which updates the model. The cycle then continues until the reaction performance meets the specified target, or the reagents are exhausted.

Shields *et al.* successfully applied this workflow to three reaction classes, in which the algorithm varied multiple reaction parameters, including the temperature, solvent and ligand (the molecule that binds to the metal centre of the catalyst). In each case, their algorithm successfully identified optimal reaction conditions using approximately 50 test experiments from a pool of up to 312,500 possible combinations of variables.

After tuning the algorithm using published data sets, the authors statistically evaluated its performance using an optimization game, in which the algorithm competed with expert chemists. The authors selected a reaction that would be optimized in the game, and then defined five reaction variables that could be altered, limiting the players to a fixed set of possibilities for each variable: 12 ligands, 4 bases, 4 solvents, 3 temperatures and 3 concentrations. The researchers then experimentally tested and measured the outcomes of all 1,728 possible combinations of variables.

Next, Shields and colleagues gave 50 expert chemists up to 100 attempts to carry out a virtual optimization: participants selected 5 combinations of variables, and were then shown the experimental outcomes, after which they could select a new batch of 5 combinations to try to achieve the maximum possible reaction yield. Likewise, the algorithm played the game 50 times, but each time starting with random experimental values. The experts made better initial choices, but the algorithm outperformed the players, on average, after the third batch of experiments (Fig. 1).

More notably, the algorithm consistently arrived at greater than 99% yields, which was possible only by using an unusual ligand that was not known to work well for the type of reaction targeted in the game. Overall, the game provided a crucial lesson in cognitive bias: most chemists ended the game early, having used only mainstream reagents, without realizing that they could have further improved the yields by making more-adventurous choices. Shields *et al.* have thus developed an accessible tool that could be used by non-experts to optimize a wide range of reactions. Importantly, the workflow carries researchers through the encoding and optimization processes for multiple chemistries without requiring changes to the code.

This empowering tool is poised to provide chemists with an alternative approach for reaction optimization, unlocking the many benefits of machine learning. Not only will it accelerate the pace of research in chemical synthesis, but it will also help to increase the range of reaction variables tested. Moreover, the tool eliminates the need for chemists to triage potential experimental variables to mitigate time and material costs. I expect this technology to be widely adopted, enabling rapid optimization of reaction conditions and discoveries off the beaten path.

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Medical research

Food for thought about the immune drivers of gut pain

Stuart M. Brierley

Debilitating gut pain is common, but the underlying cause is often unclear. It emerges that gut infection triggers localized immune responses that cause normally innocuous foods to be perceived as harmful, leading to persistent pain. **See p.151**

Pain evolved to alert us to and protect us from actual or potential tissue damage. There are three common forms: nociceptive pain, which is associated with the detection of damaging stimuli; inflammatory pain, associated with inflammation or infection; and chronic pain, which is a maladaptive, long-term form of pain[1]. On page 151, Aguilera-Lizarraga *et al.*[2] report evidence from mice and humans indicating a previously unknown mechanism that contributes to chronic gut pain.

When an individual has an obvious injury, such as a broken arm, we can readily appreciate that they are in pain. But if the injury site can't be easily pinpointed, it can be difficult to determine the origin of the pain. This is a common problem for people who have pain in their internal organs, with some forms of such pain being easier to diagnose than others. For instance, people who have inflammatory bowel disease might have easy-to-spot indicators of disease, such as gastrointestinal bleeding, inflammation of the intestinal lining, or the presence of distinctive biomarker molecules in faecal or blood samples[3]. However, people who have irritable bowel syndrome (IBS), a condition that affects 11% of the global population[4], lack such clear hallmarks of illness, and no obvious cause explains their chronic abdominal pain and concurrent constipation or diarrhoea.

> **"A bacterial gut infection can profoundly change local immune responses."**

Previous studies indicate that IBS is more common in women than in men[4], with IBS symptoms being triggered by factors such as stress[4], gastroenteritis (a disease caused by the ingestion of contaminated food or water)[4], alterations in gut microorganisms[5], and changes in communication between the gut and brain[6]. Aguilera-Lizarraga *et al.* now show that a bacterial gut infection can profoundly change local immune responses in the gut, resulting in certain foods being perceived as harmful, and thereby causing persistent gut pain (Fig. 1).

