

# How bacteria utilize sialic acid during interactions with the host: snip, snatch, dispatch, match and attach

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## Abstract

*N*-glycolylneuraminic acid (Neu5Gc), and its precursor *N*-acetylneuraminic acid (Neu5Ac), commonly referred to as sialic acids, are two of the most common glycans found in mammals. Humans carry a mutation in the enzyme that converts Neu5Ac into Neu5Gc, and as such, expression of Neu5Ac can be thought of as a 'human specific' trait. Bacteria can utilize sialic acids as a carbon and energy source and have evolved multiple ways to take up sialic acids. In order to generate free sialic acid, many bacteria produce sialidases that cleave sialic acid residues from complex glycan structures. In addition, sialidases allow escape from innate immune mechanisms, and can synergize with other virulence factors such as toxins. Human-adapted pathogens have evolved a preference for Neu5Ac, with many bacterial adhesins, and major classes of toxin, specifically recognizing Neu5Ac containing glycans as receptors. The preference of human-adapted pathogens for Neu5Ac also occurs during biosynthesis of surface structures such as lipo-oligosaccharide (LOS), lipo-polysaccharide (LPS) and polysaccharide capsules, subverting the human host immune system by mimicking the host. This review aims to provide an update on the advances made in understanding the role of sialic acid in bacteria-host interactions made in the last 5–10 years, and put these findings into context by highlighting key historical discoveries. We provide a particular focus on 'molecular mimicry' and incorporation of sialic acid onto the bacterial outer-surface, and the role of sialic acid as a receptor for bacterial adhesins and toxins.

## INTRODUCTION

Sialic acids are members of the nonulosonic acids, nine-carbon sugars found in both eukaryotes and prokaryotes [1, 2]. *N*-glycolylneuraminic acid (Neu5Gc), and its precursor *N*-acetylneuraminic acid (Neu5Ac), are the two most abundant sialic acids found in mammals (Fig. 1). Although Neu5Gc is present in most mammals, humans have a mutation in the enzyme CMP-Neu5Ac hydroxylase (CMAH) [3–6], which converts Neu5Ac to Neu5Gc. Consequently, humans are only able to produce Neu5Ac *de novo*, although several studies have demonstrated that trace levels of Neu5Gc is present in human tissues [7–10]. It is thought that the Neu5Gc present in normal human tissue may come from dietary sources such as red meat and dairy products [10, 11], although novel pathways have been proposed that may reactivate the defective CMAH protein under certain conditions [12]. Neu5Ac is present in every human cell type, and it is involved in cell–cell adhesion, signalling and immunity, discriminating 'self' from 'non-self' [13]. Additionally, Neu5Ac plays a key role in mucus, where the high abundance of negative charges provides protection against bacterial adhesion and colonization [14].

A number of bacterial species are able to directly synthesize Neu5Ac, including *Escherichia coli* [15–17], *Campylobacter jejuni* [18], *Neisseria meningitidis* [19], *Streptococcus agalactiae* [20] and *Fusobacterium nucleatum* [21]. In *E. coli*, two genes, *neuB* and *neuC*, are required for Neu5Ac biosynthesis [22–24]. NeuB catalyses the formation of Neu5Ac from *N*-acetyl-D-mannosamine and phosphoenolpyruvate [25], with NeuC responsible for the biosynthesis of the precursor of Neu5Ac, *N*-acetyl-D-mannosamine.

Bacteria are also able to synthesize additional nonulosonic acids (NulOs) that are structurally similar to sialic acid. Bacterial NulOs include Legionaminic acid, Pseudaminic acid, Fusaminic acid and Acinetaminic acid, with naming conventions

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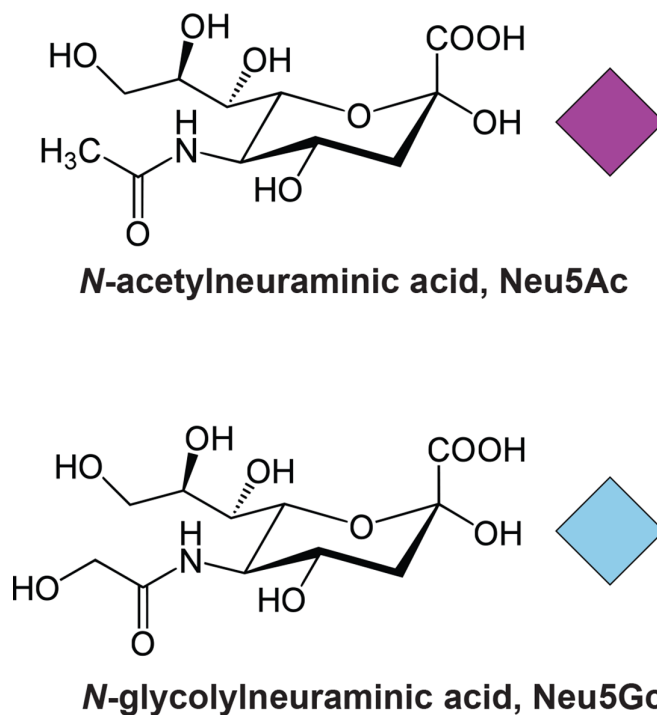
**Keywords:** sialic acid; *N*-acetylneuraminic acid; Neu5Ac; *N*-glycolylneuraminic acid; Neu5Gc; adherence; adhesin; molecular mimicry; bacterial pathogen.

