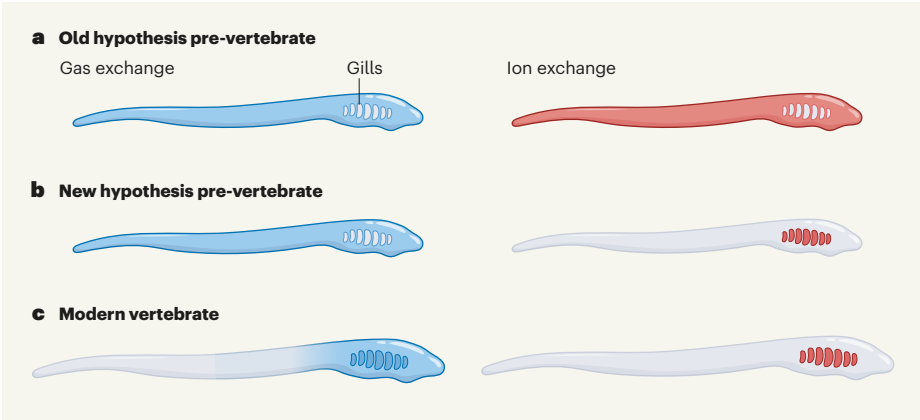

Figure 1 | An early role for gills in ion exchange. a, Small burrow-dwelling animals offer a model for understanding key steps in the early evolution of vertebrates. It has long been thought that gas exchange (the taking up of oxygen and the release of carbon dioxide) and ion regulation (transfer of ions between the body and its environment) occurred through the skin, across the length of the body, in our pre-vertebrate ancestors. **b**, Sackville *et al.*[1] present evidence for a new hypothesis, in which ion regulation was occurring predominantly at the gills much earlier in evolution, perhaps as far back as the first pre-vertebrates with gills. **c**, The authors report data suggesting that, as vertebrates evolved to become larger, more-active organisms, the gills became the dominant site for gas exchange.

gas exchange shifted towards occurring in the gills. This finding supports the theory that increased gas exchange at the gills occurred as vertebrates evolved larger body sizes. Surprisingly, the same was not true for ion regulation — which always occurred at the gills, no matter how big the ammocoetes were.

This led the authors to look further back in the animal family tree to find the roots of this unexpected characteristic. For those experiments, Sackville and colleagues used the acorn worm (*Saccoglossus kowalevskii*) and a species of amphioxus (*Branchiostoma floridae*), which are both close relatives of vertebrates. These small marine creatures have similar lifestyles to ammocoetes, living in burrows where they filter-feed with their gills.

The authors took advantage of the acorn worms' regenerative capacity and cut them in two. When they measured gas exchange over the still-living front and back halves of the worms, they found that the capacity for gas exchange was the same in the two halves. This finding supports the existing theory that, before vertebrate evolution, the dominant site of gas exchange was not the gills. When exploring ion regulation in acorn worms and amphioxus, the authors were hindered by the high ion content of seawater, which meant that they could not directly measure ion changes in the animals' environments. Instead, the authors examined the presence and location of known molecular markers for specialized cells called ionocytes, which control ion regulation in vertebrates[5]. Sackville and colleagues found that in both acorn worms and amphioxus, the levels of ionocyte markers were higher in the gills than in the skin.

The authors' beautiful microscopy analysis of the gills showed that the suspected ionocytes were found in a location similar to where they occur in fish gills. Together with the team's lamprey work, these results dash the theory that ion regulation moved to the gills along with gas exchange in early vertebrates. Instead, the findings suggest that ion regulation shifted to the gills much earlier in animal evolution, maybe even as far back as the first organisms with gills (Fig. 1). The authors speculate that the original regulatory function of the ionocytes in the gills might have been linked to feeding, with ionocytes controlling the secretion of a gel-forming substance to trap food particles.

Although Sackville and colleagues' findings are compelling, sceptics might point out that the presence of ionocyte markers does not prove that these cells function as ion regulators, and that direct measurements of ion transfer in acorn worms and amphioxus are needed. Another issue is whether the ionocytes present in vertebrate gills, as well as suspected ionocytes in the gills of non-vertebrates, are indeed evolutionarily related. It is possible that these similar cells evolved independently. This possibility could be explored by investigating how the ionocytes develop in each of these animals. If they come from the same part of an embryo and require the same genetic instructions for their development, then we will have even stronger support for a deep origin of gill ionocytes.

