



Review

Antibiotics and autoimmune and allergy diseases: Causative factor or treatment? [☆]Anna Strzēpa^a, Francis M. Lobo^b, Monika Majewska-Szczepanik^a, Marian Szczepanik^{a,*}^a Department of Medical Biology, Faculty of Health Sciences, Jagiellonian University Medical College, ul. Kopernika 7a, 31-034 Krakow, Poland^b Section of Allergy and Clinical Immunology, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT, USA

A B S T R A C T

The newborn infant emerges from an almost sterile environment into a world of bacteria. Bacteria colonize the infant's skin, lungs, and, of most importance, the gut. The process of bacterial colonization is coordinated, and each body niche acquires a unique composition of bacteria. In the gut, most bacteria belong to the *Firmicutes* and *Bacteroidetes* phyla, while *Actinobacteria* and *Proteobacteria* are far less abundant. Some of these bacteria possess strong immunoregulatory properties.

Bacterial colonization is essential to skew the newborn's immune response away from the allergy-favoring Type-2 response towards a Type-1 immune response, which is essential for pathogen elimination. Imbalance between Type 1 and Type 2 responses, however, can promote autoimmunity. In addition, the microbiota shapes immune responses in adults. Autoimmune and allergic diseases are commonly associated with an altered composition of resident bacteria, which is known as dysbiosis. Perhaps the most common cause of disruption and alteration of the bacterial colonization of newborns is the use of antibiotics. It is not known whether the dysbiosis precedes or is the consequence of allergic and autoimmune disorders, and whether antibiotics can be a trigger for these disorders, depending on the type of antibiotic used and the maturity of immune system.

In this review, we discuss the development of the microbiota in different body niches and their immunomodulatory potential. We evaluate the impact of antibiotics, both in mice and in humans, on microbial communities and how that may impact the development and manifestation of diseases through all stages of life: the prenatal period, childhood, and adulthood.

1. Introduction

Although, the antibacterial properties of mold have been noticed in ancient times, the first mold-derived antibiotic, penicillin, was discovered by Alexander Fleming only in 1929. It took more than ten years thereafter before mass production and distribution of penicillin began in 1945. For the last seventy years, antibiotics have become among the most frequently prescribed medications worldwide. It is estimated that in 2010 alone about 70 billion individual antibiotic doses were taken by

patients worldwide [1]. Such common use of various antibiotics is partly based on the belief, among physicians and patients, that antibiotics are completely safe. As with practically all other types of drugs, antibiotics have great benefits and often serious risks. The beneficial characteristics of antibiotics consist largely of their ability to treat infection and to inhibit the spread of the pathogenic microbe. However, we must remember that advances in the field of infection therapy over the last several decades have led to a drastically increasing application of antibiotics. While these antibiotics are eliminating pathogens, they

Abbreviations: ACD, allergic contact dermatitis; AD, atopic dermatitis; ADD, allergic airway disease; AHR, aryl hydrocarbon receptors; AMP, antimicrobial peptides; anti-CCP, cyclic citrullinated peptide autoantibodies; APC, antigen presenting cells; ATP, adenosine triphosphate; CD, Crohn's disease; CHS, contact hypersensitivity; CIA, collagen-induced arthritis; CNS, central nervous system; DC, dendritic cells; DNFB, 2,4-dinitrofluorobenzene; DSS, dextran sulfate sodium; EAE, experimental autoimmune encephalomyelitis; FOS, fructo-oligosaccharide; GF, germ-free; GPCR, binding to G protein-coupled receptors; HDAC, histone/protein deacetylases; IAA, indole-3-acetic acid; IBD, inflammatory bowel diseases; IDO, indoleamine 2,3-dioxygenase; ILC3, group 3 innate lymphoid cells; iMNC, mononuclear cells; iTreg, inducible T regulatory; LTA, lipoteichoic acid; MLN, mesenteric lymph nodes; MS, multiple sclerosis; OVA, ovalbumin; PP, Peyer's patches; PSA, polysaccharide A; RA, rheumatoid arthritis; RF, rheumatoid factor; ROS, reactive oxygen species; SAA, serum amyloid A; SCFA, short chain fatty acids; SFB, Segmented Filamentous Bacteria; SFP, specific pathogen-free; T1D, type 1 diabetes; TNP-Cl, picryl chloride; Treg, T regulatory; UC, ulcerative colitis

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also eliminate, partially or substantially, naturally existing commensal bacteria.

1.1. The role of microbiota in allergy and autoimmunity

Most if not all autoimmune diseases are driven by self-reactive T lymphocytes. These autoreactive T cells become activated when mechanisms of inducing tolerance to self-antigens break down, leading to activation, proliferation and generation of IFN- γ -producing Th1 and IL-17A-secreting Th17 lymphocytes. In contrast to autoimmune diseases, atopic disorders develop when the immune system responds to innocuous antigens leading to induction of Th2 lymphocytes that secrete, most importantly, IL-4, IL-5 and IL-13, supporting IgE production and eosinophilia. Elevated Th2 immunity is the feature of several allergic diseases, which develop in early childhood and proceed one another in the so called “atopic march”. The atopic march starts with hypersensitivity to food allergens, which manifests as food allergy and atopic dermatitis (AD), followed by hypersensitivity to inhalant allergens, manifesting as asthma and rhinoconjunctivitis.

The incidence of allergic disorders and of autoimmune diseases, such as type 1 diabetes (T1D), multiple sclerosis (MS) and Crohn's disease (CD), is rising. The observed steep rise correlates with lower incidence of many infectious diseases, increased “hygiene,” which most interpret as a decrease in exposure to commensal microorganisms, and widespread use of antibiotics. Increased hygiene leads to reduced exposure to microbial antigens, whereas antibiotics, apart from eliminating pathogens, also deplete natural flora, leading to long-lasting changes in the composition and quantity, or load, of microbiota in the antibiotic recipient. The initial “hygiene hypothesis” suggest that the decreasing incidence of infections over the last several decades has led to the increasing incidence of both autoimmune and atopic disorders. The more recently advanced “microbial deprivation signal hypothesis,” however, proposes that reduced exposure to or complete lack of certain bacterial signals in industrialized “hygienic” societies is causally related to the rise in autoimmunity and allergy [2]. The revised “microbial deprivation hypothesis” puts emphasis on the role of diversified bacterial signals derived from microbiota.

Until recently, most studies have paid an attention only to post-natal bacterial exposure. Due to recent findings of fetal tissues having their own microbiome, however, concerns over the role of pre-natal bacterial signals and their reduction due to antibiotics use are increasing.

2. Dynamics of microbiota composition

Although microbes reside in several anatomical locations, colonizing all surfaces covered by epithelia such as the skin, vagina, airways, and mouth, the lower gastrointestinal tract of mammals harbors the greatest density and diversity of commensal microorganisms. These include bacteria, archaea, fungi, viruses and protozoans. Bacteria, however, predominate and reach 10^{14} microbial cells in the colon [3,4].

In addition, microbial communities have been isolated from formerly “forbidden” niches, formerly thought of as sterile, such as the placenta, breast, uterus, Fallopian tubes and even semen [5]. Similar to mucosal surfaces, the skin also is populated by microbiota. The observed differences in microbiota composition and microbiota density at various body parts are conditioned by nutrient availability, humidity, temperature, pH and levels of oxygen.

2.1. Microbiota in utero

For many decades, until very recently, it has been believed that both the fetus and the placenta are germ free, and that such sterile conditions ensure normal fetal development. There are reports, however, suggesting that bacteria are present in normal placentas. Recent data prove the presence of microbes in amniotic fluid [6], umbilical cord blood [7], meconium [8], and placental membranes [9]. The placental

microbiome shares a high degree of similarity to that found in the mother's oral cavity, consisting of *Firmicutes*, *Tenericutes*, *Proteobacteria*, *Bacteroidetes* and *Fusobacteria* phyla [9]. The interaction at the immunological level between the oral cavity and the uterus has been pointed out by several studies, such as the demonstration that maternal oral infections with *Porphyromonas gingivalis* are associated with pregnancy complications such as preterm birth [10]. The mechanism by which maternal oral microbiota affects the fetus is still unknown. One possibility is the spread of oral microbiota to the placenta, leading to intrauterine dysbiosis. It has been observed that genetically modified bacteria inoculated orally into to pregnant dams could translocate to amniotic fluid [7]. Little is known about the initial interaction between the fetus and microbiota in utero and its subsequent influence on the development of fetal immune system. Placental microbiota dysbiosis, characterized by a high level of urogenital and oral commensal bacteria, is associated with chorioamnionitis, the leading cause of preterm labor [11]. Moreover, preterm delivery has been associated with higher level of *Firmicutes* and *Tenericutes* phyla, and less abundant *Actinobacteria*, in the placental basal plate [12]. The negative impact of intrauterine dysbiosis extends beyond pregnancy complications, as infections in utero [13,14] and colonization with *Staphylococci* have been associated with a higher risk of asthma in offspring [15], supporting the immunoregulatory role of intrauterine microbiota. It is quite certain, however, that the degree of postnatal microbial exposure is much greater than that occurring during prenatal life.

