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General discussion

The incidence of infants born preterm has increased to more than 10% worldwide. In the Netherlands, this is reflected by an incidence of very preterm infants (gestational age <32 weeks) of 0.63% in 1983, and 1,5% in 2013. Improvement of perinatal care has resulted in increased survival rates of preterm infants, thereby shifting the focus of research towards improving the quality of life in preterm infants and limiting the long-term complications of preterm birth. The aim of this thesis was to evaluate the development of preterm infants with a strong focus on immune development. We have analyzed "immune" protection by transplacental derived antibodies at preterm birth. Additionally we followed these infants and evaluated their vaccination responsiveness later in life. Vaccination responsiveness is an accepted and validated tool to analyze immune health. IgG antibody levels, recognized as one of the key biomarkers for immune development, have been analyzed along with other biomarkers for immune responsiveness, such as cytokine levels.

An intervention study was performed in order to investigate the relevance of dietary intervention aimed at improving general health outcomes as well as neurodevelopmental outcomes for these preterm infants.

Infection and inflammation play an important role in both short and long term health outcomes for preterm infants. Therefore the role of the immature immune system and transplacentair acquired antibodies to prevent infections was studied in this thesis. The potential of a specific dietary intervention to support the immature immune system and as a consequence lower incidence and/or severity of infections was investigated in detail.

As most of the transplacental transport of IgG takes place during the third trimester of pregnancy, the preterm infant lacks a significant part of this transport and thus suffers from too low levels of maternal derived protective antibodies. In this thesis, we confirmed that preterm infants indeed have lower ratios of transplacental transported IgG antibodies

as compared to term infants with a GA>36 weeks. This results in lower antibody levels in preterm infants at birth as compared to term infants. Several factors influence the risk of preterm infants to develop an infection with a vaccine preventable disease. Prenatally maternal antibody titres are the main predictors of antibody levels of the infant at birth.[90,98,298] Influencing maternal antibody titres might therefore be an opportunity to increase the preterm infants IgG antibody level. Most women at childbearing age will be vaccinated during childhood. Vaccinations are known to induce lower vaccine specific antibody levels compared to natural infection,[150,151] and due to the high vaccine coverage during the last decades, antibody levels will in general not be boosted by exposure to the wild-type virus.[152] The type of vaccine used to vaccinate the mothers influences the transplacental transport ratios during the pregnancy as well. Polysaccharide vaccines showed significantly lower transplacental transport ratios than protein vaccines. This difference can be explained by the different IgG subclasses (isotypes) produced after administration of these vaccines. The transplacental transfer of IgG2 isotypes, which is the main subclass induced by polysaccharides in the capsule from bacteria like Hib, MenC and pneumococci is known to be less effective compared with other IgG subclasses.[77,78] Pneumococcal, Hib and MenC antibodies are mainly IgG2 subclass antibodies. IgG2 transplacental transport is less effective compared with other IgG subclasses like IgG1 and IgG3 which are the main antibodies produced after DTaP vaccination.[77,78] However, active transport of antibodies against polysaccharides is possible as Munoz et al[122] have shown for pneumococci after maternal vaccination.

To attain higher antibody concentrations in infants at birth, a booster of the maternal antibody levels by a vaccination during pregnancy may be an option. In several countries (among others, the US, Great Britain and Belgium), it is policy to vaccinate every mother in the third trimester with DTaP.[214] For the Netherlands, there are conflicting results about cost-effectiveness of revaccinating mothers during the third trimester. Studies on maternal vaccination[107-111] were only performed in pregnancies ≥30 weeks of gestation and the antibody concentrations were only higher in the infant at birth when the vaccination was given at least 2 weeks before delivery.[112] Therefore the most vulnerable group of infants, very preterm infants with a GA< 32 weeks, will probably barely benefit from these vaccinations. Maternal vaccination during pregnancy is not an option for MMR, as this vaccine contains live attenuated viruses.[163,164]

