In: Advances in Medicine and Biology. Vol. 109 ISBN: 978-1-53610-207-9
Editor: Leon V. Berhardt

Chapter 1

SIALIC ACIDS IN EARLY LIFE AND THEIR IMPACT BEYOND INFANCY

Maria J. Martin* *and Elena Oliveros*
Abbott Nutrition R&D, Granada, Spain

ABSTRACT

Sialic acids (Sia) are key players in the cellular physiology from fecundation to aging and disease. They participate in cell-cell interactions, binding to pathogens, axon scaffolding in brain, synaptic plasticity, immune cell regulation, inflammation, cancer, and autoimmune and metabolic diseases, including cardiovascular diseases or diabetes. Their role in fetal and postnatal development in humans is also of paramount importance. Sialylated molecules, not only protein and gangliosides but also oligosaccharides, have been shown to play a pivotal role in gut maturation and microbiota development, as well as in resistance to pathogens both in gastrointestinal and respiratory tracts. Regarding brain development, a fast increase of brain gangliosides after birth seems to be related to key neuronal events as cell proliferation, neurogenesis or synaptic transmission. Moreover, brain gangliosides and glycoproteins have been associated with learning and memory abilities. Thus, Sia are essential for the infant during the first months of life and a correct sialylated status may condition the development of the baby. Question arises whether such an influence for Sia extends further and health during adulthood and aging might partly depend on the Sia

* maria.j.martin@abbott.com.

metabolism during early life. The present review will attempt to shed light on the topic from the early programming perspective.

Like old mithological Janus or the 19th century's classical horror character - Dr Jekyll and his evil alter ego Mr Hyde-, sialic acids (Sia) are described in the literature as heroes and villains. While life and development without sialic acids is impossible, its presence/increase is also bound to the development of malignancies and increased risk of suffering from grave diseases or the most severe stage of those diseases.

No other sugar has raised similar interest and literature. But Sia peculiarities are worth any word dedicated to them. Therefore, let us start the tale of Sia from the very beginning.

SIALIC ACIDS IN HUMAN DEVELOPMENT

Sperm and egg, as most mammalian cells, are fully coated by a dense glycocalix composed by glycoproteins and glycolipids [1]. Glycoconjugates on the sperm surface are highly sialylated, which has been interpreted as a way of eluding the female immune response while traversing the reproductive tract in search of the ovum. But, in order to be ready to fecundate the egg, sperm cells go through a process called capacitation. During capacitation, 20% of Sia bound to sperm membrane is lost mainly due to the action of sperm sialidases Neu1 and Neu3 [2].

After fecundation, a change in glycosylation in the zygote and developing embryo has also been reported by several authors (for an excellent review, see [1] and references within). Glycosylation seems to be crucial in the implantation phase, allowing the embryo binding to the endometrium [3]. Thus, selectin adhesion is important in the maternal-fetal interface since L-selectins on the fetal side strongly bind to the maternal sialylLe^X-decorated MECA-79 [4].

Furthermore, sialylation is critical during the early stages of development and the inactivation of the key enzyme of sialic acid synthesis –UDP-GlcNAc 2-epimerase– is lethal, as shown in a targeted mutagenesis of this gene in which no homozygous null embryos could be identified at embryonic day 10 (E10.5) [5]. Loss of protection from proteolysis and impaired cell-cell recognition were hypothesized by the authors as potential causes of lethality.

As mentioned before, Sia also plays its double hero/villain role at this early stage of life. While being essential for development of the embryo, Sia can be a hurdle when it comes to nourish that same embryo. During the first 12 weeks of human gestation endometrial glands produce secretions that, in addition to plasma filtrate, serve as a source of nutrients for the developing embryo. A loss in sialylation in certain glycoconjugates secreted by the endometrium may enhance their uptake by the developing embryo and, thus, contribute to its optimal nutrition [6].

Changes not only affect the offspring. Hence, an increase in maternal serum total Sia (TSA) as gestation progresses has been reported [7]. The study included data from women at different weeks of gestation, from 5 to 37+ weeks, showing an increase from 503 mg/L to 636 mg/L. Despite the main limitation of this study, i.e., it was not a follow-up of the same women along their pregnancies, it is worth taking into account such an increase in TSA, which authors associated to the expansion of the different maternal and fetal tissues.

Crook et al. also reported a significant increase in TSA in pregnant women when compared to non-pregnant women (991 vs 660 mg/l, $p<0.001$). TSA values remain high even 12 weeks after delivery (775 mg/L; $p<0.001$ compared to controls)[8].

This rise in TSA likely reflects an intensification of the endogenous synthesis of Sia in the mother in order to satisfy the increasing demands of the growing fetus first, and those of the lactating baby after birth. Sia is crucial for the correct development of the different tissues, mainly for the brain. While the liver is able to synthesize Neu5Ac from UDP-*N*-acetylglucosamine through a series of reactions performed in the cytosol [9], rate of synthesis may not fulfill the requirements of the infant. Under these circumstances, dietary Sia becomes critical since it can be incorporated into glycoconjugates once released into its free form during digestion [10].

Levels of TSA during postnatal development also reflect child growth. Thus, it has been reported that term newborns have around 384 mg/L TSA, while preterm babies have lower amounts (313 mg/L). The level of TSA increases during childhood up to an average value of 705 mg/L in children aged 1-15 years [11]. Other authors have reported an increase of total Sia excreted in urine, being 20.9 mg/24h in infants (3 months), 30.6 mg/24h in toddlers (age 2 years), 64.4 mg/24h in school age children (age 8 years) and 83.8 mg/24h in adolescents (age 15 years) [12], reflecting a probable increase also in serum. We may speculate that during the exponential phase of development when brain is rapidly developing, Sia requirements are so high

that very little is discarded and excreted and these high demands drop as children grow and their brains acquire a more mature and stable structure.

TSA levels during adulthood have also been studied in a subpopulation out of a broad sample of Finnish subjects from age 25 to 74 years (n=200 subjects out of 11500) [13]. TSA values are similar in men and women, when the general population is considered. However, TSA significantly increased with age in the female population while remained stable in males. A significant increase was observed around menopause, showing that TSA levels may be influenced by sexual hormones. Moreover, the same authors showed that the use of contraceptive pills and hormone treatment had a significant effect on TSA.

Regarding age differences, a study comparing 37 geriatric patients (mean age 80 years, 48% females) vs 50 younger healthy subjects (mean age 40 years, 54% females) showed a significant increase in TSA in the elderly (744.7 mg/L vs 630.4 mg/L). The main limitation of this study is that it did not take into consideration gender as a factor, although groups seemed to be well balanced [14]. Sia changes along life are summarized in Figure 1.

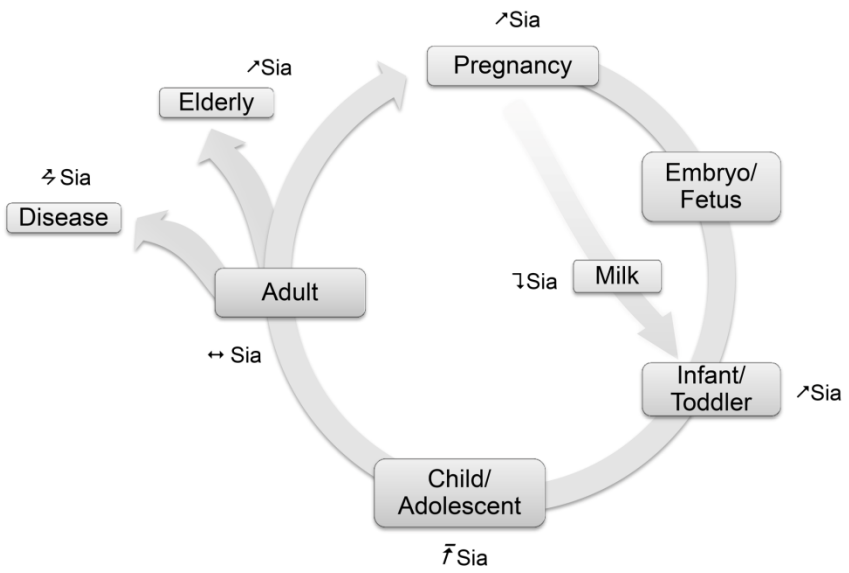

Figure 1. Life cycle of Sia. Arrows indicate the trend of Sia content in serum along that stage.

SIA AS A MARKER OF DISEASE

Increased TSA levels have been associated to malignancy and metastasis in several tumors, including ovarian carcinoma, brain tumors, leukemia, lung, oral, colorectal, glad bladder and endometrial cancers, among others [15, 16]. The highest level correlates positively with the most advanced stages of tumor and metastasis. Also, tumor cells are hypersialylated with respect to normal cells due to an overexpression of sialyltransferases, an enhanced flux rate through the biosynthetic pathway –more substrates, overexpression of genes involved-, and a differential expression of sialidases [17]. The highly sialylated tumor cell surface facilitates escape from apoptosis, contributing to the survival of the tumor and to therapy resistance [18, 19].

TSA is also a useful marker for leukemia and solid tumors in children [20]. Elevated TSA and lipid-bound Sia were observed in children with various malignancies compared to healthy children, and also in those with simultaneous cancer and infection respect to those with infections only [21].

It is well known that humans lost the ability to synthesize Neu5Gc during our species evolution [22]. However, there is evidence of the presence of Neu5Gc in glycoconjugates from tumor cells, an epitope known as Hanganutziu-Deicher (HD) antigen. The formation of HD antibodies has been shown to occur not only in patients with several malignancies, but also in those with infections and liver diseases [23]. Targeting NeuGc-containing gangliosides has been proposed as a therapy in children with high-risk neuroblastoma and other pediatric tumors [24].

Sia are also elevated in several inherited disorders that can be diagnosed by determining the Sia level in urine. Thus, sialidosis –a primary sialidase deficiency- leads to a 3-20-fold increase in Sia excretion [25]. While sialidosis or galactosialidosis affects bound Sia, Salla disease, intermediate severe Salla disease, and infantile free sialic acid storage disease (ISSD) are neurodegenerative disorders resulting from increased lysosomal storage and urine excretion of free Sia [26].

Inflammation and acute-phase response are also accompanied by an increase in TSA, and both free Sia and lipid-bound Sia have been proposed as markers for monitoring systemic inflammation, mainly associated to heart failure [27]. A 17-year follow-up study has shown that TSA is an independent risk factor for coronary heart disease, mainly in women since an increase of 25 mg/dL in serum is associated with a hazard ratio of 1.40 (1.06 in men) [28]. This study confirms the results shown in a previous 20-year follow-up study

reporting that the relative risks for CHD mortality almost doubled in the highest serum sialic acid quartile population [29].

SIA IN DIABETES AND OBESITY

The current prevalence and rising trend of diabetes is one of the most concerning public health issues worldwide. Several authors have reported an increase in TSA in diabetic patients compared to normoglycemic controls [30-34]. In a 40 years follow-up study of a large population from Sweden, it was shown that elevated TSA significantly increased the risk of hospitalizations in diabetic subjects. Moreover, the risk of suffering from diabetes was higher in those individuals with TSA above the median [35]. Other studies confirmed that high TSA in diabetic patients is associated with additional complications such as nephropathy [36, 37], retinopathy [38], and vascular disease [39].