2.2. Development of gut microbiota

Like other mammals, a human infant emerges into the world from an almost sterile environment, and that, in the first months of life, the infant is colonized gradually by bacteria. There are two periods of intestinal colonization: the phase of milk nourishment consumption (the period of breast feeding or administration of artificial preparations) and the stage of changing from a milk diet to solid foods [16].

The first microorganisms that colonize the gastrointestinal tract are facultative anaerobes, *Staphylococcus*, *Streptococcus* and *Enterobacteria*, which are replaced by obligate anaerobes, *Bacteroides* and *Clostridium*, when most oxygen in the intestines is unavailable [17]. The initial infant microbiota composition has low diversity, and is dominated by the *Proteobacteria* and *Actinobacteria* phyla, which are outnumbered as the baby becomes older by *Bacteroides* and *Clostridium* [18]. The composition of natural flora is affected by a number of factors including, e.g., country of birth, prematurity, manner of delivery (vaginal vs. caesarean section), early hospitalization, or use of antibiotics. Caesarean section delivery leads to colonization with bacteria from maternal skin, *Staphylococcus* and *Propionibacterium*, while vaginal birth leads to dominance of vaginal bacteria such as *Lactobacillus* and *Prevotella* [19]. Recent data on the infant gut microbiota prove that almost all vaginally born children have high level of *Bacteroidetes* and relatively high *Actinobacteria*, with low levels of *Firmicutes* and *Proteobacteria*. In contrast, caesarean section delivery leads to high level of *Firmicutes* and *Proteobacteria*, with low levels of *Bacteroidetes*. The microbiota profile of children delivered by caesarean section shifts to that found in vaginally born children at the age of 12 months, which is characterized by high level of *Bacteroidetes* [20]. The microbiota composition at the age of 2–5 years starts to resemble the microbiota in adults [21]. Up to that age, the child's microbiota is not stable and is very sensitive to modification by various agents, including antibiotics. This is of great significance, as it has been shown that modification of microbiota early in life modulates baby peripheral immune response.

The microbiota of the human gut in adults is composed predominantly of *Bacteroidetes* and *Firmicutes* (90%), complemented with *Actinobacteria*, *Proteobacteria* and *Verrucomicrobia* [22]. Studies conducted over many years have shown that the bacteria living in the alimentary tract have an essential role in the processes of food digestion, modulation of lipid metabolism, promotion of angiogenesis,

immunity to infections, and maintenance of immune homeostasis [4].

2.3. Skin microbiota

The skin is home to a myriad of microbes residing on the epithelial surface, as well as in associated appendages, such as hair follicles and sebaceous glands. Across the 1.8 m² of skin surface, approximately 10⁶ bacteria reside per square centimeter, for a total of over 10¹⁰ bacterial cells covering a whole human body [23,24]. The human skin microbiota is limited to a relatively small number of species, a situation dictated by the 'less friendly' living conditions compared with those prevailing within the mucous membranes of the intestines or genital tract. The skin surface is quite diverse, consisting of different micro-environments with distinct pH, temperature, moisture, sebum content, and topography. These niche-specific physiologic differences influence the resident bacteria and fungi. Oily surfaces like the forehead support lipid-loving bacteria that differ from those found at dry sites like the forearm. Sebaceous sites are dominated by lipophilic *Propionibacterium* species, while humidity-loving *Staphylococcus* and *Corynebacterium* species are abundant in moist areas [23,24]. The sites in which bacterial diversity is the highest are the dry areas, with mixed representation from the phyla *Actinobacteria*, *Proteobacteria*, *Firmicutes* and *Bacteroidetes* [24]. Fungi also are identified in the skin microbiota, with the genus *Malassezia* dominating core-body and arm sites, while foot sites are colonized by a more diverse combination of *Malassezia*, *Aspergillus*, *Cryptococcus*, *Rhodotorula*, *Epicoccum*, and others [25].

Development of skin microbiota differs from that observed during gut flora development. During the development of skin microbiota, shifts in the growing child's exposure to local microflora are associated with the exploration of new environments through crawling, walking, and increasing socialization. The skin microbiota, however, go through a later major shift during sexual maturation and the transition through puberty.

2.4. Lung microbiota

The newborn lung microbiota in mice consists mainly of *Firmicutes* and *Gammaproteobacteria*, which are outnumbered by *Bacteroidetes* as the mice get older [26]. This lung microbiota shift is associated with Th2 to Th1 skewing of immune response to airborne antigens. Neonatal mice exposed to house dust mite antigen have an exaggerated Th2 immune response and eosinophilia, which is far less robust in adult mice [26]. There are limited data regarding microbiota shifts in human neonates due to the inherently prohibitive nature of such studies.

3. Microbiota shape immune responses

The immune system of neonates is, by definition, immature and requires adequate bacterial signals for proper development [27]. The neonate's immune system resembles one found in germ-free (GF) mice. These mice have reduced numbers of immune cells in mesenteric lymph nodes (MLN) and Peyer's patches (PP). The number of germinal centers also is reduced, leading to decreased systemic antibody production, with the exception of IgE [16]. Elevated IgE production depends on Th2 cells and requires IL-4 [28], which is in high abundance in GF mice, as their immune response is skewed towards a Th2 type [29]. As evidence of this Th2 skewing, GF mice develop a milder form of collagen-induced arthritis (CIA) and experimental autoimmune encephalomyelitis (EAE), but show more severe allergic responses to food antigens [30] or airborne ovalbumin (OVA) [31]. These GF mice exposed to OVA have high levels of Th2 type cytokines and elevated IgE levels in sera [31]. Exacerbated allergic responses to foods in GF mice, however, can be prevented by colonization with selected bacterial species, demonstrating the importance of intestinal microbes in regulating allergic responses [30].

Germ-free mice colonized with the same microbiota either just after

the birth or 3 weeks after birth will demonstrate distinctly different intestinal microbiota, simply from the differing moment of inoculation, leading to differences in immune system responses. Mice colonized 3 weeks after birth show altered frequency of immune cells and cytokine concentrations [32]. These data suggest the presence of a time window that is critical for establishing a balanced mutualistic interaction between the host and the microbiota. These data also suggest that alteration of the microbiota through antibiotics use, which is the most prescribed drugs for children [33], can have a lifelong impact on immune responses.

Delivery of an infant through the vaginal canal initiates colonization of the gut, skin and lungs with complex microbial communities. Pro-inflammatory signals derived from microbes skew newborn immune responses from Th2 to Th1 and drive regulatory cell development. Th1 responses provide protection against many infections but, when dysregulated, can promote autoimmunity. On the contrary, weak Th1-mediated immunity results in Th2 polarization. Such Th2 polarity is observed in children who develop allergic diseases later in life [34].

3.1. Mechanisms involved in immunoregulation by gut microbiota

The gut microbiota regulates immune responses not only in the intestine but also in the periphery. The immune system is under the influence of large bacterial families and unique bacterial species, through their cell wall constituents, bacterial metabolites, and other mechanisms. Both bacterial-derived molecules and metabolites leak from gut into the bloodstream, and regulate immune responses in the periphery.

Immune responses in the intestine are regulated by several bacteria species: *Segmented Filamentous Bacteria* (SFB), *Bacteroides fragilis*, *Lactobacillus*, *Bifidobacterium*, *Clostridium* cluster IV and XIVa. SFB are a Gram positive spore-forming bacteria, most closely related to *Clostridium*. SFB play a crucial role in the generation of pro-inflammatory Th17 lymphocytes [35,36], as proven by experiments in GF mice inoculated with bacterial mixtures with or without SFB [36,37]. It has been shown that SFB interact closely with gut epithelial cells, supporting induction of serum amyloid A (SAA), which stimulates Th17 cell generation [35]. Although Th17 cells are proinflammatory, SFB-mediated Th17 cell induction provides protection against enteropathogenic *Citrobacter rodentium* [36]. On the other hand, when the gut bacteria composition is altered, SFB may promote gut inflammation [38].

Th17 cell differentiation is also supported by commensal-derived adenosine triphosphate (ATP). It has been shown that lower numbers of Th17 cells in the gut of GF mice can be reversed by providing exogenous ATP [38].

Aggravated Th1 and Th17 immune responses can be inhibited by other bacterial species. It has been observed that intestinal inflammation in models of dextran sulfate sodium (DSS)-induced colitis, as well as colitis induced by *Helicobacter hepaticus*, is ameliorated effectively by *Clostridium* cluster IV and XIVa [39] and *Bacteroides fragilis* [40], respectively, via induction of IL-10-producing T regulatory (Treg) cells. IL-10 production induced by *Bacteroides fragilis* involves interaction of the bacterial cell wall constituent polysaccharide A (PSA) with TLR2. Additionally, *Clostridium* cluster IV and XIVa inhibit inflammatory responses by stimulation of TGF- β -secreting dendritic cells (DC) [39].

