The role of interleukin-22 in mammalian intestinal homeostasis; friend and foe.

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Abstract

Interleukin-22 (IL-22) is an important cytokine in the intestinal environment. IL-22 is mainly produced by immune cells and targeted at non-immune cells such as epithelial and stromal cells in a broad array of tissues such as -but not restricted to- the liver and adipose tissue. IL-22 therefore connects immune functions with metabolic functions of the host, and since it is induced by the microbiota, connects host functioning to the outside environment. IL-22 induces epithelial cell proliferation aiding in rapid epithelium regeneration and wound healing. Additionally, IL-22 activates anti-apoptotic genes and DNA damage response pathways, enhancing epithelial cell survival. Recently, it has also been shown that IL-22 induces Paneth cell differentiation in humans. However, IL-22 can also contribute to intestinal epithelium damage and reduces microbial diversity in the intestine directly or indirectly by inducing excessive antimicrobial peptide production by epithelial cells. Moreover, IL-22 enhances angiogenesis and may therefore support tumorigenesis in the intestine. In conclusion, it appears that whether IL-22 has a beneficial or harmful effect in the mammalian intestine largely depends on its regulation. This review aims to provide a comprehensive overview of the current literature and emphasizes that IL-22 signalling outcome depends on the timing and duration of IL-22 production, the presence of it regulators such as IL-22BP, and the specific location of the cytokine production in the gastrointestinal tract.

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Abstract

Interleukin-22 (IL-22) is an important cytokine in the intestinal environment. IL-22 is mainly produced by immune cells and targeted at non-immune cells such as epithelial and stromal cells in a broad array of tissues such as -but not restricted to- the liver and adipose tissue. IL-22 therefore connects immune functions with metabolic functions of the host, and since it is induced by the microbiota, connects host functioning to the outside environment. IL-22 induces epithelial cell proliferation aiding in rapid epithelium regeneration and wound healing. Additionally, IL-22 activates anti-apoptotic genes and DNA damage response pathways, enhancing epithelial cell survival. Recently, it has also been shown that IL-22 induces Paneth cell differentiation in humans. However, IL-22 can also contribute to intestinal epithelium damage and reduces microbial diversity in the intestine directly or indirectly by inducing excessive antimicrobial peptide production by epithelial cells. Moreover, IL-22 enhances angiogenesis and may therefore support tumorigenesis in the intestine. In conclusion, it appears that whether IL-22 has a beneficial or harmful effect in the mammalian intestine largely depends on its regulation. This review aims to provide a comprehensive overview of the current literature and emphasizes that IL-22 signalling outcome depends on the timing and duration of IL-22 production, the presence of it regulators such as IL-22BP, and the specific location of the cytokine production in the gastrointestinal tract.

Introduction

The human intestine has a large surface area of ~$32m^2$ (reviewed in Helander & Fandriks, 2014), while the murine intestinal tract has been assessed to be close to $2m^2$ (Casteleyn et al., 2010). The intestine is home to trillions of microorganisms, collectively termed microbiota. The microbiota is separated from the hosts' immune system by a single layer of intestinal epithelial cells. There is a sophisticated communication between the microbiota and the immune system through the epithelial barrier, and this communication is a key for maintaining intestinal homeostasis. Disruption in this communication in the intestine may lead to dysbiosis, a disturbance in microbial community due to environmental factors such as food, antibiotics, and medicine, or internal host factors such as overreacting or insufficient immune responses. Intestinal epithelial cells are much more than just a physical barrier between the host and the outside world. These cells secrete mucus and antimicrobial peptides (AMPs), sense harmful and beneficial microbes, and induce and modulate immune responses. This is accomplished by various specialised cell types, such as goblet cells and Paneth cells (reviewed in Maloy & Powrie, 2011). Intestinal epithelial cells are connected by tight junction proteins, which maintain the intestinal barrier and regulate paracellular permeability. Another important function of the intestinal epithelium is to provide the host with nutrients (reviewed in Goyal et al., 2021). To perform their cell-specific functions, intestinal epithelial cells require distinct signals. These signals are often communicated by cytokines, and one of these cytokines is interleukin-22 (IL-22). IL-22 belongs to a family of interleukin-10 cytokines. IL-22 is produced by various immune cells, from both innate and adaptive immunity. Known sources of IL-22 are T helper 1 cells (Th1), T helper 17 cells (Th17), T helper 22 cells (Th22), γδ T cells, natural killer T (NKT) cells, and type 3 innate lymphoid cells (ILC3s) (reviewed in Dudakov et al., 2015). IL-22 production and bioactivity can be regulated positively as well as negatively (**Box 1**). Important described positive regulators of IL-22 production include IL-23, IL-1β, IL-7, aryl hydrocarbon receptor (AhR), and Notch (Dudakov et al., 2015). Known inhibitors of IL-22 production and activity include IL-22 binding protein (IL-22BP), TGF- β, ICOS, c-Maf, IL-27, and IL-25 (Dudakov et al., 2015). The active secreted form of human IL-22 is 146 amino acid protein which binds to membrane-bound

interleukin-22 receptor (IL-22R), a heterodimeric receptor with IL-22R1 and IL-10R2 subunits (Dudakov et al., 2015). IL-10R2 subunit is shared with other cytokines, such as IL-10, IL-26, IL-28, and IL-29 (Dudakov et al., 2015). IL-22R1 is found to be expressed on epithelial cells in a number of human tissues, such as skin, small intestine, colon, kidney, as well as pancreas (Wolk et al., 2004). However, IL-22R1 is not found to be expressed on immune cells (Wolk et al., 2004). The fact that IL-22 is produced by immune cells but it exclusively communicates to various non-immune tissues makes IL-22 signalling particularly interesting. IL-22 has been observed to be beneficial as well as harmful in the intestine, indicating that the lack or the presence of IL-22 may define the gut homeostasis. IL-22 has been reported to stimulate epithelial cell proliferation and production of mucins – the major components of mucus (He et al., 2022; Patnaude et al., 2021). Additionally, IL-22 has been shown to induce Paneth cell differentiation as well as AMP secretion by Paneth cells (He et al., 2022). Contrastingly, IL-22 has also been reported to elicit pathophysiological effects, such as diarrhoea. IL-22 increases the tight junction permeability and therefore disrupts the intestinal barrier and homeostasis (Patnaude et al., 2021; Wang et al., 2017). These opposing findings illustrate that the role of IL-22 in intestinal homeostasis is not yet clearly defined, and it is a matter of ongoing research. This review aims to elaborate on the beneficial as well as harmful effects of IL-22 in the mammalian intestine, as well as investigate the factors which define its effects on the intestinal homeostasis such as timing, concentrations, location, and regulators.