Conversely, some studies have found no differences in TSA between controls and type 1 diabetic children (aged 12-15 years) [40, 41] and young adults [42]. Young diabetic subjects recruited for those studies did not have any associated pathology, i.e., nephropathy or vascular disease, and in fact, prepuberal children seem to be protected against comorbidities. An intervention at the onset of diabetes during childhood and adolescence can prevent the development of associated vascular diseases in adults [43]. It can be hypothesized that TSA is not a marker of diabetes *per se* but a marker of associated complications that develop later in life. Thus, longer follow-up studies of type 1 diabetic patients from the onset of the disease may shed light on the true role of Sia in the evolution of the diabetes.

Mechanism

Desialylation has been shown to play a role in insulin receptor (IR) activity. Insulin receptor is a large glycosylated transmembrane protein with tyrosine kinase activity that becomes auto-phosphorylated upon binding to insulin [44], triggering a signaling cascade that eventually leads to trafficking of the glucose (Glc) transporter GLUT4 to the plasma membrane [45]. It has been shown that insulin binding to IR induced desialylation of the IR by neuraminidase Neu1 and this accompanied activation of the receptor [46]. A reduction in insulin-dependent Glc transport by 30% was reported in rat adipocytes [47] and other study showed a reduction in insulin action by 50%

when 23% of total cellular sialic acid content was released by neuraminidase treatment [48]. Haxho et al. [49] have proposed a mechanism in which insulin binding to the IR initiated a cross-talk between a G-protein-coupled receptor platform and Neu1, which is then induced to hydrolyze α2,3-sialyl residues on IRβ subunits allowing subsequent dimerization, activation and signaling [50].

Sialylated molecules seem to be crucial in the development of insulin resistance. Signaling from the insulin receptor is mainly restricted to caveolae microdomains of the plasma membrane [51]. In normal, insulin-responsive cells IRs are mainly located in the caveolae, anchored there by caveolin-1 (Cav-1) [52]. Upon insulin binding, a signaling pathway is activated and GLUT4 transfers to the plasma membrane allowing the uptake of Glc from the extracellular millieu (see Figure 2, left panel). Early reports showed that TNFα-mediated insulin resistance in adipocytes could be prevented by the use of inhibitors of GM3 synthase, one of the first enzymes in the ganglioside synthetic pathway; moreover, exogenous GM3 was able to suppress Glc uptake in cultured adipocytes to a similar extend as TNFα did [53]. Two-fold increase in GM3 was found in microdomains of adipocytes when treated with TNFα, suggesting that GM3 may be a regulator of insulin signaling [54]. Other authors have reported similar results not only in adipose tissue [55] but in muscle [56] and fibroblasts [57], thus GM3 is now considered as "a gatekeeper of obesity-associated insulin resistance" [58]. Taking all this evidence into account, Inokuchi has proposed a mechanism to explain the role of GM3 in insulin resistance and diabetes (Figure 2) [59]. In normal cells, the amount of GM3 in the microdomains is low mainly due to the activity of neuraminidase Neu3 [60], thus IR remains in the caveola and insulin signaling leads to the membrane expression of GLUT4. In pro-inflammatory conditions, the presence of TNFα triggers the synthesis of GM3 by enhancing the expression of GM3 synthase. At the same time, TNFα induces the elimination of IR from the caveola, disrupting insulin signaling. It has been shown that IR can bind to either GM3 or to Cav-1, but not to both at the same time. IR-Cav-1 complex seems to be dissociated by the interaction of a Lys residue of the IRβ to the Sia moiety of the GM3, thus removing the IR anchoring to the caveola [54].

Therefore, when GM3 levels are elevated after stimulation by TNFα, these ganglioside molecules "sequester" IR from the caveola and the cell becomes insulin-resistant [61].

Hence, GM3 has been proposed as a marker for metabolic syndrome and diabetes. Serum GM3 levels were higher in hyperglycemic patients and type 2 diabetic individuals with severe obesity [62], and further studies have even

identified GM3 molecular species that were elevated in these patients, proposing GM3(d18:1-h24:1) as the best candidate for metabolic screening [63]. Accordingly, GM3 synthase-deficient mice are protected against obesity induced by high fat diet and show enhanced insulin sensitivity [64] and obese Zucker rats exhibit lower Neu3 protein content in skeletal muscle and liver [60].

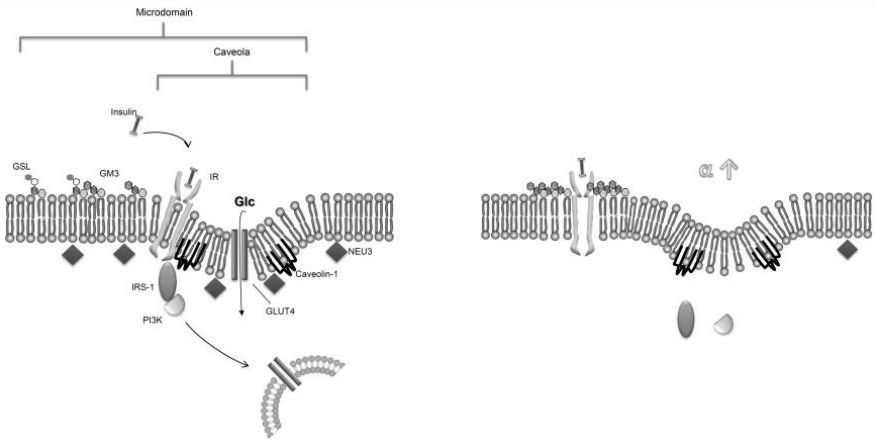

Figure 2. GM3 in insulin resistance (based on [59]). Under normal conditions (left panel), insulin receptor remains anchored to the caveola by caveolin, being able interact with IRS-1 upon insulin binding and trigger the signal that allows GLUT4 translocation to the plasma membrane for Glc uptake. GM3 levels remain low due to the action of Neu3. Under insulin resistance conditions (right panel), the excess of GM3 stimulated by TNFα keeps the insulin receptor sequestered out of the caveola. Thus, even when it binds insulin, there is no connection to IRS-1 and the signaling is blocked.

Not only GM3, but other glycosphingolipids have also been involved in the development of metabolic syndrome. Since GM3 is the precursor of many other gangliosides, an increase in GM3 may consequently lead to an increase in other gangliosides. Thus, adipose tissue from obese animals is enriched in a-series gangliosides, i.e., GM2, GM1a, and GD1a when compared with adipose from normal weighed animals [55]. Increase of a-series gangliosides, but not b-series ones, resulted in the accumulation of leptin in adipocytes due to an impairment in the secretion of this anorexigenic hormone, whose levels in obese animal serum are very low [65]. Also, an increase in GM1 on the cell surface of senescent endothelial cells caused insulin resistance [66].

Conversely to the previously cited studies, other authors have reported that an increase in Neu3 induced a reduction in the phosphorylation of IRβ and the development of insulin resistance in skeletal muscle [67, 68]. Chavez et al. have suggested that the mechanism involving GM3 may be more relevant in the adipose tissue due to the high number of caveolae on the surface of these cells, whereas myotubes contain far fewer [69].

In summary, Sia and sialylated molecules such as ganglioside GM3 are likely to play a preeminent role in the development and pathophysiology of insulin resistance and diabetes.

Obesity in Childhood

Since pediatric obesity has been described as an inflammatory condition and obese children have elevated levels of proinflammatory cytokines including TNFα [70], it is likely that the mechanism described above also works in the youngest diabetic patients.

Childhood obesity has become a global public health concern as the number of obese children has tripled in the last 3 decades in North America and other Western countries [70]. Being the aim of the present review to discuss the influence of early life Sia on posterior health and disease, we can but hypothesize about a role on diabetes and obesity since the published evidence is scarce. It is widely accepted that gestational diabetes mellitus (GDM) and maternal obesity are associated with an increased risk of obesity and Glc intolerance in the progeny, even maternal insulin resistance per se can promote metabolic disease in offspring [71]. Sia is one of the metabolites with a differential regulation in insulin resistant mothers with respect to normoglycemic mothers [71]. Others have also reported increased levels of TSA in GDM women or pregnant women with impaired Glc tolerance when compared to normoglycemic pregnant women [72]. The levels were significantly higher in those women with previous GDM than those without previous GDM [73]. Defected glycosylation of glycodelin-A –a protein found in the amniotic fluid and involved in fetomaternal defense– has been reported in GDM patients, with lower levels of Sia and loss of α2,6-sialylation with respect to α2,3-glycans [74]. Reduction of Sia on glycoproteins may explain the higher levels of TSA in GDM women. Whether sialylation in the fetus is altered as well remains to be determined, but we could speculate that normal pattern may be modified to some extent. Moreover, altered glycosylation in GDM women seems to persist beyond delivery, since changes in the normal

amount of Sia bound to lactoferrin and secretory immunoglobulin A in milk from these women have been described [75].

Microbiome, Sialic Acid and Early Programming

One of the hottest topics in bioscience in these first years of the new millennium is gut microbiome. New molecular tools including metagenomics, metatranscriptomics, and high throughput rRNA-based techniques have allowed for the identification of new microbial communities populating the human intestine [76], and thus humans are now considered as "super-organisms" with part of our metabolic capabilities depending on the millions of microbial genes located in our intestinal symbionts [77]. The intestinal microbiota consists of approximately 1000 species that mainly belong to two bacterial phyla, Firmicutes and Bacteroidetes, but other phyla such as Proteobacteria, Actinobacteria, Fusobacteria, Verrumicrobia, and Cyanobacteria [78], and even methanogenic Archaea [79] are also represented. Microbial density and diversity increase from the proximal to the distal digestive tract, being more abundant in the colon (10^{11} cell/mL effluent) [78].

The role of gut microbiota –or intestinal flora as it was termed at the time- in polysaccharide fermentation and vitamin synthesis was established decades ago [80, 81]. More recently, it was shown that microbiota modulates immune differentiation and intestinal functions including motility, it contributes to enhance nutrient accessibility, has effects on nutrient availability and energy expenditure, reduces bone mass, and affects adiposity and certain behaviors, such as anxiety [82].

In 1900, Henry Tissier described how babies acquired the first bacterial inoculum while traversing the birth canal, since the uterus was considered a sterile environment [83]. For more than a century, it was therefore thought that humans were born germ-free, and gut colonization started at birth [84]. Thus, microbiologists devoted much attention on the colonization of what it was considered a virgin territory. It was shown that delivery mode modified the microbiota in the newborn since vaginally delivered infants acquired a microbiota resembling that of their mother's vagina and feces, i.e., *Lactobacillus* and *Prevotella*, while C-section infants harbored communities similar to skin bacteria, i.e., *Staphylococcus*, *Corynebacterium*, and *Propionibacterium spp* [85]. Microbial ecosystem in the newborn colon changes rapidly during the first days of life. Due to the abundance of oxygen in the gastrointestinal tract at that time, first microbiota consists mainly in

facultative anaerobic *Enterobacteriaceae*, such as *Escherichia coli*, *Klebsiella* and *Enterobacter*. As the oxygen is consumed by these microorganisms, obligate anaerobes start thriving and replace facultative anaerobes. Thus, after 2-3 days, gut is populated mainly by bifidobacteria, lactobacilli, *Bacteroides* and *Clostridium* [84, 86].

During the 2000's decade, several authors provided evidence that utero was not as sterile an environment as it was previously thought. Bacteria could be detected in cord blood, amniotic fluid and fetal membranes in normal, non-pathological conditions demonstrating the maternal microbial transmission in utero [87]. Besides, maternal provisioning of microbes to the offspring seems a common mechanism in many animal phyla [83]. Placenta harbor a rich microbiota, composed of non-pathogenic species from Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes, and Fusobacteria phyla, being very similar to maternal oral microbiota [88]. The presence of autochthonous bacteria was also demonstrated in human meconium, being *Enterococcus fecalis* and *Staphylococcus epidermidis* the most predominant species [89]. It has also been shown that meconium clearly differentiates from first fecal samples, since Firmicutes dominate meconium microbiota while Proteobacteria are more abundant in early feces [90].

