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Growing up: A NOD2 our microbes

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In a recent report in *Science*, Schwarzer and colleagues demonstrate the growth benefits of treatment with *Lactiplantibacillus plantarum* strain WJL in a preclinical mouse model of chronic undernutrition. *L. plantarum* influences the somatotropic axis to promote growth through intestinal epithelial NOD2 sensing.

Undernutrition is a major cause of linear growth stunting, a syndrome that affects over 149 million children under five globally.[1] Stunting is associated with negative long-term outcomes, including diminished cognitive and physical development, reduced productivity, and poor health in adulthood.[2] Stunted children often suffer from suboptimal immune responses, altered gut microbial communities, chronic inflammation, and repeated enteric infections.[3] The first 1,000 days of a child's life from the time of conception to two years of age is a critical time window for therapeutic interventions to improve growth.[1] Unfortunately, current nutritional interventions typically have a modest impact in reversing early-life stunting, likely due to the multifactorial nature of this syndrome.[4] To study the consequences of early-life growth stunting, animal models have been developed employing caloric restriction, macro- and micronutrient deficiency, and/or small intestinal enteropathy.[4] To understand the role of the microbiota in undernutrition, some models complement a malnourished diet with antibiotic cocktails to disrupt the microbiome or use malnourished gnotobiotic mice.[5] Common features of these models include reduced weight gain and linear growth as well as elevated intestinal inflammation and permeability.[4]

In previous work, Schwarzer and colleagues demonstrated that the intestinal microbiota interacts with the somatotropic axis to drive systemic growth.[6] This axis was negatively affected in germ-free mice as indicated by low levels of insulin-like growth factor-1 (IGF-1), suggesting that the gut microbiota could play a role in regulating the somatotropic axis, thereby influencing host growth and development. In this same study, the authors were able to show the growth-promoting properties of the *Lactiplantibacillus plantarum* WJL (Lp^{WJL}) strain in undernourished mono-associated gnotobiotic mice.[6] These results complemented previous findings in a *Drosophila melanogaster* model of nutrient scarcity in which *L. plantarum* promoted growth through the somatotropic axis.[7] In their current work, Schwarzer et al. investigate mechanisms by which *L. plantarum* regulates IGF-1 and promotes growth in undernourished infant mice.[8]

To do so, the authors introduce a preclinical mouse model of chronic undernutrition. They demonstrate that 3-week-old male mice weaned onto a diet low in protein and fat (MAL diet) developed features of growth stunting including reduced length, weight gain, and femur growth. Furthermore, MAL-fed mice had reduced expression of *Ghr* and *Igf-1* in the liver, as well as lower circulating IGF-1 and insulin (Figure 1). Interestingly, they did not observe features of enteropathy in this model. However, MAL-fed mice did have reduced proliferating intestinal epithelial cells in jejunal crypts, suggesting altered renewal rates in the intestinal stem cell (ISC) compartment. After establishing their model, the authors next investigated whether Lp^{WJL} could induce similar growth benefits observed in previous work.[6,7] MAL-fed mice treated with Lp^{WJL} showed overall increased growth relative to the MAL placebo group over the five-week treatment. They also had elevated levels of IGF-1 in both serum and liver, suggesting Lp^{WJL} improves growth through its influence on the somatotropic axis (Figure 1). Within the gut, Lp^{WJL}-treated MAL mice had an increased number of proliferating cells in jejunal crypts compared to the placebo group, and transcriptomic profiling of jejunal tissue revealed an upregulation of genes associated with type 1 interferon signaling, suggesting LP^{WJL} treatment impacts intestinal epithelial cell proliferation and signaling. Intriguingly, the authors showed that heat killed Lp^{WJL} or cell wall components of this strain were sufficient to increase growth in MAL mice, demonstrating that colonization of recipient mice with live Lp^{WJL} was unnecessary to achieve these benefits. The growth-promoting capacity of Lp^{WJL} was also strain specific, as administration of the closely related isolate $Lp^{NIZO2877}$ did not significantly impact growth.

