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General introduction

Preterm birth occurs in 8% of all births. Birth before a gestational age (GA) of 32 weeks, or very preterm birth, occurs in 1-2% of all births in the Netherlands.[1] The number of infants that are born prematurely is increasing, and due to improved care in the last decades the survival rates of preterm infants has increased as well. In the US, factors relating to these increasing rates include an increase in maternal age and the use of reproductive assisted technology, but it is difficult to discern whether this can be generalised to other countries.[2] Directly after birth, preterm infants face several challenges; immature lungs can result in respiratory problems, brain haemorrhage can result in neurological deficits and gastro-intestinal immaturity can result in feeding problems, immune disbalance and necrotising enterocollitis (NEC) which alone or in combination can result in life threatening illness.[3,4] Preterm infants have an immature immune system, making them vulnerable for infections during the neonatal period and maybe even later in life. These short-term consequences of preterm birth necessitate intensive care treatment in highly specialised neonatal intensive care units. Although this care is essential for the survival of the infant, the intensive care treatment itself interferes with the body's defence against infection by interruption of mucosal and epithelial barriers, making the infant even more vulnerable for infections.[5] Furthermore, the microbiome of the gut plays a pivotal role in the maturation of the mucosal and even systemic immune system. A well-developed microbiome is essential to keep the immune system healthy and balanced. For this reason, delayed intestinal colonisation might be a key player in the maturation and education of the immune system in preterm infants. However it should be realised that immune maturation and/or development is influenced by immune activation as well. Potential sources of activation include infections or inflammation or even by controlled activation such as vaccinations.

An important part of the immune system is localized in the mucosal tissue of the intestine.[6] For this reason dietary interventions have the potential to access "immune" tissue, potentially leading to a change in function. The mucosal microbiome is an essential part of the first line of defence of the immune system by blocking pathogens but also as a stimulus of several immune factors. Dietary interventions that interfere with the microbiome might play a significant role in immune development. However unique dietary interventions may influence the immune system via other routes as well without involvement of the microbiome. Dietary intervention that can influence the immune system both locally in the gut and systemically is sometimes labelled with the term "immunonutrition".

Due to improvement in intensive care treatment for preterm infants over the years, the number of preterm infants surviving the initial phase of treatment and facing the long term consequences of their prematurity and this treatment is on the rise too.[7] In 2007, 52% of the extremely preterm infants (GA <27 weeks) in the Netherlands survived the neonatal period. However the downside is that almost 29% showed severe disability at 2 years of age.[8] Therefore it is of major importance to improve long-term health outcome such as neurodevelopmental and immune outcomes.

Immune system in preterm infants

The immune system is a complex network of cells, tissues, and organs that cooperate to defend the body against attacks by "foreign" invaders so called danger signals. Broadly speaking, the immune system consists of three levels of defense. The first level is the physical/chemical barrier of the skin and mucosal surfaces including an individual's unique microbiota. The second level of defense is the innate immune system, followed by a third level; the adaptive immune system. The innate immune system is the first active response to invading pathogens, classically described as a rapid, short-lived, nonspecific response, which was recently described by Levy et al. as a trainable component of the immune system.[9,10]

The adaptive immune system is the most specific part of the immune system and improves by every subsequent encounter with that particular infectious agent.[11] Every invasion will activate memory, thereby further developing the specific but also non-specific immune response. All functional levels of the immune system interact with and influence each other in a bidirectional manner. It should be realised that in all newborns the adaptive immune system still has to develop specificity and memory, which will be completed in the early childhood years and used as rationale for vaccinations.[12]

The immune system is a highly complex system, and its maturation begins in-utero and develops throughout the first years of life.[9] All infants are born with an immature immune system. Preterm infants do have a disbalanced immune system leading to higher susceptibilities for immune related disorders such as infections and inflammation. It should be realised that many factors influence the development of the immune system such as the neonatal

intensive care medical treatment including the use of antibiotics and anti-inflammatory drugs, opportunistic infections, inflammation, genes, environment and the mother.

