this effect is, in fact, dependent on SAA, as the authors suggested, it would be necessary to humanize the mouse model, simulating variable levels of SAA, perhaps by modifying carbohydrate hydrolysis with amylase supplementation of the polysaccharide-rich diet.

In summary, the study of Poole *et al*. [5] elegantly dissects the interaction between host genetics and the gut microbiome, providing solid evidence of the role of *AMY1*-CN and SAA in the oral and gut microbiomes. Their findings urge to conduct future studies to unravel the complex interactions between *AMY1* genetics, diet (starch sources), and the microbiome, and the effect of these interactions on obesity and other traits which could eventually translate into personalized nutrition.

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Spotlight

A Common Receptor Found for Echoviruses

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It has remained a puzzle why infants, during the first weeks of life, are especially prone to enterovirus infections. New work (*Proc. Natl. Acad. Sci. U. S. A.* 2019;116: 3758–3763) shines light on this matter by showing that the neonatal Fc receptor, prevalent in several tissues, acts as a pan receptor for several echoviruses.

Echoviruses belong to the genus *Enterovirus* within the family Picornaviridae [1]. Echoviruses are very small viruses, approximately 30 nm in diameter, and do not contain a lipid envelope. This feature makes these viruses quite stable as they are not as easily killed by normal hand washing and disinfectants as enveloped viruses such as influenza viruses. Echoviruses consist of 30 different serotypes and have been associated with a high number of acute infections worldwide, EV9 and EV30 being the most common serotypes. Echovirus infections can cause mild symptoms but also severe diseases such as meningitis and encephalitis [1,2]. Neonates and young infants are especially susceptible to severe enterovirus diseases that may turn fatal [1,3]. Echoviruses and their close relatives, coxsackieviruses, have also been associated with chronic diseases such as type I diabetes and atherosclerosis, further stressing their importance [2].

The transmission of enteroviruses occurs via the fecal–oral route where the mucosal cells of the gastrointestinal tract serve as the place for primary infection [4]. Carolyn Coyne's group pinpointed earlier that enterocytes promoted primary infection, based on their *in vitro* studies from human fetus-derived enteroids [5]. However, at that point, the receptor responsible for the primary infection was not yet determined [5]. From the primary infection site, enteroviruses typically cause secondary infections in other tissues such as liver, heart, pancreas, and nerve tissue, when the viruses spread systemically via the bloodstream and lymphatic system. Infection in secondary infection sites depends on the presence of specific cellular receptors and other host factors, for example, in helping to attach the virus particles onto the cell surface [4].

Several molecules have been identified to act as cell-surface receptors for enteroviruses (Table 1): poliovirus receptor (PVR) for three types of poliovirus; coxsackie–adenovirus receptor (CAR) for several coxsackie B viruses; and integrins for coxsackievirus A9 and echovirus 1 [1]. In addition to the primary receptors, enteroviruses often bind to other molecules that act as coreceptors, such as the decay-accelerating factor (DAF) – which has been found to bind some coxsackieviruses and echoviruses – or β_2 microglobulin (β2M), which was shown to be important for the entry of echovirus 1 and 7 [1,6,7]. In particular, it has been suspected that β2M has a role *in vivo* due to the strong inhibitory effect of anti-β2M antibodies on the binding of several different enteroviruses to cells, which prevents infection by those viruses *in vitro*.

Table 1. Enterovirus Receptors[a,b]

Receptor	Virus
PVR	Poliovirus (serotypes 1-3)
SCARB2	EV-A71 CV-A (serotypes 7,14, and 16)
PSGL1	EV-A71-PB CV-A (serotypes 2,7,10, 14, and 16)
Annexin II	EV-A71
Heparan sulfate	EV-A71 E-5
Sialic acid	EV-A71, CV-A24v EV-D70, EV-D68
ICAM5	EV-D68
LDLR, VLDLR, and LRP	Rhinovirus (minor)
ICAM1	Rhinovirus (major) CV-A21, CV-A24
CDHR3	Rhinovirus C
DAF	CV-A21 CV-B (serotypes 1,3, and 5) E (10+ serotypes)
CAR	CV-B (serotypes 1–6)
Integrin $\alpha_v\beta_3$	CV-A9 E-1, E-9
Integrin $\alpha_2\beta_1$ (VLA2)	E-1, E-8
FcRn	E (serotypes 5-7,9,11,13,30)

