International Pig Topics

Volume 28 Number 2 (2013)

Independent thoughts for independent minds

GUT HEALTH
The importance of intestinal cells.

NUTRITION
Reviewing the nutrient needs of modern pigs.

PERFORMANCE
Piglets benefit from monoglycerides in sow's milk.

ENVIRONMENTAL CONTROL
We look at options from around the world.

SALMONELLA
Control and monitoring tailored to specific needs.

PCV2 VACCINATION
Long lasting protection favours batch homogeneity.

Importance of intestinal cells for gut health – IPEC studies

by R. Davin, Grup de Recerca en Nutrició, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain.

There are basically two types of cell cultures: (a) primary cell cultures and (b) continuous cell lines. Primary cells are directly isolated from a subject that have limited lifespan, whereas continuous cell lines, also referred as established or immortalised, have acquired the ability to proliferate with infinite lifespan.

There are several means to achieve the immortality of a cell line: tumor cells and undifferentiated cells are inherently immortal, otherwise normal cells can be immortalised (confer tumoral ability) adding foreign genes through a virus.

Continuous cell lines form what the scientific community designate as cell lines and are one of the most used and useful models to study the effect of a specific factor (for example feed additives, drugs, micro-organisms) on a specific cell type (epithelial cells, lymphocytes, fibroblasts) at the most basic level.

Cells are seeded in a plate and are grown and maintained at an appropriate temperature, gas mixture and growth media.

IPEC-J2 is a non-transformed epithelial cell line isolated from a neonatal piglet that has been and still is used as a model of experimental challenges with pathogens, such as salmonella, E. coli and other agents.

In this article we explain the use of IPEC-J2 cell line as a study model for feed ingredients and additives in those challenging (infectious) situations.

What are IECs?

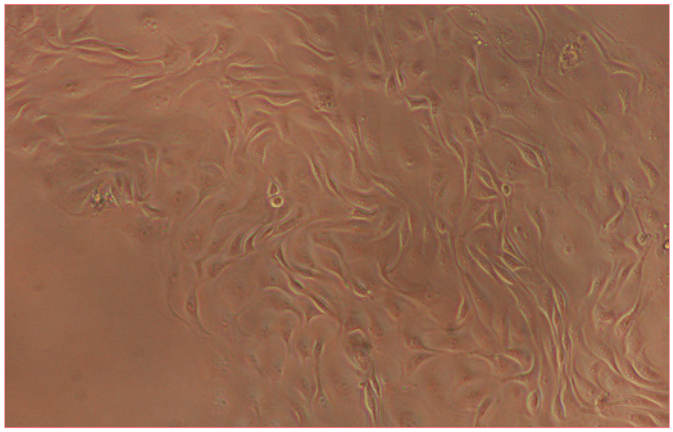
The surface of the intestinal epithelium represents an area of approximately 100m^2. It consists of a single layer of columnar epithelial intestinal cells (IECs) that separates the intestinal lumen from the external environment of the host tissue.

IEC's are involved in multiple functions of the digestive system, such as the absorption of nutrients and water, exchange of electrolytes and hormone production.

Because of its permanent exhibition to the external environment, IECs are of key importance for maintaining the integrity of the intestinal epithelium and reducing the risk of invasion by external agents, forming a physical barrier. They are also crucial for the maintenance of intestinal homeostasis.

Epithelial intestinal cells help on the physical barrier function due to two physical structures of the epithelium (microvillus and tight junctions) that separates the external environment of the organism, preventing the invasion of micro-organisms. IECs also secrete a large variety of antimicrobial peptides, cytokines and chemokines that are activated in pathological or infectious situation enhancing the inflammatory response.

Fig. 1. IPEC-J2 cells attached at the well-plate surface.