Other bacterial species such as *Lactobacillus* and *Bifidobacterium* also have potential to reduce intestinal inflammation. The anti-inflammatory effects of these bacterial species rely on their potential to induce tolerogenic DC, which produce IL-10, TGF- β and indoleamine 2,3-dioxygenase (IDO). These mediators are involved in the generation of inducible T regulatory (iTreg) cells [41], which suppress undesired immune responses in the periphery. Additionally, Th3 lymphocytes, localized in mesenteric lymph nodes, which secrete high levels of TGF- β suppress undesired immune responses to autoantigens [42].

There are many reports showing that systemic immune responses

are regulated constantly by gut microbiota. Germ free mice, which have no microorganisms living in and on them, show a reduced severity of CIA and EAE. The severity of both diseases, however, is exacerbated after inoculation of GF mice with SFB [43,44]. On the contrary, elevated immune response in the periphery is suppressed by Treg cell-inducing *Bacteroides fragilis* and *Clostridium* cluster IV and XIVa [39,45].

It has been shown that various bacterial products and metabolites breach the intestinal epithelium. Short chain fatty acids (SCFA) such as acetate, propionate, and butyrate are widely recognized modulators of immune response in the periphery. SCFAs are produced during bacterial fermentation of indigestible polysaccharides. The immunoregulatory role of SCFAs springs from their potential to inhibit histone/protein deacetylases (HDAC) and binding to G protein-coupled receptors (GPCR).

HDAC inhibition by SCFAs preserves acetyl groups of histones and proteins. Acetylated histones then promote chromatin decondensation and subsequent gene transcription. The transcription rate in turn can be enhanced by acetylation of specific transcription factors, a prominent example of which is FoxP3. HDAC inhibition increases FoxP3 expression and elevates its activity, leading to induction of Treg cells of their suppressive functions [46,47]. Interestingly, SCFAs, especially butyrate, have been shown to inhibit HDAC activity, leading to hyper-acetylation of the FoxP3 promotor and acetylation of FoxP3 transcription factor protein. Sustained acetylation of FoxP3 by the effect of SCFAs leads to enhanced Treg cell differentiation and activity, resulting in reduced airway inflammation in models of airway allergy [48,49].

A further anti-inflammatory activity of SCFAs is inhibition of NF- κ B. It has been found that neutrophils and human mononuclear cells treated with SCFAs show reduced production of proinflammatory cytokines [50,51]. This effect may be common among immune cells, as SCFAs also inhibit NF- κ B activation in macrophages and dendritic cells [49,52].

Another mechanism of regulation of host physiology by SCFAs involves GPCRs. There are four receptors that recognize SCFA; GPR41, GPR43, GPR109a and Olfr78. The first of these three receptors regulate immune responses, while the Olfr78, located in renal epithelium, modulates blood pressure.

GPR41 and GPR43 recognize all SCFAs produced during starch fermentation in the colon but the affinity of these receptors to acetate, propionate and butyrate varies. Propionate has the highest affinity for both receptors. Butyrate has higher affinity for GPR41 but lower affinity for GPR43, while the affinity of acetate is the converse. GPR109a is the only receptor that recognizes solely the SCFA butyrate [53].

GPR41 and GPR43 have different patterns of tissue expression. GPR41 is found on the surface of a wide range of cells including cells present in the colon, kidneys, blood vessels and nervous system. In contrast, GPR43 is found mostly on immune cells and endothelial cells. GPRs couple with intracellular G proteins. G(i) protein couples with GPR41 and GPR43, while G(q) protein associates only with GPR41. GPR41 activation inhibits proliferation and increases apoptosis of epithelial cells [53], whereas activation of GPR43 regulates immune responses. Mice deficient in GPR43 show elevated inflammatory responses in animal models of colitis, arthritis and asthma. The pathologies of these conditions are a consequence of elevated production of proinflammatory cytokines, many of which are dependent upon NF- κ B, which is inhibited by SCFAs [50,51]. Additionally, elevated inflammatory responses in mice deficient for GPR43 can be attributed to modulation of chemotaxis and suppression of DC maturation.

Indole derivatives are another type of molecules generated by bacterial metabolism. Indole derivatives are formed from dietary tryptophan, which is metabolized by gut bacteria to indole-3-acetic acid (IAA), or by host-derived indoleamine 2,3-dioxygenase 1 to kynurenine [54]. The indole derivatives are recognized by cytosolic aryl hydrocarbon receptors (AHR) that recognize xenobiotics, which are small chemical compounds [55,56]. Binding of AHR by indole derivatives induces the translocation of AHR to nucleus where it upregulates transcription of certain genes

involved in xenobiotic metabolism.

Apart from being involved in response to xenobiotics, AHR is a known regulator of immune responses. High levels of AHR are found in Th17 cells, and activation of AHR enhances Th17 cell differentiation [57] and promotes IL-17 production [58]. Terminally differentiated Th17 cells produce IL-22, which supports regeneration of epithelial membranes by inducing production of antimicrobial proteins. AHR activation is indispensable for IL-22 production, as this cytokine is not produced by differentiated Th17 cells in AHR-deficient animals [59]. Additionally, AHR drives IL-22 production by group 3 innate lymphoid cells (ILC3) [60]. On the other hand it has been observed that AHR supports tolerogenic immune response by enhancing differentiation of FoxP3 + Treg [58] and IL-10-producing Tr1 cells [57].

In the gut, AHR ligands are generated from dietary tryptophan only by certain species of *Lactobacilli* that have the necessary sets of enzymes. It has been found that only *Lactobacillus reuteri*, but not *Lactobacillus johnsonii*, induce IL-22 production by ILC3, providing resistance against *Candida albicans* infection [54]. Impaired IL-22 production by ILC3 cells enhances the load of yeast infection in the gut and impairs recovery from DSS-induced colitis in CARD9 deficient mice. These effects are attributed to dysbiosis, characterized by lower levels of *Lactobacilli* and impaired tryptophan catabolism [61].

Polyamines are yet another group of immunoregulatory compounds derived from intestinal bacteria. Polyamines such as putrescine, spermidine, and spermine are highly cationic molecules synthesized from arginine both by gut bacteria and by host metabolism. In mammals, arginine is converted to ornithine by arginase 1, and the product then is converted by ornithine decarboxylase to putrescine. Spermidine and spermine are created in subsequent reactions. Polyamines have been shown to have immunoregulatory properties. Spermine suppresses production of proinflammatory cytokines such as TNF- α , IL-1, IL-6, MIP-1 α , and MIP-1 β by LPS-stimulated human peripheral blood mononuclear cells [62]. Polyamines in the gut have been shown to suppress NF- κ B activation and apoptosis in intestinal epithelial cells [63].

3.2. Skin microbiota shape immune responses

The skin, the external surface of our body is host to a rich diversity of microflora. The resident commensal microflora fills a niche that otherwise could be colonized by pathogenic microorganisms that can cause infection. This, however, is not the only positive function of the resident microflora. It recently has been shown that skin microbiota modulate both innate and adaptive immune responses. The skin is equipped with a highly sophisticated immune surveillance system consisting of epithelial cells, lymphocytes, and antigen presenting cells (APC) that populate the epidermis and the dermis. It already has been shown that lipoteichoic acid (LTA) released by *S. epidermidis* in the setting of acute skin damage mitigates inflammatory responses and induces tissue repair. Additionally, it has been demonstrated that skin microbiota control the expression of various innate immune factors including antimicrobial peptides (AMPs) and components of the complement system [23]. The skin microbiota also act as endogenous adjuvants of the skin immune system by controlling the level of IL-1, a cytokine involved in the initiation and amplification of adaptive immune response [64]. Accordingly, *Propionibacteria* have been shown to have adjuvant and antitumor activities and to contribute to a more efficient immune response to infections [65].

Comparison of T cells from GF mice and from specific pathogen-free (SPF) mice reveals that the former produce lower levels of pro-inflammatory cytokines such as IL-17A and IFN- γ . Colonization of the skin of GF mice with just one bacterium, *Staphylococcus epidermidis*, was sufficient to reinstate production of IL-17A by skin resident T cells and also to develop Th1-mediated immune responses to *Leishmania major* [64]. One can speculate that changes in skin microbiota diversity, richness, and even distribution, can have an impact on skin health.