After birth, feeding choice influences the bacterial community of the colon (Figure 3). Differences in bacterial species isolated from feces of breast-fed and formula-fed infants were long established by culture [91] and more recently by molecular techniques [92]. Breastfeeding favors a simpler microbiota, dominated by bifidobacteria; while formula-fed infants harbor a more diverse community with prevalence of *Bacteroides* [93]. Using metagenomics techniques, Praveen et al. found that the formula-fed microbial community network is denser and more complex than the breastfed corresponding network, while the co-expression (host-microbiome) network in breastfed babies is denser, reflecting a higher degree of interaction between human milk bioactive compounds, infant gut, and microbiome [94]. Relationship also extends to the mother; in fact, in a study performed with seven mother-infant pairs at different times after birth some species, i.e., *Bifidobacterium breve*, *Staphylococcus epidermidis*, and *Escherichia/Shigella spp*, that appeared in maternal feces were also found in breast milk, and infant feces, demonstrating the vertical bacterial transfer mother-newborn (Figure 3) [95].

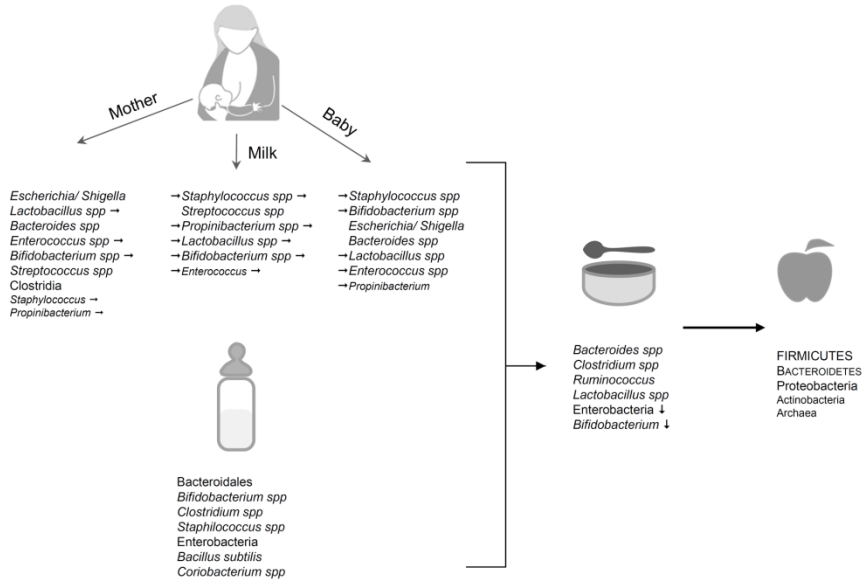

Figure 3. Microbiome in infancy and early childhood. The picture shows the main species/groups associated to each stage. Upper left, for breastfed infants, the bacterial profile in mother and baby feces and also in milk is shown. Arrows indicate those taxa that appear in the three members of the triad and less abundant genera are indicated by reduced font size. Bottom left, typical microbiota composition for formula fed infants. Paramount changes take place when solid foods are given to the baby. The apple icon represents adult-like microbiota, which is thought to be established by age 2-3y.

After millennia of co-existence, it is fair to suppose that gut microbes would have evolved and adapted to extract all the best from such a rich culture media as milk [96]. On the other hand, mammals must have taken advantage of microbial metabolism to exploit the beneficial influence of a selected microbiome on the defenseless newborns. Genome sequencing has shown that a typical species from breast-fed infants, i.e., *Bifidobacterium longum* subsp. *infantis*, has developed a special gene cluster –the 43kB HMO cluster– encoding catabolic genes, extracellular binding proteins and permeases that allow for the metabolism of milk oligosaccharides [97]. To the point, it is remarkable that milk oligosaccharides were initially known (back in the 1950's) by its "bifidogenic" effect, and in those early days they were commonly referred to as bifidus factor [98]. In general, infant-type bifidobacteria are able to subsist on milk oligosaccharides, while adult bifidobacteria, e.g., *Bifidobacterium adolescentis* and *B. catenulatum*, show no growth on lacto-N-biose, a building block for human milk oligosaccharides

(HMO), as the sole carbon source [99]. Not only bifidobacteria, but *Bacteroides* spp, such as *B. fragilis*, *B. caccae*, and *B. vulgatus*, are also able to grow in the presence of HMO [100]. All these species knit the complex network that forms the infant microbiota ecosystem, where "friendly" bacteria are favored by a continuous supply of HMOs as a carbon source and pathogen bacteria growth is hindered by acidification of the medium, colonization resistance, or antimicrobial byproducts [101].

Mature human milk contains 10-15 g/L of HMOs [102]. Conversely to other mammals, sialylated fraction is minority (1-0.4 g/L), being 3'- and 6'-sialyllactose the most abundant sialylated oligosaccharides [103, 104]. Despite neutral oligosaccharides are more abundant than sialylated ones, infant microbiota species are well adapted to sialic acid and can upregulate their sialic-acid use pathways when in the presence of both HMO and intestinal mucins [100].

Not only commensal bacteria, but pathogenic species are able to use host Sia as sources of carbon, nitrogen, and as blocks for cell wall synthesis. Moreover, certain pathogens are able to decorate themselves with Sia in a successful attempt to escape host immune system, either synthesizing its own Sia, e.g., *E. coli*, or taking it from the host, as *Neisseria gonorrhea* and *Haemophilus influenzae* [105]. In fact, it has been shown that Sia transport is essential for *H. influenzae* virulence [106].

Sia metabolic pathway has been extensively studied in E. coli, in which the Neu5Ac metabolism *nan* operon was first defined [107]. Initially, five genes were identified: *nan*A (Neu5Ac aldolase), *nan*T (transporter), *nan*E (epimerase), *yhc*I (kinase, *nan*K), and *yhc*H (no function assigned yet). Other *nan* genes also regulated by the repressor protein NanR have been added to the original operon although located in two other operons. All the three operons are now known as the *E. coli* sialoregulon. This cluster is confined to 46 bacterial species, 42 of which are mammalian colonizers [108, 109], including *Bifidobacterium spp* [110] and some Lactobacilli [109].

In order to metabolize Neu5Ac, bacteria rely on the free form released from glycoconjugates by sialidases produced either by the host or by microorganisms. According to this, two kinds of bacteria have been identified, those that are able to secrete sialidases into the medium and capture the free Neu5Ac ("Scavengers") and those that are able to transport an oligosaccharide through its outer membrane, break it down into their sugar constituents and then, transport each sugar inside ("Swallowers"). An additional third type has been identified, describing those bacteria that reject Sia while using other sugars ("Spitters") (Figure 4) [105].

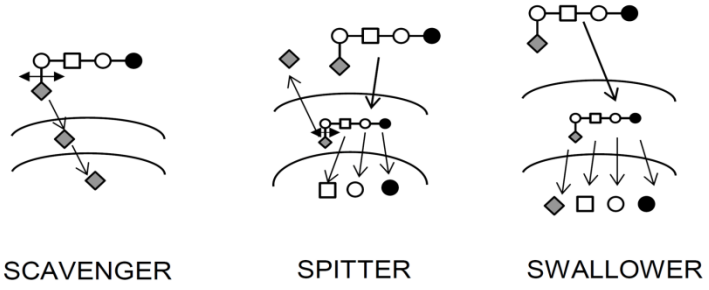

Figure 4. Types of bacteria according to the metabolism of Sia [105]. Picture shows how sialic acid or oligosaccharides pass through the outer membrane to the intermembrane space and which sugars are transported to the cytoplasm. Scavengers release Sia in the outside and transport it into the cell; Spitters transport oligosaccharides to the intermembrane space, where Sia is release and "spit" out of the bacteria, while the other sugars cross the inner membrane; Swallowers "swallow" the whole oligosaccharide to the intermembrane space, where glycosidases cut it into sugars which are transported inside the cell.

The ability to metabolize and grow on sialylated glycans released from the intestinal mucosa undoubtedly constitutes an evolutionary advantage for the bacteria, but a serious issue for the newborn who is trying to thrive and develop its own mucosal architecture. It is worth mentioning here that the fetal intestine lacks the gradient of sialylation that can be found in the adult organ and this specific glycosylation starts being built after birth [90]. A continuous supply of mucosa-like structures, free in the luminal fluid –the HMOs- is not only convenient for the bacteria, which can use them as fuel without having to release it from the cellular glycoconjugates, but also for the baby, by keeping their cellular surface "safe and intact." The benefits of a good supply of HMOs have been recently reported in a study showing that sialylated HMOs may be causally related with infant's growth, since breast milk from mothers of healthy infants in rural Malawi contains higher amount of total and sialylated HMOs when compared to breast milk from mothers of stunted infants. When colonizing germ-free young mice or gnotobiotic piglets with the microbiota of these stunted infants, the authors reported a microbiota-dependent increase of weight gain and enhanced ability to use nutrients on those animals receiving sialylated milk oligosaccharides [111].

It is broadly accepted that microbiota contributes to the maturation of newborn immune systems, both innate and adaptive. During the first years of life, the different actors of immunity are developing, i.e., immunoglobulins, NK cells, Th1 cells, and B cells are not fully mature until age 3-5y. An

appropriate bacterial colonization determines the correct development of the immune system and may reduce the risk of allergies, obesity, and diabetes later in life [112]. In a follow-up study performed in normal weighed and overweight/obese children during 7 years, it was shown that normal weight children had higher counts of bifidobacteria at early age than those becoming overweight [113].

Moreover, microbiota has been described to play a role in neurodevelopment via a bidirectional communication between brain and gut through the brain-gut-microbiota axis. A well-documented example is autism spectrum disorders (ASD), which are commonly associated with gastrointestinal issues and an abnormal microbiota composition enriched in *Clostridium*, *Bacteroides*, and *Desulfovibrio* [114].

Thus, besides modulating this microbiome influence in other systems, Sia seems to play a direct role in the development of both immune and nervous system in the infant. We will address these topics on the next sections.

SIA IN IMMUNE SYSTEM MATURATION

As noted above, human newborns are quite altricial with regards to their immune system. During the fetal stage, cross-placental maternal antibodies provide the earliest defense and later human milk is also a vehicle of passive protection, as described below. However, this defense is transitory and neonate's own immune system rapidly matures during the first months of life [115].

Newborns have an impaired adaptive response, with a clear polarization towards Th2 responses, low numbers of effector memory T-cells and a poor B-cell repertoire. This makes them more vulnerable to respiratory and intestinal infections. The innate immunity is also immature, exhibiting deficiencies in neutrophil numbers, NK cell cytotoxic capacity and IL production [116]. By contrast, inhibitory Treg cells are highly present in cord blood, which has been related to the downregulation of the fetal immune system during pregnancy in order to prevent abortion [117].

During pregnancy, maternal antibodies (IgGs) cross the placenta since the 13^{th} week of gestation, and remain in the infant circulation until 3 months of age, providing an extra protection against pathogens [118] but also hindering specific response to infection and vaccination later in life [119].

After birth, breast colostrum and milk provide the babies with additional protection against infectious diseases while contributing to maturation of the

intestine and immune system [120]. In comparison to formula-fed infants, breastfeeding has been reported to improve the mucosal and systemic immune functions in the infants, as well as promoting an anti-inflammatory environment and inducing tolerance to non-pathogenic antigens, such as food antigens or commensal bacteria [120]. Beneficial effects of breastfeeding with regards to the prevention of infections in babies have long been recognized and demonstrated [121].

