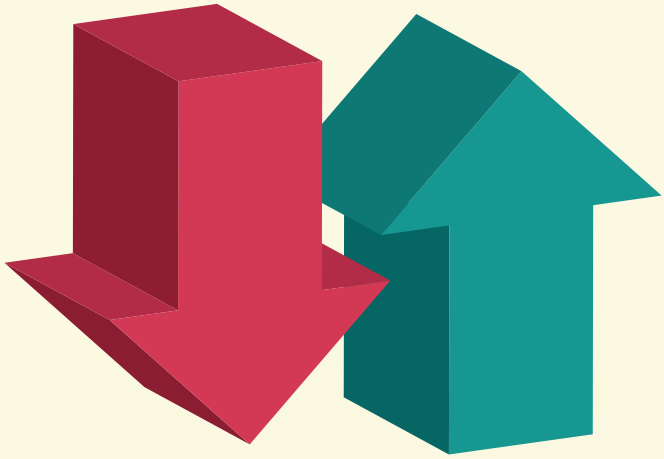


# Sides matter

Molecular biologist **Dr Steeve Boulant** explains how his research into cellular polarity is casting new light on the relationship between viruses and epithelial cells

**Can you introduce yourself and introduce your work with viral infection?**

I am the head of the Cellular Polarity and Viral Infection research group, which I established in 2012. Our research aims at better understanding how intestinal epithelial cells lining the surface of our digestive tract can simultaneously tolerate the presence of our commensal microbial flora and be fully armed to defend our body against foodborne infectious disease.

**Your laboratory is interested in the fundamental mechanisms of virus uptake, particularly clathrin-mediated endocytosis (CME). What is CME?**

Endocytosis is the general process by which cells transfer molecules from their extracellular environment to their intracellular compartments. Cells have developed multiple uptake mechanisms in order to adapt to the nature and size of the cargo molecules they want to internalise. One of the predominant pathways relies on a protein called clathrin and is therefore referred to as CME.

**What interests you about endocytic virus uptake mechanisms?**

Viruses are strict intracellular parasites; they must enter the host cell to initiate infection. In order to infiltrate a cell, the virus relies on its ability to hijack the same endocytic pathways that cells use for their normal endocytic function. As such, understanding how endocytosis is regulated in physiological conditions may help us design novel strategies to block viral entry.

**Are there specific types of diseases you are working with?**

We are studying enteric viruses, and my lab mostly uses mammalian reovirus (MRV) as a model enteric virus, as it is safe to handle. Our interest is not only the pathogen but the cellular functions underlying viral infection. Specifically, we are interested in how the innate immune system acts in intestinal epithelial cells, and how this correlates with the polarised nature of these cells.

**Using live-cell imaging combined with single-particle tracking, you have identified key steps in how MRV enters polarised cells. Can you discuss the details of this landmark achievement?**

Enteric virus infection often initiates from the apical side of intestinal epithelial cells. As viruses replicate, the barrier function of the epithelium can be lost, and viruses would gain access to the underlying tissue. To mimic this condition in the lab we use epithelial cells grown in special devices allowing us to specifically infect them from their apical or basolateral side.

We then developed novel labelling and tracking methodologies to follow viruses as they enter the cell and initiate replication. We found that the molecular mechanisms that regulate CME of viruses coming from the apical side are significantly different from the mechanisms regulating viral uptake from the basolateral side. Additionally, the outcome of viral infection was different, as infected cells make fewer viral particles if infected from their basolateral side. This observation made me realise that cellular polarity might affect virus replication at multiple levels within their life cycle. This is why I decided to focus my research group on cellular polarity and viral infection.

**You are now developing a mini-gut system that mimics the integrity of the intestinal tissue. What inspired you to take this approach? What are its benefits?**

Recently, stem cell biologist Hans Clevers from Utrecht developed organotypic cultures referred to as organoids, or mini-guts. They exploit the presence of a stem-cell niche in the crypt of the intestinal villi. When cultured appropriately with differentiation factors, these cells can grow *in vitro* and form complex structures reminiscent of the gut organisation. Moreover, they are made from normal, non-cancerous, healthy tissues.