**Abbreviations:** CDC, cholesterol dependent cytolysin; CMAH, CMP-Neu5Ac hydroxylase; LOS, lipo-oligosaccharide; LPS, lipopolysaccharide; Neu5Ac, *N*-acetylneuraminic acid; Neu5Gc, *N*-glycolylneuraminic acid; NulO, nonulosonic acid.

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**Fig. 1.** Structures of *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc), and the graphical representation of these glycans as per SNFG guidelines.

typically following from the species they were first observed in; for example, Legionaminic acid was first observed in *Legionella pneumophila* [26], responsible for the respiratory infection Legionnaires disease, and Acinetaminic acid in the multi-drug-resistant pathogen *Acinetobacter baumannii* [27]. The structure, biosynthesis, and role of these NulOs were thoroughly reviewed recently [2], and as such, this review will focus on the role of Neu5Ac ('sialic acid') in bacterial–host interactions.

## A QUICK TRIM ISN'T GOOD FOR THE HOST: SIALIDASES ENHANCE BACTERIAL VIRULENCE

Sialidases, also called neuraminidases, are enzymes that cleave sialic acid residues from complex glycan structures. This released sialic acid can then be acquired by bacteria to serve as a nutrient source, allow evasion of the immune response and the exposed underlying glycan structures may serve as points of adherence for bacterial cells. Action of viral sialidases, for example from Influenza A virus, can also serve to increase bacterial virulence; this may have been the case for the 1918 'Spanish flu' pandemic, with many fatalities a result of secondary bacterial infections [28], mainly *Streptococcus pneumoniae*. The viral neuraminidase cleaves sialic acid from host glycan structures, increasing sialic acid and sialylated mucin availability. This promotes pneumococcal growth, proliferation and migration to the lungs [29]. *S. pneumoniae* itself expresses multiple neuraminidases, NanA, NanB and NanC, with diverse roles in pathobiology. NanA removal of terminal sialic acid from human glycan structures results in the exposure of galactose, which promotes pneumococcal biofilm formation [30]. NanA cleavage of sialic acid from the T-antigen [31], can lead to pneumococcal haemolytic uremic syndrome (HUS). NanA activity also modifies the immune response. For example, NanA desialylation of platelets can lead to platelet hyper-reactivity [32], leading to increased interaction with leukocytes, and increased inflammation; NanA cleavage of sialic acid activates TGF- $\beta$  signalling leading to increased permeability of blood-brain-barrier and promoting meningitis [33]. A similar immune system modification effect is seen in the pig pathogen *Glaesserella parasuis* where desialylation leads to negative regulation of the lectin (glycan-binding protein) siglec-5 [34].

Synergy between multiple sialidases, and sialidases and other virulence factors, also potentiates virulence. For example, concerted action of the pneumococcal sialidases NanA and NanB leads to mucus escape – secreted NanB results in increased expression of NanA due to Neu5Ac stimulation of *nanA* transcription [35]. Expression of NanA results in increased cleavage of mucus, allowing *S. pneumoniae* to escape mucus binding and mucociliary clearance [35]. In *Vibrio cholerae*, the neuraminidase VcN hydrolyses sialic acids from complex gangliosides in the human gut, resulting in high amounts of the underlying GM1 structure. GM1 is the receptor for cholera toxin, Ctx [36, 37]. Thus, VcN-mediated cleavage of sialic acids from gangliosides potentiates the virulence of Ctx in the induction of diarrhoea.

*Gardnerella vaginalis*, a causative agent of bacterial vaginosis, employs multiple sialidases during pathogenesis [38, 39], with the activity of sialidases contributing to cellular invasion [40]. Similarly, the sialidase of the periodontal pathogen *Porphyromonas gingivalis* is required for attachment and invasion of oral epithelial cells by both *P. gingivalis* and other periodontal pathogens [41].

The sialidase NanI is important in *Clostridium perfringens* intestinal colonization [42] and supports survival in the presence of mucin [43], with NanI showing preference for glycan structures terminating in Neu5Ac [44]. Sialidases also allow the use of Neu5Ac as carbon sources in multiple *Mycoplasma* species [45, 46], potentiating colonization and persistence.

## TO SNATCH AND DISPATCH: ACQUISITION OF SIALIC ACID FROM THE HOST, AND CONSUMPTION AS A CARBON SOURCE

Excellent reviews have been written recently describing bacterial sialic acid uptake [47], and how sialic acid is used as a carbon source [48]. Often, sialic acid released by sialidases/neuraminidases is used as a carbon source.

