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Different capacity of *in vitro* generated myeloid dendritic cells of newborns of healthy and allergic mothers to respond to probiotic strain E. coli O83:K24:H31

Lenka Súkeníková[a], Viktor Černý[a], Olga Novotná[a], Petra Petrásková[a], Kristýna Boráková[b], Libuše Kolářová[a], Ludmila Prokešová[a], Jiří Hrdý[a,*]

[a] Institute of Immunology and Microbiology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic
[b] Institute for the Care of Mother and Child, Prague, Czech Republic

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ABSTRACT

Allergic diseases belong to one of the most common diseases with steadily increasing incidence even among young children. There is an urgent need to identify a prognostic marker pointing to increased risk of allergy development enabling early preventive measures introduction. It has been shown that administration of selected probiotic strains or mixtures could prevent allergy development. In our study, we have tested the capacity of probiotic strain *Escherichia coli* O83:K24:H31 (*E. coli* O83) to promote dendritic cell (DC) maturation and polarisation of immune responses. Increased presence of activation marker CD83 was observed on DC stimulated by *E. coli* O83 and DC of newborns of allergic mothers have significantly more increased cell surface presence of CD83 in comparison to children of healthy mothers. Increased gene expression and secretion of IL-10 was detected in DC stimulated with *E. coli* O83 being higher in DC of newborns of healthy mothers in comparison to allergic ones. Generally, increased presence of intracellular cytokines (IL-4, IL-13, IFN-gamma, IL-17A, IL-22, IL-10) was detected in CD4+ T cells cocultured with DC of children of allergic mothers in comparison to healthy ones. *E. coli* O83 primed DC significantly increased IL-10 and IL-17A in CD4 T cells of newborns of healthy mothers in comparison to the levels detected in CD4 T cells cocultured with control non-stimulated DC. We can conclude *E. coli* O83 induces dendritic cell maturation and IL-10 production in DC. Newborns of allergic mothers have generally increased reactivity of both DC and CD4 T cells which together with decreased capacity of DC of newborns of allergic mothers to produce IL-10 could support inappropriate immune responses development after allergen encounter.

1. Introduction

Allergic diseases present the most common illnesses with steadily increasing incidence over the last three decades especially in western countries [1]. Tremendous increment of allergy was observed even among young children. Identification of some prognostic markers pointing to increased risk of future allergy is highly desirable for introduction of early measures leading to allergy prevention or at least lowering the significance of clinical outcomes. Cord blood seems to be an ideal source of such prognostic markers because it is easily available at the time of delivery in a sufficient amount.

Different researcher groups tried to propose some immunological characteristics of cord blood as prognostic markers pointing to increased risk of allergy development (e.g. IgE levels [2], cytokines present in cord blood sera [3–5], the capacity of cord blood mononuclear cells (CBMC) to release cytokines [5], number and activity of T regulatory cells (Treg) [6–8]). Nevertheless, contradictory conclusions exist in the literature and it is therefore difficult to find a consensus on one or a group of reliable prognostic markers in cord blood pointing to increased allergy development. It seems that the most reliable marker indicating increased risk of future allergy development is the allergic status of the mother [9].

With growing knowledge of the effect of microbiota on immune system, it seems that modification of microbiota composition and function could be exploited in allergy prevention strategies. On the other hand, disturbances in microbiota development early in life by antibiotic administration lead to impaired immune responses and allergy development, as shown on murine model of contact hypersen-

* Corresponding author at: Institute of Immunology and Microbiology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Studničkova 7, 128 00 Prague, Czech Republic.
E-mail address: jiri.hrdy@lf1.cuni.cz (J. Hrdý).

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sitivity [10]. Research of bilateral interaction between microbiota and host immune system is now in the centre of current research [11]. Pioneering bacteria colonizing relatively sterile newborns are important for the induction of proper development of immature newborn immune system [12,13]. Newborńs immune system is known for its Th2 predominance. Balance among immune responses (Th1, Th2, Treg) is highly desirable and several reports suggest that bacteria colonizing newborn intestinal tract are able to facilitate the equilibrium [14,15]. Prolonged Th2 immune response together with delayed maturation of infant immune system could be the underlying mechanism leading to allergy origination [16].

Administration of specific bacterial strains with the capacity to promote immune system seems to be reasonable in terms of allergy prevention. There are many reports of probiotic administration either to pregnant women [17,18] or children [19] with the aim pro prevent allergy development. Unfortunately, there are works with the opposite results [20,21]. It is important to mention that a broad spectra of microorganisms are used as a probiotic in prevention of allergic diseases – various strains of lactic acid bacteria [22], bifidobacteria [23], or *E. coli* [24]. It seems that the strain selection together with the dose and time of administration play a critical role. We have reported on a protective effect of early *E. coli* O83:K24:H31 administration in allergy prevention [24,14] but the mechanism of its action is still unknown.

In the current study, we have used a simplified model to try to propose a mode of action of this strain on immature immune system of newborns. We focused on the capacity of *E. coli* O83:K24:H31 to promote maturation of monocyte derived dendritic cell and subsequent induction of immune responses (Th1xTh2xTh17xTreg).

