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Porcine IPEC-J2 Intestinal Epithelial Cells in Microbiological Investigations

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Abstract

IPEC-J2 cells are porcine intestinal columnar epithelial cells that were isolated from neonatal piglet mid-jejunum. This cell line forms polarized monolayers with high transepithelial electrical resistance when cultured on 0.4 µm pore-size filters. The cell line is unique in that it is derived from small intestinal tissue (compared to the common human colon-derived lines HT-29, T84, and Caco-2) and is not transformed (compared to the porcine small intestinal line, IPI-2I). Porcine intestinal epithelial cells more closely mimic human physiology than analogous rodent cell lines (e.g. IEC-6 or IEC-18), which is important in studies of zoonotic infections; in addition, they provide specificity to study porcine-derived infections. IPEC-J2 cells are increasingly being used in microbiological studies to examine the interactions of various animal and human pathogens, including *Salmonella enterica* and pathogenic *Escherichia coli,* with intestinal epithelial cells. The IPEC-J2 cell line has also been employed in some probiotic studies, in which the cells have been used as an initial screening tool for adhesiveness and anti-inflammatory properties of the potential probiotic microorganisms. The validity of these studies is not clear as follow-up studies to assess the efficacy of the probiotics in vivo have not been published to date. The aims of this review are to provide a comprehensive overview of the microbiological studies that have been conducted with IPEC-J2 cells and a reference guide of key cellular and immune markers that have been identified in this cell line that may prove to be useful in future studies.

Keywords

IPEC-J2; porcine intestinal epithelial cell; cell line; *Salmonella enterica*; *Escherichia coli*

Introduction

IPEC-J2 is a non-transformed columnar epithelial cell line that was isolated from neonatal piglet mid-jejunum in 1989 by Helen Berschneider at the University of North Carolina (Berschneider, 1989). Although IPEC-J2 cells grown in monolayers were first employed in transepithelial ion transport and cellular proliferation studies (Kandil et al., 1995; Rhoads et al., 1997; Rhoads et al., 1994), this primary cell line has been used increasingly to characterize epithelial cell interactions with enteric bacteria and viruses. The results of these

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investigations have provided important insights into the initial host responses to pathogenic and nonpathogenic (e.g. commensal or probiotic) microorganisms.

Properties of cultured cells

In many studies, IPEC-J2 cells have been propagated in 50% Dulbecco's Modified Eagle's Medium (DMEM) and 50% Ham's F12 Nutrient Mixture containing 5% fetal bovine serum, 1% insulin/transferrin/sodium selenite media supplement (Invitrogen, Carlsbad, CA), 5 ng/ml epidermal growth factor, and 1% penicillin/streptomycin. However, the use of antibiotics in the cell culture medium has been reported to suppress IPEC-J2 cell growth (Schierack et al., 2006). The cells become polarized, express tight junction proteins (e.g. claudins type 3 and 4, occludin), and form tight junctions when grown on 0.4 μm pore-size Transwell® filters; these cells form a single confluent monolayer manifesting high transepithelial electrical resistance (> 1 kΩ). Areas of stratification have been reported (Schierack et al., 2006). In agreement with earlier experiments by Schierack et al. (2006), a recent investigation has determined that IPEC-J2 cells grown on Transwell®-COL collagen-coated permeable supports manifest optimum transepithelial resistance and culture characteristics (Geens and Niewold, 2011a). Microvilli have been observed to form after maximum trans-epithelial resistance has been attained (Geens and Niewold, 2011a; Schierack et al., 2006). Although IPEC-J2 cells may possess a glycocalyx, they do not produce mucus (Schierack et al., 2006; van der Aa Kuhle et al., 2005). Important epithelial cell markers that have been detected in IPEC-J2 cells are listed in Table 1.

IPEC-J2 cells in microbiological investigations

IPEC-J2 cells were first employed as a model of the porcine small intestine in a study of the obligate intracellular pathogen *Lawsonia intracellularis*, the causative agent of porcine proliferative enteropathy. The bacterium was found to internalize initially within membrane-bound vacuoles and subsequently reside in the cell cytoplasm, similar to what was observed after its infection of the intact porcine intestinal mucosa (McOrist et al., 1995). *L. intracellularis* infection was relatively greater in an ileum-derived rat enterocyte cell line (IEC-18) than in IPEC-J2 cells, a finding attributed to microbial tropism for the ileal-colonic epithelium (McOrist et al., 1995).

There has been a steadily increasing use of these cells to investigate epithelial innate immune responses to a wide variety of microorganisms. These studies will be summarized in the sections below as various infection models are discussed. Complementing these investigations, the expression of several immune molecules in the cells has been examined, although detection of some mediators between investigative groups has been varied (Table 2).