After birth, preterm and term infants are protected by their maternal antibodies and through herd immunity before the first immunization. Lower maternal derived IgG concentrations indicate shorter duration of protection. The current immunization schedules in the Netherlands are divided into immunizations that start at 2 months of age (DTaP-Hib-pneumococci) and > 12 months of age (MenC and MMR) irrespective of gestational age. The low antibody concentrations of protective IgG against the different vaccine preventable diseases in infants most certainly predict a susceptibility of the infants for these types of infections and, there-

fore, the majority of infants is unprotected against pertussis before the first vaccination.[123]. For measles, mumps, rubella and varicella we showed that percentages of levels of protection were not different between preterm and term infants at birth, but that the GMC's of preterm infants were significantly lower compared to term infants (2.5-fold for measles, 1.8-fold for mumps, 1.7-fold for rubella and 2.4-fold for varicella).[44] This implies that preterm infants will lose their protective maternal antibodies approximately one month earlier than term infants. At 5 months, 99% of the preterm infants did not have protection to measles, mumps or rubella and therefore will be unprotected until the first vaccination that is at 14 months in the Netherlands.

Besides the maternal antibodies, herd immunity is an important protection to vaccine preventable diseases for all infants before their first immunizations. Herd immunity relies on high vaccination rates in the overall population preventing for outbreaks of these vaccine-preventable diseases. In recent years however, the number of parents that decide, for various reasons,[299] not to vaccinate their children, is on the rise. In the Netherlands, epidemic episodes of pertussis occur every 3-5 years.[124] In 2011–2012, a worldwide increase in pertussis notifications was observed, which is a threat for all infants who are not yet fully vaccinated, especially for the most vulnerable infants such as preterm infants.[140,141,149] New protection strategies against pertussis are therefore investigated. Besides the previous mentioned maternal vaccination, vaccination of close contacts shortly after birth of the infant (cocooning) can be used as a protective method.[300,301] However this is only effective in preterm infants when all caregivers and close contacts are vaccinated, which is known to be challenging and remains mostly incomplete.[301]

For measles it is known that infants suffer from the highest age-specific measles-incidence in Europe.[140,141,149] In addition, infants are more vulnerable for measles-related complications like otitis media, pneumonia, corneal ulcer and subacute sclerosering panencephalitis.[141,142] Although one of the goals of the WHO was to eliminate measles in 2010, measles infections still occur in infants and children in Europe.[143,144]

Small outbreaks of mumps and rubella still arise in the Netherlands, but have not affected infants in the last years.[131,145] Close monitoring for vaccine preventable diseases remains absolutely necessary.

Another option to reduce the risk of vaccine preventable diseases in preterm infants would be to start vaccination at an earlier age in life, especially for MMR. However, seroconversion is shown to be lower after early vaccination due to the presence of maternal antibodies and immaturity of the immune system.[156] Circulating maternal antibodies inhibit vaccine responses in infants by formation of immune complexes and epitope specific masking of B cell determinants. However T cell priming is not prevented by maternal antibodies.[157] Inhibition by maternal antibodies cannot be the only reason for low seroconversion, as both preterm and term infants lose their antibodies well before vaccination. Immaturity of the immune system is another reason for low serocoversion[158,159] which might be even more

important for inadequate responses to both Hib and MMR vaccinations in preterm infants when administered before 12 months of age. During the last outbreak of measles, an additional vaccination at 6 months was used in both the US and the Netherlands, followed by the routine immunization schedule at 14 months and 4-6 years. However the evidence for a positive effect in preterm infants is scarce.[160] [161] If there is no direct risk of contact with the viruses, longer protection by maternal antibodies in combination with the first vaccination after 12 months (as previously recommended) seems to be the preferred way to protect preterm infants.

