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Gut microbiota in children and altered profiles in juvenile idiopathic arthritis

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ABSTRACT

Microbial diversity plays a key role in the maintenance of intestinal homeostasis and in the development of the immune system in the gut mucosa. Maybe one of the most important function of our gut microbiota is the immune system education, in particular the discrimination of friends from foes that occurs during childhood. In addition to bacterial antigens, several metabolites of microbial origin have a crucial role in training of the immune system, such as Short Chain Fatty Acids (SCFAs).

There are many evidences on the role of the gut microbiota in rheumatic diseases, in particular modifications of microbiota composition causing dysbiosis that, in turn, can induce gut permeability, and thus immunological imbalance and trigger inflammation. In particular, immune cells can reach extra-intestinal sites, such as joints and trigger local inflammation. Childhood is a crucial period of life for development and evolution of the gut microbiota, especially for the acquisition of fundamental functions such as immunotolerance of commensal microorganisms. For this reason, gut dysbiosis is gaining interest as a potential pathogenetic factor for Juvenile Idiopathic Arthritis (JIA). Here we summarized the studies conducted on JIA patients in which a pro-arthritogenic microbial profiles has been observed; this, together with a depletion of microbial biodiversity, clearly distinguish patients' from healthy subjects' microbiota. Further studies are however needed to better clarify the role of microbiota in JIA pathogenesis.

1. Introduction

The gastrointestinal tract is the district that hosts the greatest number of microorganisms: an estimate of about 100 trillions (10^14), a number 10 times higher than the cells of the human body (10^13). The microbiota brings our idea of "human genome" to a new scale, in fact, quantitatively, the genes of the intestinal microbiota exceed about 100 times the number of genes of the human body. We can say that our biochemical functions are not only the result of the action of our genes, but also that of "our" microorganisms, so we have to study both our genome and our "metagenome" that represents the set of genes of our microbiota. Specifically, the bacterial population inhabiting the human gastrointestinal tract consists of more than 500 bacterial species belonging to four main phyla: Firmicutes and Bacteroidetes, representing almost 80–90%, while Proteobacteria and Actinobacteria about 10–20%; then there are a small part of the Archaea methanogens (mainly *Methanobrevibacter smithii*), eukaryotes (especially the yeasts) and viruses (for example phages). Thus, we are superorganisms and the microbiota is an organ, actually a "super organ".

2. The microbial community: from infancy to adulthood

For several years, it was believed that humans were sterile before birth. Recent studies have instead described that meconium of healthy newborn infants contains a simple microbial community, dominated by genera such as *Escherichia-Shigella*, *Enterococcus*, *Leuconostoc*, *Lactococcus* and/or *Streptococcus* [1–3]. The formation of the first microbial community is strictly dependent from mode of birth, which can substantially influence the composition of the neonatal gut microbiota. Children who are delivered spontaneously present an initial simplified

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gut microbiota similar to that of the mother's vaginal tract, dominated by Lactobacilli. Instead, children born by Caesarean section present a gut ecosystem populated by genera such as *Staphylococcus*, *Corynebacterium* and *Propionibacterium*, very similar to the microbiota of the mother's skin [4,5].

Immediately after birth, the human intestine is soon colonized by a series of microorganisms, with the first colonizers being facultative anaerobic bacteria; the newborn gut is an environment suitable for the colonization of strictly anaerobes, such as *Bacteroides*, *Clostridium* and *Bifidobacterium spp.*. During the first months of life, the intestinal microbiota is therefore characterized by a low richness, with a dominance of Actinobacteria and Proteobacteria phyla. Subsequently the microbiota becomes more complex with greater biodiversity, determined by the increase of Firmicutes and Bacteroidetes phyla [6–8]. Many factors influence the microbial colonization of the gastrointestinal tract; among these, genetics, gestational age, mode of birth, diet during the first months of life (breast milk or formula), and last but not least environmental factors, such as hygiene and possible antibiotic treatment [9,10].

During the first year of life, as a result of the increase in food variety and environmental exposures, microbial biodiversity increases, and so the complexity of the gastrointestinal microbiota too [11,12]. Childhood is a fundamental moment of development and evolution of the gut microbiota, especially for the acquisition of physiological functions. The infant gut microbiota seems to be specialized for the acquisition of nutrients in particular vitamin B and amino acids [12].

The first three years of life represent the most critical period for the growth and development of the child. In fact, changes of the diet (introduction of new food) and consequently intestinal microbiota modifications have the potential to deeply influence the health and host development [4,13–15]. It has been observed that the microbiota of a 3 year old child is 40%–60% similar to that of a healthy adult, reaching a microbial composition in the adolescence comparable to that of an adult [4]. Although the gastrointestinal microbiota of children and adolescents contains many of the same taxa present in adults, significant differences have been reported in the proportions of *Bacteroides* and *Bifidobacterium spp*., as well as members of the *Clostridia* class, and it has now been demonstrated that the adult intestinal microbiome remains stable from the third to the seventh decade of life [16].

Factors affecting the stability and complexity of intestinal microbiota over time, from infancy to adulthood to the elderly include genetics, birth mode, diet, geography, hygiene, stress, medications. Some of these factors may introduce perturbations leading to phenomena of microbial dysbiosis. This disturbing event introduces the concept of "resilience" which refers to the amount of perturbation that a microbial system can tolerate and the ability to restore the state of equilibrium [17]. In the healthy gut, biodiversity, resilience and stability contribute to important physiological processes including protection against pathogens and education of immune system [18].

3. Host-microbes co-evolution, intestinal homeostasis and dysbiosis

Human beings, like all animals, represent a complex structure in which various microbial ecosystems are established in the various districts of the body (e.g. skin, vagina and intestine). The intestine is the most densely populated district by microorganisms (10^14 bacteria). During evolution, changes in length and the partitioning of the digestive tract allowed vertebrates to occupy different habitats and exploit different nutritional strategies. Many of these changes in bowel physiology have been driven by the need to optimize basic physiological and biological functions, such as nutrient uptake [19]. The animal and human host and their microbiota have evolved over the millennia in a homeostatic and symbiotic relationship. The normal functioning of the digestive and immune system depends on the presence of non-pathogenic "beneficial" bacteria and the homeostasis of the intestinal microbiota is characterized by the coexistence of various commensal microbial species.

The "healthy" status is represented by a high level of intestinal microbial richness, while the disease status is characterized by the massive reduction of intestinal microbial species, as observed in several inflammatory and autoimmune diseases [20,21]. In an ecological context, biodiversity creates an ecosystem able to resist to perturbations deriving from the external environment [22]. In fact, competitive interactions between microbial species can help to maintain the stability of the intestinal microbiota [23]. Understanding how the "healthy" microbiota gives rise to a stable and resilient state would allow strategies to increase resilience in disease conditions.