Salmonella enterica studies

In their initial characterization of IPEC-J2 cells, Schierack et al. (2006) demonstrated that the cells support invasion of *S. enterica* serovars Typhimurium (*S.* Typhimurium) and Choleraesuis (*S.* Choleraesuis). These bacteria could be seen replicating in intracellular vacuoles as has been observed in other epithelial cell models of *Salmonella* infection. *S.* Typhimurium has also been shown to invade and replicate inside polarized IPEC-J2 cells better than in the non-polarized porcine intestinal cell line IPI-2I (Boyen et al., 2009). *S.* Typhimurium is internalized in IPEC-J2 cells within two minutes after bacterial exposure to the apical aspects of cell monolayers, and a rapid increase in the numbers of internalized bacteria can be detected between 15 and 60 minutes. Internalization of *S.* Typhimurium was not dependent on the GTPase Rac 1, but was decreased in the presence of both the *Rho* GTPase inhibitor mevastatin and the actin inhibitor cytochalasin D (Brown and Price, 2007). Additionally, the growth phase of *S.* Typhimurium appears to be a factor in the efficiency of

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internalization into IPEC-J2 cells (Schmidt et al., 2008). A DT104 field isolate of *S.* Typhimurium, as well as two reference strains of *S.* Typhimurium were used to demonstrate that recovery of intracellular bacteria from IPEC-J2 cells was greater for organisms in the mid-log phase of growth compared to the stationary growth phase. These results were replicated in porcine ileal explants (Schmidt et al., 2008).

Virulence factors elaborated by *Salmonella* species mediate bacterial invasion of IPEC-J2 cells. The importance of *Salmonella* pathogenicity island-1 (SPI-1) in bacterial invasion of porcine intestinal epithelial cells has been demonstrated through the use of three separate SPI-1 *S.* Typhimurium mutants. Mutations in *hilA* (a SPI-1 regulatory protein), *sipB* (a translocator/effector protein), and *sipA* (an effector protein) displayed decreased invasion in IPEC-J2 cells compared to wild-type *S.* Typhimurium (Boyen et al., 2006). Interestingly, the *sipA* mutant manifested an invasion defect in the polarized IPEC-J2 cells, but not in non-polarized IPI-2I porcine intestinal cells, confirming a similar phenomenon seen with polarized and non-polarized human cell lines. In addition, a *rfaC* mutant of *S.* Typhimurium with a defective lipopolysaccharide (LPS) core and subsequent impaired flagellar function invaded IPEC-J2 cells less efficiently compared with the wild-type strain (Crhanova et al., 2011).

Production of immunomodulatory factors by IPEC-J2 cells in response to *Salmonella* species has been examined by multiple research groups. Both *S.* Typhimurium and *S.* Choleraesuis elicited vectorial interleukin (IL)-8 and macrophage inflammatory protein (MIP) -3α secretion from IPEC-J2 cells, as well as in ileal explants obtained after oral inoculation of swine with *Salmonella* (Skjolaas et al., 2006). Interestingly, secretion of IL-8 was delayed in response to *S.* Choleraesuis (6 hrs vs. 3 hrs) and was apically polarized, compared to the more rapid basolateral response induced by *S.* Typhimurium. *S.* Typhimurium elicited increased expression of IL-8 and MIP-3α mRNA compared to *S.* Choleraesuis (Skjolaas et al., 2007). In addition, *S.* Typhimurium induced a significant mRNA increase in TNF-α after only 1.5 hours of exposure; this appears to correlate with TNF-α secretion from IPEC-J2 cells, although there is a constitutive basolateral secretion of this cytokine in uninfected cells (Burkey et al., 2009). Expression of porcine β-defensins (pBD) by IPEC-J2 cells in response to infection by these two serovars of *S. enterica* has also been examined. After a 24 hour infection of cells with *S.* Typhimurium, mRNAs encoding pBD1 and pBD2 were increased; *S.* Choleraesuis, by comparison, produced only a small increase in pBD2 mRNA (Veldhuizen et al., 2009).