Box 1. Conditions that may determine the outcome of IL-22 signaling in the intestine

Presence and expression of positive regulators

(AhR, IL-23, IL-1B, IL-7, Notch, butyrate).

Presence and expression of negative regulators

(IL-22BP, TGF-B, ICOS, c-Maf, IL-25 and IL-27, IL-10).

- IL-22R1/IL10R2 expression.
- Cell source of IL-22 (innate versus adaptive immune cells).
- Localization of IL-22 producing cells and range of action of the IL-22

(local in small intestine or colon; or systemic).

Timing, duration and amount of IL-22 production.

Diseased versus homeostatic state (see **Table 1**).

Beneficial role of IL-22 in intestinal homeostasis

The integrity of the intestinal barrier is essential for a healthy gut. There are examples of intestinal diseases in which the criteria for homeostatic barrier functioning are not met, such as inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), but also necrotizing enterocolitis (NEC). These multifactorial conditions are usually characterized by excessive or insufficient immune responses leading to unresolved inflammation and disruption in the intestinal epithelial barrier (reviewed in Sommer et al., 2021), alongside other pathological symptoms (Mihi et al., 2021; reviewed in Zhang & Li, 2014). The multifactorial nature makes these pathologies difficult to treat and there is, therefore, active ongoing research to find possible therapeutic agents, which could help restore the epithelial barrier in the affected individuals. Enhancing IL-22 signalling has been suggested as such a therapeutic agent since IL-22 appears to be a communication bridge between the immune and tissue-resident non-immune cells, such as intestinal epithelial cells (Wolk et al., 2004). Here, the examples of how IL-22 may positively influence intestinal homeostasis will be elaborated on.

IL-22 and pathologies

One disease where IL-22 may have a positive influence is necrotizing enterocolitis (NEC). NEC is an intestinal inflammatory disorder primarily affecting neonates and premature infants, and it is thought to occur because of an underdeveloped immune system (Neu, 2014). A mouse study found fewer IL-22-producing cells as well as low levels of IL-22 in the intestinal lamina propria of neonate pups compared with adult mice (Mihi et al., 2021). Mihi et al. (2021) also showed that daily intraperitoneal injections with recombinant murine IL-22 for three days led to a reduction in pro-inflammatory cytokine IL-1β as well as in reduction in disease severity in NEC-induced pups compared with phosphate-buffered saline (PBS)-treated littermates. Treatment with IL-22 was further shown to decrease the damage of ileal epithelium in mice (Mihi et al., 2021). Reduction in pro-inflammatory cytokine profile and intestinal damage during *in vivo* experiments indicates that IL-22 treatment may have restorative effects in the treatment of NEC by reducing excessive inflammation and enhancing epithelium healing.

IL-22 has been shown to be protective in a colitis model applied in mice. This colitis model uses a transfer of $CD4^{+}CD45RB^{hi}$ T cells (naïve T cells) into *Rag1*$^{-/-}$ mice to induce intestinal disease. Since these *Rag1-/-* mice lack all T cells (including regulatory T cells), there is no regulation of the transferred T cells and these cells rapidly increase in numbers, causing inflammation in the intestine. This *Rag1-/-* model has been employed to study the role of IL22 in colitis. For example, *Rag1*$^{-/-}$ or*Il22*$^{-/-}$ *Rag1*$^{-/-}$ mice received IL-22-deficient or wild-type $CD4^{+}CD45RB^{hi}$ T cells intraperitoneally, and while all mice developed colitis, the mice fully lacking IL-22 developed most severe symptoms (Zenewicz et al., 2008). This suggests that IL-22 has a protective role in this murine colitis model. Moreover, it indicates that the source of IL-22 may be important in defining whether IL-22 confers protective or harmful effect in mammalian intestine.

IL-22 and mucus

Another way IL-22 may be beneficial is by inducing mucus secretion. The mucus in the intestine provides a vital protective layer separating gut microbiota from the epithelial cells and the host's immune system, and it helps maintain the integrity of the intestinal barrier (reviewed in Okumura & Takeda, 2017). Commensal and pathogenic bacteria get trapped in the mucus and are expelled from the intestine by peristalsis, preventing excessive bacterial colonization (reviewed in Kim & Ho, 2010). Evidence suggests that IL-22 might play a role in maintaining the mucus layer. In both murine *in vivo* and human *in vitro*experiments treatment with IL-22 increased the expression of membrane-bound mucins (Patnaude et al., 2021). Membrane-bound mucins are expressed on the apical side of intestinal epithelial cells, where they form a glycocalyx (a layer consisting of glycoproteins and glycolipids) by extending 200-1500 nm above the cell surface (reviewed in Kim & Ho, 2010). Murine *in vivo* experiments showed that intravenous injections with IL-22-Fc lead to an increase in Mucin-1 in the colon (Patnaude et al., 2021). IL-22-Fc in this experiment is a murine recombinant IL-22 linked to the Fc (fragment crystallizable) region of mouse IgG1. Fc-fusion proteins are used to prolong the plasma half-life of proteins and therefore increase their therapeutic efficacy (reviewed in Czajkowsky et al., 2012). *In vitro* experiments with human colon-derived organoids showed that treatment with recombinant human IL-22 increases the gene expression of membrane bound mucins*MUC1* , *MUC4* , and *MUC13* (Patnaude et al., 2021). IL-22 treatment has also shown to increase *MUC1* expression in human jejunum- and duodenum-derived organoids (He et al., 2022). However, in mice, the expression of Mucin-2 and the presence of goblet cells have been shown to moderately decrease (although not significantly) upon treatment with IL-22 in the colon (Patnaude et al., 2021). It is possible that the effect of IL-22 on the expression of mucins may differ between the different sections of intestine. Human and mouse membrane-bound mucins have been shown to stimulate cell migration and inhibit apoptosis (reviewed in Kim & Ho, 2010). The evidence that IL-22 upregulates the expression of some of the membrane-bound mucins in human and mouse colon epithelial cells suggests that treatment with IL-22 may have a beneficial effect in conditions where patients are challenged with increased bacterial burden or a disrupted epithelial barrier, such as IBD or NEC.