Besides antibodies (IgG, IgM, and sIgA), human milk contains a variety of anti-microbial components, such as lysozyme, lactoferrin and lactoperoxidase, as well as other compounds that promote immune modulation, i.e., cytokines, nucleotides, and growth factors. Immune cells have also been found in breast milk [117, 122]. Main immune factors found in milk are listed in Table 1.

Table 1. Components of human milk with immunological capacities [117, 122]

Activity	Milk Component	
Anti-microbial	Immunoglobulins: sIgA, IgM, IgG Lactoferrin Lysozyme Lactoperoxidase Defensins Haptocorrin Complement sCD14 Gangliosides♦	Mucins♦ α-lactalbumin κ-casein♦ Lactadherin Oligosaccharides♦ Fatty acids Toll-like receptors Cells: Neutrophils, macrophages, and lymphocytes
Tolerance	Cytokines: IL-10 and TGFβ Anti-idiotipic antibodies	
Immune modulation	Macrophages T and B lymphocytes Cytokines: IL-2, IL-4, IL-5, IL-12, IFNγ LC-PUFA Bifidobacteria	Growth factors: IGF-1 Hormones: prolactin, leptin Peptides Nucleotides Gangliosides♦
Anti-inflammatory	Cytokines: IL-10, TGFβ IL-1R antagonist TNFα and IL-6 receptors Adhesion molecules Osteoprotegerin	LC-PUFA Hormones and growth factors sCD14 Lactoferrin

♦ Components containing Sia.

Immune cells express Sia-binding proteins and also the sialylated counterparts, allowing the interaction between them. Thus, leukocytes rolling and migration to inflammation sites are mainly dependent on the interaction between selectins and their ligand, Sialyl-Le^X epitope on membrane glycoproteins [123]. L-selectin expressed in leukocytes, and E-selectin from the endothelial surface, bind to the Sialyl-Le on the opposite cells allowing for rolling and arresting the circulating leukocytes at the targeted zone. In the same way, P-selectin on platelets plays an important role in the thrombus formation.

Due to their abundance on the cell surface and negative charge, Sia rule the interaction between cells, i.e., between immune cells and pathogens, or immune cells and other body cells, governing the differentiation between "self" and "non-self." Siglecs, sialic-acid binding immunoglobulin-like receptors, participate in this discrimination and contribute to the regulation of innate and adaptive immunity [124]. Siglecs are expressed on the surface of a number of immune cells, such as B cells, neutrophils, monocytes, NK cells, dendritic cells, and macrophages, but also in microglia in the brain [125].

We have previously mentioned that some bacterial species are able to decorate themselves with Sia for escaping the surveillance of Siglec-armed macrophages [126] and the complement system activation [127]. On the other hand, immune cells are able to recognize "alien" antigens on the cell surface and react against them. Neu5Gc in humans is a good example of a "non-self" molecule. Having lost the capacity for synthesizing Neu5Gc, humans are able to react against it and produce anti-NeuGc antibodies. It has been shown that human infants have anti-NeuGc IgM and IgG as soon as they are weaned and exposed to dietary Neu5Gc by 6 months of age [128].

In fact, a meat-rich diet has been postulated as the most plausible origin of the Neu5Gc that can be found in humans, being red meat and dairy products, including goat and sheep milk, the richest sources [129].

As cancer cells are able to express Neu5Gc on their surface, it has been hypothesized that this could explain the association of meat-rich diet with certain types of cancer [128]. An early exposure to Neu5Gc-rich food might increase the risk of developing malignancies later in life, but this hypothesis needs to be demonstrated.

The role of milk sialylated compounds in the prevention of infection in infants is clearer and has been described by many authors. Milk sialoconjugates serve as decoys for pathogen bacteria and bacterial enterotoxins, preventing them for binding the intestinal mucosa [130]. Also, they can promote the growth of bifidobacteria and lactobacilli [131].

Moreover, gangliosides have an effect in the activation of T cells, inducing the development of intestinal immunity by modulating the response of lymphocytes during early infancy [132].

SIA IN BRAIN AND COGNITIVE FUNCTION

If we were asked to select a single organ in the body to talk about Sia, that would be undoubtedly the brain. Not only because Sia concentration in brain is one of the highest in the body organs [133], but mainly due to the key role Neu5Ac plays in several crucial functions of brain. And despite being very abundant in other organs of non-human mammals, brain contains scarce amounts of Neu5Gc [134]. Therefore, when talking about Sia in the brain we must refer almost exclusively to Neu5Ac.

Neu5Ac content of human brain is 2-4 times higher than that of any other mammalian species [135], most of it bound to gangliosides (65%) or glycoproteins (32%) and little in free form [136]. There are some topological differences, thus Neu5Ac concentration in the left lobe is 22% higher than in the right lobe [135], being frontal cortex, cerebellar cortex, and caudate nucleus the areas with the highest concentration of gangliosides (Figure 5) [137]. Grey matter is 3 times richer in gangliosides than white matter and around 15-fold the amount present in visceral organs like liver or kidneys [138].

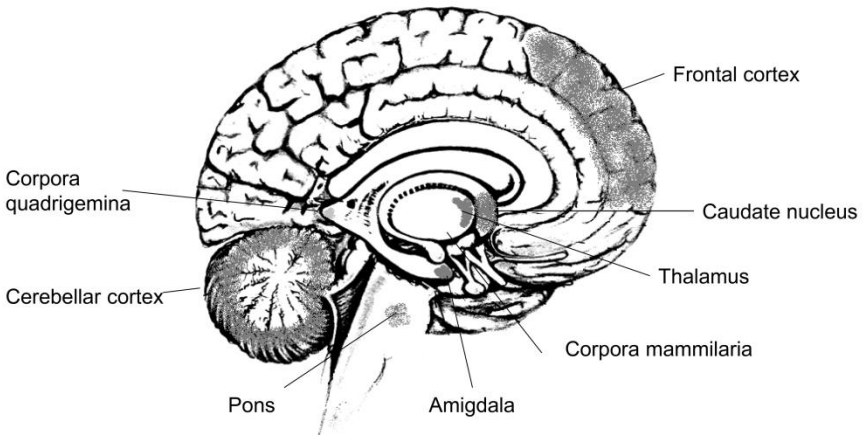

Figure 5. Brain areas with the highest Sia concentration (based on Roukema and Heijlman [135] and Kracun et al. [136]).

Concentration of gangliosides in brain rapidly increases from the 10th week of gestation until age 4-5 years; beyond this age it remains stable until age 50y when it starts decreasing [139]. A peak around the 22nd week of gestation has been reported in frontal and occipital cortices, corresponding to a period of rapid synaptogenesis [140]. In the rat -and a similar trend could be expected in humans- simplest gangliosides such as GM3 and GD3 are predominant in early embryonic stages, while GD1a and GT1b prevail during the perinatal period and GM1 and GD1b increase their expression as the brain matures [141]. Thus, GM1, GD1a, GD1b, and GT1b are the main gangliosides in adult brain. During fetal stages, 9-*O*-Acetyl-GD3 has also been found in migrating neuroblasts of the subventricular zone [142].

Complex gangliosides are required for optimal myelin formation, interaction between axon and myelin, and axonal stability. Animal studies have demonstrated that a deficiency in some of these key components due to the disruption of expression of ganglioside-synthase genes was associated to impaired cognitive skills [143] and also to alterations in the formation of neural structures [144]. Due to their location in the lipid rafts of the plasma membrane of neural cells, gangliosides are able to interact with functional protein membranes involved in neural excitability and synaptic transmission [145-147] and, by extension, in memory formation and learning. It has recently been suggested that gangliosides modulate calcium homeostasis due to its interaction with the negatively charged residues of Neu5Ac bound to gangliosides [146] and its action on Ca^{2+} dependent enzymes, ion channels (kinases), ion pumps and transport proteins (Ca^{2+}-ATPase) [148, 149]. Neurite outgrowth [150], neural cell differentiation [151], axonal sprouting and myelination [152], cell-cell communications and cell adhesion are functions currently assigned to gangliosides. Thus, brain gangliosides mediate the formation of synapses and neural circuits. Synaptic plasticity and changes on these neural circuits have been widely considered one of the major cellular mechanisms that underlie learning and memory formation. The process of long term potentiation (LTP) is regarded as one of the most important types of synaptic plasticity. In the last years, several studies have shown the effect of gangliosides in the expression of long-lasting activity-dependent synaptic modifications in response to stimuli in the CA3 hippocampal region [153].

Neu5Ac is also conjugated with glycoproteins, mainly in a polymeric form called polysialic acid (PolySia) made up of >90 units of Neu5Ac bound in α2-8. Over 95% of PolySia in the brain is linked to NCAM, but there are other polysialylated proteins, i.e., SynCAM and Neuropilin-2 [141]. PolySia is regulated during brain development via changes in the expression of the two

sialyltransferases responsible for its synthesis, ST8Sia-II and ST8Sia-IV. PolySia starts to be detected around the 3rd-4th weeks of gestation, by the time of neural tube closure and increases its expression being maximal the first week after birth, then dropping by 50% during childhood [154]. PolySia has been associated with cell migration, neurite outgrowth and fasciculation. Conversely, downregulation of PolySia induces differentiation and it is a prerequisite for optimal myelin formation. NCAM is characterized by being highly polysialylated at pre- and postnatal stages in different cerebral structures like glial cells, optic nerve, neuroblasts, postmitotics neurons, retinal ganglion cell axons or astrocytes [155].

In adults, PolySia expression has been found in areas that retain neurogenesis, such as the dentate gyrus of the hippocampus, the olfactory system, and some subventricular zones [156, 157].

One of the most important roles assigned to PolySia is the modulation of the adhesion ability of NCAM. Thus, a high rate of polysialylation implies that neural cells are not able to adhere to each other or to other cells due to the repulsion of negatively charges of Sia chains and the steric hindrance. Thus, PolySia can act as an antiadhesive glycotope blocking both homophilic and heterophilic binding [141, 146].

As described above, PolySia has functional roles enhancing neural migration, neurogenesis and synaptic connections [156]. Presynaptic cells seem to establish more synapsis with those postsynaptic neurons expressing NCAM than with those that not expressing it [152]. PolySia also promotes sprouting and branching of axons.

Different studies have highlighted that PolySia-NCAM is able to enhance LTP [156]. In fact, the complex formed by PolySia and the brain-derived neurotrophic factor (BDNF) seems to have a key role in synaptic plasticity and neurogenesis [158].

Finally, another nervous system cell adhesion molecule, the myelin-associated glycoprotein (MAG), binds to sialylated glycoconjugates like gangliosides [159]. MAG is mainly bound to two types of gangliosides, GD1a and GT1b. This glycoprotein is thought to promote the inhibition of neurite outgrowth [152] and this function is reversed when MAG is desialylated; for this reason gangliosides have been considered as functional MAG ligands [160].

Preclinical and Clinical Studies at Early Age in Relation to Cognitive Skills

As stated before, the brain rapidly grows at the embryonic and postnatal stages. Dietary Neu5Ac can be taken up during key stages in order to cover extra requirements. Several preclinical studies with rodents and piglets have demonstrated that Sia is able to cross the blood brain barrier (BBB) from the bloodstream by diffusion and also through a carrier-mediated process and thus reach the brain to be incorporated into gangliosides and PolySia [161, 162]. Therefore, a deficient food intake by the mother or the newborn could lead to a shortage of key ingredients in the formation of essential neural structures, compromising a suitable cognitive development of the baby. This is especially important in preterm infants, who are usually at an increased risk of not receiving the required amount of Sia for a proper neurodevelopment.