Several research groups have reported the expression of pathogen recognition receptors in IPEC-J2 cells (Table 3). Expression of Toll-like receptors (TLRs) 2, 3, 4, 5, 6, 8, 9, and 10 in IPEC-J2 cells has been noted (Arce et al., 2010; Burkey et al., 2009; Devriendt et al., 2010; Liu et al., 2010b; Mariani et al., 2009). *S.* Typhimurium, but not *S.* Choleraesuis or purified LPS, increased TRL2 mRNA levels in IPEC-J2 cells and porcine intestinal explants (Burkey et al., 2009). We have confirmed this finding in IPEC-J2 cells using a DT104 field isolate of *S.* Typhimurium (Brosnahan, unpublished results). *S.* Typhimurium also induced an increase in TLR4 mRNA expression in porcine gut explants, but this effect was not seen in infected IPEC-J2 cells (Burkey et al., 2009). It is important to note that the TLR expression profiling done in porcine gut explants by Burkey et al. included RNA from whole intestinal tissue and not just from intestinal epithelial cells, which may contribute to the differences seen in TLR expression between IPEC-J2 cells and explants. The authors also noted that TLR4 appeared to be constitutively expressed at a higher level in IPEC-J2 cells than the other TLRs examined, which may also contribute to the differences seen. Until recently, IPEC-J2 cells were thought not to express TLR5 (Mariani et al., 2009). However Devriendt et al. demonstrated TLR5 protein expression by Western blot analysis and showed that purified flagellin (isolated from *E. coli*) could induce IL-6 and IL-8 from IPEC-J2 cells,

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presumably through an interaction with TLR5 (Devriendt et al., 2010). We have confirmed TLR5 expression in these cells by mRNA analysis (Brosnahan, unpublished results).

Escherichia coli studies

Adherence of *E. coli* wild-type and mutant strains has been widely studied in IPEC-J2 cells using a variety of pathogenic strains. Certain strains of enteropathogenic *E. coli* (EPEC) were shown to form attaching and effacing lesions on IPEC-J2 cells, based on actin accumulation in the cells and the formation of bacterial microcolonies (Schierack et al., 2006). Multiple studies have demonstrated that F4 fimbriae (K88) is important for adherence of enterotoxigenic *E. coli* (ETEC) to IPEC-J2 cells. Both ETEC expressing F4 fimbriae, as well as purified fimbriae, were shown to be capable of binding IPEC-J2 cells (Johnson et al., 2009a; Koh et al., 2008). Additional support for the importance of F4 fimbriae in ETEC infection of IPEC-J2 cells comes from Geens and Niewold, who used isogenic strains to shown that only the strain possessing the F4 fimbriae was able to efficiently bind IPEC-J2 cells (Geens and Niewold, 2011b). Adherence of F4+ ETEC to IPEC-J2 cells was shown to be more efficient with a stable polymeric form of the adhesin (Devriendt et al., 2010). Furthermore, purified F4 fimbriae were shown to be internalized via a clathrin-mediated endocytosis pathway, resulting in transcytosis of the fimbriae (Rasschaert et al., 2010). The results of these studies using the IPEC-J2 cell line may be useful in determining how oral inoculation of piglets with purified F4 fimbriae results in a protective immune response to further challenge with F4+ ETEC, which would be extremely valuable for the swine production industry.

Conversely, two research groups have indicated that IPEC-J2 cells do not express the F18 fimbrial receptor as F18+ ETEC strains do not bind the cells (Koh et al., 2008; Rasschaert et al., 2010). F18 receptor expression in swine has been correlated with older pigs (3-23 weeks old) (Bardiau et al., 2010), however Koh et al. demonstrated that F18+ ETEC did bind another porcine small intestinal cell line, IPEC-1 cells, which were also isolated from a day old piglet (Koh et al., 2008). It is possible that F18 receptor expression occurs earlier in the ileum as the IPEC-1 cells were from a mixture of ileal and jejunal tissue, whereas IPEC-J2 cells were isolated from only jejunal tissue (Gonzalez-Vallina et al., 1996). Another study, however, demonstrated that *E. coli* O139 isolates from pigs were capable of binding IPEC-J2 cells (Sonntag et al., 2005), which is interesting because this serotype is commonly associated with the expression of F18 fimbriae (Imberechts et al., 1994). Sonntag et al. did in fact amplify the gene for the major subunit of F18, FedA, however further work elucidating the role of F18 in adherence to the cells was not performed. It is important to note that PCR amplification of *fedA* can occur in the absence of F18 expression (Imberechts et al., 1992), therefore it is possible that these porcine isolates were expressing a different adhesin that was responsible for mediating attachment to the IPEC-J2 cells.

FimH (a component of type I fimbriae) has also been studied in relation to IPEC-J2 cell adherence, however this study was done using avian pathogenic *E. coli* (APEC), which would not normally colonize pigs nor intestinal epithelium so the relevance of this study is unclear (Musa et al., 2009).

Some ETEC strains at low cell densities (~10^5 colony forming units, CFUs) induced phosphatidylserine expression and arrested metabolic activity, signs indicative of the early stages of apoptosis (Johnson et al., 2009b). This cellular damage was dependent on ETEC strains that bound the cells efficiently, and the damage appeared to increase subsequent adherence of ETEC to the cells. Trans-epithelial electrical resistance across IPEC-J2 cell monolayers was also reduced by ETEC infection, in the absence of observable changes in the tight junction proteins occludin, zonula occludens-1 (ZO-1), and claudin-1 (Johnson et

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al., 2010). Higher densities (~10^8 CFUs) of ETEC were shown to kill IPEC-J2 cells, possibly through a quorum sensing-dependent mechanism (Zhu et al., 2011).

