In mice, zinc improves regeneration of key immune organ and immune-cell recovery after bone marrow transplant

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Dudakov Lab postdoctoral fellow Dr. Lorenzo Iovino discovered two ways that zinc helps boost the immune system. Credit: Robert Hood / Fred Hutch News Service

Zinc's immune-boosting properties are well-established, but we're still untangling how it works. In a new study published in late March in the scientific journal *Blood*, Fred Hutchinson Cancer Research Center scientists reveal two different ways the mineral supports immunity. Using mice, the team discovered that zinc is required for development of a specialized type of immune cell and prompts a critical immune organ to regenerate after damage.

The study also revealed that an experimental compound that mimics zinc's action in this organ works even better than the natural mineral to promote immune recovery and could have therapeutic potential for people who receive a blood stem cell transplant as a treatment for advanced leukemia or another serious blood disease.

"This study adds to our knowledge of what zinc is actually doing in the immune system," said Hutch immunologist Dr. Jarrod Dudakov, who led the work with Dudakov Lab research associate Dr. Lorenzo Iovino.

With the experimental compound, "we could repeat everything we could do with zinc supplementation, but we could remove one of the largest barriers, which is this really prolonged period beforehand that you needed to supplement," Dudakov said.

The researchers caution that while their work sheds light on why zinc is so important for immunity, it's not a green light to begin mega-dosing with the mineral.

"We're not saying zinc is a panacea," Iovino said. "There can be serious reactions due to intoxication and accumulation. We would not recommend taking zinc randomly."

He and Dudakov are further exploring the clinical potential of compounds that recreate zinc's immune-boosting effects after acute immune damage, as well as looking at whether such treatments could also help curb the chronic immune decline that accompanies aging.

Zinc supports the immune system

There's a long history linking zinc with optimal immune function. For example, it's known that people whose zinc levels are too low have little to no infection-fighting T cells and the thymus, the organ in which T

cells develop, is nearly non-existent, Iovino said. When zinc-deficient people are given extra zinc, their thymuses grow and start pumping out these immune cells.

When chemotherapy and radiation fail to cure a patient's blood cancer, they undergo a blood stem cell transplant (also known as a bone marrow transplant) to receive new, healthy blood stem cells that will replace their cancerous blood cells and turn into infection-fighting white blood cells, oxygen-carrying red blood cells and wound-healing platelets. Prior to their infusion of blood stem cells, patients receive treatments that wipe out their own bone marrow and kill off remaining cancer cells. But these treatments, and the transplant itself, can lead to side effects that raise the risk of low zinc levels. These can include diarrhea and inflammation, but also the use of inflammation-fighting steroids. Also, said Iovino, we absorb less zinc as we age, and many blood cancer patients are over 60.

"I started to think, maybe this [blood stem cell transplant patient] population is at risk [for low zinc]," he said. "There's a lot of data on aging and zinc deficiency, but not about zinc supplementation and the role of zinc in the acute-damage setting of the immune system."

An initial study by Iovino in patients undergoing autologous stem-cell transplants (in which they receive their own blood stem cells) for the blood cancer multiple myeloma suggested that zinc could boost immune recovery.

But it didn't explain why. To figure this out, Iovino teamed up with Dudakov.

Thymic regeneration and immune function

Dudakov is a world expert on the thymus, particularly the processes it employs to regrow after injury.

Despite being so central to our defenses against infection, the thymus is quite delicate. Many stressors, including chemotherapy and infection itself, cause it to shrink and its T-cell production to plummet.

But the thymus is also resilient. After acute injury, it turns on processes that help it regenerate, regrow and begin producing new T cells again. With the goal of someday developing therapies that make use of these natural processes, Dudakov and his team have outlined the molecular pathways and cell types that govern them. Such treatments could improve vaccine efficacy and hasten thymic regeneration after stressors like chemotherapy, blood stem cell transplant and radiation exposure.

After enduring damaging pre-transplant chemotherapy regimens, the thymus may take months or even years to return to its former level of T-cell production. This leaves patients immunodeficient and vulnerable to life-threatening infections even if their cancers have been overcome. Iovino's clinical work hinted that zinc may be a key player in thymic recovery after a damaging event.

Zinc is critical for T-cell development and thymic regeneration

As in humans, Iovino and Dudakov found that the thymuses of mice deprived of dietary zinc shrink and produce notably fewer mature T cells, even after as little as three weeks of a no-zinc diet. Iovino was able to show that without zinc, T cells cannot fully mature.

