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Review

Modulation of intestinal barrier function by glucocorticoids: Lessons from preclinical models

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ABSTRACT

Glucocorticoids (GCs) are widely used drugs for their anti-inflammatory and immunosuppressant effects, but they are associated with multiple adverse effects. Despite their frequent oral administration, relatively little attention has been paid to the effects of GCs on intestinal barrier function. In this review, we present a summary of the published studies on this matter carried out in animal models and cultured cells. In cultured intestinal epithelial cells, GCs have variable effects in basal conditions and generally enhance barrier function in the presence of inflammatory cytokines such as tumor necrosis factor (TNF). In turn, in rodents and other animals, GCs have been shown to weaken barrier function, with increased permeability and lower production of IgA, which may account for some features observed in stress models. When given to animals with experimental colitis, barrier function may be debilitated or strengthened, despite a positive anti-inflammatory activity. In sepsis models, GCs have a barrier-enhancing effect. These effects are probably related to the inhibition of epithelial cell proliferation and wound healing, modulation of the microbiota and mucus production, and interference with the mucosal immune system. The available information on underlying mechanisms is described and discussed.

1. Intestinal barrier function

The gastrointestinal tract is the largest surface facing the outside environment, being in direct contact with the commensal microbiota and antigens from the diet [1]. To ensure homeostasis, the intestine acts as a permeable but selective barrier, absorbing nutrients and water to obtain energy and blocking the passage of antigens and bacteria to the inner milieu. This critical property of the gut is known as intestinal barrier function (IBF) [2]. The pivotal player in the IBF is the intestinal epithelium, which comprises mainly absorptive enterocyte cells but also goblet cells, enteroendocrine, tuft, and Paneth cells. The epithelial monolayer acts as a selective barrier regulating the bidirectional passage of substances between the lumen and the mucosal milieu. In addition to constituting a purely physical barrier, the epithelium plays an active role in the immune response, as it expresses receptors involved in the innate immune response, secretes chemokines and cytokines, and acts as a non-professional antigen-presenting cell type [1]. Other factors contributing to IBF include different elements that help to avoiding the direct contact of microbiota to the epithelial cell surface: (i) the mucus layer, the secretion of immunoglobulin A and antimicrobial peptides [3]; (ii) the mucosal immune system, which features the highest proportion of immune cells of the whole body and constitutes a critical

Abbreviations: DSS, dextran sulfate sodium; FITC, fluorescein isotiocyanate; GC, glucocorticoid; GR, glucocorticoid receptor; IBD, inflammatory bowel disease; IBF, intestinal barrier function; IEC, intestinal epithelial cells; MKP-1, MAPK phosphatase 1; MLCK, myosin like chain kinase; TEER, transepithelial electrical resistance; ZO, zonulae occludens.

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interface of vital importance for the development and regulation of the innate and adaptive immune system [4]; (iii) motility, which prevents excessive microbial luminal growth; and (iv) the composition of the microbiota. Commensal bacteria, apart from contributing to the digestion process, participate in the development and control of the immune response [3], decrease pathogen colonization, and influence epithelial dynamics [1]. The mucosal immune system is finely regulated so that a forceful response is induced against pathogens while antigens from food, commensal bacteria, and self-antigens are tolerated [3]. A failure in this complex equilibrium can cause severe intestinal disorders, such as inflammatory bowel disease (IBD), celiac disease, irritable bowel syndrome, and diseases affecting other organs, such as diabetes mellitus, sepsis, and schizophrenia, among others [2,5].

Epithelial cells are held together by specialized structures, namely tight junctions, located apically, adherens junctions, and desmosomes (see below). Regulation of paracellular permeability is exerted chiefly at the tight junction level [6]. Tight junctions are constituted by transmembrane proteins (claudins, occludins, tricellulin and the junctional adhesion molecule) and peripheral membrane associated proteins that connect to the actin cytoskeleton (zonulae occludens (ZO) 1–3, AF-6, and cingulin) [2,7]. Some claudins are pore-forming (claudin-2, 7, 10, 15, and 16) while the rest have a tightness function (claudin-1, 3, 4, 5, 8, 11, 14, and 19) [6]. Tight junctions are finely regulated by several signaling pathways, such as myosin light chain kinase (MLCK), MAPK signaling or protein kinase C, A and G signaling, among others [7]. In most cases, pathologically increased permeability, for instance, by dysregulated secretion of proinflammatory cytokines (TNF, IFNγ, IL-1β, IL-6 or IL-13) is associated with modulation of tight junctions.

Transepithelial transport may take place through transcellular or paracellular pathways. The former involves transporter-mediated uptake, endocytosis, or exocytosis of large molecules, while the paracellular pathway involves the passage of ions, water, and small-to-large molecules through intercellular spaces. The epithelial barrier may also be disrupted by breaches in its continuity as a result of direct challenges causing local injury. Transport of luminal macromolecules, including proteins, and even microorganisms (via large gaps in the epithelium), is particularly relevant for the host. Translocation at a low rate is thought to play a physiological role in inducing tolerance by allowing sampling by dendritic cells in draining mesenteric lymph nodes. In contrast, abnormal high-rate translocation may lead to overt immune stimulation and mucosal or extraintestinal inflammation.

2. Glucocorticoids and intestinal barrier function

Glucocorticoids (GCs) are substances with pleiotropic effects widely used clinically due to their well-known anti-inflammatory and immunosuppressive actions. For instance, it has been estimated that more than 1% of the population in the USA is under chronic treatment with GCs [8]. GCs are the first-line treatment for the control of IBD bouts [9] due to either ulcerative colitis or Crohn's disease, particularly prednisone, methylprednisolone, hydrocortisone, beclomethasone dipropionate and budesonide. These are multifactorial conditions characterized by chronic and relapsing intestinal inflammation episodes [10]. Although their origin remains unknown, it is generally assumed to be the result of an impaired tolerance to the intestinal microbiota, a process in which a deficient IBF may be a key factor [11–13]. The use of GCs in IBD is limited not only by their well-known side effects in long-term treatments [14], but also by the fact that 25–30% of patients do not respond to GCs [15] and by their inability to prolong the relapse periods [16]. GCs are also used profusely in the management of non-intestinal inflammatory conditions, such as sepsis and a plethora of other diseases [17,18]. It is widely accepted that the intestinal microbiota may be a relevant source of translocating microorganisms in this context [19,20], especially considering that IBF has been shown to be weakened by systemic inflammation in animal models, resulting in bacterial translocation [21]. Increased epithelial apoptosis has been suggested to be involved in IBF compromise in animal models. On the other hand, GCs, particularly dexamethasone, are applied to treat severe COVID19 cases [22,23]. IBF is reportedly affected in COVID19 patients, possibly secondary to the systemic inflammatory response associated to the so-called 'cytokine storm' rather than to direct mucosal injury [24]. In particular, IL-6 appears to play a prominent role in producing diffuse vascular damage at the intestinal mucosal/submucosal level [25]. Furthermore, COVID19 has been related to changes in intestinal microbiota, which extend beyond clinical resolution with viral negativization by PCR [26]. Thus, delineation of the exact role of GCs in regulating IBF is critical.

The impact of the treatment with GCs on IBF is not entirely understood. Exogenous GCs stimulate epidermal barrier formation during development, while cutaneous treatment in adulthood alters epidermal barrier function, causing skin atrophy (decrease skin thickness and elasticity and increased fragility) and delayed wound healing [27]. Conversely, the lack of epidermal GC receptor (GR) signaling on adult mice provokes skin barrier defects and cutaneous inflammation [28]. In the intestinal milieu, GCs may improve IBF in active IBD [29], but it is not clear whether this is a direct effect or the consequence of GC-mediated anti-inflammatory actions. On the contrary, high cortisol levels due to acute stress increase intestinal permeability [30,31]. Some authors have suggested that GCs reinforce barrier function [32,33] although others sustain that GCs increase intestinal permeability and translocation [34–36]. This review aims to present and clarify the effects of GCs on the different elements involved in the maintenance of the IBF.

3. Tight junctions, permeability and glucocorticoids – in vitro studies

The effects of GCs on tight junctions and permeability are controversial, particularly considering the complexity of comparing the results obtained under basal conditions and in the context of inflammation. To differentiate between GC immunological from epithelial actions, in vitro studies will be summarized first (see Table 1 and Fig. 1).

3.1. *Effect of GCs on intestinal epithelial cell tight junctions in basal conditions*

In basal conditions, GCs appear to reduce permeability and tighten cell junctions. Thus, in IEC6 and IEC18 cells hydrocortisone (500 nM) increased ZO-1 expression, with a higher associated transepithelial electrical resistance (TEER), which is consistent with tighter intercellular unions, formation of developed microvilli, reorganization of the endoplasmic reticulum, the trans-Golgi complex, and the cytoskeletal network [37]. In Caco 2 cells, another intestinal cell model, dexamethasone (0.1–10 μM) was able to increase claudin-4 (CLDN4) expression and its presence in cellular contacts, and at the same time, it reduced the expression and junctional location of the pore-forming claudin-2, in basal conditions. This had no significant effect in the expression of ZO-1, occludin, and claudin-1, 5, 7 and 8. Besides, MLCK expression and MLC phosphorylation were also unaffected. GC-induced alterations in tight junction dynamics were accompanied by an increase in TEER, revealing a reduced paracellular permeability to ions. Moreover, permeability to relatively small organic molecules, such as fluorescein isothiocyanate (FITC) - dextran 4 kD or Lucifer Yellow (~0.5 kD), was not altered [38]. This is consistent with a modulation of tight junctions, as these are generally impermeable to proteins, although they can permit access in certain conditions such as after cholinergic agonists [39]. The authors found that GCs act through MAPK phosphatase 1 (MKP-1) stimulation, since dexamethasone enhanced both its expression and activity, whereas IBF modulation was prevented by MPK-1 inhibition with triptolide (which is not a specific inhibitor, as it also reportedly activates caspases and inhibits TNF induced NFκB activation, among other activities). MKP-1, also known as dual specificity phosphatase 1 (DUSP1), is a phosphatase that targets the MAP kinases ERK, p38 and JNK, that has

Table 1
Effect of GCs on tight junctions and epithelial permeability in vitro.

Model	GC	Effect	Highlights	Reference
Basal				
IEC6	HC 50–500 nM DEXA 50 nM	↑TEER Growth arrest ↓ cyclin-dependent kinase 6 and p27Kip1 TJ formation Long, slender microvilli Reorganization of endoplasmic reticulum and trans-Golgi network	Antiproliferative effect obtained after lag time and with probable autocrine mechanism	[37]
Caco 2	DEXA 0.1-10 μM	↑TEER, ↑cation permeability ↑CLDN4, ↓CLDN2 TJ structure unchanged FITC-dextran 4 kD unchanged	Mechanism related to MKP-1 induction Postconfluent cells	[38]
Caco 2	PRED 1–5 μM DEXA 1 μM	No effect on TEER		[41]
Caco 2/BBE	HC 500 nM	↓CLDN1 in postconfluent cells due to reduced binding of GR to the promoter	NR3C1 and HES1 display reciprocal regulation	[42]
Caco 2	HC 36 μg/ml (~100 μM)	↓TEER, ↑ion secretion Unchanged HRP flux		[43]
Caco 2/BBE Human colon crypts Rat colonic FRC/TEX cells	HC 500 nM HC/ corticosterone	↓TEER ↑FITC-dextran 4 kD ↓occludin, CLDN1 ↑CLDN2	Lubiprostone prevents downregulation of GR and co-chaperones	[44]
With inflammatory cytokines				
Caco 2. TJ disruption by TNF.	HC 36 μg/ml (~100 μM)	Partially reverts TNF-induced TEER decrease, HRP flux, and ion secretion increase Partial recovery from TNF-induced TJ disruption (↑ZO-1 ↑occludin ↑CLDN1) No effect on MLCK		[43]
Caco 2. TJ disruption by TNF.	PRED 1–5 μM	Inhibits TNF-induced decrease in TEER Inhibits TNF-induced inulin flux	Mechanism involves inhibition of TNF-induced MLCK	[41]
T84 and endotoxin-activated monocytes	BUDE and analogues (1–100 nM)	Inhibition of elevated basal Isc, decreased TEER and inhibited forskolin secretory response	Effects correlate with inhibition of TNF release by macrophages	[47]
Caco2, postconfluent. TJ disruption by TNF.	Methylprednisolone 50 μM ± IL10	Combination reverts TNF-induced drop in TEER ZO-1, OCCLDN unchanged	Protective effects only in the presence of IL-10, which ↑GR	[45]
Caco 2	DEXA 0.1-10 μM	↓ TNF-induced CLDN2	DEXA does not prevent decline in TEER induced by IFN/TNF or IL1β but TEER is higher than in controls	[38]

BUDE, budesonide; DEXA, dexamethasone; HC, hydrocortisone; HRP, horseradish peroxidase; Isc, short circuit current; PRED, prednisolone; TJ, tight junction.

