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RESEARCH ARTICLES

The content of docosahexaenoic acid in the suckling and the weaning diet beneficially modulates the ability of immune cells to response to stimuli☆

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Abstract

The objective of the study was to isolate the effect of feeding a diet supplemented with docosahexaenoic acid (DHA) during the suckling and/or the weaning period on immune system development and function in offspring. Dams were randomized to one of two nutritionally adequate diets: control diet ($N=12$, 0% DHA) or DHA diet ($N=8$, 0.9% DHA). Diets were fed to dams throughout lactation, and then at weaning (21d), two pups per dam were randomly assigned to continue on the same diet as the dam or consume the other experimental diet for an additional 21d. At 6 weeks, splenocyte phenotypes and *ex vivo* cytokine production after stimulation with concanavalin A (ConA), lipopolysaccharide (LPS) or ovalbumin were assessed. Pups who received the control diet during both periods had the lowest production of IL-2 after ConA ($P<.05$ for interaction). Pups fed DHA during suckling had higher IL-10 production after all mitogens, regardless of the weaning diet ($P<.05$). Feeding DHA at weaning, regardless of the suckling diet, resulted in a lower production of IL-1β and TNF-α in LPS-stimulated splenocytes and a higher proportion of total CD27+ cells (all $P<.03$). Our findings suggest that providing no DHA during critical periods of immune development resulted in a less efficient Th1 response upon challenge (IL-2 production). Feeding DHA during suckling had a programming effect on the ability of splenocytes to produce the regulatory cytokine IL-10. Feeding a DHA diet during weaning led to a lower TNF-α and IL-1β response to a bacterial antigen.

Keywords: Immunology; Lactation period; Weaning period; Offspring; Development; Programming

1. Introduction

T and B lymphocytes are key components of the acquired immune system that are important for the individual or animal to appropriately and effectively deal with environmental challenges [1]. Although there is considerable development of the immune system during pregnancy, the suckling period and the early weaning period are both critical stages where the acquired immune system matures [2]. T cells can be divided into T helper Th1 cells or Th2 cells according to the different cytokines they produce. The immaturity of the immune system at birth is characterized by a predominance of Th2 cells and maturation during infancy is associated with an increase proportion of Th1 cells as well as an increase in their ability to produce cytokines including interleukin IL-2, interferon IFN-γ and tumor necrosis factor TNF-α [3].

Studies have demonstrated that both the level of fat and the balance between omega-6 (n-6) and omega-3 (n-3) long-chain polyunsaturated fatty acids (LCPUFA) in the diet modulate T cell function in different stages of the life cycle and after immune challenges [4,5]. More specifically, arachidonic acid (AA, n-6) and docosahexaenoic acid (DHA, n-3), which are found in significant amounts in breast milk, have both been suggested to have a beneficial effects on immune system development early in life [6]. Indeed, although the essentiality of AA for optimal growth is still under debate [7,8], providing adequate amount of AA early in life is hypothesized to be important for the immune system development as there is a rapid increase in the content of AA in thymocytes in the post natal period [9] that is a critical period for T cell development. Nutritional intervention studies consistently showed that infants fed formula supplemented with AA and DHA early in life have a reduce risk of developing allergic/atopic diseases (reviewed in Ref. [10]). Previous studies have also demonstrated some beneficial effects of fish oil supplementation either during pregnancy [11,12] or childhood [13] on the immune system development and/or function. The effect of diet during critical periods of development has profound implications on both the immediate biological response and the response later in life. However, this concept of nutritional programming has not been well established for immune function.