4. The microbiome and immune training

Microbial diversity plays a key role in the maintenance of intestinal homeostasis and in the development of the immune system in the gut mucosa. Maybe one of the most important functions of our microbiota is the immune system education, in particular the discrimination of friends from foes. In addition to bacterial antigens, several metabolites of microbial origin have a crucial role in training of the immune system, such as Short Chain Fatty Acids (SCFAs). SCFAs are known as fuel for colonocytes, but provide important immune protective functions. For example, acetate was found able to regulate intestinal homeostasis, by stimulation of the proliferation of Treg cells in the lamina propria [24], and butyrate have several immunological functions associated to potent regulatory effects on gene expression, promoting epithelial barrier integrity [25].

Regarding correlation between gut microbiome and immune responsivity, some studies showed important new insights on underlying molecular effects of the microbiome on immune responses [26], the role of environmental and genetic factors on T and B cell immune traits [27] and genetic and host/environmental factors influencing cytokine responses [28,29]. Combining large-scale analysis of immune variability with accompanying functional information allows examination of immunity at an unprecedented level. These studies provide for the first-time insights and reference values for future studies addressed at understanding how the microbiome affects (the interaction between) inflammation and autoimmune disorders and how the metabolome is involved in this interaction.

The breaking of the microbial balance generates intestinal dysbiosis, characterized by an altered composition of the microbial populations with consequent continuous immunological stimulation that leads to abnormal immune responses that often originate an inflammatory status. Dysbiosis has been described in many gastrointestinal diseases, such as inflammatory bowel diseases (IBD), celiac disease, irritable bowel syndrome (IBS), antibiotic-associated diarrhea, tropical enteropathy and many others [30–33]. Recently, numerous evidences suggest that the intestinal dysbiosis is not limited to gastrointestinal diseases, suggesting that intestinal bacteria can influence the systemic immune response. In fact, studies have shown intestinal dysbiosis in relation to metabolic syndromes (obesity, diabetes), chronic periodontitis, vaginosis, atopic diseases, rheumatic diseases, Alzheimer's disease, autism and others [34–37]. The loss of a correct homeostasis of the intestinal immune system, together with the increased intestinal permeability, have been reported to be closely correlated with alterations of the microbiota that make the host more susceptible to the onset of intestinal diseases [38]. During aging, processes of chronic inflammation are characterized by loss of gastrointestinal function, alteration of host immune response and consequent chronic gut inflammation. The characteristics of an unbalanced microbiota include an increase in gram-negative bacteria linked to an environment with high oxidative stress, with inflammation and production of pro-inflammatory metabolites. Often these variations appear to be diet-dependent and strongly correlated with health indicators, including susceptibility markers, as well as inflammation markers [39].

However, while disruption of intestinal homeostasis can be widely recognized, it is not clear whether dysbiosis represents a cause or a consequence of the disease. Several studies have shown differential factors contributing to dysbiosis, including host genetics (mutations in genes involved in intracellular recognition of microbes, such as the *NOD2* gene, or in genes related to regulatory or pro-inflammatory immune responses), lifestyle (diet, stress, hygiene), exposure to microorganisms and pharmacological treatments, such as antibiotics. Mutations in genes involved in mechanisms of immune regulation or in the pro-inflammatory pathways could lead to uncontrolled inflammation in the gastrointestinal tract. In presence of dysbiosis, there is an abnormal composition of the microbiota, which often results in a reduction in the number of symbionts and/or an increase in the number of pathobionts. The result is a nonspecific inflammation that can predispose the genetically susceptible individual to disease states characterized by inflammatory responses mediated by T-helper cells [40]. It is also hypothesized that inflammation alone influences the composition of the microbiota favoring the pathobionts [40].

A healthy microbiota contains a balanced microbial composition characterized by symbiotic bacteria carrying beneficial functions to the host such as commensal bacteria and permanent residents, that do not bring any benefit or harm to the host, as well as pathobiont bacteria, permanent bacteria with pathogenetic potential, but strictly under control of the symbionts and the host. During the dysbiosis, however, the number of symbionts decreases, while the number of pathobionts increases [40]. Furthermore, hygiene and excessive use of antibiotics, unable to distinguish between pathogenic or symbiotic/commensal microorganisms, could negatively affect the microbiota [40]. In this context, the “old friends” hypothesis proposed in 2003 by Rook et coworkers [41] and the “Hygiene hypothesis” [42] can explain the relationships between microbial exposure and development of autoimmune diseases. The “old friends” hypothesis refers to those microorganisms that evolved together with the human immune system, in the Paleolithic era, at the time of hunter-gatherers, and that therefore influenced the evolution and development of the human immune system [43].

The “hygiene hypothesis” (also called the “theory of biome depletion” and the “lost friends theory”) states that the reduction of important microbial patterns necessary to educate the host's immune system results in a decrease in intestinal microbial richness with consequent alterations in processes involving host-microorganism interactions, capable to promote the onset of disease in susceptible hosts [42,43]. Therefore, a sterile environment or excessive hygiene, typical of Western developed countries, allows us to protect against exposure to dangerous pathogens, but at the same time to avoid exposure to so-called “old friends” [44–46].

Many subjects living in traditional rural communities are continuously exposed to a number of deadly infectious diseases, such as malaria or gastrointestinal or respiratory tract infections. These infections may have a chronic effect on inflammation, as it has recently been shown that brief stimulation of monocytes to microbes induces a prolonged hyper-inflammatory state, which has been named trained immunity, and which is mediated by profound reprogramming of the intracellular metabolism and epigenetic reprogramming [47]. Alterations in trained immunity processes could set important stages in autoimmune disorders.

A limited exposure makes the immune system immature, especially during childhood and can promote the development of allergies and autoimmune diseases, while early exposure to ancient environmental microorganisms, the so-called “old friends”, is essential to “train” our immune system, preventing the attack of non-legitimate targets that is the starting event of autoimmune disorders.

5. The impact of diet on the intestinal microbiota

Numerous studies have shown that the mutual relationship between the intestinal microbiota and the host is strongly influenced by the diet. The consumption of various nutrients in fact affects the composition and structure of microbial communities as it provides substrates for microbial metabolism itself.

From childhood, the biggest change in the composition of the microbiota occurs when solid food is introduced. At weaning, a shift towards a more stable microbiota was observed, similar to that of an adult [11]. In this period of life, the concept of association between diet, commensal bacteria and health can be introduced. Numerous studies have described differences in the composition of the gut microbiota in formula or breastfed infants [48–50].