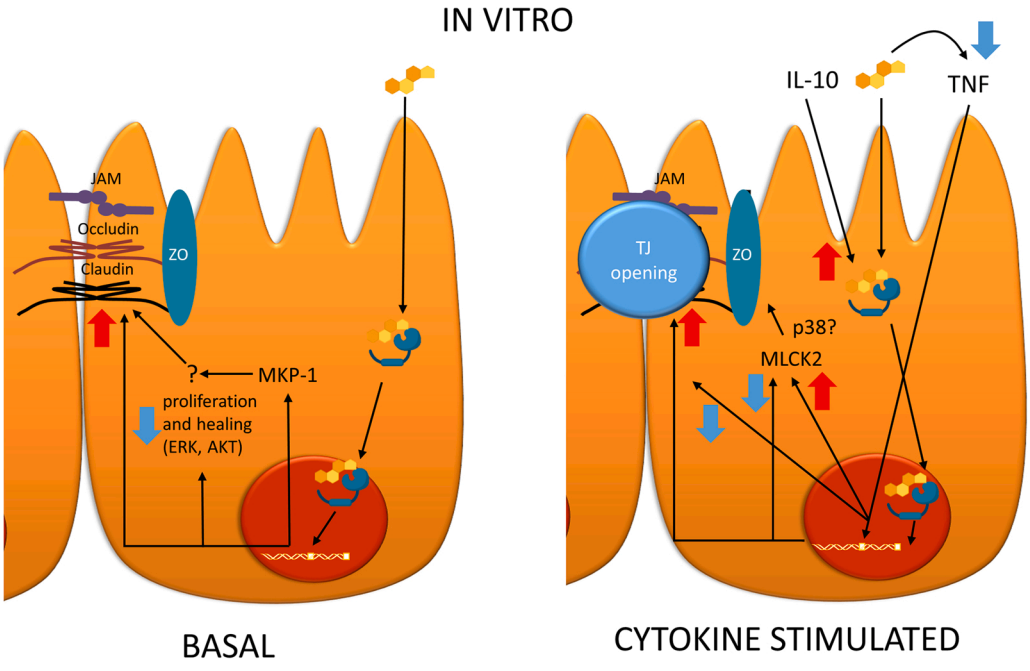

Fig. 1. Mechanisms described for GC modulation of intestinal epithelial cells based on in vitro models. In basal conditions (left), GCs may enhance barrier function via MKP-1 dependent mechanisms and inhibit proliferation and wound healing. In the presence of inflammatory cytokines (TNF, right), MLCK2 is upregulated and increases permeability, a mechanism that is counteracted by GCs. Expression of TJ tightening proteins may be also enhanced. TNF levels may be reduced by the immunomodulatory actions of GCs acting on other cell types. IL-10 may potentiate GC actions by increasing expression of the GR. The mechanisms depicted are derived from different studies and thus are not necessarily consistent.

been involved in GC anti-inflammatory actions [40]. However, in this case, the mechanism was independent of its downstream MAPK substrates p38 and p44/42 (JNK was not activated in this setting), as specific inhibitors of these kinases had no effect.

Conversely, in another study using the same cell model (Caco 2 cells), no change in IBF after prednisolone (1–5 μM) administration under basal conditions was noted [41]. The experimental conditions in these two studies differ in that Fischer et al. [38] treated the cells with dexamethasone for an extended time, and differences appeared only after the cells had undergone complete differentiation (i.e., 21 days onwards). These conditions enhance GC responsiveness because of increased expression of the GC receptor NR3C1. However, other authors have documented GC responses in undifferentiated Caco 2 cells [41,42]. In turn, GCs have also been reported to reduce rather than increase TEER in basal conditions, although a rather high concentration was used in this study (i.e., 100 μM hydrocortisone) [43]. However, a lower concentration (500 nM) augmented permeability to FITC-dextran 4 kD, decreased occludin and claudin 1, and increased claudin 2, in differentiated Caco 2 cells (BBE subclone) [44]. Taken together, these studies indicate variability in the effects of GC on intestinal epithelial cells that is enhanced by differentiation.

3.2. *Effect of GCs on tight junctions in stimulated intestinal epithelial cells*

Other in vitro studies have focused on the characterization of the effect of GCs on tight junctions and permeability in conditions of tight junction disruption by inflammatory mediators. As a rule, preservation of IBF has been reported in these conditions. TNF has been the agent more frequently used, and Caco 2 cells the most common in vitro model. Boivin et al. demonstrated that prednisolone by itself can reduce the drop of TEER and avoid the increase in inulin flux caused by TNF [41]. The mechanism is related to inhibition of MLCK promoter activity and reduced protein expression (which are stimulated by TNF). Thus, the levels of phosphorylated MLC were reduced and, therefore, the contraction of the perijunctional actomyosin ring, resulting in the decreased opening of the tight junctions [41]. Also in Caco 2 cells, hydrocortisone has been shown to partially revert the decreased expression of claudin-1, occludin and ZO-1 and the augmented horseradish peroxidase flux (indicative of transcellular permeability) and short circuit current (chloride secretion) produced by TNF [43]. In turn, dexamethasone was not able to prevent the decline in TEER induced by IFN-γ/TNF or IL-1β [38]. It should be noted however that TEER was consistently higher in GC-treated monolayers, so that partial protection was achieved. The combination of GC (methylprednisolone 50 μM) and IL-10, but not the GC alone, reverted the TEER decline due to TNF in differentiated Caco 2 cells [45]. This effect was associated to reduced phosphorylation of p38, but disappeared in the presence of the p38 inhibitor SB203580; this was interpreted by the authors as indicative that a certain degree of activation is required for GC-mediated effects on IBF. The mechanism may be related to the upregulation of GR-α expression by IL-10. As mentioned above, p38 inhibitors had no effect on the modulation of IBF by GCs [38].

The effect of GCs in epithelial cells has also been characterized in organoids stimulated by inflammatory cytokines [46]. Prednisolone (10 μM) reduced barrier dysfunction, with increased E-cadherin and immunoglobulin like domain containing receptor 1 and decreased claudin 2, MLCK and STAT1 phosphorylation. Of note, prednisolone had no effect on FITC-dextran 4 kD permeation in basal conditions.

Of course, since pro-inflammatory cytokine expression is downregulated by GCs, they may exert IBF protective effects by indirect mechanisms as well, at least in part. This was evidenced by using a T84 (human intestinal epithelial cells) – peripheral blood mononuclear cells coculture model under anti-CD3 stimulation to mimic epithelial alterations brought about by immune activation (McKay et al., 1995). Budesonide addition (≥100 nM) largely prevented the inhibition of secretory responses and the decrease in resistance and barrier function induced by activated leukocytes. This effect was exerted primarily on T lymphocytes and secondarily on macrophages, with no direct effect on IECs. Similar results were obtained with a lipopolysaccharide (LPS)-activated macrophages - T84 coculture model, where budesonide and two analogues (administered apically) counteracted the drop in TEER effectively, augmented basal short circuit current (reflecting chloride secretion), and inhibited forskolin evoked secretory response [47]. Thus, the evidence largely points to a protective effect of GCs on IBF under inflammatory conditions through direct and indirect actions. In turn, corticosterone reportedly enhanced the ethanol/acetaldehyde-induced deterioration of tight junctions in Caco 2 cells, together with redistribution of occludin and ZO-1 and of E-cadherin and β-catenin at the adherens junction level, whereas no effects were induced by corticosterone alone (up to 10 μM) [48].

4. Tight junctions, barrier function and glucocorticoids – *in vivo* studies

In contrast to the effects in vitro, treatment with GCs in vivo seems to increase intestinal permeability and bacterial translocation (see Table 2 and Fig. 2). Administration of a high dose of dexamethasone (0.8 mg/day, i.p.) for 2 days to fasted rats (possibly to reduce basal IgA secretion) resulted in reduced IgA bile levels and bacterial IgA coating, augmented bacterial adherence to cecal mucus surface, and significant translocation to mesenteric lymph nodes [49]. In another study, administration of a significantly lower dose of dexamethasone (0.1 mg/kg, s.c.) to rats also augmented intestinal permeability (lactulose:mannitol ratio, fractional excretion of sucralose, FITC-dextran 10 kD), mimicking the effect of stress [30]. The administration of dexamethasone to broilers increased the intestinal permeability to FITC 3–5 kD [50]. The prolonged administration of prednisolone to mice also resulted in a leaky gut, as suggested by the increased levels of endotoxin in serum [33]. Of note, in the same study, the IBF weakening effect was prevented by manipulation of the microbiota, namely broad-spectrum antibiotic treatment (although this would also diminish the LPS luminal content) or, alternatively, by addition of the probiotic *Lactobacillus reuteri.* Unfortunately, the contribution of these interventions was not assessed separately, i.e., in the absence of GC treatment, which complicates the interpretation of these findings.

In young animals the effects of GCs on IBF appear to be age-dependent. Thus dexamethasone (0.01–2.5 mg/kg s.c.) increased the permeability to FITC-dextran 4 kD in the colon of postnatal day 10 rat pups (a model of human preterm newborns). But it had no effect in postnatal day 20 pups (equivalent to human term newborns), owing to differences in GR expression [51]. The effect of dexamethasone mimics that of maternal separation stress in postnatal day 10 rats, which was confirmed to be due to endogenous corticosterone, as it was sensitive to mifepristone blockade. The mechanism in this case (and possibly with dexamethasone as well) involves MLCK activation, as inhibition of this enzyme with ML7 reduced the defect in permeability and bacterial translocation.

Other authors have reported little or no effect of GCs alone on IBF, but enhancement of barrier defects brought about by different stimuli. This is the case of the synergistic effect of corticosterone administration (25 mg/kg/day s.c. for 4 weeks, mimicking chronic stress) and alcohol intake [48], or of corticosterone in the context of burn injury [52,53]. In the latter study, the phenotype was associated to increased IL-18 expression in the small intestine, which was upregulated by the GC and resulted in increased neutrophil recruitment and augmented FITC-dextran 4 kD permeability [53]. It has been proposed that IL-18 mediates the exacerbating effect of stress on experimental colitis [54], and IL-18 levels are predictive of lethality in postoperative sepsis [55]. IL-18 actions are complex, however, and its role in GCs modulation of IBF is unclear at present [56–59].

Table 2
Effect of GCs on tight junctions and epithelial permeability in vivo.

Model	GC	Effect	Comments	Reference
Fasted rats	DEXA 0.8 mg/day i.p. for 2 days	↓ IgA bile levels and bacterial IgA coating ↑ bacterial adherence to mucus surface, translocation to mesenteric lymph nodes		[49]
Rats. Restrain or swimming stress.	Reproduced by DEXA administration (0.1 mg/kg s.c.)	↑ permeability (sucrose, sucralose, lactulose/mannitol and lactulose/sucralose excretion)	Abolished by adrenalectomy or mifepristone	[30]
Broilers	DEXA (1 mg/kg i.m.)	↑FITC 3–5 kDa		[50]
Mouse	PRED (subcutaneous pellet, 2.5 mg/kg/day) – 8 wk	↑Endotoxin in serum	Counteracted by antibiotics or *Lactobacillus reuteri*	[33]
Postnatal day 10 rat pups	DEXA (0.01–2.5 mg/kg s.c)	↑FITC-D FD4 in rat colon	Effect of maternal separation stress inhibited by mifepristone MLCK activation	[51]
Mouse	Corticosterone administration (25 mg/kg/day s.c., 4 wk)	↑endotoxemia With alcohol: ↑endotoxemia, TJ disruption, mucosal and systemic inflammatory markers		[48]
	Corticosterone + burn injury	Enhanced bacterial translocation		[52]
Mouse	Corticosterone 25 mg/kg s.c. 7 days	JNK and c-Src activation, TJ disruption and protein thiol oxidation in colonic mucosa	Mimics the phenotype after restraint stress and in vitro cellular stress	[91]
Rat	Corticosterone 3 mg/kg s.c., 10 days	↑PEG400 in colon (not jejunum), larger markers unaffected ↓ occludin, ZO-1, claudin 1	Mimics effects of chronic water avoidance stress	[89]
Mouse	BUDE 3 or 12 μg/day p.o., 7 days	No differences in claudin expression or intestinal permeability to FITC-dextran 4 kDa	No inflammation	[34]

BUDE, budesonide; DEXA, dexamethasone; HC, hydrocortisone; HRP, horseradish peroxidase; PRED, prednisolone; TJ, tight junction.