Based on these findings, Schwarzer et al. hypothesized that nucleotide-binding oligomerization domain-containing protein-2 (NOD2) sensing was involved in mediating the growth benefits of Lp^{WJL}. NOD2 is an intracellular innate immune sensor that detects microbial components derived from bacterial peptidoglycan and plays important roles in mucosal homeostasis.[9] To test this hypothesis, the authors used reporter cells expressing the innate immune receptors toll-like receptor (TLR) 2 and 4 as well as NOD1 and 2. Treating these cells *in vitro* with heat killed LP^{WJL} and $Lp^{NIZO2877}$ as well as with purified cell wall components revealed the ability of both strains to activate TLR2 and NOD2. However, LP^{WJL} was able to activate NOD2 to a significantly greater extent than $Lp^{NIZO2877}$, implicating

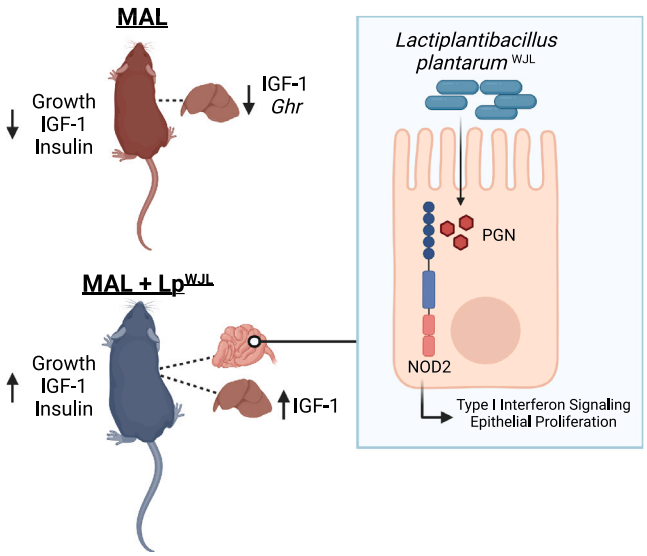

Figure 1. NOD2 sensing in intestinal epithelial cells mediates *Lactiplantibacillus plantarum*^{WJL} growth promotion in undernourished mice

Schwarzer et al. report a preclinical mouse model of chronic undernutrition that leads to reduced growth, circulating and liver IGF-1, and jejunal crypt epithelial proliferation in mice weaned into a low fat and protein diet (MAL). MAL-fed mice treated with *Lactiplantibacillus plantarum* strain WJL (Lp^{WJL}) showed improved growth and an increase in liver and serum IGF-1 levels. Results demonstrated that the ability of Lp^{WJL} to improve growth was dependent on NOD2 sensing in intestinal epithelial cells. Cell wall components, including peptidoglycan (PGN), derived from this strain activated intracellular innate immune receptor signaling, leading to epithelial proliferation in jejunal crypts and a transcriptional signature of type 1 interferon signaling. Thus, Lp^{WJL} induced growth in MAL-fed mice through intestinal NOD2 sensing to influence the somatotropic axis in this model of chronic undernutrition. Image created with Biorender.com.

signaling through this receptor as a potential explanation for their differential growth effects. Indeed, no increase in growth was observed in NOD2$^{-/-}$ mice treated with LP^{WJL}, while this strain retained its ability to improve growth in MyD88$^{-/-}$ mice, suggesting NOD2 activation is essential to the activity of LP^{WJL}. To investigate whether *L. plantarum* was sensed systemically or locally within the intestine, the authors employed cell type-specific knockouts of NOD2. Mice lacking NOD2 in intestinal epithelial cells (NOD2$^{\Delta IEC}$) did not benefit from LP^{WJL} treatment with respect to growth or IGF-1 levels. Similarly, a lack of IEC-specific NOD2 prevented crypt epithelial cell proliferation and blocked increased expression of type 1 interferon responsive genes following Lp^{WJL} treatment. On the other hand, mice lacking NOD2 in hepatocytes treated with LP^{WJL} still showed improved growth relative to placebo treated animals, suggesting that sensing of *L. plantarum* by intestinal epithelial cell NOD2 is necessary to promote growth in this model of chronic undernutrition (Figure 1).