Indeed, skin disorders such as psoriasis, AD, and acne all have been associated with dysbiosis of the skin flora. Acne, a chronic inflammatory disease of the pilosebaceous unit, is not yet completely understood, but altered bacterial colonization is considered to be one of the main elements contributing to its development [66]. Recent findings in AD patients show a significant change in microbial skin flora compared to healthy volunteers. Atopic dermatitis long has been associated with *Staphylococcus aureus* skin colonization and infection and is treated successfully with anti-Staphylococcal antibiotics in combination with corticosteroids [67].

In contrast to AD, less is known about the role of the skin microbiota in psoriasis. It has been shown that psoriatic plaques are infiltrated by IL-17A producing T cells that are associated with the pathogenesis of the disease. It has been speculated that local expansion of certain skin commensal organisms can trigger the Th17-mediated inflammatory response observed in psoriatic plaques. Indeed, it has been found that diversity of the microbial population overlaying psoriatic lesions is greater than that found in healthy skin. *Firmicutes* are predominant, whereas *Actinobacteria* are underrepresented in psoriatic lesions in comparison to healthy skin [68].

3.3. Microbiota shape immune responses in lungs

Colonization of mucosal surfaces in the lungs influences the skewing of immune responses between Th2 to Th1. Immune responses in the lungs potentially can be modulated by pulmonary exposure to specific bacteria, as observed after exposure to *Escherichia coli*, which suppresses allergic response to OVA [69] through mediation by TLR4 signaling and modulation of DC activation [69]. The *Escherichia coli* derived ligand LPS has been shown to bind to TLR4 and to activate DC, leading to induction of Th1 cells, which in turn can diminish Th2 responses [70].

Immune responses in the lungs are also under influence of gut microbiota. It has been shown that GF mice have higher numbers of iNKT cells in the gut lamina propria and lung tissues. The iNKT cell population contributes to increased mortality after induction of asthma. When GF mice are colonized with standard pathogen free (SPF) microbiota, the number of iNKT cells is decreased, leading to reduced allergic responses to OVA [71]. Additionally, colonization with SPF microbiota modulates the frequency and activation status of APC in lungs. Certain populations of pulmonary APC support conversion of Helios + Treg into Helios-Treg cells, which have been found to be responsible for decreased airway responsiveness to allergens [26]. Targeted modulation of intestinal microbiota is a potential strategy to regulate immune responses in the lungs, as supported by the finding that intragastric administration of *Mycobacterium vaccae* [72], or the stomach-resident pathogen *Helicobacter pylori* [73], reduces pulmonary allergy.

4. Influence of prenatal exposure to microorganisms on immune responses in offspring

4.1. Maternal microbiota shapes immune responses in children

According to the “hygiene hypothesis”, children living on farms have a lower incidence of allergies [74]. The protective effect of the farm environment is even more pronounced when mothers live on farms during gestation [75]. Children of mothers who were exposed to a wide range of farm microbial products during pregnancy have lower incidence of allergies at school age [75] (Fig. 1). Interestingly, bacterial supplementation in the form of the probiotic *Lactobacillus* GG, given prenatally to mothers and then for 6 months to their children, prevented development of early atopic disease in the form of eczema [76]. More studies on this subject are needed, as another study using L. GG strain from ATCC did not show any protective effects using a similar supplementation regiment [77]. The crucial role of microbial signaling in immune shaping during prenatal period is highlighted by another

study in which the mothers only were supplemented with *Lactobacillus rhamnosus* LPR and *Bifidobacterium longum* BL999, revealing that maternal probiotic supplementation during the last two months of pregnancy and the first 2 months of breast-feeding reduces the risk of eczema in children with a familial predisposition to allergy [78]. It has been shown that prenatal probiotic supplementation has long lasting effects that extends beyond infancy [79]. The mechanism by which diverse microbial signals reduce the incidence of atopy is still unknown. It has been observed that children with higher expression of TLR5 and TLR9 in cord blood are protected from atopy at the age of two [74]. Higher expression of TLR receptors has been associated positively with exposure to increased numbers of different farm animal species [75]. Both TLR5 and TLR9 activation converts tolerogenic DC into Th1 promoting APC [80]. Higher expression of TLR9, which recognizes DNA-derived motif CpG, indicates that the protective effects of increased prenatal exposures to microbial signals does not necessarily depend on live microorganism but could depend on motifs derived from environmental exposure to inanimate bacteria.

The epidemiological data are consistent with experimental animal models. When dams are exposed to LPS before conception and during pregnancy, the immune response to OVA is reduced [81]. Prenatal intranasal immunization with *Acinetobacter lwoffii* F78 suppresses allergic airways inflammation [82]. These effects are associated with higher levels of proinflammatory Th1 type cytokines in offsprings [81,82], which can countervail the atopy-favoring Th2 response. Additionally, the level of TLR messenger RNA in lungs is transiently increased, although the levels of TLRs in placenta are decreased. Decreased expression of TLRs in placenta is crucial for post-natal protection against atopy, as the protective effect is abolished in TLR2/3/4/7/9 –/– knockout mice [82]. These data stand in contrast to the elevated TLRs expression found in cord blood during infant delivery [75]. The discrepancies may be explained by the differing rates of immune system maturation in human infants and mice. They both point out, however, the importance of TLR signaling during early stages of life.

Apart from TLR-stimulating microbial signals, bacteria generate AHR and SCFA molecules that regulate offspring immune responses. When pregnant mothers receive oral acetate or a high fiber diet, which elevates acetate levels, the offspring demonstrate reduced AAD (allergic airway disease), characterized by reduced Th2 inflammation, with decreased IgE concentration, reduced eosinophil infiltration and lung inflammation, as well as increased Th1 responses. This effect is solely dependent on acetate, as mixed cohousing of pregnant dams that received high fiber diet or control diet experiments, which allow passive transfer of microbiota, is not protective. Similar correlation has been observed in humans. High intake of dietary fiber by pregnant women protects from development of cough or wheeze in infants only when the mothers have no symptoms of asthma. This effect is dependent on acetate-mediated HDAC inhibition, what induces Treg cells [83].

AHR ligands generated during transient bacterial colonization of pregnant dams have been shown to enhance maturation of the intestinal immune system in neonates. Offspring from gestationally colonized dams have a higher number of large intestinal F4/80 + CD11c + mononuclear cells (iMNCs) and NKp46 + ROR γ t + ILC3, which is dependent on AHR ligands. Additionally, transfer of bacterial molecules from mother to offspring is mediated by maternal IgG antibodies [84].

4.2. Antibiotic therapy of mothers initiates the atopic march in children

Antibiotics are prescribed commonly during pregnancy and just after the delivery. Over 40% of pregnant women receive antibiotics in the perinatal period [85]. These antibiotics alter maternal microbiota, which in turn can affect fetal microbiota. It has been demonstrated that eleven broad-spectrum antibiotics have the ability to affect the fetus after crossing the placenta [86], thus having the potential to modulate the fetal microbiota directly. As yet, there are no reports describing the impact of prenatal antibiotic administration on the composition of the

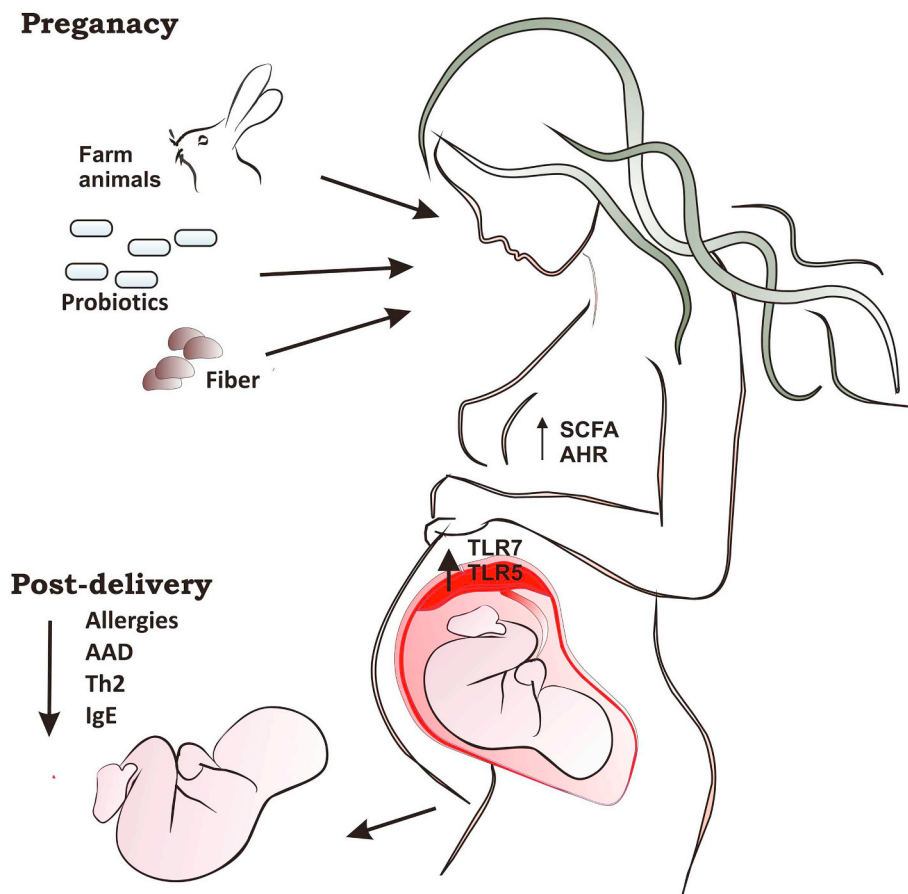


Fig. 1. Diverse bacterial signals during pregnancy reduce the risk of Th-2 associated immune responses post-natally.

prenatal microbiota. However, several reports describe the impact of prenatal antibiotic administration on postnatal immune responses. According to “the microbial deprivation hypothesis”, prenatal antibiotic administration has been implicated in the initiation of the atopic march. Atopic to indoor allergens, such as dust mites, and then outdoor allergens such as pollens. The atopic march starts with AD, food allergy, then followed by allergic rhinitis and asthma.