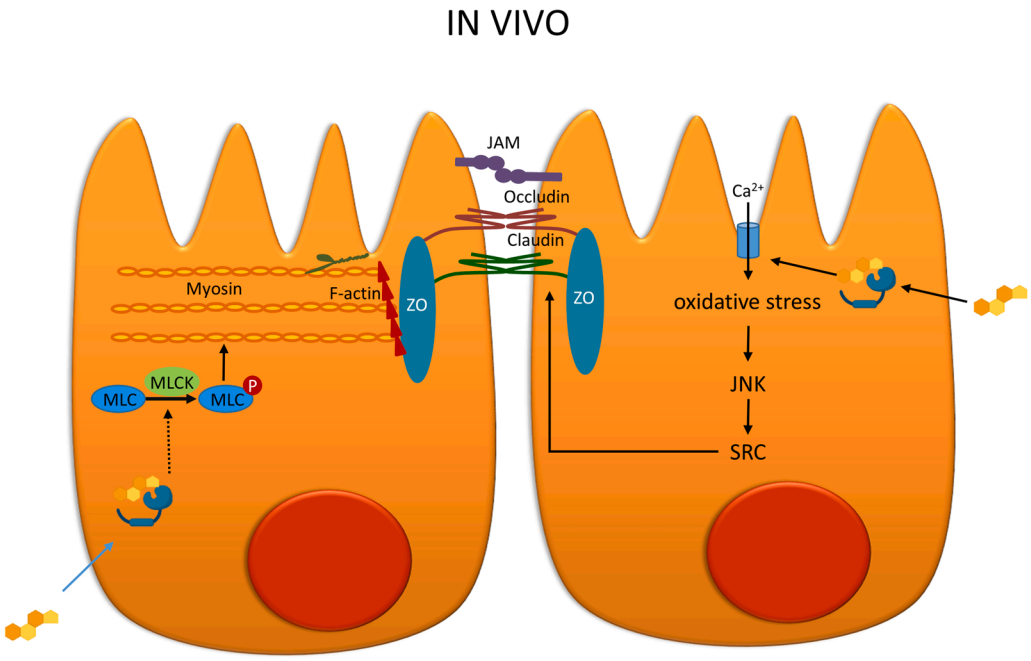

Fig. 2. Mechanisms described for GC modulation of intestinal epithelial permeability based on in vivo models. GCs may enhance epithelial permeability by increasing MLCK activity (left). A second mechanism proposed is related to activation of JNK and SRC, resulting in opening of the tight junctions. Several studies have pointed also to the involvement of the microbiota, possibly by augmenting bacterial adherence to mucus (not shown). The mechanisms depicted are derived from different studies and thus are not necessarily consistent.

4.1. Effect of GCs on tight junctions and barrier function in the inflamed intestine

Given the deleterious effects observed in vivo in the uninflamed intestine, an interesting question arises regarding the involvement of GCs in the modulation of barrier function in colitis. In mice, prednisolone (10 mg/kg/day) has been shown unable to prevent bacterial translocation despite a substantial colitis limiting effect [60]. We have found a similar lack effect on translocation by oral budesonide in rats [61]. Furthermore, we have observed increased permeability and bacterial translocation at high doses of budesonide in the dextran sulfate sodium (DSS) colitis model, with some IBF compromise even at lower doses [34]. In another study dexamethasone (1 mg/kg/day i.p. from day 3 to day 5 post DSS) had a modest, nonsignificant effect on augmented permeability to FITC-dextran 4 kD in DSS colitis [62]. In the oxazolone model of rat colitis, high doses of budesonide reduced [^{51}Cr]EDTA intestinal permeability [63]. The mechanism may be related at least in part to the modulation of IgA secretion. Thus dexamethasone (0.8 mg/kg i.p., 2 days) has been shown to enhance mucus adhesion of bacteria and increased paracellular permeability to the chemotactic peptide fMLP by downregulation of IgA secretion [64,65]. Such effects have also been reported for corticosterone (100 mg/rat as a s.c. pellet) [52]. Of note, bacterial adhesion per se promotes increased permeability. The effect on permeability appears to be fundamentally dependent on the presence of microbiota, based on sensitivity to antibiotics and the correlation between bacterial adhesion and tissue conductance [64]. In turn, the survival of translocating bacteria appears to be facilitated by increased circulating GC due to immunodepression [66].

Interestingly, GCs appear to also alleviate endoplasmic reticulum stress and to facilitate mucin secretion [67]. However, continued dexamethasone administration (0.01 and 0.05 mg/kg/day p.o. for 7 weeks) actually reduced mucin production in the rat colon [68]. This was associated with reduced diversity of the microbiota and increased inflammatory infiltration and expression of inflammatory markers (IL-1β, TNF) in the colon. A possible connection between the latter and increased Proteobacteria was suggested.

Hence GCs have variable effects on IBF in the inflamed intestine, including weakening barrier function, even in the face of antiinflammatory activity. Based on the in vitro evidence the ultimate outcome may represent the balance between protective and harmful mechanisms. Of note, the effect of GC treatment in experimental colitis is itself variable, including colitis worsening [69]. The effects of GCs on experimental colitis are beyond the scope of this review and will not be considered here in detail.

4.2. Effect of GCs on tight junctions and barrier function in sepsis

Several authors have encountered beneficial actions of GCs on IBF in sepsis, in which IBF may be compromised. Although the pathology of sepsis is complex, the gut microbiota has been claimed to be involved in disease progression as a source of microorganisms and microbe-derived molecules reaching the bloodstream because of impaired IBF. For instance, germ-free animals are protected in a model of hemorrhagic shock compared with normal control mice [70]. In experimental models of sepsis in rats, the administration of dexamethasone (between 0.1 and 1 mg/kg i.p.) reinforced the tight junctions via ZO-1, claudin 1 and occludin upregulation, reduced intestinal permeability and even decreased bacterial translocation [32,71]. Of note, the GC was administered as a single dose immediately before or after sepsis was induced. Similar results were obtained with hydrocortisone (2.8 mg/kg i.p.) in the cecal ligation and puncture model of sepsis in rats [72], with decreased mortality and inflammation and enhanced IBF. Dexamethasone administration (500 μg/mouse, roughly equivalent to 20–25 mg/kg) protected TNF challenged mice against increased permeability and bacterial translocation, and against ileal mucus depletion or endoplasmic reticulum stress in goblet cells and Paneth cells [73]. The latter cells are more relevant for IBF compromise in this model than absorptive enterocytes, even though these are strongly affected by TNF as well. Thus, GCs appear to enhance IBF in experimental sepsis.

The complex and varying effects of GCs on IBF may be due in part to changes in GR expression and function. In the specific case of claudin 1 (encoded by the *CLDN1* gene), the promoter is regulated by the transcription factors NR3C1 and the hairy and enhancer of split-1 (HES1). HES1 represses *CLDN1* expression, while GC-activated NR3C1 stimulates *CLDN1* transcription and inhibits HES1. A dynamic equilibrium between HES1 and CLDN1 exists, which occurs via intra-chromosomal regulation in order to control their expression throughout differentiation. Chronic elevated GC levels such as those caused by stress or exogenous treatment can impair colon barrier function by interfering with this intra-chromosomal communication [42]. GR expression is downregulated by stress in vivo, and by cortisol in Caco 2 cells, resulting in decreased expression of tight junction proteins [44]. Interestingly, treatment with mifepristone during stress induction reversed the reduced binding of GR to the *CLDN1* promoter [42].

4.3. Effect of Nr3c1 KO in intestinal epithelium

The impact of specific deletion of *Nr3c1* in the mouse intestinal epithelium, and therefore of the loss of epithelial GC function, has been investigated using a tamoxifen-inducible system [35,74,75]. In the first of these studies an augmented intestinal passage of FITC-dextran 4 kD in vitro and reduced localization of ZO-1 at the surface epithelium were noted, suggestive of positive effects of GCs on IBF under basal conditions [35]. Mucus production was increased, consistent with the results of other studies [68]. These effects were accompanied by mild, self-limiting colitis, which may be attributed to a proinflammatory status of IECs [35]. While this phenotype is certainly more complex than a simple permeability defect, it is clearly consistent with a prominent role of endogenous GCs in the regulation of epithelial permeability. Of note, mice with constitutive rather than inducible *Nr3c1* KO reportedly display a defect in GC-evoked glucose absorption but an otherwise normal colon, consistent with the transitory effect of receptor KO in basal conditions [76].

The phenotype of *Nr3c1*$^{IEC-/-}$ mice subjected to experimental colitis has also been studied, with widely different results. Our group found that KO mice were partly protected against DSS colitis, presumably due to enhanced proliferative capacity and to the augmented endogenous synthesis of GCs [74]. In turn, Muzzi et al. reported a completely different phenotype, characterized by aggravated colitis and augmented colonic permeability to Evans blue [75]. The reasons for this discrepancy are unknown, but may be due at least in part to differences in the protocol used, i.e. a low DSS dose (1% w/v) administered for 10 days instead of 2.5% w/v for 7 days. Since GCs exert protective and harmful actions on the epithelium, the relative balance may be critically dependent on the context. The complexity of the phenotype is highlighted by the fact that epithelial *Nr3c1* silencing may be pro- or anti-inflammatory [35,74,75], or by the observation of decreased expression of chemokines *Cxcl1*, *Cxcl5*, and *Ccl5* and lower amounts of neutrophils and macrophages in the colonic mucosa of KO mice with aggravated colitis [75].

5. Effects of GCs and stress on intestinal permeability and barrier function

Stress is the most potent stimulus for endogenous GC production. Chronic psychological stress is a risk factor for many disorders, particularly gastrointestinal conditions, such as peptic ulcer, irritable bowel syndrome and IBD. Stress has been long known to alter IBF, which has been studied extensively in animal models. Thus, stress augments intestinal permeability [30,31,51,53,77,78] and allows bacteria to reach the inner milieu [79,80], which may later result in triggering a mucosal immune response. Mucus secretion is also stimulated by stress [78]. These defects in intestinal permeability involve both the paracellular and transcellular pathways [81]. The mechanisms accounting for IBF modulation in stress are complex. In many instances, corticotropin-releasing hormone (CRH) and mast cells have been shown to play an essential role in mediating changes in intestinal permeability and transport. It is likely that the role of GCs in comparison with other pathways involving CRH, neuropeptides, or mast cells varies depending on the type of stressful stimuli and other additional factors, such as age [31,78,80,82–87]. At any rate, GCs seem to be involved in stress-induced alteration of IBF, at least in some cases. Thus, acute stress induced in rats by forced swim has been found to enhance small intestinal and colonic permeability [30]. These changes were associated with increased (3-fold) corticosterone in plasma and were prevented by adrenalectomy or inhibition of the GR (mifepristone) and, as noted above, were reproduced by dexamethasone. Similarly, acute restraint stress has been found to augment LPS permeability in the mouse colon, associated with diminished ZO-1 expression, which was reversed with mifepristone, pointing to a critical role of GR [88]. Chronic water avoidance-induced stress augmented colonic permeability to PEG400 (but not to larger markers), associated with reduced expression of occludin, ZO-1 and claudin 1, also in a mifepristone sensitive fashion [42,89]. The effect of maternal separation stress on the colonic permeability to FITC-dextran 4 kD and on bacterial translocation was also prevented by GR blockade, as noted above [51]. The mechanism appeared to involve MLCK mediated contraction of the epithelial cytoskeleton. This mechanism has also been documented in adult rats under acute stress [90].

From a mechanistic standpoint, it is interesting to note that various stressful stimuli may signal via Ca^{2+} elevation and secondary oxidative stress in colonocytes. Thus, in Caco 2 cells, intracellular Ca^{2+} chelation virtually abolished the effect of osmotic stress, DSS and cyclic stretch on barrier function [91]. The same protection was afforded by knockdown of the Ca^{2+} apical channels CaV1.3 or TRPV6, by antioxidants and, to a lower extent, by nitric oxide synthase inhibitors. The mechanism further involved activation of JNK and c-SRC. c-SRC is downstream of JNK and regulates tight junctions by occludin tyrosine phosphorylation, resulting in dissociation of the latter from ZO-1. In this study oxidative stress was of mitochondrial origin. These features were largely reproduced in mice in a chronic restraint model of stress and by repeated corticosterone administration [91] (Fig. 2).

Since GCs and stress may compromise IBF resulting in increased bacterial translocation, they may in principle have an impact on the inflammatory status of the intestine. It should be noted that there are several reports of acute and chronic stress models in which no signs of inflammation are observed along with the documented defects in IBF [30,81,92]. On the other hand, increased inflammatory markers or frank colitis have been also described. For instance, signs of colonic and jejunal inflammation have been reported in rats subjected to 14-day crowding-induced chronic stress, associated with a 3.5-fold increase in circulating corticosterone (and a yet higher peak at stress onset) and activation of mast cells [93,94]. Continued (10 d) water avoidance stress in rats results in increased colonic myeloperoxidase activity and leukocyte infiltration, associated with hypercorticosteronemia (~2-fold, 17-fold in the first hour), mucus depletion, and permeability defects [95]. Small intestinal inflammation is induced by 3-week cold exposure stress in rats (with severely depressed epithelial proliferation rate) [96] and by 4-week environmental factors stress [97]. In the subordinate colony housing model of chronic stress, colitis arises following an initial phase characterized by local immune suppression, decreased epithelial proliferation, reduced apoptosis, and mucus depletion. Adrenalectomy prevented the initial immune suppression (attenuated cytokine secretion, infiltration, IgA secretion) and the development of colitis after that [98]. This suggests that inflammation was secondary to bacterial translocation, a mechanism consistent with the increased luminal bacterial load by stress [99] and with higher sensitivity of animals to chemically induced colitis in a setting of HPA hypoactivation [100,101]. However, direct evidence of translocation in this study was not provided [102]. Signs of colitis and IBF compromise have also been found in 6 h-restraint stress model in rats [103]. Interestingly, these were counteracted by pharmacological PPARγ activation, independently of approximately 7-fold increased corticosterone plasma levels [104]. Another link between stress and intestinal inflammation is provided by the aggravation of experimental colitis by stressful stimuli [78,101] and the increased sensitivity to the colitogenic agent TNBS [105].

