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The clinical significance of sialoderiveratives in the search for novel means of antipathogen therapy

In the present issue (p 410), Scocco and Pedini (2006) describe a detailed study of the presence and localisation of sialoderivates in the horse mandibular gland. The main finding of this study is not only that different cellular populations of the mandibular glands show a different distribution of O- and N-linked glycoconjugates, but also that the sugar moieties differ from those found in the bovine salivary gland. One may ask why these findings are important for equine clinicians - as they may not seem that significant on first sight. However, recent findings in man and chicken place these results in a larger, far more important context.

More than 50 years ago, Blix, Klenk and other investigators discovered, nearly concurrently, that sialic acid, neuraminic acid, or N-acetylneuraminic acid was a major product released by mild acid hydrolysis of brain glycolipids or salivary mucins. The complete structure, chemistry, and biosynthesis of this molecule were subsequently characterised in the 1950s and 1960s by several groups, and it was agreed to use sialic acid as the family name covering all of the more than thirty derivatives of neuraminic acid, with N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc) forming the core structures (Blix *et al*. 1957).

Sialic acid is found in a wide variety of substances and tissues in animals and man, occurring most abundantly in glycoproteins and glycolipids, explaining its presence throughout human tissues. It is also found in several fluids, including serum, cerebrospinal fluid, saliva, urine, amniotic fluid and milk (for further information, see http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=glyco.chapter.988).

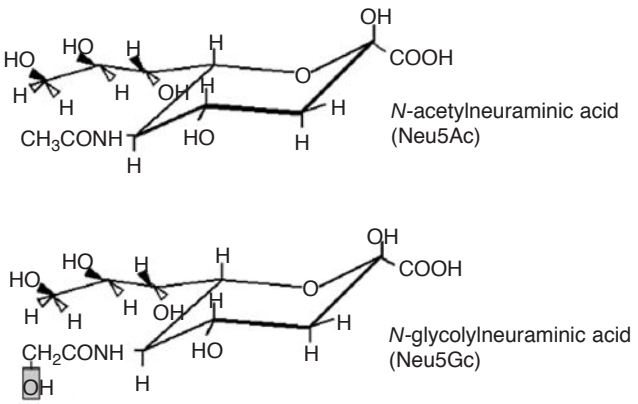

Fig 1: Molecular structures of N-acetylneuraminic acid and N-glycolylneuraminic acid.

The most common form found in man is Neu5Ac, whereas in most other animals (with the notable exception of chickens) Neu5Ac peacefully coexists with Neu5Gc, a slightly modified form of sialic acid bearing an hydroxyl group at the N-acyl position (for general structure see Fig 1).

During a long evolution, many pathogenic microorganisms have learned to explore eukaryotic cell surface glycoconjugates, i.e. glycolipids, glycoproteins and proteoglycans, as receptor molecules for cell attachment to facilitate tissue colonisation and invasion processes. In brief, specific proteins called adhesins expressed on the surface of bacteria, viruses, fungi and parasites interact with carbohydrate chains of glycoconjugates, which enable microbes to colonise mucosal surfaces and tissue lesions. In this context, the role of sialic acid in binding of pathogens to host cells has been reported over many years. Only recently, proteoglycans expressed by host cells were shown to bind many different pathogens. By removing terminal carbohydrate moieties of these various glycoconjugates with sialidase and other exoglycosidases or with glycosaminoglycan (GAG) degrading enzymes on the cells in monolayers, these structures were proven to be receptor molecules for various sialoadhesins and heparan sulphate binding proteins expressed by pathogens.

In addition to the possible role of sialic acid in brain development and rheumatoid arthritis, sialic acid is an immune moderator that affects the flow resistance of mucus which, in turn, repels bacteria, viruses and other harmful microbes. In several *in vitro* and animal studies, sialic acid derivates have been shown to inhibit infection with strains of influenza A and B viruses more effectively than any prescription antivirals (Ryan *et al*. 1995; Smith *et al*. 2001). Therefore, this helpful information is nothing to sneeze at!

