Summary

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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 key biomarkers for immune development have been analyzed, along with other biomarkers for immune responsiveness such as cytokine levels.

The primary aim of this thesis is to evaluate the development of preterm infants focusing on the immune system. Secondary aim was to investigate the relevance of a dietary intervention aimed at improving general health outcomes as well as neurodevelopmental outcomes for these preterm infants. A double-blind randomised controlled trial in 113 preterm infants on the effect of enteral supplementation of a prebiotic mixture of neutral and acidic oligosaccharides was performed to elucidate the role of neutral and acidic oligosaccharides on modulation of the immune response, during the neonatal period and later in life. The background and aims of this thesis are further addressed in Chapter 1 and 2.

In **chapter 3** a review of the literature on transplacental transport of IgG in preterm infants is presented. Preterm infants have lower antibody concentrations for all antibodies compared with term infants. Maternal and infants antibody concentrations showed a strong correlation.

In **chapter 4** we showed lower placental transfer ratios of antibodies against Diphtheria, Tetanus, Pertussis, Haemofilus influenza and Meningitis C antigens in preterm infants compared with term infants and found that polysaccharide-vaccine–specific antibodies showed lower transplacental transport ratios than protein-vaccine–specific antibodies. Maternal concentrations are the most important determinants of infant antibody concentrations against vaccine-preventable diseases.

In chapter 5 we showed that due to lower placental transfer ratios of antibodies against measles, mumps, rubella and varicella preterm infants had 1.7- 2.5 times lower geometric mean concentrations at birth compared to term infants. Therefore preterm infants benefit to a lesser extent from maternal antibodies against MMRV than term infants, posing them even earlier at risk for infectious diseases caused by these still circulating viruses.

Part II of this thesis describes the influence of enteral supplementation of a prebiotic mixture of neutral and acidic oligosaccharides during the neonatal period on both immune and neurodevelopmental outcomes. In **chaper 6** we determined the effect of enteral supplementation of a prebiotic mixture of neutral and acidic oligosaccharides on serious infectious morbidity, feeding tolerance and short-term outcome in preterm infants. Enteral supplementation of neutral and acidic oligosaccharides decreases the incidence of endogenous infections, if given in sufficient amounts. Neutral and acidic oligosaccharides did not show an effect on feeding tolerance (time to full enteral feeding) or the incidence of necrotising enterocolitis. Enteral supplementation of neutral and acidic oligosaccharides decreased the incidence of mild BPD.

Chapter 7 describes the cytokine profiles over the first year of life in preterm infants. During the neonatal period cytokine levels increased, followed by a decrease at 5 months and 12 months. Enteral supplementation of the neutral and acidic oligosaccharides decreased cytokine levels at day 7 but not at day 14, indicating a temporarily anti-inflammatory effect. In the neonatal period, serious infection before sampling increased all cytokine levels.

The response to vaccinations in preterm infants is described in **chapters 8 and 9**.Enteral supplementation of neutral and acidic oligosaccharides during the neonatal period does not improve the immunization response to DTaP-Hib, but does alter the response to pneumococcal vaccins. We showed a regulatory effect of neutral and acidic oligosaccharides on the response to conjugated polysaccharide pneumococcal vaccines with normalization of the enhanced responses in preterm infants towards levels similar to healthy term infants. Hib vaccins did show lower protection rates in preterm infants, with 27% of the infants not reaching long-term protection after the booster vaccination at 11 months.

By influencing both the gastrointestinal tract and the immune system, neutral and acidic oligosaccharides might influence the gut-brain-immune axis. As prematurity influences brain development the long-term effects of the oligosaccharides on neurodevelopmental outcomes were investigated. In **chapter 10** we showed that supplementation of neutral and acidic oligosaccharides did not affect the neurodevelopmental outcomes of the BSID at 24

months corrected age. Infections, lower bifidobacteria counts and higher serum cytokine levels during the neonatal period are associated with lower neurodevelopmental outcomes at 24 months of age indicating the relevance of microbiome and immune responses in neurodevelopmental processes.

An in-vitro fermentation study of preterm infants feces is described in **chapter 11**. The microbiota in preterm infants feces generates low amounts of SCFAs during the first weeks of life, but increases after approximately two weeks, when dietary fibers, especially scGOS/lcFOS, increase the generation of mainly acetate after fermentation. C-section delays the ability of intestinal microbiota to produce SCFA even in the presence of dietary fibers.

Finally, in chapter 12, a general discussion with a reflection on the findings described in this thesis and suggestions for the future are presented.