In this context, it is of interest to consider the possible proinflammatory effects of exogenous GCs. Budesonide has no apparent deleterious inflammatory effects at the doses of 3 and 12 μg/day p.o. in mice, with no differences in claudin expression or intestinal permeability to FITC-dextran 4 kD [34]. However, long-term dexamethasone administration induces inflammation in goats [106] and it has been reported to elicit colonic inflammation in basal conditions in mice [69]. The latter was linked to the activation of the mTOR pathway in IECs, since the phenotype was partially reversed by pharmacological inhibition (rapamycin) and epithelial knockout of mTOR. Of note, the dose used in this study was rather high, i.e., 5 and 10 mg/kg i.p., roughly equivalent to 100 and 200 μg/day.

Taken together, the analyzed information indicates that stress may produce inflammation in the colon and the small intestine, at least in certain cases, and that this effect is reproduced by GCs, with some exceptions.

6. GC effects on epithelial proliferation and wound healing

GCs have a well-known inhibitory effect on wound repair in the skin and other parts of the body. Several studies have investigated the impact of these agents on intestinal epithelial wound healing and proliferation. Dexamethasone (100–200 nM) was reported to reduce wound healing in two colonic cell lines, HT29 and HCT116 cells [107]. These experiments were carried out in the presence of deferoxamine to mimic hypoxic conditions. Han et al. described that dexamethasone (10 μM) inhibits $TGF\beta_1$-induced migration of HCT116 cells by inhibiting AKT and ERK phosphorylation as well as CYR61 (Cysteine-rich angiogenic inducer 61) expression [108]. This effect was independent of cell proliferation, which was not altered by GCs in this study. Prednisolone and budesonide were found to inhibit wound healing and proliferation in HT29 and IEC6 cells, although it should be noted that the concentrations assayed here were in the high range (i.e., >1 mM and 300 μM, respectively) [109]. Using the IEC6 cell line, a dual, concentration-dependent effect on wound healing was described [110]. Interestingly, in the same study, prednisolone and budesonide stimulated proliferation at relatively low concentrations (1 μM and 10 nM, respectively) and inhibited it at high levels (100 μM) [110]. A similar curve was reported for restitution, with enhancement observed at low concentrations. Our group has confirmed inhibition of wound healing in Caco 2, IEC18 and IEC4.1 cells induced by different GCs ([34] and unpublished data). Because GCs still have inhibitory effects in the presence of submaximal concentrations of the antiproliferative agent mitomycin C, interference with the healing process involves not only the proliferative response but also restitution.

There are also in vivo studies on epithelial proliferation. Administration of betamethasone-17-valerate reduces epithelial proliferation in the jejunum of adult rats [111]. Similar results have been reported with prednisolone (2.5 mg/kg) [112]. Differential effects were reported in one study, with decreased proliferation found with betamethasone-17-valerate vs. no effect with prednisolone [113]. Long-term administration of dexamethasone (0.2 mg/kg i.m., 21 days) to goats resulted in decreased epithelial proliferation (evidenced by reduced PCNA and cyclin D2 expression) and, as mentioned above, induced colitis [106]. Apoptosis was upregulated in the colonic mucosa in this study. In *Nr3c1*$^{IEC-/-}$ mice, our group has observed increased proliferation of colonic epithelial cells, both in basal conditions and in the context of DSS colitis [35,74]. This is consistent with an antiproliferative effect of GC and in fact is considered protective in the context of inflammation. However, conflicting studies exist [114,115]. GCs (prednisolone-21-phosphate 0.75 mg/kg p.o.) facilitate recovery from jejunal resection in the rat by augmenting IEC maturation without affecting proliferation [116]. Such effects have been also observed in vitro [117,118].

The study of the effect of long-term GC treatment on epithelial proliferation using triamcinolone acetonide 1 mg/kg i.m. per week, revealed that the jejunal epithelium exhibited lower proliferation at short term but increased mitotic activity and BrdU and PCNA positive cells in the long term (33 and 63 d) [119]. Similar results were obtained in the colon, although enhancement of proliferation took longer to ensue [120]. Since the number of cells per crypt was concomitantly decreased, it is possible that these changes reflect increased cell turnover, possibly secondary to increased apoptosis (not evaluated in the study).

In the newborn intestine GCs have a trophic action. In rats intestinal epithelial proliferation is markedly upregulated in the postnatal period, peaking around 14 d, and thereafter is reduced to adult levels, a process in which hydrocortisone may be involved [121]. As noted above, GC sensitivity is much reduced thereafter due to reduced GR expression. Adrenalectomized rat pups exhibit slowed intestinal maturation and proliferation [122]. Similarly, Tomaszweska et al. noted that prenatal administration of dexamethasone (0.03 mg/kg i.m. every other day for 45 days) resulted in increased epithelial proliferation in the small intestine, accompanied by distinct changes, including decreased claudin and cadherin expression [123]. Schaeffer et al. [124] induced

precocious sucrase-isomaltase expression by hydrocortisone (50 μg/g s.c.), which correlated with decreased TGF-$\beta_{1/2}$. As the latter reduce proliferation, this may play a role in early development intestinal maturation as well. Hydrocortisone has been reported to enhance [^{3}H] thymidine uptake from fetal small intestinal explants at 50 ng/ml (i.e., 138 nM) [117]. Overall, GCs promote epithelial proliferation and maturation in the early stages of development.

It is important to consider that the effects on epithelial proliferation may be partly indirect, as GR expression is not colocalized with the proliferative compartment, i.e., it is higher in the crypt surface than at the base. However, antiproliferative effects appear to be present in vitro if cells express the GC receptor. For instance, crypt intestinal IEC6 and IEC18 cells express the GR in basal conditions and respond to GCs with decreased proliferation, downregulation of the cell cycle regulatory proteins cyclin-dependent kinase 6 (CDK6) and p27^{Kip1}, tightened tight junctions (increased ZO-1 expression and TEER) and appearance of microvilli, consistent with differentiation (but with no induction of brush border enzymes) [37]. In this study, dexamethasone was shown to have a ~10-fold higher affinity for the GC receptor than hydrocortisone and a ~5-fold higher antiproliferative potency (a 25-fold potency is generally established for systemic effects of dexamethasone vs. hydrocortisone). Of note, hydrocortisone required a lag period of one to several days, depending on the experimental conditions, and in some cases enhanced proliferation was observed immediately after GC addition. The authors attributed the antiproliferative effect to an autocrine mechanism. Using mouse IEC4.1 cells our group has demonstrated that dexamethasone and hydrocortisone have similar potencies in the wound healing assay [34]. Thus, the relative potency of GCs may differ in IECs vs. other cell types.

At physiological concentrations, GCs generally have no effect on intestinal epithelial apoptosis, but they may be proapoptotic at high concentrations [110]. In turn, GCs have been shown to have an inhibitory effect on TNF-induced apoptosis acting via a STAT1 mechanism [125].

Clinically, GCs have been associated with an increased risk of colonic perforation [126]. They also have a well-known inhibitory effect on the recovery from intestinal anastomoses, with or without colitis or sepsis, as established in animal models [127–129] but also clinically [130]. Opposite results have been reported by other researchers [131]. Dexamethasone appears to be more potent than methylprednisolone and hydrocortisone in a rat preclinical model in this regard [132].

Therefore, in the adult intestine, GCs affect restitution and proliferation negatively in the epithelial compartment, resulting in delayed wound healing, in a manner akin to the known effect in the skin,

Table 3
Studies reporting changes in intestinal microbiota by GCs.

Model	GC treatment	Treatment period	Analyzed sample / Method	Decreased	Induced	Reference
MRL/lpr mouse Lupus erythematosus model	Prednisone 5 mg/kg/day Orally	5 weeks	Fecal samples / V3–4 region of 16S rRNA by Illumina	Proteobacteria Deferribacteres *Rikenella* *Mucispirillum* *Oscillospira* *Bilophila*	Bacteroidetes *Prevotella* *Anaerostipes* Significant alteration of 33 bacterial taxa	[134]
Mouse	Prednisolone 10 mg/kg/day Orally	2 weeks	Fecal samples / V1–3 region 16S rRNA pyrosequencing	Bacteroidetes	Firmicutes	[136]
Mouse	Prednisolone 2.5 mg/kg/day by s.c. implant (5 mg)	8 weeks	Fecal samples / V4 sequence of 16S rRNA by Illumina MiSeq	Verrucomicobiales Bacteroidales	Clostridiales	[33]
Mouse	Dexamethasone 1 mg/kg/day i.m.	10 weeks	Fecal samples / 16S rRNA sequence	Firmicutes Decrease in richness and diversity	Bacteroidetes Proteobacteria	[148]
Mouse Control and germ-free, and heterozygous and homozygous for MUC2	Dexamethasone i.p. 1 mg/kg/day (acute treatment) 5 mg/kg per 3 days (chronic treatment).	10 days (acute) / 4 week (chronic)	Fecal samples / V4–5 region from 16S rRNA by Illumina MiSeq	Bacteroidetes *Mucispirillum*	Actinobacteria Firmicutes *Bifidobacterium* *Lactobacillus*	[137]
Mouse	Dexamethasone 20 mg/kg/day Injection	7 days	Fecal samples / PCR-DGGE (intensity of bands)	Rumincococcaceae Lachnospiraceae	–	[149]
Rat	Dexamethasone 0.01 - 0.05 mg/kg/day Orally	7 weeks	Fecal samples / V3–4 region of 16S rRNA by Illumina MiSeq	Bacteroidetes Firmicutes Actinobacteria α-proteobacteria γ- proteobacteria	–	[68]
Rat	Dexamethasone 0.1, 0.5, 1, 2.5, 5 and 10 mg/kg i.p. (single dose)	48 h	Ileum / analysis of cultivable bacteria		5 and 10 mg/kg increase total aerobic and anaerobic bacteria and lactobacilli.	[150]
Rat	Hydrocortisone 40 mg/kg/day i.p.	21 days	Fecal and intestinal mucosa samples / V3–4 region of 16S rRNA by Illumina MiSeq	*Fecal samples:* Coriobacteriaceae Bacteroidaceae Bacillaceae *Bacteroides* *Lactococcus* *Ruminococcaceae* *Ruminococcaceae* *Mucosa samples:* Moraxellaceae Clostridiaceae *Lachnospiraceae* *K4A136*	*Fecal samples*: Actinobacteria Bacteroidales *Ruminococcus* *Mucosa samples:* Lachnospiraceae	[138]

although a positive modulation may be achieved at low concentrations in some cases. The impact of this effect at the clinical level is unclear.

7. GCs and the microbiota

Interestingly, some studies have suggested an implication of the microbiota in the GC-mediated modulation of barrier function, as noted above (see Table 3). Thus, endogenous corticosterone was found to be involved in IBF perturbation in a model of psychogenic stress exacerbation of nonsteroidal anti-inflammatory drug-induced small intestinal injury, and the phenotype was transmitted by cecal microbiota transplantation [133]. In experimental sepsis, hydrocortisone (2.8 mg/kg i.p., considered a 'stress dose') exerts protective effects, with lower mortality, decreased inflammation, and reduced epithelial apoptosis, plus ameliorated IBF [72]. Since these effects are comparable to those obtained by fecal transplantation of a 'normal' microbiota in the same study, a deleterious role of the 'septic' microbiota was assumed. Hydrocortisone increased the number of Paneth cells, which could be relevant to microbiota modulation as these cells are implicated in the secretion of antimicrobial peptides and therefore could be relevant to modulation of the microbiota [72]. In an interesting study, He at al. showed that the effect of prednisone in an animal model of systemic lupus erythematosus (MRL/lpr mice) was influenced by modulation of the intestinal microbiota using bromofuranone, an inhibitor of bacterial AI-2/LuxS quorum sensing [134]. This agent had no effect on the disease per se, but enhanced the effect of the GC, presumably via changes in the microbiota. However, these were admittedly complex, and differed in mice receiving bromofuranone only and the bromofuranone/GC combination. In turn, only a weak correlation was found between dexamethasone anti-inflammatory activity and reduction of permeability with changes in the microbiota, mostly a lower presence of genus *Bacteroides* and of *Escherichia* and *Shigella*, clustering at the order level closer to control mice, plus a recovery of bacterial diversity [62].

Several studies have documented that exogenous GCs alter the intestinal microbiota, although the changes are far from being documented with certainty. This aim is complicated by the high variability noted among species, subjects, and experimental designs (Table 3). Although some discrepancies have been described, an emerging common pattern is observed where exogenous GCs increase the abundance of Firmicutes [135–137] and Actinobacteria [135,137,138], while they decrease that of Bacteroidetes [68,135–137] or specifically Bacteroidales [33,138]. Interestingly, these data have been obtained using different GCs (prednisone, prednisolone, hydrocortisone, and dexamethasone), pointing to a common mechanism of action. Available data on microbiota modulation by endogenous GCs are also contradictory. For example, a higher microbiota diversity has been described in a model of stress in the rat using an incommunication box [139], while decreased diversity was shown in a limited nesting stress study [140], although in both studies an increased intestinal permeability with stress was reported.

The mechanisms accounting for the modulation of the microbiota have not been characterized in detail, but available evidence points at changes in mucus (qualitative and quantitative), alteration of IgA and antimicrobial peptides, modulation of the NOD-like receptor family pyrin domain containing 6 (NLRP6) inflammasome, etc. [49,64,65,141,142].