We have previously established [14] in our rodent model that (1) feeding a diet containing no DHA to lactating dams resulted in a breast milk fatty acid composition similar to what has been observed in human milk in the United States and Canada (0.24% DHA) and (2)

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feeding a DHA diet (0.9% of total fatty acids as DHA) increased the DHA content of breast milk (1.09% DHA) to levels found in human milk from Japan and northern Canada (≥0.8% of DHA) where fish intakes or other sources of DHA are high [15] or of women taking fish oil supplements [16]. Using our previously established rodent model, we aimed at determining the optimal timing for providing an additional physiologically achievable amount of DHA early in life (*i.e.* suckling or weaning period) and its programming effect on the immune system. The objective of the current study was therefore to determine the effect of feeding a diet supplemented with DHA while containing an adequate amount of AA (so as to achieve the content in breast milk), during the suckling (intervention on the dam) and/or the weaning period on immune system development and function in offspring. Immune function was assessed by cytokine production by splenocytes stimulated with mitogens or ovalbumin (OVA), a dietary antigen.

2. Materials and methods

2.1. Animals and diets

All animal care and experimental protocols were conducted in accordance with the Canadian Council on Animal Care and approved by the University of Alberta Animal Ethics Committee. Primiparous Sprague–Dawley rats ($n=20$) were obtained from Charles River Laboratories (Montreal, Quebec, Canada) on day 14 of gestation and were individually housed in an environment that is controlled by temperature and humidity, with a 12/12-h reversed light cycle. Dams were fed standard rat chow (Lab diet 5001; PMI Nutrition International, Brentwood, MO, USA) throughout gestation and then randomized to one of two nutritionally adequate experimental diets 24–48 h prior to parturition: control diet (0.4% AA and 0% DHA of total fatty acids, $N=12$) or DHA diet (0.4% AA and 0.9% DHA of total fatty acids, $N=8$). The litters were culled to ensure 10 pups/dam and diets were fed *ad libitum* throughout lactation. Offspring were kept with their mothers until the end of the suckling period (3 weeks of age) where dams and two pups from each dam (pooled) were terminated. Data on 3-week-old pups have been previously published in Ref. [14]. Then, one pup from each dam (only females were kept) was randomly assigned to the control diet or the DHA diet in a crossover design and the diets were fed for an additional 3 weeks. At 6 weeks, pups were terminated. This study design allowed us to specifically investigate the impact of feeding a DHA diet during the suckling period but also during the weaning period and resulted in four different diet groups as presented in Fig. 1. The immune system development in rodents and humans has been shown to share many similarities and the suckling rat has been proposed as a good model for immunonutrition studies in early life [2,17].

Both experimental diets were isocaloric and isonitrogenous, and the nutrient composition was identical differing only in the LCPUFA content (Table 1). The nonlipid nutrient composition of the experimental diets has been previously described [18]. The added fat mixture to the rodent diet was composed of flaxseed oil, sunflower oil, saturated canola oil, olive oil, a high AA oil and a DHA oil [both AA and DHA oils were provided by DSM (Nutritional Products, Columbia, MD, USA)] and each fatty acid was matched closely so the diet primarily differed only in the total n-3 content. Both diets met the essential fatty acid requirements of the rodent and had similar PUFA/SFA ratio. Diets were prepared weekly and stored at 4°C until fed; feed cups were replaced every 2–3 days to prevent oxidation. Dietary intake and body weight were recorded regularly throughout the intervention.

Table 1
Fatty acid composition of the experimental control and DHA diet fed to pups during the suckling and the weaning period adapted from Ref. [14]

Fatty acid	Control diet	DHA diet
g/100 g of total fatty acids		
C14:0	0.1±0.0	0.4±0.0
C16:0	6.7±0.3	6.2±0.1
C16:1n-7	0.2±0.0	0.2±0.1
C18:0	38.8±1.2	40.6±0.2
C18:1n-9	29.0±1.7	24.8±0.3
C18:2n-6	21.2±0.5	21.6±0.0
C20:0	0.9±0.0	0.9±0.0
C18:3n-3 (ALA)	1.7±0.1	3.3±0.1
C20:3n-6	0.4±0.1	0.4±0.1
C20:4n-6 (AA)	0.4±0.0	0.4±0.0
C22:6n-3 (DHA)	0	0.9±0.1
Other fatty acids[a]	0.8	0.4
Total SFA	46.5±0.8	48.1±0.3
Total PUFA	23.6±0.6	26.6±0.1
Total n-6	21.9±0.5	22.3±0.1
Total n-3	1.6±0.1	4.2±0.1
Total MUFA	29.1±1.7	24.9±0.4
Ratio n-6/n-3	13.3	5.3
Ratio PUFA/SFA	0.5	0.6