In healthy individuals, a process called oral tolerance results in the immune system 'ignoring' orally consumed substances[7,8]. An exception to this tolerance occurs for substances perceived by our bodies as being dangerous, such as harmful (pathogenic) bacteria, parasites and viruses. Our bodies identify foreign invaders by detecting molecular fragments called antigens, which provide a type of 'barcode' enabling our immune systems to specifically identify the intruders. Our immune systems can tag these antigens by producing antibodies that recognize them, enabling the pathogen to be quickly targeted if it appears again. Our defences should focus only on the 'bad guys', and leave the innocent bystanders alone. However, Aguilera-Lizarraga and colleagues hypothesized that a failure in oral tolerance might result in an indiscriminate targeting of both friend and foe.

To determine how this proposed breakdown in tolerance might occur in mice, the team used a model system that harnessed the

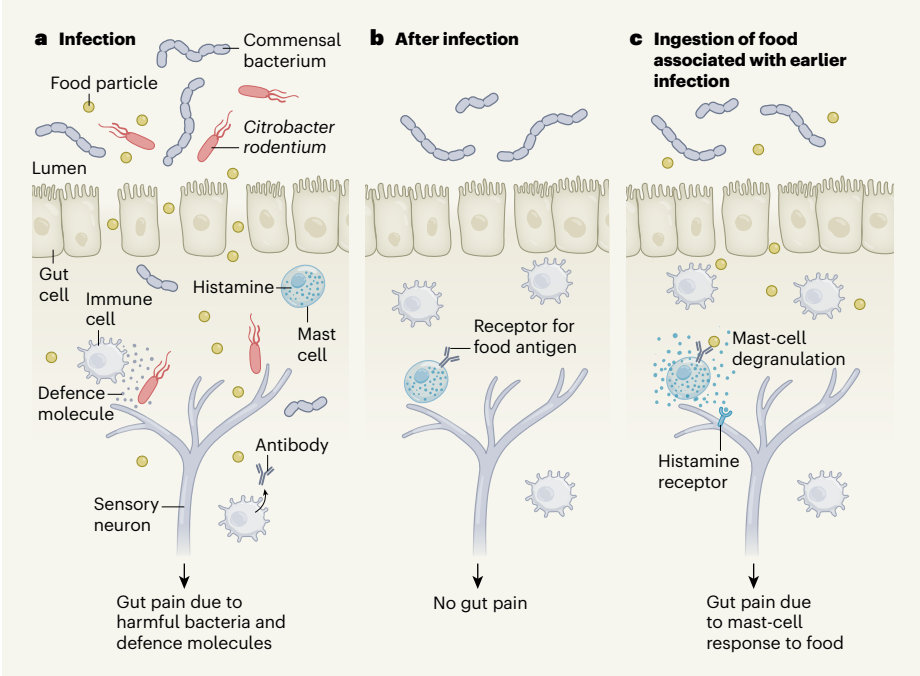

Figure 1 | An immune response to harmless food causes pain. Aguilera-Lizarraga *et al.*[2] reveal a previously unknown cause of gut pain. **a**, Immune cells in the gut, including mast cells, which contain histamine molecules, don't usually target food or microorganisms that normally reside there (commensal bacteria). If mice are infected with the bacterium *Citrobacter rodentium*, immune cells respond when the gut barrier breaks down (gut cells lose their connectivity), and food and bacteria leave the gut lumen and enter the body. Immune cells target *C. rodentium* by releasing defence molecules, and also target the harmless food present by producing antibodies that recognize it. Pain occurs as a result of the infection. **b**, After infection, repair of the gut barrier begins. Mast cells become primed to respond by moving near to neurons and expressing a receptor (generated on the basis of the antibody made previously) that recognizes a fragment of the food called an antigen. **c**, On subsequent ingestion of the food, mast cells recognize it and release histamine through a process called degranulation. Histamine binds to a receptor on sensory neurons, activating them and causing pain.

pathogenic bacterium *Citrobacter rodentium* and ovalbumin, a protein found in egg white. Repeated ingestion of ovalbumin alone did not evoke signs of gut pain, as determined by the measurement of abdominal contractions in response to distension of the animals' colon and rectum. However, the administration of ovalbumin after the mice had recovered from a *C. rodentium* infection accompanied by dietary ovalbumin caused gut pain and diarrhoea.