Different animal studies have been performed in the last decades in order to assess the role of Sia in the development of cognitive skills.

The effect of an exogenous administration of Sia at postnatal age on learning and memories abilities has been more widely studied in rodents despite the limitations of this animal model. The difficulty in starting Sia supplementation just after weaning or the stress that this intervention usually causes to rat pups are some of the disadvantages of using this model. Regarding the source of Sia administered in these studies, both conjugated forms like gangliosides [163-165] and free Neu5Ac [166, 167] have been used. The most common via of administration in these studies were intraperitoneal or subcutaneous injections while oral supplementation has seldom been used.

The most conclusive work was conducted by Morgan et al., demonstrating that an improvement on the animal behavior after weaning and at 6 month of age was associated to an increased amount of Sia in the brain of the rats that received Neu5Ac [166].

Mei and Zheng also demonstrated that intraperitoneal injections of bovine brain gangliosides in neonatal, adult or aged rats were able to improve the cognitive function [168].

In 2009, Vickers et al. confirmed that rats orally supplemented with a ganglioside-containing bovine complex milk lipid (CML) from neonatal day 10 until postnatal day 80 had better scores in novelty recognition and spatial learning tasks [169]. In this case, the behavioral effect observed cannot be exclusively assigned to gangliosides since CML also contains other milk components like phospholipids or fatty acids [153].

Supplementation to pregnant and lactating rats has also been performed to test the possible effects of Sia on the offspring behavior [170, 171]. Thus Hiratsuka et al. have recently demonstrated that feeding Neu5Ac during pregnancy and lactation in n-3 fatty acids deficient dams improved the recognition index in a novel recognition task performed in pups after weaning [170].

Piglets have been suggested as the closest animal model to human infants due to their resemblance in development and brain growth [156, 172]. Wang et al. performed a study using casein-glycomacropeptide as the source of Neu5Ac and gave it to the piglets in increasing amounts for 35 days [172]. Learning performance and memory was better in the group of animals that received the higher doses of Sia.

In 2013, Li et al. [173] also used a piglet model to test the effects of phospholipids and gangliosides on brain and cognitive development. For that purpose, a phospholipid/ganglioside supplement was given to the animals from postnatal day (PD) 8 to PD28. An improvement of spatial learning and higher weights of brain were observed in those neonatal piglets fed the supplement.

Due to ethical reasons, there are few human studies carried out to test the effects of oral Sia administration on humans cognitive performance. Most of them are indirect clues supporting that Sia may be relevant for cognitive development in infants. Hence, a study published in 2003 demonstrated that breast-fed babies showed higher amounts of Sia in brain when compared to formula-fed ones [138]. In fact, several studies have shown the influence of breastfeeding on the development of enhanced cognitive skills in children [174]. The most widely accepted hypothesis attributes this effect to certain components of human milk, such as Neu5Ac and DHA [175]. However, this hypothesis has not been demonstrated yet due to the complexity of human milk composition and the difficulties to design an ethically acceptable trial to prove it. Besides, other aspects such as maternal education, socioeconomic status, and mother-baby interaction during breastfeeding, are powerful cofounding variables to take into account.

Recently, it has been published one clinical trial led by Xu et al. that aimed at testing the effects of an oral administration of a mixture of gangliosides (GM1a, GD1a, GD1b and GT1b) on the brain functions of more than 2000 children with cerebral palsy [176]. The results showed that gangliosides administration was able to improve the neurological symptoms that feature in this disorder. However, this study has been published in Chinese and details on the design are not known to us. Therefore, similar clinical trials,

performed in other populations and more widely disseminated, might contribute to clarify the true role of Neu5Ac on cognition.

CONCLUSION

Sialic acids are "special" sugars. Due to their chemical structure, their charge, their position in the glycan chains, the variety of substitutions that constitue the family, they clearly surpass the biological capabilities of other sugars like glucose and galactose. Briefly, there are very few biological processes in which sialic acids are not involved to some extent. In this review, we have revisited sialic acid roles from the beginning of a new being through development and how sialic acids are involved in the onset of some chronic diseases such as diabetes. We have described the relationship between the host and the gut microbiota from a "sialylated" perspective and also summarized the key role of sialic acid in immunity as well as in neurogenesis and cognitive functions.

Sialic acid supply during early postnatal development has been shown to be paramount for the establishment of the neuron circuitry that ensures proper and adequate brain functions in later stages. Sialylated compounds also contribute to the establishment of the "appropriate" microbiota after birth that will predate a normal and healthy microbiota in the adult. These two are the clearest examples of the relevance of sialic acid in the early life for safeguarding health during adulthood. We can speculate that other sialic acid-mediated functions will also be influenced by the initial exposure to sialic acids, but this remains to be demonstrated. Proclaiming Sia as hero or villain is still afar.

REFERENCES

[1] Benoff S. (1997). Carbohydrates and fertilization: an overview. *Mol Hum Reprod* 3, 599-637.

[2] Ma F., Wu D., Deng L., Secrest P., Zhao J., Varki N., Lindheim S. and Gagneux P. (2012). Sialidases on Mammalian Sperm Mediate Deciduous Sialylation during Capacitation. *J Biol Chem* 287, 38073-38079.

[3] Meseguer M., Aplin J. D., Caballero-Campo P., O'Connor J. E., Martin J. C., Remohi J., Pellicer A. and Simon C. (2001). Human Endometrial Mucin MUC1 Is Up-Regulated by Progesterone and Down-Regulated In Vitro by the Human Blastocyst. *Biology of Reproduction* 64, 590-601.

[4] Achache H. and Revel A. (2006). Endometrial receptivity markers, the journey to successful embryo implantation. *Hum Reprod Update* 12, 731-746.

[5] Schwarzkopf M., Knobeloch K.-P., Rohde E., Hinderlich S., Wiechens N., Lucka L., Horak I., Reutter W. and Horstkorte R. d. (2002). Sialylation is essential for early development in mice. *Proc Natl Acad Sci U S A* 99, 5267-5270.

[6] Jones C. J. P., Aplin J. D. and Burton G. J. (2010). First Trimester Histiotrophe Shows Altered Sialylation Compared with Secretory Phase Glycoconjugates in Human Endometrium. *Placenta* 31, 576-580.

[7] Alvi M., Amer N. A. and Sumerin I. (1988). Serum 5-nucleotidase and serum sialic acid in pregnancy. *Obstetr Gynecol* 72, 171-174.

[8] Crook M., Constable S., Lumb P. and Rymer J. (1997). Elevated serum sialic acid in pregnancy. *J Clin Pathol* 50, 494-495.

[9] Warren L. and Felsenfeld H. (1962). The biosynthesis of sialic acids. *J Biol Chem* 237, 1421-1431.

[10] Rohrig C. H., Choi S. S. and Baldwin N. (2015). The Nutritional Role of Free Sialic Acid, a Human Milk Monosaccharide, and Its Application as a Functional Food Ingredient. *Crit Rev Food Sci Nutr* 26, 0.

[11] Kobayashi T., Tanaka S., Hirosawa H. and Watanabe N. (1986). Serum sialic acid in children - normal range and correlation with CRP. *Kyorin Igakkai Zasshi* 17, 403-407.

[12] Fang-Kircher S. G. (1997). Comparison of sialic acids excretion in spot urines and 24-hour-urines of children and adults. *Eur J Clin Chem Clin Biochem* 35, 47-52.

[13] Ponnio M., Alho H., Nikkari S. T., Olsson U., Rydberg U. and Sillanaukee P. (1999). Serum sialic acid in a random sample of the general population. *Clin Chem* 45, 1842-1849.

[14] Crook M., Treloar A., Haq M. and Tutt P. (1994). Serum total sialic acid and acute phase proteins in elderly subjects. *Clin Chem Lab Med* 32, 745-748.

[15] Sillanaukee P., Ponnio M. and Jaaskelainen I. P. (1999). Occurrence of sialic acids in healthy humans and different disorders. *Eur J Clin Invest* 29, 413-425.

[16] O'Kennedy R., Berns G., Moran E., Smyth H., Carroll K., Thornes R., O'Brien A., Fennelly J. and Butler M. (1991). A critical analysis of the use of sialic acid determination in the diagnosis of malignancy. *Cancer Lett* 58, 91-100.

[17] Bull C., Stoel M. A., den Brok M. H. and Adema G. J. (2014). Sialic acids sweeten a tumor's life. *Cancer Res* 74, 3199-3204.

[18] Schultz M. J., Swindall A. F., Wright J. W., Sztul E. S., Landen C. N. and Bellis S. L. (2013). ST6Gal-I sialyltransferase confers cisplatin resistance in ovarian tumor cells. *J Ovarian Res* 6, 25.

[19] Lee M., Lee H.-J., Bae S. and Lee Y.-S. (2008). Protein sialylation by sialyltransferase involves radiation resistance. *Mol Cancer Res* 6, 1316-1325.

[20] Lahdenne P. and Heikinheimo M. (2002). Clinical use of tumor markers in childhood malignancies. *Ann Med* 34, 316-323.

[21] Romppanen J., Petaja J., Heikinheimo M. and Mononen I. (1998). Total and lipid-bound serum sialic acid in children with malignancy or infections. *Anticancer Res* 18, 2793-2797.

[22] Chou H.-H., Takematsu H., Diaz S., Iber J., Nickerson E., Wright K. L., Muchmore E. A., Nelson D. L., Warren S. T. and Varki A. (1998). A mutation in human CMP-sialic acid hydroxylase occurred after the Homo-Pan divergence. *Proc Natl Acad Sci U S A* 95, 11751-11756.

[23] Malykh Y. N., Schauer R. and Shaw L. (2001). N-Glycolylneuraminic acid in human tumours. *Biochimie* 83, 623-634.

[24] Scursoni A. M., Galluzzo L., Camarero S., Lopez J., Lubieniecki F., Sampor C., Segatori V. I., Gabri M. R., Alonso D. F. and Chantada G. (2011). Detection of N-glycolyl GM3 ganglioside in neuroectodermal tumors by immunohistochemistry: an attractive vaccine target for aggressive pediatric cancer. *Clin Dev Immunol* 2011, ID245181.

[25] Waters P. J., Lewry E. and Pennock C. A. (1992). Measurement of sialic acid in serum and urine: clinical applications and limitations. *Ann Clin Biochem* 29 (Pt 6), 625-637.

[26] Pagon R. A., Adam M. P., Ardinger H. H., Bird T. D., Dolan C. R., Fong C.-T., Smith R. J., Stephens K., Adams D. and Gahl W. A. (2013). Free Sialic Acid Storage Disorders. In: RA P., MP A. and HH A., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle.

[27] Rajendiran K. S., Ananthanarayanan R. H., Satheesh S. and Rajappa M. (2014). Elevated levels of serum sialic acid and high-sensitivity C-

reactive protein: markers of systemic inflammation in patients with chronic heart failure. *Br J Biomed Sci* 71, 29-32.

[28] Knuiman M. W., Watts G. F. and Divitini M. L. (2004). Is sialic acid an independent risk factor for cardiovascular disease? A 17-year follow-up study in Busselton, Western Australia. *Ann Epidemiol* 14, 627-632.

[29] Lindberg G., Rastam L., Gullberg B. and Eklund G. A. (1992). Serum sialic acid concentration predicts both coronary heart disease and stroke mortality: multivariate analysis including 54,385 men and women during 20.5 years follow-up. *Int J Epidemiol* 21, 253-257.

