

## The intestinal epithelial response to damage

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The constant renewal of the intestinal epithelium is fueled by intestinal stem cells (ISCs) lying at the base of crypts, and these ISCs continuously give rise to transit-amplifying progenitor cells during homeostasis. Upon injury and loss of ISCs, the epithelium has the ability to regenerate by the dedifferentiation of progenitor cells that then regain stemness and repopulate the pool of ISCs. Epithelial cells receive cues from immune cells, mesenchymal cells and the microbiome to maintain homeostasis. This review focuses on the response of the epithelium to damage and the interplay between the different intestinal compartments.

**intestine, regeneration, microenvironment**

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### INTRODUCTION

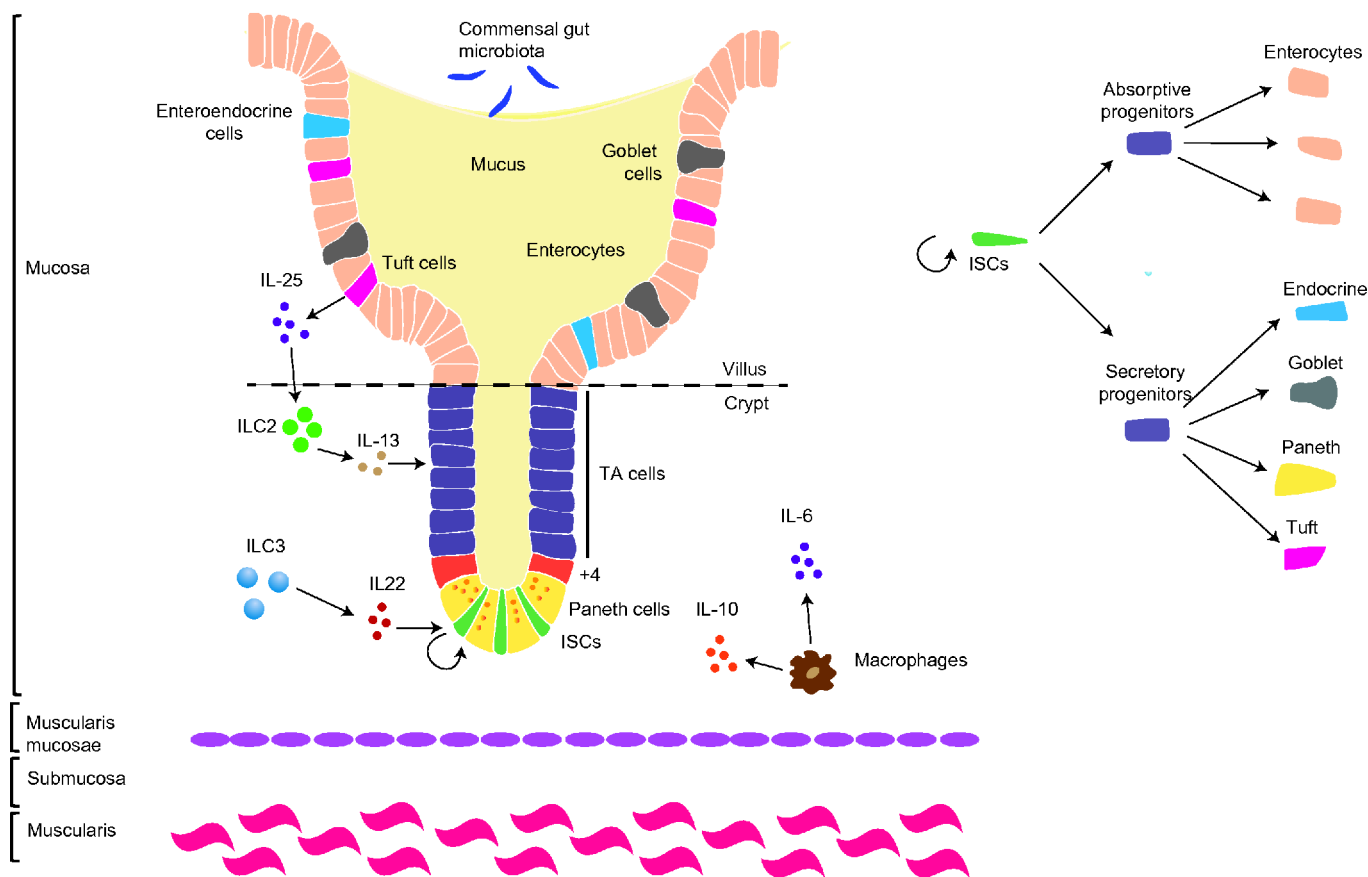
The luminal surface of the intestine is continuously exposed to a wide variety of potentially damaging environmental factors, such as infections and toxins. In order to maintain its integrity, the intestinal epithelium has developed an extraordinary capability for regeneration (Beumer and Clevers, 2016). After injury, the intestinal epithelial cells (IECs) adjacent to the wound lose columnar polarity and migrate to cover the lesion, in a process called “epithelial restitution” (Neurath, 2014). Subsequent stem cell activation, proliferation and differentiation occur in order to expand the pool of cells available for repair. This process relies on tight interactions between intestinal secretory and absorptive epithelial cells, stem cells and immune cells to prevent uncontrolled proliferation and safeguard homeostasis (Taniguchi et al., 2015).

