Winning the Fight against Influenza

Annual influenza epidemics and episodic pandemics continue to cause widespread illness and mortality. The World Health Organization estimates that annual influenza epidemics cause around 3–5 million cases of severe illness and up to 650,000 deaths worldwide. Seasonal influenza vaccination still remains the best strategy to prevent infection, but the vaccines that are available now offer a very limited breadth of protection. Human broadly neutralizing antibodies (bnAbs) that bind to the hemagglutinin (HA) stem region provide hope for a universal vaccine (Figure 1a)\textsuperscript{1,2}. Binding of these bnAbs prevents the pH-induced conformational changes that are required for viral fusion in the endosomal compartments of target cells in the respiratory tract and, hence, viral entry in our cells.

To utilize these findings on bnAbs to develop inhibitors, a multi-institutional group led by researchers from The Scripps Research Institute and Janssen Pharmaceuticals employed a wide range of systematic studies. The most recent results have been recently published in two breakthrough papers in Science. The first paper reported the use of diverse camelid single-domain antibodies on influenza virus hemagglutinin to generate multidomain antibodies with impressive breadth and potency. The multidomain antibody MD3606 protects mice against influenza A and B infection when administered intravenously or expressed locally from a recombinant adeno-associated virus vector (a collaboration with researchers at U. Pennsylvania). Crystal and single-particle electron microscopy structures of these antibodies with hemagglutinins from influenza A and B viruses reveal binding to highly conserved epitopes (Figure 1b).

The research work on bnAbs sparked interest and several bnAbs are being evaluated as passive immunotherapy in clinical trials. As antibodies are large and complex molecules, they are generally unsuited for oral delivery. Therefore, the second phase of this research was aimed at harnessing the structural details of the molecular interactions and mechanisms of HA stem bnAbs to identify an orally active small molecule that mimics the bnAb functionality.

The study screened a diverse chemical library for compounds that selectively target the group 1 HA epitope of bnAb CR6261 through a binding assay that detects displacement of a CR6261-based small protein (previously designed by David Baker’s group\textsuperscript{3}). Benzylpiperazines were identified as a major hit class, with an agent named JNJ7918 being the most promising candidate. Consistent with its binding to the functional HA stem epitope, this compound also neutralized influenza infection in vitro.
Key chemical modifications were subsequently introduced to optimize binding and neutralization potency, as well as properties dictating metabolic stability and oral bioavailability, to finally afford JNJ4796. This lead compound binds and neutralizes a broad spectrum of influenza A group 1 viruses in vitro and protects mice against lethal and sub-lethal influenza challenge after oral administration. The compound also effectively neutralizes virus infection in reconstituted three-dimensional cell culture of fully differentiated human bronchial epithelial cells (a collaboration with researchers at U. Hong Kong).

Cocrystal structures with H1 and H5 HAs reveal that JNJ4796 recapitulates the original CR6261-HA hotspot interactions and provide detailed and valuable information on the minimal epitope in the HA1-HA2 fusion region of the stem for an antiviral small molecule to neutralize influenza A group 1 viruses (Figure 1).

Further studies showed that the mechanism of action of JNJ4796 is based on the inhibition of the pH-sensitive conformational change of HA that triggers fusion of the viral and endosomal membranes and release of the viral genome into the host cell.

The researchers used SSRL and APS for the first part of their study and SSRL for the structures of the small molecule compounds bound to the HA.

References

Primary Citations

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