The animals also had a 'leaky gut', as shown by higher than normal intestinal permeability. This finding suggests that the intestinal lining did not provide its normal function as a physical barrier, and instead allowed intestinal contents to access the underlying tissue, thereby triggering an immune response and activating sensory nerves. In addition, the authors found that specific antibodies to ovalbumin were present in the colon but not elsewhere in the body.

The increased gut pain experienced by the treated animals could be prevented either by genetically engineering them to lack IgE, a type of antibody, or by giving them an anti-IgE antibody to block the actions of ovalbumin-specific IgE antibodies produced by the animals' immune system. Conversely, the presence of ovalbumin-specific IgE antibodies in the animals' colon mimicked the effect of enhanced gut pain generated after ovalbumin ingestion, in mice that had not been infected with *C. rodentium*.

Aguilera-Lizarraga and colleagues went on to unravel some molecular details underlying this pain response. They showed that after *C. rodentium* infection and ovalbumin treatment, immune cells in the mouse colon called mast cells underwent degranulation, an event that releases molecules, including histamine, that are needed for defence. If this process was blocked, either by using a drug that prevents mast-cell degranulation or by genetically engineering mice to lack mast cells, this lessened or prevented the enhanced gut pain the animals experienced on ovalbumin ingestion after infection.

The authors present evidence indicating that histamine release triggers pain by affecting sensory neurons in the gut. Supernatant liquid taken from the colons of these mice increased the *in vitro* sensitivity of sensory neurons that signal pain. This effect could be prevented either by using a drug to block the histamine H_1 receptor, which is found on sensory neurons, or by using mice genetically engineered to lack this receptor.

The authors next injected solutions of soy, wheat, gluten and milk — all of which have been linked to food allergies and can cause gut symptoms, including bloating and abdominal pain[7] — directly into the colorectum of 12 people who had IBS and 8 healthy individuals. All of those with IBS, but only two of the healthy individuals, showed signs of an immune reaction to at least one of the foods. People with IBS had more mast cells in close proximity to nerve fibres compared with healthy individuals, suggesting more-effective transfer of information between the mast cells and nerve endings of the sensory neurons.

The authors report that 23% of the faecal samples from people with IBS were positive for infection by the bacterium *Staphylococcus aureus*, compared with only 9% from healthy subjects. This finding is intriguing because *S. aureus* is one of the main microbial sources of 'superantigens' – potent antigens that have been linked to nonspecific activation of immune cells called T cells[9]. Indeed, 47% of faecal samples from people with IBS were positive for at least one superantigen, compared with only 17% of such samples from healthy volunteers. These findings might suggest that previous infection and the presence of superantigens promote enhanced gut pain in some people with IBS by priming their immune-system response.

The authors' study raises several points for further consideration. For example, the mechanisms involved were determined using ingestion of an antigen in mice, whereas the food solutions tested in humans were injected directly into the gut mucosa (mucous membranes). It would be interesting to determine whether specific human diets containing the ingredients tested recapitulate the authors' findings. Also, this mechanism of a breakdown in oral tolerance does not explain why women have a greater predisposition to developing IBS than men[4]. Although Aguilera-Lizarraga and colleagues' study has relevance for mechanisms associated with post-infectious IBS (as in gastroenteritis), it would be interesting to investigate whether this mechanism is relevant for other types of IBS, such as constipation-predominant IBS, diarrhoea-predominant IBS or IBS with mixed bowel habits.

The authors used *C. rodentium* as the pathogenic organism for their mouse model system. However, infections by other harmful microorganisms, such as *Escherichia coli*, *Salmonella*, *Giardia* and *Shigella*, can also precede the onset of IBS. Clinical epidemiology studies suggest that gut infections by these pathogens increase the likelihood that a person will develop IBS[10].

Does this mechanism apply only to the colorectum, or is it also relevant to other gut

regions such as the stomach, small intestine and proximal colon? If so, and if the same type of immune response occurs in other gut locations, different sensory nerves might be activated there, triggering different symptoms, such as nausea, discomfort and bloating, that are relevant to other gut-pain disorders, for example a condition called functional dyspepsia[4].

Aguilera-Lizarraga and colleagues' work presents numerous potential options to consider for therapeutic intervention. These include: improving intestinal-barrier function to reduce gut access to the intestinal immune system; targeting IgE antibodies that are specific to the food substance of interest; reducing mast-cell degranulation; targeting molecules released by mast cells or the receptors on which they act; and blocking the colonic sensory nerves that transmit the noxious information and cause pain.