### RESPONSE OF THE EPITHELIUM DURING DAMAGE

The intestinal epithelium is mainly composed of two types of differentiated lineages: the absorptive enterocytes and the secretory lineage (goblet, Paneth, enteroendocrine, tuft and M cells). The proportion of each of these differentiated cells varies according to the anatomical region within the small or large intestine (Barker et al., 2010). The source of these differentiated cells is intestinal stem (ISCs) that give rise to highly proliferative transit-amplifying (TA) cells, which in turn differentiate into the various cell types (Figure 1).

A major breakthrough occurred a decade ago with the identification of *Lgr5*, a Wnt target gene, as a marker of ISCs. *Lgr5* is expressed by slender cells, known as crypt base columnar cells, which are located at the base of intestinal crypts and interspersed between Paneth cells. The CBCs, in contrast to stem cells in some other epithelia, are rapidly cycling. Lineage tracing using an inducible Cre knock-in mouse line demonstrated that the *Lgr5*<sup>+</sup> CBCs yield clonal

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**Figure 1** Stem cell-fueled renewal of the intestinal epithelium. The intestinal epithelium is composed of two compartments, namely crypts and villi. The crypts harbor ISCs intermingled between Paneth cells at their base. ISCs divide each day to give rise to secretory and absorptive progenitor cells, also known as TA cells. While moving up along the crypt-villus axis, the TA differentiate into the various epithelial cells composing the villi. Homeostasis of the intestinal epithelium is maintained through the interplay between epithelial cells, immune cells, cytokine signaling and the microbiota.

ribbons that move up along the crypt-villus axis, persisting throughout the lifetime of the mouse and containing all cell lineages. These two characteristics of self-renewal and multipotency qualify CBCs as ISCs (Barker et al., 2007; Barker et al., 2012). Later, several studies focused on the gene-expression profile of fluorescence-activated cell sorting (FACS)-purified *Lgr5*<sup>+</sup> CBCs. *Ascl2*, *Olfm4* and *Smoc2* were thus identified as specific CBC markers (Muñoz et al., 2012; van der Flier et al., 2009; van der Flier et al., 2009), with *Ascl2* being essential for stem cell survival (Barker et al., 2012; van der Flier et al., 2009). An important *in vitro* achievement was the ability to culture epithelial “mini-guts” (or organoids) from a single *Lgr5*<sup>+</sup> CBC by adding R-spondin (the LGR5 ligand), EGF (Epidermal Growth Factor) and Noggin (BMP antagonist) to the medium. These organoids recapitulate the structure of *in vivo* intestinal epithelium with crypt-like structures and inter-crypt epithelium with all the differentiated cell types, further demonstrating the stemness of CBCs (Sato and Clevers, 2013; Sato et al., 2009).

While the status of *Lgr5*<sup>+</sup> CBCs as ISCs is largely accepted, the function of “+4” cells is still controversial.

Classically, cells at the +4 position (4 cells above the base of the crypt) have been viewed as being DNA label-retaining, a characteristic of quiescent cells, and extremely radiation sensitive (Potten et al., 1974). These two features were long thought to be markers for stemness and, to this day, there is still debate about whether these label-retaining cells (LRCs) are a type of “reserve” stem cell or whether they are committed cells that are able to dedifferentiate into stem cells upon injury (Barker et al., 2008).