Supplementation of neutral and acidic oligosaccharides

In particular during the first year of life preterm infants have an increased risk of nosocomial and vaccine preventable infections. Preterm birth leads to an immunological cascade resulting in an unbalanced pro-inflammatory/anti-inflammatory cytokine profile which makes preterm infants more susceptible to infections.[34] Especially the first month of life seems to represent a highly critical window in the maturation of the immune system, partly explaining why during this month most infections occur.[194] Their need for intensive care treatment and stay at NICUs causes that most serious infections in preterm infants during the first weeks of life are due to blood stream infections caused by coagulase-negative staphylococci [166], followed by infections due to endogenous bacteria, mostly originating from the gastrointestinal tract. We showed that enteral supplementation of a prebiotic mixture did not significantly decrease the risk of serious infectious morbidity in preterm infants, although there was a trend towards a lower incidence of serious infectious morbidity, especially for infections with endogenous bacteriae. The incidence of endogenous infections and ≥2 serious infections episodes was significantly lower in infants receiving a mean supplementation dose of at least 50% of the maximum supplementation dose of 1.5 g/kg/day during the study period (per-protocol analysis). This suggests that enteral supplementation of a unique prebiotic mixture consisting of neutral and acidic oligosaccharides of preterm infants may reduce the risk to serious infectious morbidity, especially endogenous infections, if given in sufficient amounts.

However it might also be hypothesized that the effect of the prebiotic mixture will become even more significant later in life, so after the peak of the first nosocomial infection. This is in part supported by our fermentation study with different types of fibers. The influence of the fibers in the stool of preterm infants became more pronounced at the third and fourth time point, after > 14 days of life, while most infections in the preterm infants of our study occurred during the second week of life.

The dose-depending effect is in line with the study of Moro et al.,[178] showing a dose dependent effect of neutral oligosaccharides on the growth of *Bifidobacteriae* and *Lactobacillus* in the intestinal tract of term born infants. However, most serious infections occur within the first 2 weeks of life, a period in which the very preterm infant is most often not

yet fully enterally fed due to limited feeding tolerance. In addition enteral feeding is only slowly increased to limit the risk of development of necrotizing enterocolitis. In this period of increasing enteral feeding, the full dose of the prebiotic mixture can not be given due to the effect of the prebiotic mixture on the osmolarity of the feeding. Therefore, in the period when most serious infections occur the dose of the prebiotic mixture may be too low to exert its full protective effect.

It has been hypothesised that each type of immune response, either pro- or anti-inflammatory, develops its own regulatory mechanisms aimed at a healthy immune balance. For a balanced immune response different types regulatory T-cell populations are essential. Overall, the analyses show that after the neonatal period serum cytokine levels decrease until the age of about one year. Causes for preterm delivery such as chorioamnionitis are known to cause significant changes in cytokine profiles.[18] In this thesis, we show that higher levels of TNF-α have been measured at birth and day 7 after a pregnancy complicated by chorioamnionitis. After birth the cytokine profiles of all measured cytokines in preterm infants are increased during or shortly after infections, which is immunologically explainable and needed to mount a proper immune response directed against the pathogens. In our study the use of antibiotics increased cytokine levels, such as IL-6 and IL-10, during the neonatal period. This is in line with the findings of Sood et al. who described higher cytokine levels during sepsis, especially in bacterial sepsis.[195] Enteral supplementation of scGOS/lcFOS/pAOS decreased cytokine levels in preterm infants at day 7 after birth, indicating a temporarily anti-inflammatory effect, which is in line with many different preclinical studies using laboratory animals.[219,233] This effect mimics the effect of breast milk feeding as shown in term infants.[196] After day 7 this effect disappears. At day 14, most cytokine levels are not different in the scGOS/lcFOS/pAOS vs. the placebo group. However IL-1b and IL-4 are still affected, possibly indicating an anti-allergic stimulus. An explanation for this finding might be that the oligosaccharides were supplemented both to breast milk feeding and preterm formula feeding. We hypothesise that since preterm infants are seldom exclusively breast fed during the first 10 days of life, the beneficial effects of human milk oligosaccharides may be exceeded by the oligosaccharides in the scGOS/lcFOS/pAOS mixture. Another possible explanation for this finding may be the fact that the peak incidence of nosocomial sepsis in preterm infants occurs one to two weeks after birth. The influence of infections is larger than the influence of the scGOS/lcFOS/pAOS on the cytokine levels and therefore abolishes this effect at day 14. This might be explained by the direct influence of infections on cytokine levels. However we hypothesise that the use of broad-spectrum antibiotics during infections in preterm infants affects cytokine levels as well by an opposite effect of neutral and acidic oligosaccharides.[43,201]