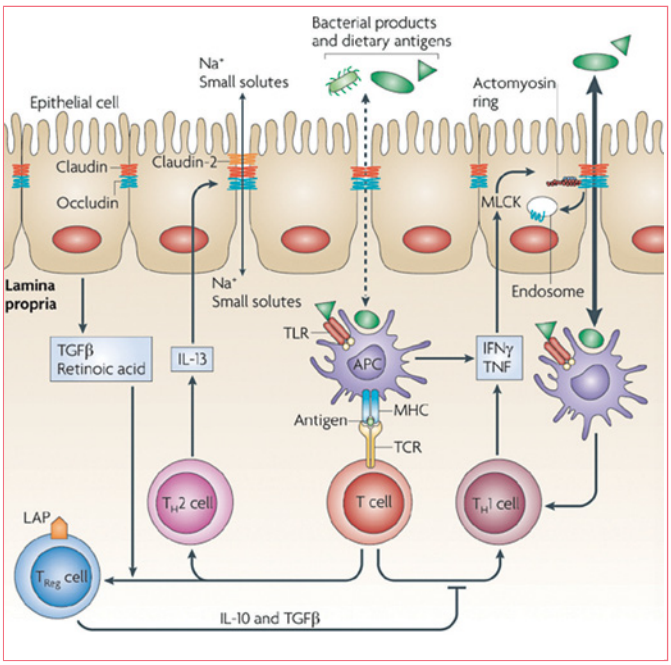


Fig. 2. Barrier function of IECs (J. R. Turner, 2008).

During an infection, when a pathogen breaches the intestinal mucosa it is recognised by toll-like receptors (TLRs). TLR are expressed at the intestinal surface playing a key role in the innate immune system, since they activate immune cell responses.

Between the IEC there are globet cells that secrete mucus. This mucus contains mucins that form a permeable barrier that protects and repairs the intestinal epithelium of any damage. Mucins can also interact with bacteria and have the ability to immobilise and eliminate them through the movements of the gut.

Intestinal cell lines

Most research has been conducted in human intestinal cell lines that are derived from a cancerous colon (for example HT-29, T84, Caco-2) or duodenum (HUTU-80). Also in small intestinal rat cell lines (for example IEC-6 and IEC-18), which are not cancerous.

However, the structure and function of the gastrointestinal tract of rodents appear to differ from that of other domestic mammals like swine.

Nowadays, there are three different porcine intestinal epithelial cell lines:

- IPI-2I cells were isolated from ileal tissue of an adult boar and were transformed with a plasmid that immortalises the cells.
- IPEC-1 cells were isolated from a mixture of ileal and jejunal tissue of a day old piglet. It is a non-transformed cell line.
- IPEC-J2 cell line was isolated from jejunal tissue of a day old piglet and is non-transformed.

The lack of intestinal epithelial cell lines not derived from tumors and the specificity for swine stands the importance of IPEC-J2 cell line, further considering that it can appropriately support infections by a large variety of micro-organisms. Most

Continued on page 9

Continued from page 7
importantly, because it has been demonstrated that results of studies performed with IPEC-J2 cells can be translated to porcine whole animal studies.

How IPEC are affected

IPEC-J2 has become more used since the characterisation done by Shierack et al., 2006. Since then, more than 15 articles have described the expression of different cellular and immune molecules, and more than 20 articles have used them as a model of the intestinal epithelium for micro-organism infections.

Before its characterisation, IPEC-J2 cells were used as a model of porcine proliferative enteropathy (Lawsonia intracellularis) infection because it internalises and proliferates on them, as it was also observed in chlamydia challenges.

When Shierack et al., 2006 characterised IPEC-J2 cells, they demonstrated that the IPEC also support invasion of different species of salmonella and adhesion of E. coli.

The ability of these bacteria to induce an inflammatory and immune response (for example expression of cytokines and TLRs) has been repeatedly demonstrated in following studies with IPEC-J2. It has also been shown that the permeability of IPEC-J2 monolayers (measured by trans-epithelial electrical resistance; TEER) is reduced by enterotoxigenic Escherichia coli (ETEC).