More recently, experiments showed that ablation of *Lgr5*<sup>+</sup> cells by knocking a diphtheria toxin receptor (DTR) gene into the *Lgr5* locus did not perturb homeostasis of the epithelium, as other cells can compensate for the loss of CBCs and eventually give rise to new *Lgr5*-expressing cells, thus fueling the hypothesis that these other cells behave as “reserve” stem cells (Tian et al., 2011). The *Lgr5*<sup>+</sup> CBCs are nevertheless indispensable for repair in the context of radiation, as ablation of *Lgr5*<sup>+</sup> cells in *Lgr5*<sup>DTR</sup> mice prior to exposing these mice to radiation severely impairs epithelial regeneration (Metcalf et al., 2014). This impaired regenerative capability, and thus the requirement for *Lgr5*<sup>+</sup>

CBCs upon damage, is radiation-specific, since *Lgr5*<sup>+</sup> cells ablation does not affect the regenerative response of the epithelium after dextran sulfate sodium (DSS)-induced injury (Metcalf et al., 2014). Additionally, several other studies have reported that *Bmi1*, *mTert*, *Hopx* and *Lrig1* mark +4 cells that behave as a reserve stem cell pool, especially in the context of injury (Montgomery et al., 2011; Powell et al., 2012; Sangiorgi and Capecchi, 2008; Takeda et al., 2011). However, this area of study has remained controversial, as other studies have reported that these markers are actually expressed throughout the crypt—and, more importantly, in CBCs—therefore challenging the notion that these genes mark reserve stem cells (Itzkovitz et al., 2012; Muñoz et al., 2012).

A number of subsequent experiments have shed light on this question by demonstrating considerable plasticity in intestinal progenitor cells, a process that might be key in the response to damage. Several groups have shown that both secretory and enterocyte progenitors are able to dedifferentiate and regain stemness upon epithelial damage and ablation of CBCs. The use of lineage tracing in *Dll1*<sup>GFP-ires-CreERT2</sup> and *Alpi*<sup>GFP-ires-CreERT2</sup> knock-in mice to label, respectively, a subset of secretory or absorptive progenitors, showed that upon CBC ablation these cells generate long-lived ribbons of progeny originating from the crypts and spreading up the flanks of the villi (Figure 2). Furthermore, *Lgr5* expression reappears at the bottom of the crypt in these labeled ribbons, demonstrating that progenitors dedifferentiate and upregulate stem-cell specific genes (Ishibashi et al., 2017; Tetteh et al., 2016; van Es et al., 2012).

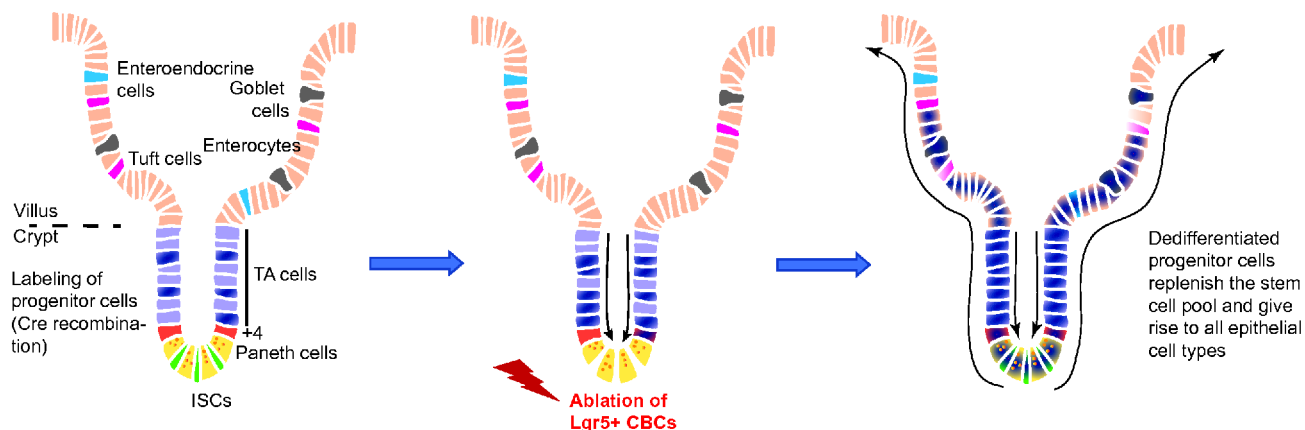
Interestingly, the dedifferentiation potential of enterocyte precursors requires physical proximity to the crypt and is lost upon crypt exit (Tetteh et al., 2016). The ability of enterocyte precursors to regenerate the damaged epithelium is particularly meaningful in light of the fact that the enterocyte lineage is the most abundant cell type of the epithelium. Another demonstration of plasticity in LRCs involved the observation that these LRCs are precursors committed to the secretory lineage and express both secretory and *Lgr5*<sup>+</sup> signatures. After intestinal injury, these cells also revert to stem cells, proliferate and give rise to cells occupying the whole crypt (Buczacki et al., 2013). Finally, the plasticity of progenitor cells is also observed through changes in chromatin accessibility. The chromatin profiles of CBCs and enterocyte progenitors are very similar, and the chromatin and RNA profiles of both secretory and enterocyte progenitors convert to the profile of CBCs upon injury. Cells labeled by *Bmi1*, one of the putative reserve stem cell markers, were also found to be pre-terminal enteroendocrine cells whose chromatin accessibility resembles that of secretory precursors and that can revert to stemness upon injury (Jadhav et al., 2017; Yan et al., 2017).