Preterm infants face multiple problems after birth, which can be explained by the immaturity of most organs, including the gastrointestinal tract. After birth, the gastrointestinal tract has to adapt and develop very quickly, including its own unique microbiome. The

gastrointestinal tract has been recognized as an important immune organ as well and is for this reason linked to many different diseases. Over the last years many publications have described the importance of a healthy microbiota presence in the developing gut. The gastrointestinal tract hosts 10^{12} bacteria per gram of content in adults and for this reason the gastrointestinal tract has an immense and very important microbiota compartment. This microbiota is constantly producing and releasing potent immunomodulatory molecules, which significantly affect the development of a healthy and balanced immune system.[183] The gastrointestinal microbiome has to develop after birth and it is well described that this process is very different and often delayed in preterm infants.[43] The gut is a highly interesting target organ in preterm infants since its immune cells and the microbiome are the first point of contact for enteral feeding including unique immunomodulating ingredients such as prebiotics. However the development of the immature gastrointestinal tract limits the amount of enteral feeding which is tolerated in the first days to weeks after birth.

Influence of scGOS/lcFOS/pAOS on vaccinations in preterm infants

In a recent study by Oh et al, Toll-like receptor 5 (TLR5)-deficient mice are unable to respond to the bacterial innate immune stimulus flagellin, as well as in germ-free mice or mice that have undergone sustained treatment with antibiotics. [182] This finding supports the hypothesis of this thesis that modulation of the microbiome in the gastrointestinal tract might have systemic “immune”-effects that could influence the response to vaccinations or infectious triggers. The TLR5-deficient mice showed a diminished response to vaccinations after antibiotics.[182] Preterm infants combine an immature gastrointestinal tract and delayed intestinal colonization with an immature immune system. Additionally broad-spectrum antibiotics during the first weeks of life affect the development as well. It is known that antibiotics can influence the gut microbiome for more than 6 months after administration,[302] which is within the period that preterm infants receive their primary series of vaccinations.

The infants in this randomized controlled trial received the prebiotic mixture during the first 30 days of life, as the first month after birth is an important period in the evolution of among others the immune system. Singhal et al. emphasize that early nutrition gives long-term health effects and the first month of life seem to be a critical window to esteem that influence.[194] By improvement of the immune system with the prebiotic mixture, we expected an elevated vaccine response on immunizations. In addition, Benyacoub et al. showed in a murine model that fructo-oligosaccharides (FOS) supplemented to the enteral feeding increased their vaccine response to an oral Salmonella vaccine. Mice receiving FOS during one week before immunization showed higher IgG levels against Salmonella than mice in the control group.[218]

Preterm infants during this study were vaccinated with DTaP-IPV-Hib and pneumococci at 2, 3, 4 and 11 months of uncorrected age, similar to term infants. We did not find any differences in the antibody responses to the DTaP-Hib vaccinations between the scGOS/

lcFOS/pAOS supplemented and the placebo group, except for the responses of pneumococci vaccinations. Whether T cell vaccine specific responses are affected, as shown in animal influenza vaccination studies[233], was not analyzed in this study.

In contradiction with our hypothesis, we found lower levels for pneumoccoal antibodies at 5 months of age in the prebiotic supplemented group. Preterm infants without oligosaccharide supplementation to the enteral feeding have a higher pneumococcal antibody response to the PCV serotypes 4, 6B, 9V, 19F and 23F than term infants at 5 months of age. Enteral supplementation of scGOS/lcFOS/pAOS mixture during days 3-30 of life in preterm infants leads to a diminished antibody response to pneumococcal polysaccharides in preterm infants, but importantly they showed similar anti-polysaccharide IgG antibody levels as term infants after the primary series immunizations. The lower IgG levels to the 7 vaccine serotypes in the scGOS/lcFOS/pAOS supplemented infants at 5 months might reflect either a direct or indirect (by modulation of the microbiota) immunomodulatory effect of the neutral and acidic oligosaccharides in the first months of life.

