Normal Development of Gut Microbiota and Dysbiosis

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Microbiota in Functional Gastrointestinal Disorders in Infancy: Implications for Management

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Abstract

The complex and diverse intestinal microbiome is recognized as important in promoting human health. An altered gut microflora, referred to as dysbiosis, is increasingly recognized as having an etiologic role in a variety of conditions, including functional gastrointestinal disorders: colic in infants and irritable bowel syndrome in older children. Probiotics are defined as live microorganisms that, if ingested in sufficient amounts, restore microbial homeostasis and have a benefit on health. Randomized controlled trials indicate that probiotics can be effective in a variety of intestinal conditions, including colic and irritable bowel syndrome. Probiotics may promote gut microbial diversity, but timing of the intervention appears crucial. Strain-specific effects on colonization resistance, epithelial barrier integrity, modulation of signal transduction, impacts on innate and adaptive immune responses, and effects on visceral hyperalgesia likely explain the observed variability in various probiotic strains. In the future, probiotics are likely to be chosen for use in a defined clinical setting based on underlying mechanism(s) of action. The precise component of the probiotic agent mediating observed effects is the subject of current research. Unresolved issues relate to optimal dosages, timing of ingestion, single versus combination formulations, maintenance of viability in storage, and the merits of employing probiotic-derived products.

Introduction

Colic is one of a series of conditions affecting babies during their first year of life that frequently calls the attention of health care providers (Table 1). As summarized in the recently published Rome IV criteria for functional gastrointestinal disorders (now also referred to as disorders of gut–brain interaction) across the
life span, colic is defined by the manifestations of recurrent (>3 times/week) and protracted (>3 h/day) periods of irritability, crying, and fussiness that begins in otherwise healthy babies during the first few months of life [1]. These infants are developmentally normal and have a normal growth velocity for age. Symptoms usually occur in the afternoon or evening hours and can be alarming for parents and other caregivers, who are concerned about underlying causes of the apparent abdominal pain and discomfort. However, colic is a transient, developmental issue that generally resolves before 6 months of age, as the baby grows older. Alarming features that should trigger a search for other underlying causes include unexplained fever suggesting an occult urinary tract infection and suboptimal growth velocity. Even though colic has been considered as an extreme variation in normal patterns of infant fussiness, the symptoms can cause considerable distress for caregivers who seek effective and safe interventions to see the baby, and themselves, through the stressful transition period.

Evidence that the Gut Microbiome Is Altered in Functional Gastrointestinal Disorders

Studies indicate that intestinal microbial dysbiosis is a feature in both children [2] and adults [3] with irritable bowel syndrome (IBS). As with many studies related to the gut microbiota in other human conditions, the unanswered question is whether the gut dysbiosis is an underlying cause of intestinal symptoms of abdominal pain, bloating, and flatulence. Studies show that colonic biopsies and colonic washes taken from adults with IBS have higher proteolytic activity and greater pronociceptive activity in a murine model of visceral sensitivity than in biopsies and washes derived from the colons of healthy, asymptomatic controls [4]. The source of protease activity mediating the observed effects could well be luminal microbes. Intervention studies that impact the gut microbiota composition and/or diversity are another approach to consider whether there is a cause-effect relationship between the gut microbiome and symptoms that are consistent with IBS.

In a study in Dutch infants, reduced diversity in bacterial species was identified in fecal samples taken from infants who went on to develop colic versus