## ROLE OF THE MICROENVIRONMENT DURING REGENERATION

The need for external growth and signaling factors when culturing *Lgr5*<sup>+</sup> CBCs *in vitro* as organoids underlies the fact that maintenance of CBCs requires cues originating from their microenvironment. Indeed, signals from the intestinal epithelium and adjacent mesenchymal cells, which include fibroblasts, immune cells, enteric neurons and capillaries, are essential to regulate stem cell activation and self-renewal (Beumer and Clevers, 2016). In particular, the balance between Wnt/ $\beta$ -catenin signaling, Notch signaling and BMP inhibition in the crypt plays a key role in the regulation of these processes (He et al., 2004; Pinto et al., 2003; Tian et al., 2015).

It has been proposed that the ISC niche is composed of Paneth cells in the crypt, which express *Wnt3*, and which were thought to be indispensable for stem cell survival (Sato et al., 2011). However, two different groups successfully demonstrated, using *Atoh1*<sup>-/-</sup> mice, a mutant strain in which all intestinal secretory lineages are eliminated, that the lack of Paneth cells did not alter the homeostasis of the crypt, with CBCs occupying the entire crypt base, thus indicating that Paneth cells are dispensable for stem-cell activity of CBCs *in vivo* (Durand et al., 2012; Kim et al., 2012). In addition, genetic ablation of intestinal epithelial Wnt activity in mice did not perturb gut homeostasis or its ability to regenerate after radiation (Kabiri et al., 2014). These data therefore point toward non-epithelial sources of essential factors for niche homeostasis. Accordingly, subepithelial telocytes, a subset of mesenchymal cells identified by the expression of Forkhead Box L1 (*Foxl1*), were recently identified as an essential source of Wnt ligands in both the small and large intestine. Upon genetic ablation in FOXL1<sup>+</sup> telocytes of porcupine, a key protein involved in the secretion of Wnt ligands, intestinal stem and transit amplifying cells stop proliferating and are decreased in number, thus impairing epithelial renewal (Aoki et al., 2016; Shoshkes-Carmel et al., 2018). Another subset of mesenchymal cells, identified by *Gli1*, are an essential source of Wnt in the colon, as shown by the loss of colonic epithelial integrity upon blocking of Wnt secretion by these cells (Degirmenci et al., 2018). These studies underlie the role of the stroma as a stem cell niche to maintain intestinal homeostasis. (Aoki et al., 2016).

A further demonstration of the importance of subepithelial fibroblasts came in regards to the epithelial response to *Salmonella typhimurium* infection. Upon infection, these cells produce IL-33 that acts directly on *Lgr5*<sup>+</sup> and progenitor cells via their ST2 receptor to promote a switch in their differentiation fate toward the secretory lineage through suppression of Notch signaling. This remodeling reinforces the barrier function of the epithelium and the secretion of antimicrobial peptides by Paneth and goblet cells, as shown



**Figure 2** Stem cell ablation and lineage tracing point to plasticity in the intestinal epithelium. Cre-mediated recombination in progenitor cells (secretory or absorptive) is indicated by gradient blue in the labeled cells. After ablation of *Lgr5*<sup>+</sup> cells, progenitor cells dedifferentiate and replenish the pool of stem cells. These cells give rise to all types of differentiated epithelial cells, as indicated by the ribbon of labeled cells going up the crypt-villus axis.

by the increase in severe tissue damage and death in *I133*<sup>-/-</sup> mice (Mahapatro et al., 2016). Finally, another subset of mesenchymal fibroblasts expressing CD34<sup>+</sup> Gp38<sup>+</sup> that also contribute to stem cell maintenance through the secretion of essential niche factors like Wnt2b, Grem1 and Rspo1 was reported. Following DSS-induced injury, these cells remain close to the crypt and up-regulate Grem1, Rspo1 and several chemokines, cytokines and growth factors involved in immune response and wound repair (Stzpourginski et al., 2017).