IL-22 and butyrate

Butyrate is one of the short-chain fatty acids (SCFAs) produced by the microbes in the gut and it is the main source of energy for intestinal colonocytes (reviewed in Martin-Gallausiaux et al., 2021). SFCAs are products of anaerobic fibre fermentation by the microbes. The presence of butyrate in the intestine has been suggested to play a role in regulating the effects of IL-22 in the intestine (Patnaude et al., 2021). Interestingly, like the addition of IL-22, the addition of butyrate to human colon-derived organoids also increased *MUC13*expression, and its effects were further enhanced when IL-22 was added together with butyrate (Patnaude et al., 2021). Interestingly, recently research performed in zebrafish uncovered a link between acetate produced by microbes and upregulation of IL22 (Liao et al., 2023). Specifically, this study showed that vitamin D induced anti-microbial peptide expression by activating IL22 signalling by increasing the abundance of acetate-producing bacteria (Liao et al., 2023).

IL-22 and intestinal barrier function

The intestinal epithelial barrier is protected by AMP production as well as rapid epithelial cell renewal. The gastrointestinal epithelium is renewed in 2-3 days in mice and in 3-5 days in humans (reviewed in Goyal et al., 2021). The rapid regenerative capacity of the intestinal epithelium allows for a symbiotic relationship between the host and the microbiota within the gut. Intestinal stem cells (ISCs) are the sole source of all other cell types found in the intestine (reviewed in Barker, 2014). It is therefore essential that the ISCs are well protected from the possibly harmful luminal contents, and that these cells receive correct signals to appropriately differentiate and proliferate. ISCs are offered protection by their location deep within the crypts, the AMPs produced by neighbouring Paneth cells, and the mucus layer (reviewed in Barker, 2014). IL-22 appears to initiate signals for ISC and epithelial cell proliferation, differentiation, and functioning. A number of *in vivo* and *in vitro* studies have shown that IL-22 has the capability to induce the proliferation of epithelial cells, but also stem cells in the intestine (Lindemans et al., 2015; Patnaude et al., 2021; Zha et al., 2019; Zhang et al., 2020). In case of colonic epithelial cells, it has been shown that treatment with recombinant human IL-22 leads to epithelial cell proliferation in human primary colon tissue organoids (Patnaude et al., 2021). In agreement with this, Patnaude et al. (2021) also reported that by injecting mice intravenously with IL-22-Fc, colon epithelial cells exhibit increased proliferation compared with the control treatment. Some studies have also shown that IL-22 positively affects the stem cell compartment in the intestine. For example, treating murine small intestine-derived organoids with exogenous recombinant IL-22 increases organoid size by inducing Lgr5+ stem cell proliferation (Lindemans et al., 2015). Likewise, another study demonstrated that by administering bacteria carrying IL-22 to irradiated mice increased Lgr5+ stem cells in the ileum (Zhang et al., 2020). However, others (He et al., 2022; Mihi et al., 2021; Zha et al., 2019) have not been able to replicate the reported intestinal stem cell expansion. Nevertheless, it has been shown that IL-22 treatment leads to proliferation of transit-amplifying (TA) cells in jejunal enteroids (Zha et al., 2019). TA cells are a cell type in a developmental stage between stem cells and fully differentiated epithelial cells (reviewed in Rangel-Huerta & Maldonado, 2017). Moreover, it has been revealed that IL-22 drives the differentiation of Paneth cells, the major producers of AMPs, in human small intestine organoids (He et al., 2022), and it has also been linked to AMP production in the intestine (He et al., 2022; Lindemans et al., 2015; Mihi et al., 2021; Patnaude et al., 2021). Specifically, *in vitro*experiments with small intestine tissue from humans and mice have shown that treatment with IL-22 increases the mRNA levels of different AMPs, such as Reg3β and Reg3γ (Lindemans et al., 2015; Mihi et al., 2021). Additionally, Reg1α, Reg1β, Reg3α, β-defensin 3, and lipocalin-2 gene expression has been observed to be upregulated in human colonic organoids as well as small intestine-derived organoids when treated with IL-22 (He et al., 2022; Patnaude et al., 2021). The aforementioned*in vitro* studies are also supported by results from murine*in vivo* experiments where Reg3β and Reg3γ expression was upregulated in the colon after treatment with IL-22 (Gronke et al., 2019; Lindemans et al., 2015; Patnaude et al., 2021). The abovementioned studies illustrate that IL-22 can be beneficial in maintaining the intestinal homeostasis by protecting the stem cells with inducing AMP production and initiating cell proliferation and differentiation in the small and large mammalian intestine.

IL-22 as therapeutic agent

As mentioned above, IL-22 can lead to proliferation of intestinal epithelial cells and could thus aid in the regeneration of the intestinal epithelium upon damage. The effects of IL-22 as a therapeutic agent have been investigated in the context of irradiation damage in the gut. Irradiation causes damage to different intestinal cell types, such as γδ T-cells and helper T-cells, but also intestinal stem cells, Paneth cells, and goblet cells (Zhang et al., 2020). γδ T-cells and helper T-cells are known sources of IL-22 (reviewed in Dudakov et al., 2015). Zhang et al. (2015) presented a novel approach to deliver IL-22 to the site of intestinal damage. They transformed *Lactobacillus reuteri*and *Escherichia coli* to carry recombinant plasmids with murine IL-22, which would be expressed by the bacteria in the intestine. They showed that treatment with *L. reuteri* and *E. coli* carrying the IL-22 transgene can increase the survival of mice up to 85%, compared to the control group, if administered by gavage 24h after total body irradiation. It was shown *in vitro* that *E. coli*secretes IL-22, whereas *L. reuteri* needs to be lysed in order to release the intracellular IL-22 (Zhang et al., 2020). While this study does not demonstrate that the irradiation damage in the intestine is entirely restored by administering IL-22-producing bacteria, Zhang et al. (2020) do show increased numbers of intestinal stem cells in the ileum, as well as Paneth cells and goblet cells in mice *in vivo*after the treatment. Using recombinant bacteria to deliver IL-22 to the gut to diminish intestinal damage after irradiation, or other type of intestinal injury, may be a safe treatment method since *E. coli*was cleared from the colon by day five after gavage, indicating that the recombinant bacteria does not colonize the intestine (Zhang et al., 2020). However, *L. reuteri* clearance is not reported. The reason why *L. reuteri* has been proposed as a vehicle to deliver the biotherapeutic agents to the intestine is because this bacteria is considered safe for the host and can be transformed to additionally carry prophages which would lyse the bacteria during the gastrointestinal transit, after which the therapeutic agent *L. reuteri* is carrying would be released (Alexander et al., 2019). It is important to bear in mind that IL-22 delivery to the intestine by bacteria carrying the IL-22 transgene would likely be beneficial only in cases when the epithelial barrier is severely disrupted, since the receptors for IL-22 are found on the basolateral side of the epithelial cells and not on the apical side (Patnaude et al., 2021; Wang et al., 2017), and IL-22 could thus not elicit its effects from the gut lumen.