[a]Modified from a review article by Baggen *et al*. [1] with further information from [8].
[b]Abbreviations: CAR, coxsackievirus–adenovirus receptor; CDHR3, cadherin-related family member 3; CV, coxsackievirus; CV-A24v, coxsackievirus A24 variant; DAF, complement decay-accelerating factor; E, echovirus; EV, enterovirus; EV-A71-PB, EV-A71-PSGL1-binder; FcRn, neonatal Fc receptor; ICAM, intercellular adhesion molecule; LDLR, low-density lipoprotein receptor; LRP, LDLR-related protein; PSGL, P-selectin glycoprotein ligand 1; PVR, poliovirus receptor; SCARB2, scavenger receptor class B member 2; Sia, sialic acid; VLA2, very-late antigen 2; VLDLR, very-low-density lipoprotein receptor.

However, it has been difficult to directly address the most relevant molecules in a physiological setting.

The recent study by Morosky *et al.* shines light on the puzzle as to which molecules may prove physiologically relevant for these serious infections that affect infants in their early life [8]. Morosky *et al.* show that the key player is the Fc receptor, which is normally in charge of IgG and albumin binding in various tissues [9]. This receptor is ubiquitously expressed in several tissues, such as the small and large intestine, and in the microvasculature of several organs such as brain and liver [9]. In placenta, the Fc receptor is responsible for transferring maternal antibodies to the fetus, thus giving rise to passive immunity in the child [9]. Importantly, the Fc receptor is highly expressed in the outermost cell layer contacting the blood from the maternal placenta. Those cells are normally resistant to viral infections, but taking into account the Fc receptor-mediated transcytosis of IgG, it may be possible that Fc receptor-bound viruses use the same transcytosis mechanism to enter the fetal blood stream.

Morosky *et al.* were examining why certain cell types were not permissive to echovirus infection when they found a role for the Fc receptor [8]. Comparison of the receptor proteins expressed in permissive and nonpermissive cell types led to the finding that the Fc receptor is one of the most downregulated receptors in the cells showing negligible infection. In order to prove that the Fc receptor is indeed the relevant receptor for several clinically relevant echoviruses, Morosky *et al.* first studied the receptor usage *in vitro*. By using human and mouse cell lines they showed that the human receptor makes nonpermissive cells prone to infection. In contrast, cell lines that are devoid of the receptor, or in which the receptor was downregulated, were not infected by echoviruses. More importantly, they showed, using a mouse model, that the Fc receptor is the missing factor for infection through the gut. They introduced the human receptor to neonatal mice and showed the expression of the receptor in several tissues, including the intestine, stomach, liver, and brain. They introduced live virus through oral administration and showed that, after 7 days, liver, brain, and stomach subepithelial muscle layers were efficiently infected by echoviruses. This was an important proof that Fc receptor could indeed promote infection through the normal oral route.

Morosky *et al.* have now an explanation as to what may be the role of β2M on echovirus infection. Namely, they show that a recombinant Fc receptor, in complex with β2M, interferes with virus binding on cells, but not the construct with β2M only. Although they did not show this with the Fc receptor alone, the results indicate that the Fc part is responsible for the actual binding. β2M is known to regulate the Fc receptor trafficking and targeting to the plasma membrane, which makes β2M a crucial player for Fc receptor function.

The connection between high prevalence of echovirus infection in neonates and young infants and expression of the Fc receptor in the placenta is compelling. Most of the enterovirus infections in neonates are presumed to originate during delivery, when the newborn is exposed to the blood and genital secretions of the mother, or due to nosocomial transmission [3,10]. However, congenital enterovirus infections during the first few hours after birth have also been documented, and enteroviruses have been identified in placentas, suggesting that the infection may be acquired *in utero* [3,10]. The finding by Morosky *et al.* also supports the possibility that vertical transmission of enteroviruses may occur during pregnancy, and provides important insights into the pathogenesis of enteroviruses.