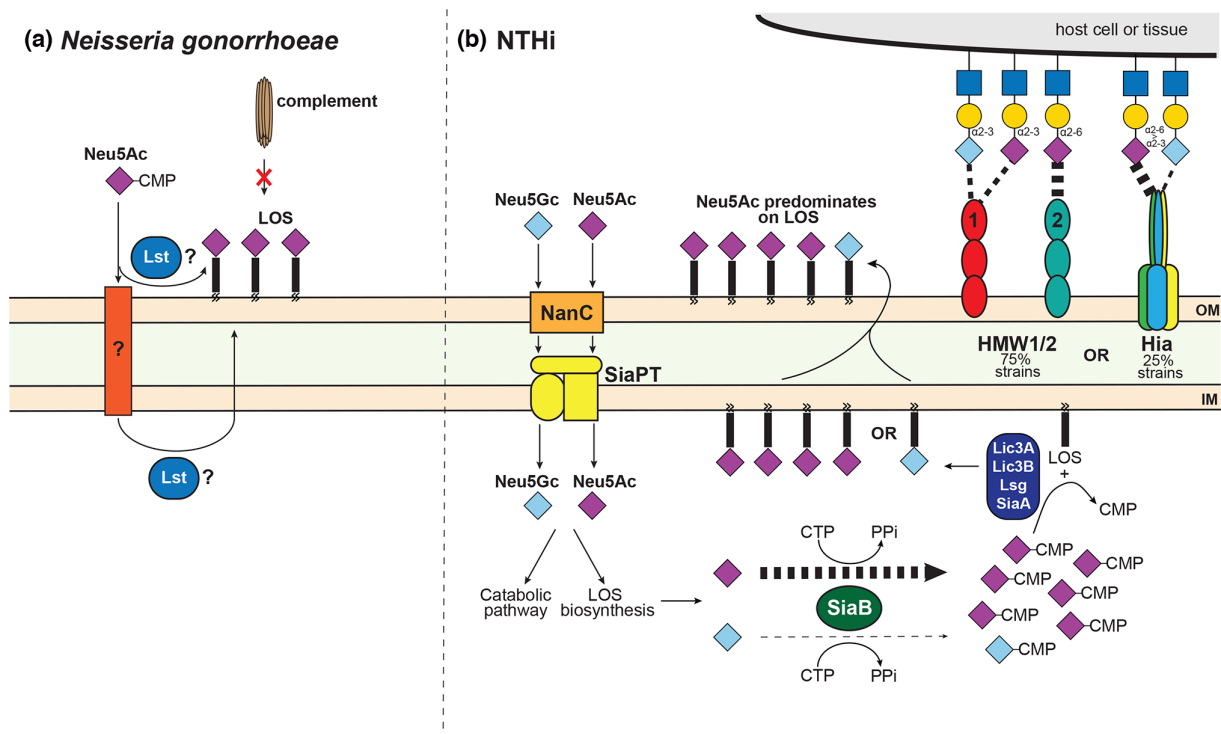
Multiple mechanisms exist in the bacterial domain to take up sialic acid from the environment. The first sialic acid transporter to be described was NanT in *E. coli*, a proton-coupled secondary transporter [49]. NanT protein homologues have been observed in multiple bacterial organisms, including *Yersinia pestis*, *Tannerella forsythia* and *Bacteroides fragilis* [47]. A further secondary transporter, named SiaT [47], a sodium solute symporter, exists in bacterial pathogens such as *Clostridium perfringens* [50] and *Staphylococcus aureus* [51]. Bacterial TRAP (tripartite ATP-independent periplasmic) transporters, made up of periplasmic substrate binding proteins and a cognate inner-membrane transporter complex [52] are also able to transport sialic acid. TRAP transporters are classed as secondary transporters as they use ion-electrochemical gradients to transport substrates [52]. The SiaPT TRAP transporter from *Haemophilus influenzae* was the first sialic acid TRAP transporter to be discovered [53, 54], is regulated by SiaR [55] and is essential for *H. influenzae* virulence [56]. Homologues of this system have been described in a diverse range of bacterial pathogens, including *Vibrio cholerae* [57] and *Pasteurella multocida* [58]. Like TRAP transporters, ABC (ATP-binding cassette) transporters also consist of periplasmic solute binding proteins and a cognate inner-membrane transporter complex. However, unlike TRAP transporters, ABC systems use ATP to directly transport solutes, and are classed as primary transporters [52]. Both Gram-negative and Gram-positive bacterial pathogens encode sialic acid ABC transporters, including *Haemophilus ducreyi*, and multiple *Clostridial* and *Streptococcal* species [59].

Following uptake, bacteria can use sialic acid in multiple ways. In addition to using sialic acid as a building-block for surface structures (reviewed in detail later in this review) a number of bacteria, both pathogens and commensals, use sialic acid directly as a carbon source. Catabolism of sialic acids, both Neu5Ac and Neu5Gc, results in formation of fructose-6-phosphate, which can then feed into glycolysis. The diversity of the systems for sialic acid catabolism and their roles are great and varied, and have been reviewed previously [48, 60]. However, a number of new examples have been described recently. *V. cholerae* motility is triggered by the presence of free Neu5Ac, and the sugar *N*-acetylglucosamine, which has a  $\text{NHCOCH}_3$  modification of the second carbon of the glucose ring. Catabolism of both these sugars into glucosamine-6-phosphate is the essential step in inducing motility [61]. The gut commensal *Ruminococcus gnavus* utilizes mucin as a nutrient source, with expression of multiple sialidases key to this utilization [62]. This illustrates that in addition to the pathogens described in the sialidase section above, members of the normal gut flora also utilise sialidases to release sialic acid as a nutrient source. Transcription of sialic acid catabolic genes is repressed by the NanR transcription factor in *Corynebacterium glutamicum* [63]. NanR is almost ubiquitously found in bacterial species that are able to catabolize sialic acid, indicating a possible common origin of the ability to break down sialic acids.

## A WOLF IN SHEEP'S CLOTHING: IMMUNE EVASION AND MOLECULAR MIMICRY

In addition to being able to cleave sialic acid to enhance virulence and to use free sialic acids as carbon and energy sources, many bacterial pathogens use sialic acids as a cell-surface decoration, and as a structural building block.

