# CRISPR screen identifies new anti-inflammatory drug target

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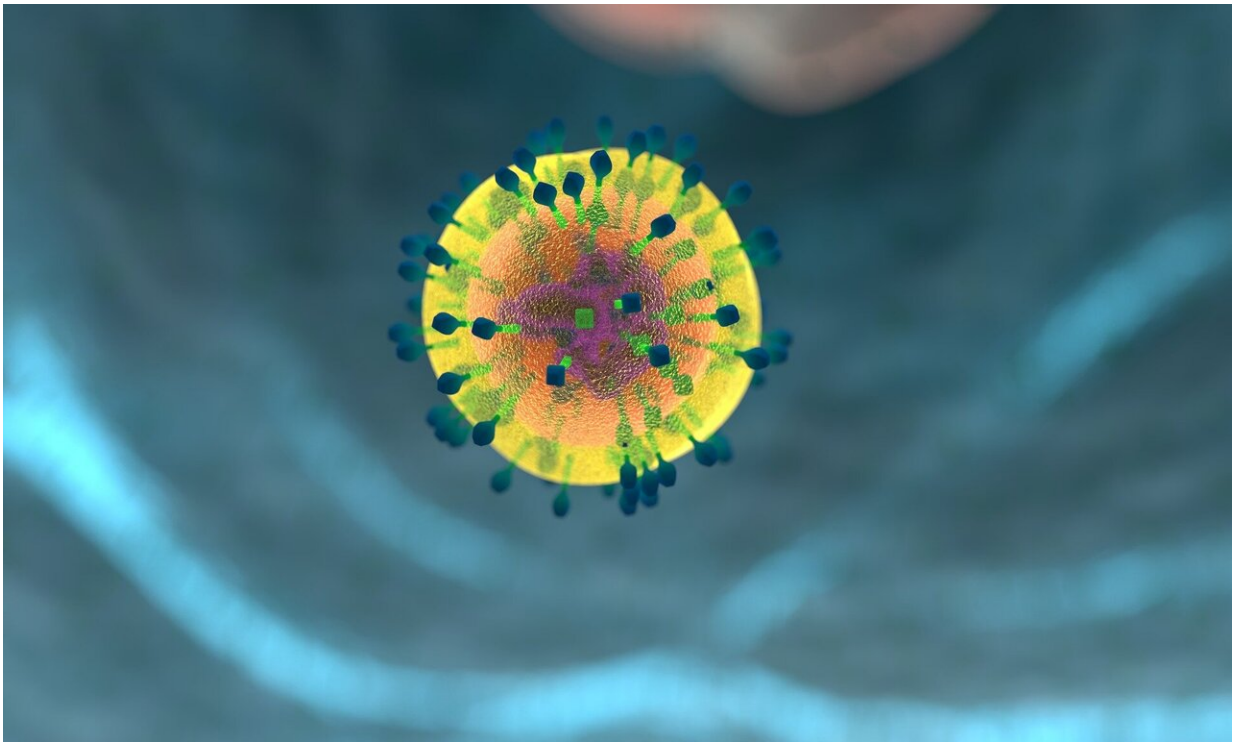

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A metabolic enzyme that has been studied in cancer biology and is important for T cell function may offer a new target for anti-inflammatory therapeutics, Vanderbilt researchers have discovered. They report Nov. 11 in the journal *Immunity* that inhibiting or genetically deleting the enzyme, called MTHFD2, reduced disease

severity in multiple inflammatory disease models.

Jeffrey Rathmell, Ph.D., Cornelius Vanderbilt Professor of Immunobiology, and his team are interested in how metabolic pathways—the chemical reactions that sustain life—influence immune cell function. In the current studies, they focused on "one-carbon" metabolism, a series of reactions that generates chemical building blocks for the biosynthesis of DNA and other molecules.

