

Review article

Role of intestinal epithelial cells in the innate immune defence of the pig intestine

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Abstract – The intestinal epithelium serves as a dynamic barrier, which in the course of its normal function, maintains regulated uptake of nutrients and water while excluding potential pathogens. Over the past decade many studies have also revealed the immunological importance of intestinal epithelial cells (IEC). IEC have developed a variety of mechanisms to reduce the risk of infection by invasive pathogens or damage by toxic compounds. The effective maintenance of a physical barrier function is dependent on the establishment of well-organised intercellular junctions and a constant state of regeneration/renewal of the epithelium. IEC also participate in the innate immune responsiveness of the intestine by their ability to secrete mucus and antimicrobial peptides. IEC are also able to secrete cytokines and to respond to exogenous chemokines. This review summarises the current knowledge of the innate immune mechanisms developed by porcine IEC.

pig / epithelial intestinal cells / innate immune defence

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1. INTRODUCTION

The intestine is the most important route of entry for foreign antigens [8, 48, 59]. The antigens that may enter the body from the intestine include food proteins, natural toxins, commensal gut flora, and invading pathogens. The gastrointestinal tract (from the stomach to the colon) is lined by a continuous monolayer of epithelial cells. A pri-

mary function of these intestinal epithelial cells (IEC) is to act as a physical barrier, separating the contents of a harsh luminal environment from the layers of tissue comprising the interior milieu [21].

As a consequence of their exposed location, IEC have developed a variety of mechanisms besides the maintenance of barrier function, which act to reduce the risk of infection by invasive foreign agents. Such

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mechanisms include those that act directly to inhibit bacterial colonisation along the exposed surface of the monolayer and those that function through an interactive process with components of the underlying immune system [56]. In the latter case, IEC can be defined as playing an important role in the natural immune system, which mediates the non-specific acute inflammatory response [13, 62]. This review attempts to outline some of the mechanisms by which IEC participate in the innate immune response of the intestine (barrier function, mucus secretion, antibacterial peptide synthesis, and participation in the cytokine/chemokine network) and to review what is currently known for porcine IEC.

2. ROLE OF IEC IN THE BARRIER FUNCTION OF THE INTESTINAL EPITHELIUM

The intrinsic mechanisms of immunity are derived from the presence of a physical barrier formed by the specialised epithelial cells. Epithelial integrity is critical in maintaining a physical but selective barrier between external and internal environments. This barrier function is maintained by well-organised intercellular structures including tight junctions, adherence junctions and desmosomes surrounding the apical region of epithelial cells [23]. Tight junctions of epithelial cells consist in a narrow belt-like structure in the apical region of the lateral plasma membrane wrapped around each cell, adjoining its neighbours. Cell-cell adhesion at this junction is mediated by the interaction of the multiple membrane-spanning proteins claudin and occludin, proteins protruding from the plasma membrane of adjacent IEC. Other proteins, such as Zonula Occludens-1 (ZO-1), ZO-2 and cingulin are also involved in the regulation of tight junctions. Adherence junctions consist of interactions mediated by the homophilic adhesion of single membrane-spanning proteins E-cadherin [24] and des-

mosomes at points where extra-cellular and intra-cellular filaments co-associate.

The trans-epithelial electrical resistance (TEER) of cell monolayers can be considered as a good indicator of the degree of organisation of the tight junctions within the cell monolayer and epithelial integrity [25]. As indicated by a decreased TEER, several physical and chemical factors can dynamically alter the tight junctions. These include internal factors such as hormones, neurotransmitters, proteases, cytokines, but also food-derived contaminants, bacterial toxins, mycotoxins and xenobiotics [6, 33].