The key role for sialic acid in bacterial pathogenesis was first described in the sexually transmitted pathogen *Neisseria gonorrhoeae*, when it was discovered that CMP-Neu5Ac was the mystery substance that was acquired from the host, by a still as yet unknown mechanism, to render *N. gonorrhoeae* serum resistant [64, 65]. Addition of Neu5Ac in *N. gonorrhoeae* occurs at the terminus of lipo-oligosaccharide, LOS, a truncated version of LPS that lacks the extended O-antigen, with addition catalysed by the sialyltransferase Lst. Whether Lst is located in the cytoplasm or extracellularly is still not clear (Fig. 2). Neu5Ac addition to the LOS of *N. gonorrhoeae* prevents killing by complement [66]. Interestingly, addition of non-sialic acid NulOs to *N. gonorrhoeae* LOS by Lst resulted in an *increased* sensitivity to serum [67], illustrating the exquisite requirement of sialic acid for mediating this resistance and providing some insights into the mechanism of serum resistance. The increased Neu5Ac addition to the bacterial surface, which results in recruitment of more Factor H, inhibits the alternative complement pathway and decreases activation of the classical complement pathway [67]. *N. gonorrhoeae* LOS can also be sialylated on inner heptose residues, with loss of Neu5Ac on these residues attenuating virulence in a mouse model [68, 69].



**Fig. 2.** The role of sialic acid in the pathobiology of (a) *Neisseria gonorrhoeae* and (b) nontypeable *Haemophilus influenzae* (NTHi). (a) The role of sialic acid in serum resistance was first described in *Neisseria gonorrhoeae*, with Neu5Ac-CMP the host-acquired molecule, which resulted in this resistance. Addition of Neu5Ac to the LOS of *N. gonorrhoeae* is catalysed by the enzyme Lst, with Neu5Ac-CMP transported by an as yet unidentified transporter. Whether Lst acts extracellularly, or in the cytoplasm, is also unclear. Incorporation of Neu5Ac into LOS also allows ‘molecular mimicry’ of the host by adding the human specific sugar to a key bacterial surface feature, masking the bacteria from the immune response. (b) NTHi is one of the best studied bacterial pathogens as regards use of sialic acid during pathobiology, and displays several examples of evolution of host-adaptation. Whilst NTHi can take up both Neu5Ac and Neu5Gc with equal affinity via NanC and the TRAP transporter SiaPT, NTHi shows a marked preference for decorating LOS with Neu5Ac, with the bias being at activation of sialic acids for addition to LOS; the enzyme SiaB, which adds a CMP to sialic acid, shows 4000-fold higher affinity for Neu5Ac over Neu5Gc. In addition, the major NTHi adhesins HMW1/2 and Hia have both evolved to interact specifically with sialic acid containing receptors in the human airway, despite never being found in the same strains of NTHi. HMW 1 shows equal affinity for both  $\alpha$ 2,3-sialyllactosamine (2–3 SLN) containing either Neu5Ac or Neu5Gc, whereas HMW2 shows high affinity for  $\alpha$ 2,6-sialyllactosamine (2–6 SLN) containing Neu5Ac. Hia shows a marked preference for 2–6 SLN containing Neu5Ac, but also binds 2–6 SLN containing Neu5Gc, and 2–3 SLN. This convergent evolution of different adhesins for the same receptor means that all NTHi strains can colonize the human airway. The weight of the dashed line indicates specificity for particular sialic acid-containing structures.

A second aspect of sialic on the bacterial surface is the ‘cloaking’ effect of expressing molecules identical to host glycans. Such ‘molecular mimicry’ [70] of host structures allows bacterial pathogens to subvert the host immune response and survive.

The human-adapted pathogen non-typeable *Haemophilus influenzae* (NTHi), like *N. gonorrhoeae*, is a human-specific pathogen where the role of sialic acid in LOS has been extensively studied. Addition of sialic acid to the terminal position of LOS in NTHi promotes survival *in vivo* [71], and can occur via multiple enzymatic activities, including SiaA, LsgB, Lic3A and Lic3B. Growth in the presence of sialic acids also results in a decrease of complement-mediated killing; in this instance, increased sialic acid addition to LOS prevents binding of IgM antibodies, which results in decreased activation of the classical complement pathway [72]. In addition to using Neu5Ac as a substrate, LsgB can add the sugar ketodeoxyoctanoate (KDO) as a terminal structure of LOS [73]. The addition of KDO occurs when the concentration of free Neu5Ac is low, for example in certain *in vivo* nutrient-restricted niches and growth in a biofilm [73], and highlights the complex nature of NTHi LOS in immune-evasion. The underlying glycan structures of sialylated NTHi LOS also serve to protect the bacterium from serum IgM and complement [74]. By selectively inhibiting LOS sialylation, it is possible to increase serum sensitivity of NTHi, indicating a possible future treatment strategy for a major human pathogen [75]. Further, in an example of exquisite host-adaptation, NTHi has evolved preferential use of Neu5Ac over Neu5Gc [76] in decorating its LOS. Both Neu5Gc and Neu5Ac are taken up and used as sole carbon sources equally well, but Neu5Gc is incorporated much less efficiently into NTHi LOS. This bias occurs in the enzyme SiaB, a CMP-Neu5Ac synthetase, which activates Neu5Ac by addition of a CMP group. SiaB in NTHi has ~4000-fold-higher catalytic efficiency for Neu5Ac than for Neu5Gc [76] (Fig. 2).