On the other hand, the intestinal microbiota regulates GC signaling, at least at the systemic level. Thus, the HPA axis is effectively modulated by the presence of a normal microbiota, as evidenced by the exaggerated response to acute stress in germ-free mice and rats [143–145]. For instance, an exacerbated neuroendocrine and behavioural response to acute stress is observed in germ free F344 compared to specific pathogen free F344 male rats [144], associated with increased levels of corticosterone in plasma. Germ-free mice subjected to restraint stress have been shown to present increased levels of corticosterone, also indicating an increased response to stress [146]. In animal models, exposure to probiotics early after birth provides protection against such enhanced HPA responses and to protracted IBF dysfunction [79]. Regulation of stress and GC responses to specific components of the microbiota has been described in rats subjected to maternal separation stress that received *Bifidobacterium bifidum* G9–1 (BBG9–1) [147]. In this study, stress induced hypercorticosteronemia at postnatal day 20, together with enhanced intestinal permeability and changes in the microbiota profile, and these alterations were prevented by treatment with BBG9–1. The effect of the administration of this prebiotic remained at day 56 of life in terms of protection against hypersensitivity to restraint stress (hypercorticosteronemia and increased defecation frequency), although no differences in basal corticosterone levels or the microbiota profile were found.

The available evidence thus points to a complex interaction between endogenous and exogenous GCs and the intestinal microbiota, whose details are still poorly defined.

8. Conclusions

For a drug type as widely used as GCs for systemic and intestinal diseases, it is remarkable that there are relatively few available studies focused on their impact on IBF. The analyzed evidence indicates that GCs have direct effects on the tight junctions of intestinal epithelium, which result in enhanced TEER and lower permeability in vitro in basal conditions. They also display indirect actions by decreasing the release of IBF damaging inflammatory mediators such as TNF. In turn, GC administration appears to have IBF weakening effects in vivo. The modulation of these latter effects by antibiotics and probiotics suggests that the microbiota is involved in this differential outcome. Mechanistically, this may be related to reduced IgA secretion, by changes in mucus production, or by modulation of the microbiota itself. As a result, GCs may have a limited beneficial benefit in barrier function in the inflamed intestine, despite a positive anti-inflammatory effect, or may evoke inflammation per se via altered IBF. Interestingly, GCs appear to be more clearly beneficial in models of sepsis. This may be related in part to the use of a single dose, compared with the protocols used in other studies in vivo, but in at least one study, hydrocortisone was protective using a 7 day period of administration [72]. In nearly all cases, except for newborns, GCs have negative effects on epithelial proliferation and wound healing, which is probably more relevant for permeability and certainly for bacterial translocation in vivo than in vitro.

Since this review was focused almost exclusively on preclinical studies, it is not possible to extrapolate the analysis to clinical practice. Nevertheless, it seems clear that GCs exert both beneficial and deleterious actions on IBF (Figs. 3 and 4), and it is tempting to speculate that the latter may account for some of the limitations of GC therapy, further investigations are still needed to unravel to what extent the findings obtained in vitro and animal models could be applicable to humans.

CRediT authorship contribution statement

MAA, MTG and CM performed the bibliographical search. OMA and FSM came up with the design of the review. All authors were responsible for manuscript writing and discussion.

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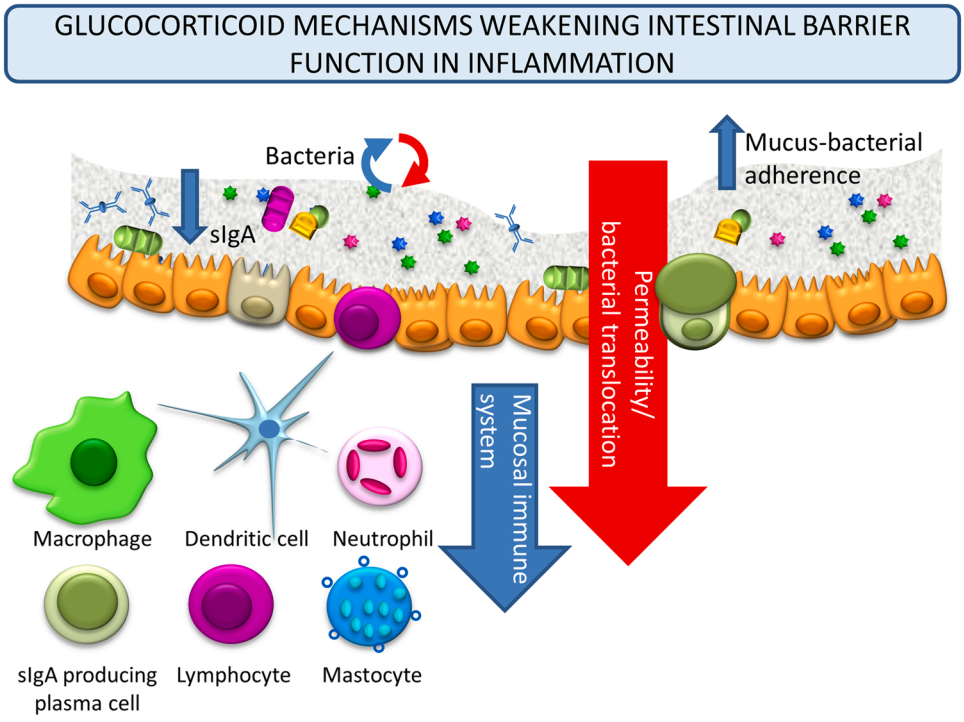

Fig. 3. Scheme of the elements of intestinal barrier function affected by GCs in the inflamed intestine. GCs tend to augment epithelial permeability and to inhibit the proliferative response and wound healing that are critical in a context of inflammatory injury. The mucosal immune system is debilitated. These conditions favor the translocation of bacteria. IgA secretion is diminished by GCs, which can result in increased bacterial adherence to mucus and contribute to IBF weakening. Changes in the microbiota have been documented with GC use, but the relevance of this factor is uncertain at present.

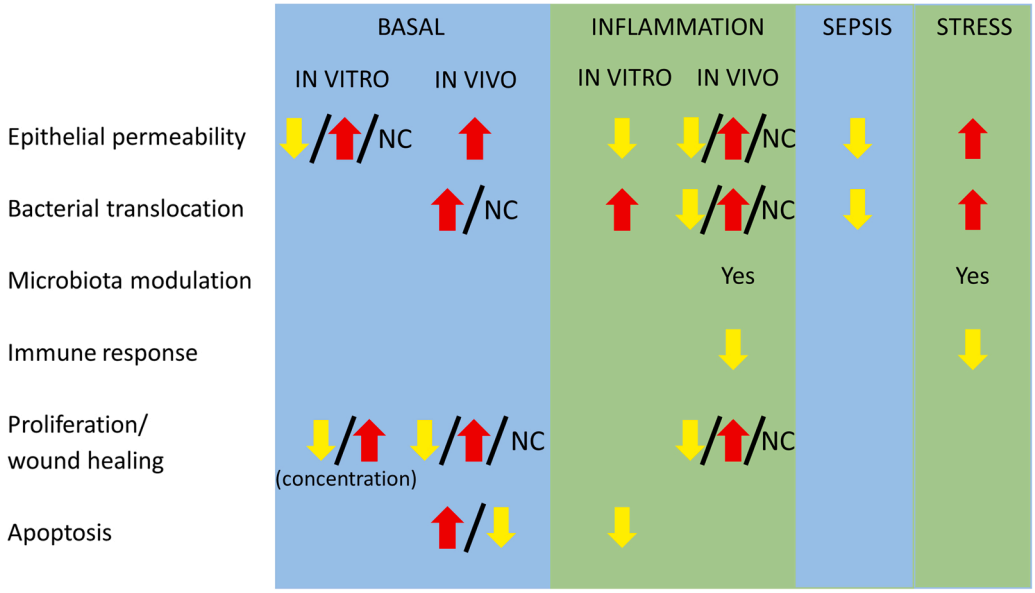

Fig. 4. GCs have both beneficial and deleterious actions on intestinal barrier function. NC: no change. Inflammation refers to actual inflammation or an inflammatory milieu in vitro. Changes in the microbiota have been documented but their relevance to the phenotype is uncertain.

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Declaration of Competing Interest

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References

[1] E. Salvo Romero, C. Alonso Cotoner, C. Pardo Camacho, M. Casado Bedmar, M. Vicario, The intestinal barrier function and its involvement in digestive disease, Rev. Esp. Enferm. Dig. 107 (11) (2015) 686–696.

[2] F. Sanchez de Medina, I. Romero-Calvo, C. Mascaraque, O. Martinez-Augustin, Intestinal inflammation and mucosal barrier function, Inflamm. Bowel Dis. 20 (12) (2014) 2394–2404.

