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Police in Seattle, Washington, wear masks to protect themselves during the 1918 flu pandemic that killed nearly 50 million people.

# THE GHOST OF INFLUENZA PAST

*A child's first flu infection shapes her response to all later ones. Now, researchers are realizing how important this 'imprint' is.*

BY DECLAN BUTLER

By the time she is about three years old, a child has usually endured her first influenza infection. If it's a nasty bout, her temperature will rise and her muscles will ache. She's probably young enough that she won't recall the illness — but her immune system will.

When the virus enters her body, its presence prompts a pool of immature, unprogrammed immune cells to start competing to become the flu's tracker and assassin. The winners — cells that bind most strongly to the virus — store a memory of the pathogen, ready to recognize and attack it the next time it strikes.

But influenza is an inveterate shape-shifter. Regions of its outer proteins can mutate as it replicates, allowing it to avoid immune detection. When infections with new flu strains occur later in life, the immune system will mount a response based on that first encounter, reacting strongly to recognized regions of the virus, but not to any that have changed. Immune cells can't tailor any novel antibodies that could help.

How exactly the immune system 'imprints' on its first-encountered strains presents a tantalizing puzzle to flu researchers — and solving it could help to combat the virus and improve vaccines.

Scientists suspect that understanding how imprinting works could help them to predict who will suffer most from seasonal strains and pandemics. Mounting evidence suggests that some people fare worse in deadly flu pandemics because their first childhood exposure was to a different version of the virus. Researchers think that this is why young adults experienced higher mortality than other age groups during the deadly 1918 pandemic[1], in which an estimated 50 million people died worldwide.

Knowledge of imprinting could help virologists to develop more-effective seasonal vaccines that could counteract circulating strains for several years, and a long-sought universal flu vaccine that could protect people for life against entirely new — and potentially pandemic-provoking — subtypes of flu. Imprinting seems to offer some immunity to flu strains related to the first infection. This broad immunity is often seen as a sign that the immune system could be coaxed into offering wide protection. "It does give us hope that we may be able to elicit a broadly protective immune response," says Aubree Gordon, an epidemiologist at the University of Michigan in Ann Arbor.

Existing flu vaccines could certainly do with some help. Their effects wear off after a few months, and they aren't very effective even in that brief window; during the 2017–18 flu season in the United States, people who received the vaccine were only 36% less likely to contract flu than those who weren't immunized, although vaccination can lessen the severity of symptoms in those who do become ill.

Imprinting might help to explain these shortfalls. But right now, the mechanisms behind this process are poorly understood, says Jennifer Nayak, a paediatric immunologist at the University of Rochester Medical Center in New York. Getting to grips with imprinting will be important to researchers who hope to tailor a universal vaccine to fit people with different past flu exposures, says Scott Hensley, a viral immunologist at the University of Pennsylvania in Philadelphia. "The same vaccine given to different people will likely elicit different immune responses, depending on their history," he says.

In April, the US National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Maryland, called for researchers to pitch projects that would explore the effects of imprinting on immunity, as part of a wider effort to fund research into a universal flu vaccine. The agency plans to spend US$5 million on a large cohort study that will recruit and monitor infants from birth for at least three flu seasons to explore at the molecular level how their immune systems respond to initial exposure and subsequent flu infections and vaccinations. Immunizations are usually recommended for babies over 6 months of age.

Studying the virus can offer only so much; better protection will also depend on studying people. Researchers are realizing that the body can mount a surprisingly broad response, even against a shape-shifter like the flu. "Influenza is one of the best studied viruses on the planet," says Katelyn Gostic, an epidemiologist at the University of California, Los Angeles (UCLA). "We're discovering a whole new continent in a world that we thought was already well mapped."

## FLU FOUNDATIONS

The concept of imprinting was first proposed by the late Thomas Francis, a virologist and epidemiologist at the University of Michigan, whose studies in the 1940s and 1950s were the first to show that individuals generate stronger antibody responses to the first flu strain they encounter, compared with those they're exposed to later in life[2].