Vertebrate evolution is a certainly a puzzle that researchers will continue to explore, being driven by a curiosity to understand our own origins. Sackville and colleagues have taken a creative approach to challenge our current understanding of this process, and have provided new avenues for exploration.

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Immunology

How immune cells ignore harmless gut bacteria

Mark A. Travis & Chiara Romagnani

The immune system must be actively controlled to prevent inflammatory bowel disease. Cell populations have been found that promote immunosuppressive regulatory T cells of the immune system in the gut. **See p.737, p.744 & p.752**

Our immune system must respond quickly to dangerous disease-causing agents but also needs to ignore our own cells and anything benign. This balancing act is precarious in the gut, which is home to a multitude of harmless microorganisms, termed the microbiota. These microbes should not attract the attention of the immune system, but if the immune system fails to ignore them and targets them instead, this can cause inflammation and the development of inflammatory bowel disease[1]. Writing in *Nature*, Lyu *et al.*[2] (page 744), Kedmi *et al.*[3] (page 737) and Akagbosu *et al.*[4] (page 752) shed light on a key process that is required to dampen immune responses directed against the microbiota.

Immune cells called regulatory T (T_{reg}) cells have a crucial role in ensuring that the

microbiota is ignored. This suppressive subset of T cells expresses the protein CD4 and the transcription-factor protein FOXP3. T_{reg} cells can dampen self-harming immune responses, thereby enabling what is described as immune tolerance in various ways, such as by interacting with pro-inflammatory cells or sequestering factors that promote an immune response[5]. T_{reg} cells called thymic T_{reg} cells arise from developing T cells in the thymus gland. They form when cells called medullary thymic epithelial cells present fragments of proteins normally present in the body (self-antigens) to the developing T cells (also known as naive T cells). This presentation of self-antigens on the cells' surface is dependent[6] on the expression of a protein called AIRE. T_{reg} cells produced outside the thymus in the body's periphery, at sites such as the gut, are called peripheral T_{reg} (pT_{reg}) cells. When pT_{reg} cells form, they express FOXP3 in response to signalling mediated by the protein TGF-β (Fig. 1).

Gut pT_{reg} cells that express the transcription-factor protein RORγt emerge early in life and have a central role in promoting tolerance to the microbiota when microbes colonize the gut after birth[7]. The generation of pT_{reg} cells in the gut can be mediated by subsets of immune cells called dendritic cells[8] that express the protein CD103 and present antigens on the surface of their cells using type II major histocompatibility complex (MHCII) molecules. These cells can be described as conventional dendritic cells.

However, some reports have highlighted the ability of other antigen-presenting cells that express MHCII and RORγt to mediate the processes leading to peripheral tolerance, although their specific role in mediating tolerance wasn't completely clear[9–13]. These antigen-presenting cells have been described as extra-thymic subsets of cells that express AIRE (known as eTACs or Janus cells) and also as group 3 innate lymphoid cells (ILC3), although the exact relationship between these different cell types is unclear[9–13]. Such cells have been shown[10,11] to have a role in immune tolerance because mice develop gut inflammation if RORγt-expressing cells that are not T cells are engineered to lack MHCII. This suggests that RORγt-expressing cells need to present antigens to T cells to enable the immune tolerance that allows the gut microbiota to be ignored. The identity of the antigen-presenting cells that promote the differentiation of RORγt-expressing pT_{reg} cells to aid immune tolerance has remained unclear, and such information could inform strategies for the prevention and treatment of inflammatory bowel disease.

The three new studies shed light on the antigen-presenting cells that promote the generation of RORγt-expressing pT_{reg} cells in the gut. All three exclude a role for conventional dendritic cells in inducing these pT_{reg} cells,

