Finally, the work by Thongsomboon *et al.* will benefit efforts to find new applications for bacterially synthesized cellulose and develop new cellulose-based compounds. *Gluconacetobacter*-produced cellulose microfibers and crystals, commonly referred to as nanocellulose, have numerous applications (*2*). The apparent biocompatibility—lack of toxicity, immunogenicity, and proinflammatory response—of unmodified cellulose makes it an attractive choice for a variety of biomedical applications, such as drug delivery, wound dressing, replacement of blood vessels, and tissue engineering of bone and cartilage (*2*, *3*). Thus, pEtN-modified cellulose would have to undergo rigorous biocompatibility testing. Nevertheless, the availability of genetic tools to manipulate *E. coli* opens numerous possibilities for using cellulose synthase genes for synthetic biology. A particularly interesting development could be the ability to produce entirely new kinds of cellulose films for applications ranging from optoelectronics to packaging.

In principle, one could imagine production of cellulose microfibers with new modifications. The catalytic domain of BcsG is a metalloenzyme of the alkaline phosphatase and sulfatase superfamily (*14*), which is anchored in the membrane by five predicted transmembrane helices (see the figure). Replacing the catalytic domain of BcsG with a different enzyme, such as an acyltransferase or a glycosyltransferase, could allow biosynthesis of cellulose nanocrystals with new optical properties, increased conductivity, or the ability to bind metal ions. ■

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MICROBIOLOGY

Taking down defenses to improve vaccines

A new approach to generating influenza virus vaccines could improve responses

By **John R. Teijaro**[1] *and* **Dennis R. Burton**[1,2]

Vaccines have been spectacularly successful in durable protection against a range of pathogens. However, they have been less successful against pathogens that have evolved immune escape mechanisms (*1*). For example, the influenza virus surface glycoprotein hemagglutinin (HA), which is the main target (antigen) for protective antibodies, shows enormous sequence diversity between different strains, meaning that antibodies induced by immune responses to one strain of the virus tend to be either inefficient or ineffective against other strains. This observation is often associated with the need for a new influenza vaccine every year. However, the escape mechanisms of influenza virus extend beyond antigenic variation of surface proteins. For example, wild-type viruses typically encountered in natural infection can suppress the host type I interferon (IFN-I) response, which provides the first line of defense against viral infections and promotes stimulation of an optimal immune response (*2*). On page 290 of this issue, Du *et al.* (*3*) describe the generation of a variant influenza virus that, in contrast to the wild type, is hyper–interferon-sensitive (HIS) and therefore attenuated (reduced in virulence). Attenuated viruses typically have lower immune responses than their wild-type counterparts but, in this case, the level of attenuation still resulted in robust immune responses. The authors propose that the HIS approach could form the basis for a more effective influenza vaccine.

Following infection, viral nucleic acids are sensed by the host innate immune system through multiple pattern recognition receptors. Among the first antiviral proteins produced are IFN-I, a family of cytokines that signal to surrounding host cells and induce multiple interferon-stimulated genes (ISGs) that act to prevent viral amplification and dissemination. In addition to inhibiting viral replication, IFN-I signaling also promotes the optimal induction of both the innate and adaptive arms of the immune response. To counteract a potent IFN-I response, many viruses encode proteins that inhibit IFN-I production and/or signaling, highlighting the evolutionary importance of host IFN-I signaling in controlling early virus infection (*4*). Influenza virus is no exception and encodes nonstructural protein 1 (NS1), which exerts potent anti–IFN-I effects and is essential for viral fitness (*5*).

> ***"The authors argue that the approach may be broadly applicable in creating efficacious live attenuated vaccines for a plethora of viral infections."***

To investigate the possibility of harnessing IFN-I sensitivity for attenuated vaccine design, Du *et al.* employed a quantitative high-throughput genetic mutagenesis system coupled to next-generation sequencing (*6*, *7*) to simultaneously measure loss-of-function mutations in influenza virus in the presence or absence of IFN-I signaling. Using this approach, they identified multiple IFN-I–sensitive mutations across the influenza virus genome, including mutations outside of the gene encoding NS1. The authors then combined an assortment of eight IFN-I–sensitive mutations in multiple viral genes to create a HIS virus. The HIS influenza virus increased both IFN-I sensitivity and production, attenuated viral fitness in vitro and in vivo, and did not produce disease pathology after high-dose administration to mice and ferrets (commonly used models of influenza infection). Importantly, despite multiple mutations in viral genes in the HIS virus, enough antigenic determinants were conserved to generate antiviral T and B cell–mediated immune responses (which involve antibodies) to promote protection after challenge with multi-