Subepithelial myofibroblasts, another mesenchymal cell type, are also an important component of the stem cell niche. They secrete angiopoietin-like protein 2 (ANGPTL2) that helps regulate the balance between  $\beta$ -catenin and BMP signaling in the niche. Although the intestine of *Angptl2*<sup>-/-</sup> mice develops normally in homeostatic conditions, epithelial regeneration is impaired upon DSS-induced colitis (Horiguchi et al., 2017). Another component of the niche whose importance in the colonic epithelial response to damage was recently demonstrated is the extracellular matrix. Using a DSS-injury model, the colonic epithelium in mice was shown to suppress the expression of adult stem cell markers and to reprogram into a primitive state with fetal-like properties. These authors also showed that the mechanical sensors YAP and TAZ are activated by extracellular matrix remodeling induced by DSS and are crucial for inducing this reprogramming process (Yui et al., 2018). The same phenomenon of epithelial reprogramming to a fetal-like signature was demonstrated in the small intestine in a context of parasitic infection with *Heligmosomoides polygyrus*, thus raising the possibility that fetal reprogramming might be a broader feature of the intestinal response to injury (Nusse et al., 2018). Interestingly, although the role of epithelial Wnts has been debated, in the context of a rotavirus infection, which affects differentiated cells and spares the intestinal crypts, epithelial Wnt becomes necessary and non-redundant

for repair. Upon such infection, the *Lgr5*<sup>+</sup> CBCs are activated and proliferate to replenish the pool of differentiated cells and repair the injury. However, after epithelial Wnt ablation, the ISC response and crypt expansion are no longer observed upon rotavirus infection (Zou et al., 2018).

The influence of the nervous system as a niche component during intestinal response to damage has been less extensively studied. However, several studies indicate that enteric glial cells (EGCs), the major component of the nervous system in the gut, contribute to epithelial homeostasis and response to injury. EGC activation through stimulation of the vagus nerve prevents blunting of villi associated with severe burn injury (Costantini et al., 2010). Another group confirmed the role of EGCs during epithelial regeneration using a transgenic mouse model of EGC ablation. They showed that EGCs promote wound repair by increasing spreading of intestinal epithelial cells through proEGF secretion (Van Landeghem et al., 2011).

Besides niche derived signals, the intestinal epithelium is also sensitive to host nutritional state. Accordingly, both calorie restriction (CR) and high-fat diet (HFD) induce an expansion in the pool of ISCs and a reduction in radiation-induced crypt injuries, but through different mechanisms. CR decreases the length of the villi and the number of differentiated cells while increasing the pool of ISCs and Paneth cells. Crypts from these mice are more likely to form organoids *in vitro*, a capacity that is maintained even after returning to a nutrient-rich media. The effects of CR on the crypt were initially thought to be mediated by mTORC1 signaling. However, studies showed that CR has opposite effects on the mTOR pathway in Paneth cells and CBCs. It is thus the nearby influence of Paneth cells on the ISCs that guides their response to CR, rather than a direct response of these stem cells to CR (Igarashi and Guarente, 2016; Yilmaz et al., 2012). In contrast, HFD reduces the number of Paneth cells while still increasing ISC activity and resistance to in-

jury, such that ISCs become independent of the niche, likely due to an increase in Wnt activity mediated by the activation of the nuclear receptor PPAR- $\delta$  and to the expression by ISCs of their own Notch ligands (Beumer and Clevers, 2016; Beyaz et al., 2016).

