EFFECTS OF ANTIBIOTIC USE ON THE MICROBIOTA OF THE GUT AND ASSOCIATED ALTERATIONS OF IMMUNITY AND METABOLISM

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ABSTRACT

The excessively widespread use of antibiotics has created many threats. A well-known problem is the increasing bacterial resistance to antibiotics, which has clearly become a worldwide challenge to the effective control of infections by many pathogens. But, beyond affecting the pathogenic agents for which it is intended, antibiotic treatment also affects the mutualistic communities of microbes that inhabit the human body. As they inhibit susceptible organisms and select for resistant ones, antibiotics can have strong immediate effects on the composition of these communities, such as the proliferation of resistant opportunists that can cause accute disease. Furthermore, antibiotic-induced microbiota alterations are also likely to have more insidious effects on long-term health. In the case of the gut microbiota, this community interacts with many crucial aspects of human biology, including the regulation of immune and metabolic homeostasis, in the gut and beyond. It follows that antibiotic treatments bear the risk of altering these basic equilibria. Here, we review the growing literature on the effects of antibiotic use on gut microbiota composition and function, and their consequences for immunity, metabolism, and health.

Keywords: Antibiotics, human microbiome, gut microbiota, pathogenic bacteria, infection, immunity, autoimmunity, immunotolerance, atopy, inflammation, metabolism, obesity, metabolic syndrome.

INTRODUCTION

The gut harbours the most dense and complex microbiota of the human body, which contributes importantly to several basic physiological functions, including nutrition, defence against pathogens, and metabolic and immune homeostasis. Consequently, disturbances in the composition and function of the gut microbiota, i.e. dysbioses, can have severe consequences for health at several different levels.[1,2] In particular, evidence is mounting for the involvement of dysbioses in the broad variety of health problems associated to immune and metabolic malfunctions. Antibiotics are one of the main factors causing dysbiosis and therefore play a significant role in generating the associated suite of undesirable health effects. In this review, we will first address the direct effects of antibiotics on the gut microbiota, and we will then discuss how antibiotic-induced dysbioses relate to immune and metabolic health, and the underlying mechanisms likely to be responsible for such relationships.

EFFECTS OF ANTIBIOTICS ON THE GUT MICROBIOTA

Numerous works employing different technologies have explored the effects of antibiotics on the

composition of the gut microbiota. These studies have been consistent in demonstrating that dysbiosis can ensue upon antibiotic administration. In the case of broad-spectrum antibiotics, deep 16S rRNA gene pyrosequencing showed that the abundances of roughly one-third of the bacterial taxa in the gut microbiota could be affected, decreasing the taxonomic richness, diversity, and evenness of the community.[3] Moreover, the effect of antibiotic administration on gut microbiota composition is rapid, as drastic losses of diversity and abundance shifts can occur within 3 days of drug initiation.[4] After termination of antibiotic treatment, the gut microbiota presents a certain degree of resilience, in that it is usually capable of returning to a composition more similar to the one it had before. However, a complete return to the initial state is often not achieved. Several studies have followed-up the progress of gut microbiota composition during months to years after treatment, demonstrating that differences can remain after long periods of time.[3-6] Mouse models have similarly revealed long-lasting gut microbiota alterations induced by different antibiotics, including ampicillin, cefoperazone or vancomycin.[7,8]

More recently, the characterisation of the effects of antibiotics on the gut microbiota has been extended to multi-omic analyses, demonstrating that antibiotic treatment not only alters the taxonomic composition, but also the overall gene expression, protein activity and metabolism of the community. Monitoring of the gut microbiota during and after 14-days of β-lactam therapy revealed that the metabolome was maximally altered by day 6 after the initiation of treatment, whereas maximum change in taxonomic composition was not reached until day 11, when richness and diversity were lowest.[9] This shows that the gut microbiota can modify its metabolic activity in response to antibiotic stress much faster than the relative survival and growth of different bacterial taxa, which will affect community structure. Further to this, the carbohydrate-degrading enzymatic activities of the total gut microbiota from β-lactam-treated patients were experimentally measured, showing that these subjects had high and unbalanced sugar anabolic capacities, similar to those observed in obese individuals.[10] In a different approach, the short-term alteration of microbial physiological state and activity was analysed after *ex vivo* incubation of faecal samples with different antibiotics.[11] In most cases, antibiotic exposure increased the proportion of gut microbiota cells with damaged membranes, particularly for cell wall synthesis inhibitors, such as ampicillin and vancomycin. These antibiotics also affected which fractions of the gut microbiota were more or less active, as judged by the amount of nucleic acids detected within the cell. In particular, after ampicillin treatment the proportion of Bacteroidetes increased within the most active gut microbiota fraction. Antibiotics also affected community-wide gene expression, with increases in the expression of genes for antibiotic resistance, stress response, and phage induction. In addition, those antibiotics that act by inhibiting translation, such as tetracycline and the macrolides, also resulted in an increased expression of genes related to genetic information processing (e.g. transcription and translation). Overall, these works clearly demonstrate that antibiotics alter not only the taxonomic composition, but also the functioning of the gut microbiota, and therefore stress their potential to impact on the numerous human physiology processes that rely on microbial activities.