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Forum

Influenza Neuraminidase: Underrated Role in Receptor Binding

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Influenza virus neuraminidase cleaves sialic acid groups from cell glycoproteins, enabling release of the virus from host cells. Neuraminidase also contributes to virus binding to the sialic acid groups of cell glycoproteins, which could complement the receptor-binding function of hemagglutinin, enhancing enzymatic activities of neuraminidase, and facilitate virus infection.

Influenza A Tropism through Binding Affinity

Influenza A viruses (IAVs) infect humans and a wide range of animal species, including birds, pigs, horses, dogs, and marine mammals (e.g., seals and whales). The initial step in viral infection is the binding to sialic acid receptors, typically avian-like receptors (sialic acid- α2,3-galactose linkage, SA2,3Gal) and human-like receptors (sialic acid-α2,6-galactose linkage, SA2,6Gal) on the host cell surface glycoproteins. The last step in viral infection is the cleavage of sialic acid receptors from glycoproteins and glycolipids by neuraminidase (NA) and release of virus particles after budding from the host cell plasma membrane. Gaining binding affinity to human-like SA2,6Gal receptors is considered a key prerequisite for avian IAVs that cause pandemics or epidemics among humans. The IAV glycoprotein hemagglutinin (HA) attaches to host receptors during virus entry, and the receptor-binding specificity of HA is well documented to affect IAV host tropism. We review recent studies demonstrating that NA, the other IAV glycoprotein, contributes to virus binding to the sialic acid receptors at host cell glycoproteins and that, such receptor-binding contributions can complement those of HA, enhance NA enzymatic activities, and facilitate virus infection and host adaptation.

Receptor Binding of IAV NA

The NA of IAVs belongs to the exosialidase family of enzymes which cleave the α-ketosidic linkage between synthetic substrates and the terminal sialic acid residues in oligosaccharides, glycoproteins, glycolipids, or colominic acids. It is generally accepted that the functions of HA and NA are, to some extent, contrary: HA binds to sialic acid receptors on host cells and mediates virus attachment and entry, whereas NA cleaves the α-ketosidic linkage between the terminal sialic acid residues and the remaining substructures. However, it has been reported that, in addition to their enzymatic functions, NA proteins, such as N1, N2, and N9, also have sialic acid receptor-binding functions. For example, subtype N9 from A/tern/Australia/G70C/75(H11N9) alone hemagglutinates animal erythrocytes by using a second receptor-binding site (SRBS), which is also referred to as the hemadsorption site, that is distinct from the NA catalytic site [1].

Structural analyses suggested that the SRBS of NA consists of three loops (367–372, 400–403, and 432) that interact with the Neu5Ac moiety in chair conformation, which is similar to that of the receptor binding site (RBS) of HA [2]. In contrast, the NA catalytic site interacts with the sialic acid receptor in a twisted boat conformation [2]. Sequence analyses showed that these residues in the SRBS of NA are mostly conserved among avian IAVs but not in human and swine IAVs [2]. Of interest, the sialic acid binding function of the SRBS of NA has been so far identified primarily in avian IAVs [3]. On the other hand, sequence diversity in the SRBS across different NA subtypes, or even within the same NA subtype, is well documented to affect the receptor-binding ability of NA. For example, exchange of amino acids in the loops at positions 368–370 and positions 399–403 of N9 into those corresponding positions in the N2 by site-directed mutagenesis made N2 acquire the hemagglutination activity as shown in N9 [4]. The pandemic viruses A/Singapore/1/57(H2N2) and A/Brevig Mission/1/18 (H1N1) differed from their avian counterpart precursors with abolished or reduced hemadsorption activity due to variations in the SRBS of NA [3].

Current studies suggest that, through its glycan binding, the SRBS of NA can increase the enzymatic activities on multivalent substrates but not monovalent substrates [3]. The NA of a human H7N9 virus enhanced overall virus binding to SA2,6Gal but not to SA2,3Gal via the SRBS of NA when the whole virus is used in the assays [5]. Interestingly, another study, when using recombinant NA proteins, showed that the NA of human