The role of gut microbiota in juvenile idiopathic arthritis

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ABSTRACT
Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in childhood, with prevalence of 16–150 cases per 100,000 children. The etiopathogenesis of JIA is a challenge incorporating a complex network with only 18% attributed to genetic factors. The remaining part should therefore be explained by non-hereditary factors. Given that around 70% of the immune cells are located in the gut, the potential role of the gut microbiota in the etiopathogenesis of JIA has been recently investigated. The aim of this review is to discuss the complexity of the link between gut microbiota and JIA, the different methods for identifying bacteria, the shape-up of the microbiota from birth to adulthood. The objectives are to discuss various pathways involved in this process: changes in the microbiota contents in healthy individuals and JIA patients, increased gut permeability, influence on T-cell differentiation and proliferation. Factors that have been associated with dysbiosis: diet, pathogens and drug use, are discussed. JIA is not a benign disease, it is a chronic disease and an important cause of short- and long-term disability—significant joint contractures, leg-length inequalities and uveitis, which can lead to impaired vision. It is known that at least one-third of children will have ongoing active disease into their adult years, and many will have some limitation in their daily life activities. A deeper understanding of the pathways by which disturbances in the microbiome may evolve to disease may open doors to the development of new treatment or prevention strategies in the future.

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Introduction
The link between the microbiota and immune-mediated diseases has been a subject of great interest. The microbiota is a collective term for the trillions of commensal microorganisms (bacteria, archaea, viruses and unicellular eukaryotes) that inhabit our epithelial surfaces, including the gut, respiratory tract and skin. Whilst the role of viruses, archaea, and unicellular eukaryotes is relatively understudied, in recent years the bacterial components of the microbiota and its role in modulating immune responses have attracted intense investigation. The gut microbiota has recently received increasing attention as a potential contributing factor to the development of a wide variety of diseases [1]. Owing to some powerful tools, we can now generate extensive analyzes of the microbial communities, revealing that they may contribute to the pathogenesis of immune-mediated diseases, including arthritis. Different studies have revealed that the gut microbiota influences the systemic autoimmunity through various pathways: changes in the microbiota contents, increased gut permeability, influence on T-cell differentiation and proliferation.

The human intestine contains over 1000 bacterial species and $10^{14}$ bacterial cells. The collection of genes in the microbiota is referred to as the ‘microbiome’. Encoding 9.8 million non-redundant genes and 100-fold more proteins than the human genome [2], the gut microbiome provides an enormous source of antigenic variation.

Methods for identifying bacteria: from past to present
It has been more than a century since Elie Metchnikoff suggested that alterations in the intestinal milieu may promote improved overall health. The oldest method of identifying bacteria is culture. While this remains an

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important tool in clinical medicine, it is an ineffective means of identifying the contents and relative abundances of complex communities of organisms, many of which are difficult if not impossible to culture. The majority of gut microbiota (over 80%) could not be technically cultivated with the culture-based methods. The development of DNA-based culture-independent methods has been fundamental for deepening our understanding of the bacterial species that constitute the gut microbiota. Two DNA-based sequencing methods have been used to investigate the taxonomic identity and function of the gut microbiota. The first one focuses on the highly conserved 16S ribosomal RNA (rRNA) gene sequence of bacterial microorganisms. The method of 16S rRNA sequencing has been extremely useful for phylogenetic classification of particular species within the gut microbiota. However, analysis of the 16S rRNA gene alone does not provide any information about the functional capacity of the gut microbiome. The second widely-used technique, whole-genome shotgun next-generation DNA sequencing (NGS), has been used to overcome this problem. Whole-genome shotgun sequencing analyzes every gene within a given sample, allowing functional profiles to be assigned to the gut microbiota based on the genes that are present. It is a technique currently applied for identification of the metabolic and enzymatic pathways present in these microbial communities.

