RESEARCH HIGHLIGHT OPEN

Microbial metabolite facilitates virus control: inosine and T cells curb early-life influenza infection

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In a recent publication in *Cell*, Stevens et al. uncovered that microbiota disruptions reduced inosine levels and impaired $CD8^+$ T cell immunity against influenza infection in early life. Treatment with inosine-producing bacteria or inosine alone restored T cell responsiveness through a nuclear factor interleukin 3 (NFIL3)-dependent mechanism.[1]

To model early-life dysbiosis, the authors pretreated pregnant dams with broad-spectrum antibiotics (ABX). Microbiota transfer from these mothers to their offspring was impaired, with notably reduced transmission of *Lactobacillaceae* and *Bifidobacteriaceae*. Infection of dysbiotic infant mice with influenza A (H1N1) revealed a diminished virus-specific $CD8^+$ T cell effector response in the lungs and draining lymph nodes concomitant with lung damage and mortality. Moreover, rechallenge in recovered 8-week-old mice with H1N1 or the heterosubtypic, $CD8^+$ T cell cross-reactive strain H3N2 further demonstrated an impaired viral-specific immune response later in life. This emphasizes the importance of perinatal microbiota-mediated immune priming to effectively protect against viral[1]- and bacterial pathogens.[2] Still, ABX treatment during a critical developmental window may also directly impact T cell function and warrants further investigation.

This finding was strengthened by analyses of human lung tissue samples. T cells isolated from post-mortem human tissue of ABX-exposed infants showed impaired T cell differentiation and effector function compared to ABX-naïve infants. Indeed, a strong correlation between human and murine infants was detected in the $CD8^+$ T cell transcriptome under dysbiotic conditions. Remarkably, influenza-specific T cell abundance, proliferation, and activation were greatly increased in $CD8^+$ T cells from ABX-naive compared to ABX-exposed human tissues.

The observed microbiota-dependent immune functions may arise through altered microenvironment-, T cell intrinsic- or stochastic events occurring during infection. Thus, the authors isolated distinctly labeled, virus-specific $CD8^+$ T cells from dysbiotic or control mice and transferred an equal mixture of CFSE-labeled cells into age-matched recipients. Following H1N1 infection, virus-specific $CD8^+$ T cells from dysbiotic and control mice were then evaluated in the same recipient. $CD8^+$ T cells from the dysbiotic donor proliferated less, showed reduced interferon (IFN)-γ and CD44 expression compared to the transferred control $CD8^+$ T cells. Adoptive transfer of either dysbiotic or control $CD8^+$ T cells into separate donor mice and consequent H1N1 infection revealed a drastic weight loss of recipient mice receiving dysbiotic $CD8^+$ T cells. Together, this elegant approach identified a $CD8^+$ T cell intrinsic mechanism as a driver of impaired antiviral immunity.

Single-cell RNA sequencing, pseudo time trajectory- and protein analysis highlighted a pronounced shift towards naïve $CD8^+$ T cells in dysbiotic mice, whereas control mice were featured by an increase in memory T cells and exhausted T progenitor cells. Gene regulatory network analysis, as well as validation experiments on the protein level, identified NFIL3 as a central protein within a regulon that governs T cell effector differentiation and function. A T cell-specific NFIL3-deficient mouse model clearly demonstrated a similar impairment of T cell differentiation and viral defense as observed in dysbiotic mice. Analysis of histone tri-methylation linked NFIL3 to repressed transcription factor 7 (*Tcf7)* and lymphoid enhancer-binding factor 1 (*Lef1)* gene transcription, two well-known regulators of T cell proliferation and effector differentiation.

One critical issue remained: How does the microbiome induce NFIL3-dependent $CD8^+$ T cell viral response? Metagenomic sequencing from dysbiotic or control mouse and human fecal samples revealed *Haemophilus*, *Enterococcus*, *Lactobacillus*, and *Bifidobacterium* as significant discriminators between groups. Metagenomic-predicted metabolite analysis indicated inosine as a highly depleted metabolite in the dysbiotic microbiome state. The authors thus tested whether *Bifidobacterium pseudolongum* – a known inosine producer[3] – or inosine treatment would improve T cell immunity related to influenza infection. *B. pseudolongum* monocolonization increased the T cell effector subset as observed in ABX- untreated and NFIL3-proficient mice. Inosine treatment in dysbiotic mice increased virus-specific $CD8^+$ T cells, repressed *Tcf1* transcription, and protected from excessive weight loss following H1N1 infection. Comparison of human and mouse T cells from lungs of dysbiotic and control states linked inosine treatment to increased presence of $NFIL3^+$ T cells, which was dependent on adenosine 2A (A_{2A}) receptor signaling and induced phosphorylation of the downstream transcription factor cAMP response element-binding protein (CREB). Inosine also enhanced T cell proliferation and activation, thus unequivocally demonstrating a protective role of inosine treatment upon influenza infection (Fig. 1).