He also found that zinc deficiency slows recovery of T-cell numbers after mice receive immune-destroying treatments akin to those given to patients about to receive a blood stem cell transplant. After treating mice with full-body irradiation, the scientists saw that the numbers of T cells climbed more slowly in zinc-deficient mice. They also saw fewer of the

supportive cells that help T cells grow.

Conversely, extra zinc sped up immune recovery. T-cell numbers of mice that received extra zinc for three weeks prior to radiation treatment rebounded better than in mice that received a standard amount of zinc in their diet. Iovino saw similar effects in a mouse model of blood stem cell transplant in which mice receive donated stem cells.

"So we had a consistent result of a better reconstitution of the thymus and also a better reconstitution of T cells in the peripheral blood [after zinc supplementation]," Iovino said. "But the mechanism was not there yet."

Previous work by Dudakov and others in his lab had revealed that a specialized group of cells in the thymus release factors that nudge the T cell-supporting cells to repair and regrow.

Iovino first looked at whether zinc directly stimulates these cells to grow, but found no effect. Then he looked at whether zinc could be influencing regenerative processes by effecting release of a repair factor called BMP4.

He found lower levels of BMP4 in thymuses from zinc-deficient mice after radiation treatment than in those on a standard diet. But thymuses from mice who'd received extra zinc in the weeks leading up to their radiation treatment had even higher levels of BMP4. These findings suggested that zinc connects to BMP4-dependent regeneration pathways. But how?

"At that point, we were tearing our hair out, because zinc interacts with something like 300 proteins, and probably many more indirectly," Dudakov said.

More than a dozen molecules funnel zinc into cells, while another 10 funnel it out—but Iovino was able to rule them out. Dudakov's previous work had revealed which cells in the thymus make BMP4. The researchers wondered: Does changing the intracellular levels of zinc in these cells increase BMP4? The answer was no.

Instead, Iovino saw that zinc levels outside cells increased after thymic damage, suggesting it was the change in zinc levels around BMP4-releasing cells that seemed to be key. T cells accumulate zinc as they develop, but release it after a damaging event—such as a burst of radiation—kills them off. This flood of zinc activates a renewal pathway when BMP4-releasing cells sense a change in external zinc levels that implies large-scale T-cell death.

Cells use a molecule called GPR39 to sense a change in external zinc. Iovino saw that levels of GPR39 in BMPR-releasing cells increased after radiation damage. He was then able to promote BMP4 release and thymic regeneration with an experimental compound that mimics rising zinc external levels by stimulating GPR39.

"What we think is going on is, as you give zinc supplementation, that gets accumulated within the [developing T cells]. It gets stored and stored and stored, then the damage comes along and the zinc is released," Dudakov said. "Now you have more zinc than you normally would, and it can instigate this regenerative pathway. But [with the experimental compound] we can just directly target that receptor and basically get the same effect without any of that pretreatment."

Getting to the clinic

There's still a lot to learn before they can turn their findings to therapeutic strategies, the scientists said.

Transplant patients already receive mineral supplements, so if extra zinc were to be incorporated into their treatment regimens, it would be important to make sure that anyone receiving it is truly zinc-deficient. Iovino thinks many patients might be, but right now there isn't a good test to assess this. He's currently working on developing one, which would first be used to help researchers determine whether patients' zinc status correlates with immune recovery after blood stem cell transplant.

Dudakov is excited to pursue GPR39-stimulating compounds as therapies to improve thymic recovery after acute injuries like pre-transplant radiation. The team is currently screening similar compounds to find any that may be more effective. Evidence also suggests that injury to the thymus may play a role in graft-vs.-host disease, a sometimes debilitating and even deadly transplant complication in which transplanted donor immune cells attack a patient's healthy tissue. Dudakov hopes that a treatment that helps heal the thymus could also help alleviate this condition, which can affect up to 70% of patients who receive donated stem cells.

He and Iovino are also working to determine whether such compounds could help with thymic regeneration in other settings. Unfortunately, our thymuses also slowly shrink and reduce their T-cell output as we age. Dudakov and Iovino would also like to know whether this chronic degeneration could be slowed by boosting the organ's regenerative processes. (Dudakov thinks ingesting extra zinc would be unlikely to prevent long-term thymic decline, as that ebb isn't caused by a single damaging event that also spikes extracellular zinc levels.)

"I'm extremely excited about [activating GPR39] as a potential therapeutic strategy. It just works really, really well," Dudakov said.

More information: Lorenzo Iovino et al, Activation of the Zinc-sensing receptor GPR39 promotes T cell reconstitution after

hematopoietic cell transplant in mice, *Blood* (2022). DOI: 10.1182/blood.2021013950. ashpublications.org/blood/arti … 182/blood.2021013950

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