As the mini-guts form, stem cells differentiate into most – if not all – cell types found in the intestinal epithelium. As such, an important difference between classical monotypic cell culture and mini-guts is that the organotypic cultures not only recapture the 3D organisation and cellular polarity state of the intestinal epithelium, but also reproduce the functional complexity intrinsic to their cell type composition.

**How are you using mini-guts?**

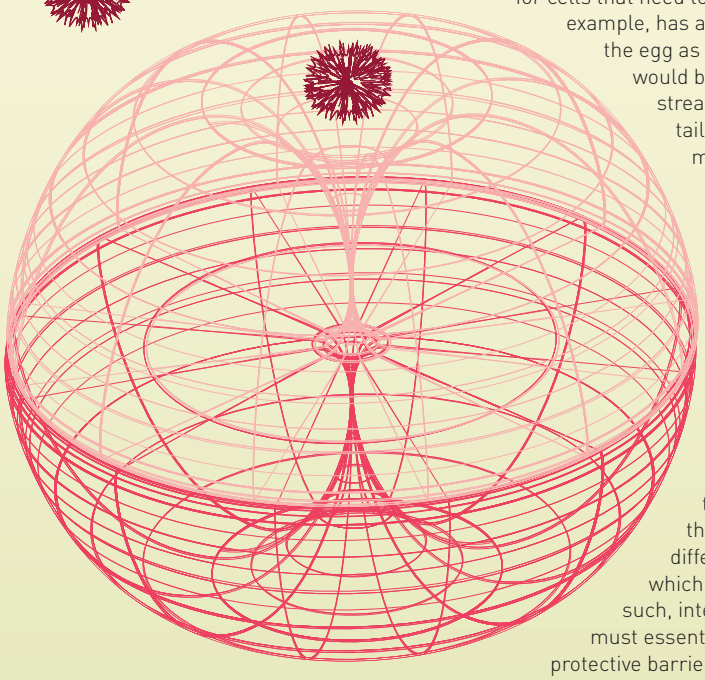
We are currently adapting mini-guts to study virus infection. We can already recapitulate infection of intestinal epithelial cells with several enteric viruses, and we have found that the signalling pathways induced during virus entry and the antiviral response are significantly different compared to the signalling observed in classical cell culture systems.

I believe this line of research will allow us to understand how gut homeostasis is achieved and maintained, which in turn will offer us a new perspective to study the body's relationship with commensal flora, which was recently found to be involved in many aspects of our development. Similarly, it might help us understand situations where commensal flora is misrecognised by the epithelial cells and induce a devastating inflammatory response (eg. inflammatory bowel diseases such as Crohn's disease and ulcerative colitis).

# Do viruses infect cells differently if they are upside-down?

Microbiologists at the **University Hospital of Heidelberg** and the **German Cancer Research Center** in Germany are conducting groundbreaking research into how viruses infect cells when they approach the cell from different sides; their discoveries could have resounding implications for health

Boulant and his team have developed their own epithelial models using polarised cells – allowing them to study the impact of viral infection on epithelial barrier function



**AS A HUMAN** being moving around in a relatively large world, the idea of polarity might seem like a concept to be taken for granted. The top of our heads (apical) are clearly opposite the bottom of our feet (basal), and the left side of our bodies definitely have different features when compared to our right – as your heart is probably slightly to the left of centre, and you are more likely to favour your stronger right arm. Moreover, you might sleep on your stomach – a ventral aspect of your body – but you mostly certainly use a dorsal aspect to sit in a chair.

These three types of fundamental polarities, which all exist in different planes, enable many of the complex behaviours we can perform. When it comes to single cells, however, scientists have shown that this characteristic that we often utilise without question is more difficult to define – although most cells have been shown to have an apex and a base.