From a dietary point of view, can oral tolerance, once lost, be reacquired? In this regard, food-allergy studies suggest that eliminating the offending foods from people's diets, and then gradually reintroducing them, can improve the long-term prognosis[11]. Exclusion diets are increasingly popular for remedying gastrointestinal symptoms, including gluten-free diets for coeliac disease and, for IBS, diets low in a group of carbohydrates that are not completely digested or absorbed in the intestine (called FODMAPs — fermentable molecules of oligosaccharides, disaccharides, monosaccharides and polyols)[12]. Aguilera-Lizarraga and colleagues' study provides information on the mechanisms underlying abdominal pain, and gives added meaning to the saying, 'you are what you eat'.

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Developmental biology

A molecular handbook for human development

Felicia Kuperwaser & Itai Yanai

A large-scale, high-resolution cell atlas of gene expression and regulation in human embryos enables innovative investigation of development through multi-organ and multi-modal analysis.

Charles Darwin developed his theory of natural selection by comparing features between individuals and species. A comparative approach is also crucial to establish the cellular taxonomy that underlies human physiology. Technological advances in single-cell genomics have facilitated the production of numerous cell atlases that, through comparative analysis, define the full set of cells that constitute a system of interest — usually a whole organ[1]. Extending the scope of an atlas from organ to whole organism increases the power of this approach by capturing data across physiological systems. To this end, two papers in *Science* present the comprehensive molecular characterization of cell types across nearly all organs during human fetal development[2,3]. They reveal previously unidentified cell subtypes, and define cell-differentiation pathways through analysis of gene expression and chromatin (the DNA–protein complex into which a cell's genetic material is packaged).

The work is a remarkable feat, both technically, in terms of the complexity of the paired data, and because of the scale of the studies, which involved analysis of 15 organs from human fetuses between 72 and 129 days after conception. In the first of the papers, Cao *et al.*[2] generated gene-expression profiles (transcriptomes) from 4 million single cells across these organs. Analysis of these profiles revealed 77 main cell types, defined with reference to existing single-organ atlas data.

In the second of the papers, Domcke *et al.*[3] presented an improved method for assessing chromatin accessibility, an analysis that provides insight into how genes are regulated during development. Loosely packaged chromatin regions are thought to be more accessible to regulatory proteins such as transcription factors, and are often involved in regulating gene expression and in establishing and maintaining cell identity. The authors' approach enabled the analysis of 800,000 cells from the same samples as those used by Cao and colleagues, which led to the identification of 54 of the same cell types.

The considerable data collected allowed both groups to define highly expressed 'marker' genes and corresponding transcription factors unique to each cell type. The authors also integrated their atlases with existing mouse atlas data[4], making each a more robust and complete reference. Combining these data sets enables validation of how cell types are characterized in each species and will help researchers to better design experiments that use mouse models to investigate human physiology. Together, the papers constitute a substantial resource, which is openly available on an interactive website (descartes.brotmanbaty.org).

The authors developed an analytical framework that led to interesting biological insights, demonstrating the potential of this body of

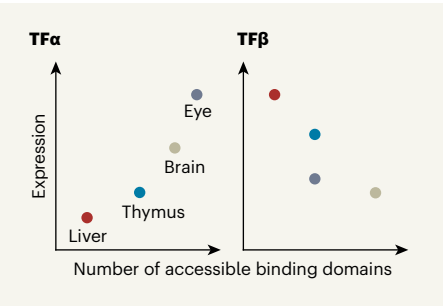

Figure 1 | Combined data types in a cell atlas for human development. A new cell atlas of human development combines data on gene expression[2] and chromatin accessibility[3] (chromatin is the DNA–protein complex in which DNA is packaged — 'looser' packaging makes DNA accessible to regulatory proteins such as transcription factors) in cells across 15 developing organs. The researchers combined these data to study the roles of transcription factors in human development, by cataloguing the expression of transcription factors of interest and the presence of their binding domains in accessible chromatin in each cell type. Transcription factors for which expression positively correlated with the presence of binding domains (such as TFα in this example) were assigned as transcriptional activators, whereas a negative correlation (as with TFβ) suggested a role as transcriptional repressors.