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Original article

High fecal IgA is associated with reduced *Clostridium difficile* colonization in infants

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Abstract

Colonization of infants with *Clostridium difficile* is on the rise. Although better tolerated by infants than adults, it is a risk factor for future allergic disease. The present study describes associations between infant fecal immunoglobulin A (IgA) and colonization with *C. difficile* in 47 infants enrolled in the Canadian Healthy Infant Longitudinal Development (CHILD) study.

C. difficile colonization was observed in over half (53%) of the infants. Median IgA was lower in infants colonized with *C. difficile* (10.9 μg versus 25.5 μg per g protein; $p = 0.18$). A smaller proportion of infants with IgA in the highest tertile were colonized with *C. difficile* compared to the other tertiles (31.3% versus 64.5%, $p = 0.03$). In unadjusted analysis, odds of colonization with *C. difficile* was reduced by 75% (OR 0.25 95% CI 0.07, 0.91 $p = 0.04$) among infants with IgA in the highest tertile compared to those in the other tertiles. Following adjustment for parity, birth mode and breastfeeding, this association was even stronger (aOR 0.17, 95% CI 0.03, 0.94, $p = 0.04$). Our study provides evidence that high fecal IgA, independent of breastfeeding, is associated with reduced likelihood of *C. difficile* colonization in infancy.

Keywords: Clostridium difficile; Immunoglobulin A; Infant

1. Introduction

Alongside its global rise as a cause of a debilitating infection [1], the enteric pathogen, *Clostridium difficile*, is being detected at an earlier age and in a greater number of infants than observed in the 1980s [2]. Up to 75% of healthy infants are colonized by *C. difficile* and, in some communities, 26% of them with toxic-producing strains [3]. In contrast to older children and adults who can develop severe diarrhea and colitis, carriage of *C. difficile* is well tolerated by infants [4]; they manifest no obvious symptoms from the toxins released by this anaerobe. The organism can be acquired in infancy from environmental contamination in the nursery or home environment [3]. Between 12 and 24 months of age, *C. difficile* abundance diminishes in gut microbiota. Yet, studies have

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shown that infants colonized with *C. difficile* are at increased risk for atopic outcomes later in childhood, including allergic sensitization, atopic dermatitis, recurrent wheeze and asthma [5–7]. Still other research suggests that associations with atopic disease are not due to *C. difficile* per se, but rather its presence may be a marker for lower colonization resistance. Changes in the ecosystem of gut microbiota in infants, such as higher abundance with genus *Ruminococcus* and *Klebsiella*, and lower abundance of *Bifidobacterium*, have been observed in the presence of *C. difficile* [8]. In adults, these changes are brought on with antibiotic use [9]; among infants, prior exposure to antibiotics is also risk factor for *C. difficile*, as is reduced exposure to human milk or lack of food diversity [3,10].

Although infants are better able to tolerate *C. difficile*, not all infants are colonized with this microbe; it is detected in only one third of infants under the age of 6 months [3]. What factors prevent colonization? Immunoglobulin A (IgA), in its secretory form (sIgA), is important in immune exclusion and the development of oral tolerance [11,12]. During the first weeks of life an infant's immune system is immature and its ability to produce IgA is limited [13]. Evidence from animal and human studies has demonstrated interactions between IgA and commensal gut bacteria whereby microbial exposure stimulates IgA production and in turn, IgA regulates the composition and activity of the microbiota (Fig. 1) [14–16]. Animal studies have shown that an absence of IgA in the intestine alters gut microbial composition and intestinal colonization of germ-free mice induces IgA responses [17,18]. Kukkonen et al. demonstrated a trend towards higher fecal IgA concentration following pre and probiotic treatment (*Lactobacillus* and *Bifidobacterium* species) of infants; antibiotic treatment was found to abolish the increase in fecal IgA in infants at 6 months following the administration of probiotics [19]. In a study on 64 Swedish infants, Sjögren et al. reported higher salivary IgA concentrations at 6 and 12 months following colonization with species of bifidobacteria [20]. In the same study, colonization at one month with *C. difficile* and lactobacilli was associated with increased IgA levels in children at age 1 and 5 years.