In human milk, there are important bioactive compounds, in particular the indigestible oligosaccharides such as fructooligosaccharides (FOS) and galacto-oligosaccharides (GOS) that contribute to the absorption and digestion of nutrients, to the immune protection and to the selective promotion of the growth of the *Bifidobacterium* genus [48,49,51–53]. *Bifidobacterium* is linked to the fortification of the intestinal mucosal barrier, to the protection against pathogens and to the modulation of the intestinal immune system [54–56]. Another difference is that aerobic microorganisms appear to be more prevalent in the feces of breast-fed infants, whereas the facultative anaerobic and anaerobic microorganisms, which preferentially use anaerobic glycolysis, are more commonly found in the feces of formula-fed infants [11].

The different food sources have therefore guided the evolution of human beings where co-evolution with symbiotic microorganisms has given rise to genuine mutualistic relationships. A comparison between the intestinal microbiota between different primates and mammals has shown that humans are grouped together with omnivorous primates compared to non-primate species [19], suggesting how the variety of foods in an omnivorous diet is the most important factor of the gut microbiota evolution.

Significant variations in the composition of the microbiota have been associated with the consumption of dietary fibers of fruits and other vegetables, compared to a diet rich in animal proteins, simple sugars and lipids. In controlled dietary experiments in healthy volunteers, variations in the intake of resistant starches or non-starch polysaccharides have been linked variations of specific bacterial taxa, such as *Ruminococcus bromii* and *Eubacterium rectale* [57]. By *in vitro* analysis of human fecal samples, these taxa have been shown to selectively metabolize specific substrates consisting of indigestible carbohydrates [58]. The model systems have in fact shown that an important function of the microbiota of the intestine consists in its ability to ferment complex carbohydrates and polysaccharides, with the consequent production of SCFAs [59]. Additional sources of glycans for the metabolism of the intestinal microbiota are also represented by the mucus at the level of the intestinal mucosa produced by the host.

In recent years, thanks to studies carried out on different populations all over the world in non-urbanized environments, it has been possible to understand the role of diet in determining the composition of the intestinal microbiota. In several studies fecal microbiota from traditional populations living in rural, non-industrialized societies were compared with that of populations characterized by a typically Western lifestyle [12,60–64].

Our study [60] showed for the first time that the intestinal microbiota of children living in the African rural village of Burkina Faso was completely different from the microbiota of children living in Italy, as representative of a modern Western country. By using the Next Generation Sequencing technique, we analyzed the composition of the intestinal microbiota of healthy children aged 1–6 years who live in a rural African village in Burkina Faso, whose life conditions are very similar to those of Neolithic. The diet of these children is mainly vegetarian, consisting of cereals such as millet, sorghum, vegetables and legumes. The results were compared with those obtained from a population of Italian children of the same age having a typically Western diet. The microbiota of children from Burkina Faso have greater biodiversity compared to Italians, especially bacteria that are able to digest

cellulose (*Prevotella, Xylanibacter, Butyrivibrio* and *Treponema*). These bacterial genera allow to maximize the extraction of energy from non-digestible food polysaccharides, and return beneficial compounds for our intestines, such as SCFAs, in particular butyrate, a powerful natural anti-inflammatory. Moreover, despite the fact that these children live in precarious hygiene conditions and are subject to a high rate of infectious diseases, they present a reduced number of potentially pathogenic bacteria, such as *Escherichia coli/Shigella*, which are instead present in the feces of Italian children.

Furthermore, we have also shown how feeding is responsible for the composition of microbiota; in fact, children of 1–2 years old still breastfed, belonging to both populations, showed a similar microbial profile with a predominance of *Bifidobacterium*.

Subsequently, other studies demonstrating the impact of diet on the intestinal microbiota were conducted on geographically isolated populations residing in Amazonian regions (Venezuela), rural Malawi populations [12], and on Bangladeshi children [65], as well as a study of populations of Papua New Guinea in comparison with residents of United States of America [63]. An interesting work carried out on the last hunter-gatherer populations, the Hazda from Tanzania, reconfirmed how the diet plays a key role in the determination of the intestinal microbial structure and how specific microbial profiles are acquired with specific metabolic functions [62].

In order to understand how long the diet is able to modify the composition of the intestinal microbiota, David and coworkers [66], performed a study on 10 volunteers who followed a strictly vegetarian diet for 5 days, moving then on to a strict carnivorous diet in the 5 days later. Interestingly, in 24–48 h the composition of the microbiota changed significantly: during the vegetarian diet, bacterial species able to ferment complex carbohydrates prevail, while during the diet based on animal proteins bacterial species are selected, such as *Bilophila wadsworthia*, able of metabolizing proteins and toxic compounds deriving from the burning of meat, which have a well-known strong pro-inflammatory potential.

More recently, our study on Burkina Faso populations [61] has examined the effect of diet, environment and lifestyle when people move from a rural environment to urban areas, experience improved socio-economic conditions and are exposed to a “globalized” Western type diet. Once again, we have shown that urbanization and changes of dietary habit play a role in shaping gut microbiota, independently to ethnicity. Furthermore, we demonstrated that ancient microorganisms, especially fiber-degrading bacteria, are at risk of being eliminated by the fast-paced globalization of foods and by the advent of westernized lifestyle. Thus, these results can help to understand the role of microbiota in the epidemiology of non-communicable diseases.

In addition, the diet has also been associated with other types of intestinal microorganisms, such as Archaea and fungi [67,68]. For example, carbohydrate consumption is associated with the abundance of *Methanobrevibacter* [68]. This archaeon can increase the production of gas and SCFAs by metabolizing hydrogen by fermentation of carbohydrates, and thus playing an anti-inflammatory role [69]. Likewise, the diet could also influence intestinal fungal communities, which have been associated with the pathogenesis of IBD. Evidence has shown that alteration of immune response to fungi could contribute to chronic inflammation, as occurs in Crohn's disease [70–72].

Moreover, the MetaHIT Consortium proposed to categorize the intestinal microbiota in “enterotypes” [73]. People can be classified as having a predominance of *Prevotella*, *Bacteroides* or *Ruminococcus* in the intestinal microbiota. These enterotypes were related to a diet rich in animal protein and fat (*Bacteroides*) and a diet rich in carbohydrates and simple sugars (*Prevotella*) [74]. There has been considerable discussion about enterotypes; some data sets support the existence of these categories, while others do not [17], suggesting that the detection of enterotypes depends on the computational approach used to analyze data sets [75]. It is suggested that a better term could be “enterogradient”, based on the dominance of *Bacteroides* or *Prevotella*. It seems that these two genera largely do not exist in equal proportions in the human gut [76]. A larger proportion of *Prevotella* in the human intestinal microbiota could be considered a marker of rural or traditional culture, while a higher percentage of *Bacteroides* is associated with industrialized countries [73]. Overall, these studies demonstrated the co-evolution between diet and microbiota in rural areas and urban societies, showing the effect of Westernization on the loss of traditional microbes [60,66,74,77] and supporting the hypothesis of a consequent depletion of bacterial wealth in relation to metabolism (obesity, insulin resistance, dyslipidemia) and inflammatory disorders.