In addition to *N. gonorrhoeae* and NTHi, a number of other major human pathogens also decorate their LOS with Neu5Ac. For example, sialic acid found in the LOS of the gastric pathogens *V. cholerae* and *Campylobacter jejuni* [18, 57]. In *C. jejuni*, this molecular mimicry of host epitopes leads to the autoimmune disease Guillain–Barre syndrome, where anti-Neu5Ac antibodies, elicited by *C. jejuni* sialylated-LOS, attack the hosts own gangliosides containing these structures [77]. In the related organism *Campylobacter coli*, loss of LOS biosynthetic enzyme function means only a small fraction of strains (1%) are able to sialylate their LOS, meaning infection with *C. coli* is much less likely to lead to autoimmune disorders [78]. This is another nice example of niche adaptation: the ‘molecular mimicry’ benefits of LOS sialylation of a human pathogen (*C. jejuni*) results in severe disease sequelae for the host, whereas an organism that rarely infects humans (*C. coli*) does not need to sialylate its LOS in order to survive.

Several bacterial organisms express LPS, lipo-polysaccharide, instead of LOS. LPS contains the core LOS structure plus an extended polysaccharide, the O-antigen [79]. O-antigens typically contain Neu5Ac, and are critical virulence factors in many organisms such as *Pseudomonas aeruginosa* [80] and *Salmonella enterica* serovar Typhimurium [81]. The LPS of *Vibrio vulnificus* is decorated with sialic acid [82], with the inability to synthesize this molecule leading to a 300-fold lower chance of survival compared with a wild-type strain. In addition, sialic acid plays a critical role in *V. vulnificus* biofilm formation, motility, and protects the bacterium from the host immune response [83]. *Fusobacterium nucleatum*, a potential cancer-causing bacterium that inhabits the human oral cavity, incorporates sialic acid into its LPS [84], and sialylation of the LPS of *P. gingivalis* LPS reduces inflammation [85], allowing long-term periodontal colonization.

Many bacterial pathogens also express a capsule. Capsules are extracellular structures that are almost entirely glycan based, and serve to mask bacteria from the immune system. Serogroup B *Neisseria meningitidis*, in addition to decorating its LOS with sialic acid like the closely related organism *N. gonorrhoeae* [86], produces a capsule containing multiple Neu5Ac residues, as does *E. coli* K1. In both instances, these polysialic acid capsules confer resistance to complement mediated killing and phagocytosis [86–89]. Nearly all Streptococci express a polysaccharide capsule, but only *Streptococcus agalactiae* (Group B Strep, GBS), responsible for neonatal meningitis and urinary tract infections, and *Streptococcus suis*, a major pig and zoonotic pathogen, are known to contain sialic acid [90]. Sialylation of the GBS capsule is key to immune evasion [91, 92], likely through sialic acid on the GBS capsule binding to host Sia-recognizing Ig superfamily lectins (Siglecs). This blocks neutrophil and macrophage activation, and suppress platelet activation [93]. In *S. suis*, the role of capsular sialic acid is less clear. Not all *S. suis* capsular serotypes contain sialic acid (e.g. serotypes 2 and 14 contain sialic acid, serotype 9 does not) [94] but the interactions between strains expressing all three capsular types and host cells are similar and serotype 9 capsule is critical for bacterial survival in blood and development of clinical disease [94]. Confoundingly, sialic acid has been shown to be crucial for *S. suis* capsule synthesis, whereas for GBS, capsule synthesis may occur in the absence of a sialic acid moiety [95]. The linkage of sialic acid in the *S. suis* capsule occurs via an  $\alpha$ 2,6-linkage. These types of linkage are recognized by the haemagglutinins of both human and swine influenza viruses (demonstrating the zoonotic nature of *S. suis* infections). Because of this interaction, adherence to and invasion of airway epithelial cells is enhanced when *S. suis* encounters influenza virus-infected cells [96]. Adherence and invasion efficiency of GBS is also enhanced when cells were pre-infected by influenza A virus with  $\alpha$ 2–3 linked neuraminidase specificity [97]. These recent findings indicate understanding synergy between viral and bacterial pathogens is key to combatting disease.

## ATTACHING TO THE HOST: SIALIC ACID AS A CELLULAR RECEPTOR FOR BACTERIAL ADHESINS

As one of the most abundant and wide-spread host surface molecules, sialic acid or glycan structures containing a sialic acid moiety, are ideal receptors for bacteria to bind to during colonization and disease.

NTHi, in addition to evading the host via addition of Neu5Ac onto LOS, expresses multiple adhesins that have high specificity for sialic acid (Fig. 2). NTHi express the adhesins HMW (HMW1 and HMW2) and Hia, but individual strains only encode genes for HMW1/2 (75% of strains) [98], or Hia (25% of strains) [99], with this discrete strain distribution likely the result of an ancient strain divergence event. HMW1 binds to  $\alpha$ 2,3-sialyllactosamine (2–3 SLN) [100] that contains terminal Neu5Ac or Neu5Gc via an  $\alpha$ 2,3 linkage [101]. HMW2, which is ~65% identical to HMW1, binds the related glycan  $\alpha$ 2,6-sialyllactosamine (2–6 SLN), with high specificity for 2–6 SLN containing Neu5Ac [101]. Hia binds 2–6 SLN with a ~10-fold preference for Neu5Ac over Neu5Gc [102], with preference for these structures located in Hia binding domain 1 (BD1), the high-affinity substrate binding region of Hia [102]. Hia will also bind 2–3 SLN containing either a Neu5Ac or Neu5Gc with equal affinity, but this binding is not mediated by BD1. Intriguingly, 2–3 SLN is found mainly in the lower human respiratory tract, whereas 2–6 SLN is found throughout the entire respiratory tract, but predominates in the upper airway [103]. Thus, NTHi strains are able to colonize the entire human airway as strains have independently evolved the ability to bind human-airway-specific glycans via either the HMW1/2 proteins or the Hia protein. *Moraxella catarrhalis* is a respiratory tract pathogen often found in co-infections with NTHi. Binding of *M. catarrhalis* to mucus occurs via bacterial binding to Neu5Ac and Neu5Ac-linked glycan structures (e.g.  $\alpha$ 2–6 sialyllactose; Neu5Ac linked to lactose) within mucus, with this binding correlating with increased inflammation [104]. The human gastric pathogen *Helicobacter pylori*, responsible for stomach ulcers and gastric cancer, expresses multiple adhesins