[30] Khan S. A. and Rao J. R. (2014). Sialic acid a marker of diabetic complication. *Int J Pharm Biol Sci* 4, 70-77.

[31] Debry G., Bourdillat P., Lombardo Y. and Mullor J. (1968). Distribution of sialic acid in serum proteins in diabetes. *C R Soc Biol* 162, 2298-2301.

[32] Ekin S., Mert N., Gunduz H. and Meral I. (2003). Serum sialic acid levels and selected mineral status in patients with type 2 diabetes mellitus. *Biol Trace Elem Res* 94, 193-201.

[33] Gavella M., Lipovac V., Car A., Vucic M., Sokolic L. and Rakos R. (2003). Serum sialic acid in subjects with impaired glucose tolerance and in newly diagnosed type 2 diabetic patients. *Acta Diabetol* 40, 95-100.

[34] Ozben T., Nacitarhan S. and Tuncer N. (1995). Plasma and urine sialic acid in non-insulin dependent diabetes mellitus. *Ann Clin Biochem* 32 (Pt 3), 303-306.

[35] Khalili P., Sundstrom J., Jendle J., Lundin F., Jungner I. and Nilsson P. M. (2014). Sialic acid and incidence of hospitalization for diabetes and its complications during 40-years of follow-up in a large cohort: the Varmland survey. *Prim Care Diabetes* 8, 352-357.

[36] Shivananda Nayak B., Duncan H., Lalloo S., Maraj K., Matmungal V., Matthews F., Prajapati B., Samuel R. and Sylvester P. (2008). Correlation of microalbumin and sialic acid with anthropometric variables in type 2 diabetic patients with and without nephropathy. *Vasc Health Risk Manag* 4, 243-247.

[37] Prajna K., Kumar J. A., Rai S., Shetty S. K., Rai T., Shrinidhi, Begum M. and Shashikal M. D. (2013). Predictive value of serum sialic Acid in type-2 diabetes mellitus and its complication (nephropathy). *J Clin Diagn Res* 7, 2435-2437.

[38] Crook M. A., Tutt P., Simpson H. and Pickup J. C. (1993). Serum sialic acid and acute phase proteins in type 1 and type 2 diabetes mellitus. *Clin Chim Acta* 219, 131-138.

[39] Deckert T., Yokoyama H., Mathiesen E., Ronn B., Jensen T., Feldt-Rasmussen B., Borch-Johnsen K. and Jensen J. S. (1996). Cohort study of predictive value of urinary albumin excretion for atherosclerotic vascular disease in patients with insulin dependent diabetes. *BMJ* 312, 871-874.

[40] Kurtoglu S., Atabek M. E., Muhtaroglu S. and Keskin M. (2006). The association of serum total sialic acid/total protein ratio with diabetic parameters in young type 1 diabetic patients. *Acta Diabetol* 43, 1-5.

[41] Moussa M. A. A., Alsaeid M., Refai T. M. K., Abdella N., Al-Sheikh N. and Gomez J. E. (2004). Association of serum sialic acid with cardiovascular metabolic risk factors in Kuwaiti children and adolescents with type 1 diabetes. *Metabolism* 53, 638-643.

[42] Crook M., Cartwright K., Lumb P. and Worsley A. (2000). Serum sialic acid in young type-1 diabetic patients. *Diabetes Research and Clinical Practice* 47, 119-122.

[43] Verrotti A., Catino M., Di Ricco L., Casani A. and Chiarelli F. (1999). Prevention of microvascular complications in diabetic children and adolescents. *Acta Paediatr Suppl* 88, 35-38.

[44] Hubbard S. R. (2013). The Insulin Receptor: Both a Prototypical and Atypical Receptor Tyrosine Kinase. *Cold Spring Harb Perspect Biol* 5, a008946.

[45] Leto D. and Saltiel A. R. (2012). Regulation of glucose transport by insulin: traffic control of GLUT4. *Nat Rev Mol Cell Biol* 13, 383-396.

[46] Dridi L., Seyrantepe V., Fougerat A., Pan X., Bonneil E., Thibault P., Moreau A., Mitchell G. A., Heveker N., Cairo C. W., Issad T., Hinek A. and Pshezhetsky A. V. (2013). Positive regulation of insulin signaling by neuraminidase 1. *Diabetes* 62, 2338-2346.

[47] Ciaraldi T. P. (1989). Neuraminidase treatment of isolated rat adipocytes and differential regulation of basal and insulin-stimulated glucose transport. *Diabetes* 38, 951-958.

[48] Salhanick A. I. and Amatruda J. M. (1988). Role of sialic acid in insulin action and the insulin resistance of diabetes mellitus. *Am J Physiol* 255, E173-179.

[49] Haxho F., Alghamdi F., Neufeld R. and Szewczuk M. (2014). Novel Insulin Receptor-Signaling Platform. *Int J Diabetes Clin Res* 1, 005.

[50] Blaise S., Romier B., Kawecki C., Ghirardi M., Rabenoelina F., Baud S., Duca L., Maurice P., Heinz A., Schmelzer C. E., Tarpin M., Martiny L., Garbar C., Dauchez M., Debelle L. and Durlach V. (2013). Elastin-derived peptides are new regulators of insulin resistance development in mice. *Diabetes* 62, 3807-3816.

[51] Saltiel A. R. and Pessin J. E. (2003). Insulin Signaling in Microdomains of the Plasma Membrane. *Traffic* 4, 711-716.

[52] Gustavsson J., Parpal S., Karlsson M., Ramsing C., Thorn H., Borg M., Lindroth M., Peterson K. H., Magnusson K.-E. and StralfordS P. (1999). Localization of the insulin receptor in caveolae of adipocyte plasma membrane. *FASEB J* 13, 1961-1971.

[53] Tagami S., Inokuchi J.-I., Kabayama K., Yoshimura H., Kitamura F., Uemura S., Ogawa C., Ishii A., Saito M., Ohtsuka Y., Sakaue S. and Igarashi Y. (2002). Ganglioside GM3 participates in the pathological conditions of insulin resistance. *J Biol Chem* 277, 3085-3092.

[54] Kabayama K., Sato T., Kitamura F., Uemura S., Kang B. W., Igarashi Y. and Inokuchi J.-i. (2005). TNFa-induced insulin resistance in adipocytes as a membrane microdomain disorder: involvement of ganglioside GM3. *Glycobiology* 15, 21-29.

[55] Tanabe A., Matsuda M., Fukuhara A., Miyata Y., Komuro R., Shimomura I. and Tojo H. (2009). Obesity causes a shift in metabolic flow of gangliosides in adipose tissues. *Biochem Biophys Res Commun* 379, 547-552.

[56] Zhao H., Przybylska M., Wu I. H., Zhang J., Siegel C., Komarnitsky S., Yew N. S. and Cheng S. H. (2007). Inhibiting glycosphingolipid synthesis improves glycemic control and insulin sensitivity in animal models of type 2 diabetes. *Diabetes* 56, 1210-1218.

[57] Nagafuku M., Sato T., Sato S., Shimizu K., Taira T. and Inokuchi J. (2015). Control of homeostatic and pathogenic balance in adipose tissue by ganglioside GM3. *Glycobiology* 25, 303-318.

[58] Lipina C. and Hundal H. S. (2015). Ganglioside GM3 as a gatekeeper of obesity-associated insulin resistance: Evidence and mechanisms. *FEBS Lett* 589, 3221-3227.

[59] Inokuchi J.-i. (2014). GM3 and diabetes. *Glycoconjugate* J 31, 193-197.

[60] Lipina C., Nardi F., Grace H. and Hundal H. S. (2015). NEU3 sialidase as a marker of insulin sensitivity: Regulation by fatty acids. *Cell Signal* 27, 1742-1750.

[61] Inokuchi J. (2011). Inhibition of ganglioside biosynthesis as a novel therapeutic approach in insulin resistance. *Handb Exp Pharmacol* 203, 165-178.

[62] Sato T., Nihei Y., Nagafuku M., Tagami S., Chin R., Kawamura M., Miyazaki S., Suzuki M., Sugahara S.-I., Takahashi Y., Saito A., Igarashi Y. and Inokuchi J.-I. (2008). Circulating levels of ganglioside GM3 in metabolic syndrome: A pilot study. *Obes Res Clin Pract* 2, I-II.

[63] Veillon L., Go S., Matsuyama W., Suzuki A., Nagasaki M., Yatomi Y. and Inokuchi J. (2015). Identification of Ganglioside GM3 Molecular Species in Human Serum Associated with Risk Factors of Metabolic Syndrome. *PLoS One* 10, e0129645.

[64] Yamashita T., Hashiramoto A., Haluzik M., Mizukami H., Beck S., Norton A., Kono M., Tsuji S., Daniotti J. L., Werth N., Sandhoff R., Sandhoff K. and Proia R. L. (2003). Enhanced insulin sensitivity in mice lacking ganglioside GM3. *Proc Natl Acad Sci U S A* 100, 3445-3449.

[65] Ji S., Ohkawa Y., Tokizane K., Ohmi Y., Banno R., Furukawa K., Kiyama H. and Furukawa K. (2015). b-series gangliosides crucially regulate leptin secretion in adipose tissues. *Biochem Biophys Res Commun* 459, 189-195.

[66] Sasaki N., Itakura Y. and Toyoda M. (2015). Ganglioside GM1 Contributes to the State of Insulin Resistance in Senescent Human Arterial Endothelial Cells. *J Biol Chem* 290, 25475-25486.

[67] Alghamdi F., Guo M., Abdulkhalek S., Crawford N., Amith S. R. and Szewczuk M. R. (2014). A novel insulin receptor-signaling platform and its link to insulin resistance and type 2 diabetes. *Cell Signal* 26, 1355-1368.

[68] Sasaki A., Hata K., Suzuki S., Sawada M., Wada T., Yamaguchi K., Obinata M., Tateno H., Suzuki H. and Miyagi T. (2003). Overexpression of plasma membrane-assocd. sialidase attenuates insulin signaling in transgenic mice. *J Biol Chem* 278, 27896-27902.

[69] Chavez J. A., Siddique M. M., Wang S. T., Ching J., Shayman J. A. and Summers S. A. (2013). Ceramides and glucosylceramides are independent antagonists of insulin signaling. *J Biol Chem* 289, 723-734.

[70] Sacheck J. (2008). Pediatric obesity: an inflammatory condition? *JPEN J Parenter Enteral Nutr* 32, 633-637.

[71] Isganaitis E., Woo M., Ma H., Chen M., Kong W., Lytras A., Sales V., DeCoste-Lopez J., Lee K.-J., Leatherwood C., Lee D., Fitzpatrick C., Gall W., Watkins S. and Patti M.-E. (2014). Developmental programming by maternal insulin resistance: hyperinsulinemia, glucose

intolerance, and dysregulated lipid metabolism in male offspring of insulin-resistant mice. *Diabetes* 63, 688-700.

[72] Findik R. B., Yilmaz F. M., Yilmaz G., Yilmaz H. and Karakaya J. (2012). Sialic acid levels in the blood in pregnant women with impaired glucose tolerance test. *Arch Gynecol Obstet* 286, 913-916.

[73] Sriharan M., Reichelt A. J., Opperman M. L. R., Duncan B. B., Mengue S. S., Crook M. A. and Schmidt M. I. (2002). Total sialic acid and associated elements of the metabolic syndrome in women with and without previous gestational diabetes. *Diabetes Care* 25, 1331-1335.