IL-22 to combat genotoxic stress

The intestine is challenged daily by potentially pathogenic bacteria and possibly genotoxic food items ingested by the host. Genotoxicity is the ability of a substance to damage DNA, and possibly lead to mutations and tumours. One such source of genotoxic stress is glucosinolate-containing cruciferous vegetables, such as broccoli and cabbage (Schumacher et al., 2014). While glucosinolates themselves are not genotoxic to mammalian cells, some of their breakdown products (by bacterial enzymes), such as 1-methoxy-3-indolylmethyl alcohol (1-MIM-OH), can be (Schumacher et al., 2014). A study demonstrated that stimulating murine intestinal cells*in vivo* by gavage with 1-MIM-OH led to an increase in DNA adduct formation in the caecal tissue compared to the controls (Gronke et al., 2019). DNA adducts are formed when substances bind to DNA, leading to nucleotide mispairings and eventually mutations. Remarkably, it was shown that 1-MIM-OH treatment increased IL-22$^+$ ILC3s and γδ T cells, as well as IL-22 production by these cell types (Gronke et al., 2019). 1-MIM-OH treatment also led to an increase in the expression of a known IL-22 response gene, $P\epsilon\gamma 3\gamma$, in primary colon epithelial cells *in vivo* (Gronke et al., 2019). This experiment shows that even though glucosinolate metabolites may have a genotoxic effect on intestinal cells, they significantly increase the amount of IL-22$^+$ cells as well as IL-22 in the intestine. It is noteworthy that the concentrations of 1-MIM-OH used in the experiment are considerably higher than the average estimated daily intake of glucosinolates in men and women (Schumacher et al., 2014). Thus, humans would have to consume kilograms of cruciferous vegetables per day to attain harmful concentrations of glucosinolate metabolites in their intestines. Nevertheless, IL-22 may protect against genotoxic stress by inducing DNA damage response (DDR) pathways (Gronke et al., 2019). DDR is a safety mechanism containing a network of activated genes which protect the cells from accumulating mutations when challenged with genotoxic stress. Mice subjected to a glucosinolate-free diet exhibited a significant reduction in DDR gene expression as well as IL-22 production in their primary colon epithelial cells (Gronke et al., 2019). On the contrary, DDR effector gene expression and

IL-22 production were upregulated in mice who received glucosinolate-containing diet (Gronke et al., 2019). These results indicate that IL-22 may be necessary for an appropriate initiation of DDR in the mammalian intestine.

Taken together, IL-22 appears to participate in many processes involved in maintaining intestinal homeostasis. It has been shown to protect against genotoxic stress, decrease the damage to ileal epithelium, and decrease the expression of proinflammatory cytokine during induced NEC. IL-22 has also been demonstrated to increase the production of membrane-bound mucins as well as AMPs by intestinal epithelial cells. Additionally, it has been reported that IL-22 can increase the proliferative capacity of intestinal stem and epithelial cells. All the above-mentioned processes are necessary for the maintenance and restoration of intestinal homeostasis, and it appears that IL-22 can shift the homeostasis in the mammalian gut towards a positive direction. However, the role of IL-22 in intestinal homeostasis is not as explicit. IL-22 has also been demonstrated to have detrimental effects in the intestine. All studies referring to the beneficial effects of IL-22 are summarized in **Table 1** .

Harmful role of IL-22 in intestinal homeostasis

IL-22 has several properties that make it suitable as a therapeutic agent due to its role in maintaining intestinal homeostasis. In the next section, however, the focus will be on the harmful effects of IL-22 in the intestine and why it could be appealing to therapeutically target, in other words inhibit, IL-22 instead.

IL-22 and intestinal stem cells

Contrastingly to the results presented about the beneficial effect of IL-22 on ISC proliferation, some studies report IL-22 reducing the numbers of ISC. Zha et al. (2019) found that treating murine jejunal enteroids with murine recombinant IL-22 reduces the number of Lgr5+ ISCs. These *in vitro* results were supported by *in vivo*experiments. Intraperitoneal injections with IL-22 for seven days (1 µg/day) led to a decrease in Lgr5+ stem cell population in jejunum as well as ileum in the treated mice (Zha et al., 2019). Another study reported that IL-22 treatment reduced human small intestine organoid formation efficiency, organoid budding, and increased cell death (He et al., 2022). Since ISCs are the source of all types of intestinal epithelial cells (reviewed in Barker, 2014), their maintenance is critical to maintaining intestinal homeostasis. IL-22 is upregulated in CD and UC (Andoh et al., 2005), and the results from the abovementioned studies suggest that too much IL-22 could be detrimental for intestinal epithelial regeneration and could be one of the factors responsible for impaired mucosal regeneration and impaired healing observed in IBD (reviewed in Sommer et al., 2021).

IL-22 and epithelial barrier integrity

Although we have discussed the beneficial effects of IL-22 in intestinal barrier integrity, some studies indicate that IL-22 may decrease the barrier integrity. Experiments with epithelial cell monolayers have shown that IL-22 treatment increases the paracellular permeability and reduces transepithelial electrical resistance (TEER) (Patnaude et al., 2021; Wang et al., 2017). Reduction in TEER indicates reduced epithelial integrity. Experiments with human colorectal Caco-2 cells demonstrated that treating the monolayer with recombinant human IL-22 for 72h from the basolateral side significantly decreased TEER compared with the control treatment (Wang et al., 2017). To confirm that IL-22 is responsible for reducing TEER, the monolayer was treated with recombinant human IL-22BP, a known negative regulator of IL-22. IL-22BP treatment counteracted the TEER-reducing effect of IL-22 on Caco-2 cell monolayer (Wang et al., 2017). Similar results were obtained in experiments with the human colon-derived T-84 cell line, where a significant decrease in TEER was observed 48h post-treatment with human recombinant IL-22 (Patnaude et al., 2021). IL-22

signalling from the exclusively basolateral side of the epithelial cells is also confirmed in experiments with T-84 cells by Patnaude et al. (2021). IL-22 exhibited no effect on TEER, nor the expression of IL-22-inducible genes, such as *REG1A* and *REG3G* , when administered from the apical side of the abovementioned cell lines (Patnaude et al., 2021; Wang et al., 2017). Moreover, cell viability was also not affected by IL-22 treatment (Patnaude et al., 2021; Wang et al., 2017), indicating that IL-22 had the TEER-reducing effect via basolaterally expressed receptors in these cell lines. Interestingly, it was shown that butyrate is able to override the disruptive effects of IL-22 on TEER (Patnaude et al., 2021). This indicates once again that the presence of butyrate in the (large) intestine might influence the effects IL-22 has in the mammalian gut.