2. Material and methods

2.1. Subjects

Healthy and allergic mothers without any substantial complication during the course of pregnancy and children delivered by caesarean section at full term in the Institute for the Care of Mother and Child in Prague, Czech Republic from August 2015 to December 2016 were included into the study after signed written informed consent. Mothers involved in the current study were from the capital and suburban areas. The age was not significantly different between non-allergic mothers (NA) (32.8 ± 4.8 years) and allergic mothers (A) (32.7 ± 3.7 years). The length and birth weight of newborns were not different either (on average 49.4 ± 2.3 cm A, 49.9 ± 2.4 cm NA; 3.488 ± 508.5 kg A, 3.227 ± 395.1 kg NA). The length of the pregnancy was similar as well (39 w 3/7 ± 0.5 days A and 39 w 1/7 ± 8.6 days NA). Other factors possibly influencing cord blood mononuclear cell reactivity, like smoking, diet (vegetarian, raw, etc.) did not differ between healthy and allergic groups. The diagnosis of allergy in mothers was based on the clinical manifestation of allergy persisting for longer than 24 months (allergy against respiratory and food allergens manifested by various individual combinations of hay fever, conjunctivitis, bronchitis, asthma, eczema or possible other allergic manifestation), monitored by an allergist, positive skin prick tests or positive specific IgE antibodies and anti-allergic treatment before pregnancy. Detailed list of type or combination of allergies of allergic mothers involved in the study is provided in Table 1. Only two women used anti-allergic drugs during the whole course of pregnancy (from Table 1 patient 2 used Claritine and patient 16 used Zyrtec (cetirizine)). The mothers with previous miscarriages, diabetes, transplantation, transfusion, serious surgery and any health problem during the course of pregnancy were excluded from the study. The study was approved by the Ethical Committee of the Institute for the Care of Mother and Child (Prague, Czech Republic). A total of 65 maternal–child pairs were included in the current study. Based on maternal allergy status, the children were divided into two groups: 43 children NA (22 females, 21 males) and 22A (11 females, 11 males).

Table 1
List of mothers included in allergy group and kind of confirmed allergy.

Allergic mother	Type of alllergy/combination of allergies
1	Drugs (antibiotics), food allergy, pollen
2	Pollen, dust, mites
3	Drugs (antibiotics), pollen (grass)
4	Pollen, dust, dander (cat)
5	Atopic dermatitis, pollen (spring season)
6	Food allergy, metals, contact dermatitis
7	Metal
8	Pollen, mites, dander (cat, dog)
9	Pollen, dust, mites
10	Dust, pollen, insect venom (bee)
11	Pollen, cat dander
12	Pollen
13	Dust, pollen (grass)
14	Pollen, bee venom, drugs
15	Mites, feathers
16	Food, allergy, mites, pollen, bee venom
17	Insect
18	Dust, pollen
19	Pollen
20	Pollen
21	Dust, mites
22	Drugs, pollen

2.2. Cord blood sampling

Approximately 15 ml of cord blood was collected after thorough cleaning of the cord in sterile heparinized tubes (10 U heparin/ml) for further analyses and cord blood cell separations.

2.3. Cord blood cell isolation and dendritic cell generation

Cord blood mononuclear cells (CBMC) were isolated by gradient centrifugation using Histopaque (Sigma-Aldrich). Dendritic cells (monocyte derived dendritic cells; moDC) were derived from adherent fraction of CBMC as described previously [25]. Briefly, after 1 h cultivation of CBMC in cell culture flasks in the incubator with regulated CO_2 atmosphere, nonadherent CBMC were washed out and adherent CBMC were cultured for 6 days with rhIL-4 and rhGM-CSF.

2.4. Induction and characterisation of maturation of moDC

In vitro generated moDC were seeded on day 6 at a concentration of 1×10^6 cells/ml in 12-well plates and stimulated with LPS (1 μg/ml, *Escherichia coli*, Sigma), or probiotic bacteria *E. coli* O83:K24:H31 in the ratio: 10 bacterial cells: 1 moDC for 24 h.

2.4.1. Flow cytometry analyses of maturational status of moDC

Maturational status was estimated according to the presence of activation markers on moDC by flow cytometry. moDC were cultivated with *E. coli* O83 or LPS for 24 h and then stained with anti-CD40 (cat. no. 1F-416-T100); anti-CD80 (PC-287-T100), anti-CD83 (1P-677-T100), anti-CD86 (1A-531-T100), anti-MHCII (343310, BioLegend) anti-CD11c (T7-529-T100), all Exbio, plc. and analysed by BD FACS Canto II flow cytometer with BD FACS Diva version software 6.1.2. (Becton Dickinson, Franklin Lakes, NJ). To ensure the quality and reproducibility of data, Cytometer setup & tracking beads were used to check the instrument performance and to assure its stability and validity of data analysed on different days.

2.4.2. Detection of cytokines produced by moDC

Cytokines released by nonstimulated and stimulated moDC during 24 h of incubation were detected by ELISA in culture supernatants. Reagents for IL-6 (primary antibodies – cat. no. MAB 206, biotinylated

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