RELEVANCE OF THE MICROBIOME IN AUTOIMMUNE-INFLAMMATORY DISEASES

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LAY SUMMARY

The human body is a vast and complex ecosystem, teeming with billions of organisms that are invisible to the naked eye. These microorganisms, collectively known as the microbiome, play a crucial role in our health, development, and overall well-being.

Recent studies have revealed that the composition of the microbiome could be one of the factors that lead to several common autoimmune diseases such as ulcerative colitis, Crohn's disease, multiple sclerosis, rheumatoid arthritis, type 1 diabetes, and systemic lupus erythematosus.

However, the field is still beginning, and it's difficult to interpret such a huge amount of data because various factors linked to lifestyle, including diet and exercise, influence the microbiome composition.

Combining data from clinical groups of people with neurological, gastrointestinal and systemic autoimmune diseases may help us shed some light on microbiota's role in many severe human conditions and develop new and personalized treatments.

SCIENTIFIC SUMMARY

The human body, including the gastrointestinal tract, skin, and other mucosal environments, is inhabited by many microorganisms known as the microbiome. Over the last twenty years, several studies using sequencing-based approaches have revealed that the microbiota is strictly related to many autoimmune diseases (ADs), such as inflammatory bowel disease, including ulcerative colitis and Crohn's disease.

It has also been observed that changes in the composition of the gut microbiome, which is influenced by genetic and environmental factors such as lifestyle (smoking and diet) and certain specific bacterial taxa and their metabolites, show associations with clinical indices in patients with ADs, such as multiple sclerosis, rheumatoid arthritis, type 1 diabetes, and systemic lupus erythematosus.

There is evidence that alteration in the microbiome strongly affects the production of serotonin, thus influencing the levels of IL-6 and IL-17, two pro-inflammatory cytokines that stimulate immune cell activation and further cytokine production.

Moreover, an intriguing new theory suggests a direct link between the well-known "molecular mimicry" and the alterations of the gut-associated lymphoid tissue suggesting a link between several pathogens such as EBV and multiple sclerosis.

Nevertheless, the field is still developing, and analyzing and interpreting such a huge amount of data on the constitution of the microbiome can be challenging. However, combining data

from clinical groups of people with neurological, gastrointestinal and systemic autoimmune diseases may help us to shed some light on the role of the microbiome in many severe human conditions and to develop new and personalized treatments.

INTRODUCTION

Over the last decades, research into the human microbiome, which is made up of several organisms that interact with each other and the immune system, has been quickly advancing, showing that it is strictly related to human health.

The interest in the microbiota, its composition and its effects on human health and disease have thus extended into every area of medicine.

Autoimmune-inflammatory diseases were the first conditions in which the microbiome research helped understand some of the possible factors that could lead to the disease's development and identify novel treatment avenues.

This review summarizes recent progress in understanding how the gut microbiome is involved in several intestinal and extra-intestinal diseases.

We first describe how the microbiota can influence immune function through complex interactions in the gut. We then discuss the evidence that dysbiosis, which is the alteration of the microbiome's composition, is associated with autoimmune-inflammatory diseases. In conclusion, we consider potential microbiome-targeting therapeutic strategies.

MICROBIOTA AND THE IMMUNE SYSTEM

Autoimmune diseases occur when the body's natural defense system, the immune system, cannot distinguish between its cells and foreign cells, causing the body to attack normal cells mistakenly.

Over the past decades, several hypotheses have been made up for developing this type of disease, some related to the microbiota.

The microbiota can vary in composition due to several genetic and environmental factors, in particular, linked to lifestyle and eating habits. It is believed that alterations in the microbiome's composition can affect immune functions in different ways.[1-3]

SEROTONIN

The first process that is considered to be influenced by bacterial metabolites is the production of serotonin, which has well-known critical roles in the brain and gut.[1,2]

Functions of serotonin

In the brain, it acts as a neurotransmitter involved in behaviour, cognition and locomotor activation. In the gut, it affects intestinal peristalsis, motility, mucus secretion, vasodilatation and absorption of nutrients.

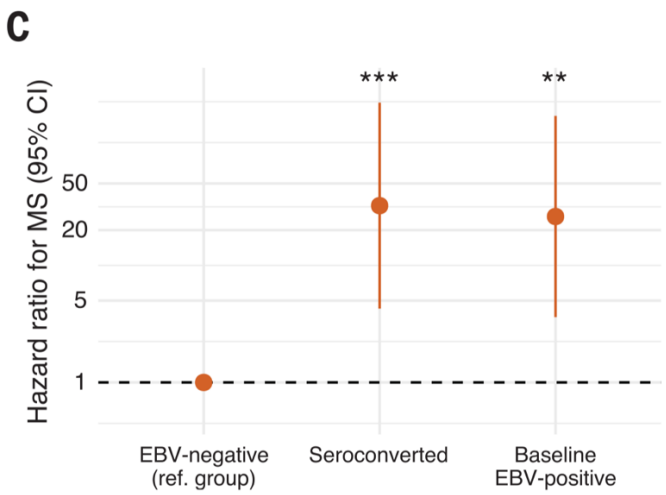
Additionally, serotonin shows several immune-regulatory functions through receptors expressed on innate and adaptive immune cells. It controls the production of cytokines by immune cells, regulates the engagement of neutrophils and activation of T cells, and can stimulate autoreactive T cells to produce Interleukin-6 and Interleukin-17.
Interleukins, like IL-6 and IL-17, are pro-inflammatory cytokines that stimulate immune cell activation and further cytokine production. Their dysregulation has been linked with several autoimmune diseases: for example, IL-17-secreting T cells (Th17 cells) were relevant in several autoimmune diseases. Blood levels of IL-17 were significantly higher in patients with MS than in healthy controls, and IL-17 serum levels and the frequency of relapses in MS were strongly correlated. [1,2,9]