recognizing glycan structures containing terminal Neu5Ac residues. The SabA adhesin has specificity for terminal  $\alpha$ 2,3-linked Neu5Ac, including the sialyl Lewis<sup>x</sup> antigen [105] and gangliosides in the human stomach [106]. The *H. pylori* adhesin HP0721 specifically recognizes sialyllactose residues [107, 108] with a preference for Neu5Ac over Neu5Gc. Flagella and pili are also involved in binding to host sialic acid. For example, *P. aeruginosa* pili and flagellum interact with GM1 and GM2 gangliosides containing  $\alpha$ 2,3-linked Neu5Ac residues [109]. In an interesting example of sialylation *not* increasing adherence to the underlying glycans, the Ata adhesin of the multi-drug-resistant pathogen *A. baumannii* interacts with high-affinity to *N*-Acetyllactosamine, which consists of a galactose and GlcNAc joined by a  $\beta$ 1–4 linkage [110]. Sialylation of the GlcNAc with Neu5Ac to form sialyllactosamine did not change the affinity of the interaction [110]. This suggests that Neu5Ac may not be ‘seen’ by Ata, with the underlying structure of *N*-acetyllactosamine, or the individual components of this structure, more important to binding, and that not all human pathogens have an affinity for sialic acids.

Many pathogenic Streptococci specifically recognize sialic acid-linked structures on multiple human cells and tissues. *Streptococcus pyogenes* (Group A Strep, GAS), a major human pathogen responsible for a throat and skin infections, and a number of severe invasive infections, interacts with sialic acid during colonization. This interaction occurs via the outer-surface M-protein [111], a major virulence factor in this bacterium, and which displays extreme variability between GAS strains. The M1 protein variant has varying affinity for ABO(H) blood group antigen structures [112]. Colonization of human oral epithelial cells by GAS expressing the M3 and M12 variants is also mediated by blood group antigens, as well as the sialic acid containing Lewis (Le) antigen. Removal of linkage-specific fucose, galactose, *N*-acetylgalactosamine and sialic acid, modulated GAS colonization [113] indicating a dependence on sialic acid, but that the sialic acid can be contained in multiple arrangements and structures. *S. suis* also adheres to sialic acid containing glycans via the adhesin SssP1, a fimbria-like structure, which promotes adherence to host cells [114]. Multiple Streptococcal species are able to cause endocarditis, an infection of the heart tissue that can be life threatening. Adherence to sialic acid containing structures via their Sialic acid-binding serine-rich repeat adhesins, which contain SLBRs (Siglec-like binding regions), is key to this interaction in *Streptococcus gordonii* and *Streptococcus sanguinis* [115]. Both species show a preference for  $\alpha$ 2–3 sialoglycan structures, with these structures present on multiple plasma proteins that have been cited as biomarkers for vascular disease [115]. The structure of these SLBRs is very similar to mammalian siglecs, but the glycan ligand they interact with quite different [116], suggesting it will be possible to target inhibitors to the bacterial proteins to treat infections. *S. gordonii* expresses a second adhesin that interacts with sialic acid, Hsa, with this protein having a role in biofilm formation in this species [117]. *Streptococcus oralis*, a human oral bacterium that can also cause meningitis, binds platelets via recognition of a terminal sialic acid by the adhesin Fap1 [118]. *S. oralis* also produces a neuraminidase, NanA, which cleaves this terminal sialic acid with the exposed underlying  $\beta$ 1,4-linked galactose also bound by the Fap1 adhesin [118]. AsaA, a second sialic-acid binding adhesin characterized in *S. oralis*, helps bacteria bind to platelets and contributes to the development of endocarditis [119]. *S. oralis* subsp. *dentisani* produces a serine-rich repeat protein fibril, FapC, which binds sialic acid in saliva, and which aids oral colonization [120].