"One-carbon metabolism has been a target for drug development for years and years, but it really hasn't been explored in an unbiased way," said Rathmell, who is also director of the Vanderbilt Center for Immunobiology. The immunosuppressant drug methotrexate, for example, inhibits an enzyme in the one-carbon metabolism pathway, but it may not be the "right target or the right drug" for optimal therapeutic activity, he said.

To systematically study the pathway in T cells—white blood cells that respond to specific antigens (such as surface proteins on viruses)—Ayaka Sugiura, an MD-Ph.D. student in Rathmell's group, developed a screening strategy using the genome editing technology CRISPR. She designed CRISPR "guides" to selectively inactivate each gene in the one-carbon metabolism pathway and introduced this "library" into isolated T cells, carefully controlling the experimental conditions so that each cell had only one (or no) inactivated gene.

By studying the modified cells in an animal model of asthma, Sugiura was able to identify genes important to T cell function during the disease process. She then examined the expression of each identified gene during T cell development and in patients with a variety of inflammatory diseases.

MTHFD2 stood out. It was highly expressed in disease states and during

embryonic development, but it was expressed at low levels, or not at all, in adult tissues, Sugiura said.

MTHFD2 had previously been a target for anti-cancer drug development because of its overexpression in many tumors. Although preclinical studies did not support further anti-cancer development of MTHFD2 inhibitors, Sugiura was able to use a well-characterized inhibitor in her studies.

"MTHFD2 is important for nucleotide synthesis not only for DNA, but also for proper signaling required for T cell function," Sugiura said. Inhibiting MTHFD2 with a drug or genetically eliminating it reduced overall proliferation of CD4 T cells (a particular type of T cell the group studied) and blunted immune responses, she said.

The researchers discovered, however, that the effects of MTHFD2 inhibition were different for subsets of CD4 T cells that are generated in response to antigen stimulation. Inhibiting MTHFD2 promoted the activity of regulatory CD4 T cells (Treg), which suppress the immune response. But inhibiting MTHFD2 blocked inflammatory CD4 T cells (Th17) and actually converted them to an anti-inflammatory phenotype.

"This was pretty surprising," Rathmell said. "Ayaka was able to show that inhibiting MTHFD2 doesn't just stop an immune response, it actually switches it from inflammatory to anti-inflammatory."

In animal models for multiple sclerosis, inflammatory bowel disease, and a general allergic response, inhibiting or eliminating MTHFD2 reduced disease severity, supporting its potential as a therapeutic target for anti-inflammatory drug development. The Rathmell group is working with collaborators to develop inhibitors with improved clinical characteristics.

The researchers also were encouraged to find that giving an MTHFD2

inhibitor in a vaccination model did not impair the immune response to a vaccine.

"It was promising that while the inhibitor suppressed inflammation in multiple disease models of hyperactive T cell activity, it did not affect desirable T cell responses, such as the response to vaccination," Sugiura said.

The findings suggest that immune cell subsets rely on one-carbon metabolism—and MTHFD2 function—in different ways, the researchers noted.

And although MTHFD2 inhibitors were not successful as anti-cancer agents in general, they might be useful for cancers driven by inflammation, such as colorectal cancer. An MTHFD2 inhibitor would be expected to slow down cancer cell proliferation and also block "the specific inflammatory T cells that can promote that type of cancer," Rathmell said.

The Rathmell group is using the CRISPR-based screen Sugiura developed to explore multiple sets of genes in various disease models and is working to build a core resource for other Vanderbilt investigators. "This screening strategy and whole approach to look for important disease genes, which might be therapeutic targets, in an unbiased way is really valuable and has been very impactful for our group," Rathmell said.

**More information:** Jeffrey C. Rathmell, MTHFD2 is a Metabolic Checkpoint Controlling Effector and Regulatory T Cell Fate and Function, *Immunity* (2021). DOI: 10.1016/j.immuni.2021.10.011. www.cell.com/immunity/fulltext … 1074-7613(21)00448-9

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