Emerging and re-emerging infectious diseases are a continuing threat to human health and to the domestic animal populations of the world. Man continues to influence global ecology with an ever-growing demand on land use and intensified farming to feed an increasing population that has developed means of transportation permitting rapid global transportation, and therefore rapid global spread of infectious diseases. Each of these factors, and many others, has implications for the emergence of novel disease agents, such as severe acute respiratory syndrome (SARS) coronaviruses, the global threat in 2003, and H5N1 influenza virus, the possible global threat in the years to come.

Influenza virus infects the upper respiratory tract of many mammals. The H1N1 strain was responsible for the Spanish Flu pandemic, one of the worst epidemics in human history, killing more than 20 million people in 1918–1919.

Influenza particles are surrounded by a lipid bilayer acquired from the host cell membrane. In this envelope there are 2 glycoproteins: haemagglutinin (HA) and neuraminidase (NA). Whereas HA in the viral envelope binds to sialic acid on host cells in order to allow for fusion to the cell membrane and subsequently entrance into the host cell, NA cleaves sialic acid from the host cell, allowing the virion to escape from the host cell during the budding process. Changes in the HA and NA surface antigens of influenza can occur in 2 ways, allowing for different HxNx strains: 1) antigenic drift is the gradual accumulation of point mutations leading to minor changes in the surface antigens and 2) antigenic shift is a major change in the genetic composition of the virus, frequently by shuffling of RNA strands within a host cell infected by 2 or more viral strains. Since adaptive immune responses are highly specific for viral surface antigens, antigenic drift and shift can generate enough variability to make an individual susceptible to recurrent influenza infections.

However, for a virus to emerge successfully in a human population it must achieve 2 features. The first feature is replication in human cells. This replication requires a virus to accomplish at least 5 different, sequential steps: 1) contact with a human host, 2) entry into the appropriate cell type, 3) production of more copies of itself, 4) overcoming any immediate host response and 5) exiting from the cell and transmitting to another. The second feature is human-to-human transmission. It is obvious from the number of viruses that have achieved the first feature, but not the second, that the adaptive changes necessary for a virus to replicate in a foreign host are independent of, but necessary for, those required for successful transmission between individuals. In this context, RNA viruses, such as the Nipah virus, Hendra virus, SARS coronavirus, H5N1 influenza A virus and Ebola virus are of great concern as they have all jumped from animals to man, but have yet to achieve the next step of successful establishment.

The emergence of influenza A viruses in a new host population seems, at least in part, to be mediated by virus-receptor interactions. The main reservoir of influenza A viruses are aquatic birds, from which viruses sporadically transmit to other hosts in whom they can adapt and form stable lineages. Influenza viruses from avian and human sources preferentially bind different forms of sialic acid, the virus receptor, on the host cell. Avian influenza viruses have a preference for sialic acid that is linked to the galactose unit in an α2-3 conformation (SAα2,3Gal), whereas human viruses preferentially bind those with an α2-6 linkage (SAα2,6Gal; for review see Suzuki 2005). This binding preference makes biological sense when one considers the host environment in which these viruses grow. The respiratory tract of avian species contains predominantly SAα2,3Gal, in contrast to the abundance of SAα2,6Gal in the human respiratory tract. Correspondingly, it has been shown that human viruses replicate poorly in avian hosts and, conversely, avian viruses replicate poorly in human individuals. However, receptor specificity does not seem to be an absolute barrier to infection, and viral mutations may result in increased disease severity (Stevens *et al*. 2006).

Although more than 100 people have been infected by the current H5N1 influenza A virus strain, human-to-human transmission seem to be a rare event (Beigel *et al*. 2005). Given the possibility that once influenza virus has crossed the species barrier, human-to-human transmission should result in a higher incidence rate in man, the question to answer seems to be: what are the limiting barriers?