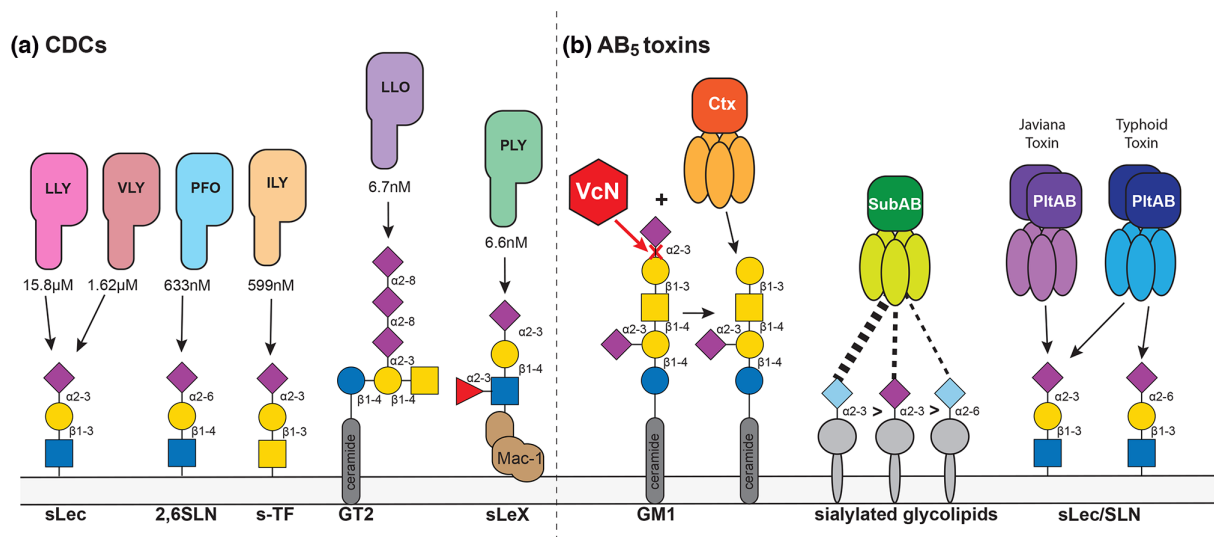
Mycoplasmas are host-adapted, reduced genome pathogens. Each vertebrate has its own specific *Mycoplasma* pathogens, where they cause a range of diseases. *Mycoplasma genitalium*, a human sexually transmitted pathogen, binds sialic acid containing receptors via the adhesin P110 [121], which together with protein P140 forms the Nap adhesion complex [122]. P110 binds  $\alpha$ 2–3- or  $\alpha$ 2–6-sialyllactose, with this interaction critical to initiating attachment to human cells [121]. *Mycoplasma pneumoniae* is a common cause of human respiratory tract infections, including bronchitis and atypical pneumonia. *M. pneumoniae* binds glycoprotein receptors having terminal sialic acid residues via the P1 adhesin protein [123], with no preference for either  $\alpha$ 2,3- or  $\alpha$ 2,6-sialyllactose. Mycoplasmas are also able to traverse surfaces via a characteristic ‘gliding’ motility, with *M. pneumoniae* specifically gliding via adherence to  $\alpha$ 2,6-sialyllactose [123]. *Mycoplasma mobile*, a fish pathogen, also uses sialylated glycans as a binding partner for gliding motility [124] via the adhesin Gli349 [125].

## A SUGAR COATING CAN KILL YOU: SIALIC ACID AS RECEPTORS FOR BACTERIAL TOXINS

Multiple bacterial species produce toxins, secreted protein effectors that damage host cells and tissues, releasing nutrients and potentiating virulence. Just like adhesins, bacterial toxins have evolved to recognise sialic acid residues as receptors to mediate host cell binding.

The CDCs, cholesterol-dependent cytolysins, are a major class of pore-forming toxin produced by Gram-positive bacterial pathogens [126]. In a recent study, all major bacterial CDCs were shown to bind host cell glycan structures as high-affinity receptors, and that some CDCs have no measurable binding affinity for cholesterol [127]. CDCs from *C. perfringens* (PFO), *G. vaginalis* (VGO), *Listeria monocytogenes* (LLO), *Streptococcus pneumoniae* (PLY), *Streptococcus mitis* (LLY) and *Streptococcus intermedius* (ILY) all have high affinity (in the nM– $\mu$ M range) for glycan structures containing terminal Neu5Ac sialic acid residues [127] (Fig. 3), with the activity of these toxins blocked via addition of their corresponding glycan receptor.

Gram-negative bacteria produce a class of toxins known as the AB<sub>5</sub> toxins, consisting of a multiprotein complex of A and B subunits [128]. The A subunits are the toxin effector, whereas the B subunits contain the cognate ligand binding function



**Fig. 3.** Two major groups of bacterial toxins have evolved to specifically recognize sialic acid containing glycans. The CDCs (cholesterol dependent cytotoxicity), produced by Gram-positive bacteria, and the AB<sub>5</sub> toxins, made by Gram-negative bacteria, both utilize host glycans as cellular receptors, with many specifically recognizing sialic acid containing glycan structures. Where available, the  $K_D$  (dissociation constant) is shown for the glycan structure with the highest affinity binding to each toxin. The action of Ctx, produced by *Vibrio cholerae*, requires the action of the neuraminidase VcN to cleave Neu5Ac in order to expose the underlying glycan structure, which is the preferred receptor. Host-evolution is evident for the AB<sub>5</sub> toxins, produced by Shiga-toxin producing *E. coli* (STEC; SubAB), and *Salmonella* spp. SubAB binds both Neu5Ac and Neu5Gc containing glycan structures, with a preference for Neu5Gc containing structures, demonstrating the broad mammalian host range of STEC; Typhoid toxin (PltAB), produced by the human-restricted pathogen *Salmonella* Typhi, binds Neu5Ac linked via an  $\alpha 2,6$ - or  $\alpha 2,3$ -linkage, whereas Javiana toxin, produced by *Salmonella* Javiana, shows a marked preference for  $\alpha 2,3$ -linked Neu5Ac. These differences in receptor specificity strongly influence disease severity resulting from infections by these organisms.