The pig tight junction has been poorly characterised; this is mainly due to the lack of available porcine epithelial cell lines. To our knowledge, only three porcine intestinal epithelial cell lines (namely IPI-2I, IPEC-1 and IPEC-J2) have been characterised [22, 29, 57]. Among them only the IPEC-1 line developed a TEER upon *in vitro* culture [1, 7] as observed in human IEC lines. *Ex vivo* studies, based on the TEER measurement of pig intestinal samples mounted in Ussing chambers, have indicated that total parenteral nutrition adversely affects gut barrier function in neonatal piglets [30]. However in these animals the decreased TEER was not associated with an increased bacterial translocation or a change in Claudin-1, occludin and ZO-1 expression [30]. In a model of gut injury, it has been demonstrated that prostaglandins mediated the recovery of barrier function through an effect on the regulation of the assembly of tight junction by phosphatidylinositol 3-kinase [5, 35].

3. INVOLVEMENT OF IEC IN MUCUS LAYER PROTECTION

The mucus layer is part of the various protection systems developed at the intestinal epithelial cell level. It is composed of mucins associated with other proteins and lipids. It forms a continuous gel into which a bicarbonate-rich fluid is secreted, maintaining a neutralising pH at the epithelial

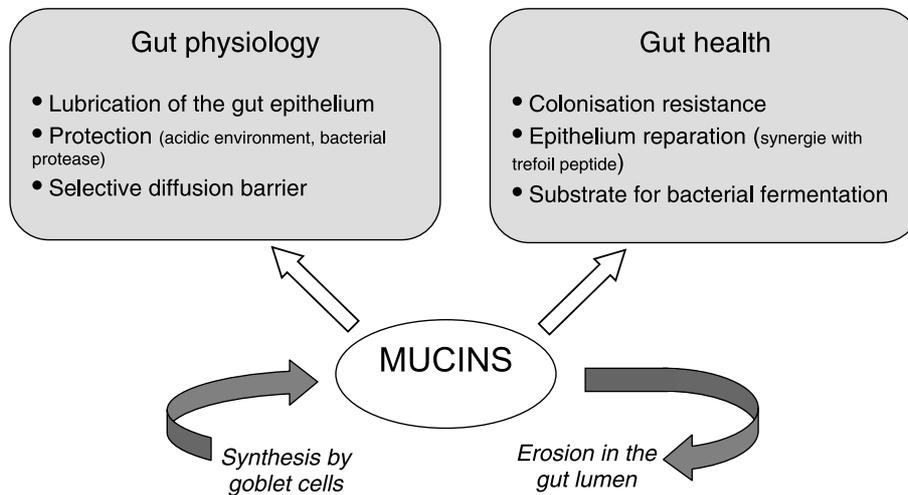


Figure 1. Functional properties of digestive mucins (adapted from [44]).

surface. Mucin subunits are comprised of a protein backbone and a large number of carbohydrate side chains that contain neutral sugars including hexosamines. Mucins are usually subdivided into two groups: the membrane-anchored (gel-forming) and the secreted mucins (non gel-forming) groups [11]. They are synthesised and secreted by Goblet cells through baseline secretion and active exocytosis [52]. The tight adherence of mucin to the apical surfaces of the epithelium is due to a specific complex between mucin oligosaccharides and a mucin binding protein on the apical side of epithelial cells.

The mucus layer is in a dynamic balance between mucin synthesis and secretion from goblet cells of the underlying epithelium and erosion on the luminal side releasing mucin into the gut lumen. Many substances, including food contaminants, hormones, neuropeptides, and inflammatory mediators such as cytokines and lipids, can regulate mucin synthesis and induce its exocytosis [10, 52].

Mucins are involved in gut physiology since they constitute a selective diffusion barrier permeable to nutrients and not to

macromolecules (Fig. 1). They also participate in lubrication of the gut epithelium and in its protection against an acidic environment and proteases from endogenous or bacterial origin [44]. Because of their ability to fix commensal bacteria, their synergistic action with trefoil peptides in epithelium repair and their interaction with other protective components such as IgA, various growth factors or cytokines, mucins are also involved in gut health [44]. Mucins are poorly digested before reaching the large intestine, where they are fermented by enteric bacteria and thus influence the gut microbial environment (Fig. 1).