Table 1. Functional gastrointestinal disorders in the first year of life

<table>
<thead>
<tr>
<th>Disorder</th>
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<tbody>
<tr>
<td>Colic</td>
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<tr>
<td>Functional constipation</td>
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<tr>
<td>Dyschezia</td>
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<tr>
<td>Functional diarrhea (variously referred to as chronic nonspecific diarrhea of infancy)</td>
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<tr>
<td>Regurgitation</td>
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1 Adapted from: Benninga et al. [1].
age-matched controls [5]. It should be noted that gender, birth weight, route of delivery, and duration of breastfeeding did not differ between the two study groups. In this study of 12 colicky babies and an equal number of asymptomatic subjects, microbial diversity was lower in stools obtained at 2 weeks of age in the study group with excessive crying. In particular, numbers of bifidobacteria and lactobacilli were reduced in babies who went on to develop colic. Since *Bifidobacterium* species and lactic acid-producing bacteria are frequently employed as probiotic preparations, there appears to be a rationale to use such agents to increase bacterial subpopulations that are reduced in numbers and thereby enhance gut microbial diversity. Validation of these findings in a larger number of infants in various parts of the world is now required.

**Evidence that Altering the Gut Microbiota Impacts Symptoms in Irritable Bowel Syndrome**

Increasing experimental and clinical evidence supports the existence of a bidirectional and modifiable microbiome-gut-brain axis [6]. As shown in Figure 1,
the behavior of germ-free mice differs from that observed of specific pathogen-free animals. Mice whose gut bacteria are disrupted by antibiotic treatment early in life had increased visceral sensitivity in adulthood, even though the composition of the gut microbiota had long been normalized [7]. Moreover, delivery of beneficial microbes (probiotics) has an impact on altering murine behavior that is mediated, at least in part, via vagal afferents because surgical vagotomy blocks the observed effects [8]. Microbial reconstitution can be undertaken, either by employing fecal microbial transplantation or by using a more targeted approach with either a combination of microbes or a single strain of what is considered to be a beneficial organism [9].

Probiotics are defined as live microorganisms which, if taken in sufficient amounts, have a benefit on health [10]. Increasing evidence shows that probiotics can reduce the symptoms of IBS in both children and adults. A meta-analysis of 23 randomized controlled trials evaluating the effectiveness of various probiotic strains in adults with IBS showed that the microbes are more effective than placebo (relative risk reduction of IBS of 0.79; 95% confidence intervals: 0.70–0.89; number needed to treat just 7; 95% confidence interval: 4–13) [11]. However, which individual microbial species and strains are the most beneficial awaits further direct head-to-head comparative trials.

Probiotics as a Management Strategy for Infant Colic

As summarized in Table 2, a number of trials indicate that the probiotic *Lactobacillus reuteri* (strain DSM 17928) provided at a dose of $1 \times 10^8$ bacteria, delivered as 5 drops once daily in a hydroscopic oil suspension for 3–4 weeks, is more effective than placebo in alleviating symptoms of fussiness and irritability in babies with colic [12]. Outcome measures include duration of infant crying and

<table>
<thead>
<tr>
<th>Reference No.</th>
<th>Country</th>
<th>Duration, days</th>
<th>Change in crying duration, min/day</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Italy</td>
<td>28</td>
<td>-66.8</td>
<td>-78.4, -55.2</td>
</tr>
<tr>
<td>15</td>
<td>Italy</td>
<td>21</td>
<td>-125.0</td>
<td>-172.0, -78.0</td>
</tr>
<tr>
<td>16</td>
<td>Poland</td>
<td>21</td>
<td>-55.2</td>
<td>-60.2, -50.2</td>
</tr>
<tr>
<td>17</td>
<td>Australia</td>
<td>28</td>
<td>-11.0</td>
<td>-78.6, 100.6</td>
</tr>
<tr>
<td>18</td>
<td>China</td>
<td>28</td>
<td>-55.1</td>
<td>-55.1, -50.9</td>
</tr>
<tr>
<td>19</td>
<td>Canada</td>
<td>21</td>
<td>-39.8</td>
<td>-50.9, -28.7</td>
</tr>
<tr>
<td>Summary</td>
<td></td>
<td></td>
<td>-55.9</td>
<td>-64.4, -47.3**</td>
</tr>
</tbody>
</table>