Various components of the innate immune system are immature after birth, which reduces the capacity to respond to infections in an effective way. Among these components are deficiencies in non-cellular responses to pathogens leading to reduced phagocytosis,[13] and a relative monocytopenia and neutropenia.[14] In particular, the immune signaling and the link between innate and adaptive immune system is diminished in preterm infants.[15] The adaptive immune system shows differences in cytokine responses and B- and T-cell memory.[11]

Cytokines

Cytokines are a key part of the immune system, which are vital for both immune cell signalling and function. They play a pivotal role in both the susceptibility but also immune-defence towards almost all danger signals, including infections. In utero, fetal cytokine responses are skewed towards a Th2 and anti-inflammatory phenotype, to prevent mother-fetal rejection.[11] In cord blood, pro-inflammatory cytokines such as Interleukin (IL)-2, IL-4, Il-5, IL-6, IL-8, IL-10 and tumor necrosis factor α (TNF-α) are higher in preterm infants compared to term infants, suggesting that a pro-inflammatory milieu might be involved in preterm deliveries. For some pro-inflammatory cytokines there are conflicting results regarding higher or even lower levels in cord blood of preterm infants compared to term infants.[16,17] Levels of pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, TNF-α and IFN-γ are higher in cord blood of preterm infants who were prenatally exposed to infection.[18] IL-10, IL-18, IFN-γ, TGF-β and TNF-α might differentiate between infants with fungal and bacterial sepsis, later in the neonatal period.[18] Preterm infants with systemic infections have lower levels of IL-17 and higher levels of IL-6 and IL-8. The highest blood cytokines levels in preterm infants are found on the day of birth.[19] Levels of cytokines at birth or in the neonatal period (until 21 days post delivery) in preterm infants are possible predictors of bronchopulmonary dysplasia, white matter brain damage and cerebral palsy.[20–24] It is unknown whether specific cytokine levels are different in preterm infants with a high susceptibility for infections compared to preterm infants with a low susceptibility for infections.

IgG antibodies

Another protective mechanism in infants is IgG antibody isotypes. Every infant is born with detectable maternal Immunoglobulin G (IgG), transported over the placenta (transplacental transport) by an active, receptor mediated process during pregnancy. Maternal IgG can protect infants against vaccine preventable diseases until they receive their first vaccinations and are in that respect extremely important.[25] Previous studies showed that the degree of transplacental transport of IgG is dependent on the duration of gestation.[26–28] During the first trimester, a small amount of IgG is transported to the fetus.[29,30] Whereas the fetal IgG

is approximately 10% of the maternal concentration at 17-22 weeks of gestation, it rises to 50% at 28-32 weeks of gestation as determined by chordocentesis.[25,31] The increase in fetal IgG concentration between 29 and 41 weeks of gestation is two times as high as between 17 and 28 weeks of gestation, therefore term infants at birth often haven higher IgG concentrations than their mothers.[30] In general, higher IgG concentrations at birth are associated with longer protection. Diminished maternally derived IgG mediated protection poses the preterm infants earlier at risk for vaccine preventable infections.

Immunisation guidelines within the Dutch National Immunisation Programme

During the first months after birth, infants are protected against vaccine-preventable infectious diseases by maternal antibodies only.[32-34] The primary series of Diphtheria-Tetanus-acellular Pertussis-inactivated Polio-Haemophilus influenza type B- vaccines (DTaP-IPV/Hib) was administered in combination with pneumococcal immunisations at the age of 2, 3 and 4 months in the Netherlands during the study period. These vaccines are followed by a booster vaccination at 11 months. The first immunisations against measles, mumps, rubella (MMR) and meningococcal C (MenC) are only administered at 14 months (Table 1.1).

Preterm infants are recommended to receive their immunisations according to the standard guidelines of the Dutch National Immunisation Programme (NIP), not corrected for gestational age or birth weight.[35] However, there is scientifically no solid reason for this approach.

Table 1.1. Dutch National Immunisation Programme during study period[35]
Vaccinations for preterm infants are administered according to the uncorrected age.

Age	Injection 1	Injection 2
At birth	HBV[a]	
2 months	DTaP-IPV/Hib (± HBV)[b]	Pneumococcal[e]
3 months	DTaP-IPV/Hib (± HBV)[b]	Pneumococcal[c,e]
4 months	DTaP-IPV/Hib (± HBV)[b]	Pneumococcal[e]
11 months	DTaP-IPV/Hib (± HBV)[b]	Pneumococcal[e]
14 months	MMR	Pneumococcal[e]
4 years	DTaP-IPV	
9 years	DT-IPV	MMR
12 years[d]	HPV	

a Only for infants of whom the mother tested positive for HBsAg.
b Only for infants of whom the mother tested positive for HBsAg until 2011, every infant receives HBV since 2011.
c Deleted from the current schedule (2015)
d Only for girls; three doses at 0 days, 1 month, 6 months.
e 7-valent pneumococcal vaccine in 2006-2010, 10-valent since 2010.