Toxins have also been thoroughly studied using IPEC-J2 cells; most importantly their roles in bacterial adherence have been documented. Heat-labile enterotoxin (LT) made by ETEC was capable of increasing bacterial adherence to IPEC-J2 cells. This was attributed, at least partly, to the toxin's ability to increase cAMP levels in host cells (Johnson et al., 2009a). Shiga toxin 2e (Stx2e) -producing *E. coli* strains (STEC) isolated from piglets bound IPEC-J2 cells, whereas STEC isolates from humans did not, underscoring the usefulness of this cell line for species-based studies (Sonntag et al., 2005). In support of this host specificity, porcine ETEC strains were shown to bind to porcine gut epithelial cell lines (e.g. IPEC-J2 and IPEC-1) better than to human-derived intestinal INT-407 cells (Koh et al., 2008). These authors also noted that polarization of the IPEC-J2 cells did not affect the binding patterns compared to the same cells when not polarized. Additionally, some strains of human-derived ETEC, but not porcine-derived ETEC, seem to be inhibited by a heat-stable factor that is secreted by IPEC-J2 cells (Brown et al., 2007). This factor was only generated by the cells when they were grown in medium supplemented with serum, and it seemed to be specific for ETEC, as the attachment of multiple enterohemorrhagic *E. coli* (EHEC) O157:H7 strains remained unaffected.

Shiga toxin (verotoxin) was shown to play a role in adherence of EHEC O157:H7 to IPEC-J2 cells and porcine ileal loops (Yin et al., 2009a). Shiga toxin may also act to induce higher levels of β1-integrin in IPEC-J2 cells, which may mediate the increased adherence seen with Shiga toxin-positive strains of EHEC (Liu et al., 2010a).

Purified heat-stable enterotoxin b (STb) from ETEC was shown to associate with IPEC-J2 cells, but was not readily internalized despite the presence of sulfatide, a known STb receptor, on the surface of the cells (Albert et al., 2011). Additionally, a polysaccharide isolated from marine algae, λ-carragenan, which is already used in the food industry as a thickener, was shown to inhibit STb-induced permeabilization of IPEC-J2 cells (Goncalves et al., 2008). The authors speculated that this compound could be given to piglets in their feed during the period of time when they are most susceptible to the effects of STb to prevent diarrheal disease.

Studies with other pathogens

IPEC-J2 cells support infection and proliferation by various species of *Chlamydia* (Schierack et al., 2006). Two viral infection models have also been established in the cell line: vesicular stomatitis virus (VSV) (Botic et al., 2007) and rotavirus (Liu et al., 2010b). Similar to studies done with bacterial strains derived from different hosts, Liu et al. demonstrated that a porcine rotavirus strain more readily infected the IPEC-J2 cells than a human-derived strain (Liu et al., 2010b).

Recently, IPEC-J2 cells were used to demonstrate that high concentrations of deoxynivalenol, a toxin made by the fungus *Fusarium*, reduced the viability of porcine intestinal epithelial cells, especially when applied basolaterally (Danicke et al., 2010; Diesing et al., 2011a; Diesing et al., 2011b). This is significant because this toxin often contaminates cereal grains, which when fed to pigs leads to diarrhea and weight loss.

In an attempt to study oral transmission of prion-based diseases, IPEC-J2 cells were shown to only partially support co-localization of PrP^{BSE} (from bovine spongiform encephalopathy) with the 37kDa/67kDa laminin receptor LRP/LR, a known receptor for PrP, on the epithelial cell surface (Kolodziejczak et al., 2010). The cells did not support co-localization of either PrP^{CWD} (from chronic wasting disease) or ovine PrP^{SC}. Since the

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IPEC-J2 cells express laminin receptors at a relatively low level, higher receptor expression on enterocytes in vivo may be required for oral transmission.

Probiotic studies

The IPEC-J2 cells have been increasingly used to study potential probiotic microorganisms. These studies generally focus on either the adhesiveness of the probiotic to the cells or the ability of the probiotic to inhibit pathogen-induced inflammatory responses.

