Understanding immune responses to the influenza vaccine

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The quest to improve influenza vaccines is aided by research into the immune response that they generate. Two recent studies have focused their attention on the specificities of antibodies induced after vaccination with conventional inactivated influenza vaccines.

Vaccines that stimulate antibodies to the viral hemagglutinin (HA) have been a cornerstone of the public-health response to seasonal epidemics and, infrequently, pandemics of influenza-induced morbidity and mortality. Current influenza vaccines are multivalent, containing antigens to the circulating A(H3N2), A(H1N1 pdm2009) and either one or both lineages of influenza B viruses. Some of the primary challenges in this regard are the continued evolution of the viruses in circulation and the need to select the appropriate strains to incorporate into a vaccine some 6 months before its actual use. Compounding this issue is the fact that the vaccine induces protective immunity only to viruses that are antigenically similar to the vaccine strains. Although the effectiveness of available vaccine formulations is a point of intense study, the consensus is that there is ample room for improvement. One approach that has been undertaken is to detail the immune response to vaccination and infection in humans, in hope of learning what an ideal and broadly reactive immune profile might look like. In this issue of *Nature Medicine*, two studies carry out this approach. One study provides a cautionary tale of the effect of developing vaccines in chicken eggs, and the other improves understanding of the type of antibody response that we would wish to induce to protect against infection (**Fig. 1**).

Studies that examine the responses of single B cells to influenza vaccination, for example[1], have shed new light on the specificities of antibodies induced. Here Lee *et al.*[2] were able to use an elegantly different tactic, in which they profiled the antibodies themselves rather than the cells that produce them. The team used a high-resolution proteomics approach, combined with B cell–receptor transcript sequencing, to examine the antibody responses of a cohort of young adults before and after vaccination with an inactivated trivalent influenza vaccine. The abundance and properties of the antibodies before and after vaccination were compared. They found that the majority of the response to vaccination was dominated by pre-existing, rather than new, antibodies, and that only a small number of antibody clonotypes dominated the response. Their data also support a number of epidemiologic studies that have observed a less robust neutralizing response to infection in

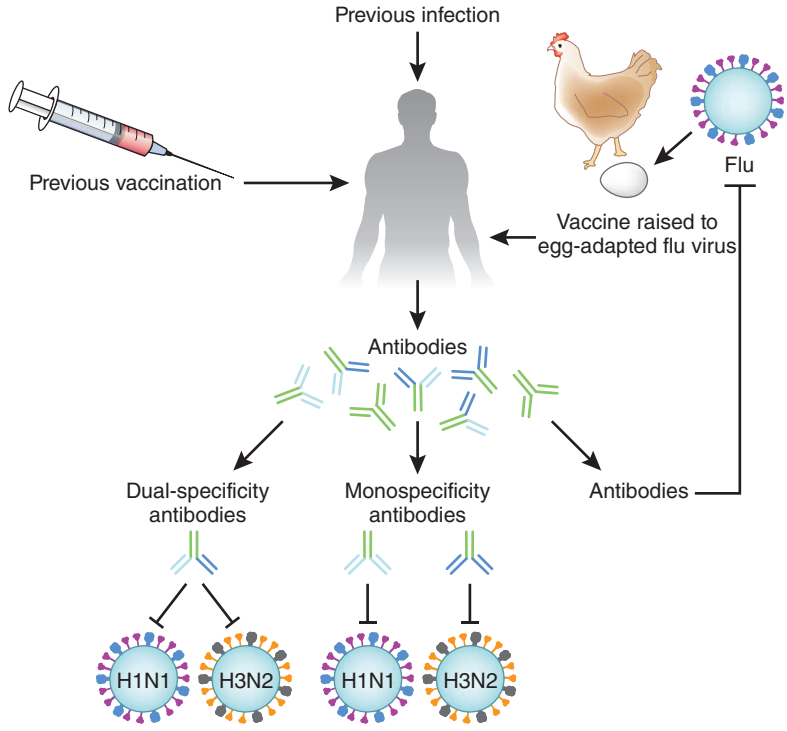

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Figure 1 Analysis of the immune response to the influenza vaccine. The kinds of antibodies raised in response to the influenza vaccine depend on a multitude of factors. Lee *et al.*[2] show that in response to vaccination, antibodies are raised that can target either a multitude of strains or just one strain. Raymond *et al.*[6] show that vaccination with an egg-adapted flu strain can result in antibodies that specifically target antigens from these adapted viruses.