For preterm infants with a relatively immature immune system and lower transplacental transport, the period between total weaning of maternal antibodies and the first protection by vaccination is longer than in term infants, posing them at higher risk for vaccine-preventable diseases. As preterm infants have lower levels of maternal antibodies at birth and loss of these antibodies is similar to term born infants, the postnatal age at which preterm infants are deprived of protection by these antibodies is much younger then in term born infants. Even in term infants after one vaccination, the response to pertussis vaccination gives only (partial) short term protection, therefore the period that preterm infants are unprotected is even longer.[36] Furthermore, preterm infants are known to be commonly vaccinated later than term infants, especially very low birth weight infants.[37,38] The response to vaccinations in preterm infants may be diminished compared to term infants, due to the immaturity of the preterm immune system.

Microbiota and the immune system

The gut plays a major role in the development and function of the immune system. Normal initial colonisation of the gut is an important event in the adjustment of the newborn to the extrauterine environment.[39] The gut microbiota are key to develop a healthy barrier function and mucosal integrity. In addition it plays a pivotal role in the development of the mucosal and systemic immune system.[40] The composition of the gut microbiota is very complex and is important for human health.[41]

Immunomodulatory commensal bacteria are known to control for example the Th17:Treg balance and can affect the homeostasis of effector immune cells locally in the lamina propria, which will dictate the nature of the host immune response during an environmental challenge, such as intestinal infection.[42] The bacteria can influence the immune system by secretion of unique metabolic factors, of which short chain fatty acids (SCFA) are an example. These factors modify host or other bacterial matrix to generate new molecules with unique signalling properties or by secreting enzymes that modify host or other microbial metabolic pathways.[42]

It is known that preterm infants have a delayed colonisation of the gut, especially with beneficial bacteria, like certain bifidobacteria, which have a favourable influence on the immune system.[43] Several factors play a role in the delayed colonisation of the gut in preterm infants. They have a higher likelihood to be delivered by cesarean section, bypassing the maternal vaginal and colonic microbiota, have less exposure to maternal skin flora due to intensive care treatment, receive less maternal human milk and receive more antibiotics and other medication influencing the microbiota.[40,44] This results in a less differentiated microbiota, a relatively unstable profile and delayed acquisition of adult profiles.[40,43]

As a consequence macrophages in the lamina propria of the intestine produce proinflammatory cytokines, which are important for effective immune responses, but are also known to induce epithelial injury.[45]

Feeding in preterm infants

Enteral feeding is known to influence the colonisation of the gut, and the immature immune system of preterm infants.[46] Feeding, especially in the window of the first month of life, therefore offers a unique opportunity to influence the development of the immune system. Human milk contains a wide range of health promoting elements including nucleotides, fatty acids, immunoglobulins, cytokines, immune cells, lysozyme, lactoferrin, microbes or microbial fragments, growth factors and human milk oligosaccharides.[47] Over 200 types of these human milk oligosaccharides have been identified of which 80% are neutral and 20% of these oligosaccharides are acidic.[48] The many beneficial elements in human milk are making this the preferred type of feeding in all infants. However maternal human milk is not always available for preterm infants because of late or unsuccessful initiation of lactation in mothers of preterm infants.[49]

Neutral and acidic oligosaccharides

Formula feeding is used when maternal human milk is not available. Non-human milk oligosaccharides are developed to mimic the function of human milk oligosaccharides. Short chain galacto-oligosaccharides and long chain fructo-oligosaccharides are neutral oligosaccharides. In the past, research has mainly focussed on supplementation of neutral oligosaccharides, which showed advantageous effects in term and preterm infants.[50,51] These effects included stimulation of a bifidogenic intestinal flora,[52,53] reduction of pathogens in the intestine,[54] production of beneficial fermentation metabolites such as immunomodulatory short chain fatty acids (SCFA),[51] a decrease of stool pH,[54] improved intestinal physiology (stool characteristics, motility),[55] and less infection and atopy.[56,57] Acidic oligosaccharides (AOS) can be derived from carrots with their active component pectin. The combination of acidic and neutral oligosaccharides may have several advantageous effects [51,58–60] as stimulation of a bifidogenic intestinal flora, preventing adhesion of pathogens to epithelial tissues and stimulation of the immune system, as shown by the response to immunisations. Therefore in this study, a combination of 80% neutral oligosaccharides (scGOS/lcFOS) and 20% acidic oligosacccarides (pAOS) has been used.