We previously reported on pre and postnatal predictors of fecal IgA levels in infants at 3–4 months of age [21]. To extend this work by better understanding the interactions between *C. difficile* and host immunity, we examined the association between infant fecal IgA concentration at this age and fecal colonization by *C. difficile*, independent of breastfeeding as a main source of IgA.

2. Materials and methods

2.1. Study design

A sub-sample of 47 infants (36–46 weeks gestation) from the Vancouver and Winnipeg sites of the Canadian Healthy Infant Longitudinal Development national population-based birth cohort (www.canadianchildstudy.ca) were included in the study for whom fecal samples were available for analysis [22]. Mothers of these infants were enrolled during pregnancy between September 2008 and January 2009. Stool samples were collected at mean age of 3.9 months (range 2.9–5.3) using a standard protocol as part of a scheduled home visit. Samples were refrigerated in the home immediately following collection and during transport and then stored at −80 °C for later use. At this time mothers were asked to report on breastfeeding status using a standardized questionnaire and breastfeeding was categorized as any breastfeeding (yes/no) and by degree of breastfeeding exposure (none, partially breastfed, exclusively breastfed). Information on other covariates were obtained from hospital records (infant sex, mode of delivery, birth weight, gestational age and maternal antibiotics

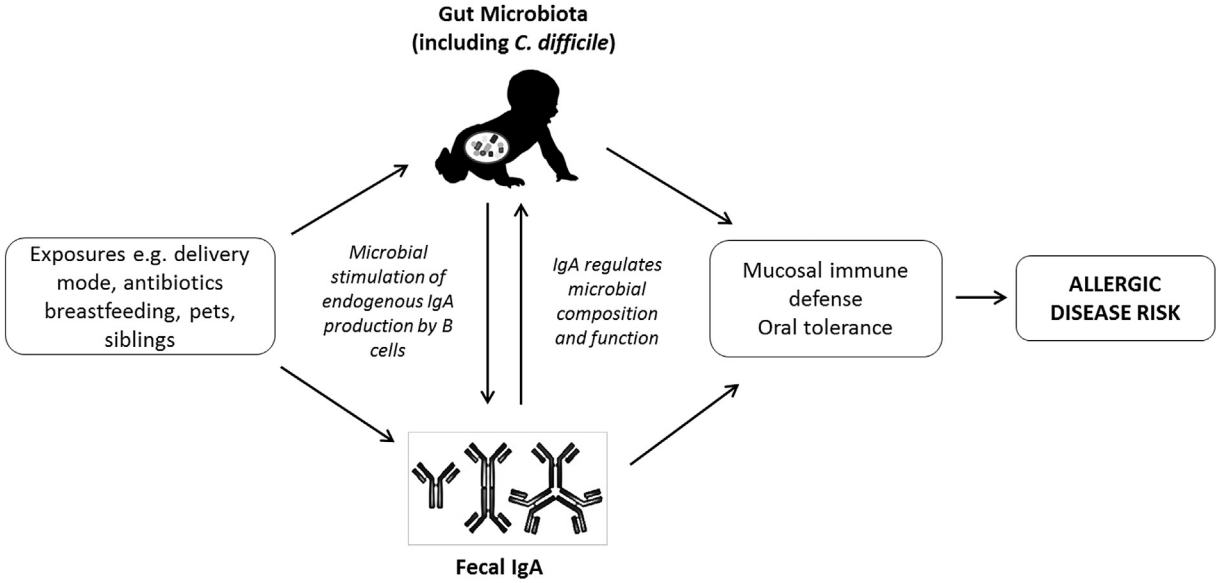

Fig. 1. Schematic representation of the potential inter-relationship between breastfeeding, gut microbiota, fecal IgA and allergic disease.