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individuals with higher prevaccination titers[3]. More specifically, individuals with high levels of influenza-specific antibodies prevaccination produced fewer new antibodies in response to vaccination than individuals with lower prevaccination titers. The model proposed by Lee *et al.*[2] is that pre-existing antibodies will bind the incoming vaccine antigen and blunt the ensuing immune response, but ways to overcome this phenomenon by alternative vaccination strategies are not immediately clear.

The authors also identified an unexpectedly high level of both neutralizing and non-neutralizing broadly reactive antibodies induced by the vaccine. Conventional wisdom is that the antibody response to inactivated influenza vaccines is narrow, limited to the vaccinating antigen and closely related viruses. However, recent data have pointed to the presence of broadly reactive HA-stalk-reactive antibodies that can be induced by various vaccination approaches[4]. Among the broadly reactive antibodies that they detected, Lee *et al.*[2] identified not only the expected HA-stalk-reactive antibodies, but also cross-reactive antibodies that bind to the receptor-binding site (RBS) of the HA monomer of both A(H1) and A(H3) viruses. These RBS-binding antibodies were not active in an HA inhibition assay—the workhorse assay for the assessment of serologic responses to influenza—but they were able to protect mice from lethal challenge when passively transferred. Why do they not, then, seem to confer a cross-protective effect as part of the vaccination response? Is it due to the levels or potency of these broadly reactive antibodies? Is it because of their limited duration or that they are in the periphery and not at the site of infection? Or is it the lack of sensitivity of epidemiologic studies to detect the protection—or some as-of-yet unknown deficiency?

It seems necessary to examine this phenomenon further by continuing the work to characterize not only the antibodies, but also the vaccinated population, so as to more fully evaluate the role of antibody type in the severity and spread of epidemic influenza. The authors also noted that a number of the influenza-B-specific antibodies produced bound to both lineages of circulating viruses, which suggests that quadrivalent vaccines containing both influenza B lineages may not be substantially more efficacious than trivalent vaccines that contain only one. Overall, the message is clear: it is possible that we are underestimating the role of cross-reactive antibodies in molding seasonal influenza impact. This has obvious implications for vaccine design.

In the study by Raymond *et al.*[5], the authors were able to identify a weakness in the current influenza-vaccine production process that leads to misdirected antibodies. Influenza viruses will variably grow to high titers in chicken eggs, and this approach has thus been the backbone of our influenza vaccine pipeline since its inception. Although newer, recombinant products, such as Flublok, or cell-culture-grown products, such as Flucelvax, are now licensed in some countries, the ability to deliver influenza vaccine on time and in sufficient quantities on a global scale relies on the capacity of the individual vaccine components to replicate in eggs. Vaccine manufacturers enhance this property by carrying out serial passage of viruses on this substrate. However, there is a long-recognized underlying issue[6]: the adaptation of human influenza viruses to grow in eggs can be accompanied by changes in residues around the RBS that alter the immunogenic profile of the virus. Forced adaptation to eggs has resulted in antigenic differences between vaccine antigen and circulating viruses, and in the 2012–13 Northern Hemisphere influenza season, this was associated with reduced vaccine effectiveness as compared to previous years[7].

Raymond and colleagues examined the plasmablast response of a recipient of an MF59-adjuvanted monovalent (H1N1 pdm2009)-based influenza vaccine produced by using the vaccine virus X-181, a vaccine virus derived from the wild-type virus A/California/07/2009. They noted that a number of antibodies were produced that were less able to neutralize the wild-type virus than the egg-adapted version X-181. Epitope mapping of these antibodies identified the RBS as the targeted site, and the presence of a key amino acid residue associated with egg adaptation correlated best with neutralization activity. Although analysis of polyclonal serum showed that there was substantial vaccine-induced immunity generated to the wild-type virus, its extent was less than that generated to the vaccine virus. Of note, targeting of the immune response to inappropriate epitopes present on vaccine, but not circulating, viruses might also have been responsible for the lower efficacy of A(H1N1 pdm2009) vaccine components in certain age groups in recent influenza seasons[8]. In fact, there was an update of the recommended vaccine virus away from those based on A/California/07/2009 (ref. 9). Whether the newly nominated viruses for vaccine development develop egg-adaptive changes remains to be seen.

These two studies remind us of the benefits and perils of inducing antibodies that target the influenza virus HA RBS. Such considerations are not new, but a new level of complexity and detail is now revealed, showing the power of modern molecular immunologic studies. The challenge now is to use this new detail to design novel vaccination approaches that could harness the benefits but limit the perils.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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