PRIZE ESSAY

MICROBIOME

Can microbes combat neurodegeneration?

Identifying a new link between microbiome and metabolites in amyotrophic lateral sclerosis

By **Eran Blacher**

Millions of people worldwide suffer from neurological disorders such as Alzheimer's disease (AD), Parkinson's disease, and amyotrophic lateral sclerosis (ALS). By gradually destroying motor abilities, communication skills, memory, and clear thinking, these devastating diseases rob patients of their independence and take a heavy toll on family members and caregivers.

The exact causes of neurodegeneration remain unclear. Only 10% of ALS cases and 5% of AD cases are familial, whereas the vast majority are of unknown etiology (*1*). In 1993, mutations in the Superoxide dismutase-1 (*Sod-1*) gene were shown to cause ALS and now account for 18.9% of familial ALS cases. Since that discovery, sequencing-based studies revealed additional relevant disease-associated mutations, but limited progress has been made in explaining the molecular mechanisms of neurodegeneration (*2*). With so little causative understanding of neurodegeneration, we must ask: Do environmental factors—such as nutrition, commensal bacteria, and their metabolites—play a role in neurological disorders?

The past decade has witnessed a paradigm shift in brain research. A transition from a dogmatic brain-focused approach toward a holistic conception of health that integrates key signaling hubs of the human body—such as the gut and its microbial populations, the peripheral immune system, and other mucosal barrier surfaces—is increasingly acknowledged as necessary to understand and cure neurodegenerative diseases.

I studied the role of the gut microbiome and its associated molecules in ALS as a postdoctoral fellow in Eran Elinav's laboratory at the Weizmann Institute of Science. The results of this study suggest that gut microbes may secrete small-molecule metabolites that potentially have unexpected regulatory functions in ALS progression both in mouse models and in human patients (*3*).

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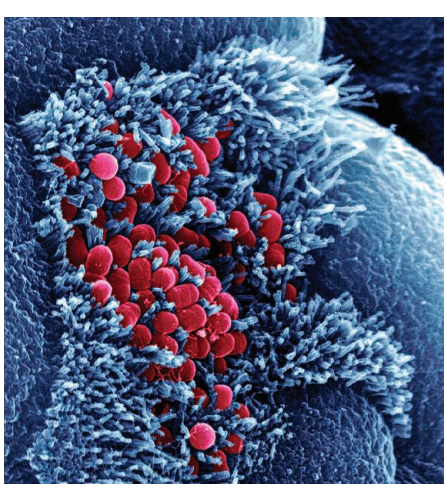
NERVOUS SYSTEM–MICROBIOME CROSS COMMUNICATION

Recent evidence suggests that the human brain constantly communicates with the gut microbiome—an ecosystem of thousands of bacterial species that inhabit the gastrointestinal tract along a "microbiome-gut-brain axis" (*4, 5*). Cross-talk on this axis can be mediated by small-molecular metabolites secreted by gut bacteria and absorbed into the blood stream. These metabolites can then access the central nervous system through the choroid plexus, where it is believed they reprogram transcriptional responses of brain cells (*6*). The gut microbiome responds quickly to environmental factors and represents a central component in their impact on the host's physiology. Therefore, we hypothesized that gut bacteria influence ALS pathogenesis.

We began our investigation by depleting the microbiome of *Sod1*-transgenic (*Sod1*-Tg) mice through wide-spectrum antibiotic treatment. Microbiome depletion resulted in a substantial exacerbation of ALS symptoms (*3*). We then compared the gut microbiome of antibiotic-treated *Sod1*-Tg mice to that of wild-type littermate controls raised in several specific-pathogen–free facilities. We discovered vivarium-dependent dysbiosis and microbiome-driven alteration in systemic metabolite's configuration that preceded clinical motor symptoms. We then demonstrated that several key bacterial genes that encode for biosynthetic enzymes of nicotinamide and its precursor, tryptophan, were reduced in the microbiome of *Sod1*-Tg mice.



Shown is a colored scanning electron micrograph (SEM) of *Escherichia coli*, one of hundreds of bacterial species residing in the human gut. Research is now revealing cross-talks between those microbes and distant organs, such as the brain, in health and disease.