Recent studies by Shinya *et al*. (2006) may provide a simple and rational explanation for why H5N1 at present rarely infect and spread between human individuals - and may also place the results presented by Scocco and Pedini (2006) in a different scientific context. As described above, human influenza viruses with a SAα2,6 receptor preference almost exclusively infect nonciliated human tracheal cells during the early phase of replication, whereas avian viruses with a SAα2,3 preference target mainly ciliated cells. Thus, the host range barrier for replication of viruses in man may not be necessarily at the level of binding, but rather at the level of targeting the appropriate cell type. This assumption has now been confirmed by Shinya *et al*. (2006). Using lectins binding specifically to SAα2,6Gal, this group showed that this sialic acid form is dominant on nasal mucosal cells, whereas SAα2,3Gal was predominantly found on non-ciliated cuboidal bronchiolar cells in the human respiratory tract (Shinya *et al*. 2006). Human influenza viruses bound extensively to epithelial cells and, to a lesser extent, alveolar cells expressing SAα2,6Gal. In contrast, avian influenza viruses, preferentially recognising SAα2,3Gal bind less to bronchial epithelial cells, but more to alveolar cells. Thus whereas a transmission of H5N1 from birds to man is possible, this viral strain can only replicate successfully in cells of the lower respiratory tract where the avian-virus receptor is expressed. These findings could explain why a successful, higher interhuman transmission rate of this H5N1 viral strain has so far not been observed; they are further supported by the fact that the H5N1 strain A/Hong Kong/213/03, recognising both forms of SA-Gal, bound extensively in both areas of the respiratory tract, allowing an easier spread by sneezing (Shinya *et al*. 2006).

The findings by Scocco and Pedini (2006) now show, even if so far only in the equine salivary gland, the presence of different SA-forms in different cells and at different loci. Whether a similar finding as described for the human respiratory tract may be found in horses, remains to be seen, but is highly likely. Therefore, the work of Scocco and Pedini may provide the first step in identifying the equine

respiratory tract area necessary for equine influenza virus survival and, therefore, could subsequently provide new ways to treat this infectious disease in horses.

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References

Beigel, J.H., Farrar, J., Han, A.M., Hayden, F.G., Hyer, R., de Jong, M.D., Lochindarat, S., Nguyen, T.K., Nguyen, T.H., Tran, T.H., Nicoll, A., Touch, S. and Yuen, K.Y. (2005) Avian influenza A (H5N1) infection in humans. *N. Engl. J. Med.* **353**, 1374-1385.

Blix, F.G., Gottschalk, A. and Klenk, E. (1957) Proposed nomenclature in the field of neuraminic and sialic acids. *Nature* **179**, 1088.

Ryan, D.M., Ticehurst, J. and Dempsey, M.H. (1995) GG167 (4-guanidino-2, 4-dideoxy-2, 3-dehydro-N-acetylneuraminic acid) is a potent inhibitor of influenza virus in ferrets. *Antimicrob. Agents. Chemother.* **39**, 2583-2584.

Scocco, P. and Pedini, V. (2006) Horse mandibular gland: *in situ* characterisation of sialoderivatives. *Equine vet. J.* **38**, 410-415.

Shinya, K., Ebina, M., Yamada, S., Ono, M., Kasai, N. and Kawaoka, Y. (2006) Avian flu: influenza virus receptors in the human airway. *Nature* **440**, 435-436.

Smith, B.J., Colman, P.M., Von Itzstein, M., Danylec, B. and Varghese, J.N. (2001) Analysis of inhibitor binding in influenza virus neuraminidase. *Protein Sci.* **10**, 689-696.

Stevens, J., Blixt, O., Tumpey, T.M., Taubenberger, J.K., Paulson, J.C. and Wilson, I.A. (2006) Structure and receptor specificity of the hemagglutinin from an H5N1 influenza virus. *Science* **312**, 404-410.

Suzuki, Y. (2005) Sialobiology of influenza: molecular mechanism of host range variation of influenza viruses. *Biol. Pharm. Bull.* **28**, 399-408.