In the gut, nervous cells are the 'eyes and ears' of the immune system

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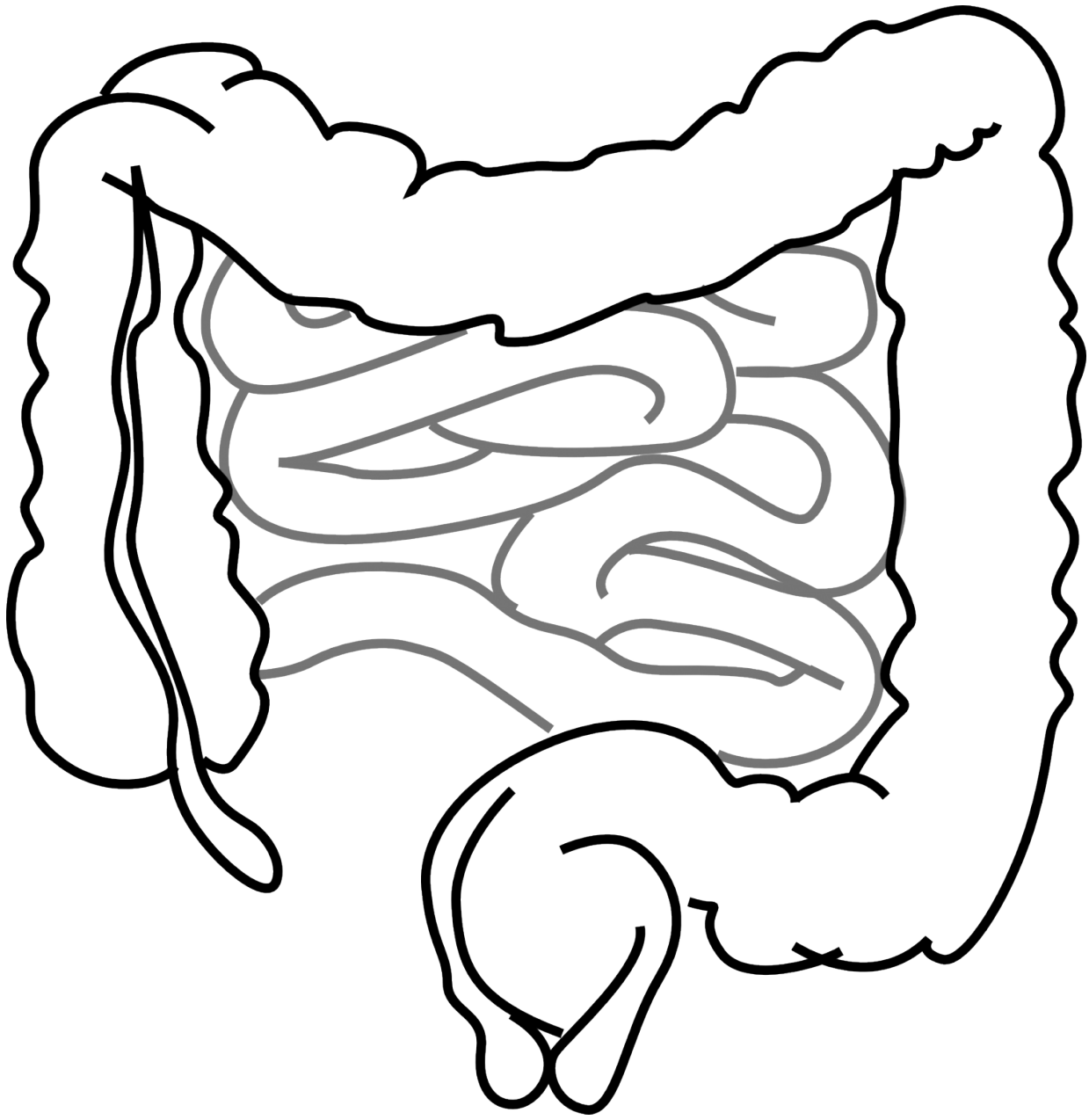

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A team of scientists in Portugal has discovered, in the mouse gut, a novel process that protects the bowel's lining against inflammation and microbial aggressions - and fights them when they arise. And, most surprisingly, they have shown that this mechanism is under the control of the intestinal nervous system - the so-called "second brain".

The sheer size of the network of nervous cells that reside in the vertebrate gut has earned it that nickname in recent years. Now, judging by the research led by Henrique Veiga-Fernandes, at Instituto de Medicina Molecular, in Lisbon, it appears that it is actually a well-deserved one. The results are published today (July 13th) in *Nature* magazine.

"Our study reveals that the nervous system acts as the 'eyes and ears' of the immune system", says Veiga-Fernandes, who is currently moving his lab to the Champalimaud Centre for the Unknown (also in Lisbon). "Nervous cells receive alerts from the gut and then give specific instructions to the immune system to repair the damage."

It is already known that there is a relationship, a dialog, between neurons in the gut and the immune system. In particular, a study published very recently by a team at Rockefeller University (USA) showed that certain neurons can induce a type of immune cells (macrophages) to produce substances that protect the gut.