Collectively, the authors have demonstrated the growth-promoting benefits of Lp^{WJL} in undernutrition through its ability to impact the somatotropic axis in multiple model systems.[6–8] These results are particularly timely given that another *L. plantarum* strain was recently investigated as a therapeutic agent in undernourished children.[10] Further work is needed to investigate the ability of LP^{WJL} to improve post-weaning growth in the context of intestinal inflammation, which could substantially impact the consequences of NOD2 signaling. It also remains to be determined whether the presence of distinct microbial communities harboring potential pathogens and/or pathobionts, such as those commonly found in undernourished children, may influence the ability of LP^{WJL} to impact growth. Recapitulating the altered gut microbial communities that contribute to undernutrition will add insight necessary to fully understanding the potential benefits of *L. plantarum* as a treatment. In addition to highlighting a potential probiotic therapy, this work also further defines a link between innate immune sensing and regulation of host growth pathways. There is a growing recognition that growth stunting often begins before birth with poor maternal health and reduced fetal growth, and investigating the ability of these gut-derived signals to promote healthy pregnancy could be a promising avenue. Beyond these conceptual advances, these findings open the door to the development of new therapeutic agents that directly target intestinal signals to stimulate growth in undernourished children. This work marks an important contribution toward understanding microbial functions and host pathways that positively impact growth in early life to improve the health of children worldwide.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Robertson, R.C., Manges, A.R., Finlay, B.B., and Prendergast, A.J. (2019). The Human Microbiome and Child Growth – First 1000 Days and Beyond. Trends Microbiol. *27*, 131–147.

2. Victora, C.G., Adair, L., Fall, C., Hallal, P.C., Martorell, R., Richter, L., and Sachdev, H.S.; Maternal and Child Undernutrition Study Group (2008). Maternal and child undernutrition: consequences for adult health and human capital. Lancet *371*, 340–357.

3. Bourke, C.D., Berkley, J.A., and Prendergast, A.J. (2016). Immune Dysfunction as a Cause and Consequence of Malnutrition. Trends Immunol. *37*, 386–398.

4. Cowardin, C.A., Syed, S., Iqbal, N., Jamil, Z., Sadiq, K., Iqbal, J., Ali, S.A., and Moore, S.R. (2023). Environmental enteric dysfunction: gut and microbiota adaptation in pregnancy and infancy. Nat. Rev. Gastroenterol. Hepatol. *20*, 223–237. https://doi.org/10.1038/s41575-022-00714-7.

5. Blanton, L.V., Charbonneau, M.R., Salih, T., Barratt, M.J., Venkatesh, S., Ilkaveya, O., Subramanian, S., Manary, M.J., Trehan, I., Jorgensen, J.M., et al. (2016). Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children. Science *351*.

6. Schwarzer, M., Makki, K., Storelli, G., Machuca-Gayet, I., Srutkova, D., Hermanova, P., Martino, M.E., Balmand, S., Hudcovic, T., Heddi, A., et al. (2016). Lactobacillus plantarum strain maintains growth of infant mice

during chronic undernutrition. Science *351*, 854–857.

7. Storelli, G., Defaye, A., Erkosar, B., Hols, P., Royet, J., and Leulier, F. (2011). Lactobacillus plantarum Promotes Drosophila Systemic Growth by Modulating Hormonal Signals through TOR-Dependent Nutrient Sensing. Cell Metab. *14*, 403–414.