6. Alterations to the gut microbiota induced by antibiotic treatment

Several concerns have risen from the antibiotic use in treating pathogenic infections, due to the capability of alteration of the diversity and functional capacity of the dominant gut microbiota [78,79]. Although benefits that antibiotics have brought to medicine are undiscussed, their use represents an example of environmental pressure on resident microbiota, able to largely disrupt the microbial environment, promote secondary infections by opportunistic pathogens and spread antibiotic resistance. In fact, antibiotics do not selectively target pathogens, but they also unavoidably affect the viability of non-pathogenic and resident species that contribute to host health.

For example, Dethlefsen and coauthors [80] describe how ciprofloxacin treatment influenced the abundance of a third of the gut bacterial taxa, decreasing the taxonomic richness, diversity, and evenness of the community, though inter-individual variation in the response to this antibiotic was observed. Others antibiotics, such as fluoroquinolones and β-lactams, administrated for seven days, were responsible of significantly decreasing microbial diversity and the core phylogenetic microbiota [81]. Moreover, in infants, combination of ampicillin and gentamicin for 4 weeks induced a significant increase of Proteobacteria and reduction of Actinobacteria compared to an untreated group [82].

Intergenerational transmission of bacteria during birth initiates the natural successional development of the intestinal microbiota in all mammals. Aloisio and collaborators [83] evaluated the effects of intrapartum antibiotic prophylaxis (IAP) on gut microbiota of newborn, routinely used in a group B Streptococcus positive-women to prevent the infection of infants. The authors found significant differences in the microbial composition of neonates born to mothers who had received IAP, with a lower abundance of Actinobacteria (in particular *Bifidobacterium spp.* strains) and Bacteroidetes, as well as an over-representation of Proteobacteria.

Korpela and collaborators [84] indicate that it is possible to correct undesired changes in microbiota composition and function caused by antibiotic treatments in infants by supplementation of a probiotic mixture together with at least partial breastfeeding.

More recently, Tulstrup et al. [85] demonstrated that the development of the gut microbiota can be disrupted by antibiotic exposure, potentially affecting early-life microbiota-dpendent metabolic programming. In particular, the authors demonstrated that in animal model early-life exposure to an antibiotic-perturbed and low-diversity microbiota is sufficient to cause changes in body weight persisting into adulthood.

Only recently it was appreciated the role of microbiota on host physiology and the associations between antibiotic use and numerous diseases. Antibiotic treatment may disrupt microbe–microbe, as well as microbe–host interactions that represent key mechanisms for the maintenance of homeostasis within this ecosystem. Commensal bacterial communities confer protection to the host through the inhibition of growth of pathogenic bacteria and competition for ecological niches, and through stimulation of host immune system that enhances its antimicrobial activity. Direct inhibition of pathogen expansion by commensal bacteria can derive by: (i) competition for nutrients, (ii)

production of antimicrobial peptides and toxins, (iii) stimulation of host defense mechanisms [86,87].

In a review of Arvonen and coworkers [88], overview of human studies evaluating long-term changes to the microbiota following exposure to antibiotics was described. Although methods of investigation of gut microbiota alteration were different among the studies, in many instances it resulted that antibiotics do impact the microbiota long-term, even up to two years [87], and it seems that antibiotics directed against anaerobic microorganisms have a lasting impact than others.

Expansion of pathogenic microorganisms can be suppressed by the presence of surrounding commensal populations that compete for similar nutrient sources. The expansion of antibiotic-resistant strains and/or the acquisition of antibiotic resistance can contribute to permanent alterations of the microbiota composition, potentially leading to loss of beneficial and/or increase of deleterious species. Moreover, reduced stimulation of the immune system by commensal bacteria could damage the correct education/training of the immune system, leading to the development of immune responses against self or harmless antigens, thus predisposing the host to autoimmunity.

Atopic, inflammatory and autoimmune diseases have been linked to gut microbiota dysbiosis [89–93] and, in some cases, significant associations have been found between antibiotic use and these diseases during early life, a critical period for maturation of the immune system and establishment of immunological tolerance [94,95]. Despite the relative paucity of data related to antibiotic exposures and autoimmune disorders, some studies showed that the number of courses of antibiotics administered during childhood is associated with risk of juvenile rheumatoid arthritis [96,97] and of inflammatory bowel disease [98]. In the review of Arvonen et al. [88], the coauthors also analyzed the two registry-based case controls studies [96,97] on JIA patients and healthy controls with the aim to evaluate whether antibiotic use affects subsequent risk of JIA, and performed a regression analysis of the data, observing that exposure to different antibiotic categories seems to be associated to higher risk of JIA compared to exposure to a limited number of antibiotics. One of the main objectives of scientific research in pediatric dysbiosis will be to understand how broad spectrum of antibiotic usage in children may disrupt normal development of the gut microbiota, and consequently the immune system, potentially leading to increased risk of inflammatory and autoimmune diseases.

7. The gut microbiota and autoimmune diseases

In economically developed countries, a progressive increase of metabolic, inflammatory and autoimmune diseases, such as obesity, type I diabetes, inflammatory bowel disease (IBD), allergies, multiple sclerosis, autism, arthritis and rheumatic diseases, have been recorded, particularly in pediatric populations. Modern lifestyles, in particular “Western” diet (high-fat/high-sugar diet), processed and refining food, urbanization, pollution, sanitation and antibiotic treatment have modified the gut microbiota composition and related metabolic functions, with respect to diet and lifestyle and gut microbial profiles of traditional and isolated populations worldwide [60,61,66,99–104].

Evidence showed that loss of beneficial symbionts, deriving by dysbiotic microbiomes, predispose to non-communicable diseases [63,105–107]. In the last decade, alteration of gut microbiota was observed in rheumatic disease and arthritis, most notably rheumatoid arthritis (RA), psoriasis, and the related spondyloarthritides (SpA), including ankylosing spondylitis (AS) and reactive arthritis (ReA), similarly to IBD, in which gut bacteria have a role in the etiopathogenesis [104,108,109]. In early life, some studies showed that artificial feeding is associated with an increased risk of autoimmune disease, such as ankylosing spondylitis (SpA) [110]. While, breast-feeding appears to be protective against JIA, as showed in some studies involving JIA children and healthy subjects [111–113].

The intestinal microbiota can modulate either local mucosal immune system or influence the systemic immune response. Of great interest is the understanding if dysbiosis is as a trigger or a reflection of autoimmune and inflammatory disorders [114,115].