Pig mucins have been characterised at the genomic and biochemical levels [14, 16, 55, 73]. Many dietary factors including fibre, protein and anti-nutritional factors have been shown to influence secretion of mucin and/or erosion of the mucus in the pig intestine [43, 50, 72]. Fibres increase endogenous protein synthesis as well as excretion of mucin at the terminal ileum [43, 50]. They mainly act through an abrasive action, scraping mucin from pig mucosa as it passes down the gut wall [32]. In addition to this physical aspect, fibres may participate in mucin erosion through

their effect on the activities and distribution of proteolytic enzymes in the intestinal lumen. They also increase mucin content and/or composition through another indirect mechanism involving short-chain fatty acids, which are formed in the colon following the fermentation of dietary fibre by bacteria [71]. Although less intensively studied than dietary fibre, protein has also been shown to increase the recovery of mucin in the pig intestine [36]. Finally, anti-nutritional factors such as tannin, lectin and protease inhibitors are other dietary compounds with the ability to increase the recovery of endogenous protein including mucin [34]. If the mechanism by which tannin modulates mucin secretion remains unknown, the ability of lectin to bind to glycoprotein at the surface of intestinal cells may account for decreased mucin hydrolysis by digestive enzymes in the small intestine. Alternatively, lectin could act through its ability to induce the release of histamine, which is a known mucus secretagogue [44].

Microbial infections could also modulate mucin secretion (review in [10]). In pigs, mostly parasitic infections such as *Oesophagostomum dentatum* or *Trichinella spiralis*, have been documented to increase mucin accumulation in the proximal and the distal colon [53, 70]. This increased mucin concentration may relate to the production and release of short-chain fatty acids in the large intestine [58] and also to a more rapid secretion of goblet cell mucins as suggested by the lower volume of mucin granules in the caecum and proximal colon of infected animals [53]. Mucins may also serve as a mechanism of defence for parasitic infection by binding and entrapment of helminthes [41, 69].

4. ROLE OF IEC IN THE SECRETION OF DEFENSINS AND OTHER ANTIBACTERIAL PEPTIDES

Another mechanism of mucosal defence is the secretion of agents exhibiting anti-

microbial properties. Antimicrobial peptides act by disrupting the integrity of the microbial membranes through their net-positive charge and their ability to fold into amphipathic structures. In mammals, two main families of antimicrobial peptides can be distinguished: the defensins (α , β and θ type) and the cathelicidins. Recent works have also established angiogenins as an important family of endogenous antimicrobial proteins [26]. The defensin structure is based on a common β sheet core stabilised by three disulfide bonds; the structure of cathelicidins is very diverse. All mammals examined so far produce cathelicidins [75]. They are mainly produced by myeloid-derived cells and β -defensins are synthesised by epithelial cells lining the respiratory, gastrointestinal and urogenital tracts [15]. The α -defensins, which have only been identified in humans, monkeys and rodents, are also expressed by different cell types, including granule-containing granulocytic leukocytes and intestinal Paneth cells. Defensins are direct effectors of antimicrobial activity, but they also regulate innate and adaptive antimicrobial immunity. Indeed, they enhance phagocytosis, promote neutrophil recruitment, enhance the production of pro-inflammatory cytokines, suppress anti-inflammatory mediators and regulate complement activation; they can also be chemokines and adjuvants (reviewed by [74]). They also facilitate the maturation as well as antigen uptake of dendritic cells.

In pigs, currently more than a dozen distinct anti-microbial peptides have been identified and a majority belong to the cathelicidin family [79].

PR-39 was isolated originally from bulk homogenates of porcine small intestines [2] but further studies demonstrated that this cathelicidin was derived from neutrophils and not from intestinal epithelia [68]. PR-39 is active mainly against gram-negative bacteria [2, 63] and increases significantly in pig sera during the onset of salmonellosis [76]. In addition, PR-39 specifically attracts polymorphonuclear cells [27], accumulates

in wound fluid [20] and inhibits the assembly of the phagocyte NADPH oxidase complex thereby limiting the production of reactive oxygen species [63]. All of these activities are tightly integrated and finely tuned in pigs during injury, infection, and wound healing.