Although we had expected that antibody responses after pneumococcal vaccination would be lower in preterm infants than term infants, the antibody responses of preterm infants in the placebo group were actually higher at 5 months than those in healthy term infants in a Dutch serosurveillance population-based study in the same time period. Preterm infants supplemented with scGOS/lcFOS/pAOS however, had similar antibody responses to the term infants of the Dutch population study. This may indicate that the preterm infants in general show a more abundant vaccination response to PCV7 at 5 months of age and that supplementation with scGOS/lcFOS/pAOS attenuates the increased vaccine response. An excessive vaccine response may be explained by the immature immune system of preterm infants, which may lead to a less balanced reaction on conjugate vaccinations. Van den Biggelaar et al.[243] previously showed that in term neonates the response to a neonatal PCV-7 vaccine (given at birth) is more Th-2 skewed, but becomes more Th-1 skewed as the vaccination is administered later in life. The immature immune system of preterm infants is known to be more Th-2 skewed and might therefore react more abundantly to this Th-2 biased vaccine, even at 5 months of age.[244] In a murine model, enteral scGOS/lcFOS/pAOS induces immune modulation with a prominent role of CD25+ Tregs towards enhanced Th1 vaccine responsiveness, possibly by suppression of the Th2 vaccine responsiveness.[200] This would make the response to a Th-2 biased vaccine of the more Th2- skewed preterm infants receiving scGOS/lcFOS/pAOS more comparable to the more Th1 skewed term infants. There were no differences between the scGOS/lcFOS/pAOS and placebo group for the combined Diphtheria, Tetanus and Pertussis vaccine responses at 5 months of age, although there was a trend towards lower levels in scGOS/lcFOS/pAOS group for Haemophilus influenze type B vaccine responses, also a conjugate vaccine.[247] We also did not detect any differences in vaccine responses between the Dutch population group and the preterm infants in our study, except for lower levels of the pertussis antigen Peractin in preterm infants.

Hib was the only vaccination that showed low protection in the preterm infants. In total, 27% of the preterm infants vaccinated following the Dutch national immunization schedule did not reach the international assigned protective antibody level for Hib after their booster vaccination at 12 months. This means that they are still vulnerable for Hib infection after completion of the immunization schedule. This might be related to the disbalanced immune system of preterm infants, but additionally the delayed intestinal colonization or the use of broad-spectrum antibiotics (as described in mice) during the neonatal period might play a role in the very different response to the Hib vaccine in preterm infants.

Brain development

Preterm birth disrupts the fetal brain development and long-term effects on neurodevelopment as a consequence of prematurity. We used this cohort of preterm infants to investigate the effect of the nutritional intervention on neurodevelopmental outcomes as well. Additionally, a possible association between the observed effects on cytokine levels and brain development was studied. The trend towards a decrease in endogenous infections and a decrease in cytokine levels IL-1β, IFN-γ and TNF-α in preterm infants after supplementation of scGOS/lcFOS/pAOS may have an influence on the brain development, as many studies have shown that there is a connection between the gut and the brain in the neonatal period.[61,67,237] However the changes in cytokine levels between the two groups might not have been evident enough to induce substantial changes in neurodevelopment as assessed by the BSID test at 24 months of corrected age. However, we showed association between both bifidobacteria and cytokine levels during the neonatal period and the BSID at 24 months of corrected age.