Studies in germ-free (GF) and specific pathogen-free (SPF) animal models [116,117], together with a wide body of literature on effect of administration of antibiotics [118] or probiotics in modulation of gut microbiota, and recently the fecal microbiota transplantation model [119,120] have highlighted the role of the microbiota in immune homeostasis. In particular, microbial communities have the potential to shape the innate and adaptive immune responses [121–124].

In animal models, mice reared in germ-free conditions are healthy, the introduction of some bacterial species can have inflammatory or anti-inflammatory effect directly promoting on inhibiting the development of inflammatory disease, such as autoimmune disease or IBD. It was hypothesized that, during intestinal inflammation, increased mucosal uptake of gut bacteria and their membrane molecules, such as Lipopolysaccharides (LPS), peptidoglycan-polysaccharides (PG-PS) lead to systemic diffusion of these bacterial components and subsequent extra-intestinal inflammation, such as arthritis [125]. This hypothesis was also supported by historical studies showing reactivation of arthritis by systemically injected LPS or PG-PS [126,127] and the founding of bacterial antigens, such as that of *Yersina spp*. and *Salmonella spp.*, within synovial leukocytes in patients with B27-associated reactive arthritis [128,129].

Some studies demonstrated that, not necessarily pathogenic bacteria, but also gut microbial communities are sufficient to induce joint inflammation [122,130]. For example, Rath and collaborators [122] identified subpopulations of intestinal bacteria playing a crucial role in the pathogenesis of spontaneous colitis and peripheral arthritis in genetically susceptible B27 transgenic rats. In particular, in animal models of arthritis, upon germ-free conditions, the re-introduction of *Bacteroides spp.*, especially *B. vulgatus* and *B. fragilis*, reactive articular inflammation [122,131]. Recently, mice colonized with *Prevotella copri,* a bacteria species found abundant in new onset RA patients, showed increased sensitivity to dextran sodium sulfate (DSS)-induced colitis and increased inflammation [132].

Moreover, in autoimmune and inflammatory diseases, the gut microbiota can induce alteration of mucosal permeability and reduction of immune tolerance to commensals [123,133–135]. Tailford and colleagues [136] showed that *Bacteroides spp.* and *Akkermansia muciniphila* were able to increase access of the bacteria to the intestinal immune system, promoting inflammatory processes. The former bacterial genus, thanks to enterotoxin production [137], and, the latter, through mucin degradation, can increase mucosal permeability and internalization of enteric bacteria in the mucosa [138–140]. Along those lines, increased intestinal permeability has been found both in JIA patients [40] and in adults with ankylosing spondylitis [141].

The gut microbiota also plays an important role in the adaptive immune system. An animal model of spontaneous arthritis, the K/BxN mouse, explained the potential role of the microbiota and the missing links with systemic immunity and autoimmune arthritis [116]. K/BxN mice reared in GF conditions showed reduction of arthritis. Commensal bacteria can induce lamina propria Th17 cells and activate inflammation on the joints by migration of T cells into the peripheral lymphoid tissue [142]. Secretion of IL-17, in turn, acts directly on B cells to induce production of autoantibody that, finally, lead to the development of disease. It was reported that segmented-filamentous bacteria (SFB) into GF K/BxN mice drive autoimmune arthritis through induction of the Th17 cells [116].

Therefore, although it was hypothesized that gut bacteria induce systemic immune response through soluble factors circulating from the gut into the periphery, the study on K/BxN mice showed that microbiota-derived products could influence the immune system at distal sites without leaving the gut. Thus, T cells whose functions are dictated by intestinal commensal bacteria can be effectors of pathogenesis in tissue-specific autoimmune disease.

As mentioned above, diet is one of the main factor contributing to

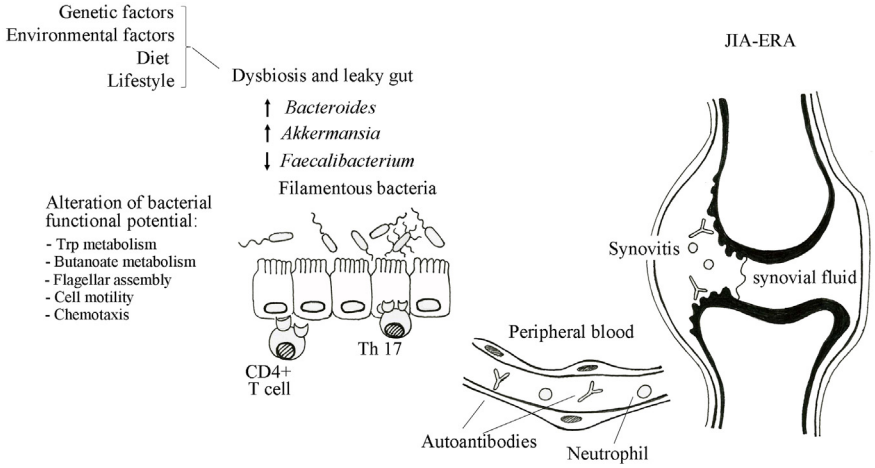

Fig. 1. Role of gut microbiota in development of JIA-ERA. Genetics and environmental factors, diet and lifestyle affect the stability and complexity of the gut microbial profiles causing dysbiosis and gut epithelial permeability. Dominance of pathobionts could be responsible of alteration of both microbial community composition and bacterial functions. Colonization of pathobionts in the gut induce differentiation of Th17 cells producing IL-17, which subsequently migrate from the gut to peripheral lymphoid tissue. IL-17, in turn, acts directly on B cells promoting differentiation of germinal center B cells in systemic organs with production of autoantibody that can circulate into target organ joints, contributing, finally, to the development or maintenance of inflammation.

modulation of gut microbiota composition.

Some disorders, such as celiac disease and obesity, have an evident association with diet and with triggering of arthritis and rheumatic disease [143,144]. Evidences on the role of the gut microbiota in rheumatic diseases lead to suppose that diet, affecting the microbiota composition, in turn, can influence these disorders [145,146].

A great body of literature showed that changing of dietary habits and lifestyle led to modifications of microbiota composition, causing dysbiosis that, in turn, can induce gut permeability, and thus immunological unbalance and trigger of gut inflammation. In arthritis condition, immune cells can reach extra-intestinal sites, such as joints and trigger local inflammation (Fig. 1) [147,148].

