Microbial Pathogenesis 95 (2016) 68–76

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Neonatal malnutrition programs the oxidant function of macrophages in response to *Candida albicans*

Thacianna Barreto Da Costa [a, c, *], Natália Gomes De Morais [b], Amanda Lúcia F. Pedrosa [c], Suênia Da Cunha G. De Albuquerque [d], Maria Carolina A.B. De Castro [d], Valéria Rêgo A. Pereira [d], Milena De Paiva Cavalcanti [d], Célia Maria M.B. De Castro [a, c]

[a] *Department of Tropical Medicine, Federal University of Pernambuco, Av. Prof. Moraes Rego, 1235, Cidade Universitária, Recife, Pernambuco, 50670-901, Brazil*
[b] *College of Medicine, Federal University of São Francisco Valley, Avenida José de Sá Maniçoba – Centro, Petrolina, Pernambuco, 56304-917, Brazil*
[c] *Keizo Asami Laboratory of Immunopathology, Federal University of Pernambuco, Av. Prof. Moraes Rego, 1235, Cidade Universitária, Recife, Pernambuco, 50670-901, Brazil*
[d] *Department of Immunology, Aggeu Magalhães Research Center, Oswaldo Cruz Foundation, Av. Prof. Moraes Rego, 1235, Cidade Universitária, Recife, Pernambuco, 50670-901, Brazil*

ARTICLE INFO

Article history:
Received 12 July 2015
Received in revised form
17 February 2016
Accepted 21 February 2016
Available online 19 March 2016

Keywords:
Malnutrition
Programming
Alveolar macrophage
Oxidative stress
Cell death
Candida albicans

ABSTRACT

Experimental maternal nutrition restriction models are used to investigate short or long-term consequences of nutritional deficiency on puppies' growth. By assuming that the immune function is directly related to host's nutritional status, the current study aims to investigate the effects of neonatal malnutrition on oxidative stress and on the cell death of the alveolar macrophage after *in vitro* infection by *Candida albicans*. *Wistar* rats were suckled by mothers fed on diets containing 17% protein (Nourished group) or 8% protein (Malnourished group) in the current assay. Both groups received the standard diet used in the vivarium until adulthood, after weaning. The results showed that the offspring from mothers fed on low-protein diet presented lower body weight from 5 days of life on. Their low weight remained until adulthood when it was compared to that of rats in the nourished group. Superoxide and nitric oxide production was lower in malnourished animals and it was accompanied by low inducible nitric oxide synthase gene expression levels in systems in which the alveolar macrophages were challenged by immunogenic stimulus. No significant differences were observed in comparisons performed between the nourished and malnourished groups in any of the analyzed cell viability (apoptosis/necrosis) parameters. The fungal inoculum-stimulated system induced higher oxidative stress and cell death by necrosis. The current study demonstrated that dietary restriction during lactation alters the oxidant function of alveolar macrophages in puppies; It happens from the gene transcription step to the release of mediators, thus compromising the host's defenses against *Candida albicans*. It raises the possibility that *Candida albicans* may cease to be a commensal fungus to become a pathogen in offspring that have suffered nutritional deficiency during critical developmental periods, due to impaired immune responses.

1. Introduction

Poor nutrition during the critical pregnancy and neonatal maturation periods may affect the development and differentiation of the immune system [1]. Knowing that the immune system develops in stages and that the basic ontogeny defenses are limited to the early life, it is plausible that nutritional deficiencies during these early stages may permanently alter the populations and/or organogenesis of specific immune cells [2]. The explanation mechanism suggested for these effects is called “programming” - the phenomenon in which a stimulus or insult at a critical period of development can have long-term effects on the organism [3].

The causal relationship among malnutrition, immunesuppression and infection has been a frequent research target [4,5].

* Corresponding author. Keizo Asami Laboratory of Immunopathology, Federal University of Pernambuco, Av. Prof. Moraes Rego, 1235, Cidade Universitária, Recife, Pernambuco, 50670-901, Brazil.
E-mail addresses: thaciannabc@gmail.com, thaciannacosta@yahoo.com.br (T.B.D. Costa).

http://dx.doi.org/10.1016/j.micpath.2016.02.012
0882-4010/

Abbreviations

AM	Alveolar macrophages
ANOVA	Analysis of Variance
ATCC	The American Type Culture Collection
BW	Body weight
C−	Negative Control
C+	Positive Control
CEEA	Ethics Committee on Animal Experimentation
COBEA	Brazilian College of Animal Experimentation
Ct	Threshold cycle
FITC	Fluorescein Isothiocyanate
G3PD	Glyceraldehyde-3-phosphate dehydrogenase
iNOS	Inducible nitric oxide synthase
LPS-Ec	Lipopolysaccharide- *E. coli*
M	Malnourished
N	Nourished
NLRP3	Nod like receptor-3
NO	Nitric Oxide
O2-	Superoxide
PBS	Phosphate-buffered saline
PMA	Phorbol 12-myristate 13-acetate
RT-PCR	Reverse Transcription Reaction, followed by Polymerase Chain Reaction
R^2	Correlation coefficient
T	Test
TLR	Toll like receptor