Escherichia coli, salmonella and chlamydia are important pathogens in the swine industry as well as representatives with highly different infection strategies; that is one of the most important reasons why IPEC-J2 cell line represents an excellent model for the interactions of these pathogens with the porcine intestinal epithelia.

ETEC toxins have also been completely studied on IPEC-J2, their roles in bacterial adherence have been presented by Johnson et al., 2009. Adherence is a key point as the adhesion of ETEC to the intestinal mucosa is a prerequisite step for its colonisation.

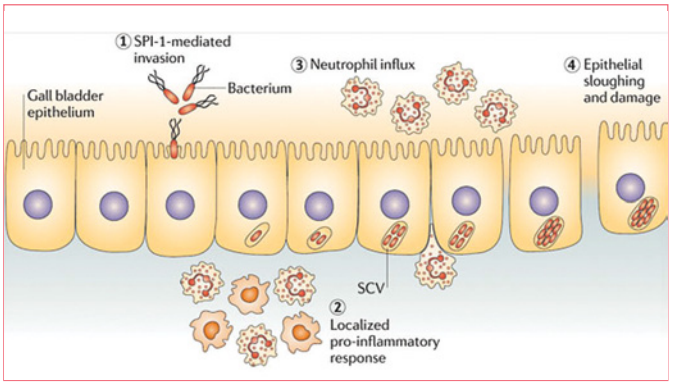


Fig. 4. Salmonella can invade epithelial cells (step 1). This intracellular infection leads to a local inflammatory response (step 2) mediated by neutrophils (step 3), with subsequent tissue damage and epithelial sloughing (step 4). (Gonzalez-Escobedo et al., 2011).

More recently, IPEC-J2 cells were used to demonstrate that high concentrations of deoxynivalenol, a toxin released by the fungus fusarium, reduced the viability of porcine intestinal epithelial cells. This is interesting on swine nutrition because deoxynivalenol often contaminates cereal grains, which cause diarrhoea and weight loss in pigs.

The IPEC-J2 cells have been increasingly used to study potential probiotic micro-organisms focusing on either the adhesiveness of the probiotic to IPEC-J2 cells or the ability of the probiotic to inhibit the inflammatory response induced by a specific pathogen.

Some examples of these probiotics are Enterococcus strains, Saccharomyces strains or multiple species of lactobacillus. The validity of these studies remains to be seen as articles that use the tested probiotics in vivo have yet to be published.

In a recent publication different feedstuffs (wheat bran, casein glycomacropeptide, mannan-oligosaccharides, locust bean extract and Aspergillus oryzae fermentation extract) were able to reduce the adhesion of ETEC to IPEC-J2 and interfere on the innate inflammatory response. Another study demonstrates that phytic acid decreased the negative effects of deoxynivalenol on the membrane integrity of the intestinal epithelial cell line. Among the various feedstuffs or additives used in IPEC studies, we will merit special attention to those related to the use and influence of zinc in the intestinal epithelium.

Zinc dose and source

Use of in-feed pharmacological levels of zinc oxide (ZnO, 2500-3000ppm) is widely accepted in the pig industry to reduce the incidence and severity of post-weaning diarrhoea in piglets, in particular that caused by ETEC K88.

However, the therapeutic mechanism is not yet well understood. In a first in vitro study conducted with the human intestinal cell line Caco-2 it was demonstrated that increasing concentrations of ZnO reduced ETEC K88 adhesion and internalisation to the cell line. It was also found that ZnO prevent the increase in tight junction permeability induced by ETEC and the ETEC-induced expression of several inflammatory response genes.

