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FIRST REACTIONS

Coating the Flu with Sticky Polymers to Look for New Drugs

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In the search for effective drugs against influenza, screening against viral neuraminidases (NAs) is a common strategy. However, this ignores the actual physical environment where the drugs function, immersed in a thick mucus barrier at the surface of the host's cells which the virus must penetrate to get to the cell. Godula and co-workers have taken on this challenge with a distinctly innovative approach: using synthetic glycopolymers to wrap up virus particles in a synthetic mucus barrier which may provide a new tool to identify drugs which may be able to prevent infection.[1]

By attaching a synthetic mucus barrier onto the virus particle itself, the authors hope to assist in identifying prophylactic drugs that function *before* virus cell entry.

The early stages of influenza A infection involve hemagglutinins (carbohydrate binding proteins) on their surface binding to sialic acids on the host cells. Fortunately, our bodies have evolved a strategy to reduce this infection mode by secreting a mucus which coats common points of entry (such as lung or nasal passages) as it is also rich in sialic acids. This results in the virus becoming trapped and through the natural movement of mucus (which maybe readers don't want to think about too much!) provides a natural clearance route. As is typical of the constant host/invader battle, influenza in turn produces NA (sialic acid cleaving) enzymes to break through this barrier to enable infection[2] (as well as their later role in virus escape from cells). Widely used drugs such as oseltamivir (to which there are now reports of resistance[3]) are potent NA inhibitors but are actually designed to treat infection by preventing release of replicated virus, *after* infection has already occurred. Hence, in the search for new drugs, NA screens are widely employed, and lead compounds with low activity or that have cross-reactivity with human NA are eliminated[4] leading to the identification of potent antivirals with *in vivo* activity.[5]

When influenza infects human cells it can get trapped in a mucus shell. Godula and co-workers mimic this process to provide a better drug screening platform.

In this issue, Godula et al. report on a new approach to enable the impact of drugs that alter the mucus entrapment of the influenza virus. By attaching a synthetic mucus barrier onto the virus particle itself, the authors hope to assist in identifying prophylactic drugs that function *before* virus cell entry. To accomplish this in a typical experiment, the host cells (or a cell line) need to be coated with human (or animal) mucus from a fresh source. Herein lies the challenge; mucins are highly heterogeneous materials in terms of both physical properties and chemical composition. Synthetic glycopolymers offer a convenient alternative to natural glycoproteins as they have a macromolecular structure and enable high valences of the glycans to be presented and have been used for a range of applications including synthetic mucus.[6,7] Godula et al. synthesized sialic acid rich glycopolymers with a lipid tail that could insert into the influenza A membrane (building on previous work on cell-inserting polymers[8]) generating a thick sialic acid rich layer around the virus particle to mimic a mucus-trapped virus, Figure 1. This tool can then enable the viral infection assay directly onto host cells without using heterogeneous mucus from a primary source.

This methodology could then be employed to screen for viral entry inhibitors based on NA inhibition; if the compounds have no activity, the viral NAs cleave the sialic acid units and infection proceeds, Figure 2. It was seen here that known NA inhibiting drugs functioned well in this assay (e.g., oseltamivir), but the more interesting result came from a small number of flavonoids that were also screened. Although these have low NA activity (μM versus nM for most antivirals), they were found to be relatively potent viral entry inhibitors (using the modified viruses). This observation suggests that taking into account the

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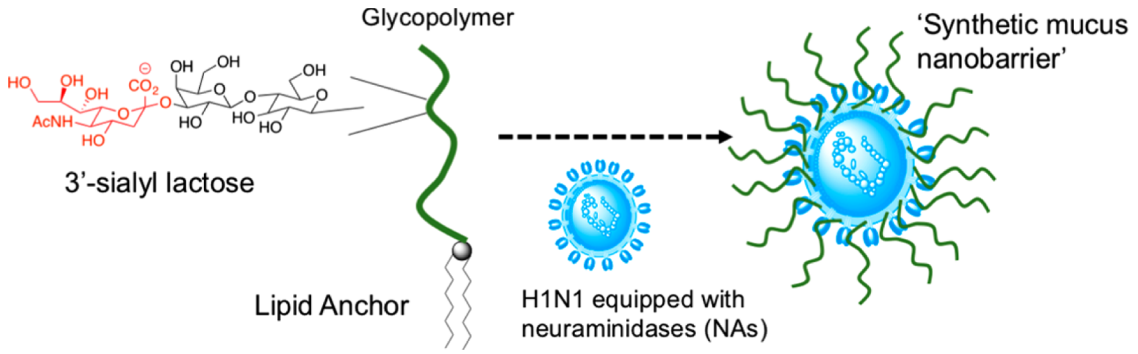

Figure 1. Insertion of poly(3′sialyl lactose) polymer into influenza virus using a lipid anchor to generate a mucus-like nanobarrier.