[74] Lee C. L., Chiu P. C., Pang P. C., Chu I. K., Lee K. F., Koistinen R., Koistinen H., Seppala M., Morris H. R., Tissot B., Panico M., Dell A. and Yeung W. S. (2011). Glycosylation failure extends to glycoproteins in gestational diabetes mellitus: evidence from reduced alpha2-6 sialylation and impaired immunomodulatory activities of pregnancy-related glycodelin-A. *Diabetes* 60, 909-917.

[75] Smilowitz J. T., Totten S. M., Huang J., Grapov D., Durham H. A., Lammi-Keefe C. J., Lebrilla C. and German J. B. (2013). Human milk secretory immunoglobulin a and lactoferrin N-glycans are altered in women with gestational diabetes mellitus. *J Nutr* 143, 1906-1912.

[76] Hamady M. and Knight R. (2009). Microbial community profiling for human microbiome projects: Tools, techniques, and challenges. *Genome Res* 19, 1141-1152.

[77] Kinross J. M., von Roon A. C., Holmes E., Darzi A. and Nicholson J. K. (2008). The human gut microbiome: implications for future health care. *Curr Gastroenterol Rep* 10, 396-403.

[78] Walter J. and Ley R. (2011). The human gut microbiome: ecology and recent evolutionary changes. *Annu Rev Microbiol* 65, 411-429.

[79] Gaci N., Borrel G., Tottey W., O'Toole P. W. and Brugere J.-F. (2014). Archaea and the human gut: New beginning of an old story. *WJG* 20, 16062-16078.

[80] Salyers A. A., Palmer J. K. and Wilkins T. D. (1978). Degradation of polysaccharides by intestinal bacterial enzymes. *Am J Clin Nutr* 31, S128-S130.

[81] Santiago L. (1954). [Provision of vitamins by the intestinal flora]. *Bol Cult Inf Cons Gen Col Med Esp* 16, 37-40.

[82] Sommer F. and Backhed F. (2013). The gut microbiota -masters of host development and physiology. *Nat Rev Microbiol* 11, 227-238.

[83] Funkhouser L. J. and Bordenstein S. R. (2013). Mom Knows Best: The Universality of Maternal Microbial Transmission. *PLoS Biol* 11, e1001631.

[84] Morelli L. (2008). Postnatal development of intestinal microflora as influenced by infant nutrition. *J Nutr* 138, 1791S-1795S.

[85] Dominguez-Bello M. G., Costello E. K., Contreras M., Magris M., Hidalgo G., Fierer N. and Knight R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A* 107, 11971-11975.

[86] Albenberg L. G. and Wu G. D. (2014). Diet and the intestinal microbiome: associations, functions, and implications for health and disease. *Gastroenterology* 146, 1564-1572.

[87] Payne M. S. and Bayatibojakhi S. (2014). Exploring preterm birth as a polymicrobial disease: an overview of the uterine microbiome. *Front Immunol* 5, 595.

[88] Aagaard K., Ma J., Antony K. M., Ganu R., Petrosino J. and Versalovic J. (2014). The placenta harbors a unique microbiome. *Sci Transl Med* 6, 237-265.

[89] Jimenez E., Marin M. L., Martin R., Odriozola J. M., Olivares M., Xaus J., Fernandez L. and Rodriguez J. M. (2008). Is meconium from healthy newborns actually sterile? *Res Microbiol* 159, 187-193.

[90] Rodriguez J. M., Murphy K., Stanton C., Ross R. P., Kober O. I., Juge N., Avershina E., Rudi K., Narbad A. and Jenmalm M. C. (2015). The composition of the gut microbiota throughout life, with an emphasis on early life. *Microb Ecol Health Dis* 26, 26050.

[91] Stark P. L. and Lee A. (1982). The microbial ecology of the large bowel of breastfed and formula-fed infants during the first year of life. *J Med Microbiol* 15, 189-203.

[92] Harmsen H. J., Wildeboer-Veloo A. C., Raangs G. C., Wagendorp A. A., Klijn N., Bindels J. G. and Welling G. W. (2000). Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *J Pediatr Gastroenterol Nutr* 30, 61-67.

[93] Castanys-Muñoz E., Martin M. J. and Vazquez E. (2016). Building a benefial microbiome from birth. *Adv Nutr* 7, 323-330.

[94] Praveen P., Jordan F., Priami C. and Morine M. J. (2015). The role of breast-feeding in infant immune system: a systems perspective on the intestinal microbiome. *Microbiome* 3, 1-12.

[95] Jost T., Lacroix C., Braegger C. P., Rochat F. and Chassard C. (2014). Vertical mother-neonate transfer of maternal gut bacteria via breastfeeding. *Environ Microbiol* 16, 2891-2904.

[96] Sela D. A. and Mills D. A. (2014). The marriage of nutrigenomics with the microbiome: the case of infant-associated bifidobacteria and milk. *Am J Clin Nutr* 99, 697S-703S.

[97] Sela D. A., Chapman J., Adeuya A., Kim J. H., Chen F., Whitehead T. R., Lapidus A., Rokhsar D. S., Lebrilla C. B., German J. B., Price N. P., Richardson P. M. and Mills D. A. (2008). The genome sequence of Bifidobacterium longum subsp. infantis reveals adaptations for milk utilization within the infant microbiome. *Proc Natl Acad Sci U S A* 105, 18964-18969.

[98] Gyorgy P., Norris R. F. and Rose C. S. (1954). Bifidus factor. I. A variant of Lactobacillus bifidus requiring a special growth factor. *Arch Biochem Biophys* 48, 193-201.

[99] Xiao J. Z., Takahashi S., Nishimoto M., Odamaki T., Yaeshima T., Iwatsuki K. and Kitaoka M. (2010). Distribution of in vitro fermentation ability of lacto-N-biose I, a major building block of human milk oligosaccharides, in bifidobacterial strains. *Appl Environ Microbiol* 76, 54-59.

[100] Marcobal A., Barboza M., Sonnenburg E. D., Pudlo N., Martens E. C., Desai P., Lebrilla C. B., Weimer B. C., Mills D. A., German J. B. and Sonnenburg J. L. (2011). Bacteroides in the Infant Gut Consume Milk Oligosaccharides via Mucus-Utilization Pathways. *Cell Host Microbe* 10, 507-514.

[101] Jost T., Lacroix C., Braegger C. and Chassard C. (2015). Impact of human milk bacteria and oligosaccharides on neonatal gut microbiota establishment and gut health. *Nutr Rev* 73, 426-437.

[102] Bode L. (2015). The functional biology of human milk oligosaccharides. *Early Hum Dev* 91, 619-622.

[103] Martin-Sosa S., Martin M. J., Garcia-Pardo L. A. and Hueso P. (2003). Sialyloligosaccharides in human and bovine milk and in infant formulas: variations with the progression of lactation. *J Dairy Sci* 86, 52-59.

[104] Newburg D. S., Chen C., Cline A. and Morrow A. L. (2016). Acidic Human Milk Oligosaccharides Vary Across Populations and Over Lactation. *FASEB J* 30, 275.275.

[105] Vimr E. R., Kalivoda K. A., Deszo E. L. and Steenbergen S. M. (2004). Diversity of microbial sialic acid metabolism. *Microbiol Mol Biol Rev* 68, 132-153.

[106] Vimr E., Lichtensteiger C. and Steenbergen S. (2000). Sialic acid metabolism's dual function in Haemophilus influenzae. *Mol Microbiol* 36, 1113-1123.

[107] Plumbridge J. and Vimr E. (1999). Convergent Pathways for Utilization of the Amino Sugars N-Acetylglucosamine,N-Acetylmannosamine, and N-Acetylneuraminic Acid by Escherichia coli. *J Bacteriol* 181, 47-54.

[108] Vimr E. R. (2013). Unified theory of bacterial sialometabolism: how and why bacteria metabolize host sialic acids. *ISRN Microbiol* 2013, 816713.

[109] Almagro-Moreno S. and Boyd E. F. (2009). Insights into the evolution of sialic acid catabolism among bacteria. *BMC Evol Biol* 9, 118.

[110] Egan M., Motherway M. O. C., Ventura M. and van Sinderen D. (2014). Metabolism of sialic acid by Bifidobacterium breve UCC2003. Appl *Environ Microbiol* 80, 4414-4426.

[111] Charbonneau Mark R., O'Donnell D., Blanton Laura V., Totten Sarah M., Davis Jasmine C. C., Barratt Michael J., Cheng J., Guruge J., Talcott M., Bain James R., Muehlbauer Michael J., Ilkayeva O., Wu C., Struckmeyer T., Barile D., Mangani C., Jorgensen J., Fan Y.-m., Maleta K., Dewey Kathryn G., Ashorn P., Newgard Christopher B., Lebrilla C., Mills David A. and Gordon Jeffrey I. (2016). Sialylated Milk Oligosaccharides Promote Microbiota-Dependent Growth in Models of Infant Undernutrition. *Cell* 164, 859-871.

[112] Martin R., Nauta A., Ben Amor K., Knippels L., Knol J. and Garssen J. (2010). Early life: gut microbiota and immune development in infancy. *Benef Microbes* 1, 367-382.

[113] Kalliomaki M., Collado M. C., Salminen S. and Isolauri E. (2008). Early differences in fecal microbiota composition in children may predict overweight. *Am J Clin Nutr* 87, 534-538.

[114] O' Mahony S. M., Stilling R. M., Dinan T. G. and Cryan J. F. (2015). The microbiome and childhood diseases: Focus on brain-gut axis. *Birth Defects Res C Embryo Today* 105, 296-313.

[115] Burgio G., Ugazio A. and Notarangelo L. (1990). Immunology of the neonate. *Curr Opin Immunol* 2, 770-777.

[116] Basha S., Surendran N. and Pichichero M. (2014). Immune responses in neonates. *Expert Rev Clin Immunol* 10, 1171-1184.

[117] M'Rabet L., Vos A. P., Boehm G. n. and Garssen J. (2008). Breast-Feeding and Its Role in Early Development of the Immune System in Infants: Consequences for Health Later in Life. *J Nutr* 138, 1782S-1790S.

[118] Palmeira P., Quinello C., Silveira-Lessa A. L., Zago C. A. and Carneiro-Sampaio M. (2012). IgG placental transfer in healthy and pathological pregnancies. *Clin Dev Immunol* 2012, 985646.

[119] Battersby A. J. and Gibbons D. L. (2013). The gut mucosal immune system in the neonatal period. *Pediatr Allergy Immunol* 24, 414-421.

[120] Kelly D. and Coutts A. (2000). Early nutrition and the development of immune function in the neonate. *Proc Nutr Soc* 59, 177-185.

[121] Newburg D. S. (2009). Neonatal protection by an innate immune system of human milk consisting of oligosaccharides and glycans. *J Anim Sci* 87, 26-34.

[122] Field C. J. (2005). The immunological components of human milk and their effect on immune development in infants. *J Nutr* 135, 1-4.

[123] Varki A. (1994). Selectin ligands. *Proc Natl Acad Sci U S A* 91, 7390-7397.

[124] Paulson J. C., Macauley M. S. and Kawasaki N. (2012). Siglecs as sensors of self in innate and adaptive immune responses. *Ann N Y Acad Sci* 1253, 37-48.

[125] Macauley M. S., Crocker P. R. and Paulson J. C. (2014). Siglec-mediated regulation of immune cell function in disease. *Nat Rev Immunol* 14, 653-666.

[126] Varki A. and Gagneux P. (2012). Multifarious roles of sialic acids in immunity. *Ann N Y Acad Sci* 1253, 16-36.