8. Schwarzer, M., Gautam, U.K., Makki, K., Lambert, A., Brabec, T., Joly, A., Šrůtkova, D., Poinsot, P., Novotna, T., Geoffroy, S., et al. (2023). Microbe-mediated intestinal NOD2 stimulation improves linear growth of undernourished infant mice. Science *379*, 826–833.

9. Strober, W., and Watanabe, T. (2011). NOD2, an intracellular innate immune sensor involved in host defense and Crohn's disease. Mucosal Immunol. *4*, 484–495.

10. Kamil, R.Z., Murdiati, A., Juffrie, M., and Rahayu, E.S. (2022). Gut Microbiota Modulation of Moderate Undernutrition in Infants through Gummy Lactobacillus plantarum Dad-13 Consumption: A Randomized Double-Blind Controlled Trial. Nutrients *14*, 1049.

MABTRAINS: Numerous anti-infective modalities ride together

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In this issue of *Cell Host & Microbe*, Buckley et al. report a biological entity called a mAbtyrin, which combines various antimicrobial functions. The authors demonstrate through *in vitro* and *in vivo* experiments that this approach can lead to highly effective antimicrobial action against *Staphylococcus aureus*.

Bacterial infections are a global health concern, causing significant morbidity and mortality worldwide.[1] The rise of antibiotic resistance has made these infections even more challenging to treat. In low- and middle-income countries, bacterial infections such as tuberculosis, pneumonia, and diarrheal diseases continue to be a significant cause of death, particularly in children. In high-income countries, healthcare-associated infections, such as methicillin-resistant *Staphylococcus aureus* (MRSA), are a growing concern. To combat this problem, it is essential to promote responsible use of antibiotics, improve infection prevention and control practices, and especially to develop new treatments and diagnostic tools. Without urgent action, bacterial infections will continue to pose a significant threat to global public health.

A promising strategy is boosting the host immune system through antibodies, either indirectly via vaccination or directly via the administration of therapeutic antibodies.[2] For *S. aureus*, several vaccine candidates have been developed, but none have been approved for clinical use.[3] Thanks to the success of antibody therapies in cancer, virology, and autoimmunity, there is now great interest in development of similar therapies against bacterial infections. Another advantage of immunotherapy is that it avoids the use of antibiotics, which can lead to antibiotic resistance. However, developing monoclonal antibody therapy is often hampered by the problem of target selection. Translation from virology teaches us that one target could be sufficient. However, the redundant attack of bacteria with their numerous virulence mechanisms makes it challenging to eliminate or disarm a bacteria with only one target. As such, we need ways to tackle more targets simultaneously.

We know from the combined literature of the past few decades that *S. aureus* is a master of disguise, using a multitude of virulence strategies. *S. aureus* secretes an array of immune-evasion molecules that target our immune system by inhibiting complement activation, inhibiting phagocytes, blocking opsonin receptors, and degrading or neutralizing several innate immune components. Furthermore, *S. aureus* secretes many toxins that largely functional similarly, killing all immune cells that have a role in destroying invading bacteria. Some of these molecules act on the inside of a phagolysosome, some in the context of extracellular replicating bacteria.

Thus, *S. aureus* produces a large array of virulence factors that contribute to the highly complex pathophysiology of staphylococcal infections.[4] This multitude of virulence factors causes a redundant attack on our immune system and the only way to answer that therapeutically is with a redundant counterattack. Either we need a multitude of monoclonal antibodies or vaccine candidates, or we need therapeutics that harbor different modalities in one molecule. Single monoclonal antibodies are theoretically a good strategy but remain insufficient. Thousands of companies all over the world are working to copy the success of antibody treatment by developing an increasing number of monoclonal antibodies. Yet, what we need is a more original and creative way to counteract the observed redundancy.

Centyrins are a class of alternative scaffold proteins based on a consensus fibronectin domain.[5] Centyrins, which can provide the specificity of an antibody, are developed to new targets of interest using *in vitro* display. With a molecular weight of just 10 kDa, centyrins are an ideal size for applications requiring a short half-life,