8. Altered microbial profiles in juvenile idiopathic arthritis

Pediatric rheumatic diseases consist of a heterogeneous group of autoimmune conditions, most of which are chronic systemic illnesses that can determine serious morbidity and long-term disability [149]. The pathogenesis of rheumatic disorders is still under investigation, and currently it is believed to originate from a complex interaction between host genetics, immunological dysregulation and environmental influences [149]. Intestinal microbiota affects many physiological processes, having a regulatory effect on intestinal permeability and a modulatory effect on host immune system and self-tolerance acquisition [150]. As above showed, altered composition and function of gut microbiota are deemed to play an important role in the development of many autoimmune chronic diseases, including Inflammatory Bowel Diseases (IBD) [151,152], Type 1 Diabetes [92,153] and chronic arthritis, such as rheumatoid arthritis (RA) and ankylosing spondylitis (AS) [132,154]. In the field of Pediatric Rheumatology, gut dysbiosis is gaining interest as a potential pathogenetic factor for Juvenile Idiopathic Arthritis (JIA).

JIA is the most common chronic rheumatic disease of childhood, with a reported prevalence between 16 and 150 per 100.000 [155]. The term JIA does not refer to a single disease but rather encompasses a group of related conditions characterized by chronic joint inflammation and variable systemic involvement [155].

According to current classification [156], there are seven different JIA categories: oligoarticular (involving less than 4 joints at onset); polyarticular (involving more than 4 joints at onset) rheumatoid factor positive and negative; psoriatic; enthesitis related arthritis (ERA); systemic onset JIA; undifferentiated arthritis. Picco and coworkers [157] have indirectly suggested a potential role of intestinal dysregulation in JIA, demonstrating an increased gut permeability in JIA patients. Moreover, an association between antibiotic use and development of JIA has been proven and may be related to alteration in host microbiota [96,97].

In the era of *Next Generation Sequencing,* studies have focused on the composition of fecal microbiota in a particular subset of JIA- ERA, as summarized in Table 1. ERA accounts for 10–20% of JIA and it is considered the equivalent of adult SpA. Clinically, it is characterized by the association of arthritis of large joints in lower extremities, entheses inflammation and common involvement of sacroiliac joints and axial skeleton [155]. ERA shows strong correlation with the presence of Human Leukocyte Antigen-B27 (HLA-B27) and with a family history of HLA-B27 related diseases, namely AS, acute anterior uveitis, sacroiliitis related to IBD [156]. Interestingly, some degree of intestinal inflammation is present in almost two thirds of pediatric SpA patients [158] and, given this association, alteration in fecal microbiota has been identified as a potential trigger for ERA that could drive gut and joint inflammation (Fig. 1).

The most of the studies conducted so far and proposing relationships between gut microbial profiles and autoimmune diseases are mostly associative. Stoll and colleagues [115] evaluated the content of fecal microbiota in a cohort of 25 children with ERA and 13 healthy controls. The study demonstrated a decreased presence of *Faecalibacterium Prausnitzii* in ERA patients compared to controls, and an increased abundance of *Bifidobacterium*, mostly *B. adolescentis*. These findings are consistent with previously reported data on IBD patients [159,160], as well as in other diseases [37] and could suggest some similarities in the pathogenesis of these conditions.

Moreover, two other bacterial strains, *Akkermansia muciniphila* and *Bacteroides* showed an increased abundance in patients versus controls, though not statistically significant. *A. muciniphila* is considered a commensal microorganism that does not cause inflammatory diseases, but is able to degrade intestinal mucin, thus increasing intestinal permeability and contributing to arthritic inflammation through disruption of tolerogenic state between the mucosal immune system and intestinal microbiota [138].

In a recent study of Stoll and coworkers [161], fecal samples were collected from 30 ERA patients and 19 healthy children, as well as 11 SpA and 10 healthy adults. Decreased levels of *F. prausnitzii* A2-165 strain were confirmed in ERA patients, and similar trends were observed in SpA adult patients. Findings of Stoll and coworkers suggest that depletion of *F. prausnitzii* could play a role in the pathogenesis of ERA and SpA. As a matter of fact, *F. prausnitzii* is a producer of SCFAs including butyrate, a compound with known anti-inflammatory and regulatory effects [162]. Whole Genome Sequencing study by Stoll and collaborators [161], analyzing the gut microbial communities at functional level, revealed a decreased representation of the butanoate pathway in microbiota of ERA patients, indicating a decreased capacity to synthesize this anti-inflammatory agent.

Furthermore, *Bacteroides* levels were similar in ERA children and controls, but *B. fragilis* was four times increased in ERA, consistently with their previous findings, while opposite trends were described for

Table 1
Summary of studies on fecal microbiota profiles in JIA in comparison with healthy children and adult SpA and controls.

Cohorts	Summary of fecal microbiota profiles	Findings on gut microbial functional profiles	References
25 children with JIA-ERA and 13 healthy subjects from USA	in ERA patients: - Reduction of *Faecalibacterium prausnitzii*, - Increase of *Bifidobacterium adolescentiis* - Trend of abundance in *Akkermansia muciniphila* and *Bacteroides*	not performed	Stoll ML et al., 2014 [115]
30 new onset JIA patients from Finland	in JIA patients: - Low abundance of Firmicutes and high abundance of Bacteroidetes (*Bacteroides* genus). - High abundance of Actinobacteria and Fusobacteria. In controls: - Trend of abundance of *Veillonella*, *Streptococcus*, *Coprococcus*, *Lachnobacterium* and *Anaerostipes.*	not performed	Tejesvi MV et al., 2015 [167]
29 JIA (19 ERA vs 10 polyarticular-JIA; nERA) and 29 healthy subjects from Italy	in both JIA subtypes: - Abundance of *Ruminococcaceae* compared to healthy subjects. in ERA patients: - Reduction in *Clostridiaceae* and *Peptostreptococcaceae* - Increase of *Clostridium cluster XIVb* in JIA-nERA: - Increase in *Veillonellaceae* - Trend of decrease in *Faecalibacterium*. HLAB27 positive patients: Abundance of *Bilophila, Parvimonas, Oscillibacter*. HLAB27 negative patients: Abundance of *Haemophilus* and *Eggerthella.*	JIA-ERA metagenome was enriched in bacterial functions related to cell motility, flagellar assembly and chemotaxis– > promotion of bacterial migration and invasiveness through mucosal barrier	Di Paola M et al., 2016 [168]
99 new onset, treatment–naïve JIA patients from Italy (n = 78) and Holland (n = 21)	In Italian JIA children: - Increase of *Erysipelotrichacea, F. prausnitzii, Parabacteroides, Ruminococcaceae, Phascolarctobacterium* and *Dorea.* - Decrease of *Allobaculum, Gemellaceae, Propionibacterium acnes, Enterococcus, Turicibacter, Blautia*and *Barnesiellaceae* in Dutch JIA patients: - Eggerthella *lenta, Rikenellaceae, Coprobacillus* and *Mogibacteriaceae* discriminate from healthy controls, but no significant differences were found. No significant differences between active and inactive disease.	not performed	van Dijkhuizen P., et al., 2018 [177]
24 JIA-ERA patients (14 with new diagnosis) and 19 controls	no significant differences in microbial profiles were found between ERA and controls	in ERA patients: - down-regulation of tryptophan catabolism: metabolites of tryptophan pathway play an immunological role by altering CD4 T cell function and promoting development or maintenance of inflammation in ERA.	Stoll ML et al., 2016 [178]
33 JIA-ERA patients and 14 healthy controls from India	in ERA patients: - Abundance of *Bacteroides*, *Enterobacteriaceae* (*Klebsiella* genus) and *Enterococcaceae.* - Reduction of *Prevotella.*	not performed	Aggarwal A et al., 2017 [163]
30 JIA-ERA patients (n = 23 Caucasian, n = 3 African-American, n = 2 Asian) and 19 healthy children (n = 17 Caucasian, n = 2 African-American), 11 SpA patients (n = 9 Caucasian, n = 2 African-American) and 10 healthy adults (n = 6 Caucasian, n = 4 African-American)	in ERA patients and in SpA adult patients: - reduction of *F. prausnitzii A2-165 strain.* in ERA patients: - four-fold increased abundance of *Bacteroides fragilis* compared to adult healthy subjects	in microbiota of ERA patients: decreased representation of the butanoate pathway– > reduction of SCFAs production	Stoll ML et al., 2018 [161]