This study did not show an antimicrobial effect of ZnO, and provides alternative mechanisms for the reduced incidence of post-weaning diarrhoea in ZnO supplemented pigs. However, as stated above, Caco-2 is a human intestinal cell line, while ETEC K88 is a porcine-specific pathogen. Some recent studies aimed to investigate the effect of ZnO dose and source on IPEC-J2 challenged with ETEC, approaching the investigation to a better research porcine model. IPEC-J2 cells were used as an in vitro model of intestinal ETEC infection with or without supplemented ZnO.

Genomic analysis identified increased expression of a variety of innate immune response genes (for example cytokine genes) in response to ETEC exposure but it was reduced when cells were simultaneously exposed to ZnO.

Metabolic effects showed that ZnO may modify IPEC-J2 cell morphology at low concentrations, and reduces cell viability at higher values. When ETEC adhesion to the host cell was measured after only two hours, ZnO supplementation did not show any effect.

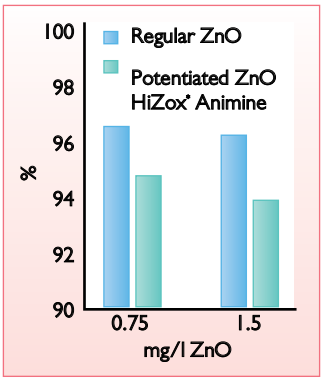


Fig. 5. Reduction of ETEC adhesion to IPEC-J2 cells.

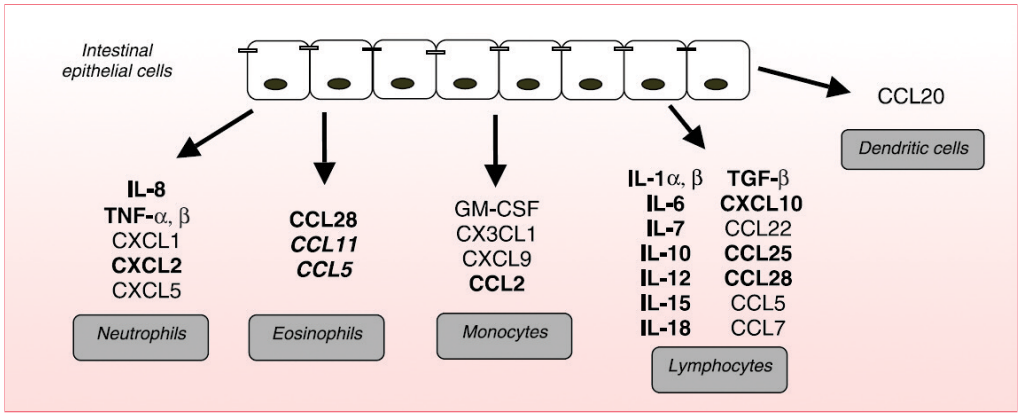
However, when it was measured on a longer period, ZnO at concentrations which do not affect IPEC-J2 morphology decreased ETEC adhesion. These findings suggest that the dose and source of ZnO have a significant impact on both IPEC-J2 and pathogen metabolism and also reduce the expression of inflammatory genes when IPEC-2 were challenged.

In conclusion:

- Intestinal epithelial cells are the first line of defence from the external environment and also have an important role on the inflammatory response.
- The use of epithelial cell lines as an in vitro model has been increasing in recent years.
- IPEC-J2 cell line provides a good specific swine model to study the effect of feedstuffs on the inflammatory response caused by specific porcine pathogens.
- The use of IPEC-J2 and other cell lines is expected to increase in the future. IPEC-J2 can be an excellent model to investigate the ZnO therapeutic mechanism. ■

References are available from the author on request

Fig. 3. Summary of the cytokines and chemokines produced by IECs.



Reprinted from International Pig Topics, published by Positive Action Publications Ltd, PO Box 4, Driffield, East Yorkshire YO25 9DJ, UK. Tel: +44 1377 241724 Fax: +44 1377 253640 Email: info@positiveaction.co.uk

HiZox®

The novel Zinc source

for Piglet

119 Animine

Not only trace minerals

www.animine.eu