[128]. The subtilase AB<sub>5</sub> toxin, SubAB, of Shiga toxin-producing *E. coli* (STEC) [129] and damage the internal organs, with STEC infection frequently resulting in acute kidney failure and haemolytic anaemia [130]. The highest affinity receptors for SubAB are sialylated glycolipids with terminal  $\alpha 2,3$ -Neu5Gc, to which the affinity is 20 times higher than to  $\alpha 2,3$ -Neu5Ac, and 30 times higher than to  $\alpha 2,6$ -Neu5Gc [131]. Although this toxin receptor preference indicates adaptation to non-human Neu5Gc expressing hosts, the lack of Neu5Gc-containing body fluid receptor competitors in humans may confer susceptibility to the gastrointestinal and systemic toxicities of SubAB [131]. In recent years, cellular receptors for other AB<sub>5</sub> toxins have been demonstrated to be host glycan structures [132] (Fig. 3). *Salmonella* Typhi, the causative agent of typhoid fever in humans, produces the AB<sub>5</sub> Typhoid toxin PltAB, in an A<sub>2</sub>B<sub>3</sub> arrangement. The B subunit, binds Neu5Ac [133–135], is reported to bind to a galactose (Gal)  $\beta 1-3/\beta 1-4$  linked N-acetylglucosamine (GlcNAc) or glucose, with either an  $\alpha 2,3$ - or  $\alpha 2,6$ -linkage to the terminal Neu5Ac [136]. In contrast, the PltB from nontyphoidal *Salmonella* (NTS) *Salmonella* Javiana displays a strong preference for  $\alpha 2,3$ -linked Neu5Ac [136]. This difference in receptor preference is consistent with the resulting disease presentation: *S. Typhi* causes life-threatening typhoid fever, with Typhoid toxin targeting multiple cell types including epithelial, endothelial and immune cells and involves both local and systemic infection; Javiana toxin binds epithelial cells found in the human gastro-intestinal (GI) tract [136], which display a high relative amount of  $\alpha 2,3$ -linked Neu5Ac over  $\alpha 2,6$ -linked Neu5Ac, and consequently results in self-limiting GI infections (non-typhoidal *Salmonella* infection).

Cholera toxin, Ctx, the AB<sub>5</sub> toxin produced by *Vibrio cholerae*, also recognizes sialylated glycan structures, with specificity for GM1 polysialylated gangliosides [137]. However, as described in the sialidase section above, the action of the VcN sialidase is required for Ctx to bind to the underlying glycan structures, rather than sialic acid itself (Fig. 3). In a humanized mouse model of cholera using a CMAH deficient mouse that can only produce Neu5Ac, the action of Ctx is enhanced, indicating that VcN prefers Neu5Ac over Neu5Gc, and that Neu5Gc impedes VcN-mediated generation of the underlying glycans in gangliosides [138]. Human epithelial cells artificially expressing Neu5Gc were also less susceptible to Ctx binding and CtxA intoxication following VcN treatment. Therefore, it appears that *V. cholerae* evolved into a human-specific pathogen by adapting to the loss of Neu5Gc in humans [138].

Multiple additional types of bacterial toxin have evolved to specifically recognise and bind human sialic acid (Neu5Ac) containing glycan structures. For example, the *Clostridium botulinum* neurotoxin BoNT/DC specifically recognizes sialic acid moieties on the Sialyl-T antigen, but not to other moieties in gangliosides [139]. The *E. coli* heat-labile enterotoxin LT-IIb shows high-affinity for gangliosides with a neo-lacto core, that can contain either Neu5Gc or Neu5Ac residues [140].

## CONCLUDING REMARKS

Bacteria are able to utilize sialic acids in multiple ways. The advantage of being able to use an abundant carbon and energy source present in all mammalian hosts is clear. The concomitant ability to acquire sialic acids, and develop an ability to release this molecule from complex glycan structures – sialidases – goes hand in hand with the ability to use sialic acids in metabolism and biosynthesis. Using a highly prevalent molecule to bind to host cells and tissues makes a lot of evolutionary sense, and many bacterial pathogens have evolved multiple adhesins that specifically recognize host sialic acids. Co-evolution of specificity for Neu5Ac is evident in several human-adapted pathogens, and has likely occurred during loss of the ability of humans to make Neu5Gc, marking Neu5Ac as the ‘human specific’ sialic acid. The close interplay of multiple factors occurs during bacterial pathogenesis, with the synergy of *Vibrio cholerae* VcN sialidase and Ctx toxin a clear demonstration of human–bacterial co-evolution. The most pernicious way bacterial pathogens utilize sialic acid is as a means to evade and subvert the immune system, starkly illustrated by the incorporation of Neu5Ac in multiple outer-surface structures such as LOS, LPS and the polysaccharide capsule. This ‘molecular mimicry’ is perhaps best studied in NTHi and the pathogenic *Neisseria*. The bias for Neu5Ac over Neu5Gc in NTHi LOS incorporation occurs at a single point, the activation of sialic acid by the enzyme SiaB into the nucleotide sugar Neu5Ac-CMP for incorporation into LOS, in another stark illustration of co-evolution of bacteria and host.

An abundance of research is uncovering further roles for sialic acid in bacterial pathogenesis and host-bacterial interactions, building on initial characterisation of these systems in multiple bacterial pathogens. The advantage gained by the use of sialic acid in immune evasion and host binding means that it is a clear target for development of new antimicrobials; block the interactions required to colonize, or those needed for toxins to bind, and disease is prevented, or at the very least decreased. Many toxins and adhesins that recognize host sialic acid are key virulence factors, and highly expressed during pathogenesis. Therefore, in addition to being targets for novel antimicrobials, these proteins are also ideal vaccine candidates, and understanding their role is key to the development of rationally designed subunit vaccines against several major human pathogens.

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### Author contributions

M.P.J. writing – review and editing. C.J.D. writing – review and editing. J.M.A. conceptualization; project administration; supervision; visualization; writing – original draft; writing – review and editing.

### Conflicts of interest

The authors declare that there are no conflicts of interest

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