[127] Bull C., den Brok M. H. and Adema G. J. (2014). Sweet escape: sialic acids in tumor immune evasion. *Biochim Biophys Acta* 1846, 238-246.

[128] Amon R., Reuven E. M., Leviatan Ben-Arye S. and Padler-Karavani V. (2014). Glycans in immune recognition and response. *Carbohydr Res* 389, 115-122.

[129] Tangvoranuntakul P., Gagneux P., Diaz S., Bardor M., Varki N., Varki A. and Muchmore E. (2003). Human uptake and incorporation of an immunogenic nonhuman dietary sialic acid. *Proc Natl Acad Sci U S A* 100, 12045-12050.

[130] Martin-Sosa S., Martin M. J. and Hueso P. (2002). The sialylated fraction of milk oligosaccharides is partially responsible for binding to enterotoxigenic and uropathogenic Escherichia coli human strains. *J Nutr* 132, 3067-3072.

[131] Nakano T., Sugawara M. and Kawakami H. (2001). Sialic acid in human milk: composition and functions. *Acta Paediatr Taiwan* 42, 11-17.

[132] Rueda R. (2007). The role of dietary gangliosides on immunity and the prevention of infection. *Br J Nutr* 98, S68-S73.

[133] Svennerholm L. (1958). Quantitative estimation of sialic acids. 3. An anion exchange resin method. *Acta Chem Scand* 12, 547-554.

[134] Davies L. R. and Varki A. (2015). Why Is N-glycolylneuraminic acid rare in the vertebrate brain? *SialoGlyco Chemistry and Biology I: Springer*; p 31-54.

[135] Wang B., Miller J. B., McNeil Y. and McVeagh P. (1998). Sialic acid concentration of brain gangliosides: variation among eight mammalian species. *Comp Biochem Physiol A* 119A, 435-439.

[136] Brunngraber E. G. (1972). Biochemistry, function, and neuropathology of the glycoproteins in brain tissue. *Adv Exp Med Biol* 32, 109-133.

[137] Roukema P. A. and Heijlman J. (1970). The regional distribution of sialoglycoproteins, gangliosides and sialidase in bovine brain. *J Neurochem* 17, 773-780.

[138] Wang B., McVeagh P., Petocz P. and Brand-miller J. (2003). Brain ganglioside and glycoprotein sialic acid in breastfed compared with formula-fed infants. *Am J Clin Nutr* 78, 1024-1029.

[139] Svennerholm L., Bostrom K., Fredman P., Mansson J. E., Rosengren B. and Rynmark B. M. (1989). Human brain gangliosides: developmental changes from early fetal stage to advanced age. *Biochim Biophys Acta* 1005, 109-117.

[140] Kracun I., Rosner H., Drnovsek V., Heffer-Lauc M., Cosovic C. and Lauc G. (1991). Human brain gangliosides in development, aging and disease. *Int J Dev Biol* 35, 289-295.

[141] Schnaar R. L., Gerardy-Schahn R. and Hildebrandt H. (2014). Sialic acids in the brain: gangliosides and polysialic acid in nervous system development, stability, disease, and regeneration. *Physiol Rev* 94, 461-518.

[142] Miyakoshi L., Todeschini A., Mendez-Otero R. and Hedin-Pereira C. (2012). Role of the 9-O-acetyl GD3 in subventricular zone neuroblast migration. *Mol Cell Neurosci* 49, 240-249.

[143] Yu R. K., Tsai Y. T. and Ariga T. (2012). Functional roles of gangliosides in neurodevelopment: an overview of recent advances. *Neurochem Res* 37, 1230-1244.

[144] Wang X.-Q., Lee S., Wilson H., Seeger M., Iordanov H., Gatla N., Whittington A., Bach D., Lu J.-y. and Paller A. S. (2014). Ganglioside GM3 Depletion Reverses Impaired Wound Healing in Diabetic Mice by Activating IGF-1 and Insulin Receptors. *J Invest Dermatol* 134, 1446-1455.

[145] Nowycky M. C., Wu G. and Ledeen R. W. (2014). Glycobiology of ion transport in the nervous system. *Adv Neurobiol* 9, 321-342.

[146] Wang B. (2012). Molecular mechanism underlying sialic acid as an essential nutrient for brain development and cognition. *Adv Nutr* 3, 465S-472S.

[147] McJarrow P., Schnell N., Jumpsen J. and Clandinin T. (2009). Influence of dietary gangliosides on neonatal brain development. *Nutr Rev* 67, 451-463.

[148] Jiang L., Bechtel M. D., Bean J. L., Winefield R., Williams T. D., Zaidi A., Michaelis E. K. and Michaelis M. L. (2014). Effects of gangliosides on the activity of the plasma membrane Ca2+-ATPase. *Biochim Biophys Acta* 1838, 1255-1265.

[149] Ledeen R. W. and Wu G. (2002). Ganglioside function in calcium homeostasis and signaling. *Neurochem Res* 27, 637-647.

[150] Ferreira A., Busciglio J., Landa C. and Caceres A. (1990). Ganglioside-enhanced neurite growth: evidence for a selective induction of high-molecular-weight MAP-2. *J Neurosci* 10, 293-302.

[151] Nakatsuji Y. and Miller R. H. (2001). Selective cell-cycle arrest and induction of apoptosis in proliferating neural cells by ganglioside GM3. *Exp Neurol* 168, 290-299.

[152] Yang L. J., Zeller C. B., Shaper N. L., Kiso M., Hasegawa A., Shapiro R. E. and Schnaar R. L. (1996). Gangliosides are neuronal ligands for myelin-associated glycoprotein. *Proc Natl Acad Sci U S A* 93, 814-818.

[153] Palmano K., Rowan A., Guillermo R., Guan J. and McJarrow P. (2015). The role of gangliosides in neurodevelopment. *Nutrients* 7, 3891-3913.

[154] Hildebrandt H. and Dityatev A. (2013). Polysialic acid in brain development and synaptic plasticity. *SialoGlyco Chemistry and Biology I: Springer;* p 55-96.

[155] Bartsch U., Kirchhoff F. and Schachner M. (1990). Highly sialylated N-CAM is expressed in adult mouse optic nerve and retina. *J Neurocytol* 19, 550-565.

[156] Wang B. (2009). Sialic acid is an essential nutrient for brain development and cognition. *Annu Rev Nutr* 29, 177-222.

[157] Eriksson P. S., Perfilieva E., Bjork-Eriksson T., Alborn A.-M., Nordborg C., Peterson D. A. and Gage F. H. (1998). Neurogenesis in the adult human hippocampus. *Nature Med* 4, 1313-1317.

[158] Muller D., Djebbara-Hannas Z., Jourdain P., Vutskits L., Durbec P., Rougon G. and Kiss J. Z. (2000). Brain-derived neurotrophic factor restores long-term potentiation in polysialic acid-neural cell adhesion

molecule-deficient hippocampus. *Proc Natl Acad Sci U S A* 97, 4315-4320.

[159] Collins B. E., Yang L. J., Mukhopadhyay G., Filbin M. T., Kiso M., Hasegawa A. and Schnaar R. L. (1997). Sialic acid specificity of myelin-associated glycoprotein binding. *J Biol Chem* 272, 1248-1255.

[160] Vyas A. A. and Schnaar R. L. (2001). Brain gangliosides: functional ligands for myelin stability and the control of nerve regeneration. *Biochimie* 83, 677-682.

[161] Wang B., Downing J. A., Petocz P., Brand-Miller J. and Bryden W. L. (2007). Metabolic fate of intravenously administered N-acetylneuraminic acid-6-14C in newborn piglets. *Asia Pac J Clin Nutr* 16, 110-115.

[162] Nohle U., Beau J. M. and Schauer R. (1982). Uptake, metabolism and excretion of orally and intravenously administered, double-labeled N-glycoloylneuraminic acid and single-labeled 2-deoxy-2,3-dehydro-N-acetylneuraminic acid in mouse and rat. *Eur J Biochem* 126, 543-548.

[163] Wainwright P. E., Lomanowska A. M., McCutcheon D., Park E. J., Clandinin M. T. and Ramanujam K. S. (2007). Postnatal dietary supplementation with either gangliosides or choline: effects on spatial short-term memory in artificially-reared rats. *Nutr Neurosci* 10, 67-77.

[164] Silva R. H., Bergamo M. and Frussa-Filho R. (2000). Effects of neonatal ganglioside GM1 administration on memory in adult and old rats. *Pharmacol Toxicol* 87, 120-125.

[165] Fagioli S., Rossi-Arnaud C. and Ammassari-Teule M. (1991). Open field behaviours and spatial learning performance in C57BL/6 mice: early stage effects of chronic GM1 ganglioside administration. *Psychopharmacology* (Berl) 105, 209-212.

[166] Morgan B. L. and Winick M. (1980). Effects of administration of N-acetylneuraminic acid (NANA) on brain NANA content and behavior. *J Nutr* 110, 416-424.

[167] Carlson S. E. and House S. G. (1986). Oral and intraperitoneal administration of N-acetylneuraminic acid: effect on rat cerebral and cerebellar N-acetylneuraminic acid. *J Nutr* 116, 881-886.

[168] Mei Z. T. and Zheng J. Z. (1993). Effects of exogenous gangliosides on learning and memory in rats. *Jpn J Physiol* 43 Suppl 1, S295-299.

[169] Vickers M. H., Guan J., Gustavsson M., Krageloh C. U., Breier B. H., Davison M., Fong B., Norris C., McJarrow P. and Hodgkinson S. C. (2009). Supplementation with a mixture of complex lipids derived from

milk to growing rats results in improvements in parameters related to growth and cognition. *Nutr Res* 29, 426-435.

[170] Hiratsuka S., Honma H., Saitoh Y., Yasuda Y. and Yokogoshi H. (2013). Effects of dietary sialic acid in n-3 fatty acid-deficient dams during pregnancy and lactation on the learning abilities of their pups after weaning. *J Nutr Sci Vitaminol* 59, 136-143.

[171] Gustavsson M., Hodgkinson S. C., Fong B., Norris C., Guan J., Krageloh C. U., Breier B. H., Davison M., McJarrow P. and Vickers M. H. (2010). Maternal supplementation with a complex milk lipid mixture during pregnancy and lactation alters neonatal brain lipid composition but lacks effect on cognitive function in rats. *Nutr Res* 30, 279-289.

[172] Wang B., Yu B., Karim M., Hu H., Sun Y., McGreevy P., Petocz P., Held S. and Brand-Miller J. (2007). Dietary sialic acid supplementation improves learning and memory in piglets. *Am J Clin Nutr* 85, 561-569.

[173] Liu H., Radlowski E. C., Conrad M. S., Li Y., Dilger R. N. and Johnson R. W. (2014). Early supplementation of phospholipids and gangliosides affects brain and cognitive development in neonatal piglets. *J Nutr* 144, 1903-1909.

[174] Kafouri S., Kramer M., Leonard G., Perron M., Pike B., Richer L., Toro R., Veillette S., Pausova Z. and Paus T. (2013). Breastfeeding and brain structure in adolescence. *Int J Epidemiol* 42, 150-159.

[175] Wang B., Brand-Miller J., McVeagh P. and Petocz P. (2001). Concentration and distribution of sialic acid in human milk and infant formulas. *Am J Clin Nutr* 74, 510-515.

[176] Xu X. Z. Z., T.C. (2005). Effect of ganglioside in repairing the neurological function of children with cerebral palsy:analysis of the curative efficacy in 2230 cases. *Chin J Clin Rehab* 9, 122-123.