adult patients. Interestingly, *Bacteroides* abundance was increased in ERA patients also in an Indian study by Aggarwal and colleagues [163]. Increased *Bacteroides* in pediatric patients, but not in adults, could suggest that this bacterial strain plays a role in JIA due to its influence on immune system development, as already hypothesized for other autoimmune pediatric diseases [164]. In their study, Aggarwal and collaborators also found higher levels of *Enterobacteriaceae*, especially *Klebsiella* genus, consistent with previous data on AS [165], and of *Enterococcaceae*, similarly to findings in IBD mouse models [166]. Moreover, *Prevotella* was less abundant, but this was likely related to differences in diet of the Indian population. In this study, oral probiotic were administered to a small sample of ERA patients in order to evaluate possible modifications in gut microbial profile, but no differences were identified after a period of four months of treatment.

Furthermore, in a Finnish work the authors evaluated [167] microbiota profiles of fecal samples from 30 new onset JIA patients, that were not treated with corticosteroid and disease-modifying anti-rheumatic drugs, finding a lower relative abundance of *Firmicutes* and higher presence of *Bacteroidetes,* especially bacteria belonging to *Bacteroides* genus, in JIA, in agreement with the previously discussed results from Stoll [115] and Aggarwal [163] in JIA-ERA. The authors found also high abundance of *Actinobacteria* and *Fusobacteria* in JIA patients and *Lentisphaerae* in controls, suggesting that JIA patients present specific microbiota profile.

In our recent study on Italian children [168], we compared fecal microbiota of patients affected by two different categories of JIA (19 ERA vs 10 polyarticular-JIA) with a cohort of 29 healthy subjects. We found that microbial biodiversity between the two JIA categories was markedly reduced with respect to healthy subjects, suggesting a correlation between disease status and biodiversity depletion, as previously reported in psoriatic arthritis and IBD patients [152,169]. Regarding the taxonomic differences, *Ruminococcaceae* were found increased in both JIA categories, while *Peptostreptococcaceae* and *Clostridiaceae* were reduced only in ERA patients, and *Veillonellaceae* abundant in JIA-non ERA, consistent with previously reported data on AS [134]. Enrichment of *Clostridium* genus abundance was found in ERA patients, while in the oligo JIA a decrease in *Faecalibacterium* genus was found compared to ERA and healthy subjects, though not statistically significant. The latter finding partially differs from previously discussed Stoll's findings, where *Faecalibacterium* was found decreased in ERA patients, but still remarks a possible pro-inflammatory role of this component of microbiota in the pathogenesis of chronic arthritis. We also discovered differential microbial profiles and intra-group variability between active disease and remission, suggesting instability of microbial ecosystem in autoimmune diseases with respect to healthy status.

Interestingly, by performing prediction analysis of metabolic functions, we found that JIA-ERA metagenome was differentially enriched in bacterial functions related to cell motility and chemotaxis, suggesting selection of potential virulence traits. These indications of enrichment in potentially pathogenic invasiveness-related traits in JIA-ERA metagenome could suggest a potential improved ability of microbial components to pass through the gastrointestinal barrier or migrate in other districts, also responding to nutrient gradients, and activate the immune responses. Moreover, given that in mice models immunogenicity of flagellin CBir1 was observed, with consequent induction of colitis, and antibodies anti-CBir1 were found in CD patients with complicated disease [170], we cannot exclude the potential effect of flagellar-assembly proteins of some components of microbiota on host immune system of JIA patients.

Furthermore, we observed differentially abundant taxa discriminating for HLA-B27 status. HLA-B27 allele represents a genetic marker strongly associated with spondyloarthropathies. At family level, increased *Lactobacillaceae* in HLA-B27positive-JIA patients, and *Pasturellaceae* in HLA-B27 negative-JIA patients were found. Of the increased genera in HLA-B27 positive JIA patients, we observed *Bilophila*, a sulphite-reducing bacterium known to be involved in murine colitis [171] and in intestinal inflammatory disorders in humans [172,173], via H_2S production, *Parvimonas*, commonly observed in periodontitis and appendicitis [174], and *Oscillibacter*, involved in gut barrier integrity in mice [175], while *Haemophilus* and *Eggerthella* were differentially enriched in HLA-B27 negative patients. When considering only the JIA-ERA group, in addition *Lactobacillus*, *Clostridium cluster XI*, a well-known pro-inflammatory and colitis inducing-bacterium, and *Dialister* frequently found in periodontitis and other infections [176], were enriched in HLA-B27 positive patients, while only *Haemophilus* discriminated for HLA-B27 negative status.

In the studies discussed so far, populations were often quite heterogeneous, due to the variable disease duration of the patients and to the different therapeutic regimens. Conversely, some other studies focused only on new onset, treatment-free JIA patients.