[3] Y. Belkaid, O.J. Harrison, Homeostatic immunity and the microbiota, Immunity 46 (4) (2017) 562–576.
[4] A.M. Mowat, W.W. Agace, Regional specialization within the intestinal immune system, Nat. Rev. Immunol. 14 (10) (2014) 667–685.
[5] J.R. Turner, Intestinal mucosal barrier function in health and disease, Nat. Rev. Immunol. 9 (11) (2009) 799–809.
[6] S. Menard, N. Cerf-Bensussan, M. Heyman, Multiple facets of intestinal permeability and epithelial handling of dietary antigens, Mucosal Immunol. 3 (3) (2010) 247–259.
[7] Y.M. Lee, J.S. Ayres, Decoding the intestinal epithelium cell by cell, Nat. Immunol. 19 (1) (2018) 7–9.
[8] R.A. Overman, J.Y. Yeh, C.L. Deal, Prevalence of oral glucocorticoid usage in the United States: a general population perspective, Arthritis Care Res. (Hoboken) 65 (2) (2013) 294–298.
[9] K. Dubois-Camacho, P.A. Ottum, D. Franco-Munoz, M. De la Fuente, A. Torres-Riquelme, D. Diaz-Jimenez, M. Olivares-Morales, G. Astudillo, R. Quera, M. A. Hermoso, Glucocorticosteroid therapy in inflammatory bowel diseases: from clinical practice to molecular biology, World J. Gastroenterol. 23 (36) (2017) 6628–6638.
[10] Y.Z. Zhang, Y.Y. Li, Inflammatory bowel disease: pathogenesis, World J. Gastroenterol. 20 (1) (2014) 91–99.
[11] K.L. Madsen, D. Malfair, D. Gray, J.S. Doyle, L.D. Jewell, R.N. Fedorak, Interleukin-10 gene-deficient mice develop a primary intestinal permeability defect in response to enteric microflora, Inflamm. Bowel Dis. 5 (4) (1999) 262–270.
[12] L.C. Yu, J.T. Wang, S.C. Wei, Y.H. Ni, Host-microbial interactions and regulation of intestinal epithelial barrier function: From physiology to pathology, World J. Gastrointest. Pathophysiol. 3 (1) (2012) 27–43.
[13] D.K. Podolsky, Inflammatory bowel disease, N. Engl. J. Med. 347 (6) (2002) 417–429.
[14] H. Schacke, W.D. Docke, K. Asadullah, Mechanisms involved in the side effects of glucocorticoids, Pharmacol. Ther. 96 (1) (2002) 23–43.
[15] J. Tung, E.V. Loftus Jr., D.K. Freese, M. El-Youssef, A.R. Zinsmeister, L. J. Melton 3rd, W.S. Harmsen, W.J. Sandborn, W.A. Faubion Jr., A population-based study of the frequency of corticosteroid resistance and dependence in pediatric patients with Crohn's disease and ulcerative colitis, Inflamm. Bowel Dis. 12 (12) (2006) 1093–1100.
[16] A.C. Ford, C.N. Bernstein, K.J. Khan, M.T. Abreu, J.K. Marshall, N.J. Talley, P. Moayyedi, Glucocorticosteroid therapy in inflammatory bowel disease: systematic review and meta-analysis, Am. J. Gastroenterol. 106 (4) (2011) 590–599.
[17] D. Annane, E. Bellissant, P.E. Bollaert, J. Briegel, D. Keh, Y. Kupfer, Corticosteroids for treating sepsis, Cochrane Database Syst. Rev. 12 (2015), Cd002243.
[18] S.D. Reichardt, A. Amouret, C. Muzzi, S. Vettorazzi, J.P. Tuckermann, F. Lühder, H.M. Reichardt, The role of glucocorticoids in inflammatory diseases, Cells 10 (11) (2021) 2921.
[19] M. Gatt, The role of the gut in sepsis, Surg. - Oxf. Int. Ed. 33 (11) (2015) 534–541.
[20] B.W. Haak, W.J. Wiersinga, The role of the gut microbiota in sepsis, Lancet Gastroenterol. Hepatol. 2 (2) (2017) 135–143.
[21] C.J. Carrico, J.L. Meakins, J.C. Marshall, D. Fry, R.V. Maier, Multiple-organ-failure syndrome, Arch. Surg. (Chic., Ill.: 1960) 121 (2) (1986) 196–208.
[22] T.R.C. Group, Dexamethasone in Hospitalized Patients with Covid-19, N. Engl. J. Med. 384 (8) (2020) 693–704.
[23] A. Papamanoli, J. Yoo, P. Grewal, W. Predun, J. Hotelling, R. Jacob, A. Mojahedi, H.A. Skopicki, M. Mansour, L.A. Marcos, A.P. Kalogeropoulos, High-dose methylprednisolone in nonintubated patients with severe COVID-19 pneumonia, Eur. J. Clin. Investig. (2020), e13458.
[24] H. Hoel, L. Heggelund, D.H. Reikvam, B. Stiksrud, T. Ueland, A.E. Michelsen, K. Otterdal, K.E. Muller, A. Lind, F. Muller, S. Dudman, P. Aukrust, A.M. Dyrhol-Riise, J.C. Holter, M. Trøseid, Elevated markers of gut leakage and inflammasome activation in COVID-19 patients with cardiac involvement, J. Intern. Med. 289 (2021) 523–531.
[25] V. Cardinale, G. Capurso, G. Ianiro, A. Gasbarrini, P.G. Arcidiacono, D. Alvaro, Intestinal permeability changes with bacterial translocation as key events modulating systemic host immune response to SARS-CoV-2: a working hypothesis, Dig. Liver Dis. 52 (12) (2020) 1383–1389.
[26] T. Zuo, F. Zhang, G.C.Y. Lui, Y.K. Yeoh, A.Y.L. Li, H. Zhan, Y. Wan, A.C.K. Chung, C.P. Cheung, N. Chen, C.K.C. Lai, Z. Chen, E.Y.K. Tso, K.S.C. Fung, V. Chan, L. Ling, G. Joynt, D.S.C. Hui, F.K.L. Chan, P.K.S. Chan, S.C. Ng, Alterations in gut microbiota of patients with COVID-19 during time of hospitalization, Gastroenterology 159 (3) (2020) 944–955.e8.
[27] P. Pérez, Glucocorticoid receptors, epidermal homeostasis and hair follicle differentiation, Dermatoendocrinol 3 (3) (2011) 166–174.
[28] L.M. Sevilla, V. Latorre, A. Sanchis, P. Perez, Epidermal inactivation of the glucocorticoid receptor triggers skin barrier defects and cutaneous inflammation, J. Investig. Dermatol. 133 (2) (2013) 361–370.
[29] G.E. Wild, K.A. Waschke, A. Bitton, A.B. Thomson, The mechanisms of prednisone inhibition of inflammation in Crohn's disease involve changes in intestinal permeability, mucosal TNFalpha production and nuclear factor kappa B expression, Aliment Pharmacol. Ther. 18 (3) (2003) 309–317.
[30] J.B. Meddings, M.G. Swain, Environmental stress-induced gastrointestinal permeability is mediated by endogenous glucocorticoids in the rat, Gastroenterology 119 (4) (2000) 1019–1028.
[31] T. Vanuytsel, S. van Wanrooy, H. Vanheel, C. Vanormelingen, S. Verschueren, E. Houben, S. Salim Rasoel, J. Tomicronth, L. Holvoet, R. Farre, L. Van Oudenhove, G. Boeckxstaens, K. Verbeke, J. Tack, Psychological stress and corticotropin-releasing hormone increase intestinal permeability in humans by a mast cell-dependent mechanism, Gut 63 (8) (2014) 1293–1299.
[32] A.B. Ribeiro, H. Giusti, A.P.T. Souza, C.R. Franci, R.S. Saia, Dexamethasone prevents lipopolysaccharide-induced epithelial barrier dysfunction in rat ileum, Shock 49 (3) (2018) 334–344.
[33] J.D. Schepper, F. Collins, N.D. Rios-Arce, H.J. Kang, L. Schaefer, J.D. Gardinier, R. Raghuvanshi, R.A. Quinn, R. Britton, N. Parameswaran, L.R. McCabe, Involvement of the gut microbiota and barrier function in glucocorticoid-induced osteoporosis, J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. (2019).
[34] B. Ocon, C.J. Aranda, R. Gamez-Belmonte, M.D. Suarez, A. Zarzuelo, O. Martinez-Augustin, F. Sanchez de Medina, The glucocorticoid budesonide has protective and deleterious effects in experimental colitis in mice, Biochem. Pharmacol. 116 (2016) 73–88.
[35] C.J. Aranda, M. Arredondo-Amador, B. Ocon, J.L. Lavin, A.M. Aransay, O. Martinez-Augustin, F. Sanchez de Medina, Intestinal epithelial deletion of the glucocorticoid receptor NR3C1 alters expression of inflammatory mediators and barrier function, FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol. 33 (12) (2019) 14067–14082.
[36] S. Kiziltas, N. Imeryuz, T. Gurcan, A. Siva, S. Saip, A. Dumankar, C. Kalayci, N. B. Ulusoy, Corticosteroid therapy augments gastroduodenal permeability to sucrose, Am. J. Gastroenterol. 93 (12) (1998) 2420–2425.
[37] A. Quaroni, J.Q. Tian, M. Goke, D.K. Podolsky, Glucocorticoids have pleiotropic effects on small intestinal crypt cells, Am. J. Physiol. 277 (5 Pt 1) (1999) G1027–G1040.
[38] A. Fischer, M. Gluth, F. Weege, U.F. Pape, B. Wiedenmann, D.C. Baumgart, F. Theuring, Glucocorticoids regulate barrier function and claudin expression in intestinal epithelial cells via MKP-1, Am. J. Physiol. Gastrointest. Liver Physiol. 306 (3) (2014) G218–G228.
[39] A.J. Kiliaan, P.R. Saunders, P.B. Bijlsma, M.C. Berin, J.A. Taminiau, J.A. Groot, M.H. Perdue, Stress stimulates transepithelial macromolecular uptake in rat jejunum, Am. J. Physiol. 275 (5) (1998) G1037–G1044.
[40] J. Hoppstädter, A.J. Ammit, Role of dual-specificity phosphatase 1 in glucocorticoid-driven anti-inflammatory responses, Front. Immunol. 10 (1446) (2019).
[41] M.A. Boivin, D. Ye, J.C. Kennedy, R. Al-Sadi, C. Shepela, T.Y. Ma, Mechanism of glucocorticoid regulation of the intestinal tight junction barrier, Am. J. Physiol. Gastrointest. Liver Physiol. 292 (2) (2007) G590–G598.
[42] G. Zheng, G. Victor Fon, W. Meixner, A. Creekmore, Y. Zong, K.D. M, J. Colacino, P.H. Dedhia, S. Hong, J.W. Wiley, Chronic stress and intestinal barrier dysfunction: Glucocorticoid receptor and transcription repressor HES1 regulate tight junction protein Claudin-1 promoter, Sci. Rep. 7 (1) (2017) 4502.
[43] L. Nahidi, A.S. Day, D.A. Lemberg, S.T. Leach, Differential effects of nutritional and non-nutritional therapies on intestinal barrier function in an in vitro model, J. Gastroenterol. 47 (2) (2012) 107–117.
[44] Y. Zong, S. Zhu, S. Zhang, G. Zheng, J.W. Wiley, S. Hong, Chronic stress and intestinal permeability: Lubiprostone regulates glucocorticoid receptor-mediated changes in colon epithelial tight junction proteins, barrier function, and visceral pain in the rodent and human, Neurogastroenterology and motility: the official journal of the European Gastrointestinal Motility Society 31(2) (2019) e13477.
[45] V. Loren, E. Cabre, I. Ojanguren, E. Domenech, E. Pedrosa, A. Garcia-Jaraquemada, M. Manosa, J. Manye, Interleukin-10 enhances the intestinal epithelial barrier in the presence of corticosteroids through p38 MAPK activity in Caco-2 monolayers: a possible mechanism for steroid responsiveness in ulcerative colitis, PloS One 10 (6) (2015), e0130921.
[46] P. Xu, M. Elizalde, A. Masclee, M. Oierik, D. Jonkers, Corticosteroid enhances epithelial barrier function in intestinal organoids derived from patients with Crohn's disease, J. Mol. Med. 99 (5) (2021) 805–815, https://doi.org/10.1007/s00109-021-02045-7.
[47] M. Zareie, R. Brattsand, P.M. Sherman, D.M. McKay, M.H. Perdue, Improved effects of novel glucocorticosteroids on immune-induced epithelial pathophysiology, J. Pharm. Exp. Ther. 289 (3) (1999) 1245–1249.
[48] P.K. Shukla, A.S. Meena, K. Dalal, C. Canelas, G. Samak, J.F. Pierre, R. Rao, Chronic stress and corticosterone exacerbate alcohol-induced tissue injury in the gut-liver-brain axis, Sci. Rep. 11 (1) (2021) 826.
[49] J. Alverdy, E. Aoys, The effect of glucocorticoid administration on bacterial translocation. Evidence for an acquired mucosal immunodeficient state, Ann. Surg. 214 (6) (1991) 719–723.
[50] E.A. Vicuña, V.A. Kuttappan, R. Galarza-Seeber, J.D. Latorre, O.B. Faulkner, B. M. Hargis, G. Tellez, L.R. Bielke, Effect of dexamethasone in feed on intestinal permeability, differential white blood cell counts, and immune organs in broiler chicks, Poult. Sci. 94 (9) (2015) 2075–2080.
[51] N. Moussaoui, V. Braniste, A. Ait-Belgnaoui, M. Gabanou, S. Sekkal, M. Olier, V. Theodorou, P.G. Martin, E. Houdeau, Changes in intestinal glucocorticoid sensitivity in early life shape the risk of epithelial barrier defect in maternal-deprived rats, PloS One 9 (2) (2014), e88382.
[52] W.G. Jones 2nd, J.P. Minei, R.P. Richardson, T.J. Fahey 3rd, S.E. Calvano, A. C. Antonacci, G.T. Shires 3rd, G.T. Shires, Pathophysiologic glucocorticoid elevations promote bacterial translocation after thermal injury, Infect. Immun. 58 (10) (1990) 3257–3261.
[53] X. Li, S.N. Rana, M.G. Schwacha, I.H. Chaudry, M.A. Choudhry, A novel role for IL-18 in corticosterone-mediated intestinal damage in a two-hit rodent model of alcohol intoxication and injury, J. Leukoc. Biol. 80 (2) (2006) 367–375.
[54] H. Matsunaga, R. Hokari, T. Ueda, C. Kurihara, H. Hozumi, M. Higashiyama, Y. Okada, C. Watanabe, S. Komoto, M. Nakamura, A. Kawaguchi, S. Nagao, A. Sekiyama, S. Miura, Physiological stress exacerbates murine colitis by