NK-lysin is another antimicrobial peptide purified from the porcine small intestine based on its antibacterial activity and that has been shown to be a new effector molecule of cytotoxic T and NK cells [3, 66]. This antimicrobial peptide shows activity against various bacteria and fungi, including *Escherichia coli*, *Bacillus megaterium*, *Acinetobacter calcoaceticus*, *Streptococcus pyogenes*, and *Candida albicans* [3]. It lyses certain tumour cells, but not erythrocytes. Antimicrobial and tumourolytic activities of NK-lysin are believed to arise from its interaction with lipids and ability to form pores in the cell membrane because of its alpha-helical structure [3].

The only member of the defensin family identified in pigs thus far is the porcine β -defensin [77]. Although α -defensins are the most abundant anti-microbial peptides in granules of polymorphonuclear cells or intestinal Paneth cells in many mammalian species [79], they have not been found in pigs. The porcine β -defensin mRNA is expressed abundantly in tongue epithelia and to a lesser extent throughout the respiratory and digestive tracts. Despite its resemblance to many inducible beta-defensins in amino acid sequence, gene structure, and sites of expression, the porcine β -defensin gene failed to upregulate in response to both in vitro stimulation and in vivo infection of pigs with *Salmonella typhimurium* or *Actinobacillus pleuropneumoniae* [78]. The constitutive expression of porcine β -defensin in airway and oral mucosa suggests that it may play a surveillance role in maintaining the steady state of microflora on mucosal surfaces. Recombinant porcine β -defensin peptide has potent antibacterial activity against both gram-positive and -negative bacteria as

well as fungi, including *Escherichia coli*, *S. typhimurium*, *Listeria monocytogenes*, and *Candida albicans* [64]. Moreover, a synergy between β -defensin, PR-39 and protegrin 3, in killing bacteria has been demonstrated in pigs [64].

5. ROLE OF IEC IN THE SECRETION OF CHEMOKINES AND CYTOKINES

Cytokines are small peptide molecules, which are important mediators in the regulation of the immune and inflammatory responses. They are produced by cells belonging to the immune system (lymphocytes, macrophages, dendritic cells...) but also by cells not traditionally considered as part of the immune system such as IEC. Indeed the IEC can produce cytokines and chemokines, crucial for the recruitment and activation of immune system cells (Fig. 2). Several cytokines including TGF- α , IL-1, IL-10, IL-15, and IL-18 are constitutively expressed by the IEC and may play a role in the basal influx of immune cells into the mucosa, in epithelial cell growth and homeostasis [65]. Other cytokines such as IL-1 α or β , IL-6, IL-8, TNF- α , MCP-1, CCL20, and GM-CSF are also expressed by normal epithelial cells but are markedly up-regulated in response to microbial infection [28, 65]. These patterns of cytokine expression have been analysed in cell lines derived from the small intestine (IEC-6 murine cells) or the colon (HT-29, T-84 or Caco-2 human cells) and in cultured small intestinal biopsies [4]. Studies have also been performed in vivo in different parts of the intestine of rodents but also of domestic animals [9, 45, 54].

Intestinal epithelial cells are not only able to secrete chemokines that will have an effect on the underlying immune cell functioning but can also respond to chemokines synthesised by themselves or by other immune cells. Indeed, IEC express a number of chemokine receptors at their apical surface. For example, IEC could respond to