Ten animal-derived *Enterococcus* strains, which are commonly used in livestock animals as probiotics, demonstrated 2-4% adhesion rates (number adhered compared to number added) on IPEC-J2 cells (Marcinakova et al., 2010). The authors also noted that the addition of 200 mM calcium increased the adhesiveness of all strains. Field isolates of *Lactobacillus plantarum* isolated from olives were also shown to adhere to IPEC-J2 cells (Bevilacqua et al., 2010). Certain low-adhesive *Saccharomyces* strains, isolated from food products, were shown to inhibit pathogenic *E. coli*-induced IL-1α secretion from the cells, indicating that high adhesion of the probiotic may not be necessary to exert beneficial effects (van der Aa Kuhle et al., 2005). Supporting this theory, multiple species of *Lactobacillus* inhibited EPEC O138 adherence roughly to the same extent despite differences in binding efficiencies of the lactobacilli (Larsen et al., 2007). These authors speculated that that the inhibition was likely due to steric hindrance, but did not consider the potential anti-inflammatory actions of the lactobacilli in their system. Conversely, the widely-used probiotic *E. coli* Nissle 1917 was shown to inhibit adhesion and subsequent invasion of IPEC-J2 cells by *S.* Typhimurium in a manner that was dependent on the ability of the probiotic to adhere to the monolayer (Schierack et al., 2011). *E. coli* mutants deficient in F1C fimbriae or flagellae that were unable to properly adhere to the cells were also unable to inhibit *S.* Typhimurium infection.

Instead of examining adhesiveness of potential probiotics, other research groups have investigated the ability of a potential probiotic to inhibit the inflammatory response to pathogenic organisms. Pre- or co-incubation with a lab strain of *Bacillus licheniformis,* or with various *B. licheniformis* and *Bacillus subtilis* strains isolated from the commercial feed product BioPlus 2B, significantly inhibited IL-8 secretion from IPEC-J2 cells in response to *S*. Typhimurium infection (Skjolaas et al., 2007). Varying results have been obtained with the potential probiotic *Lactobacillus reuteri*: a porcine-derived strain was reported to have no effect on the IL-8 response of IPEC-J2 cells to *S*. Typhimurium infection (Aperce et al., 2010; Skjolaas et al., 2007), however three human-derived *L. reuteri* strains were reported to inhibit IL-8 responses of IPEC-J2 cells to purified LPS (Liu et al., 2010c).

Probiotics have also been studied for their anti-viral effects in IPEC-J2 cells. *Bifidobacterium* and *Lactobacillus* species were shown to reduce the infectivity of VSV when incubated prior to or simultaneous with virus addition to IPEC-J2 cells (Botic et al., 2007). Following along these lines, a rotavirus infection model in the IPEC-J2 cells was developed in order to examine how *Lactobacillus* probiotics affected the infectivity of, and the immune response to, the virus. The authors demonstrated that preincubation of the cells with *Lactobacillus acidophilus* actually increased rotavirus viral titers, as well as the IL-6 response of the cells to the viral infection, whereas *Lactobacillus rhamnosus* GG, administered after infection with rotavirus, was capable of decreasing the IL-6 response (Liu et al., 2010b).

IPEC-J2 cells appear to provide a great tool for initial screening of potential probiotics, allowing research groups to analyze adherence and/or anti-inflammatory actions of the organisms being tested in the absence or presence of pathogens. The validity of these studies remains to be seen as articles that use the tested probiotics in vivo have yet to be published.

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Concluding remarks

The development of useful models to study the interaction of bacterial, viral, and fungal pathogens and their toxins with host cells is becoming increasingly important. Cell lines allow researchers to characterize host:microbe interactions at the most basic level, which can inform higher-level studies involving tissue explants, whole organ systems, and living organisms.

The scarcity of intestinal epithelial cell lines that are not derived from tumors, can form a polarized monolayer of columnar epithelial cells, and adequately support infections by a wide variety of microorganisms highlights the important position that the IPEC-J2 cell line holds in microbiological investigations, particularly those in veterinary medicine. In fact, IPEC-J2 cells are one of the few cell lines created from small intestinal tissue from any animal. The majority of human intestinal cell lines (e.g. HT-29, T84, Caco-2) are derived from the colon and most are cancerous. In fact, the only widely available small intestinal human cell line is HUTU-80, which was isolated from the duodenum, but is also cancerous. There are a few small intestinal rat cell lines (e.g. IEC-6 and IEC-18), which are not cancerous, however the translation of results from these lines to other animals is unclear as gastrointestinal structure and function of rodents appear to differ from that of other domestic mammals and humans (Sanger et al., 2011). The two other porcine intestinal epithelial cell lines, IPEC-1 and IPI-2I, have been compared to IPEC-J2 cells elsewhere (Arce et al., 2010; Koh et al., 2008), but can be summarized as follows.

