Answers to microbiome mysteries in the gills of rainbow trout

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While many immunologists use mouse models to conduct their research, J. Oriol Sunyer of Penn's School of Veterinary Medicine has made transformational scientific insights using a very different creature: rainbow trout.

In a paper featured on the cover of the journal *Science Immunology*,

Sunyer and colleagues developed a method to manipulate the trout immune system to reveal a new understanding of how the animals defend against infection while promoting a healthy microbiome. The work addresses a decades-old question of whether mucosal antibodies—those present on mucosal surfaces of the body such as the gut, or in the case of fish, the gills—evolved to fight pathogens, or to preserve a healthy microbiome. As it turns out, mucosal immunoglobulins coevolved both roles from very early on during vertebrate evolution.

"You might be thinking, 'Rainbow trout? We fish for them; we eat them,'" says Sunyer. "But it turns out they can also tell us a lot about some fundamental biomedical, evolutionary, and immunological questions."

Specifically, Sunyer and colleagues found that a mucosal antibody, an immunoglobulin known as IgT, is critical both in controlling pathogens and in regulating the microbiome of fish gills, a tissue type that shares similarities with several mucosal surfaces of mammals, such as the intestines.

"We found that IgT is playing two paradoxical roles—on the one hand reducing bad microbes, and on the other hand promoting the presence of certain beneficial bacteria," says Sunyer. "Fish are the earliest bony vertebrates to possess a mucosal immune system, and so the fact that fish possess a specialized immunoglobulin that does both jobs suggests that these two processes are so fundamentally important for vertebrate survival that they arose concurrently, early on in evolution."

For nearly 20 years, Sunyer's lab has contributed a steady stream of discovery regarding the evolution and roles of the immune system using fish as model species. In 2010, a seminal paper in *Nature Immunology* featured on the journal cover identified the role of IgT. It was the first

time that fish were shown to have a form of mucosal immunity—a more specialized response to pathogens that enter the body from the environment; in this case, through the gills, skin, and fish gut.

"Before that we thought only four-legged animals, or tetrapods, had mucosal immunity," Sunyer says. That study demonstrated the induction of potent IgT responses upon infection with a mucosal pathogen.

The group also showed that IgT coats a large portion of the bacteria that are part of the fishes' microbiome, the community of bacteria and other microbes that dwell on various tissues of the animals' bodies. That got the researchers thinking about which function arose first for vertebrate mucosal immunoglobulins: fighting pathogens or preserving a healthy microbiome.

"In mammals, the immunoglobulin IgA seems to have analogous function to IgT in fish," Sunyer explains. "In the last few years there have been some key studies showing that IgA is required to keep the mammalian microbiome in check. In mice and humans lacking IgA, their microbiome changes: The beneficial bacteria go down and the potentially disease-causing bacteria go up."

A weakness of these studies in mammals lacking IgA, Sunyer notes, is the inability to tease apart the precise role of IgA in preserving a balanced microbiome, since the lack of IgA from birth precludes the establishment of a healthy microbiota in these animals.

To better understand the roles of mucosal immunoglobulins in preserving a healthy microbiome, Sunyer and colleagues developed a model in adult fish where researchers could temporarily deplete them of IgT, lasting about two months.

By doing so they could study the role of IgT in preserving, rather than

establishing, a healthy microbiome, while also evaluating the susceptibility to pathogens of fish lacking IgT.

When they depleted IgT, the researchers found that levels of a mucosal parasite greatly increased, underscoring the immunoglobulin's role in defending against harmful invaders. But they also saw a dramatic impact on the microbiome composition: IgT-depleted fish lost the IgT coating on the bacterial community in their gills and had more bacteria "escape" from gill surfaces and enter the tissue layer beneath, leading to tissue damage and inflammation.

Looking closely at the bacteria coated by IgT in normal animals, the research team found that IgT targeted specific species over others. These species included bacteria associated with both health and disease states in fish—similar to what had been found with IgA in mammals.

Critically, the authors found that the overall microbiome in IgT-depleted fish was significantly altered, in a shift known as dysbiosis. The overall diversity of bacteria present decreased, numbers of beneficial bacteria such as those producing short-chain fatty acids—critical for the maintenance of tissue integrity and immune homeostasis—also decreased, while disease-associated species increased.

"We see that there seems to be specific microbes that have to be controlled," says Sunyer. "Either they are harmful and tend to escape and cause problems in the nearby tissue in the absence of IgT, or perhaps they are beneficial but require IgT to colonize the mucosal surfaces. In both fish and mammals, it now seems apparent that their respective mucosal immunoglobulins do these jobs."

One great benefit of the researcher's IgT depletion technique is that it's temporary and performed in adult animals. After several weeks of depletion, the fish IgT levels return to normal. Thus the researchers were

able to track the microbiome as IgT came back, observing what amounted to recovery; the microbes in the gill regained IgT coating, the microbiome was restored to its initial composition, and the tissue damage and inflammation that had been seen around the gills was reversed.

"In microbiome studies, recovery is a very important point," Sunyer says. "When you take an antibiotic, you can perturb your microbiome to the extent that recovery may take a very long time, but the perturbation we used, of removing IgT, had a profound but transient effect on the microbiome composition, which underwent a speedy recovery."

As more and more scientific studies identify links between the microbiome and various aspects of health from maintaining a healthy weight to the risk of cancer or even neurological conditions like Alzheimer's and schizophrenia, Sunyer is hopeful that his fish model will find even more applications.

"Studying only mammalian models is not going to be enough to understand the role of the microbiome in all of these physiological processes," says Sunyer.

Because the symbiotic relationship between vertebrates and their microbiome is very ancient, and one which first flourished with the emergence of mucosal immunoglobulins in fish, Sunyer says that "rainbow trout will help us discover the underlying mechanisms by which the interactions between immunoglobulins and the microbiome influence immunity, metabolism, cancer, and much more."

These studies, Sunyer adds, will have a crucial impact on the potential uses of specific species of fish bacteria as probiotic agents that may stimulate the immune system to protect against pathogens. With every other fish that we eat deriving from fish farming, an industry plagued

with emerging pathogens, novel therapies, such as probiotics, are in urgent need.

More information: Zhen Xu et al, Specialization of mucosal immunoglobulins in pathogen control and microbiota homeostasis occurred early in vertebrate evolution, *Science Immunology* (2020). DOI: 10.1126/sciimmunol.aay3254

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