

INSIGHTS
Inhibiting neuraminidase can make the difference

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Immunogens inducing antibodies against the stem of influenza virus hemagglutinin are promising candidates for the development of universal vaccines. In this issue of *JEM*, Kosik et al. (<https://doi.org/10.1084/jem.20181624>) report that inhibition of neuraminidase by anti-stem antibodies contributes to their broadly neutralizing activity.

Influenza virus carries the proteins hemagglutinin (HA) and neuraminidase (NA) on its surface. HA is composed of a variable globular head and a more conserved stalk domain. The head contains the receptor-binding domain for sialic acid. NA is an enzyme that cleaves sialic residues on glycoproteins and allows virus entry and release. The head portion of HA is immunodominant and induces antibodies that provide sterilizing immunity by blocking receptor binding and viral entry. However, the head is also highly variable, undergoes periodic drifts and shifts, and induces mostly strain-specific protection. Because of the high variability of HA, new vaccines are produced every year, and vaccine effectiveness hinges on the prediction of strains that will dominate the influenza season.

Periodically, seasonal vaccines do not match circulating strains, and this results in

poorly performing vaccines with important health and economic consequences. Universal influenza vaccines aim to protect against several, if not all, influenza infections (Erbelding et al., 2018). In 1993, a first study described a broadly neutralizing monoclonal antibody specific for an epitope in the conserved region of the HA stalk which was also able to block virus-mediated cell-cell fusion (Okuno et al., 1993). This initial observation was confirmed 15 yr later by the isolation of many monoclonal antibodies cloned from human memory B cells that recognized conserved epitopes in the HA stalk (Corti et al., 2011; Ekiert et al., 2011; Dreyfus et al., 2012). So far, the available vaccine technologies have been used to induce stalk-specific antibodies and increase their poor immunogenicity with the scope to develop a universal influenza vaccine (Krammer et al., 2013; Impagliazzo et al., 2015; Yassine et al., 2015;

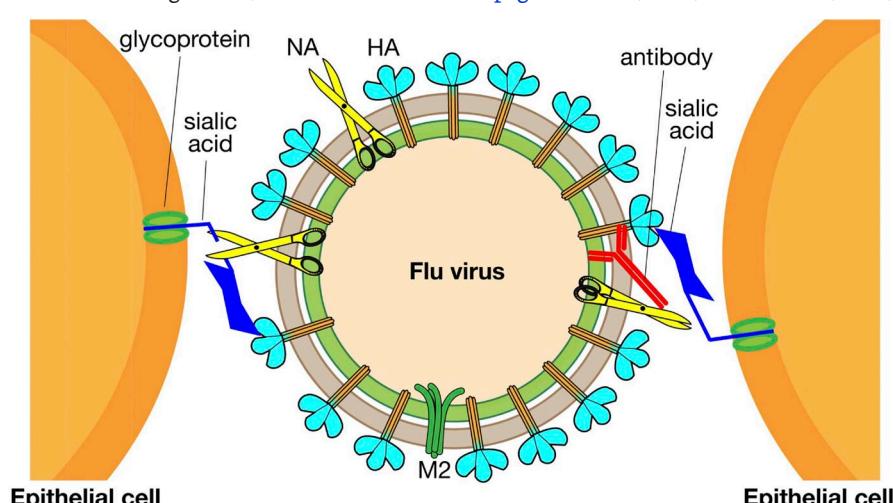


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Pardi et al., 2018). The anti-stalk monoclonal antibodies have been shown to provide protection by several mechanisms, including prevention of viral fusion with endosomal membranes during entry, impairment of viral egress from infected cells (Yamayoshi et al., 2017), and activation of antibody-dependent cytotoxicity via the engagement of the Fc gamma receptor (Mullarkey et al., 2016). These antibodies also have proven effective in vivo by preventing weight loss and mortality in mice upon challenge with influenza virus (Jacobsen et al., 2017).

In this issue of *JEM*, Kosik et al. provide further characterization of the mechanistic insights of the antiviral activity mediated by anti-stalk antibodies. Kosik et al. (2019) uncovered that anti-stalk antibodies inhibit NA activity by steric hindrance and suggested that this mechanism contributes to antibodies' broadly neutralizing activity in vitro and in vivo. These data are in line with recent findings reported in another in vitro study (Chen et al., 2018).

After demonstrating that available group I/II cross-reactive stem antibodies were capable of inhibiting NA enzymatic activity, Kosik et al. (2019) observed that the extent of NA inhibition was variable between stains



Schematic representation of the influenza virus showing that the HA binds the sialic acid receptor on the surface of eukaryotic cells. Left: Sialic acid is normally cleaved by NA during infection to allow the virus to enter the endosome and, after infection, to release viral particles produced by infected cells. Right: Anti-stalk antibodies prevent the access of NA to sialic acid, thus preventing viral entry and egress.

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and was inversely correlated with NA stalk length. To further support these findings, recombinant PR/8 viruses harboring NAs of different length were generated. [Kosik et al. \(2019\)](#) observed that a shortened NA stalk increased the ability of anti-stalk antibodies to inhibit NA, while a longer NA stalk resulted in the opposite effect. An increased neutralization ability of anti-stalk antibodies was observed when Madin-Darby Canine Kidney SIAT1 cells were infected with recombinant virus expressing the shortened NA stalk. Furthermore, [Kosik et al. \(2019\)](#) observed that this effect was more prominent when multicycle infection was enabled and demonstrated that NA inhibition impacted viral release. Importantly, these findings were equally significant *in vivo*. After passive immunization with anti-stalk antibodies, [Kosik et al. \(2019\)](#) observed that mice

infected with virus harboring the shortened NA stalk displayed reduced weight loss and lung pathology (alveolar inflammation and lymphocyte infiltration), which was the result of an increased neutralizing activity. [Kosik et al. \(2019\)](#) propose that the *in vivo* protection of anti-stalk antibodies was partially mediated by the ability of antibodies to interfere with impairment of Fc γ R-based cell activation mediated by NA. Therefore, NA inhibition resulted in increased antibody-dependent cytotoxicity. The most important question at this point is whether these promising data of stalk immunity will translate to protection in humans and whether the same mechanisms may apply.

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