1. IPEC-1 and IPEC-J2 cells are both non-transformed cell lines, whereas IPI-2I cells have been transformed with an SV40 plasmid.
2. Although all three lines were isolated from small intestinal tissues, IPI-2I cells were isolated from ileal tissue; IPEC-J2 cells were isolated from jejunum; and IPEC-1 cells were isolated from a mixture of ileal and jejunal tissue.
3. IPEC-1 and IPEC-J2 cells were both isolated from day old piglets, whereas IPI-2I cells were isolated from an adult boar (Berschneider, 1989; Gonzalez-Vallina et al., 1996; Kaeffer et al., 1993).

One functional difference that it is important to note is that both IPEC-J2 and IPEC-1 cells bound porcine ETEC strains equally, with the exception of F18+ ETEC, which only bound to IPEC-1 cells (Koh et al., 2008). Another functional difference between these lines is that the transformed IPI-2I cells were shown to have a higher level of expression of TLRs after stimulation with LPS in comparison to IPEC-J2 cells (Arce et al., 2010). The IPI-2I cell line also has a high level of constitutive IL-8 expression, making it difficult to interpret changes in expression of this cytokine induced by experimental procedures (Arce et al., 2010; Veldhuizen et al., 2006).

The ability of studies done with IPEC-J2 cells to translate to porcine whole tissue or whole animal studies has been examined in three papers. Our research group demonstrated that *S.* Typhimurium invasion of IPEC-J2 cells and porcine ileal mucosal explants was similar (Schmidt et al., 2008). Pigs orally inoculated with *S.* Typhimurium and IPEC-J2 cells infected with *S.* Typhimurium were compared by Skjolaas et al. (2006). Responses to infection were found to be similar in vitro and in vivo: variable IL-8 and MIP-3α induction, while macrophage migratory inhibitory factor (MIF) and osteopontin (OPN) were both unchanged (presumably due to high constitutive expression of these molecules in the intestinal epithelium) (Skjolaas et al., 2006). TLR expression in the distal ileum of pigs and IPEC-J2 cells was also compared after infection with *S.* Typhimurium; TLR2 was found to be upregulated in response to infection in vitro and in vivo, while TLR9 remained unchanged (Burkey et al., 2009). TLR4, on the other hand, was increased in response to *S.*

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Typhimurium infection in vivo, but no change was noted in IPEC-J2 cells. The authors noted that IPEC-J2 cells had a high level of constitutive expression of TLR4, so it is possible that further upregulation was not possible. Overall, the results obtained with IPEC-J2 cells have strong reproducibility in mucosal explants and in vivo, however Yin et al. described differences in adherence to IPEC-J2 cells and porcine ileal loops for various EHEC mutants, indicating that different model systems may not translate as readily (Yin et al., 2009a; Yin et al., 2009b). Nonetheless, the ability of some EHEC mutants to adhere to IPEC-J2 cells and ileal loops was highly correlated.

Because of the close similarity between swine and human intestinal function, studies with these cells can also provide valuable insights into the pathogenesis of zoonotic enteric infections that also affect humans. For example, many studies have demonstrated a similar IL-8 response to *S.* Typhimurium in IPEC-J2 cells, compared to what McCormick et al. (1993) originally demonstrated in human colonic T84 cells. In response to *S.* Typhimurium infection, the IPEC-J2 cells secreted 1,000-5,000 pg/ml IL-8 basolaterally, while the T84 cells were shown to secrete around 2,000 pg/ml IL-8 basolaterally, therefore indicating a similar response between the different species (pig versus human) and locations (small intestine versus large intestine) of the cell lines (McCormick et al., 1993; Schierack et al., 2006; Skjolaas et al., 2006, 2007). Additionally, the cytokine profile for IPEC-J2 cells that has been shown here (Table 2) is similar to that described by Eckmann et al. (1993) for the four main human intestinal cell lines (HT29, T84, Caco-2, and SW620). All of the cells have been shown to be negative for IL-2, IL-4, and IFN-γ, while mRNA for IL-1α, IL-8, and TNF-α is variable among the human cell lines, but is present in IPEC-J2 cells (Eckmann et al., 1993). Expression of IL-1β and TGF-β is also variable among the human cell lines, and it is unclear whether IPEC-J2 cells are capable of producing these molecules as different research groups have produced contrasting results.

The IPEC-J2 cell line provides researchers with a unique tool: specificity for swine based infection studies, but also a potential model of the intestinal epithelium for comparative investigations of zoonotic infections, in which strains of specific microorganisms (e.g. *Salmonella enterica* serovar Typhimurium) may similarly colonize the small intestines of both pigs and humans. Given the high homology in porcine and human intestinal structure and function, and presumably in enterocytes as well, studies performed with IPEC-J2 cells may provide valuable insights on microbial interactions with the human intestinal mucosa.