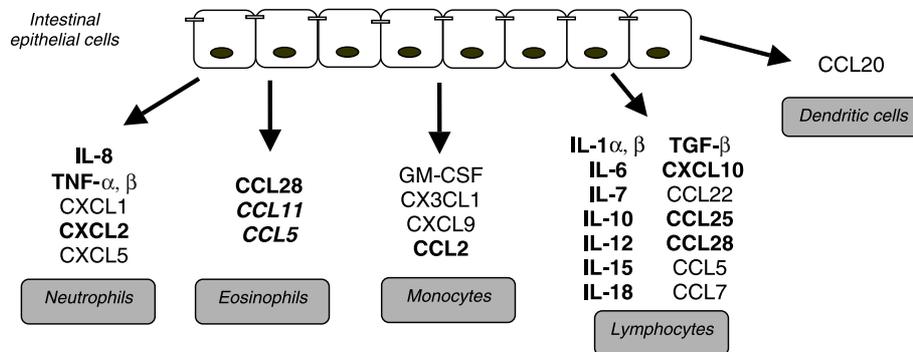


Figure 2. Summary of the cytokines and chemokines produced by intestinal epithelial cells. The usual target of each cytokine is shown although in some cases the same cytokine may act on more than one cell type. The figure includes constitutive and induced cytokine. In bold are indicated the cytokine cloned in the pig, in italic the ones partially cloned (adapted from [65]). IL: interleukin; CCL/CXCL: Chemokine (C-C/C-X-C motif) ligand; TGF: transforming growth factor.

IL-1 via their IL-1 receptors and thus amplify the effects of IL-1 during the inflammatory response [39]. Taken together, these findings indicate that IEC play a pivotal role in local immune responses and that chemokines mediate immune cross-talk between IEC, leukocytes, and adjacent cells in the surrounding mucosal environment.

In pigs many cytokines have been cloned and characterised [18, 46, 47]. For several cytokines, specific antibodies are also available, or cross-reacting antibodies have been identified allowing the determination of the protein [61]. In addition several DNA arrays have been created to specifically study the pig immune system [12, 31, 49]. The rapidly expanding status of cytokine reagents available in swine reflects the current interest in porcine immunology and disease pathogenesis, the potential of pigs as organ donors for xenotransplantation in humans, and the use of swine in biomedical research.

Both physiological and pathological situations have been shown to modify the intestinal secretion/expression of cytokines in pigs. For example, weaning has been associated with a transient increase in inflammatory cytokines such as IL-1 β , IL-6 and TNF- α [37, 38, 54] as well as an increase

of TGF- β [40] in different parts of the pig intestine. Similarly, infection with *Escherichia coli*, *Salmonella* species, *Schistosoma mansoni*, *Toxoplasma gondii* or *Ascaris suum* increased the intestinal expression of inflammatory cytokines [17, 19, 42, 51, 67]. However, in these studies the cytokine expression/synthesis has been analysed in whole intestinal samples and the specific involvement of intestinal epithelial cells relative to other cytokine producing cells (dendritic cells, intraepithelial lymphocytes...) has not been described. The limited availability of porcine intestinal epithelial cell lines has also restricted in vitro studies of the interaction between pathogens and porcine intestinal epithelial cells, since it has largely been documented with human or murine intestinal cell lines [60].

6. CONCLUSION

In this review the importance of IEC in the innate immune response of the intestinal tract has been underlined. Indeed IEC form a physical barrier and secrete a variety of compounds such as mucins, antimicrobial peptides and cytokines that participate in the innate immune defence. In addition,

IEC also take an active part in the induction of adaptive immune surveillance at the mucosal surface. This involves the collaboration of IEC with antigen-presenting and lymphoid cells and occurs mainly in the follicle-associated epithelium. These mucosal adaptative immune mechanisms encompass a complex array of both humoral factors, particularly secretory IgA, and cellular factors, including lymphocytes unique to the gut-associated lymphoid tissue such as intra-epithelial lymphocytes and lamina propria lymphocytes. In pigs, because of the limited availability of intestinal epithelial cell lines, most of the data concerning the role of IEC in the innate and adaptive immune response of the intestine have been obtained from *in vivo* and *ex vivo* experiments. However, given the complex intercellular signalling that occurs within the gut, the coordinated interplay between the different mediators, these experiments are more difficult to interpret than *in vitro* experiments on IEC.

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