A 2002 study by McKeever et al. using a cohort of 24,690 children, shows that antibiotic treatment during pregnancy is associated with an increased incidence of AD, asthma, and hay fever. It also demonstrates that the risk of allergy rises with increasing numbers of antibiotic courses, as two courses of treatment results in a higher incidence of atopy when compared to one cycle of treatment [13,14].

It has been found that prenatal antibiotic exposure is a risk factor for the development of AD. A study evaluating 1128 children exposed to antibiotics prenatally shows an increased risk of AD not only at the age of 1 year [87] but also at the age of 4, an effect that is independent of post-natal antibiotic exposure [88]. Another study, involving only 102 children at the age of 1, shows that the effects of antibiotic treatment depend on treatment timing. A recent study shows that antibiotics administered during the first trimester of gestation has no influence on eczema development, but antibiotics given during the second or third trimester insignificantly increases the risk of eczema [89]. Another study of 411 children shows that antibiotics administered during third trimester does not change the incidence of AD in the children of atopic mothers [90,91]. On the other hand, a large Danish study involving 62,560 mother-children pairs demonstrates that the mother's atopy increases the risk of eczema in children at the age of 18 months only when antibiotics had been given during the pregnancy [92].

Use of antibiotics during pregnancy also is linked to development of asthma [15,93,94]. In a study involving 3000 women, antibiotics use

any time during pregnancy has been found to increase the risk of an asthma diagnosis at the age of 4 to 5 [15]. In a small pilot study examining 100 children, the use of antibiotics during second or third trimester increases the risk of persistent wheeze [89]. Another study carried out in the Chicago urban area, shows that perinatal antibiotic exposure during second to third semester significantly increases the risk of asthma, but is weakly associated with wheezing [95]. In contrast to previous findings, some studies show no association between perinatal use of antibiotics and recurrent wheeze [88]. This inconsistency may be associated with the time course of antibiotic therapy and the way in which different antibiotics modulate maternal immune response. Interestingly, the increased risk for childhood asthma is observed not only when antibiotic treatment occurs prenatally, but also if antibiotics are given 80 weeks before conception and up to 80 weeks after delivery [94].

Another factor that may impact the severity and relative risk of asthma is mother's health status. In asthmatic mothers, antibiotic use during pregnancy exacerbates the severity of asthma in children [91]. It has been reported that treatment of non-respiratory tract infections with antibiotics is a powerful risk factor for asthma in children [91]. A further study has shown that antibiotics used for treatment of respiratory tract infections increase the risk of asthma, and that the risk of asthma is even higher if mothers are treated with antibiotics for respiratory and urogenital infections [93].

Cow's milk is one of the first food products introduced to baby. An increased risk of allergic responses to cow's milk has been observed in mothers treated with cephalosporins, macrolides, and extended spectrum penicillins during breastfeeding. Tetracycline administration also increases the risk of allergies to cow's milk when given before pregnancy [96].

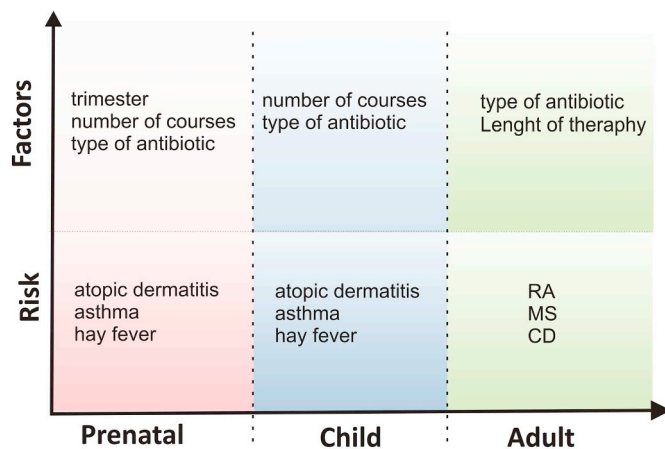


Fig. 2. The impact of antibiotic treatment increases the risk of certain diseases in an age dependent manner.

5. Early childhood

5.1. The impact of early life antibiotics use on immune responses in mice

Antibiotics given to neonatal mice, but not to mature mice, heighten the allergic response to OVA applied intranasally [97] or transdermally [98], to food allergens [30] and in spontaneous allergic responses in the skin of NC/Nga mice [99] (Fig. 2). Individual antibiotics differ in their effect on immune responses. Murine pups treated with vancomycin, but not streptomycin, develop aggravated asthma after intranasal immunization with OVA [97]. Likewise, kanamycin, but not polymyxin B, is effective in inducing atopic dermatitis-like skin lesions in NC/Nga mice [99]. The differential impact of different antibiotics on the allergic response may be associated with their differential potential to modulate intestinal microbiota. Treatment with vancomycin and kanamycin, which, contrary to streptomycin, enhance allergic responses, not only reduces intestinal microbiota but also induces microbiome dysbiosis. It is evident that broad spectrum antibiotics like enrofloxacin [98], or antibiotic mixtures such as kanamycin, gentamicin, colistin, metronidazole, and vancomycin [97] have a greater impact on the developing neonatal immune system.

It has been shown that treatment with antibiotics reduces the number of bacterial molecules inside the gut and in the circulation. Bacterial associated molecular patterns are recognized by TLR receptors. TLR activation generates proinflammatory signals, which support induction of Th1 immune response and enhance immune cell proliferation. Interestingly, expression of TLR2, TLR4 and TLR5 in the colon is the highest in 2 week old mice compared to mature 6 week old mice [100], indicating that pups are more prone to generate a proinflammatory Th1 response, which may impair Th2 response in newborns. It has been found that treatment with a mixture of broad-spectrum antibiotics (streptomycin, ampicillin, metronidazole and vancomycin) reduces TLR expression in two week old pups [100]. These changes in TLR expression are associated with an increased level of IL-4 and a decreased level of IL-17A in peripheral lymphocytes, indicating impaired skewing towards the Th17 immune response [100]. We have observed that the broad spectrum antibiotic enrofloxacin, provided through breastfeeding, reduces the number of intestinal bacteria and also impairs Th1 skewing in mice. The enrofloxacin recipients show reduced production of IFN- γ and IL-17A and increased production of Th2 type cytokines, IL-4, IL-13 and IL-10. Altered cytokine production influences antigen specific antibody secretion, leading to lower levels of OVA-specific IgG2a antibody and higher production of antigen-specific IgE, which is a hallmark of atopy [98]. These changes are not observed when enrofloxacin was provided to adult mice [98], suggesting the presence of a window of time early in life when the

immune system is markedly responsive to microbial signaling. These timeframes still are not defined, but antibiotic treatment of 3 weeks old mice still is sufficient to affect Th2 to Th1 skewing and increase synthesis of IgE [101].

Antibiotics not only decrease the total number of bacteria but also modify microbiota composition. Vancomycin, which exacerbates experimental asthma, reduces bacteria of the *Bacteroides* group and a few *Clostridia* belonging families such as *Clostridiales*, with an observed increase in the *Lactobacillus* group [97]. Similarly, the antibiotic mixture of kanamycin, gentamicin, colistin, metronidazole, and vancomycin significantly reduces *Bacteroidetes*, *Firmicutes* and *Proteobacteria*, allowing outgrowth of *Lactobacillaceae* [97]. A similar type of dysbiosis is observed after enrofloxacin treatment of adult mice [102].