Our in vitro study with an improved combination of scGOS/lcFOS without pAOS showed that microbiota in preterm infants stools produced low SCFA during the first weeks of life, but this production increased after approximately two weeks, when dietary fibers, especially scGOS/lcFOS, show the ability to increase the production of cumulative SCFA after fermentation in vitro. The microbiota in the stools showed low potential to generate the butyrate or propionate during the first weeks of life, with scGOS/lcFOS only increasing butyrate after 4 weeks. A Cesarean section and longer initial antibiotic course diminishes the production of SCFA. Our data suggests that feeding supplementation within first 2-3 weeks of delivery in premature infant and specially those born by cesarean section might not provide the desired outcome such as increased production of SCFA. Furthermore, as little is known about the SCFA receptor maturation in (preterm) infants and their sensitivity for the different SCFA during the neonatal period. It was suggested by several scientists that SCFA receptors might have an influence in preterm birth, as fetal membrane expression of GPR43 was significantly higher in women delivering prematurely with evidence of infection.[295] Furthermore it is suggested that SCFA receptors might offer therapeutic opportunities later in life.[296] More investigation in the maturation of SCFA receptors is therefore needed.

Changes in method

The studies performed in this thesis have several methodological considerations. The main methodological consideration to discuss is the sample size of the vaccination studies. Sample size calculation was based on the primary outcome of the main trial; the number of infections during the first 80 days of life.[61] The sample size of 113 infants in this trial was calculated on the basis of the differences in incidences in infectious morbidity (76% and 50%, respectively) in a previous study. Based on these figures and a 2-tailed $\alpha = 0.05$, $\beta = 0.20$, and a sample size of $2 \times [2 \times 7.85 \times 0.63 (0.37)]/(0.26)^2 = 2 \times 54$ infants were calculated. This resulted in a sample size at follow-up that was relatively small and made the vaccination study susceptible to a type II error. This type of error is known as a "false negative" error, indicating a test of poor sensitivity.

An optimal supplementation dose of 1.5 g/kg/day was reached at a median postnatal age of 11 days due to restriction of the maximal osmolarity of the enteral feeding. Therefore, infants may not have received an optimal dose of scGOS/lcFOS/pAOS-supplementation to reach the maximal effect at postnatal day 7 or 14. This might have had an influence on the incidence of infections, changes in cytokine profile and the overall influence of the scGOS/lcFOS/pAOS on the immune system, covering an increased effect on the immune response. Furthermore, the effect on vaccination responses might be more pronounced if the period of oligosaccharides supplementation was extended to include the period in which preterm infants receive their first vaccinations.

Antibiotics and the scGOS/lcFOS/pAOS mixture have counteractive effects on the intestinal microbiota as the scGOS/lcFOS/pAOS mixture is developed to stimulate the normal development of the intestinal microbiota[62], especially growth of so-called health promoting bacteria such as bifidobacteria, whereas antibiotics inhibit the normal development of the intestinal microbiota, by reducing the number of bacteria, including numbers of bifidobacteria.[225] Antibiotics also alter the composition of the intestinal microbiota and might affect the T cell function.[303] The use of broad-spectrum antibiotics causes alteration of the composition of the microbiota that can persist for a longer period following a short course of antibiotics.[226,227] We hypothesize that frequent use of broad-spectrum antibiotics in our NICU, which was the case in 75% of our study-infants, could have reduced the effect of the scGOS/lcFOS/pAOS mixture on the intestinal microbiota. This might reduce the bacterial growth during the first 30 days of life as described by Westerbeek et al,[62] thereby masking a possible effect of growth of Bifidobacteria bacteria or other health promoting bacteria.

For the best comparison between the preterm and term infants in the vaccination study, we should have included term infants with the same follow up as the preterm infants. We used a Dutch population study, conducted in the same year, as comparison, but these infants did not receive the same booster vaccination.

Prymula et al. described that prophylactic administration of paracetamol impaired the immunization response to pneumococcal vaccination.[228] In our study, we did not record

information on the number of infants who received prophylactically paracetamol before the immunization but there is no indication that this might be different between the groups and prophylactically paracetamol use is usually low in the Netherlands.[249]

Conclusions

Transplacental transport of maternal IgG against measles, rubella, varicella-zoster, mumps, *Haemophilus influenza* type B, diphtheria, pertussis and tetanus is lower in preterm infants than in term infants. IgG levels of preterm infants decrease earlier in life below the protective cut-off level than term infants, which puts preterm infants at higher risk for vaccine-preventable diseases even at an earlier age.