Stevens et al. add another facet to microbe-derived inosine as a potent immunomodulatory agent, in this particular case as an activator of $CD8^+$ T cell-dependent antiviral immunity. Similarly, inosine has been highlighted as a potent adjuvant for checkpoint blockade and, more recently, in chimeric antigen receptors (CAR) T cell therapy in various cancer entities.[3,4] We anticipate that future efforts will focus on how inosine may be used in the treatment of viral infections and other indications. Notably, the first patent for

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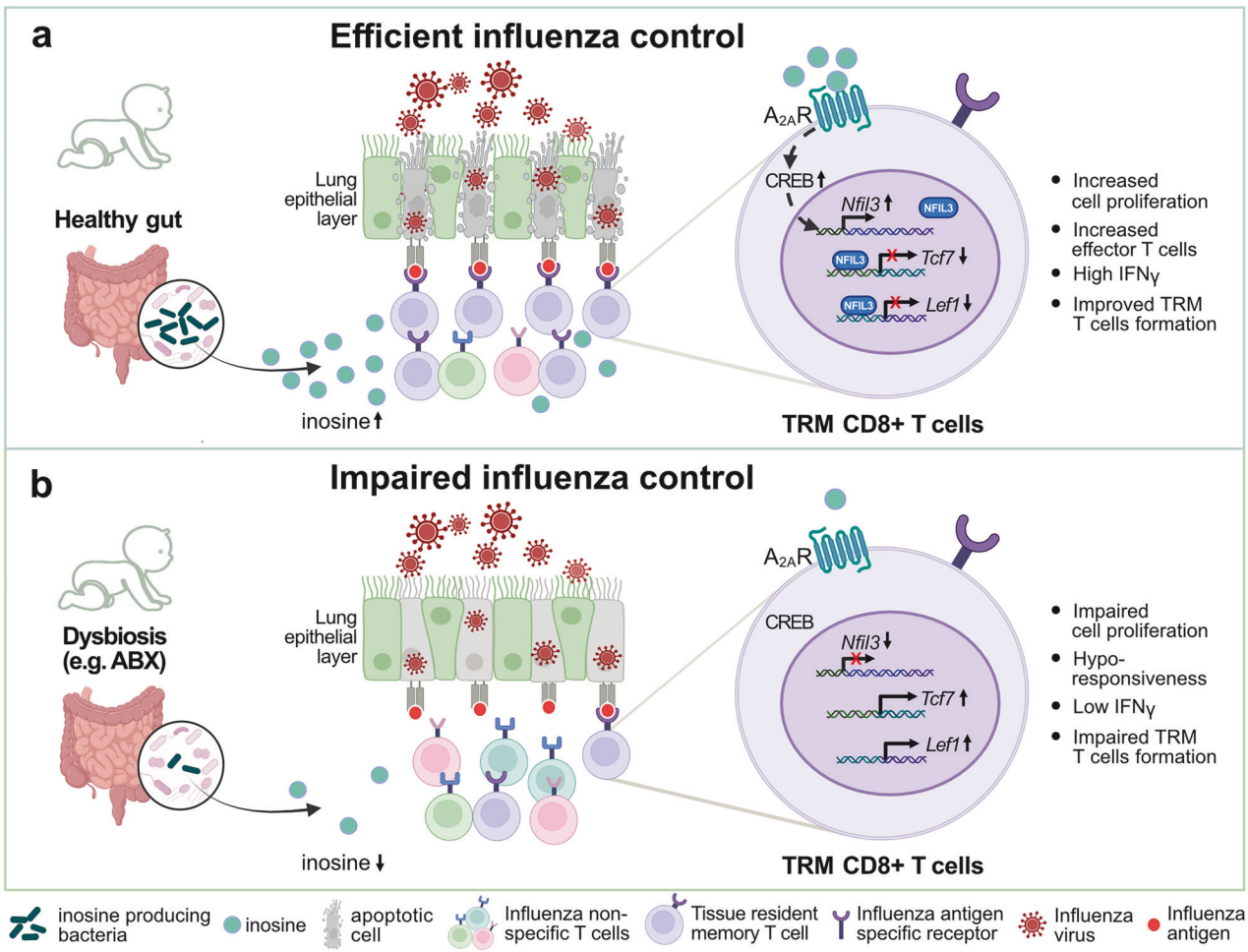