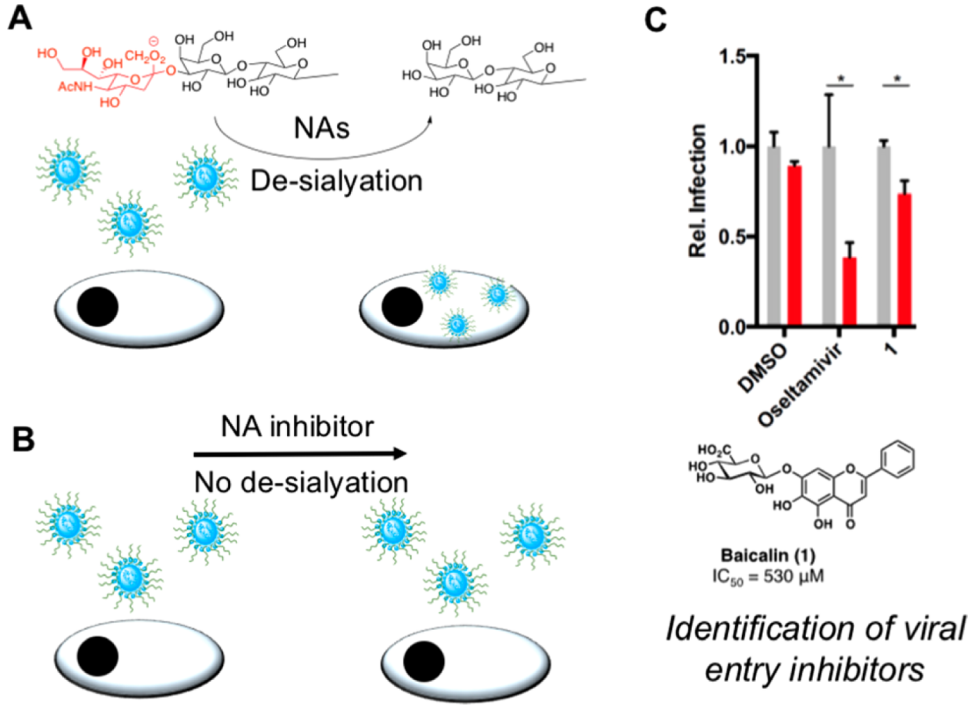

Figure 2. Mucus-like nanobarriers to screen for new infection inhibitors. (A) In the absence of NA inhibitors the NA present on influenza disialylate the polymers enabling cell infection to proceed, in analogy to when cells are coated with natural mucus; (B) addition of NA inhibitors (even with weak NA activity) prevent desialyation and infection is inhibited; (C) snapshot of infection data showing that mM inhibitors of NAs (compared to nM for oseltamivir) can still inhibit infection.

mucus permeation/entrapment into screens for influenza inhibitors could reveal new hits for preventative (prophylactic) treatments which function before cell entry has occurred—this is in contrast to current NA inhibiting drugs which treat infection by preventing intracellular virus release postreplication.

A possible benefit of this approach is that it might enable the use of lower activity, but highly selective, neuraminidase inhibitors in preventative medicine.

A possible benefit of this approach is that it might enable the use of lower activity, but highly selective, NA inhibitors in preventative medicine. The flavonoids identified in this work, using a hemagglutination model, supported this idea.

Of course, there are challenges to take this research further. Key is to demonstrate whether the mucus barrier constructed here is actually sufficiently similar to nature's mucus—a polymer brush on the surface of a particle will behave differently from the gel-like mucus phase which has complex material properties. As the rate of clearance and turnover of the mucus is related to these properties, it is not clear whether this assay is oversimplified—as with any new discovery.

These results also open up a new challenge to glycan-mimetic chemists; can one make a self-enclosed barrier, similar to the approach shown here, but with the necessary complexity and physical properties to better reproduce the

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mucus barrier? It highlights the increasing convergence of materials science with glycoscience to replicate the high valancy and precise presentation associated with natural glycans for anti-infective treatments.[9] I am sure many will take up this challenge!

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REFERENCES

(1) Cohen, M.; Senaati, H. P.; Fisher, C. J.; Huang, M. L.; Gagneux, P.; Godula, K. *ACS Cent. Sci.* **2016**, DOI: 10.1021/acscentsci.6b00191.
(2) Cohen, M.; Zhang, X.-Q.; Senaati, H. P.; Chen, H.-W.; Varki, N. M.; Schooley, R. T.; Gagneux, P. *Virol. J.* **2013**, *10*, 321.
(3) Hayden, F. G.; de Jong, M. D. *J. Infect. Dis.* **2011**, *203*, 6−10.
(4) von Itzstein, M. *Nat. Rev. Drug Discovery* **2007**, *6*, 967−974.
(5) Kim, J.-H.; Resende, R.; Wennekes, T.; Chen, H.-M.; Bance, N.; Buchini, S.; Watts, A. G.; Pilling, P.; Streltsov, V. A.; Petric, M.; et al. *Science (Washington, DC, U. S.)* **2013**, *340*, 71−75.
(6) Jones, M. W.; Otten, L.; Richards, S.-J.; Lowery, R.; Phillips, D. J.; Haddleton, D. M.; Gibson, M. I. *Chem. Sci.* **2014**, *5*, 1611−1616.
(7) Tang, S.; Puryear, W. B.; Seifried, B. M.; Dong, X.; Runstadler, J. A.; Ribbeck, K.; Olsen, B. D. *ACS Macro Lett.* **2016**, *5*, 413−418.
(8) Huang, M. L.; Smith, R. A. A.; Trieger, G. W.; Godula, K. *J. Am. Chem. Soc.* **2014**, *136*, 10565−10568.
(9) Bhatia, S.; Camacho, L. C.; Haag, R. *J. Am. Chem. Soc.* **2016**, *138*, 8654−8666.

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