In vitro models to analyse nutritional and microbial antigens at the intestinal mucosal surface

Elena Mengheri, INRAN, Rome, Italy

Isabelle P. Oswald, INRA, Toulouse, France

Hermann-Josef Rothkötter, Otto-von-Guericke-University, Magdeburg, Germany
Objectives

- Systems to analyse the effects of nutrients, toxins and probiotics on the cellular regulation and transport function of the epithelium
- Basis is the animal
- Ex vivo or in vitro systems are a first step to understand the benefit of the food additives, e.g. probiotics
Probiotics are live microorganisms which when ingested in certain numbers and confer health benefits on the host (FAO/WHO)
Advantages and disavantages of each model

**In vivo**
- representative of cell complexity
- but objectives: decrease the number of in vivo experiments

**Ex vivo**
- reduction in the number of animals used
- multiplying the number of conditions tested on identical tissues

**In vitro**
- only single cell type
- away from the real situation and cell complexity
Effects of the mycotoxin Deoxynivalenol (DON) and probiotics on jejunal explants

- Pig jejunum (conservation in william medium)
- Realization of explants
- Incubation of explants at 39°C during 4h
- Fixation Formaldehyde freezing -80°C
- Western Blot, ELISA and PCR
Effect of probiotic bacteria (L. sobrius) on DON induced inflammation

**Approach ex vivo**

- Preparation of intestinal explants (lamina propria)
- -/+ 10 µM DON
- -/+ 3 nM T-2
- Washing of toxins
- + Lactobacillus sobrius 1 x 10⁹ CFU/ml
- Transcriptomic analysis

**Pro-inflammatory response:** IL-1 beta, IL-1 alpha, IL-8, TNF-alpha

**Effect of probiotic bacteria (L. sobrius) on DON induced inflammation**

No effect of L. sobrius on the pro-inflammatory response
Effect of probiotic bacteria (L. sobrius) on cell signaling after epithelial stimulation via TLR-4

➢ To evaluate whether mucosal inflammation induced by enterotoxigenic E.coli K88 was mediated by activation of toll-like receptor (TLR)-4 signaling leading to NFkB activation and therefore to inflammatory cytokine expression.

➢ To evaluate whether Lactobacillus amylovorus DSM 16698 could inhibit this signaling
Inflammatory pathway induced by TLR4 activation
In vitro model: Transwell™ System

apical:
IPEC-1 or IPEC-J2 or Caco-2

basolateral:
Effect of probiotic bacteria (L. sobrius) on cell signaling after epithelial stimulation via TLR-4

Caco-2 cells: well characterized human intestinal cells, that reproduce in vitro the small intestinal mucosa. They differentiate as mature enterocytes after 17-21 days of culture

Pig intestinal explants (prepared by INRA Toulouse)

NF-kB Inflammatory cascade was analysed by Western Blot in Caco-2 cells and pig intestinal explants, both treated with ETEC K88 and/or L. amylovorus.

apical: Caco-2

L. amylovorus DSM 16698 (5x10^7/ml)

ETEC K88 (5x10^6/ml)
# Activators of TLR4 signaling

In Caco-2 cells and pig intestinal explants

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<tr>
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<th>ETEC K88</th>
<th>L. amylovorus</th>
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<tbody>
<tr>
<td>TLR4</td>
<td>↑</td>
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<tr>
<td>MyD88</td>
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<tr>
<td>P-p65/NFkB</td>
<td>↑</td>
<td>No increase</td>
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Inhibitors of TLR4 signaling
In Caco-2 cells and pig intestinal explants

ETEC K88

TOLLIP

IRAK-M

L. amylovorus

No decrease
Cytokine secretion by Caco-2 cell

EAAP, 29.08.2013, Session 47, E. Mengheri, I.P. Oswald, H.J. Rothkötter
CONCLUSIONS TLR4 signaling

- ETEC activates the TLR4 signaling
- L. amylovorus is able to inhibit the TLR4 inflammatory cascade induced by ETEC likely through activation of the inhibitor TOLLIP
- Similar results were obtained in intestinal explants from piglets
in vitro-culture: recent improvements
In vitro model:  IPEC C cells

Intestinal Porcine Epithelial Cell lines
- **IPEC-1**: (R. Gonzalez-Vallina et al. 1996)
  - jejunal and ileal epithelia
  - polarised cells with apical microvilli
- **IPEC-J2**: (P. Schierack et al. 2005)
  - jejunal epithelia
  - polarised, apical microvilli
  - thin apical mucus layer

Air-Liquid-Interface cultures
Air–liquid interface cultures enhance the oxygen supply and trigger the structural and functional differentiation of intestinal porcine epithelial cells (IPEC)

Constanze Nossol · A.-K. Diesing · N. Walk · H. Faber-Zuschratter · R. Hartig · A. Post · J. Kluess · H.-J. Rothkötter · S. Kahlert
**Fig. 1** Influence of cell culture method on morphology and expression of tight junction protein ZO-1 in intestinal porcine epithelial cell lines IPEC-1 and IPEC-J2. Immunofluorescence staining of cells cultured on (a) impermeable dish (dish), (b) porous membrane (1-µm pore size) applying a conventional culture procedure (conv) and (c) porous membrane applying air–liquid interface (ALI) culture conditions with enriched access to oxygen. (d) Section of porcine intestinal mucosa showing ZO-1 distribution in three villi in x–y layer (flat section villus 1, arrow) and in z–y layer (villus II and III, cross-section, arrowhead). (e) Low magnification scheme of section d. Scale bar 20 µm.
Fig. 2 Ultrastructural analysis of IPEC-1 and IPEC-J2 cell lines cultured with different supports and conditions. IPEC-1 cells (a, c, e) and IPEC-J2 cells (b, d, f) were cultured conventionally on impermeable support (a, b), on 1 μm membrane (c, d) and according to the ALI protocol on 1 μm membrane (e, f). A section of porcine jejunum is shown for comparison (g). Apical side of the cell layer is on the top. Microvilli structures (mv) are located on apical membrane (e–g) and basolateral intercellular spaces are shown (c–g). Nucleus (nu). Scale bar 4 μm (a–f)
Summary

- Ex vivo and in vitro methods are necessary to observe the intestinal response to nutrients, toxins and probiotics.

- Caco-2, IPEC-1 and IPEC-J 2 obviously are suitable cell-culture systems.

- Intestinal explants provide the basis for studying the first hours after an intestinal stimulus.

- Bacteria (pathogens or probiotic) affect the barrier via humoral mediators or via direct contact.
Thank you!