ANTIBIOTIC-INDUCED GUT MICROBIOTA

Alterations on the Immune System and Related Health Problems

The impact of antibiotics on the gut microbiota has short and long-term effects on the development and operation of the immune system that can generate a variety of health problems. Such problems relate mainly to a decreased resistance to infection or to a disrupted immune homeostasis, which may result in atopic, inflammatory or autoimmune disease.

Decreased Resistance to Infection

In the short-term, drastic alterations of gut microbiota composition and function can affect the immediate risk for intestinal infection due to the acquisition and spread of incoming pathogens or to the opportunistic pathogenic behaviour of some resident members of the gut microbiota. An important example of this problem is the prevalence of antibiotic-associated diarrhoea (AAD), caused by intestinal overgrowth of common nosocomial pathogens such as *Klebsiella pneumoniae*, *Staphylococcus aureus* and, most frequently, *Clostridium difficile* (*C. difficile*).[12-15]

Importantly, *C. difficile* can cause a varying degree of ailments from a single, self-limiting episode of diarrhoea to more intractable, long-term problems with recurrent infections.[1,16] The gut microbiota of patients infected with *C. difficile* has decreased diversity,[17] and a mouse model has shown that treatment with clindamycin produces long-lasting changes in the small and large intestinal microbiota, with loss of approximately 90% of the cecal taxa, which can be followed by a state of chronic *C. difficile* infection.[18,19] It has been postulated that AAD may also result from *Candida* overgrowth,[20-22] although elevated numbers of intestinal *Candida* can be a consequence of antibiotic treatment or diarrhoea per se rather than a direct cause of AAD.[23]

The effects of antibiotics on the capacity of the immune system to battle infection probably proceed through several related ways, involving both innate and adaptive immunity. Antibiotic-induced gut microbiota changes can alter the type and diversity of microbial-associated molecular patterns (MAMPS) that are encountered by receptors such as the cytosolic NOD1 and the membrane-spanning Toll-like receptors (TLRs), present in various intestinal epithelial cells (IECs) and innate immunity cells. In turn, the altered stimulation of these receptors can impact numerous processes, from the development of intestinal lymphoid tissues to the differentiation of T cell subtypes, the priming of neutrophils for bacterial killing, the production of antibacterial molecules, and the release of cytokines and pro-cytokines with a variety of functions.[24] For instance, treatment of mice with metronidazole, neomycin and vancomycin diminishes expression of Reg3γ, a lectin with bactericidal activity against Gram-positives, the expression of which is normally induced through the interaction of MAMPS with TLRs present in the surface of T cells and IECs; as a result, vancomycin-resistant *Enterococcus* (VRE) colonisation of the small and large intestine is facilitated, potentially leading to a state where >97% of the gut microbiota is VRE.[8,25,26] Similarly, administration of a cocktail of antibiotics containing ampicillin, neomycin, metronidazole, and vancomycin depletes the gut microbiota and diminishes the level of the MAMP peptidoglycan, which reduces the neutrophil-mediated killing of *Streptococcus pneumoniae*.[27] Also, immune responses against viral infection can be affected by the antibiotic-induced alteration of pro-cytokine expression; for example, neomycin, an antibiotic that predominantly kills Gram-negative bacteria, decreases the expression of the pro-interleukins (IL) pro-IL-1β and pro-IL-18, impairing responses against the influenza virus.[28] Regarding adaptive immunity, amoxicillin-induced gut microbiota changes have been shown to reduce expression of Major Histocompatibility Complex (MHC) Class I and Class II genes in the small and large intestine, as well as serum levels of immunoglobulin G (IgG).[29]