After birth, preterm infants have a delayed intestinal colonisation. Together with an immature gastrointestinal tract and immature immune system, this increases the risk in preterm infants to develop serious infectious morbidity during the first weeks of life. Enteral supplementation of a prebiotic mixture consisting of neutral and acidic oligosaccharides does not significantly decrease the risk of serious infectious morbidity in preterm infants. However there was a trend towards a lower incidence of serious infectious morbidity, which was significant for infections with endogenous bacteria, if the supplementation was given in sufficient amount. The neutral and acidic oligosaccharides decrease most cytokine levels at day 7, indicating a temporary anti-inflammatory effect. Most cytokines decrease after the neonatal period.

Short term enteral supplementation of a prebiotic mixture consisting of scGOS/lcFOS/pAOS during day 3-30 of life did not improve the immunization response in preterm infants to the DTaP-Hib vaccination, but showed differences in the response to the pneumococcal vaccinations. Preterm infants supplemented with scGOS/lcFOS/pAOS showed lower pneumococcal antibody levels compared to the placebo group, but these levels were more comparable to the levels found in term infants.

The Hib vaccination was the only vaccination that showed low protection in preterm infants in our study. After the booster vaccine at 12 months 27% of the infants did not reach the long-term protective level for Hib.

Long-term effects of the scGOS/lcFOS/pAOS supplementation were not shown in the neurodevelopmental outcomes of preterm infants at 24 months of corrected age. Cytokine levels and bifidobacterial counts in the neonatal period might be associated with later neurodevelopmental outcomes in preterm infants, but our cohort was not large enough to draw firm conclusions. Further research to the influence of immunomodulatory feeding on brain development is therefore needed.

Future

Immunomodulatory nutrition is an interesting research area especially for very vulnerable infants such as very preterm infants. Unfortunately, although the type of feeding for very

preterm infants can be well controlled, the amount of nutrition that these infants actually receive is rather low, especially during the first weeks of postnatal life. The fermentation part of the study clearly showed that in preterm infants the effect of immunomodulatory dietary fibers on SCFA is limited during the first and very important week(s) to modulate and balance the immune system. Thus in order to induce more pronounced immunomodulatory effects more aggressive methods to improve the intestinal microbiome might be necessary to establish an environment that is optimal for maturation of the immune system in preterm infants. A mixture consisting of prebiotics and probiotics (combined called symbiotics) is likely to have a more marked effect on the immune system than prebiotics alone. Probiotics alone have shown to reduce NEC in preterm infants,[240] they induce an increased vaccination response in term infants.[241] Studies in healthy term infants supplemented with a probiotic during the first 6 months of life support this finding[242] .

In adults fecal microbiome transplants are used in cases where microbiome dysbiosis is the cause of disease, especially for recurrent Clostridium difficile infection.[304] Fecal microbiome transplant, transplanting the microbiome from a donor to a patient, is one of the most dramatic ways of changing the microbiota and might therefore be effective. Furthermore, it raises the question what kind of donor should be used. Maternal microbiome is used previously in a 13-month-old infant,[305] but as it is known that the microbiome is both age and diet-dependent[306] and a mother who just delivered prematurely, which might be related to inflammation,[307] other donors such as healthy term breast-fed infants, should be considered. However, in preterm infants a fast introduction of microbiome might also overwhelm the immature gastrointestinal tract, thereby inducing a negative spiral of inflammation and negative effects on the immature gastrointestinal tract.

With increasing numbers of parents that refuse certain vaccinations, the risk for infections with vaccine preventable diseases rises for all infants that did not yet receive their first vaccinations, especially for preterm infants that are born with lower transplacental maternal antibody levels. Methods to increase the maternal antibody levels even before pregnancy by boostering all woman of childbearing age or prevention of infection after birth by methods like cocooning offer opportunities to increase the safety of these very preterm infants.

Our cohort showed that responses to Hib are lower in preterm infants and suggest the need for further investigation of the possibilities of an extra booster, especially for polysaccharide vaccines These vaccines seem to request more of an immature immune system than protein vaccins.