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Table 1

Cellular Molecule Expression in IPEC-J2 Cells

Molecule	mRNA	Protein	Reference/s
β1-integrin	+	ND	2009 Yin (RT-PCR, data not shown), 2010a Liu (RT-PCR)
Claudin-1	ND	+	2010 Johnson (IHC, Western blot)
Claudin-3	+	+	2006 Schierack (IHC); 2009 Mariani (RT-PCR); 2011a Diesing (Western blot, IHC)
Claudin-4	+	+	2006 Schierack (IHC); 2009 Mariani (RT-PCR)
Claudin-14	ND	-	2006 Schierack (IHC);
Claudin-16	ND	-	2006 Schierack (IHC);
Cytokeratin 18	+	+	2006 Schierack (RT-PCR, IHC);
Endothelial nitric oxide synthase (eNOS)	ND	-	2010 Bauchart (Western blot)
FABP1 (fatty acid binding protein 1)	-	ND	2011b Geens (microarray, RT-PCR)
FABP2/I-FABP (intestinal fattacy acid binding protein 2)	-	-	2011b Geens (microarray, RT-PCR, ELISA)
Gb3 synthase	+/-	ND	2009 Yin (RT-PCR, negative, data not shown); 2010a Liu (RT-PCR, positive)
Gb4 synthase	+	ND	2009 Yin (RT-PCR, data not shown)
Inducible nitric oxide synthase (iNOS)	ND	+	2010 Bauchart (Western blot)
Keratin 8 (KRT8)	+	ND	2009 Mariani (RT-PCR)
Laminin receptor (37kDa/67kDa LRP/LR)	ND	+	2010 Kolodziejczak (flow cytometry, low expression)
Mammalian target of rapamycin (mTOR)	ND	+	2010 Bauchart (Western blot)
Mucin 1 (MUC1)	+	ND	2009 Mariani (RT-PCR)
Mucin 2 (MUC2)	-	ND	2006 Schierack (RT-PCR);
Mucin 3 (MUC3)	ND	+	2006 Schierack (suggested by periodic acid-Schiff reaction staining of cell membrane); 2010b Liu (ELISA)
Nucelolin	+/-	ND	2009 Yin (RT-PCR, negative, data not shown); 2010a Liu (RT-PCR, positive)
Occludin	+	+	2006 Schierack (IHC); 2009 Mariani (RT-PCR); 2010 Johnson (IHC)
Osteopontin (OPN)	+	ND	2006 Skjolaas (RT-PCR, high constitutive expression)
Sulfatide		+	2011 Albert (IHC)
Villin (VIL1)	+	ND	2009 Mariani (RT-PCR)
Zonula occludens-1 (ZO-1)	ND	+	2010 Johnson (IHC); 2011a&b Diesing (Western blot, IHC)

+, present; -, absent; +/-, detected in some studies, but not in others; ND, not determined.