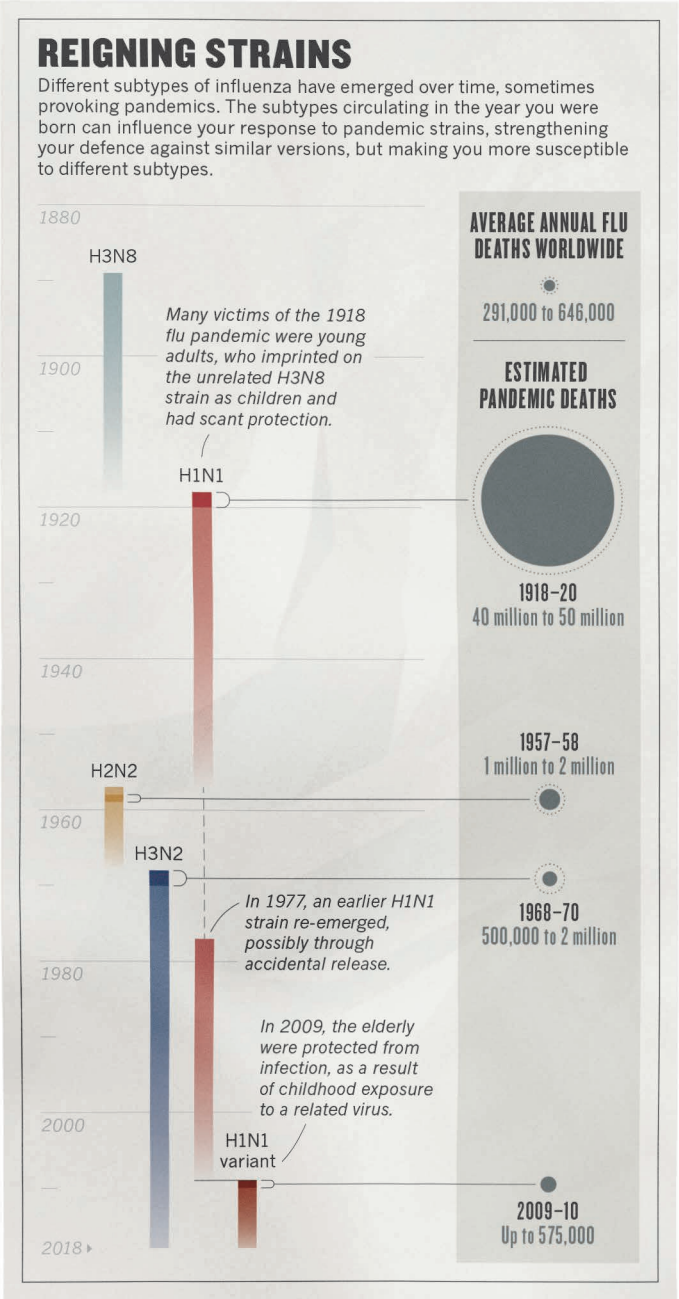
Researchers have since refined the concept. In a study of more than 150 people aged 7–81 in southern China, scientists measured antibody levels against several different strains of flu virus, looking at how their immune systems responded to strains they would have encountered at different times in their lives. The researchers found that after the first infection, subsequent strains have a progressively dwindling influence on the immune response[3], explains Justin Lessler, an epidemiologist at Johns Hopkins Bloomberg School of Public Health in Baltimore, Maryland, and a co-author of the study. "While immune imprinting plays a critical role, a focus on that alone can lead us to miss important aspects of how influenza immunity develops across multiple exposures," he says.

In 2009, a new flu variety emerged in Mexico, resulting in a pandemic that gave researchers one of their best chances yet to study imprinting using modern immunological methods. A series of studies[4,5] suggests that the virus prompted such a strong immune response that it 'awoke' in people who contracted it a broad immunity that had lain dormant since early imprinting. Many individuals generated antibodies that could attack not only the new strain but also members of its wider family.

Flu viruses come in a few flavours. The major version that causes illness in humans has many subtypes, which are named after proteins on their surface: there are 18 known forms of the haemagglutinin (HA) protein, and 11 of the neuraminidase (NA) protein. Each virus subtype has an HA and an NA variant. Bolting them together gives each subtype its name — such as H1N1 or H3N2. Some have been found to infect only certain animal groups, but others can morph into new versions capable of infecting humans.

In a *Science* paper[6] in 2016, Gostic and her colleagues analysed all known human cases of two subtypes of bird flu, H5N1 and H7N9, in circulation in six countries. The two viruses afflicted different age groups. H5N1 mostly infected young people, whereas almost all cases of H7N9 were in older people. By looking at the year of birth of each individual with the flu, they found that susceptibility abruptly changed in 1968, with people born before then more vulnerable to H7N9, and those born after more vulnerable to H5N1.

These people hadn't met either subtype before. But depending on when they were born, they had encountered related varieties. Flu subtypes can be divided into two groups according to certain characteristics of their HA protein. H5N1 belongs to the same broad group as H1N1



SOURCE: P. R. SANDERS-HASTINGS AND D. KREWSKI *PATHOGENS* **5**, 66 (2016).

CAROLYN KASTER/AP/SHUTTERSTOCK

**Frozen flu-virus strains are stored at the US National Institutes of Health.**

and H2N2 — strains that circulated seasonally before 1968.

Anyone born before that year would have been imprinted with one of these group 1 strains, and so protected from H5N1. But in 1968, everything changed: a pandemic of H3N2 struck, and became the only seasonal subtype. Most people born after that time were thus imprinted with the H3N2 strain, a group 2 virus. The H7N9 variant belongs to the same group — so many people born after 1968 were protected against it.

The finding suggests that imprinting with a virus from one of the two HA groups might offer broad cross-protection against new subtypes in the same group, challenging many public-health experts' assumption that most people would have little or no protection in pandemics, which are usually caused when new subtypes of flu emerge.