IL-22 is thought reduce epithelial integrity by altering tight junction structures, namely by claudin-2 up-regulation (Patnaude et al., 2021; Wang et al., 2017). Claudin-2 is a junctional protein expressed in the gastrointestinal tract in humans (Wang et al., 2017). Claudin-2 forms paracellular channels to allow the passage of solutes, such as Na^+ (Tanaka et al., 2017). However, overexpression of claudin-2 may lead to excessive transepithelial paracellular leakage in the intestine, which can be harmful to the host. Upregulation of claudin-2, as well as IL-22, is seen in intestinal diseases, such as CD and UC (Andoh et al., 2005; Wang et al., 2017). Treating Caco-2 cells with IL-22 for 24, 48, 72, and 96 hours showed increased expression of *CLDN2* , a gene coding for claudin-2 protein, but no other measured tight junction protein-coding genes (Wang et al., 2017). Claudin-2 expression was also upregulated upon IL-22 treatment in human primary intestinal epithelium cells (Wang et al., 2017), as well as in mice *in vivo* and human organoids derived from healthy and UC donors (Patnaude et al., 2021). To demonstrate that claudin-2 upregulation is indeed induced by IL-22, the Caco-2 cell monolayer was subsequently treated with IL-22BP, and the results showed that IL-22BP completely abrogated the effects of IL-22 on claudin-2 expression (Wang et al., 2017). Additionally, knocking down the *CLDN2* gene in Caco-2 cells confirmed that claudin-2 causes the reduction of TEER (Wang et al., 2017). Taken together, in the experimental setting of epithelial cell monolayers, it has been shown that IL-22 can be detrimental by upregulating junctional protein claudin-2 and thereby reducing TEER. The epithelial integrity in the intestine is essential for proper functioning and the evidence that IL-22 can harmfully act upon this barrier illustrates that IL-22 may also be detrimental. However, it is important to realize that these experiments were conducted on cell monolayers and may not reflect physiological conditions present in the mammalian intestine. Other models such as intestinal organoids or even whole organism may be better to evaluate the effect of IL-22 on intestinal epithelial barrier.

IL-22 in colitis models

In some murine colitis models, IL-22 has been shown to have a harmful role in the intestine. In the innate colitis model, mice were injected with anti-CD40 antibodies to induce colitis. In an experiment with *Il-23*$^{-/WT}$*Rag*$^{-/-}$ mice, it was shown that neutralization of endogenous IL-22 with anti-IL-22 antibody after anti-CD40 injections leads to a significant reduction in weight loss, colitis scores, and colon pathology (Eken et al., 2014). When IL-22 levels were restored with IL-22-expressing plasmid injections in *Il-23*$^{-/-}$*Rag*$^{-/-}$ mice, they again developed severe colitis upon injections with anti-CD40 (Eken et al., 2014). Injection of empty plasmids alone did not lead to colitis development. These observations suggest that in mice, IL-22 has a pathological effect in anti-CD40-induced acute innate colitis. However, colitis developed in *Il-23*$^{-/-}$*Rag*$^{-/-}$ mice only after anti-CD40 injection, indicating that IL-22 plasmid injections alone do not cause colitis (Eken et al., 2014). Additionally, it was reported that *Il-23*$^{-/-}$*Rag*$^{-/-}$mice who received IL-22 plasmid after anti-CD-40 injections had significantly more neutrophils in the colonic lamina propria compared with empty-vector recipients. The authors suggested that IL-22 may facilitate colitis pathology by recruiting neutrophils to the site of intestinal damage (Eken et al., 2014). Neutrophils produce neutrophil extracellular traps (NETs) to bind pathogens, but too many NETs may also be harmful to the host (reviewed in Castanheira & Kubes, 2019). Excessive neutrophil recruitment in the intestine may lead to enhanced NET production and induce inflammation in the colon. The experiments by Eken et al. (2014) indicate that IL-22 on its own is likely not disruptive, but rather it initiates signals which recruit other cell types or cytokines, which lead to harmful outcomes.

IL-22 was also shown to have a detrimental effect in a different experimental colitis model. $CD4^+CD45RB^{hi}$ T cells (naïve) or T_{reg} cell-depleted $CD4^+CD45RB^{lo}$ T cells (memory/effector) derived from wild-type (WT) mice were transferred into *Rag1*$^{-/-}$ mice to induce colitis (Kamanaka et al., 2011). Specifically, Kamanaka et al. (2011) found increased IL-17 and IL-22 mRNA expression after disease development in the colons of *Rag1*$^{-/-}$ mice who received T_{reg}cell-depleted $CD4^+CD45RB^{lo}$ T cells in comparison to mice who received the naive $CD4^+CD45RB^{hi}$ T cells. They additionally report that transfer of IL-22 knock-out (KO), but not IL-17 KO T cells ($CD4^+CD45RB^{lo}$ T and T_{reg} depleted) to *Rag1*$^{-/-}$ mice reduced weight loss and colitis scores compared with mice who received WT T cells. This indicates that memory/effector T cell-derived IL-22 might be involved in the pathogenicity of the colitis model used in this study. Possible mechanisms by which IL-22 can influence colitis is by inducing epithelial hyperplasia as there is evidence that IL-22 promotes epithelial cell proliferation in the colon (Kamanaka et al., 2011; Patnaude et al., 2021). Additionally, the levels of Reg3γ were significantly reduced in IL-22 KO memory-effector transfer mice, suggesting that IL-22 may drive colitis by inducing Reg3γ, which may alter the microbiota composition and lead to dysbiosis (Kamanaka et al., 2011). Interestingly, the results of the study by Kamanaka et al. (2011) again emphasize that the source of IL-22 might be determining the effects in its target tissues in view of the fact that IL-22 was protective in the $CD4^+CD45RB^{hi}$(naïve T cell transfer) colitis model (Zenewicz et al., 2008).