Analysis by GLC of $n=2$ batches, mean±SEM; AA, arachidonic acid; ALA, α-linolenic acid; DHA, docosahexaenoic acid; MUFA, monounsaturated fatty acids; n, omega; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

[a] Other fatty acids refer to fatty acids that contributed for less than 0.1% in the diet that included trace of 10:0, 12:0, 20:2n-6, 20:5n-3, 22:0, 22:4n-6 and 22:5n-3.

2.2. Tissue collection

At 6 weeks, pups were euthanized by CO_2 asphyxiation and subsequent cervical dislocation. Spleens were collected aseptically and immune cells were isolated (see below). Intestines were removed and the length recorded.

2.3. Immune cell isolation

Immune cells were isolated from spleens as previously described [19]. Briefly, single cell suspensions were obtained by disrupting tissue through a nylon mesh screen in sterile Krebs–Ringer Hepes buffer with bovine serum albumin (5 g/L) (Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada). Erythrocytes were lysed with ammonium

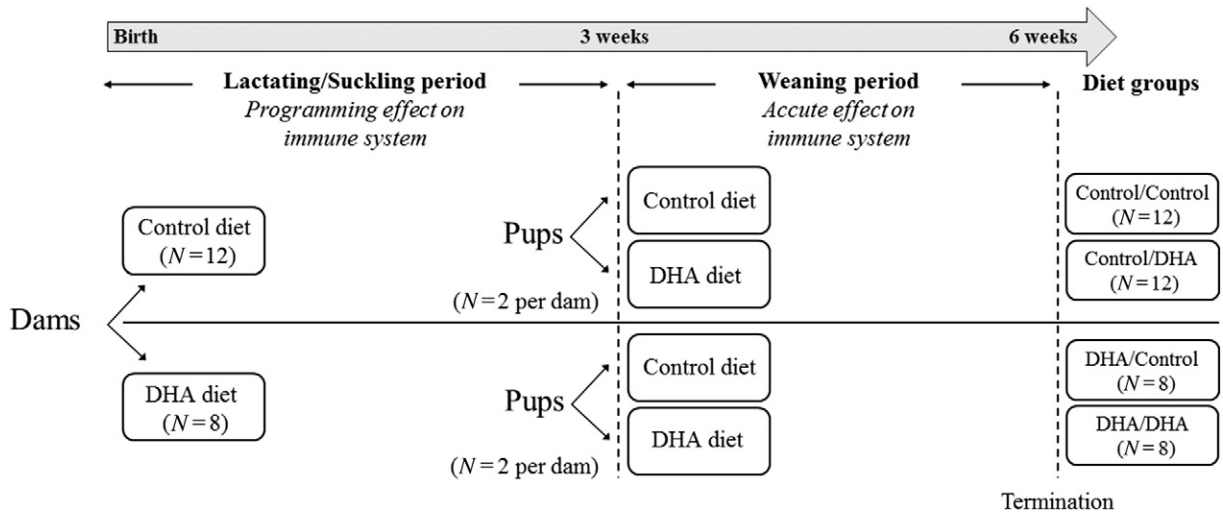

Fig. 1. Animal study design.Dams were randomly assigned to the control diet or the DHA diet for the duration of the lactating/suckling period. At 3 weeks, 2 pups from each dam were then randomly assigned to the control diet ($N=1$ per dam) or the DHA diet ($N=1$ per dam) in a crossover design and the diets were fed for an additional 3 weeks. At 6 weeks, pups were terminated and since the dams are the experimental unit in this study design the number of observation within each group is equal to the number of dams.