The gut microbiota has been largely investigated in an effort to demonstrate its vast inter-individual diversity and its role in autoimmunity [3]. Two large-scale NGS studies provide useful information about the identity and functionality of the gut microbiota [2,4]. Although the gut is densely populated with species, there is a rather small variety in the investigated populations. It is now well known that up to 90% of the intestinal gut microbiota is represented by Firmicutes and Bacteroidetes with only minor presence of other phyla as Actinobacteria, Proteobacteria, Fusobacteria and Verrucomicrobia [5].

**Shape-up of the microbiota from birth to adulthood**

We also know that there is no microbiome in a developing foetus. The initial shape-up of the different microbial colonies of the gut begins as early as childbirth. The newborn gets exposed to microorganisms that inhabit the maternal genital tract during normal vaginal delivery (first encounter), maternal faecal bacteria or the skin in the case of Caesarean section. The foetal microbiota gets additionally shaped up by the skin as well as by the breast milk microbiota. At birth the sterile neonatal gastrointestinal tract is colonized by facultative aerobes, with bacteria appearing in the faeces within a few hours of birth. Gradually consumption of oxygen by these bacteria allows the colonization of strict anaerobes, which make up the majority of the adult gut microbiota. Initial colonizers of the gut consist of aerobic organisms, such as *Enterococcus* and *Streptococcus*, which are then replaced with anaerobes such as *Bifidobacterium* and *Lactobacillus* [6]. Subsequently, the infant begins to develop a more mature (adult-like) microbiota dominated by the Bacteroidetes and Firmicutes phyla. The age at which this transition begins to take place is variable and appears to be affected by when the child transitions from a formula/breast-milk diet to table foods.

Many outside factors can influence the colonization event at birth. The type of delivery affects the initial colonization and may have long-term implications for the microbiota. The initial microbiota of infants delivered vaginally bears strong resemblance to their maternal microbiota, whereas that of infants delivered via C-section is more consistent with an adult skin microbiota [7]. Vaginal delivery promotes the infant gut to become colonized with Bifidobacteria. In contrast, infants delivered by C-section harbour bacterial communities found on the mother’s skin surface such as *Staphylococcus*, *Corynebacterium* and *Propionibacterium* spp. [8,9]. Several studies have evaluated whether the mode of delivery is associated with autoimmune disease risk with conflicting results. A Canadian study [10] did not show any such effect, whereas a Danish registry study [11] showed that C-section was associated with small yet significant increases in the risk of a variety of conditions, including inflammatory bowel disease (IBD) and juvenile idiopathic arthritis (JIA).

Juvenile idiopathic arthritis is the most common rheumatic disease in childhood with a prevalence of 16–150 cases per 100,000 children [12]. By definition, it is characterized by arthritis of unknown origin persisting for more than 6 weeks, and starting before the age of 16 years.

The etiopathogenesis of JIA is a challenge incorporating complex network of genetic susceptibility and environmental factors. Exposure to environmental triggers may lead to local tissue damage and/or the release of self-antigens, finally resulting in chronic synovial inflammation. Recently, a large genome-wide association study with more than 2800 JIA cases and more than 13,000 healthy controls, however, indicated
that only 18% of JIA pathogenesis could be attributed to genetic factors [13]. The remaining part should therefore be explained by non-hereditary, possibly environmental influences these children commonly encounter.

**Gut microbiota and the immune system: influence on T-cell differentiation and proliferation and its association with JIA**

It is known that the gut microbiota has the potential to effectively stimulate and direct the host innate and adaptive immune responses in humans by triggering instructional signals to the immune cells, particularly regulatory T-cell (Treg) and T-helper (Th) cell differentiation. On the other hand, immune responses towards self-antigens are hypothesized to be a central event in the development of JIA [12]. Reportedly, there appears to be clustering of memory T cells around antigen-presenting cells in the synovium of JIA patients [12,14,15], with Th1 and IFN$\gamma$+ cells being the most abundant [14]. Other cells detected in the joints of children with JIA are Th17 cells [16]. Apparently various sets of effector T cells play a role in the pathogenesis of JIA [17]. Regulatory T cells (Tregs) have also been implicated in JIA on the basis of their increased presence at the site of inflammation [18]. As previously reviewed [19], these findings have suggested a disease concept in which the balance between regulatory and effector T cells is disturbed, resulting in chronic joint inflammation in JIA [18,20]. Taking into account the well-documented interplay of the gut microbiota with T-cell differentiation and proliferation, the contribution of the microbiome to JIA pathogenesis seems plausible [19].