Fig. 1 Bacterial-derived inosine induces NFIL3-T cell-dependent antiviral immunity in early life. Gut microbiome-derived inosine and consequent elevated systemic levels of inosine (e.g., plasma and lung) activate the adenosine 2 A receptors ($A_{2A}R$) on $CD8^+$ T cells. Receptor activation enhances cAMP response element-binding protein (CREB) activation and increases the expression of the transcription factor nuclear factor interleukin 3 (NFIL3). NFIL3 represses the transcription of transcription factor 7 (*Tcf7)* and lymphoid enhancer-binding factor 1 (*Lef1*). This promotes $CD8^+$ T cell proliferation, effector differentiation, IFN-γ production, and supports the development of tissue resident memory (TRM) cells in the lung. Collectively, this strengthens antiviral immunity and protects newborns from influenza infection. **a** Depicting sufficient inosine abundance leading to efficient influenza virus control. **b** Depicting reduced inosine abundance, leading to impaired influenza virus control. Figure was created with BioRender, Affinity Designer 2 and Photoshop

the use of inosine in cancer immunotherapy has been assigned (IMMUNOSPARKLE BIOSCIENCE LLC). Furthermore, inosine levels at the onset of viral infection or cancer may be valuable as a predictive- or patient stratification biomarker.

Some unresolved aspects of inosine remain mysterious. First, inosine treatment has previously been shown to repress pro-inflammatory immune function in various immune cells, including T cells.[5] The availability of co-stimulator agents may control context-dependent effects and could underlie diverging observations.[3] *Bifidobacterium pseudolongum*-derived inosine induces T cell activation against virus-infected and malignant cells.[1,3] Thus, we anticipate future investigations to identify secondary microbe-derived factors that act in concert with inosine. Second, inosine pranobex (inosine combined with dimepranol acedoben), an antiviral agent, has been in use since 1971. Whether dimepranol acedoben acts as a secondary co-stimulatory agent for inosine to induce virus control, acts in a related or even distinct manner, warrants further investigation. Third, inosine alters the function of various immune cells, including $CD4^+$ T and B cells that are crucial for virus control. We expect B cells, $CD4^+$- and interactions with $CD8^+$ T cells in the context of inosine treatment as another upcoming topic in the field.

Together, the study by Stevens et al. highlights a critical pathway by which *Bifidobacterium pseudolongum*-derived inosine promotes NFIL3-dependent $CD8^+$ T cell immunity against influenza virus infection (Fig. 1). This work paves the way for future studies, including large patient cohorts probing the use of microbiome restoration strategies for high-risk infants —e.g., born preterm or exposed to antibiotics— and inosine-based therapies aimed at preventing severe disease progression.

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ADDITIONAL INFORMATION

Competing interests: L.F.M. has submitted a patent for the use of bacteria, including *B. pseudolongum*, in the treatment of cancer. L.S. and T.L. declare no competing interests.

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REFERENCES

1. Stevens, J. et al. Microbiota-derived inosine programs protective CD8(+) T cell responses against influenza in newborns. *Cell*, (2025).
2. Zegarra-Ruiz, D. F. et al. Thymic development of gut-microbiota-specific T cells. *Nature* **594**, 413–417 (2021).
3. Mager, L. F. et al. Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. *Science* **369**, 1481–1489 (2020).
4. Klysz, D. D. et al. Inosine induces stemness features in CAR-T cells and enhances potency. *Cancer Cell* **42**, 266–282.e268 (2024).
5. He, B. et al. Resetting microbiota by Lactobacillus reuteri inhibits T reg deficiency-induced autoimmunity via adenosine A2A receptors. *J. Exp. Med* **214**, 107–123 (2017).