But Veiga-Fernandes' team has gone further: "What is totally new in our work", he says, "is that not only did we discover the phenomenon, but we also described the molecular mechanisms at play".

It all started when he and his colleagues identified the presence of a receptor protein, called Ret, on the surface of a type of immune cells called innate lymphocytes (lymphocytes are white blood cells), which are among the most important regulators of inflammation and infection at mucous membranes. Ret acts, in fact, as a switch which can be turned on or off by the signals it receives.

Lymphocytes parading as neurons

Scientists also knew that the same Ret protein is present on the surface of nervous cells in the gut, where it regulates the function of these cells by picking up, as if it were an antenna, chemical signals (called neurotrophic factors) coming from outside the cells. "Suddenly, we were seeing a type of lymphocytes parading as neurons", says Veiga-Fernandes. "This was a big surprise. What was the Ret protein doing on those lymphocytes?"

To try to elucidate this enigma, the team started by locating the innate lymphocytes that expressed the Ret receptor in the gut of laboratory mice, which had been genetically modified so that their cells glowed green when carrying Ret on their surface. And they discovered that, immediately beneath the intestinal mucosa, there are, in fact, thousands of cellular clusters, each containing 100 to 200 innate lymphocytes expressing Ret.

The next step consisted in determining what could be the function of the protein in those lymphocytes. "We then showed that the Ret protein controls the production, by the innate lymphocytes in the gut, of interleukin-22 (IL-22), a molecule that is extraordinarily important for the repair of the gut epithelium [or wall]", says Veiga-Fernandes.

In fact, they confirmed that transgenic mice which did not express Ret on their innate lymphocytes had an altered intestinal epithelium that was

less able to regenerate and to express the genes that promote repair.

These results led to another idea: proving that those animals, given their altered epithelium, were prone to various inflammatory pathologies and infections of the gut. "We tested this idea in mice infected with gut bacteria or in which we had induced a chronic bowel inflammation", says Veiga-Fernandes. "And what we saw was that the animals that did not express Ret were highly susceptible to both things and died very quickly."

On the other hand, transgenic mice in which the expression of Ret had been boosted to higher-than-normal levels proved to be "totally resistant" to these pathologies.

The next step was to figure out how Ret is activated in the innate lymphocytes. In other words, to identify the cells that send the necessary neuroregulatory signals to the Ret proteins on the innate lymphocytes, thus inducing these immune cells to produce the key molecule of intestinal repair IL-22. To answer this question, the team used high-resolution microscopy to search for cells in the vicinity of the innate lymphocytes that could be responsible for turning on the Ret switch.

Multi-cellular troika

"We then discovered that all the cluster of innate lymphocytes were very close to glial cells, a type of nervous system cell", says Veiga-Fernandes. "In fact, these are the cells that make the neurotrophic factors that activate the Ret protein on the innate lymphocytes." Glial cells are not neurons, but they are a crucial component of the nervous system.

Next question: how do glial cells detect intestinal threats in order to activate Ret in the innate lymphocytes at the right time?

"Actually, what enables the glial cells to make these Ret activators is the fact that they are able to detect signals of bacterial presence and of damage to intestinal tissue", says Veiga-Fernandes. "These signals are either produced by bacteria or substances called alarmins, which are signals that are emitted by any cell when it is in trouble."

"To summarize, we identified a multi-cellular 'troika' [innate lymphocytes, glial cells, gut epithelial cells], orchestrated by neurotrophic factors, that protects the intestine", says Veiga-Fernandes. "And we found that changes in this cellular and molecular axis lead to intestinal inflammatory disease and to the incapacity of eliminating intestinal infections."

A future application of these results may be the development of new preventive and therapeutic strategies against chronic bowel inflammations - such as Crohn disease and ulcerative colitis - and even against intestinal cancer, according to Veiga-Fernandes.

The team is now exploring ways of activating the innate lymphocytes directly, without the help of glial cells. "We want to manage to do the glial cells' job in their place", says Veiga-Fernandes.

Henrique Veiga-Fernandes graduated in Veterinary Medicine at Universidade de Lisboa, Portugal. He was awarded a PhD in molecular and cellular biology from Université René Descartes, in Paris, France. He developed his post-doctoral research at Institut Necker in Paris, France, and at the National Institute for Medical Research, in London, UK.

He started his own group in 2009 at Instituto de Medicina Molecular (IMM), in Lisbon, and in 2014 he became member of the IMM's board of directors. More recently, his lab joined the new Biology of Systems and Metastasis research programme at the Champalimaud Centre for the

Unknown, also in Lisbon.

Henrique Veiga-Fernandes made ground-breaking contributions to the understanding of immunological memory, innate lymphoid cells and hematopoietic stem cell biology. Among other distinctions, he received three European Research Council (ERC) awards, he has been elected as EMBO member and he is Commander of the Ordem Militar de Sant'Iago da Espada of Portugal.

More information: Glial-cell-derived neuroregulators control type 3 innate lymphoid cells and gut defence, *Nature*, DOI: 10.1038/nature18644

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