Another colitis model often used to investigate intestinal inflammation mechanisms is by inhibiting, knocking down, or knocking out IL-10 in mice. IL-10 inhibits the expression of pro-inflammatory cytokines, such as TNF-α, IL-6 and IFN-γ (Gasche et al., 2000), and therefore regulates inflammation. Gunasekera et al. (2020) found that IL-22 levels, as well as a number of antimicrobial IL-22-target gene mRNA levels, were significantly higher in *Il-10*$^{-/-}$ mice compared to the WT mice. IL-22 protein levels were also increased in the colon and small intestine of *Il-10*$^{-/-}$ colitic mice compared to the WT mice (Gunasekera et al., 2020). These observations suggest that IL-10 negatively regulates IL-22 expression in the intestine. Since it is known that IL-22 upregulates AMPs in the intestine, the diversity of microbiota was evaluated in *Il-10*$^{-/-}$ mice. *Il-10*$^{-/-}$ mice had less diverse microbiota compared with WT mice, as well as *Il-10*$^{-/-}$*Il-22*$^{-/-}$, and *Il-22*$^{-/-}$ mice (Gunasekera et al., 2020). Reduced microbiota diversity is usually correlated with gut dysbiosis. It is possible that IL-22 driven AMP upregulation in the intestine leads to dysbiosis and consequently to intestinal disorders. Several Reg-family AMPs, such as Reg1α/β, Reg3, and Reg4 are known to be overexpressed in the intestines of humans with UC and CD (Granlund et al., 2011; Tsuchida et al., 2017). To determine a more specific role of IL-22 in this colitis model, *Il-10*$^{-/-}$*Il-22*$^{-/-}$ mice were used. *Il-10*$^{-/-}$*Il-22*$^{-/-}$, nor *Il-22*$^{-/-}$ mice develop chronic colitis, indicating that IL-22 is involved in chronic colitis development (Gunasekera et al., 2020). While *Il-10*$^{-/-}$colitic mice exhibited rectal prolapse, as well as ulcerations, crypt abscesses, and mucosal hyperplasia in the colon, *Il-10*$^{-/-}$*Il-22*$^{-/-}$, and *Il-22*$^{-/-}$ mice did not develop any of the symptoms (Gunasekera et al., 2020). However, it is important to bear in mind that in this study exogenous IL-22 in *Il-10*$^{-/-}$*Il-22*$^{-/-}$ mice was not used to show that indeed IL-22 is the aberrant factor. Taken together, these data indicate that IL-10 is an important negative regulator of IL-22 and its downstream genes in the intestine, and without appropriate regulation, IL-22 may become aberrant and cause pathology in the intestine.

IL-22 regulation and its role in tumorigenesis

While IL-22 is seen as beneficial in the intestine by promoting epithelial cell proliferation, it may also be detrimental for the same reason. IL-22 is found excessively expressed in human colon cancer tissues compared to healthy donor tissues, and *in vitro* experiments have shown that IL-22 enhances tumour proliferation (Jiang et al., 2013). Tumours are formed by uncontrolled cell proliferation, and IL-22 has been shown to promote epithelial cell proliferation in human and mouse models (Lindemans et al., 2015; Patnaude et al., 2021; Zha et al., 2019). It is therefore hypothesized that when IL-22 lacks correct inhibiting signals in the intestine during a steady state, for instance by IL-22BP, it may initiate tumour formation by signalling epithelial cells to continuously proliferate. IL-22BP, a potent IL-22 inhibitor produced by dendritic cells (Martin et al., 2014), is upregulated during homeostatic conditions and is downregulated in the colon upon mechanical damage, whereas IL-22 levels exhibit the opposite (Huber et al., 2012). It has been shown in murine models that IL-22 and IL-22BP exhibit inverse expression patterns (Huber et al., 2012). Additionally, *Il22bp-/-*

mice showed increased epithelial cell proliferation during the DSS-induced colitis recovery phase whereas in WT mice, the cell proliferation during the recovery phase had reduced to a rate similar to steady-state conditions (Huber et al., 2012). Moreover, they showed that lack of IL-22BP lead to accelerated development and higher number tumours in the colon in comparison with WT mice (Huber et al., 2012). The study by Huber et al. (2012) illustrates how important the tight regulation of IL-22 is in the intestine. Tumour formation is unquestionably a multifactorial process and IL-22 alone certainly is not responsible for this process, but without appropriate regulation it may enhance tumorigenesis by initiating excessive epithelial cell proliferation.

IL-22BP is not only important in preventing IL-22 signalling in tumour formation but also in continuous regulation of IL-22 signalling in the intestine of healthy individuals where it helps to maintain homeostasis. IL-22BP is constitutively produced by dendritic cells (Martin et al., 2014), and IL-22BP levels are usually upregulated during steady-state conditions and downregulated during inflammation. It allows to maintain the levels of IL-22 low in a healthy gut and elevated during intestinal damage. However, both IL-22 and IL-22BP have been observed to be increased in human CD and UC (reviewed in Zenewicz, 2021). These observations emphasize that IL-22 is a potent cytokine which is actively upregulated during inflammation, and that elevated IL-22BP levels may not always be enough to inhibit IL-22 signalling.

While the study by Huber et al. (2012) clearly shows that it is important to appropriately inhibit IL-22 to suppress tumour formation, they do not elaborate on the mechanisms by which IL-22 enhances tumour growth. Others have suggested to therapeutically target (and thus inhibit) IL-22 since there is evidence that it promotes tumour angiogenesis (Protopsaltis et al., 2019). Protopsaltis et al. (2019) showed that IL-22 enhances human endothelial cell proliferation, survival, and migration in a dose-dependent manner *in vitro* . Using the *ex vivo* murine choroid explant model, they also showed that IL-22 treatment promotes vessel outgrowth significantly more than the controls. Finally, they demonstrated that blocking IL-22 significantly reduces the volume of tumours induced by EL4 T cell lymphoma cell line in $Rag^{-/-}$ and C57BL/6 mice*in vivo* compared to controls. Even though the experiments by Protopsaltis et al. (2019) did not show the harmful effect of IL-22 in the intestine, their findings could also be relevant to types of cancer found in the intestine since the induction of angiogenesis is one of the hallmarks of cancer (Hanahan & Weinberg, 2011). Human colon cancer patients have been observed to have significantly higher levels of IL-22 in their cancer tissue than healthy controls (Jiang et al., 2013). It is thus possible that IL-22 enhances intestinal, as well as other tissue, tumour growth and development by promoting angiogenesis.

Taken together, there are many examples of IL-22 having a negative impact on intestinal homeostasis in mammals, when dysregulated. *In vivo* and *in vitro* experiments have demonstrated that IL-22 leads to a reduction of intestinal stem cells and intestinal epithelial barrier integrity, but also enhances paracellular permeability via upregulation of junctional protein claudin-2. It has also been shown that IL-22 can exacerbate colitis in some murine models. Lastly, it was shown that IL-22 promotes tumour angiogenesis by enhancing endothelial cell proliferation, survival, and migration. All studies referring to harmful effects of IL-22 are summarized in **Table 2** .