5.2. Influence of antibiotics on development of atopic diseases

Antibiotics are the most commonly prescribed drugs for children [33]. A retrospective study by Farooqi et al. evaluating 1934 subjects demonstrates that antibiotic treatment during first two years of life has a strong association with development of eczema, asthma, and hay fever later in life [103] (Fig. 2). A similar trend also has been observed in Japanese children, in whom antibiotic use in the first two years of life increases the risk of atopic disorders such as asthma, AD, and allergic rhinitis at the age of 5 [104]. The association between antibiotic use and allergic diseases has been confirmed by some authors [13,14,105,106] but rejected by others [107]. A recent multicenter study evaluating almost 200,000 children from more than twenty countries confirms the association between antibiotics use during the first year of life and the development of asthma, AD and rhinoconjunctivitis at the age of 6 to 7 years [108]. Antibiotics use in children has not been found to be related to atopy in the mother, which is a strong predictor of atopy in children [103].

The variability in outcomes between these studies may be due to the mechanism of microbiota modulation by various antibiotics. Microbiota composition is influenced by the type of antibiotic used, namely, broad vs. limited-spectrum antibiotics, the number of courses of antibiotic treatment, and other external factors that impact microbiota composition, such as pets, probiotics, and the number of siblings. Generally, the more often the child is exposed to broad-spectrum antibiotics, the higher is the chance of a detrimental shift in the microbiota.

An increased incidence of atopy is associated with repeated courses of antibiotics during the first year of life [13,14]. For example, five courses of antibiotics double the risk of developing asthma [109]. Antibiotics not only reduce gut flora, but also make the flora less diverse, with many species dominated by a single strain. The bacterial communities also are less stable, as antibiotic treatment results in the loss of similarity between the microbiota of the children in a family. The composition of gut microbiota also is more variable between children [20]. Antibiotic use results in long-lasting changes in microbiota composition, such that 12 to 24 months were required to restore the microbiota diversity after an antibiotic course [110]. Some bacterial species never recover. The changes in microbiota compositions and the severity of these changes also are influenced by the type of antibiotic used. Antibiotics, both for treatment of urinary tract infections and upper respiratory tract infections, increase the odds ratio for the development of atopic disorders later in life. The use of penicillin, cephalosporin, and macrolides increases the risk of development of atopic disorders [103]. The incidence of specific atopic disorders, however, could depend on type of antibiotic used. In Japan, the two most common antibiotics prescribed are cephem and macrolide. Use of cephem increases the risk of asthma and allergic rhinitis, while the use of macrolide is associated with development of AD at the age of five [104]. Antibiotics also have variable potential in modulating the microbiota profile and inducing dysbiosis. For example, two drugs that are commonly used for respiratory tract infections, macrolides and penicillins, generate variable risks of asthma. An increased risk of asthma is

associated with a prominent microbiota shift after use of macrolides. Penicillin use is less threatening to microbiota composition and associated with a lower risk of asthma [110]. In countries where penicillin is the primary antibiotic medication for respiratory tract infections, the incidence of asthma is lower comparing to countries where use of more diverse antibiotics is more common. Broad-spectrum antibiotics are prescribed in Estonia, while penicillin is the predominant antibiotic in Sweden, which may correlate with an increased risk of asthma in Estonia as opposed to Sweden [111]. It therefore appears that there is an increased risk of developing atopic disorders associated with the use of broad spectrum antibiotics [103].

5.3. The influence of antibiotics on atopic diseases in humans

For the sake of this review and its scrutiny we include only few most prominent studies to emphasize the importance of research on the role of antibiotics in development of asthma, eczema and hay fever (Fig. 2).

5.3.1. Asthma

McKeever TM, et al. demonstrate that, although there is an association between early-life antibiotic use and asthma, asthmatic children have more respiratory tract infections before their asthma diagnosis than healthy controls [13,14]. Some studies show that the risk of developing asthma is still significant after adjusting for the use of antibiotics for upper and lower respiratory tract infections [112], or just lower respiratory tract infections [113], while, in another study, adjustment for the respiratory tract infection led to loss of significance in the risk of developing asthma [172].

Other studies reveal an association between the type of antibiotic used and asthma. In one study, only antibiotics for lower respiratory tract infections are associated with development of asthma [114]. In contrast, another study shows that antibiotics for non-respiratory infections are associated with asthma development [115]. Other studies find that the risk of asthma is increased after treatment with broad-spectrum antibiotics such as cephalosporins, sulfonamides, trimethoprim, macrolides, and amoxicillin [93,113].

5.3.2. Eczema

An early study by Farooqi et al. shows an association between antibiotics use in the first and second year of life and eczema [103]. While a later study demonstrates an increased risk of eczema when antibiotics are used during first year of life [108], other investigators point out the lack of an association between antibiotic use early in life and eczema at 5 to 10 years of age [106], 3.5 years of age [116] and 3 years of age [15,90]. Moreover, antibiotics use early in life does not increase the risk of eczema even in children with parental history of atopy [116]. However, meta-analysis points out that exposure to antibiotics early in life increases the risk of developing AD by up to 40% [117]. Interestingly, a stronger association has been observed for broad spectrum antibiotics [117], such as cephalosporins or macrolides [118]. Additional antibiotic exposure has been shown to add an extra 7% risk increase of eczema development [117]. Interestingly, very brief antibiotic exposure, lasting less than 24 h, during vaginal delivery does not increase the risk of eczema, while an intrapartum antibiotic exposure lasting longer than 24 h, has been shown to increase the risk of AD [119].

5.3.3. Allergic rhinitis

The association of antibiotics use early in life and eczema and asthma has been studied meticulously. These studies show that antibiotics used during childhood also are linked to development of allergic rhinitis [120] and food allergy at school age [121].

5.3.4. Hypersensitivity reactions

Although the evidence is scarce that perinatal modification of gut microbiota influences the intensity of various inflammatory diseases,

including autoimmune disorders, the association seems to be valid. For instance, perinatal exposure to streptomycin dramatically increases the severity of hypersensitivity pneumonitis in a model of Th1/Th17 cell-mediated disease induced by intranasal administration of *Saccharopolyspora rectivirgula* antigen. The mechanism by which perinatal microbiota influence Th1/Th17 responses depends on type of the resultant dysbiosis. It has been shown that dominance of *Bacteroidetes*, observed after streptomycin treatment, enhances peripheral Th1/Th17 diseases, while a dominance of *Firmicutes* following vancomycin treatment has no impact on Th1/Th17 disorders [122].

6. Adult life

Allergic diseases typically start in childhood, while autoimmune disorders develop later in life. The prevalence of both types of diseases increases continuously and is linked to increased hygiene and antibiotic use. Although the studies evaluating the impact of antibiotic exposure early in life on the incidence of autoimmune diseases are limited and have been difficult to conduct, many studies have examined the association between gut microbiota composition in adults and autoimmunity. Since many patients who suffer from autoimmune diseases have altered microbiota composition, it is difficult to determine whether the causative factor is the dysbiosis or the disease itself (Fig. 3). It is certain, however, that antibiotics create vast and long-lasting changes in microbiota composition. For instance, treatment with ciprofloxacin for 5 days decreases diversity, richness, and evenness of fecal microbiota, after which four weeks are required for the gut microbiota to recover, and persistent deficiency of some bacterial taxa after half of a year [123]. It has been found that altered microbiota composition increases susceptibility to colonization with pathogenic *Clostridium difficile* [124]. Similar long-term alterations also have been observed in mice [125]. It is apparent that changes in the microbiota due to antibiotics use, such as dysbiosis, loss of a key taxa and biodiversity, outgrowth of pathogens, influence the induction and severity of autoimmune as well as atopic diseases in adults.

As application of antibiotics ameliorates the severity of several autoimmune diseases, it is important to note that there are other ways by which antibiotics influence immune responses. Although the main intended action of antibiotics is to kill or inhibit bacterial growth, many antibiotics possess direct immunomodulatory and anti-inflammatory properties. Antibiotics such as clindamycin, macrolides, metronidazole, rifampin and tetracyclines, including tetracycline, doxycycline, minocycline, and lymecycline, demonstrate activities distinct from the killing or growth inhibition of bacteria [126]. Antibiotics modulate both the innate and the adaptive arm of the immune response. It has been observed in different animal models that antibiotics influence neutrophils and macrophages by inhibiting complement-mediated chemotaxis, phagocytosis, reactive oxygen species (ROS)-production, and pro-inflammatory cytokine production. Additionally, antibiotics inhibit T cell responses and T cell proliferation [126]. An example of the anti-inflammatory properties of antibiotics is the ameliorating effect of minocycline on the severity of inflammatory diseases such as rheumatoid arthritis and scleroderma. Minocycline also has been found to confer benefits not clearly related to immunity, namely, neuroprotection in models of ischemia, of traumatic brain injury, and of neurodegenerative diseases such as Parkinson's Huntington's diseases, multiple sclerosis, Alzheimer's disease, and spinal cord injury [127].