**Evidence of involvement of gut microbiota in development of arthritis**

Animal models have been critical for understanding how the gut microbiota influences the immune system development [21–25]. Studies of germ-free mice show that the small intestinal mucosal immune system fails to develop properly: T-cell subsets in the gut are abnormal in germ-free mice and there are reductions in Th17 cells in the lamina propria of the small intestine [23] and in Tregs in the colonic lamina propria [24]. Those experiments in germ-free mice revealed that the gut microbiota shapes the intestinal immune system.

One of the first mouse models used to demonstrate that commensal bacteria influence peripheral autoimmunity was the K/BxN spontaneous mouse model of arthritis. K/BxN mice do not develop arthritis if housed in a germ-free environment, which is paralleled by a decrease in the production of Th17 cells compared to conventionally housed K/BxN mice [25]. In this model, monoclonization with segmentous filamentous bacteria alone is sufficient to restore arthritis by inducing the differentiation of Th17 cells in the lamina propria, which can recirculate to the joint to cause arthritic inflammation. Therefore, the gut microbiota is thought to be an important environmental factor in the development of arthritis.

**Relationship between the microbiota contents and JIA**

Multiple studies have addressed the potential role of the microbiome in JIA in particular. The most straightforward explanation by which the microbiota might predispose to JIA is related to its contents. It is known that certain bacteria appear to have the capacity to promote an inflammatory process, while others appear to be protective. Preliminary studies have demonstrated that the composition of the microbiome may be altered in JIA.

Picco et al. [26] used the urinary lactulose/mannitol test to demonstrate altered intestinal permeability in children with JIA. They found it was higher in children with JIA compared to healthy control individuals [26]. Still, data on microbiome composition were lacking at this stage.

Some recent studies using 16S sequencing shed light on the constituents of the faecal microbiota in children with JIA. The first one, performed by a Dutch group, was a pilot study among eight Disease-Modifying-Anti-Rheumatic-Drug (DMARD) – naive polyarticular JIA patients and 24 age-matched healthy controls [27]. The study revealed that the intestinal microbiome diversity within the phylum Firmicutes is significantly lower in children with DMARD naive polyarticular JIA compared to healthy controls. The authors did not find differences regarding the phyla Bacteriodetes and Proteobacteria between the two studied subgroups [27].

Another study evaluating the faecal microbiota in children with newly diagnosed JIA in comparison to healthy controls revealed significantly lower proportion of bacteria belonging to the phylum Firmicutes, as well as a modest, but statistically significant, increase in the Bacteroides genus in JIA [28]. The potential for this genus to demonstrate pathogenicity in arthritis was illustrated by animal models of arthritis, in which the disease is absent in the germfree...
Increased gut permeability.

In addition, Scher et al. [30] identified abundant *Prevotella copri* in many newly diagnosed rheumatoid arthritis patients, demonstrating as well that these bacteria could directly trigger inflammatory responses in mice. This study tested the effect of monocolonization of mice with *P. copri*, in an experimental model of arthritis. The *P. copri*-colonized mice were more susceptible to experimental arthritis.

Furthermore, the role of the microbiome in the pathogenesis of enthesitis-related arthritis (ERA)—another distinct subtype of JIA—was also studied [31–33]. Among children with JIA, alterations in the *Faecalibacterium prausnitzii* levels appear to be at least partially specific to the ERA subgroup [33]. Stoll et al. [33] reported statistically significant reduced levels of *F. prausnitzii* in children with ERA compared to healthy control individuals. This finding of decreased *F. prausnitzii* was consistent with observations that this organism may have anti-inflammatory properties through direct effects on cytokine production [34], as well as through increased production of short-chain fatty acids (SCFAs) such as butyrate. Butyrate and other SCFAs appear to promote regulatory T-cell function [35]. SCFAs serve as major sources of energy for the intestinal enterocytes and also regulate the differentiation of T cells, promoting a regulatory phenotype [36]. Therefore, decreased abundance of *F. prausnitzii* may result in decreased regulation of inflammation. In addition, Stoll et al. [33] identified two distinct subsets of patients, one with markedly elevated abundance of Bacteroides genus and the other with high abundance of *Akkermansia muciniphila*. The significance of the increased abundance of *A. muciniphila* in a subset of JIA/ERA patients, is unclear. Derrien et al. [37] postulated that as this species is known for its capacity to degrade intestinal mucins, decreased production of mucin could potentially be damaging to the gut wall, resulting in increased permeability.