Discussion

Intestinal homeostasis depends on the intricate signalling network between gut microbiota, the host's immune system, and the intestinal epithelium integrity. It is clear that even with the knowledge there is about IL-22 today, it's role remains yet contradictory in the mammalian intestine. Some studies report highly beneficial effects of IL-22 in the intestine, while others show the opposite. Moreover, IL-22 appears to have an opposing role also in other tissues, such as the skin, lungs, and kidneys (reviewed in Dudakov et al., 2015). The fact that IL-22 is produced by plethora of immune cells and it has a systemic effect in the mammalian organism indicates that this cytokine and its production must be strictly regulated.

One of the beneficial effects designated to IL-22 in the intestine is the promotion of AMP secretion by

intestinal epithelial cells. Several studies have shown that IL-22 induces epithelial cells to increase the production of AMPs, such as β-defensin 3, lipocalin-2, and a number of Reg-family peptides (Gronke et al., 2019; Gunasekera et al., 2020; He et al., 2022; Lindemans et al., 2015; Patnaude et al., 2021). AMPs, as the name indicates, target microbes and are thus an integral part of the protection at the mucosal surfaces against intestinal inhabitants. AMPs are produced mainly by Paneth cells that reside in the bottom of small intestinal crypts (reviewed in Goyal et al., 2021). Importantly, IL-22 has been revealed to drive the differentiation of human Paneth cells (He et al., 2022). The location of Paneth cells creates a concentration gradient of AMPs in the crypts, with the highest concentration of AMPs in the bottom of the crypt, protecting the most important cell types within the intestine – stem cells. IL-22, therefore, enhances the protection of the source of all cell types in the intestine and thus contributes to the maintenance of intestinal homeostasis. However, several of the Reg-family AMPs are found to be overexpressed during UC and CD in humans (Tsuchida et al., 2017). AMP overexpression may lead to a significant decrease, or even elimination, of some bacterial species resulting in an ecological disbalance of intestinal microbiota, allowing possibly harmful bacterial species to thrive and cause damage to the host. Additionally, elevated IL-22 in the intestine has been linked to reduced microbial diversity because of an IL-22-induced increase in AMP production (Gunasekera et al., 2020). Taken together, it appears that when appropriately regulated, IL-22 induces epithelial cells to secrete just the right amount of AMPs to ward off intestinal microbes at the mucosal surfaces without causing dysbiosis. However, excessive amounts of IL-22 may lead to overexpression of AMPs in the intestine which, as a consequence, trigger microbial dysbiosis possibly leading to intestinal disorders, such as UC and CD.

IL-22 has been reported to increase the proliferative capacity of the intestinal epithelial cells in a number of studies (Lindemans et al., 2015; Patnaude et al., 2021; Zha et al., 2019). This feature of IL-22 certainly makes it beneficial in the mammalian intestine since the rapid renewal of the epithelium is the foundation of the symbiotic relationship between gut microbiota and the host. Without the fast regeneration of the epithelium, the intestinal microbes would likely overwhelm the host and its immune system. Because IL-22 appears to induce epithelial regeneration in the intestine, it has been suggested to use this cytokine as a therapeutic agent in dysbiotic and intestinal inflammatory disorders, such as IBD or NEC. However, the effect IL-22 has on ISC proliferation remains controversial. There are studies reporting an increase in ISC numbers upon IL-22 treatment (Lindemans et al., 2015; Zhang et al., 2020), while one reports the opposite (Zha et al., 2019). Intriguingly, the positive and negative effects of IL-22 on the intestinal stem cell population was observed in vitro and in vivo (Lindemans et al., 2015; Zha et al., 2019; Zhang et al., 2020). The discrepancy in these results could be due to differences in tissues used to derive organoids, the concentration of IL-22 used in the experiments, the expression of IL-22 receptor, or the time period the of IL-22 treatment. It is noteworthy that while Zha et al. (2019) report a reduction in Lgr5+ active stem cell population upon IL-22 treatment, TA cell proliferation in the intestine was promoted by IL-22 in mice.

Furthermore, it has been demonstrated that IL-22 specifically induces Paneth cell differentiation and proliferation in human, but not murine, small intestine derived organoids (He et al., 2022). This is a major difference in the effects IL-22 has in human and murine intestinal models. It is, thus, possible that the differences reported in effects of IL-22 in the intestine could be due to differences in the experimental setups. Differences such as, the species of origin (human vs mouse) of tissues and cell lines used for *in vitro* studies, the specific intestinal sections examined, or the timing and the duration of IL-22 treatment. It is also important to recognize that while intestinal organoids are an excellent *in vitro* model to study molecular interactions under various physiological conditions, they have limitations. For instance, IL-22 may potentially be differently regulated in different parts of the intestine (small vs large intestine), or in structures not found in the organoids. Furthermore, the presence of IL-22 regulators, such as IL-22BP varies between *in vivo* and *in vitro* experiments. *In vitro*models usually do not include such compounds, whereas they are likely present in *in vivo* systems. This can consequentially lead to different outcomes of the experiments. Therefore, a whole organism still remains to be the best model to study complex immunological interactions.

Another aspect that might lead to difference in IL-22 signalling outcome is a difference in the cell types that produce IL-22. This is well illustrated by the T cell transfer colitis model in mice. It has been shown that IL-22 originating from memory/effector T cells induces pathogenicity (Kamanaka et al., 2011), whereas