Previous results of intervention with neutral and acidic oligosaccharides in preterm infants

Our group previously reported on the effects of supplementation of scGOS/lcFOS/pAOS in the first 30 days of life. If scGOS/lcFOS/pAOS is given in sufficient amounts, it decreases the incidence of endogenous infections (see also chapter 6).[61] Furthermore, the intestinal colonisation as reflected by the total bacterial count was increased at day 14 after birth; however no differences were found in percentages of health-promoting bacteria.[62] scGOS/lcFOS/pAOS supplementation decreased the stool pH and stool viscosity in preterm infants.[63] However, scGOS/lcFOS/pAOS supplementation did not affect feeding tolerance, nor

faecal IL-8 and faecal calprotectin levels and intestinal permeability in the first week of life in preterm infants.[61,64,65]

Follow up until the age of 1 year for allergic and infectious diseases showed no effect of scGOS/lcFOS/pAOS on allergic and infectious diseases in the first year of life.[66]

Gut-brain-immune axis

Recently the influence of the gut on the brain is extensively reviewed.[67] Brain development is not complete at birth, and is particularly underdeveloped in immature preterm infants. Reviews showed that the intestinal microbiota modulates different brain development pathways.[68–70] This connection is a two direction pathway, as both the brain can influence intestinal development and the intestinal microbiota can influence the development of the brain. One of the pathways by which microbiota influence the brain development is by gastrointestinal inflammation. In the setting of gastrointestinal inflammation, intestinal epithelial cells become more permeable and enterochromaffin cells, lymphocytes, mast cells and dendritic cells secrete a plethora of neuroimmune factors that can stimulate enteric nerves. Preterm infants are known to have an impaired gut barrier function and therefore an increased intestinal permeability.[64,71]

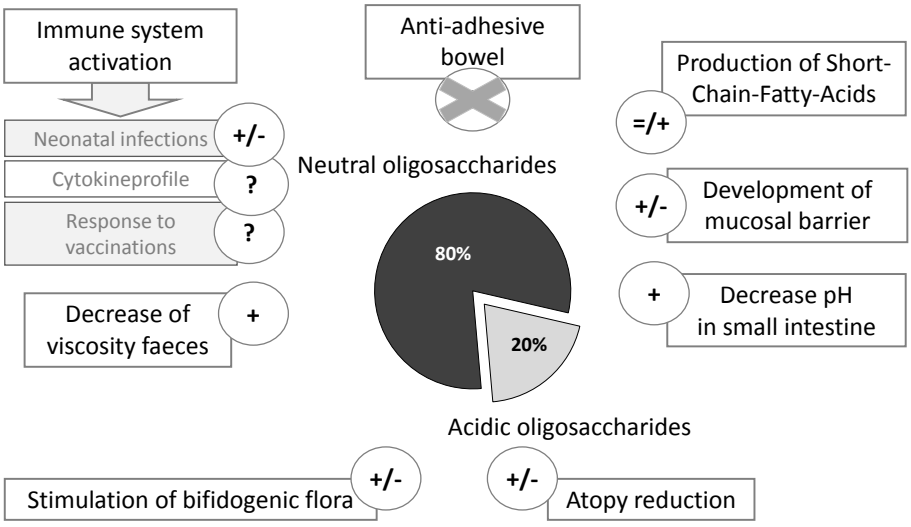

Figure 1.1. Functions of non-human neutral and acidic oligosaccharides in the CARROT study. Previous measured effects are showed, anti-adhesive bowel is not measured in this study.

Preterm infants are also more vulnerable to (gastrointestinal) infection, making them susceptible to altered development of brain pathways. In addition to the stimulation of enteric nerves, cytokines and lymphocytes will enter the circulation due to increased intestinal permeability. Subsequently, lymphocytes and serum cytokines (IL-1β, IL-6, IFN-γ, TNF-α) can pass the blood-brain barrier and some cytokines (IL-1β, TNF-α) can even bind to brain endothelial cells inducing an immune response in the brain.[72] The immune response in the brain can consist of an increased number of lymphocytes and cytokines. Additionally, cytokines can be produced by neuroglia, resulting in changed neuronal homeostasis.[73] Influences from the gut may also influence memory formation, emotional arousal and affective behaviors.[73,74]