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VIRAL INFECTION

Stemming influenza viruses

> vaccination strategies that elicit antibodies directed against the stem region of HA are protective against multiple influenza strains

A key goal in influenza research is the design of a universal flu vaccine that affords protection against multiple viral subtypes. Now, two studies report new vaccination strategies that engineer the stem region of haemagglutinin (HA) glycoprotein to elicit antibodies that protect against multiple influenza subtypes in animal models of infection.

A large number of flu infections in humans are caused by influenza A viruses, predominantly by strains with HA subtypes H1 and H3, but other subtypes (including H2, H5 and H7) that are able to infect humans can arise from animal reservoirs. Protective immunity against influenza largely relies on antibodies, and following natural infection, or current vaccination strategies, antibodies are primarily directed against the head region of HA. However, the head region of HA is highly variable both within and between different influenza subtypes, which means that antibody responses directed against one strain do not necessarily protect against unrelated strains or subtypes. By contrast, the stem region of HA is relatively conserved even across influenza subtypes, and the discovery of broadly neutralizing antibodies (bnAbs) that target this region suggests that the generation of immune responses targeting the stem could protect against multiple influenza subtypes (that is, heterosubtypic protection).

To generate immunogenic versions of the stem region of HA that are capable of heterosubtypic protection, Yassine *et al.* performed six iterative cycles of structure-based design by which they systematically removed the HA head group from H1 HA proteins while optimizing protein folding, trimerization and exposure of the stem region that is recognized by bnAbs. The resulting 'H1 HA stabilized-stem proteins' (HA-SSs) were further fused to the ferritin subunit from *Helicobacter pylori* to create self-assembling nanoparticles (termed 'H1-SS-nps'), a strategy that has been shown to increase the ability of HA to elicit antibodies. Impagliazzo *et al.* used a different structure-based strategy to generate 'stable HA stem antigens' ('mini-HAs'); in a five-step process, they started with H1 HA proteins and generated soluble and stable HA monomers that lack the HA head group, maximized exposure of the stem region that is recognized by bnAbs, and then engineered the stem monomers to create stable trimeric mini-HAs.

Yassine *et al.* tested the protective efficacy of H1-SS-nps in mice and ferrets. Immunization with these nanoparticles elicited broad antibody responses against group 1 HA subtypes (including H1, H2, H5 and H9) in both mice and ferrets, whereas antibody responses against group 2 HA subtypes (H3 and H7) were observed in mice. Furthermore, mice and ferrets immunized with H1-SS-nps were protected against lethal infection with a heterosubtypic H5N1 virus; whereas all animals immunized with empty nanoparticles died, all mice and four out of six ferrets immunized with H1-SS-nps survived. Interestingly, the antibody-dependent protection elicited by the H1-SS-np vaccine seemed to be mediated by effector mechanisms other than viral neutralization.

Impagliazzo *et al.* tested their mini-HAs for the ability to confer protection in mice and in non-human primates. In mice, immunization with the mini-HAs induced the generation of antibodies against group 1 (including H1, H5 and H9) and group 2 (including H3 and H7) virus strains. Furthermore, immunization with mini-HAs provided heterosubtypic protection against lethal infection with an H5N1 virus in all of the vaccinated mice. In cynomolgus monkeys, animals vaccinated with mini-HAs had reduced fever after infection with a heterologous H1N1 virus compared with mock-vaccinated monkeys, which was similar to what was observed in monkeys that had received the seasonal flu vaccine. However, vaccination with mini-HAs induced the generation of high titres of antibodies with the ability to neutralize H5N1, whereas vaccination with the seasonal flu vaccine did not.

Finally, both studies confirmed the key role of antibodies in mediating heterosubtypic protection by transferring immunoglobulin from mice immunized with either H1-SS-nps or H1 mini-HAs, followed by infection with an H5N1 virus. In both cases, mice receiving the immunoglobulin from the immunized mice survived the challenge with a lethal infectious dose, whereas mice that received control immunoglobulin succumbed to infection.

Collectively, these studies demonstrate that vaccination strategies that elicit antibodies directed against the stem region of HA are protective against multiple influenza strains and pave the way for the development of a universal flu vaccine.

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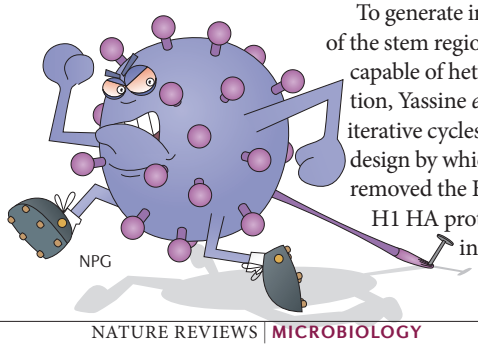

ORIGINAL RESEARCH PAPERS Impagliazzo, A. *et al.* A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen. *Science* http://dx.doi.org/10.1126/science.aac7263 (2015) | Yassine, H. M. *et al.* Hemagglutinin-stem nanoparticles generate heterosubtypic influenza protection. *Nat. Med.* http://dx.doi.org/10.1038/nm.3927 (2015)