6.1. Rheumatoid arthritis

Rheumatoid arthritis (RA) affects up to 1% of population. Due to a theory in the second half of the 20th century that RA is caused by infection by microorganisms, it had been treated with antibiotics [128]. Present studies, however, indicate that RA is an autoimmune disease that is under the influence of gut microbiota. Experiments in GF mice show that the complete lack of microbiota results in a reduced severity

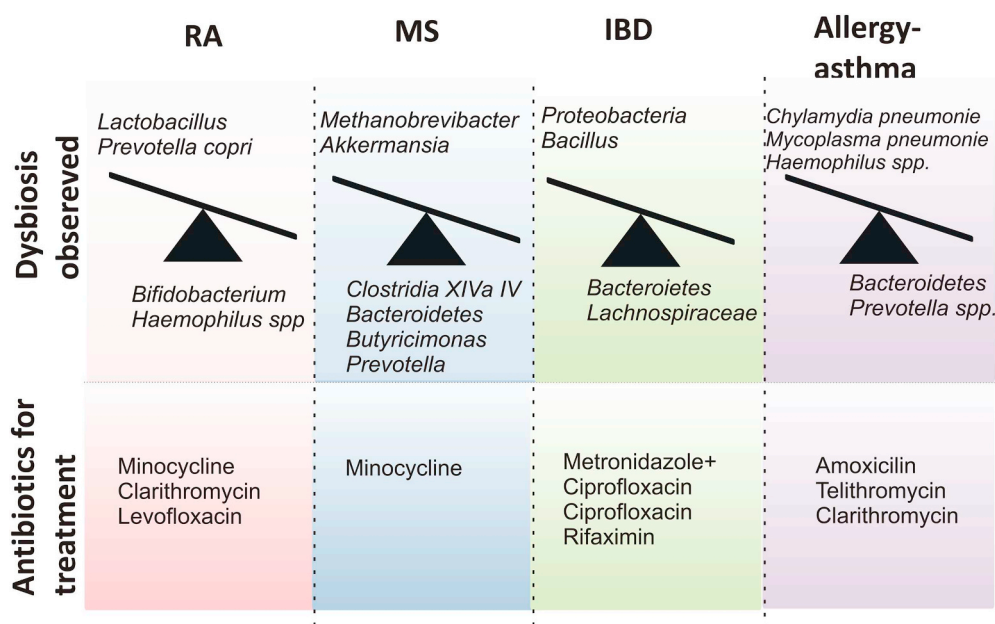


Fig. 3. Autoimmune diseases and allergy are associated with dysbiosis, which could be potentially normalized by antibiotic therapy.

of autoimmune arthritis [44] and that this situation can be reversed by inoculation of a single SFB bacterial species, which promotes development of Th17 cells involved in development of RA. Interestingly, an altered microbiota profile is observed in patients with early and fully developed RA. Early stages of RA are characterized by a decrease in *Bifidobacterium* and the *Bacteroides-Porphyromonas-Prevotella* group as well as the *Eubacterium rectale-Clostridium coccoides* groups [129], and increase in *Lactobacillus* [130]. Enrichment of a single bacterial species, *Prevotella copri*, has been observed in patients with new onset of RA prior to treatment [131]. Patients with fully developed RA have an altered gut, dental and salivary microbiota profile [132]. In general, RA patients have increased number of Gram positive and reduced number of Gram negative bacteria in the intestinal microbiota. The decrease of *Haemophilus spp.* and increase of *Lactobacillus salivarius* might be of particular importance in the pathogenesis of RA, as the abundance of unclassified *Haemophilus spp.* negatively correlates with anti cyclic citrullinated peptide autoantibodies (anti-CCP) and rheumatoid factor (RF) [132], while high numbers of *Lactobacillus salivarius* are seen in very active RA. In addition, dysbiosis is observed in the oral cavity of RA patients which is characterized by an increase in anaerobic bacteria, such as pathogenic *Porphyromonas gingivalis* [132], the DNA of which also has been found in synovial fluid [133].

Early studies have evaluated the impact on RA of tetracyclines, which are broad spectrum antibiotics, acting on both Gram-positive and Gram-negative bacteria, as well aerobic and anaerobic bacteria. Treatment of RA patients at early stage of disease with minocycline results in improved outcomes compared to the hydroxychloroquine treated group [134]. Patients with early stage of RA also have been treated with macrolides, which are mostly used against Gram-negative bacteria. It has been observed that treatment with clarithromycin for 6 months leads to disease amelioration [135,136]. Addition of clarithromycin to standard RA therapy, methotrexate and methylprednisolone, leads to remission 2 months earlier compared to standard RA treatment alone [137]. In another study in active RA, standard methotrexate therapy has been supplemented with levofloxacin, which belongs to the fluoroquinolone class. Treatment with levofloxacin results in a significant reduction of many secondary outcomes of RA [135,136]. Interestingly, treatment with another fluoroquinolone, enrofloxacin, aggravates disease in CIA, a mouse model of RA [138]. Enrofloxacin applied before CIA induction significantly reduces the

number of aerobic and anaerobic bacteria. These discrepancies regarding the timing of antibiotics use and effect on disease should be explored in more controlled manner, as they could answer the question of whether dysbiosis is the cause or the consequence of RA. Up to the present, the results suggest that the development of RA creates a dysbiosis which may be normalized by antibiotics, while antibiotics use before disease development of disease may exacerbate it.

6.2. Multiple sclerosis (MS)

MS is a chronic inflammatory autoimmune disease characterized by the presence of cellular infiltrates consisting primarily of lymphocytes and macrophages as well as localized areas of demyelination in the central nervous system (CNS). MS is thought to be initiated by self-reactive CD4⁺ T cells with specificity for a variety of antigens present within the myelin sheath.

The prevalence of MS depends on geographical location. MS affects up to 1% of the population in Europe and America, and up to 0.05% in Sub-Saharan Africa. This variability suggests that environmental factors, such as microorganisms, or factors that modify them, play a role in the pathogenesis of MS. Indeed, studies employing the animal model of MS, EAE, show that natural gut microbiota drive T cell responses against myelin antigens, initiating autoimmune response in the CNS. It has been observed that mice devoided of microbiota have significantly reduced severity of EAE [43]. Patients with relapsing-remitting MS have distinct microbiota profiles, characterized by decreased numbers of *Clostridia* belonging to clusters XIVa and IV, as well *Bacteroidetes*. Although some *Clostridia* species are known inducers of Treg cells, the *Clostridia* species reduced in MS patients do not show this potential [139]. *Clostridia* also are producers of SCFAs, which exert their anti-inflammatory activity through GPCR receptors present on immune cells. Additionally, it has been observed that SCFA-producing *Butyricimonas* and *Prevotella* are decreased in MS patients, while *Methanobrevibacter* and *Akkermansia* are increased in MS patients [140]. Interestingly, transplantation of human microbiota from MS patients to GF mice lead to a significantly higher incidence of spontaneous EAE compared to the microbiota from healthy-controls. MS patient microbiota transplanted to GF mice have significantly lower levels of *Sutterella* compared to healthy-control microbiota transplants [141].

A study conducted in the United Kingdom evaluating 163 MS

patients shows that short-term penicillin therapy increases the risk of developing MS. Penicillin treatment exceeding 2 weeks, however, and occurring at least three years prior to first symptoms of MS, reduces the risk of the disease [142]. To confirm this observation, a much larger study examining 3259 MS cases from the Danish Multiple Sclerosis Registry has been conducted. This study not only confirms that penicillin use increases the risk for MS but also shows that the use of any kind of antibiotics increases the risk of MS in similar way [143]. Although antibiotic use appears to increase the risk of developing MS, antibiotics use after MS disease initiation may be protective. It has been found that treatment of MS patients after a first focal clinical demyelinating event with minocycline, which is a broad-spectrum antibiotic belonging to tetracycline class, reduces the risk of conversion to fully diagnosed MS [144].

Contrary to clinical data, murine models indicate that the use of antibiotics and antibiotics mixtures before induction of disease can be protective. Mice treated orally with an antibiotic mixture containing ampicillin, neomycin, metronidazole, and vancomycin for one week before EAE induction show significantly reduced severity of EAE. Amelioration of EAE is mediated in part by induction of Treg cells [145]. Additionally, treatment with antibiotics mixture increases the frequency of IL-10 producing CD5⁺B cells that skew Th1/Th17 proinflammatory towards an antiinflammatory Th2 immune response [146]. Interestingly, application of rifampicin starting at the immunization day has been shown to be equally effective in reducing EAE severity. Rifampicin treatment reduces the blood-brain-barrier permeability and inhibits differentiation of Th17 cells, resulting in lower levels of IL-17A in the serum [147]. Similarly, treatment of mice with the β -lactam antibiotic ceftriaxone starting at the time of myelin basic protein immunization or at the onset of EAE show a reduced severity of disease and more rapid recovery from disease, respectively [148]. Treatment with ceftriaxone suppresses antigen presentation by DCs, leading to a reduction of T cell proliferation, cytokine production, and T cell migration to the CNS [148]. Although these data indicate that antibiotic treatment suppresses EAE, some studies point out an opposite effect. Rats treated with macrolides (clarithromycin or azithromycin) starting two days before EAE induction develop more severe disease, which may be explained by reduced expression of nitric oxide synthase iNOS [149].

