Aims and design

Adapted from BMC Pediatr 2008;8(46):46.
Aims and design

Aims of this thesis

The primary aim of this thesis is to evaluate the development of preterm infants focusing on the immune system. This begins with analysing protection by transplacental derived antibodies at preterm, following up these infants to evaluate their vaccination responsiveness later in life, seen and accepted as a highly relevant readout for immune health. IgG antibody levels were key biomarkers for immune development analyzes although other biomarkers such as cytokine levels were analyzed and investigated as well. An intervention study was performed in order to investigate the relevance of dietary intervention aimed at health and neurodevelopmental improvement for preterm infants.

Study design of the intervention study

Between May 2007 and December 2008, infants born with a gestational age (GA) <32 weeks and/or birth weight (BW) <1500 g were eligible for participation in the CARROT study. Exclusion criteria were: infants with GA >34 weeks, major congenital or chromosomal anomalies, death <48 hours after birth and transfer to another hospital <48 hours after birth. The infants were randomly allocated to treatment <48 hours after birth to receive either an enteral supplement of 80% scGOS/IcFOS and 20% pAOS or placebo powder (maltodextrin). The randomisation code was broken after data analysis was performed. Supplementation of the scGOS/IcFOS/pAOS or placebo was administered in increasing doses between days 3 and 30 of life according to body weight (1.5g/kg/day), and was supplemented to either own maternal breast milk or preterm formula. For each infant in the study, a feeding schedule was proposed based on birth weight (BW) and the guidelines as mentioned previously. Per 100 mL, the preterm formula (Nenatal Start Nutricia Netherlands) provided 80 kcal, 2.4 g protein (casein-whey protein ratio 40:60), 4.4g fat and 7.8g carbohydrate. The preterm
Chapter 2

114 infants included in the Carrot study

- 55 infants in scGOS/IcFOS/pAOS group
- 59 infants in placebo group

1 exclusion: syndrome

Birth

- 53 blood samples (53/55)
- 56 blood samples (56/58)

Day 3: start of supplementation

- 1 infant †

Day 7

- 52 blood samples (52/55)
- 55 blood samples (55/57)

Day 14

- 49 blood samples (49/55)
- 53 blood samples (53/56)

6 infants †

Day 30: end of supplementation

2, 3, 4 months
DTaP-Hib-Pneu vaccination

5 months

- 41 blood samples (41/49)
- 47 blood samples (47/50)

11 months
DTaP-Hib-Pneu booster vaccination

12 months

- 42 blood samples (42/49)
- 41 blood samples (41/49)

Figure 2.1. Carrot study
formula did not contain any oligosaccharides. When infants were transferred to another hospital before the end of the study, the protocol was continued under supervision of the principal investigator.\textsuperscript{61}

Serum samples were collected within 48 hours after birth (birth), at postnatal day 7 (day 7), day 14 (day 14), 4-6 weeks after the last vaccination of the primary series of DTaP-IPV-Hib and pneumococcal vaccinations at 2,3,4 months (5 months) and 4-8 weeks after the DTaP-IPV-Hib and pneumococcal booster vaccination at 11 months (12 months). The study design is summarized in figure 2.1. Serum samples of all time points were analyzed for levels of IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-17, IFN-γ and TNF-α. Serum samples of birth, 5 months and 12 months were analyzed for IgG levels of DTaP-Hib and pneumococcal serotypes. All cytokine and IgG levels were measured using a fluorescent bead-based multiplex immuno assay (MIA) (Luminex xMAP technology).

Outline of this thesis

Part I (chapter 3-5) of this thesis focuses on the transplacental transport of IgG antibodies in preterm and term infants.

In chapter 3, a review of the literature on the transplacental transport of IgG antibodies of diphtheria, tetanus, pertussis, measles, rubella, varicella-zoster, mumps, Haemophilus influenza type B, and polio to preterm infants gives an overview of the available limited results before the start of this study.

To further investigate the effect of prematurity on transplacental transport, a study on transplacental transport of IgG in very preterm and term infants was conducted. In chapter 4 and 5, we present two studies on the transport of IgG antibodies specific for pertussis, diphtheria, tetanus, haemophilus influenza type b, and Neisseria meningitidis serogroup C and the transport of IgG antibodies specific for measles, mumps, rubella and varicella zoster.

Part II (chapter 6-10) focuses on the effects of dietary intervention with a prebiotic mixture. In this intervention study, the influence of a unique prebiotic mixture (i.e. scGOS/lcFOS/pAOS) on the immune system was analyzed. The outline of part II is summarized in Figure 2.2.

In chapter 6, we describe a double-blind randomised controlled trial that is designed to determine the effect of enteral supplementation of neutral and acidic oligosaccharides on serious infectious morbidity in preterm infants during the neonatal period.

In chapter 7, we focus on the cytokine levels in preterm infants during the first 12 months of life. Additionally, we show the effect of scGOS/lcFOS/pAOS on cytokine levels in preterm infants.

In chapter 8 and 9, we present the results of the follow up study to vaccine responses in preterm infants after supplementation of scGOS/lcFOS/pAOS. We describe in two parts the effect of scGOS/lcFOS/pAOS on Diphtheria-Tetanus-acellular Pertussis-Haemophilus

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Influenza type B vaccination and on the heptavalent pneumococcal vaccination after the primary vaccination and the booster vaccination.

In chapter 10, we focus on the effect of different potential immunomodulatory nutritional interventions and their effect on stool fermentation.

In chapter 11, the long-term outcomes of the preterm infants in the study, assessed with the Bayley Scales of Infant and Toddler Development, are presented. The influences of supplementation of scGOS/lcFOS/pAOS, microbiome development and cytokine levels during the neonatal period are correlated to outcomes of neurodevelopment at the age of 24 months.

In the final chapter, a general discussion (chapter 12), all results are linked and evaluated in a broader perspective.