## BEAUTIFUL ASYMMETRY

Just as for humans, polarity can be important for cells that need to move; a sperm cell, for example, has a pressing need to reach the egg as fast as possible, and it would be unable to do so without a streamlined head and wriggling tail. Similarly, cells that make up tissues that divide different compartments of an organism – such as epithelial cells – would not work so effectively if their basal and apical sides did not behave differently.

To an extent, this is common sense. Epithelial cells make up the lining of the digestive tract, and the inside of this part of the body is so different from the outside, which is harsh and acidic. As such, intestinal epithelial cells must essentially serve two purposes: protective barrier functions and selective exchange interface. Despite this obvious difference, however, it was not until fairly recently that the importance of polarity in epithelial cells was recognised and began to be the subject of earnest study.

It was in 2010 that Dr Steeve Boulant began his work on this subject – and, specifically, started to investigate endocytosis in epithelial cells. "I quickly realised that this asymmetric functionalisation of epithelial cells is not only important for cellular processes, but also during pathogen infection," he explains – and it was this realisation that led to the development of a research programme that he is still pursuing today. First investigating polarity in epithelial cells as a Postdoctoral Associate at Harvard Medical School, he has since gone on to head the Cellular Polarity and Viral Infection research group at the University Hospital of Heidelberg in Germany.

## UP THE DOWN CELL

Researching the effects of polarity on cell function is not without its difficulties. Typically, virologists rely on immortalised cell lines when it comes to *in vitro* experimentation; these cells are not polarised, and therefore do not capture the true complexity of the host system. What is more, as Boulant's research has progressed, his group has shown that polarised cells behave very differently to their directionless counterparts – recently, for example, the scientists demonstrated that mammalian reovirus (which is often used as a model for enteric viruses, since its symptoms when it infects humans are subclinical) kills cells in traditional 2D cultures, but actually encourages survival in polarised cells. "This result highlights the importance of using an appropriate cellular model, as different cells can drastically change the outcome of infection," Boulant opines.

Accordingly, Boulant and his team have developed their own epithelial models using polarised cells – allowing them to study the impact of viral infection on epithelial barrier function and discern how infection leads to symptoms like diarrhoea. Over time, they have identified more and more differences between

the behaviour of their own model and that of other experimental systems. While the overall reasons for this great distinction between polarised cells and non-polarised cells remains a mystery, their evidence suggests that it may be due to a difference in distribution of proteins between the apical (facing the lumen of the gut) and basal (facing the surrounding environment) sides of the cell.

## INTRUDER ALERT

The experiments Boulant and his colleagues have conducted have turned up intriguing results – especially with regard to the different ways in which cells behave following viral intrusion at opposing ends of the cell. Basolateral infection, they found, actually led to the creation of fewer new virus particles than did apical infection; basal infection, it emerged, was actually triggering a greater antiviral immune response upon infection than apical infection.

Curiously, this suggests that epithelial cells have a mechanism that enables them to recognise the difference between viruses that enter the intracellular compartment via different routes. "We have accumulated evidence that this polarised immune response is linked to the polarised nature of the cell," Boulant concludes.

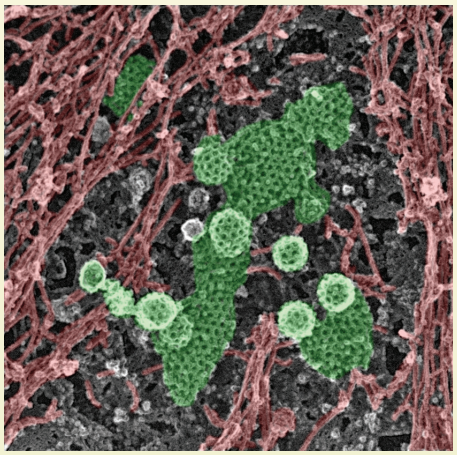
So what is the explanation for this behaviour? The answer is unclear – but Boulant and his collaborators have their suspicions. They discovered that the same proteins that govern polarity also play a pivotal role in modulating the innate immune response to viral intruders, and since a more extreme response seems to be encouraged at the basal side of the cell, the scientists hypothesise that this system has evolved to ensure that the cells can tolerate the commensal flora of the gut. On the apical side, epithelial cells are in constant contact with these benign microorganisms – and an immune response would not only damage the bacterial community, but also the cells themselves, over time.