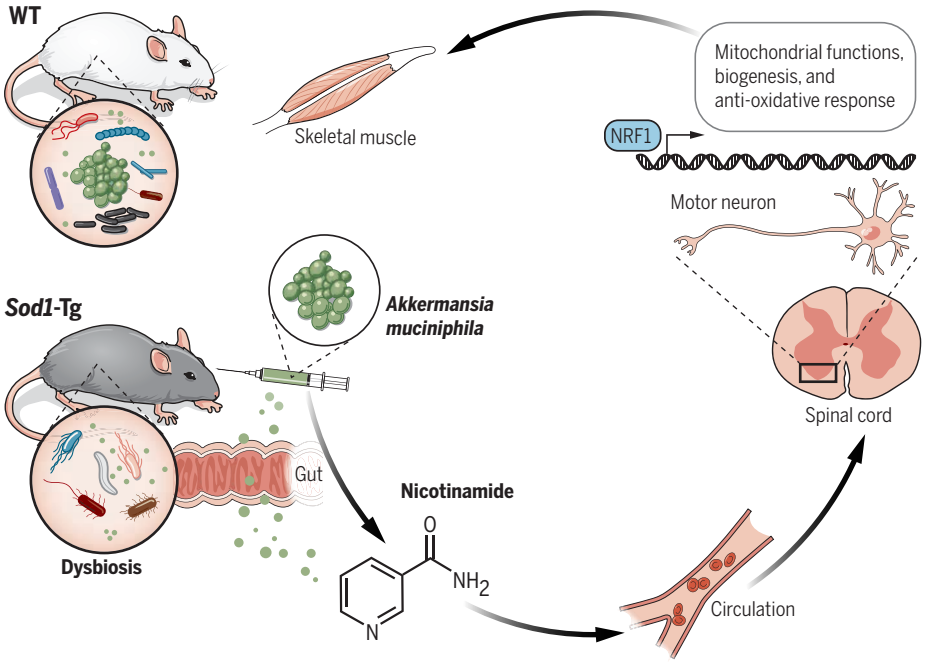
PHOTO: STEPHANIE SCHULLER/SCIENCE SOURCE

DISTINCT MICROBIAL TRANSPLANTATION AMELIORATES MOUSE ALS

Using a comprehensive metagenomic assessment throughout disease progression, we identified 11 distinct microbial strains that were correlated to disease severity. To test their clinical effects on ALS severity, we adopted a "probiotic" approach in which we anaerobically cultured individual strains and administered them to *Sod1*-Tg mice pretreated with antibiotics. Supplementation of these strains demonstrated that *Akkermansia muciniphila* ameliorated whereas *Ruminococcus torques* and *Parabacteroides distasonis* exacerbated ALS symptoms in the mice. We then used metabolomic approaches to characterize bacterial-associated metabolites in the *A. muciniphila*–treated *Sod1*-Tg mice and found that supplementation with the bacterium significantly increased nicotinamide concentrations in the nervous system. Direct administration of nicotinamide, through subcutaneously implanted osmotic pumps, also substantially improved motor abilities and spinal cord gene expression patterns in *Sod1*-Tg mice. These findings highlight nicotinamide as a potential therapeutic agent for ALS. Treating *Sod1*-Tg mice with either the bacterium *A. muciniphila* or with its associated metabolite nicotinamide enriched the expression of neuroprotective genes involved in mitochondrial structure and function, nicotinamide adenine dinucleotide^{+} (NAD^{+}) homeostasis, and removal of superoxide radicals in the spinal cord—functions that are known to be disrupted in ALS (see the figure).

Sod1-Tg ALS mice harbor preclinical dysbiosis

Treatment with *Akkermansia muciniphila*, or with nicotinamide, ameliorates amyotrophic lateral sclerosis symptoms and elicits neuroprotective transcriptional program in the spinal cord. WT, wild type.



CREDITS: (GRAPHIC) ADAPTED FROM E. BLACHER BY N. CARY/*SCIENCE*; (PHOTOS, TOP TO BOTTOM) COURTESY OF NADIA BELKIND; COURTESY OF EREZ BARUCH; MASSIMO DEL PRETE/EMBL

MICROBIOME AND NICOTINAMIDE CHANGE IN ALS PATIENTS

To determine whether our findings could be translated into a potential cure for human ALS, we sequenced the gut microbiome metagenomes of ALS patients and healthy family members that shared the same household environment. This observational study showed that the composition and function of the microbiome of ALS patients substantially differed from that of healthy family members. Moreover, we found a significant reduction in nicotinamide concentrations in both sera and cerebrospinal fluids of ALS patients. We posit that these findings are linked to our previous observations in mice and may lay the foundation for a larger clinical study in the future.

THE FUTURE: MICROBIOME-METABOLOME–BASED THERAPIES?

Harnessing rapidly developing microbiome sequencing, culturing, and computational technologies enabled us to identify a skewed metabolic pathway involved in ALS pathogenesis in mice that is highly affected by the composition and function of the gut microbiome. Similarly, studies performed during my graduate work in the laboratory of Reuven Stein showed that inhibition of CD38, the most efficient NAD^+-consuming enzyme, is a promising strategy to treat brain pathologies (*7*–*10*). Disrupted microbial metabolites profiles may also contribute to neurodegeneration, as we demonstrated in *Sod1*-Tg ALS mice (*3*). These results exemplify how microbiome profiling can be used to identify disease-modifying metabolites.

Further research implementing mass-spectrometry informatics with molecular networking has the potential to reveal the mechanisms behind microbiome-associated phenotypes (*11*, *12*). This approach may pave the way to rationally genetically engineer a transplantable metabolome that would hopefully assist in delaying or even preventing detrimental age-related illnesses. ■

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Eran Blacher received undergraduate degrees and a PhD from Tel Aviv University, Israel, and performed a postdoctoral fellowship at the Weizmann Institute of Science, where he studied the role of the microbiome-gut-brain axis in the context of neurodegenerative diseases. He is currently a senior postdoctoral fellow at Stanford School of Medicine studying the immune system–gut–brain axis in aging and neurological disorders.

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