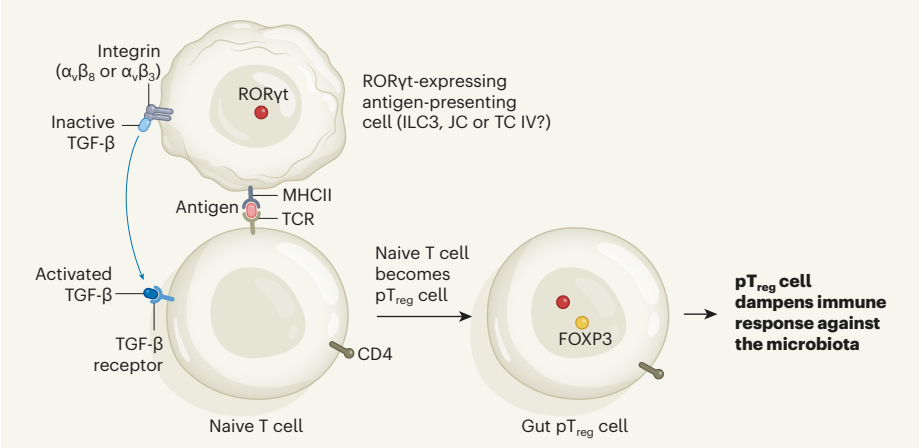

Figure 1 | The formation of immune cells that protect gut microorganisms. Lyu *et al.*[2], Kedmi *et al.*[3] and Akagbosu *et al.*[4] investigated how a type of gut immunosuppressive T cell called a pT_{reg} cell forms. Its development requires a cell that presents peptide fragments (or antigens) on a protein complex called a type II major histocompatibility complex (MHCII) – such antigens can then be recognized by a T-cell receptor (TCR) on a T cell. The authors investigated the gene-expression characteristics of several cell types proposed to be RORγt-expressing antigen-presenting cells – group 3 innate lymphoid (ILC3), Janus (JC) and type IV Thetis cells (TC IV). The exact relationships between these proposed cell populations remain incompletely understood. A RORγt-expressing antigen-presenting cell activates the protein TGF-β through an integrin protein (comprising either $\alpha_v\beta_8$ or $\alpha_v\beta_3$ subunits). Activated TGF-β can be received by the TGF-β receptor on a T cell called a naive T cell (which expresses the protein CD4). In response, the naive T cell becomes a pT_{reg} cell and expresses the transcription-factor proteins FOXP3 and RORγt. These pT_{reg} cells prevent immune responses against harmless bacteria (termed the microbiota) in the gut.

consistent with previous work[14]. The authors of all three studies investigated whether antigen-presenting cells that expressed RORγt could be responsible instead. Indeed, deletion of MHCII in cells expressing RORγt resulted in decreases in gut RORγt-expressing T_{reg} cells. Moreover, this shortfall of T_{reg} cells was paralleled by a rise in a type of T cell that mediates inflammatory immune responses (effector T helper 17 (T_H17) cells) in the gut,

"Immunologists are only beginning to understand how the host and its microbiota learn to live together."

suggesting that different antigen-presenting cells are responsible for the induction and maintenance of gut pT_{reg} and effector T_H17 cells.

Furthermore, the expression of MHCII by antigen-presenting cells that express RORγt was in itself sufficient for the development of gut RORγt-expressing pT_{reg} cells. Assessments of gene expression and the ease with which different genes are expressed (epigenetic analysis) in cells taken from certain immune sites – called lymph nodes – that drain the gut identified different subsets of RORγt-expressing cell lineages. In addition to ILC3 cells, these subsets included cells previously reported as Janus cells and, as described by Akagbosu *et al.*, four subsets of Thetis cells (called TC I to TC IV). However, how these Thetis cells relate to the previously described Janus cells requires further work. The studies highlight the complexity of RORγt-expressing antigen-presenting cells and provide insights into the similarities and differences in gene-expression signatures, developmental requirements and markers that distinguish them.

The authors aimed to identify the mechanisms responsible for the formation of these RORγt-expressing pT_{reg} cells in the gut. A key factor that promotes T_{reg} cells is TGF-β, which needs to be activated – for example, by a protein called integrin $\alpha_v\beta_8$ – after it is secreted by cells. The current studies report a reduction in the number of RORγt-expressing pT_{reg} cells in the mouse gut when either the α_v subunit (as shown by Lyu *et al.* and Kedmi *et al.*) or the β_8 subunit (as reported by Akagbosu *et al.*) was absent from RORγt-expressing cells, or – as described by Kedmi and colleagues –when mice were treated with an antibody targeting β_8. However, all three studies report that ILC3 cells seem to express little or no β_8, indicating that β_8 expression on a non-ILC3 RORγt-expressing cell population is probably important in promoting RORγt-expressing T_{reg} cells through integrin $\alpha_v\beta_8$. The most probable cell type responsible for this seems to be TC IV, which expressed higher levels of integrin β_8 than the other RORγt-expressing cell populations analysed, as Akagbosu and colleagues report.