Serotonin and the microbiota

More than 90% of serotonin occurs in the gut, where microbiota strongly affects its production. The commensal gut microbiota plays an essential role in producing short-chain fatty acids via the fermentation of dietary fiber. These specific short-chain fatty acids intensify the tryptophan hydroxylase 1 (TPH1) activity in some cells of the gastrointestinal epithelium. This enzyme (TPH1) then increases the transformation of dietary tryptophan into serotonin.
Any alterations in intestinal homeostasis thus affect these processes: in gut dysbiosis, a reduction in short-chain fatty acids can reduce the activity of THP1, which compromises serotonin production, leading to lower levels of serotonin and higher levels of tryptophan; this dysregulation contributes to abnormal systemic immune response and has been implicated in several autoimmune diseases.[1-3]

MOLECULAR MIMICRY

A second relevant mechanism that links microbiota dysregulation and autoimmune diseases is “molecular mimicry”. According to this theory, a foreign antigen shares sequence or structural similarities with self-antigens leading to the activation of adaptive immune cells.
For instance, a recent study conducted on more than 10 million young adults on active duty in the US military, 955 of whom were diagnosed with MS during their service, has revealed that the risk of MS increased 32-fold after infection with EBV.
A significantly higher number of individuals who later developed MS were EBV-positive in the second (28 of 33 MS patients) and third (32 of 33 MS patients) samples compared with individuals who did not develop MS (second sample: 40 of 90 controls; third sample: 51 of 90 controls, $p<0.001$). [1,2,8]



The risk ratio for MS according to EBV status. EBV seroconversion by the time of the third sample and EBV seropositivity at the time of the first sample was associated with a 32-fold and 26-fold increased risk of developing MS, respectively, in matched analyses.
(Adapted from Ascherio et al. Science 2022)

Therefore, a recent intriguing theory suggested that EBV that colonizes the gut microbiota activates the B-cell within the gut-associated lymphoid tissue (GALT) and that, then, the structural similarity between EBV viruses and the myelin cells would lead the immune system to attack not only the external agent, which in this specific case is the EBV, but also its cells. Other studies showed then that also the Pc-P27 peptide derived from *Prevotella copri,* a bacterium part of the human gut microbiota, stimulates T cells and B cells in patients with RA but not in healthy individuals. The presence of two autoantigens specific for RA both in the synovial tissue and in the microbial peptides from *Prevotella* sp., would lead the immune system, after being activated presumably in the GALT by Pc-P27, to recognize the cells of the synovial tissue as external agents and so attack them. [1,2,7,8]
A similar process has been detected then also for Ro60, an RNA-binding protein expressed in human commensals such as *Propionibacterium propionicum* and *Bacteroides thetaiotaomicron*, which is considered an autoantigen in systemic lupus erythematosus. [1,2,8]

THERAPEUTIC IMPLICATIONS

Considering the effects of the microbiota on the immune system and the evidence that alterations to the microbiota's composition play a pivotal role in autoimmune disease, targeted interventions to control and stabilize the gut microbiota look promising as therapeutic agents. Various approaches are under investigation, the main ones will be generally presented in the following section. [1,4,7]

Indigenous microbiome manipulation

There are mainly three potential approaches to manipulating the microbiota:

(1) direct modulation by adding or eliminating specific bacterial strains or communities.
(2) indirect modulation through diet, medication or other environmental factors.

(3) a complete replacement of the indigenous microbiota through fecal microbiota transplantation from a suitable donor.

Although all these approaches have been shown to positively affect the progress of autoimmune diseases in experimental autoimmune encephalomyelitis mice, corresponding trials in humans are mainly inconclusive. [1,2,7]

Diet

The most straightforward approach might be changing the lifestyle.

Dietary interventions can alter the microbiota composition quickly in humans.

Unfortunately, it is unclear how long they need to be maintained to achieve a permanent alteration of the microbiota, and they are notoriously difficult to enforce. More extensive and rigorous clinical studies are, therefore, a critical unmet need. [2,3]

CONCLUSIONS

The microbiota is vital for developing and maintaining immune function. However, as shown in this review, convincing evidence has allowed the identification of gut microbes correlated or associated with Ads. However, the functional role of these microbes on disease development and progression remains to be elucidated. More translational and clinical studies are required to clarify the mechanisms linking the microbiome to ADs and identify possible non-pharmacological and personalized treatments.

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