Disrupted Immune Homeostasis and Tolerance

Beyond increasing the risk for infection, antibiotic-induced alterations of the gut microbiota can affect basic immune homeostasis with body-wide and long-term repercussions. Atopic, inflammatory, and autoimmune diseases have been linked to gut microbiota dysbiosis. For example, a metagenomic approach applied to Crohn´s disease (CD) patients highlighted a reduction in Firmicutes (particularly *Clostridium leptum*) and an increase of some Gram-negative bacteria (Porphyromonadaceae) often responsible for inflammatory processes.[30,31] In the case of irritable bowel syndrome (IBS), which is the most common functional gastrointestinal disorder in Western countries, alterations in the gut microbiota have also been detected,[32-34] accompanied by an over-secretion of microbial organic acids.[35] Although no consensus has been reached regarding the association between specific bacteria and IBS, the gut microbiota of IBS patients has a reduced diversity. Moreover, IBS often follows bouts of gastrointestinal infection (post-infectious IBS) and there is evidence to suggest that antibiotics may play a role in the pathogenesis of the disorder.[36]

The effects of dysbiosis will be ever more relevant if they occur early in life, when the immune system is maturing and immunological tolerance is being established. For instance, CD has been shown to increase in children treated with antibiotics during their first 5 years.[37] Also, it has been known for decades that the specific composition of the gut microbiota during infancy and early childhood is linked to the relative occurrence of atopic diseases.[38-40] In this respect, a protective role against atopy has often been reported for lactic acid bacteria (LAB), mainly *Bifidobacteria* and *Lactobacillus*,[41-43] whereas high abundances of *Escherichia coli* and other enterics have been linked to eczema and other

allergies.[40,44-46] Furthermore, some relationships between gut microbiota composition and atopic disease, such as the association between eczema and a low-diversity microbial community dominated by enterobacteria, may extend back to the intrauterine stage, since this type of microbiota is more prevalent in the meconium of newborns who later on will develop this disease, and in those whose mothers are affected by it.[47] However, the link between allergies and antibiotic use during early life has yet to be firmly established, as associations have been found in some epidemiological studies but not in others.[48-50]

At the cellular and molecular level, the mechanisms by which gut microbiota species interact with components of the immune system to impact the development of immunotolerance are currently debated.[39,51-54] Until recently, the most critical factor in maintaining immune homeostasis was thought to be the balance between the adaptive immunity Th1 and Th2 helper cell subsets. Indeed, excessive Th1 or Th2 activation results in chronic inflammatory and autoimmune disease or in allergic disease, respectively.[55,56] However, new lines of evidence indicate that other factors are important for immune balance, including a major role for regulatory T cells (Treg) and their anti-inflammatory actions. In this view, an inadequate microbial colonisation of the gut results in an imbalance between Treg cells and their effector targets, the different Th cells, and the subsequent deregulation of immune responses could promote inflammation, autoimmunity or the onset of atopies.[40,51,57-59] Experimental work in mice has demonstrated that crosstalk between the gut microbiota and the immune system is indeed obligatory for the generation of Tregs within the intestine and the avoidance of pathological intestinal inflammation.[60] The picture of immune regulation has also grown more complex due to the discovery of the IL-17-producing Th cells (Th17),[61] which are providing new insights into the cellular and molecular mechanisms of immunity and have been shown to be important in diseases that had classically been defined as Th1 or Th2-mediated.[55,62,63] In this context, different commensal microbes will induce the differentiation of naïve T cells into different subtypes with different roles. For instance, experimental work in mice has shown that *Bacteroides fragilis*[64] and *Clostridium* species belonging to phylogenetic groups IV and XIV[65] promote the differentiation of T cells into anti-inflammatory Tregs, while the segmented filamentous bacteria (SFB) induce the development of the pro-inflammatory Th17.[66]

Studies in mice have also provided specific results regarding how antibiotics affect the balance of T cell subtypes and the homeostasis of the immune system. In agreement with the roles of *Clostridium* species and SFB just described, treatment with vancomycin, an antibiotic that kills Gram-positive bacteria, reduces the numbers of Treg cells in the colon lamina propia, and impairs the induction of Th17 cells.[65] Moreover, administration of antibiotics during early life has been shown to be a determinant of allergic sensitisation. Administration of kanamycin to 3-week-old mice reduced Peyer's patch cellularity and induced skewing of immune responses towards Th2 (increased IgE and IgG1 and stimulated IL-4 production) while reducing Th1 responses (interferon-γ production). These changes could be reversed by colonisation with *Enterococcus faecalis* and attenuated by *Lactobacillus acidophilus*, but were exacerbated by *Bacteroides vulgatus*, underscoring the importance of specific types of bacteria in maintaining immune balance.[67] Similarly, 2-week-old mice treated with a cocktail of antibiotics had decreased expression of TLRs and produced cytokine profiles that maintained a Th2 phenotype.[68] These experiments clearly show that early antibiotic administration can bias immune development towards an atopy-prone state.

