’s first natural IAV infection?

Our findings are consistent with the known potential for repeated infection by seasonal IAV subtypes. Group-matched imprinting, like other broadly protective IAV immune responses, is expected to protect against severe disease but not necessarily against infection (8, 12, 14). This parallels the reduced severity observed for repeat infections with seasonal strains (22, 23, 25). Furthermore, reexposure to a seasonal subtype typically elicits memory responses against the immunodominant HA head, which mask subdominant broadly protective responses involved in group-level imprinting (26).

For any country with suitable contact and demographic data, the methods shown here can provide rolling estimates of which age groups would be at highest risk for severe disease should particular novel HA subtypes emerge. Such projections could guide cohort- or region-specific prevention, preparation, or control. Quantitative projections of changes in \( R_{eff} \) and hence pandemic risk—will require further research into the protection arising from matched imprinting: Is some fraction of cases prevented entirely, and by what factor is infectivity reduced in mild cases arising in protected individuals?

Our findings show that emergence risk cannot be considered in isolation, even for “novel” pathogens that have not circulated in humans before. These pathogens are commonly assumed to have a bland slate of immunologically naïve humans to infect, but cross-protection from related pathogens can generate substantial population immunity. When this community of related pathogens undergoes major shifts, as during influenza pandemics, the landscape of population immunity changes accordingly. Thus, emergence of novel pathogens can be governed by bottom-up control, with population immunity acting in an important and predictable manner to modulate the widely recognized effects of virological and ecological risk factors. This perspective opens new frontiers for quantitative and mechanistic analysis of emergence risk.

References and Notes

Atomic features are dynamically formed and disassembled by laser irradiation. Although unstable, the formation of additional surface atoms in a plasmonic hotspot triggers optical field gradients that switch the Raman activity localization volume~10 nm (1,2). This extreme enhancement enables vibrational spectroscopy within small volumes, even down to single molecules (2,3). For many years, lateral resolution was believed to be ~10 nm (4); however, recent experiments have resolved the atomic structure of single molecules using tip-enhanced Raman spectroscopy (TERS) (3) and have demonstrated direct sequencing of RNA strands (5). Atomistic simulations also suggest that plasmonic confinement to atomic scales is possible (6).

Here, we show that light-activated mobilization of surface atoms in a plasmonic hotspot triggers the formation of additional “picocavities” bounded by a single gold atom. Because of strong optical field gradients that switch the Raman selection rules, the ultrasmall localization of light in these cavities alters the number and variety of vibrational modes of trapped molecules observed. The resulting cascaded ultrastrong plasmonic confinement pumps specific molecular bonds, thereby creating nonthermal vibrational populations and constituting an optomechanical resonator. Remarkably, the control of the plasmonic nanometric cavity allows for systematic and stable monitoring of Raman activity formation and disassembly. We thus demonstrate the possibility of resolving the dynamics of individual bonds within molecules. The existence, monitoring, and selective control of these picocavities will be important not only in photochemistry and photophysics but also as a platform for optomechanics, coherent control, and quantum information devices.

To produce stable, robust picocavities, we bypass complex scanning tip spectroscopies, instead using straightforward self-assembly to create “nanoparticle-on-mirror” (NPoM) geometries (Fig. 1A). Individual gold nanoparticles are spaced above a planar gold substrate by a nanometer-thick self-assembled monolayer (SAM) of biphenyl-4-thiol (Fig. 1B). Both the scattering and surface-enhanced Raman spectroscopy (SERS) signals from individual constructs are highly reproducible. All measurements are recorded at cryogenic temperatures, using a modified dark-field microscope and laser pumping at 633 nm (7). Low-temperature time-series SERS spectra from a typical gold nanoparticle (Fig. 1C) show vibrational modes that can be divided into two sets: A first set of vibrational modes is ever-present with constant intensity (“persistent lines”) while a second “blinking” set of lines appears, disappears, and changes intensity over time.

Comparing the observed spectra with density functional theory (DFT) simulations confirms that both types of lines originate from the biphenyl-4-thiol SAM. However, in the blinking set, the relative intensities are altered, and normally Raman-inactive lines (infrared (IR) absorption lines) are mixed into the SERS spectrum. Each realization of this fluctuating state displays different lines for each enhancement, indicating selective excitation of specific vibrational modes (see fig. SI3 for additional spectra). By contrast, SERS intensities in the persistent set are unaffected by the appearance of blinking lines. As shown below, this suggests that the blinking lines originate from a very small volume inside the plasmonic gap, containing only a single molecule. Such tight localization yields extremely high field gradients, accounting for the observation of Raman-inactive IR modes (8). Additional evidence implies that these small hotspots are actually sub-nm² volumes that we term “picocavities,” each consisting of only one gold atom. Full electromagnetic simulations (Fig. 1, D and E) show that picocavities locally boost the near-field intensity, leaving the rest of the plasmonic hotspot unaffected. The high field intensity within the extremely small (~1 nm²) local hotspot (Fig. 1, D and E) markedly enhances the SERS intensity of nearby molecules. We find that picocavities are spontaneously formed and destroyed under laser illumination but can be stabilized.

Picocavities are atomic-scale subnanometer structures forming an extreme class of optical localization that pushes electromagnetic coupling to the limit. To exploit and monitor their optical activity and to experimentally estimate the picocavity localization volume $V_L$, we explore how they modify the Raman scattering when exciting a molecular vibration. This process is greatly amplified by the extreme confinement of the incident light in the plasmonic gap. As a result, the population $n$ of excited vibrational states at frequencies

**REFERENCES**


**ACKNOWLEDGMENTS**

We thank the Lloyd-Smith lab and the Woroby lab for helpful comments. C. Viboud for providing insight into historic influenza data. T. Mega and S. Wu for assistance compiling data. B. Cowling for sharing poultry exposure data. P. Horby for sharing Vietnam contact data. K.M.G. is supported by the National Institute of General Medical Sciences of the National Institutes of Health (T32GM08185). M.A. is supported by the National Science Foundation Graduate Research Fellowship (DGE-1144087). M.W. is supported by the David and Lucille Packard Foundation. J.O.L.-S. is supported by the National Science Foundation (EF-0902860): the Research and Policy for Infectious Disease Dynamics (RAPIDD) program of the Science and Technology Directorate, Department of Homeland Security; and Fogarty International Center, National Institutes of Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The authors declare no competing financial interests. Case data and code for model fitting are available as supplementary data files. Requests for materials should be addressed to M.W. or J.O.L.-S.

**SUPPLEMENTARY MATERIALS**

www.sciencemag.org/content/354/6313/722/suppl/DC1

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Figs. S1 to S12

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14 May 2016; accepted 3 October 2016

10.1126/science.aag1322
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Science 354 (6313), 722-726.
DOI: 10.1126/science.aag1322

Lifelong protection against severe influenza

The first influenza attack that a child suffers can affect the way that their lifelong immunity to the virus builds up. A wide range of influenza A virus subtypes infect humans. Subtype H5 belongs to HA group 1 (which also includes H1 and H2 subtypes), and subtype H7 belongs to HA group 2 (which also includes the H3 subtype). Gostic et al. found that birth-year cohorts that experienced first infections with seasonal H3 subtype viruses were less susceptible to the potentially fatal avian influenza H7N9 virus (see the Perspective by Viboud and Epstein). Conversely, older individuals who were exposed to H1 or H2 subtype viruses as youngsters were less susceptible to avian H5N1-bearing viruses. A mathematical model of the protective effect of this imprinting could potentially prove useful to predict the age distribution and severity of future pandemics.

Science, this issue p. 722; see also p. 706