enhancing proinflammatory cytokine expression that is dependent on IL-18, Am. J. Physiol. Gastrointest. liver Physiol. 301 (3) (2011) G555–G564.
[55] K. Emmanuilidis, H. Weighardt, E. Matevossian, C.D. Heidecke, K. Ulm, H. Bartels, J.R. Siewert, B. Holzmann, Differential regulation of systemic IL-18 and IL-12 release during postoperative sepsis: high serum IL-18 as an early predictive indicator of lethal outcome, Shock 18 (4) (2002) 301–305.
[56] O.J. Harrison, N. Srinivasan, J. Pott, C. Schiering, T. Krausgruber, N.E. Ilott, K. J. Maloy, Epithelial-derived IL-18 regulates Th17 cell differentiation and Foxp3+ Treg cell function in the intestine, Mucosal Immunol. 8 (6) (2015) 1226–1236.
[57] R. Nowarski, R. Jackson, N. Gagliani, M.R. de Zoete, N.W. Palm, W. Bailis, J. S. Low, C.C. Harman, M. Graham, E. Elinav, R.A. Flavell, Epithelial IL-18 equilibrium controls barrier function in Colitis, Cell 163 (6) (2015) 1444–1456.
[58] O. Allam, S. Samarani, V. Mehraj, M.A. Jenabian, C. Tremblay, J.P. Routy, D. Amre, A. Ahmad, HIV induces production of IL-18 from intestinal epithelial cells that increases intestinal permeability and microbial translocation, PloS One 13 (3) (2018), e0194185.
[59] Z. Pu, Y. Che, W. Zhang, H. Sun, T. Meng, H. Xie, L. Cao, H. Hao, Dual roles of IL-18 in colitis through regulation of the function and quantity of goblet cells, Int J. Mol. Med 43 (6) (2019) 2291–2302.
[60] S. Fiorucci, E. Distrutti, A. Mencarelli, M. Barbanti, E. Palazzini, A. Morelli, Inhibition of intestinal bacterial translocation with rifaximin modulates lamina propria monocytic cells reactivity and protects against inflammation in a rodent model of colitis, Digestion 66 (4) (2002) 246–256.
[61] P. Martinez-Moya, M. Ortega-Gonzalez, R. Gonzalez, A. Anzola, B. Ocon, C. Hernandez-Chirlaque, R. Lopez-Posadas, M.D. Suarez, A. Zarzuelo, O. Martinez-Augustin, F. Sanchez de Medina, Exogenous alkaline phosphatase treatment complements endogenous enzyme protection in colonic inflammation and reduces bacterial translocation in rats, Pharmacol. Res. 66 (2) (2012) 144–153.
[62] H. Zhou, X. Zeng, D. Sun, Z. Chen, W. Chen, L. Fan, Y. Limpanont, P. Dekumyoy, W. Maleewong, Z. Lv, Monosexual cercariae of schistosoma japonicum infection protects against DSS-induced colitis by shifting the Th1/Th2 balance and modulating the gut microbiota, Front. Microbiol. 11 (3356) (2021).
[63] G.M. Ekstrom, S.E. Andersson, Plasma exudation, hyperaemia, and epithelial permeability in rats with oxazolone-induced colitis: modulatory effects of budesonide, Scand. J. Gastroenterol. 35 (2) (2000) 190–197.
[64] J. Spitz, G. Hecht, M. Taveras, E. Aoys, J. Alverdy, The effect of dexamethasone administration on rat intestinal permeability: the role of bacterial adherence, Gastroenterology 106 (1) (1994) 35–41.
[65] J.C. Spitz, S. Ghandi, M. Taveras, E. Aoys, J.C. Alverdy, Characteristics of the intestinal epithelial barrier during dietary manipulation and glucocorticoid stress, Crit. Care Med. 24 (4) (1996) 635–641.
[66] W.G. Jones 2nd, A.E. Barber, S. Kapur, A.J. Hawes, T.J. Fahey 3rd, J.P. Minei, G. T. Shires 3rd, S.E. Calvano, G.T. Shires, Pathophysiologic glucocorticoid levels and survival of translocating bacteria, Arch. Surg. (Chic., Ill.: 1960) 126 (1) (1991) 50–55.
[67] I. Das, C.W. Png, I. Oancea, S.Z. Hasnain, R. Lourie, M. Proctor, R.D. Eri, Y. Sheng, D.I. Crane, T.H. Florin, M.A. McGuckin, Glucocorticoids alleviate intestinal ER stress by enhancing protein folding and degradation of misfolded proteins, The, J. Exp. Med. 210 (6) (2013) 1201–1216.
[68] T. Wu, L. Yang, J. Jiang, Y. Ni, J. Zhu, X. Zheng, Q. Wang, X. Lu, Z. Fu, Chronic glucocorticoid treatment induced circadian clock disorder leads to lipid metabolism and gut microbiota alterations in rats, Life Sci. 192 (2018) 173–182.
[69] Z. Zhang, L. Dong, A. Jia, X. Chen, Q. Yang, Y. Wang, Y. Wang, R. Liu, Y. Cao, Y. He, Y. Bi, G. Liu, Glucocorticoids Promote the Onset of Acute Experimental Colitis and Cancer by Upregulating mTOR Signaling in Intestinal Epithelial Cells, in: Cancers, 12, Basel,, 2020.
[70] B.F. Rush Jr., J.A. Redan, J.J. Flanagan, J.B. Jr, J. Heneghan, T.F. Hsieh, S. Murphy, G.W. Smith, Machiedo, Does the bacteremia observed in hemorrhagic shock have clinical significance? A study in germ-free animals, Ann. Surg. 210 (3) (1989) 346–347, 342-5; discussion.
[71] L. Wang, Y.L. Cui, Z. Zhang, Z.F. Lin, D.C. Chen, Rhubarb monomers protect intestinal mucosal barrier in sepsis via junction proteins, Chin. Med. J. 130 (10) (2017) 1218–1225.
[72] S.F. Assimakopoulos, I. Papadopoulou, D. Bantouna, A.L. de Lastic, M. Rodi, A. Mouzaki, C.A. Gogos, V. Zolota, I. Maroulis, Fecal microbiota transplantation and hydrocortisone ameliorate intestinal barrier dysfunction and improve survival in a rat model of cecal ligation and puncture-induced sepsis, Shock 55 (5) (2021) 666–675.
[73] F. Van Hauwermeiren, R.E. Vandenbroucke, L. Grine, S. Lodens, E. Van Wonterghem, R. De Rycke, N. De Geest, B. Hassan, C. Libert, TNFR1-induced lethal inflammation is mediated by goblet and Paneth cell dysfunction, Mucosal Immunol. 8 (4) (2015) 828–840.
[74] M. Arredondo-Amador, C.J. Aranda, B. Ocón, R. González, O. Martínez-Augustin, F. Sánchez de Medina, Epithelial deletion of the glucocorticoid receptor protects the mouse intestine against experimental inflammation, Br. J. Pharm. 178 (2021) 2482–2495.
[75] C. Muzzi, N. Watanabe, E. Twomey, G.K. Meers, H.M. Reichardt, H. Bohnenberger, S.D. Reichardt, The glucocorticoid receptor in intestinal epithelial cells alleviates colitis and associated colorectal cancer in mice, Cell Mol. Gastroenterol. Hepatol. 11 (5) (2021) 1505–1518.
[76] S.D. Reichardt, M. Foller, R. Rexhepaj, G. Pathare, K. Minnich, J.P. Tuckermann, F. Lang, H.M. Reichardt, Glucocorticoids enhance intestinal glucose uptake via the dimerized glucocorticoid receptor in enterocytes, Mediat. Inflamm. 153 (4) (2012) 1783–1794.
[77] N. Moussaoui, J.P. Jacobs, M. Larauche, M. Biraud, M. Million, E. Mayer, Y. Tache, Chronic early-life stress in rat pups alters basal corticosterone, intestinal permeability, and fecal microbiota at weaning: influence of sex, J. Neurogastroenterol. Motil. 23 (1) (2017) 135–143.
[78] C.J. Pfeiffer, B. Qiu, S.K. Lam, Reduction of colonic mucus by repeated short-term stress enhances experimental colitis in rats, J. Physiol., Paris 95 (1–6) (2001) 81–87.
[79] M.G. Gareau, J. Jury, G. MacQueen, P.M. Sherman, M.H. Perdue, Probiotic treatment of rat pups normalises corticosterone release and ameliorates colonic dysfunction induced by maternal separation, Gut 56 (11) (2007) 1522–1528.
[80] M.G. Gareau, J. Jury, P.C. Yang, G. MacQueen, M.H. Perdue, Neonatal maternal separation causes colonic dysfunction in rat pups including impaired host resistance, Pediatr. Res. 59 (1) (2006) 83–88.
[81] J.D. Söderholm, M.H. Perdue, Stress and gastrointestinal tract. II. Stress and intestinal barrier function, Am. J. Physiol. Gastrointest. liver Physiol. 280 (1) (2001) G7–g13.
[82] B.L. Bonaz, C.N. Bernstein, Brain-gut interactions in inflammatory bowel disease, Gastroenterology 144 (1) (2013) 36–49.
[83] C.N. Bernstein, The brain-gut axis and stress in inflammatory bowel disease, Gastroenterol. Clin. North Am. 46 (4) (2017) 839–846.
[84] J.E. Mawdsley, D.S. Rampton, Psychological stress in IBD: new insights into pathogenic and therapeutic implications, Gut 54 (10) (2005) 1481–1491.
[85] F. Smith, J.E. Clark, B.L. Overman, C.C. Tozel, J.H. Huang, J.E. Rivier, A. T. Blikslager, A.J. Moeser, Early weaning stress impairs development of mucosal barrier function in the porcine intestine, Am. J. Physiol. Gastrointest. liver Physiol. 298 (3) (2010) G352–G363.
[86] F. Barreau, C. Cartier, M. Leveque, L. Ferrier, R. Moriez, V. Laroute, A. Rosztoczy, J. Fioramonti, L. Bueno, Pathways involved in gut mucosal barrier dysfunction induced in adult rats by maternal deprivation: corticotrophin-releasing factor and nerve growth factor interplay, J. Physiol. 580 (Pt 1) (2007) 347–356.
[87] S. Komatsu, M.B. Grisham, J.M. Russell, D.N. Granger, Enhanced mucosal permeability and nitric oxide synthase activity in jejunum of mast cell deficient mice, Gut 41 (5) (1997) 636–641.
[88] K. Matsuo, X. Zhang, Y. Ono, R. Nagatomi, Acute stress-induced colonic tissue HSP70 expression requires commensal bacterial components and intrinsic glucocorticoid, Brain, Behav., Immun. 23 (1) (2009) 108–115.
[89] G. Zheng, S.-P. Wu, Y. Hu, D.E. Smith, J.W. Wiley, S. Hong, Corticosterone mediates stress-related increased intestinal permeability in a region-specific manner, Neurogastroenterology and motility: the official journal of the European Gastrointestinal Motility, Society 25 (2) (2013) e127–e139.
[90] A. Ait-Belgnaoui, S. Bradesi, J. Fioramonti, V. Theodorou, L. Bueno, Acute stress-induced hypersensitivity to colonic distension depends upon increase in paracellular permeability: role of myosin light chain kinase, Pain 113 (1–2) (2005) 141–147.
[91] R. Gangwar, A.S. Meena, P.K. Shukla, A.S. Nagaraja, P.L. Dorniak, S. Pallikuth, C. M. Waters, A. Sood, R. Rao, Calcium-mediated oxidative stress: a common mechanism in tight junction disruption by different types of cellular stress, The, Biochem. J. 474 (5) (2017) 731–749.
[92] P.R. Saunders, U. Kosecka, D.M. McKay, M.H. Perdue, Acute stressors stimulate ion secretion and increase epithelial permeability in rat intestine, Am. J. Physiol. 267 (5 Pt 1) (1994) G794–G799.
[93] M. Vicario, C. Alonso, M. Guilarte, J. Serra, C. Martinez, A.M. Gonzalez-Castro, B. Lobo, M. Antolin, A.L. Andreu, E. Garcia-Arumi, M. Casellas, E. Saperas, J. R. Malagelada, F. Azpiroz, J. Santos, Chronic psychosocial stress induces reversible mitochondrial damage and corticotropin-releasing factor receptor type-1 upregulation in the rat intestine and IBS-like gut dysfunction, Psychoneuroendocrinology 37 (1) (2012) 65–77.
[94] M. Vicario, M. Guilarte, C. Alonso, P. Yang, C. Martínez, L. Ramos, B. Lobo, A. González, M. Guilà, M. Pigrau, E. Saperas, F. Azpiroz, J. Santos, Chronological assessment of mast cell-mediated gut dysfunction and mucosal inflammation in a rat model of chronic psychosocial stress, Brain, Behav., Immun. 24 (7) (2010) 1166–1175.
[95] J.D. Soderholm, P.C. Yang, P. Ceponis, A. Vohra, R. Riddell, P.M. Sherman, M. H. Perdue, Chronic stress induces mast cell-dependent bacterial adherence and initiates mucosal inflammation in rat intestine, Gastroenterology 123 (4) (2002) 1099–1108.
[96] S. Kaushik, J. Kaur, Effect of chronic cold stress on intestinal epithelial cell proliferation and inflammation in rats, Stress 8 (3) (2005) 191–197.
[97] L.M. Wilson, A.L. Baldwin, Environmental stress causes mast cell degranulation, endothelial and epithelial changes, and edema in the rat intestinal mucosa, Microcirculation 6 (3) (1999) 189–198.
[98] S.O. Reber, S. Peters, D.A. Slattery, C. Hofmann, J. Scholmerich, I.D. Neumann, F. Obermeier, Mucosal immunosuppression and epithelial barrier defects are key events in murine psychosocial stress-induced colitis, Brain, Behav., Immun. 25 (6) (2011) 1153–1161.
[99] M.T. Bailey, S.E. Dowd, N.M. Parry, J.D. Galley, D.B. Schauer, M. Lyte, Stressor exposure disrupts commensal microbial populations in the intestines and leads to increased colonization by Citrobacter rodentium, Infect. Immun. 78 (4) (2010) 1509–1519.
[100] M. Million, Y. Taché, P. Anton, Susceptibility of Lewis and Fischer rats to stress-induced worsening of TNB-colitis: protective role of brain CRF, Am. J. Physiol. 276 (4) (1999) G1027–G1036.
[101] M. Gué, C. Bonbonne, J. Fioramonti, J. Moré, C. Del Rio-Lachèze, C. Coméra, L. Buéno, Stress-induced enhancement of colitis in rats: CRF and arginine vasopressin are not involved, Am. J. Physiol. 272 (1 Pt 1) (1997) G84–G91.