In addition to their effects on the balance of T cell subtypes, antibiotic-induced dysbioses are also likely to influence immunotolerance through their impact on other processes that affect the general inflammatory tone of the intestine. Among these, dysbioses can reduce the production of the non-inflammatory IgA that contributes to pathogen and allergen exclusion in the intestinal epithelia, mucus and lumen.[40,51] Importantly, metronidazole has been shown to reduce the intestinal expression of Muc2, the major component of the mucin layer,[69] and the ensuing thinning of this layer may increase contact between epithelial cells and the gut microbiota, thereby enhancing innate immune stimulation and elevating inflammation.

EFFECTS ON METABOLIC HEALTH

The gut microbiota is increasingly considered an important factor in the regulation of host metabolism, in particular as it relates to energy homeostasis and adiposity. This is not surprising, given that intestinal microbes consume non-digestible carbohydrates and produce short-chain fatty acids (SCFA), which play several important metabolic roles. SCFA modulate secretion of the hormone GLP1, which in turn improves insulin secretion,[70] and regulates fat deposition through interaction with G-protein-coupled receptors (GPCRs).[71] Intestinal microbes also convert primary bile acids, synthesised in the human liver, into secondary bile acids, which bind to the GPCR TGR5 to promote glucose homeostasis.[72]

Several metabolic disorders have recently been linked with disbalances of the gut microbiota. Notably, obesity has been shown to be associated with phylum-level changes in the gut microbiota, reduced bacterial diversity, and altered representation of bacterial genes and metabolic pathways, differences that endow the obesity–associated microbiota with an increased capacity to harvest energy from the diet.[73-75] This is in line with the fact that long-term exposure to antibiotics is associated with increased body mass index, both in humans[76-78] and in farm animals, where low-dose antibiotics have long been used to promote weight gain.[79] Moreover, recent work in mice has shown that early antibiotic exposure can cause obesity even with normal dietary intake.[80] Antibiotic use is therefore emerging as an important risk factor for the development of obesity.

Overweight and obesity can progress to metabolic syndrome, a complex of metabolic abnormalities leading to an increased risk for cardiovascular disease, fatty liver disease, steatohepatitis, and type 2 diabetes.[81] Although the pathophysiological mechanisms that lead from obesity to metabolic syndrome are still unclear, they likely include the generation of a state of chronic low-grade inflammation associated with excess adipose tissue, which seems to be at least partly mediated by the gut microbiota. In this respect, high-fat diets (HFD) have been shown to alter the gut microbiota of mice with an increase of lipopolysaccharide (LPS)-containing bacteria, which leads to higher amounts of this pro-inflammatory bacterial cell wall component in blood serum. Interestingly, continuous subcutaneous infusion of LPS, mimicking the HFD state, has been shown to induce some of the aspects of metabolic syndrome.[82] Similarly, influx into the portal vein of bacterial components agonistic of TLR4 and TLR9 promotes the progression of fatty liver disease to steatohepatitis by enhancing hepatic expression of the pro-inflammatory cytokine TNFα.[83] On the other hand, TLR5 deficiency results in a dysbiotic state that promotes metabolic syndrome signs, such as obesity, insulin resistance and dyslipidaemia, and transplantation of the gut microbiota from TLR5-deficient mice into germ-free recipients can transmit the phenotype, suggesting that the gut microbiota alone can mediate disease.[84] Moreover, wild-type mice containing the altered gut microbiota had higher intestinal levels of pro-inflammatory TNFα and IL-1β, suggesting that the transplanted gut microbiota did contribute to the observed metabolic disorders through the induction of intestinal inflammation. Because antibiotic use can lead to gut microbiota alterations that promote inflammation,[51,69,85] it could exacerbate the progression from obesity to metabolic syndrome.

CONCLUDING REMARKS

Antibiotic-induced dysbioses have a variety of negative effects on health, some of which can remain for long periods of time after antibiotic administration. Beyond admonishing against the unnecessary, excessive or inefficient use of antibiotics, this realisation should promote research into potential strategies to minimise the negative consequences of antibiotics when their administration is required. Promising approaches involve the use of probiotic bacteria or of bacterial ligands of innate immune receptors to re-establish the interactions impeded by the antibiotic-induced alterations of the original gut microbiota.[24] However, the effective display of such approaches will necessitate much further research into the manners in which specific bacteria interact with the different components of the immune system to maintain its balance.

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