Malnutrition compromises hosts' immune defenses, therefore it facilitates the development of infectious processes. On the other hand, the repeated infections affect the nutritional status [6]. According to this decreased immune function scenario, malnourished individuals are more susceptible to infections and to opportunistic pathogens [7].

The incidence of opportunistic fungal infections in immunocompromised patients has significantly increased in recent decades. These high rates are directly related to the advances in the treatment of many diseases, especially to the use of antineoplastic agents, immunosuppressants and of broad spectrum antibiotics [8]. Thus, fungal infections are characterized as a major public health problem in many countries [9].

Candida albicans is the species that is most often isolated from fungal infections [10]. Invasive infections caused by this microorganism can become a serious clinical complication, since they frequently involve vital organs such as brain, liver and kidneys [11,12]. The host's defense against candidiasis comprises the intake and disposal of fungal structures by cells in the innate immune system, mainly neutrophils and macrophages [13]. These phagocytic cells are the key aspect in the antimicrobial response, which is the generation of oxygen and nitrogen reactive species [14,15]. However, the macrophage-microorganism interaction may cause the collapse of the balance between survival and pro-apoptotic cellular pathways and it leads to the death of these phagocytes' cells [16].

Pulmonary complications represent a major morbidity and mortality cause among immunocompromised patients [17]. In addition, a significant association between the colonization of the respiratory tract by *Candida* spp. and hospital mortality has been reported [18]. Therefore, it is important to notice that alveolar macrophages' phagocytic capacity is a major defense mechanism of the respiratory tract against invading micro-organisms and other inhaled particles [19]. Alveolar macrophage activity *in vitro* evaluation methods are important tools to investigate the functional integrity of these cells, as well as to study the interaction between micro-organisms and hosts [20].

Although previous studies have shown the relationship between malnutrition and impaired macrophage function in adulthood [5,21], there are few studies examining the neonatal malnutrition effect, followed by nutritional supplementation, on functionality of immune cells in infectious processes involving opportunistic micro-organisms. Thus, the current study aims to analyze this malnutrition model implication on superoxide and nitric oxide production, on inducible nitric oxide synthase expression and on cell viability in the alveolar macrophages after *in vitro* infection caused by *Candida albicans*, in order to help better understanding the mechanisms involving infections caused by opportunistic pathogens in malnourished individuals.

2. Methods and materials

2.1. Ethical considerations

The current study was approved by the Ethics Committee on Animal Experimentation of Biological Sciences Center, at Federal University of Pernambuco (CEEA-UFPE) (Pernambuco, Brazil; Protocol nº 23076.053096/2011-91.). All experiments were based on ethical recommendations from the Brazilian College of Animal Experimentation (COBEA) and from the *National Institute of Health Guide for Care and Use of Laboratory Animals.*

2.2. Animals and diets

A total of twenty-four *Wistar* male albino rats were used in the experiments; they were obtained in the Nutrition Department at Federal University of Pernambuco. The animals were kept in cages under controlled temperature (22 ± 1 °C), light-dark cycle of 12:12 h (Light, 6–18 h; dark, 18-6 h), and free access to water and food up to the experimental manipulation day.

Six male offspring were kept with their mothers after birth. At the first day of life the animals in the litters were divided into two groups: Nourished (N) - pups nursed by mothers subjected to diet containing 17% casein protein used as protein source, n = 12; and malnourished (M) - pups nursed by mothers subjected to diet containing 8% protein, based on casein as protein source, n = 12 (Table 1).

Animals in both groups were breastfed during the first 21 days of life. Throughout this period were daily recorded (in digital electronic scale - Mars, S-4000 model, 0.1 g sensitivity, São Paulo, Brazil) the body weight (BW) of each animal to monitor BW during nutritional manipulation. The animals were separated from their mothers after weaning (22 days of life) and kept in collective cages containing three rats each. Then, they began to be treated with Labina® (Labina - Purina Brazil S/A, São Paulo, 23% protein). Body weight was measured three times a week in this second phase of life (post-breastfeeding) in order to follow animals' nutritional supplementation until adulthood (90 days of life).

2.3. Determining Candida albicans inoculum concentration

Candida albicans (ATCC 10231) was the selected reference strain