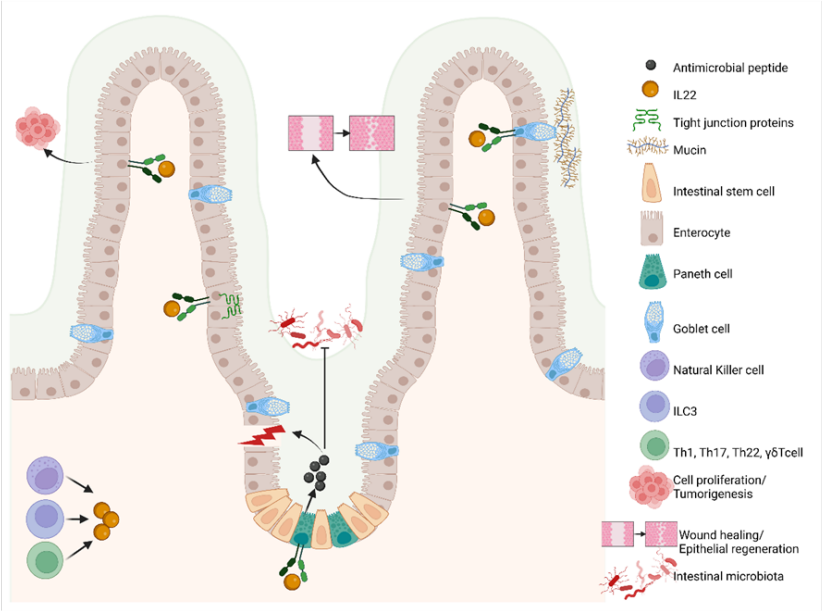
IL-22 produced by naïve T cells had a protective role in the T cell transfer colitis model (Zenewicz et al., 2008). Although, it is also possible that the effects on the intestinal tissue result from the activity of naïve T cells themselves instead of IL-22. Next to this, different immune cell types arrive at the site of the infection at different times. Neutrophils are among the first immune cells to arrive at the site of infection and are known to produce IL-22 (reviewed in Dudakov et al., 2015). However, adaptive immune cells, such as T cells, arrive at the site of infection much later. It allows for a sustained production of IL-22 during different stages of intestinal infection or damage, but it may also define the outcome of IL-22 signalling in the intestine. This might also hold true for normal early life development. Mao and colleagues showed that during early life, the sequential development of innate and adaptive immunity influences IL22 signalling outcome. Innate cells (such as ILC3s) might be activated by the microbiota before an effective adaptive immune response develops. In their research, the authors show differences in IL-22-dependent IEC signalling between wild-type and Rag1-/- adult mice. They showed that the developing adaptive immune cells (CD4+ T cells), silenced the ILC3-induced pSTAT3 signalling (as a result of IL22R binding) in the epithelial cells of wildtype mice. They also showed that in Rag-/- mice, in the absence of a dominant adaptive immune response, the persistent activation of ILC3s resulted in impaired lipid metabolism. Together these findings indicate that a possible delay or alteration in adaptive immunity might influence IL22 effector functions in terms of lipid metabolism as well as host microbe interactions. A host-microbiome feedback loop also seems to exist in mice and humans in which the microbiome can influence IL-22 production via synthesis (of precursors) of AhR ligands and IL-22 in turn influences the microbiota composition through its actions on AMP and mucus (Mar et al, 2023).

Moreover, the presence of other cytokines can affect how adaptive immune cells are activated. For instance, IL-22 together with IL-10 may have a different effect on T cell activation compared to IL-22 together with IL-17. These are important details to keep in mind when finding answers to questions related to IL-22 signalling. Next to temporal differences in IL-22 production in the intestine, there is also spatial variability. For example, ILC3s – potent IL-22 producers – are more abundant in the small intestine than in the large intestine (reviewed in Kim et al., 2016). The location of IL-22-producing and IL-22 receptor-expressing cells may therefore affect the outcome of IL-22 signalling. Moreover, the presence of IL-22BP in the intestine is highly important in defining the outcome of IL-22 signalling. Additionally, the presence and the expression of IL-22 regulators differs between intestinal sections; for example butyrate is solely produced in the colon upon microbial fermentation. Furthermore, IL-22BP is highly expressed in the colon compared to small intestine in healthy mice (Huber et al., 2012). This can significantly change the results as well as interpretations of *in vivo* experiments. Another important point to consider is how far-reaching the IL-22 signalling is. When IL-22 production is elicited at the location of damage in one specific segment of the intestine and the cytokine remains at that site, it is likely beneficial as it induces cell proliferation and healing where needed. However, if IL-22 moves to another site in the intestine, or other tissues, where there is no damage, it may induce needless epithelial cell proliferation, leading to possibly pathological conditions.

IL-22 has been linked to increased paracellular permeability and decreased epithelial integrity by upregulating claudin-2 protein (Wang et al., 2017; Zha et al., 2019). This may lead to the flux of water and solutes into the gut lumen, and this is an efficient way to remove parasites and other pathogens from the intestine. However, a persistent and uncontrolled flux of solutes and water into the gut may cause diarrhoea and dehydration. Furthermore, increased permeability allows the microbes to leave the intestinal environment and enter the tissue surrounding the intestine, potentially leading to (acute) inflammation. On top of that, IL-22-induced paracellular permeability, and not proliferation of cells, has been suggested to be the reason why organoids increase in size during *in vitro* experiments because of increased claudin-2 expression (He et al., 2022; Zha et al., 2019). Thus, the technique used to evaluate organoid growth – surface area or proliferative capacity of the cells – could lead to different interpretations of results.

An additional important factor in the intestine affecting IL-22 is butyrate, a SCFA produced by some types of intestinal bacteria, and a main energy source for intestinal epithelial cells (reviewed in Martin-Gallausiaux et al., 2021). Interestingly, butyrate has been shown to increase MUC13 expression in human colon organoids synergistically with IL-22 (Patnaude et al., 2021), suggesting that it may enhance the beneficial effects of

IL-22 in mammalian intestine. Furthermore, butyrate was shown to over-ride the disruptive effects to the epithelial barrier elicited by IL-22 (Patnaude et al., 2021). The effects butyrate has on IL-22 may be seen as a therapeutic opportunity. Treating patients with intestinal pathologies with probiotics and/or prebiotics, which stimulate butyrate-producing bacteria, together with IL-22 may increase beneficial effects of IL-22 in the intestine. But also, by supplementing only pre- and/or probiotics it may be possible to diminish the pathological effects that IL-22 elicits in the intestine. The effects of butyrate on IL-22 illustrate the diversity of signals and interactions involved in regulating IL-22 and maintaining intestinal homeostasis.



All in all, IL-22 has been shown to have both positive and negative effects in the mammalian intestine, suggestive of a complex regulation of this cytokine to ensure homeostasis in the gut (summarized in Figure 1). The conditions defining the outcome of IL-22 signalling in the intestine include the location in the intestine, the concentration of IL-22, timing and duration, the cellular source of IL-22, and the presence of IL-22 regulators. Additionally, it matters whether IL-22 is present on the apical or basolateral side of epithelial cells. To conclude, IL-22 is an appealing therapeutic agent as well as a target to help restore intestinal homeostasis, but careful consideration should be given when adopting IL-22 or IL-22 signalling targeted strategies as there are many conditions which define its effect in the mammalian gut.

Figure 1 : Beneficial and harmful effects of IL22 in the intestine. IL-22 is produced by various immune cells at the intestinal epithelium. Here, it acts on enterocytes by binding to the IL-22 receptor complex to initiate its effects. These include inducing cell proliferation/tumorigenesis, wound healing, and induction of mucins by Goblet cells and antimicrobial peptides by Paneth cells. If produced in excessive amounts, these antimicrobial peptides may damage the intestinal epithelium and reduce microbial diversity. IL-22 induces tight junction proteins, modulating epithelial barrier permeability. IL-22 also induces Paneth cells differentiation in humans. IL-22 is tightly regulated by several factors (Box 1)(figure created with BioRender).

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