"The strength of the protective effect against severe H5N1 and H7N9 infection was shocking," says disease ecologist James Lloyd-Smith, co-author of the paper and also at UCLA. Using modelling, the researchers showed that childhood imprinting gave 75% protection against severe disease and 80% against death from these avian flu viruses.

Variations in susceptibility among different age groups have been observed in other pandemics. In the 1918 pandemic, perpetrated by an H1N1 subtype, those most severely affected were young adults with broad protection against H3N8, which circulated between 1889 and 1918 when they were children. H3N8 belongs to a different group from H1N1 (see 'Reigning strains'). The 2009 pandemic was caused by a variant of H1N1, but even so, there were very few cases in the elderly, who would have imprinted on the earlier version of H1N1 that circulated after the 1918 pandemic, says Patrick Wilson, an immunologist at the University of Chicago in Illinois. An H1N1 virus also appeared in the 1970s: it was so similar to a previous strain that scientists think it was accidentally released from a laboratory or a vaccine trial[7]. "It's sort of fun to look at when you were born and sort of infer what your first imprint was," Hensley says.

The priority now is to work out how the human body is imprinting on the first strains it sees. "We need to tease out what the immunological basis of that is," says Hensley.

Over the past decade, researchers have been building a palette of techniques for studying imprinting at the molecular level. It's easy to test the level of all antibodies generated in response to a bout of flu, for instance, but getting to the roots of imprinting requires being able to focus on the subsets of antibodies that generate broad immunity.

For example, researchers are now able to sort and analyse hundreds of thousands of single cells, and they can use single-cell sequencing to characterize the major players of the immune system before and after the cells respond to their first infection. Scientists would like to know how those cells engineer such a long-lasting response to future flu.

"Now, our tools are much more refined, providing an extremely granular look at what is occurring upon first exposure, and repeated exposure, to influenza and influenza vaccine," says Buddy Creech, director of the Vanderbilt Vaccine Research Program at Vanderbilt University Medical Center in Nashville, Tennessee. He is co-directing the Universal Influenza Vaccine Initiative, a multi-university project that launched last October to study the immune response to flu and how broad immunity might be evoked. Once those mechanisms are better understood, they might be recapitulated to help make vaccines more broadly active, says Nayak.

## PEOPLE POWER

For researchers wishing to apply these tools, funders such as the US National Institutes of Health and the Bill & Melinda Gates Foundation are stepping in to help.

The Gates Foundation announced a $12-million tranche of funding in April, which it plans to put towards pilot projects that aim to develop universal flu vaccines; the call mentions imprinting and other features of the host's immune response, and will prioritize higher-risk ventures.

In the same month, the NIAID issued its $5-million call for proposals to follow large numbers of children over at least three flu seasons and potentially for years afterwards. The ultimate goal of the study, according to the NIAID, is to provide information that will help researchers to design long-lasting, universal vaccines.

Until now, research into childhood exposure has been limited, so the NIAID call is welcome news, says Nayak. Most studies of flu in children have been small, and haven't characterized each individual's exposure history firmly enough, she says. "This makes it impossible to even address whether imprinting is occurring, much less determine the mechanism responsible."

Part of the problem has been in tracking an infant's immune system, which requires repeated blood draws. As recently as 5 years ago, assays required drawing 10–20 millilitres of blood, making immunological monitoring of young babies impractical (a 3-kilogram newborn has only 240 millilitres of blood). But advances in technology have overcome that obstacle. "With these single-cell assays, you can do strong immunological work-ups with just 1 to 2 millilitres of blood," says Hensley, who has applied to run a study using cohorts in the United States and Hong Kong.

These techniques will enable researchers to catalogue an infant's exposures and vaccinations precisely over time, and to sketch a detailed picture of how immunity differs when stimulated by natural infection compared with vaccination.

The NIAID call aims to complement other cohort studies of flu around the world. The agency already supports influenza research through cohorts in Nicaragua, Hong Kong and New Zealand, but none focuses on childhood imprinting. Gordon runs the Nicaraguan cohort, which is studying the incidence and severity of flu in children. Hers is the only large cohort set up to enrol and follow children from birth, and so is well placed to study imprinting. She has applied for NIAID funding as part of a consortium, to enable her team to incorporate the specialized immunology expertise needed.

Nayak already has a small pilot imprinting study in progress, which has so far enrolled 129 children since it started in late 2016. She, too, has put in a bid to the NIAID, involving the University of Rochester and the University of Minnesota, with cohort sites in the United States and Australia. Having multiple sites hedges against the risk of a few quiet flu seasons, or a few seasons dominated by just one flu flavour.

For scientists who want to chase the elusive universal flu vaccine, the cohort studies are one strand of a multi-pronged strategy. They will also need to research basic viral biology and find fresh ingredients for vaccines, says Creech. "We really have to work the problem from both sides." ■

**Declan Butler** *is a senior reporter for* Nature.

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