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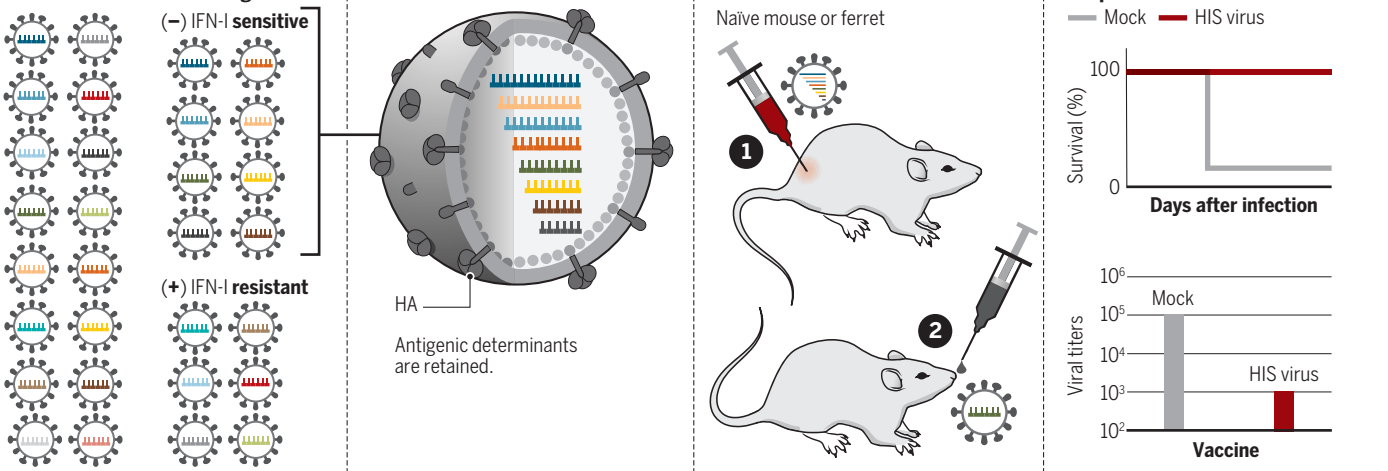
Generating live attenuated HIS virus vaccines

HIS viruses confer improved vaccination against influenza virus strains. This approach could be used to generate more effective live attenuated vaccines against other viruses.



Libraries of mutant influenza virus strains are screened for IFN-I sensitivity. IFN-I–sensitive strains are selected and sent for RNA sequencing.

IFN-sensitive strains are sequenced and mutations incorporated into a single virus, creating a HIS virus that is attenuated in vitro and in vivo.

1 Naïve mice or ferrets are vaccinated with the HIS influenza virus. **2** Mice or ferrets are challenged with homologous or heterologous virus strains.

Animals challenged with HIS virus exhibited superior protection compared to mock challenged animals with respect to both reduced mortality and viral loads.

ple strains of influenza virus when mice were vaccinated with HIS viruses derived from the H1N1 subtype and subsequently challenged with either H1N1 or H3N2 subtypes in mice and ferrets (see the figure). The protection generated by the HIS influenza virus vaccine is likely mediated through the generation of cross-reactive T cells, which can react with multiple viral strains. This is especially likely to be the case because mice vaccinated with the H1N1-HIS virus and subsequently challenged with heterologous H3N2 influenza viruses produce potent protective antibodies against the homologous H1N1 virus that will not have substantial cross-reactivity with H3N2 viruses.

The authors argue that the approach may be broadly applicable in creating efficacious live attenuated vaccines for a plethora of viral infections. Although a concern with live attenuated vaccines is often their potential to revert to virulence (*8*), attenuating the virus with multiple point mutations, as in Du *et al.*, is likely to avert this outcome. In addition to increasing safety, the use of mutations scattered throughout the viral genome should provide a barrier to the development of viral resistance. Moreover, the avoidance of mutations in HA should help preserve robust antibody responses.

The holy grail of the influenza virus vaccine field is a universal influenza vaccine (*9*). Such a vaccine would obviate the need for an annual vaccine. Furthermore, if providing durable protection, such a vaccine could be given earlier in life, when the induction of immune responses is more optimal, rather than later in life, when susceptibility to serious infection is higher (*10*) and vaccine efficacy significantly declines (*11*). The approach of Du *et al.* may be a step toward a universal influenza vaccine in that it will be safe and retain sufficient antigenic determinants to promote immune protection to multiple viral strains. However, many challenges remain. Data on cross-protection are limited to exposure to a small set of strains from the H1N1 and H3N2 subtypes of influenza viruses. It would be valuable to test additional viruses, including highly virulent avian subtypes such as H5N1 and H7N9, during subsequent challenge studies.

Another limitation is that the approach relies heavily on T cell immunity for cross-strain protection. However, much evidence suggests that B cell immunity in the form of antibodies can be very important in protection against influenza virus and indeed antibodies are the oft-used correlate of likely vaccine efficacy (*12*). In terms of the HIS approach and antibody-mediated responses, the diversity of the HA molecule is a problem because the approach is largely expected to induce strain-specific antibodies and the HA molecule rapidly mutates under immune selection pressure. It might be beneficial to combine the HIS approach with one that targets the induction of broadly neutralizing antibodies (bnAbs), which can bind and neutralize diverse influenza virus strains. For example, one could use emerging constructs being developed that promote the generation of HA bnAbs that recognize the stem region of the HA molecule, which is relatively conserved for different viral strains (*9*). Using a combined approach might increase the titer of difficult-to-induce bnAbs. Moreover, the added T cell immunity could further improve overall efficacy in humans. Indeed, there is evidence that both T and B cell immunity can contribute to vaccine protection against viral infections (*12*).

It will also be important to determine whether the approach of Du *et al.* can be extended to the generation of more effective live attenuated vaccines against other viruses. As part of such studies, the genetic analysis could be applied to other critical innate immune signaling pathways such as mutating viral proteins that attenuate the viral RNA–sensing retinoic acid–inducible gene–I (RIG-I) and melanoma differentiation associated protein–5 (MDA-5) (*13*) as well as viral proteins that antagonize IFN-I signaling. ■

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