Intriguingly, Lyu and colleagues report data

indicating that ILC3 cells promoted the survival of RORγt-expressing T_{reg} cells by expressing a different integrin, called integrin $\alpha_v\beta_3$, which hasn't previously been linked to the induction of pT_{reg} cells. Integrin $\alpha_v\beta_3$ can bind to and activate TGF-β, but also engages several other proteins. Therefore, more work is needed to identify the RORγt-expressing cells that promote RORγt-expressing T_{reg} cells through integrin $\alpha_v\beta_8$, and to investigate the *in vivo* contribution of ILC3 cells to the promotion of RORγt-expressing T_{reg} cells through integrin $\alpha_v\beta_3$.

A key question is which RORγt-expressing antigen-presenting cell population — if any — has an essential (non-redundant) role in promoting the generation of pT_{reg} cells that express RORγt and FOXP3 and that enable tolerance to the microbiota. Different approaches to abolish the expression of MHCII in ILC3 cells gave opposing results in two studies: the work by Lyu and colleagues indicates that this deficiency caused a reduction in intestinal RORγt-expressing pT_{reg} cells, whereas Akagbosu and co-workers found no effect. These results leave open the question of whether ILC3 cells have key roles in promoting RORγt-expressing pT_{reg} cells. A genetic system to specifically remove Thetis cells is still lacking.

Analyses of mice lacking MHCII in AIRE-expressing cells, or ones that lack AIRE in RORγt-expressing cells, showed no decreases in gut RORγt-expressing pT_{reg} cells compared with control animals. However, these results might not be completely conclusive regarding the role of Thetis cells and AIRE, because it seems that AIRE is deleted in only a fraction of Thetis cells by the currently available genetic tools in mice. Furthermore, AIRE might be dispensable for the generation of microbiota-specific T_{reg} cells. Alternatively, peripheral expression of AIRE by Thetis cells or dendritic cells could have a role in peripheral tolerance to other antigens not related to the microbiota or by promoting functions not associated with the induction of pT_{reg} cells, as suggested by other work[15].

These cellular interactions that have a crucial role in the regulation of tolerance to the microbiota in mice might be evolutionarily conserved in humans. A population of dendritic cells that express AIRE in human tonsils was described previously[16]. Interestingly, by analysing previously published single-cell data for samples from fetal, child and adult human intestines and gut-draining lymph nodes[17], Akagbosu and colleagues identified a gene-expression signature comparable to that of TC III and TC IV in a group of cells previously defined as dendritic cells in human fetal samples. Whether ILC3, dendritic or Thetis-cell-like cells might have an antigen-presenting role in promoting the formation of pT_{reg} cells in humans needs to be assessed.

Immunologists are only beginning to understand how the host and its microbiota learn to live together. Answering the remaining questions might set the stage for new opportunities to boost gut health and prevent the onset of immune-mediated disorders.

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Nuclear astrophysics

Underground route to grasping the oldest stars

Marco Pignatari & Athanasios Psaltis

Nuclear-fusion experiments performed deep under Earth's surface reveal one possible scenario that could have resulted in the chemical abundances found in an ancient star in the Milky Way. **See p.656**

When the first stars in the Milky Way formed around 13 billion years ago, they consisted mainly of hydrogen and helium. But other chemical elements — the heaviest being calcium — have been detected in the atmosphere of one of the oldest-known stars, an amazing object known as SMSS0313-6708 that lies just 1,800 parsecs from Earth[1]. Astronomers and astrophysicists were puzzled, and started to look for ways in which calcium and the other elements could have been made. The solution, it seems, might be found under Earth's surface. On page 656, Zhang *et al.*[2] report nuclear-physics experiments that could support one explanation for the chemical abundances found in SMSS0313-6708 — with implications for our understanding of other stars in the Universe.

Stars are giant nuclear-fusion reactors that initially generate energy by burning hydrogen in their cores and converting it to helium. Depending on the initial size of the star, the helium nuclei can then fuse to produce carbon and oxygen, followed by more fusion stages that produce heavier elements as the star evolves[3]. Stars that are around eight to ten times more massive than the Sun[4] end this cycle with powerful explosions called supernovae[5], ejecting the new chemical elements at high velocities into the interstellar space, and seeding the surrounding area with gas that will form the next generation of stars. The oldest stars in the Galaxy today therefore retain the chemical fingerprints of these first supernovae.

Finding these stars and measuring their tiny elemental abundances are key goals for astronomy, because such measurements reveal the main properties of the first generation of stars[6]. But computational experiments can also be informative about stellar properties by simulating the generation and evolution of stars. And as in real stars, nuclear reactions provide the fundamental ingredients for the production of elements in simulated stars. For this reason, understanding the rates at which these reactions occur can improve the precision with which stellar simulations can predict the abundances expected in stars such as SMSS0313-6708.