**p < 0.001. ¹ Adapted from: Harb et al. [12].
at least a 50% reduction in crying time versus baseline measures [13]. These prospective clinical studies were undertaken in Italy [14, 15], Poland [16], Australia [17], China [18], and Canada [19]. Another study conducted in Italy evaluated the effects of *L. reuteri* (strain DSM 17938) versus placebo over the first 90 days of life. At 3 months, crying time was significantly reduced in colicky babies receiving the probiotic (38 min/day) compared to infants randomized to the placebo arm of the study (71 min, *p* < 0.01). Moreover, there was a reduction in financial costs incurred for the families of infants with colic who received the probiotic agent [20]. A meta-analysis of the trials indicated that the active agent was more effective that placebo, with 5 of the 6 studies included in the evaluation having an impact that reached statistical significance [12]. A possible explanation for the negative trial undertaken in Australia could relate to a lower rate of exclusive breastfeeding compared to other studies. It should be noted that even though the total number of babies entered into all of the clinical trials is relatively small, there have been no serious adverse events reported.

**How Might Probiotics Relieve Symptoms?**

The underlying mechanism(s) that account for colic remain poorly understood. Nevertheless, as summarized in Figure 2, a compelling body of literature emphasizes that there is an active, dynamic, and bidirectional communication between the trillions of microbes colonizing the lumen and the mucous layer of the gut, with epithelial cells lining the intestine, and the underlying mucosal immune system and both the peripheral enteric and central nervous systems [21]. This has been termed the diet-microbiome-gut-brain axis. Recent evidence indicates that lactic acid-producing bacteria capable of catabolizing dietary tryptophan into a variety of indole derivatives mediate anti-inflammatory effects, both in the gut and in the central nervous system, via activation of the aryl hydrocarbon receptor and increased production of interleukin-22 by gut mucosa-resident T immune cells [22]. An accompanying editorial suggests that *L. reuteri* might be an effective probiotic choice to enhance the pathway of tryptophan metabolism as a novel anti-inflammatory strategy [23]. An additional mechanism that might be considered is that in an animal model of intestinal injury early in life, *L. reuteri* DSM 17938 increases the number of anti-inflammatory Foxp3+ regulatory T cells present in the intestinal mucosa [24].

Another potential consideration is that certain probiotic strains have a direct effect on visceral sensation. For instance, Perez-Burgoz et al. [25] reported that the firing frequency of the pronociceptive TRPV (transient receptor potential vanillloid) 1 channel in mesenteric spinal afferent nerve bundles in response to either small bowel luminal distension or capsaicin is reduced in the presence of bacterial cell-free culture supernatants derived from *L. reuteri* DSM 17928.
Interestingly, the observed effects are strain specific with culture supernatants prepared from other lactic acid-producing bacteria having no antinociceptive effects. Alternatively, \textit{L. reuteri} DSM 17928 may reduce symptoms of colic by mediating effects on gut motility and intestinal transit times [26]. Another possibility to consider as a potential underlying mechanism of action is provided by recent evidence showing that probiotics colonizing the gut of both rodents [27] and humans [28] can have a direct effect on the activity of neurotransmitters in the central nervous system.

**Conclusions**

Level 1 evidence secured from a number of prospective randomized controlled clinical trials shows that irritability in breastfed infants with colic can be better managed with probiotics than sham intervention alone. Issues related to the
definition of entry criteria (that is, how best to define colic in a reproducible manner across centers and between studies) and measures employed as measures of primary outcome have appropriately been raised as issues of concern that warrant attention [29]. Nevertheless, the body of evidence accumulated to date and summarized in this review appears quite convincing. The studies have been undertaken in various countries in Europe, Asia, and North America with trials employing a single probiotic strain from the same commercial source. Dose escalation studies and head-to-head comparisons with other widely available probiotic strains and mixtures of strains in breastfed and formula-fed infants are now warranted [30]. Whether such an intervention early during the course of the life span will change the short- and long-term composition of the gut microbiome, mucosal immune function, and sensations of visceral pain requires ongoing study and continued surveillance of those subjects already entered into these clinical trials.

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