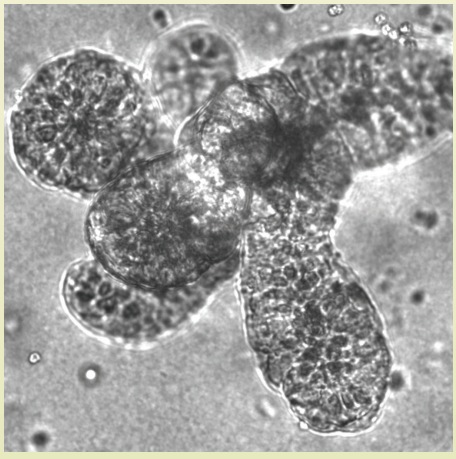
## TURNING SCIENCE UPSIDE-DOWN

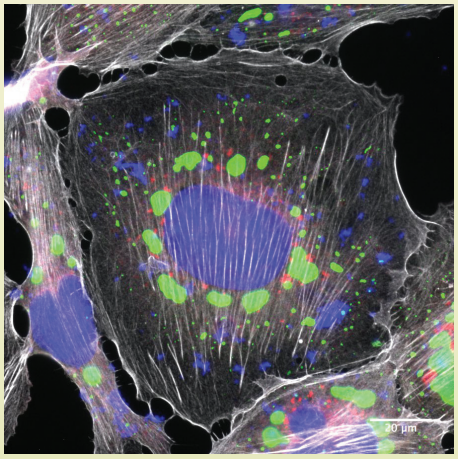
However, the epithelial cells still have to act as a barrier to infections originating in the gut – and this leads to a delicately balanced scenario in which the immune response must be fine-tuned, even within a single cell. When the pathogen infects from the basal side, this implies that the integrity of the wall of the gut has broken down – and in this situation, the strong immune response will serve the epithelial cells well. This asymmetrical, purpose-built immune response is fundamental for maintaining gut homeostasis – but it turns upside-down many experimental models and results that have been generated in the past.

Currently, the researchers are using mammalian reovirus to test their epithelial model's response and then validating the behaviours they observe with enteric viruses of greater clinical relevance – such as norovirus, rotavirus, enterovirus, coxsackievirus and poliovirus.

At the same time, the Boulant lab is working to adapt a mini-gut system developed by researchers in the Netherlands for the study of viral infection. "We are now genetically modifying and editing these organoids to better understand the fine interconnection between cellular polarity and innate immunity," Boulant enthuses – a process which, when combined with new technology the group is developing, could allow the Boulant laboratory to shed light on the mechanisms responsible for the symptoms of enteric viral infections.








**Above:** Clathrin structures. **Below left**: Intestinal organoid (mini-gut). **Below**: Reovirus infected cells.

### APPLYING THE EPITHELIUM MODEL TO INFECTIOUS DISEASES

**OBJECTIVE**

To elucidate spatiotemporal aspects of viral infection at epithelial surfaces, from entry of viral particles and intracellular trafficking to antiviral innate immune response.

**KEY COLLABORATORS**

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**DR STEEVE BOULANT** earned his Bachelor's, Master's and PhD (with excellence) in Molecular Biology and Biochemistry from Lyon-1 University. Upon graduating, he took up a post as a postdoctoral associate in the Medical Research Council Virology Unit Glasgow, UK, and in 2006 he took up the post of Marie curie Postdoctoral fellow in the same Unit. In 2008, he moved to Harvard Medical School as a postdoctoral associate and after four years, he started his current post as Group Leader at the CHS Foundation at University Hospital Heidelberg.