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Table 2

Immune Molecule Expression in IPEC-J2 Cells

Molecule	mRNA	Protein	Reference(s)
CCL2	-	ND	2009 Mariani (RT-PCR)
CD16b (FCGR3B)	+	ND	2009 Mariani (RT-PCR)
CD47	+	ND	2009 Mariani (RT-PCR)
CD58	+	ND	2009 Mariani (RT-PCR)
GM-CSF (granulocyte macrophage colony stimulating factor)	+	ND	2006 Schierack (RT-PCR); 2009 Mariani (RT-PCR);
ICAM1 (intracellular adhesion molecule)	+	ND	2009 Mariani (RT-PCR)
IFN-γ	-	ND	2006 Schierack (RT-PCR);
IL-1α	+	ND	2005 van der Aa Kuhle (RT-PCR); 2006 Schierack (RT-PCR); 2009 Mariani (RT-PCR)
IL-1β	+/-	ND	2006 Schierack (RT-PCR, negative); 2009 Mariani (RT-PCR, negative); 2010 Arce (RT-PCR, positive); Brosnahan unpublished results (RT-PCR, negative)
IL-2	-	ND	2006 Schierack (RT-PCR); 2009 Mariani (RT-PCR);
IL-4	-	ND	2006 Schierack (RT-PCR);
IL-6	+	+	2006 Schierack (RT-PCR); 2009 Mariani (RT-PCR); 2010 Devriendt (ELISA); 2010b Liu (ELISA); Brosnahan unpublished results (RT-PCR, ELISA)
IL-7	+	ND	2006 Schierack (RT-PCR); 2009 Mariani (RT-PCR)
IL-8	+	+	2005 van der Aa Kuhle (RT-PCR); 2006 Schierack (RT-PCR, ELISA); 2007 Skjolaas (RT-PCR, ELISA); 2009 Mariani (RT-PCR); 2010 Aperce (ELISA); 2010 Arce (RT-PCR); 2010 Devriendt (ELISA); 2010b Liu (ELISA); 2010c Liu (ELISA); 2011 b Geens (microarray, RT-PCR); Brosnahan unpublished results (RT-PCR, ELISA)
IL-10	-	ND	2006 Schierack (RT-PCR); 2009 Mariani (RT-PCR); Brosnahan unpublished results (RT-PCR)
IL-12A (p35)	+/-	ND	2006 Schierack (RT-PCR, negative); 2009 Mariani (RT-PCR, positive)
IL-12B (p40)	+	ND	2006 Schierack (RT-PCR); 2009 Mariani (RT-PCR)
IL-15	-	ND	2009 Mariani (RT-PCR); 2010b Liu (RT-PCR, data not shown)
IL-18	+	ND	2006 Schierack (RT-PCR); 2009 Mariani (RT-PCR)
IRG6 (inflammatory response protein 6, antiviral)	+	ND	2011b Geens (microarray, RT-PCR)
MCP-1 (monocyte chemotactic protein-1)	+/-	ND	2006 Schierack (RT-PCR, negative); 2010 Arce (RT-PCR, positive)
MHC I (major histocompatability complex I)	ND	+	2006 Schierack (flow cytometry);
MHC II (major histocompatability complex II)	ND	-	2006 Schierack (data not shown, flow cytometry);
MIF (macrophage migration inhibitory factor)	+	ND	2006 Skjolaas (RT-PCR, high constitutive expression)
MIP-3α (CCL20)	+	ND	2006 Skjolaas (RT-PCR); 2007 Skjolaas (RT-PCR); 2009 Mariani (RT-PCR);
MyD88 (myeloid differentiation primary response gene 88)	+	ND	2009 Mariani (RT-PCR)
NFκBI (nuclear factor of kappa light polypeptide gene enhancer in B cells 1)	+	ND	2009 Mariani (RT-PCR)

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Molecule	mRNA	Protein	Reference(s)
NOD1 (nucleotide-binding oligomerization domain containing 1)	+	ND	2009 Mariani (RT-PCR)
pBD-1 (porcine beta defensin 1)	+	ND	2005 Sang (RT-PCR); 2009 Mariani (RT-PCR); 2009 Veldhuizen (RT-PCR)
pBD-2 (porcine beta defensin 2)	+	ND	2009 Mariani (RT-PCR); 2009 Veldhuizen (RT-PCR)
pPGRP-L1 (peptidoglycan recognition protein-long 1)	+/-	+	2005 Sang (RT-PCR, IHC, positive); 2009 Mariani (RT-PCR, negative)
pPGRP-L2 (peptidoglycan recognition protein-long 2)	+	+	2005 Sang (RT-PCR, IHC); 2009 Mariani (RT-PCR)
TGF-β1	+/-	ND	2006 Schierack (RT-PCR, negative); 2009 Mariani (RT-PCR, positive)
TGF-β3	+	ND	2009 Mariani (RT-PCR)
TNF-α	+	ND	2006 Schierack (RT-PCR); 2007 Skjolaas (RT-PCR); 2009 Mariani (RT-PCR); 2010 Arce (RT-PCR)
TNF-β	-	ND	2006 Schierack (RT-PCR);

+, present; -, absent; +/-, detected in some studies, but not in others; ND, not determined.

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Table 3

Toll-like Receptor Expression in IPEC-J2 Cells

Molecule	mRNA	Protein	Reference(s)
TLR1	+		2010 Arce (RT-PCR)
TLR2	+	+	2009 Burkey (RT-PCR); 2010 Arce (RT-PCR); 2010b Liu (flow cytometry); Brosnahan unpublished results(RT-PCR)
TLR3	+	+	2010 Arce (RT-PCR); 2010b Liu (flow cytometry); Brosnahan unpublished results (RT-PCR)
TLR4	+	ND	2009 Burkey (RT-PCR); 2009 Mariani (RT-PCR); 2010 Arce (RT-PCR); Brosnahan unpublished results (RT-PCR)
TLR5	+/-	+	2009 Mariani (RT-PCR, negative); 2010 Devriendt (Western blot); Brosnahan unpublished results (RT-PCR, positive)
TLR6	+	ND	2010 Arce (RT-PCR)
TLR8	+	ND	2010 Arce (RT-PCR)
TLR9	+	+	2009 Burkey (RT-PCR); 2010 Arce (RT-PCR); 2010b Liu (flow cytometry); Brosnahan unpublished results (RT-PCR)
TLR10	+	ND	2010 Arce (RT-PCR)

+, present; -, absent; +/-, detected in some studies, but not in others; ND, not determined.