6.3. Colitis

The natural gut flora primes and shapes immune responses in the intestines. It has been observed that lack of natural microbiota in GF mice reduces the severity of experimental colitis. In conventional mice, the severity of this disease is strictly associated with gut microbiota composition [150]. In humans, inflammatory bowel diseases (IBD), such as ulcerative colitis (UC) and Crohn's disease (CD), are complex chronic inflammatory diseases of largely unknown cause that affect approximately 3.6 million people in Europe and the USA, mainly of Caucasian descent [151]. The pathogenesis of IBD is not fully understood, but it is well accepted that CD is a Th1/Th17-T cell-driven process, whereas UC is a Th2-like T-cell-driven process [152]. IBD patients have modified gut bacteria profiles, characterized by a decrease in *Bacteroidetes* and *Lachnospiraceae*, belonging to *Firmicutes*, and increase of *Proteobacteria* and *Bacillus* [153]. Because of this strict relationship between gut microbiota and the severity of intestinal inflammation, IBD seems to be an ideal disease to be treated by supplementation with proper microorganisms.

Several mouse models indicate that treatment with broad spectrum antibiotics is an efficient method to reduce the severity of experimental colitis. Prophylactic and therapeutic application of broad-spectrum mixture of vancomycin and imipenem reduces the severity of DSS-induced colitis [150] and spontaneous colitis in IL-10^{-/-} mice [154]. When mice in both models are treated either with ciprofloxacin or metronidazole alone, the suppressive effect on colitis is observed when antibiotics are given prior to diseases induction [150]. A similar pattern

has been observed in another study, in which IL-10^{-/-} mice treated with a mixture of neomycin and metronidazole show a significantly reduced severity of colitis, while treatment with the single antibiotic ciprofloxacin does not alleviate the disease [155].

Although murine studies suggest that broad spectrum antibiotics seem to have a beneficial influence on intestinal inflammation, several clinical studies indicate that the beneficial role of antibiotics is limited to CD only, as UC patients are refractory to antibiotic therapy. Vancomycin [156], ciprofloxacin [157], and rifaximin [158], given to UC patients along standard therapy, does not improve disease severity compared to a control group that received standard therapy only. On the other hand, the severity of CD can be reduced by antibiotic treatment. Additionally, metronidazole combined with ciprofloxacin [159], ciprofloxacin alone [160], and rifaximin [161] all have been shown to be effective alternative treatments for CD.

6.4. Allergic contact dermatitis

A cell-mediated hypersensitivity reaction in the skin to small chemical molecules called haptens underlines allergic contact dermatitis (ACD) in humans and contact hypersensitivity (CHS) in mice. ACD is a significant medical and economic problem, accounting for 30% of all occupational diseases [162]. It is worth noting that the immune response in the skin is under the influence of gut microbiota. It has been observed that mice fed with fructo-oligosaccharide (FOS), which increases the number of *Bifidobacterium pseudolongum*, or mice supplemented with *Bifidobacterium pseudolongum*, develop less severe CHS to 2,4-dinitrofluorobenzene (DNFB) [163]. Our recent work shows that partial depletion of gut microbiota with the broad-spectrum antibiotic enrofloxacin, significantly reduces the severity of CHS to picryl chloride (TNP-Cl) [102]. The suppression of CHS reaction is due to gut dysbiosis, characterized by an increased proportion of *Clostridium coccoides* (cluster XIVa), *Clostridium coccoides*–*Eubacterium rectale* (cluster XIVab), *Bacteroidetes*, and *Bifidobacterium* spp., but decreased SFB [102]. These changes in microbiota composition support the induction of cells with regulatory potential in the periphery that suppress CHS reactions in the skin [102].

6.5. Atopic disorders

Atopy is a genetically linked predisposition to produce IgE antibodies to innocuous environmental antigens. Atopic persons have a high chance to develop one or more atopic diseases such as asthma, eczema and hay fever. Although atopic diseases are more common among children, their incidence in adults is still very high, affecting up to 10%–15% of the population depending on type of atopic disease [164]. As discussed earlier, the development of atopic diseases in children is linked tightly to the deprivation of microbial signals due to increased hygiene. Moreover, antibiotics use prenatally or postnatally increases the chances of developing asthma and other forms of allergy. There does not seem to be a similar association in adults. We have found that treatment of adult mice with the broad-spectrum antibiotic enrofloxacin two weeks before and one week during epicutaneous immunization with OVA does not increase production of OVA-specific IgE antibody. Interestingly, production of OVA-specific IgE antibodies is decreased although not-significantly [98]. However, partial depletion of microbiota leaves an empty niche that can be colonized by yeasts, which stimulate Th2 type immunity that could mask the effect of antibiotic treatment. Indeed, partial depletion of the microbiota with 5 days cefoperazone treatment enables colonization with *Candida albicans*, which results in the production of prostaglandin-like immune modulators. In this study, altered gut microbiota promote the allergic response to OVA in lungs [165]. Our previous work has demonstrated that microbiota modification with antibiotics not only modulates IgE production but also affects cytokine production. Treatment of adult mice with enrofloxacin for three weeks reduces the production of Th1,

Th2 and Th17 type cytokines [98]. Another study shows that mice with atopic dermatitis treated with cefazolin and enrofloxacin have reduced serum concentration of IL-1 β , IL-6, IL-13, IL-17A and IL-17F [166], supporting the antiinflammatory potential of antibiotics. Interestingly, asthmatic patients treated with clarithromycin for 6 weeks have reduced levels of IL-5, IL-12, and TNF- α in the lungs [167].

The influence of antibiotic use on the development of atopic disorders in adults is less significant than in children. In adults, gut dysbiosis, characterized by reduced *Clostridiales*, increased *Bacteroidales*, and decreased microbiota diversity, is associated with seasonal allergies [168]. Asthmatic adults also have an altered pulmonary microbiota, characterized by increased abundance of *Proteobacteria*, particularly *Haemophilus* spp., and decreased frequency of *Bacteroidetes*, particularly *Prevotella* spp. [169]. The presence of two atypical pulmonary pathogens, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*, is also linked with asthma. Additionally, skin microbiota changes during onset of AD. It has been observed that colonization with *Staphylococcus aureus* drives eczema development, while colonization with *Corynebacterium bovis* induces robust Th2 type immune responses [166].

Altered microbiota profiles in atopic adults indicate that antibiotics could be potential therapeutic agents for atopy, through normalizing gut, lung, or skin microbiota. This subject has been investigated by only a limited number of studies that mostly focused on antibiotics as a potential therapy for asthma in adults. Early study show that amoxicillin to be an ineffective treatment for asthma exacerbation [170], as later confirmed by others. Since asthmatic patients are colonized with atypical bacteria, such as *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*, other investigators have evaluated the therapeutic potential of macrolides, which have the best coverage for atypical bacteria. In a multicenter clinical study examining 278 patients with acute asthma treated with telithromycin for 10 days, antibiotic treatment significantly improves asthma symptoms compared to placebo-treated patients, who needed 42 days to recover to similar status [171]. In another study, asthmatic patients treated with clarithromycin for 6 weeks show significant improvement in asthma symptoms, but only in patients who were positive for *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* [167].

Antibiotic treatment also is effective therapy for AD in mice. Mice that lack Adam17 in Sox9-expressing tissues, such as epidermidis, develop spontaneous AD. The inflammation is driven by colonization with *Staphylococcus aureus* and *Streptococcus bovis*, and can be suppressed by antibiotic treatment. Oral cefazolin and enrofloxacin almost completely reverse dysbiosis and eliminate skin inflammation [166].

7. Conclusions

Antibiotics are crucial medications that save millions of human lives. For millennia, we have coevolved with certain types of microorganisms, creating a mutually beneficial cooperation. Antibiotics do not discriminate between our symbionts and pathogenic guests, killing both good and bad bacteria, leading to dysbiosis. As a crucial function of our naturally residing flora is to shape immune responses, it is not surprising that altered composition of the microbiota is linked with immune-mediated diseases, including allergic and autoimmune disorders. Antibiotics can either promote or suppress the development of immune-mediated disorders depending on the time of life during which they are given. Antibiotics use in children promotes the development of allergic disorders, whereas antibiotics use in adults seems to ameliorate inflammatory responses and reduce the severity of autoimmune diseases. This picture is much more complex, however, due to the prominent immunoregulatory functions of certain bacterial species. More studies are needed to clarify further the role of specific bacteria species in the development of autoimmune and allergic diseases, and how antibiotics affect them.

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