[102] S.O. Reber, L. Birkeneder, A.H. Veenema, F. Obermeier, W. Falk, R.H. Straub, I. D. Neumann, Adrenal insufficiency and colonic inflammation after a novel chronic psycho-social stress paradigm in mice: implications and mechanisms, Endocrinology 148 (2) (2007) 670–682.
[103] A. Ponferrada, J.R. Caso, L. Alou, A. Colón, D. Sevillano, M.A. Moro, I. Lizasoain, P. Menchén, M.L. Gómez-Lus, P. Lorenzo, E. Cos, J.C. Leza, L. Menchén, The role of PPARgamma on restoration of colonic homeostasis after experimental stress-induced inflammation and dysfunction, Gastroenterology 132 (5) (2007) 1791–1803.
[104] Z. Wu, N.L. Bucher, S.R. Farmer, Induction of peroxisome proliferator-activated receptor gamma during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBPbeta, C/EBPdelta, and glucocorticoids, Mol. Cell Biol. 16 (8) (1996) 4128–4136.
[105] A.L. Colón, J.L.M. Madrigal, L.A. Menchén, M.A. Moro, I. Lizasoain, P. Lorenzo, J. C. Leza, Stress increases susceptibility to oxidative/nitrosative mucosal damage in an experimental model of colitis in rats, Dig. Dis. Sci. 49 (10) (2004) 1713–1721.
[106] L. Cai, C. Hua, Y. Geng, Q. Chen, L. Niu, S. Tao, Y. Ni, R. Zhao, Chronic Dexamethasone exposure activates the TLR4-mediated inflammation pathway and induces epithelial apoptosis in the goat colon, Biochem Biophys. Res Commun. 518 (1) (2019) 7–13.
[107] J.H. Kim, Y.J. Hwang, S.H. Han, Y.E. Lee, S. Kim, Y.J. Kim, J.H. Cho, K.A. Kwon, J.H. Kim, S.H. Kim, Dexamethasone inhibits hypoxia-induced epithelial-mesenchymal transition in colon cancer, World J. Gastroenterol. 21 (34) (2015) 9887–9899.
[108] S. Han, N.T. Bui, M.T. Ho, Y.M. Kim, M. Cho, D.B. Shin, Dexamethasone inhibits TGF-β1-induced cell migration by regulating the ERK and AKT pathways in human colon cancer cells via CYR61, Cancer Res. Treat. 48 (3) (2016) 1141–1153.
[109] S. Jung, S. Fehr, J. Harder-d'Heureuse, B. Wiedenmann, A.U. Dignass, Corticosteroids impair intestinal epithelial wound repair mechanisms in vitro, Scand. J. Gastroenterol. 36 (9) (2001) 963–970.
[110] M.N. Göke, M. Schneider, W. Beil, M.P. Manns, Differential glucocorticoid effects on repair mechanisms and NF-kappaB activity in the intestinal epithelium, Regul. Pept. 105 (3) (2002) 203–214.
[111] J. Scott, R.M. Batt, Y.E. Maddison, T.J. Peters, Differential effect of glucocorticoids on structure and function of adult rat jejunum, Am. J. Physiol. 241 (4) (1981) G306–G312.
[112] N.A. Wright, H.S. Al-Dewachi, D.R. Appleton, A.J. Watson, The effect of single and of multiple doses of prednisolone tertiary butyl acetate on cell population kinetics in the small bowel mucosa of the rat, Virchows Arch. B Cell Pathol. 28 (4) (1978) 339–350.
[113] R.M. Batt, J. Scott, Response of the small intestinal mucosa to oral glucocorticoids, Scand. J. Gastroenterol. Suppl. 74 (1982) 75–88.
[114] P.J. Tutton, Proliferation of epithelial cells in the jejunal crypts of adrenalectomized and adrenocortical hormone treated rats, Virchows Arch. B Cell Pathol. 13 (3) (1973) 227–232.
[115] R.M. Batt, T.J. Peters, Effects of prednisolone on the small intestinal mucosa of the rat, Clin. Sci. Mol. Med 50 (6) (1976) 511–523.
[116] J. Scott, R.M. Batt, T.J. Peters, Enhancement of ileal adaptation by prednisolone after proximal small bowel resection in the rat, Gut 20 (10) (1979) 858–864.
[117] P. Arsenault, D. Ménard, Influence of hydrocortisone on human fetal small intestine in organ culture, J. Pedia Gastroenterol. Nutr. 4 (6) (1985) 893–901.
[118] P. Arsenault, D. Ménard, Comparative study of the effect of hydrocortisone and thyroxine on suckling mouse small intestine in organ culture, Comp. Biochem Physiol. A Comp. Physiol. 77 (4) (1984) 721–725.
[119] A.G. Gunin, D.V. Nikolaev, Effect of acute and chronic glucocorticoid treatments on epithelial cell proliferation in the esophagus and small intestine of rats, J. Gastroenterol. 34 (6) (1999) 661–667.
[120] A.G. Gunin, D.V. Nikolaev, Two-month glucocorticoid treatment increases proliferation in the stomach and large intestine of rats, Digestion 61 (3) (2000) 151–156.
[121] J.P. Buts, R. De Meyer, J. Kolanowski, Ontogeny of cell proliferation and DNA synthesis in rat colon: role of glucocorticoids, Am. J. Physiol. 244 (5) (1983) G469–G474.
[122] T. Miyata, Y. Minai, M. Haga, Impaired growth of small intestinal epithelium by adrenalectomy in weaning rats, Acta Histochem Cytochem 41 (4) (2008) 83–88.
[123] E. Tomaszewska, P. Dobrowolski, I. Puzio, Postnatal administration of 2-oxoglutaric acid improves the intestinal barrier affected by the prenatal action of dexamethasone in pigs, Nutrition 28 (2) (2012) 190–196.
[124] C. Schaeffer, M. Diab-Assef, M. Plateroti, F. Laurent-Huck, J.M. Reimund, M. Kedinger, C. Foltzer-Jourdainne, Cytokine gene expression during postnatal small intestinal development: regulation by glucocorticoids, Gut 47 (2) (2000) 192–198.
[125] M. Ballegeer, K. Van Looveren, S. Timmermans, M. Eggermont, S. Vandevyver, F. Thery, K. Dendoncker, J. Souffriau, J. Vandewalle, L. Van Wyngene, R. De Rycke, N. Takahashi, P. Vandenabeele, J. Tuckermann, H.M. Reichardt, F. Impens, R. Beyaert, K. De Bosscher, R.E. Vandenbroucke, C. Libert, Glucocorticoid receptor dimers control intestinal STAT1 and TNF-induced inflammation in mice, J. Clin. Invest 128 (8) (2018) 3265–3279.
[126] A.L. Warshaw, J.P. Welch, L.W. Ottinger, Acute perforation of the colon associated with chronic corticosteroid therapy, Am. J. Surg. 131 (4) (1976) 442–446.
[127] P. Myrelid, S.Y. Salim, T. Darby, S. Almer, S. Melgar, P. Andersson, J. D. Söderholm, Effects of anti-inflammatory therapy on bursting pressure of colonic anastomosis in murine dextran sulfate sodium induced colitis, Scand. J. Gastroenterol. 50 (8) (2015) 991–1001.
[128] A. Polat, A. Nayci, G. Polat, S. Aksöyek, Dexamethasone down-regulates endothelial expression of intercellular adhesion molecule and impairs the healing of bowel anastomoses, Eur. J. Surg. 168 (8–9) (2002) 500–506.
[129] I. Mantzoros, I. Kanellos, H. Demetriades, E. Christoforidis, D. Kanellos, M. G. Pramateftakis, T. Zaraboukas, D. Betsis, Effects of steroid on the healing of colonic anastomoses in the rat, Tech. Coloproctol. 8 (1) (2004) s180–s183.
[130] A. El-Hussuna, J. Andersen, T. Bisgaard, P. Jess, M. Henriksen, J. Oehlenschlager, O. Thorlacius-Ussing, G. Olaison, Biologic treatment or immunomodulation is not associated with postoperative anastomotic complications in abdominal surgery for Crohn's disease, Scand. J. Gastroenterol. 47 (6) (2012) 662–668.
[131] M.J. Koruda, R.H. Rolandelli, Experimental studies on the healing of colonic anastomoses, J. Surg. Res 48 (5) (1990) 504–515.
[132] T.R. Eubanks, J.J. Greenberg, P.B. Dobrin, F.J. Harford, R.L. Gamelli, The effects of different corticosteroids on the healing colon anastomosis and cecum in a rat model, Am. Surg. 63 (3) (1997) 266–269.
[133] K. Yoshikawa, C. Kurihara, H. Furuhashi, T. Takajo, K. Maruta, Y. Yasutake, H. Sato, K. Narimatsu, Y. Okada, M. Higashiyama, C. Watanabe, S. Komoto, K. Tomita, S. Nagao, S. Miura, H. Tajiri, R. Hokari, Psychological stress exacerbates NSAID-induced small bowel injury by inducing changes in intestinal microbiota and permeability via glucocorticoid receptor signaling, J. Gastroenterol. 52 (1) (2017) 61–71.
[134] Z. He, X. Kong, T. Shao, Y. Zhang, C. Wen, Alterations of the gut microbiota associated with promoting efficacy of prednisone by bromofuranone in MRL/lpr mice, Front. Microbiol. 10 (2019) 978.
[135] X.Z. Qi, X. Tu, J.W. Zha, A.G. Huang, G.X. Wang, F. Ling, Immunosuppression-induced alterations in fish gut microbiota may increase the susceptibility to pathogens, Fish. Shellfish Immunol. 88 (2019) 540–545.
[136] J. Tourret, B.P. Willing, S. Dion, J. MacPherson, E. Denamur, B.B. Finlay, Immunosuppressive treatment alters secretion of ileal antimicrobial peptides and gut microbiota, and favors subsequent colonization by uropathogenic Escherichia coli, Transplantation 101 (2017) 74–82.
[137] E.Y. Huang, T. Inoue, V.A. Leone, S. Dalal, K. Touw, Y. Wang, M.W. Musch, B. Theriault, K. Higuchi, S. Donovan, J. Gilbert, E.B. Chang, Using Corticosteroids to Reshape the Gut Microbiome: Implications for Inflammatory Bowel Diseases, 2015, pp. 963–972.
[138] D. Cheng, H. Chang, S. Ma, J. Guo, G. She, F. Zhang, L. Li, X. Li, Y. Lu, Tiansi Liquid modulates gut microbiota composition and tryptophan–kynurenine metabolism in rats with hydrocortisone-induced depression, Molecules 23 (2018) 2832.
[139] J.D. Galley, M.C. Nelson, Z. Yu, S.E. Dowd, J. Walter, P.S. Kumar, M. Lyte, M. T. Bailey, Exposure to a social stressor disrupts the community structure of the colonic mucosa-associated microbiota, BMC Microbiol. 14 (2014) 1–13.
[140] S. Geng, L. Yang, F. Cheng, Z. Zhang, J. Li, W. Liu, Y. Li, Y. Chen, Y. Bao, L. Chen, Z. Fei, X. Li, J. Hou, Y. Lin, Z. Liu, S. Zhang, H. Wang, Q. Zhang, H. Wang, X. Wang, J. Zhang, Gut microbiota are associated with psychological stress-induced defections in intestinal and blood–brain barriers, Front. Microbiol. 10 (2020) 1–12.
[141] Y. Sun, M. Zhang, C.C. Chen, M. Gillilland 3rd, X. Sun, M. El-Zaatari, G. B. Huffnagle, V.B. Young, J. Zhang, S.C. Hong, Y.M. Chang, D.L. Gumucio, C. Owyang, J.Y. Kao, Stress-induced corticotropin-releasing hormone-mediated NLRP6 inflammasome inhibition and transmissible enteritis in mice, Gastroenterology 144 (7) (2013) 1478–1487, 1487.e1-8.
[142] P.K. Anand, R.K. Malireddi, J.R. Lukens, P. Vogel, J. Bertin, M. Lamkanfi, T. D. Kanneganti, NLRP6 negatively regulates innate immunity and host defence against bacterial pathogens, Nature 488 (7411) (2012) 389–393.
[143] G. Clarke, S. Grenham, P. Scully, P. Fitzgerald, R.D. Moloney, F. Shanahan, T. G. Dinan, J.F. Cryan, The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner, Mol. Psychiatry 18 (6) (2013) 666–673.
[144] M. Crumeyrolle-Arias, M. Jaglin, A. Bruneau, S. Vancassel, A. Cardona, V. Daugé, L. Naudon, S. Rabot, Absence of the gut microbiota enhances anxiety-like behavior and neuroendocrine response to acute stress in rats, Psychoneuroendocrinology 42 (2014) 207–217.
[145] N. Sudo, Y. Chida, Y. Aiba, J. Sonoda, N. Oyama, X.N. Yu, C. Kubo, Y. Koga, Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice, J. Physiol. 558 (2004) 263–275.
[146] K. Vagnerová, M. Vodička, P. Hermanová, P. Ergang, D. Šrůtková, P. Klusoňová, K. Balounová, T. Hudcovic, J. Pácha, Interactions between gut microbiota and acute restraint stress in peripheral structures of the hypothalamic–pituitary–adrenal axis and the intestine of male mice, Front. Immunol. 10 (2019) 1–10.
[147] H. Fukui, T. Oshima, Y. Tanaka, Y. Oikawa, Y. Makizaki, H. Ohno, T. Tomita, J. Watari, H. Miwa, Effect of probiotic Bifidobacterium bifidum G9-1 on the relationship between gut microbiota profile and stress sensitivity in maternally separated rats, Sci. Rep. 8 (1) (2018) 12384.
[148] J. Li, M. Yang, C. Lu, J. Han, S. Tang, J. Zhou, Y. Li, T. Ming, Z.J. Wang, X. Su, Tuna bone powder alleviates glucocorticoid-induced osteoporosis via coregulation of the NF-κB and Wnt/β-catenin signaling pathways and modulation of gut microbiota composition and metabolism, Mol. Nutr. Food Res. 64 (5) (2020), e1900861.
[149] H. Fang, F. Meng, F. Piao, B. Jin, M. Li, W. Li, Effect of taurine on intestinal microbiota and immune cells in Peyer's patches of immunosuppressive mice, Adv. Exp. Med. Biol. (2019) 13–24.
[150] H. Ünsal, M. Balkaya, C. Ünsal, H. Bıyık, G. Başbülbül, E. Poyrazoğlu, The short-term effects of different doses of dexamethasone on the numbers of some bacteria in the ileum, Dig. Dis. Sci. 53 (2008) 1842–1845.