Finally, we report results of a multicenter study conducted in Italy and in the Netherlands, [177]. A cohort of 99 new onset, treatment–naïve JIA patients (78 Italians and 21 Dutch) was followed longitudinally with collection of samples at baseline and after one year, which allowed to obtain fecal microbial profiles at different disease stages. JIA patients (n = 99) were compared with age- and geography-matched healthy controls (n = 107). In Italian JIA children, either at baseline or in inactive disease, enrichment of *Erysipelotrichaceae* and *Faecalibacterium prausnitzii* and depletion of *Allobaculum* and *Propionibacterium acnes* were observed with respect to healthy controls. At baseline but not in inactive disease, differences in *Enterococcus, Gemellaceae, Parabacteroides, Ruminococcaceae* and *Turicibacter* were found. Whereas *Bacteroides caccae*, *Barnesiellaceae* and *Dorea* were differentially enriched in inactive disease samples, but not at baseline. In addition, *Eggerthella lenta*, *Rikenellaceae*, *Coprobacillus* and *Mogibacteriaceae* discriminated Dutch patients compared to healthy controls, but no evidence of statistically significant differences were observed. Both in inactive and in persistent activity disease samples, microbiota richness was significantly reduced, as previously reported by our study [168]. No significant differences were instead found between active and inactive disease. The authors suppose that gut microbiota profiles are associated to inflammation in JIA, but not to disease activity status. In addition, this study showed differences in microbiota composition between Dutch and Italian samples, suggesting that differences in dietary habits and other environmental factors could affect the gut microbiota composition in geographically different populations.

Lastly, Stoll and collaborators have applied metabolomic analysis to fecal samples of JIA to evaluate the functional potential of gut microbiota in inflammatory process [178]. A total of 24 ERA patients were enrolled and divided into two cohorts; one of 14 patients with new diagnosis and another of 10 children with more longstanding ERA. Although by taxonomic analysis, no significant differences were found between ERA and healthy subjects, nano-liquid chromatography – mass spectroscopy (LC-MS) analyses, performed on fecal water, demonstrated overall a decreased variety of metabolites and altered tryptophan metabolomics profile in both patient cohorts compared to healthy controls. The essential amino acid tryptophan can be metabolized to a variety of different byproducts, some of which could be relevant for immune responses, such as kynurenine that appears to favor the development of regulatory T cells [179]. Down-regulation of tryptophan metabolism, as observed in ERA patients of this study and already described in synovial fluid of patients with RA [180]. Although metabolomic studies on arthritis so far are associative, alteration of tryptophan could play an immunological role by altering CD4 T function and promoting development or maintenance of inflammation in RA and JIA-ERA. These findings are consistent with data on CARD9 deficient mice, an animal model of colitis, and in which levels of fecal indole-3-acetic (IAA), deriving by metabolism of tryptophan, were reduced [181]. Additionally, a study of mice with DSS- induced colitis also showed decreased fecal IAA [182], further suggesting a contribution in the reduction of regulatory T cells.

9. Macrophage Activation Syndrome

Macrophage Activation Syndrome (MAS) is a potentially life threatening complication of rheumatologic diseases [183–185]. MAS is typically associated with Systemic Juvenile Idiopathic Arthritis (SJIA); overt symptoms are present in almost 10% of SJIA patients, while subclinical presentation can occur in another 30–40%. MAS has been also associated with other conditions such as pediatric Systemic Lupus Erythematosus (pSLE) and Kawasaki Disease (KD) [186].

Clinically, the syndrome is characterized by fever, cytopenias, severe hyperferritinemia, coagulopathy, liver dysfunction, and hypertriglyceridemia. Bone marrow biopsy often shows the presence of macrophages with hemophagocytic activity. Many features are shared with Hemophagocytic Lymphohistiocytosis (HLH), and MAS is indeed classified as a secondary form of HLH [184]. In the pathophysiology of MAS, excessive cellular activation of $CD8^+$ T lymphocyte and macrophages leads to a significant production of proinflammatory cytokines, the so-called ‘cytokine storm’, which sustains a marked hyperinflammatory state [184,185].

In a state of active disease, MAS may be triggered by infectious agents or changes in therapy, but sometimes it is triggered without a detectable cause.

Weaver and colleagues [163] developed a murine model of TRL9

induced MAS by intraperitoneal administration of CpG1826 into antibiotic-treated and control mice, to test whether broad-spectrum antibiotics are protective in this condition. The model showed that germ-free antibiotic treated mice did not develop the syndrome; disease protection was not mediated by defective baseline TLR9 responses and was indeed related to failed induction of myelopoiesis. Hence, such model suggests that microbiota-dependent myelopoiesis can play a central role in the regulation of inflammation, and that in the future microbiota could be targeted for therapeutic benefit for rheumatic diseases.

Amlani and collaborators [187] reported a case of a 69-year-old woman with antisynthetase syndrome, who developed ANCA associated vasculitis and HLH after a fecal microbiota transplant (FMT). In this patient, alteration of microbiota could have acted as a trigger for both vessel inflammation and the development of cytokinic storm leading to HLH, suggesting again that microbiota composition and alterations can affect significantly immune responses and inflammation.

10. Conclusions

Current evidences suggest that perturbation of gut microbiota, dysbiosis, could contribute to development of JIA. Studies conducted so far showed that JIA patients seem to have pro-arthritogenic microbial profiles, together with a depletion of microbial biodiversity that are clearly distinguished from microbiota of healthy subjects. Furthermore, findings of altered functional potential of gut microbiota, such as cell motility, flagellar assembling [168], that affect the gut mucosal barrier and favor microbial dissemination, or altered metabolism of tryptophan [178] and butanoate [161], that reduce tolerogenic immune response, seem suggest a functional contribution of the gut microbiota to inflammation status in JIA.

It is was hypothesized that alteration of single bacterial genus could have direct impact on driving inflammation, as suggested for *Bacteroides, Akkermansia* or the anti-inflammatory *F. prausnitzii,* which is found to be depleted in ERA patients. Studies in JIA have shown to share some features reported for other chronic immune diseases, such as IBD and RA, strengthening the hypothesis that alteration of microbial communities and dysbiosis, are involved in the pathogenesis of immune mediated disorders.

However, results from JIA studies are not always completely in line with each other; this can be explained by the fact that the populations considered were very heterogeneous with regard to size of cohorts, disease status and treatment and, importantly, that JIA represents a group of many conditions with diverse clinical features, inflammation and autoimmune indices. In addition, the different geographic origins and consequently environments and dietary habits of the studied cohorts, not least the inter-individual variability could affect the gut microbiota composition and explain the observed heterogeneity of microbial profiles potentially involved in JIA inflammation.

Despite the growing evidences of the role of dysbiosis in JIA, research in this field is only at an initial stage. So far, investigation of gut microbiota composition and alteration in JIA compared to healthy subjects are only descriptive and the microbial potential functional is still predictive/hypothetical, making it difficult to prove causative link between altered microbiome and JIA. Further investigations are thus needed to clarify the role of pro-arthritogenic microbial profiles and the functional implications contributing to the inflammation in each JIA category, as well as the interactions among genetics and environmental factors concurring in the development of these pathologies. Finally, research in this direction should include the understanding of the possible beneficial effect of immunomodulatory strategies through modification of gut microbiota or restoration of microbial equilibrium and maintenance of microbial biodiversity and immune homeostasis, including the relationships with epithelial barrier function.

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Appendix A. Supplementary data

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