**Relationship between microbiota and increased gut permeability**

The intestinal microbiota may also have local effects on mucosal integrity and intestinal immunity. The intestinal mucosa limits the access of gut bacteria to the lymphoid tissues, thereby preventing dysregulated activation of the local innate and adaptive immune system. As discussed above, increased levels of Bacteroides have been reported in children with JIA [28]. Multiple species in the Bacteroides genus degrade mucin, an important component of primary mucosal defense. It is reasonable that mucin degradation can increase the access of the bacteria to the intestinal immune system, promoting an inflammatory process. Bacteroides, an important group of the anaerobic bacteria dominating the gut microbiota, has been implicated to be essential for the maintenance of functional stability of the human gut.

**Dysbiosis, antibiotics and risk of JIA**

Four major factors are considered to be implicated in dysbiosis: diet, lifestyle, pathogens, and drug (especially antibiotics) use. Significant dose-dependent association between antibiotic exposure and consecutive development of JIA has been found [38,39]. Data from three Finnish national registers showed the association, and demonstrated that the risk increases with repeated exposure. The strongest associations appear to be those with treatment with lincosamides (e.g. clindamycin) and cephalosporins [38]. A study from the United Kingdom showed a similar trend and confirmed that the risk is proportional to the cumulative antibiotic exposure, especially to antibiotics taken within 1 year prior to diagnosis. However, antimicrobial agents targeting pathogens other than bacteria are not likely associated with the development of JIA [39].

**Gut microbiota as a drug target**

Multiple lines of evidence support the potential pathogenic role of gut dysbiosis in JIA which makes gut microbiota a possibly promising new territory for drug targeting [40]. Restoring the balance of the gut microbiota might contribute to the improvement of disease symptoms. The main approaches that have been used to modify the microbiota are probiotics [41–43], the administration of single or multiple strains of beneficial bacteria or yeasts, dietary modification and the use of prebiotics in the form of fibre supplementation and faecal microbial transplantation. Nevertheless, in a study performed by Malin et al. [43], no clinically beneficial effect was found when administering *Lactobacillus* GG strain, daily for 14 days in 30 children with JIA.

In studies performed by Berntson et al. [44,45], the role of exclusive enteral nutrition (EEN) was investigated in JIA. EEN was given for three to eight weeks to seven children with a flare of JIA. The authors concluded that EEN had a significant anti-inflammatory
effect on active joints, Juvenile Arthritis Disease Activity Score 27-JADAS27 and morning stiffness. Bernston et al. [44,45] suggest that a possible explanation for this response to EEN could be that the subclinical inflammation in the gut which also immunologically affects the joints has been treated with EEN. Another important part of the response could be that EEN changes the microbiota to an immunologically more preferable flora. Furthermore, next to the gut microbiome, there are now indications that also the microbiome from other body sites, such as the oral cavity and respiratory tract, may play a role [46].

Conclusions

The current concept for JIA pathogenesis is that certain environmental factors trigger a complex inflammatory response in a genetically susceptible individual. Still, the etiopathogenesis of JIA is a challenge incorporating a complex network with only 18% attributed to genetic factors. The microbiome is an interesting factor that might contribute in the process of autoimmunity through influence on T-cell differentiation and proliferation. A growing body of evidence supports the pathogenic role of gut dysbiosis in JIA, which makes gut microbiota a promising new area for drug targeting. Nevertheless, further longitudinal cohort studies are needed in order to unravel the association between the microbiome and JIA.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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