## ROLE OF IMMUNE CELLS

Innate immune cells are key players in inducing a swift response to injury. It is therefore not surprising that several cytokines secreted by these cells are involved in the intestinal epithelial response to damage. IL-22 is one of the main cytokines secreted in response to damage that has a direct influence on epithelial regeneration, and it is mostly secreted by group 3 innate lymphoid cells (ILCs), a group of cells from the lymphoid lineage lacking recombined-antigen receptor and located in the lamina propria (Spits et al., 2013). *In vivo*, IL-22 has a protective effect on the epithelium and decreases the morbidity induced by graft versus host disease or the chemotherapeutic drug methotrexate (Aparicio-Domingo et al., 2015; Hanash et al., 2012). Additional studies demonstrated that IL-22 induces the phosphorylation of STAT3 in CBCs, which enhances organoid growth without activating the Wnt or Notch pathway. These effects are independent of Paneth cells and are induced by a direct effect of the cytokine on the CBCs that upregulate IL-22R after damage, since IL-22 no longer induces organoid growth after depletion of *Lgr5*<sup>+</sup> cells using the *Lgr5*<sup>DTR</sup> allele (Lindemans et al., 2015; Pickert et al., 2009). Interestingly, STAT3 is also one of the many transcription factors activated by IL-6 signaling. As observed for IL-22, epithelial damage induces an increased secretion of IL-6. Furthermore, IL-6 is also involved in epithelial regeneration, since *Il6*<sup>-/-</sup> mice are more susceptible to DSS-induced colitis with shortening of the colon, loss of crypt structure and higher epithelial apoptosis than their wild-type counterpart (Grivennikov et al., 2009). Of note, IL-6 also triggers the activation of YAP through its co-receptor gp130, and this pathway confers protection to DSS-induced colitis as well, thus showing that this protective effect is not specific to the activation of STAT3 (Taniguchi et al., 2015).

Lastly, IL-10 is an anti-inflammatory cytokine that is also rapidly secreted by infiltrating macrophages upon intestinal injury. IL-10 promotes epithelial proliferation and wound repair through epithelial cAMP response element-binding protein (CREB) signaling and subsequent WNT1-inducible signaling protein 1 (WISP-1) secretion, a protein involved in cell proliferation and extracellular matrix production (Quiros et al., 2017).

The function of adaptive immune cells during intestinal damage has not been extensively examined. In a DSS-induced colitis model,  $\gamma\delta$ T cells have been shown to be ne-

cessary for recovery, as demonstrated by the death of *TCR $\delta$* <sup>-/-</sup> mice from severe colitis even after removal of DSS (Tsuchiya et al., 2003). However, in a biopsy-injury model, *Rag1*<sup>-/-</sup> mice, lacking mature lymphocytes, are able to repair the wound, indicating that adaptive immune cells seem dispensable in that setting (Quiros et al., 2017).

Besides their role in epithelial regeneration upon injury, cytokines secreted by the epithelium itself also help to prevent epithelial injury during bacterial or parasitic infections. For example, Tuft cells constitutively produce IL-25 and increase their IL-25 secretion during helminthic infection, in order to activate group 2 ILCs (ILC2s). Once activated, ILC2s in turn secrete IL-13, which acts on non-committed intestinal progenitors to shift their differentiation toward tuft and goblet cells, therefore inducing a tuft and goblet cell hyperplasia (von Moltke et al., 2016). Goblet cell hyperplasia and IL-13 signaling during helminthic infection are indeed important factors to help clear these infections (McKenzie et al., 1998).

## ROLE OF THE MICROBIOME

The intestinal tract harbors the largest bacteria pool in the body, and the impact of the microbiome on health and development of metabolic and auto-immune diseases is becoming increasingly clear (Wu et al., 2015). The influence of the microbiome in radiation-induced enteritis is one area that has been studied. When comparing the response to  $\gamma$ -irradiation of germ-free (GF) mice with both conventionally raised mice or GF mice that acquired microbiota in adulthood, the GF mice are resistant to radiation enteritis, and this resistance is lost upon microbiota colonization (Crawford and Gordon, 2005). Interestingly, GF mice remain radio-resistant even after colonization with specific strains of obligate and facultative anaerobes, indicating that only a subset of bacteria support susceptibility to radiation-induced enteritis. Another group studied the response of GF mice to DSS-induced colitis and observed that GF mice are in fact more prone to DSS-induced rectal bleeding, epithelial damage and mortality than conventional mice. However, GF mice display less signs of colonic inflammation. This heightened susceptibility is probably linked to weakening of the intestinal barrier (i.e. less expression of tight junction proteins), reduced secretion of cytokines known to be involved in epithelial proliferation, like IL-6 and IL-22, and lack of bacterial stimulation of pathogen recognition receptors on IECs (Hernández-Chirlique et al., 2016).

In conclusion, the intestinal epithelium response to damage is a complex process relying on plasticity of progenitor epithelial cells and essential signals emanating from the intestinal niche. In addition, the constant exposure of the epithelium to trillions of bacteria and its tight relation to the

immune and nervous system are also essential components involved in the appropriate response to injury. Therefore, while to date researchers have mainly focused on the response of a given compartment to injury, in the future, a more interdisciplinary approach integrating these different layers of regulation will be key to better understand complex diseases and develop appropriate treatments for patients suffering from acute or chronic intestinal injuries.

**Compliance and ethics** *The author(s) declare that they have no conflict of interest.*

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