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

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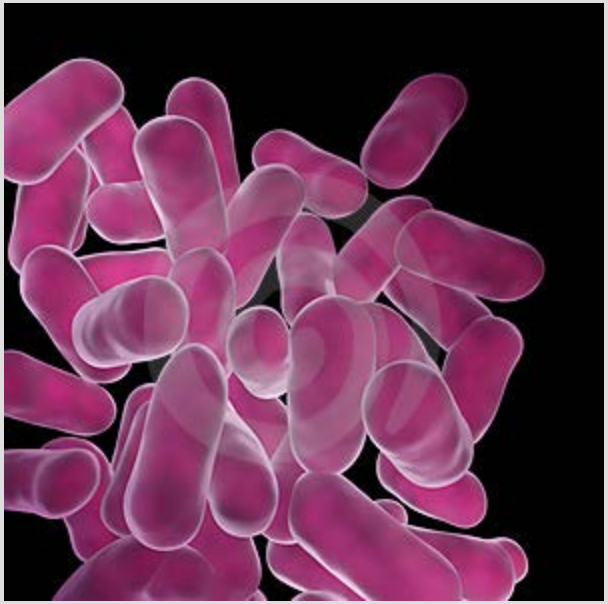
Objectives

- 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

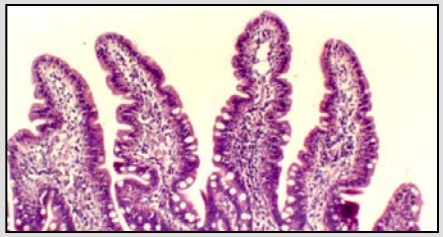
Probiotics are live microorganisms which when ingested in certain numbers and confer health benefits on the host (FAO/WHO)



Advantages and disavantages of each model

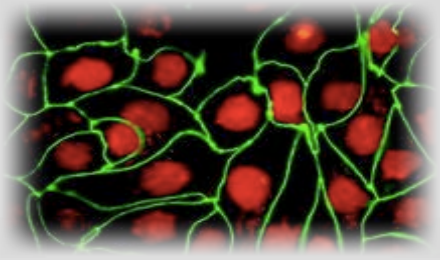
In vivo

- 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

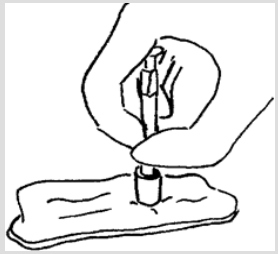


In vitro

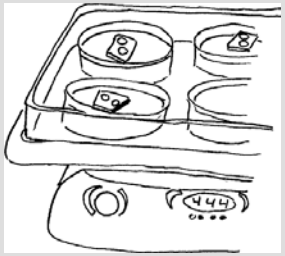
- only single cell type
- away from the real situation and cell complexity

Effects of the mycotoxin Deoxynivalenol (DON) and probiotics on jejunal explants

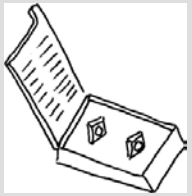
Pig jejunum (conservation in william medium)



Realization of explants



Incubation of explants at 39° C during 4h



Fixation Formal-dehyde

freeziing -80° C Western Blot, ELISA and PCR

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

Approach *ex vivo*

n = 6

Preparation of intestinal explants (lamina propria)

1h

-/+ 10 µM DON
-/+ 3 nM T-2

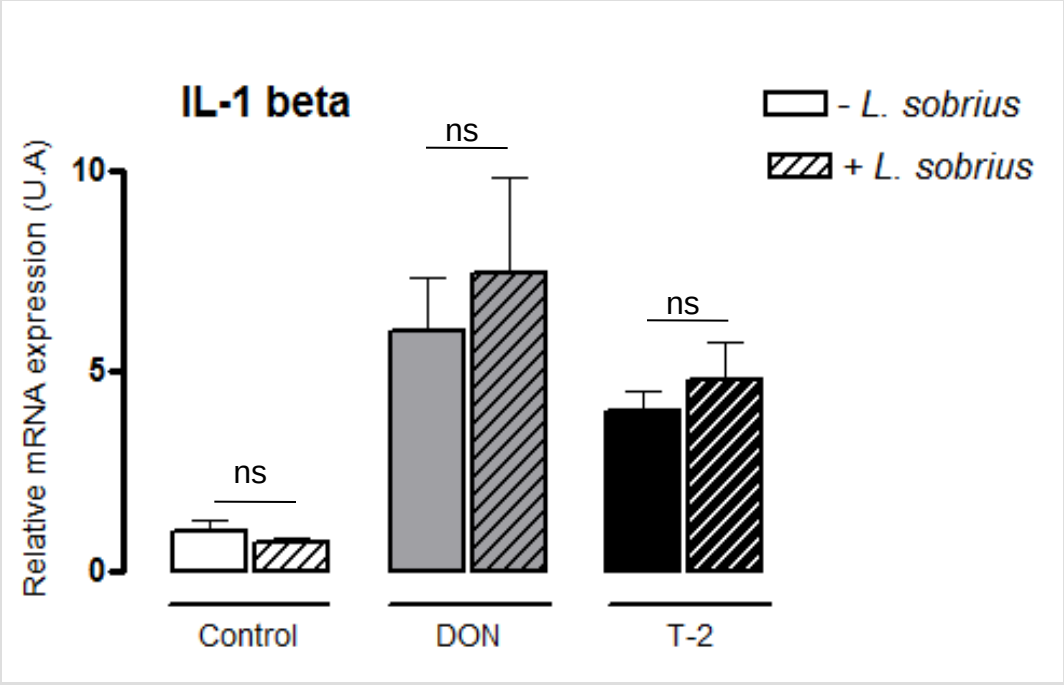
Washing of toxins

3h

-/+ *Lactobacillus sobrius*
1 x 10⁹ CFU/ml

Tanscriptomic analysis

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

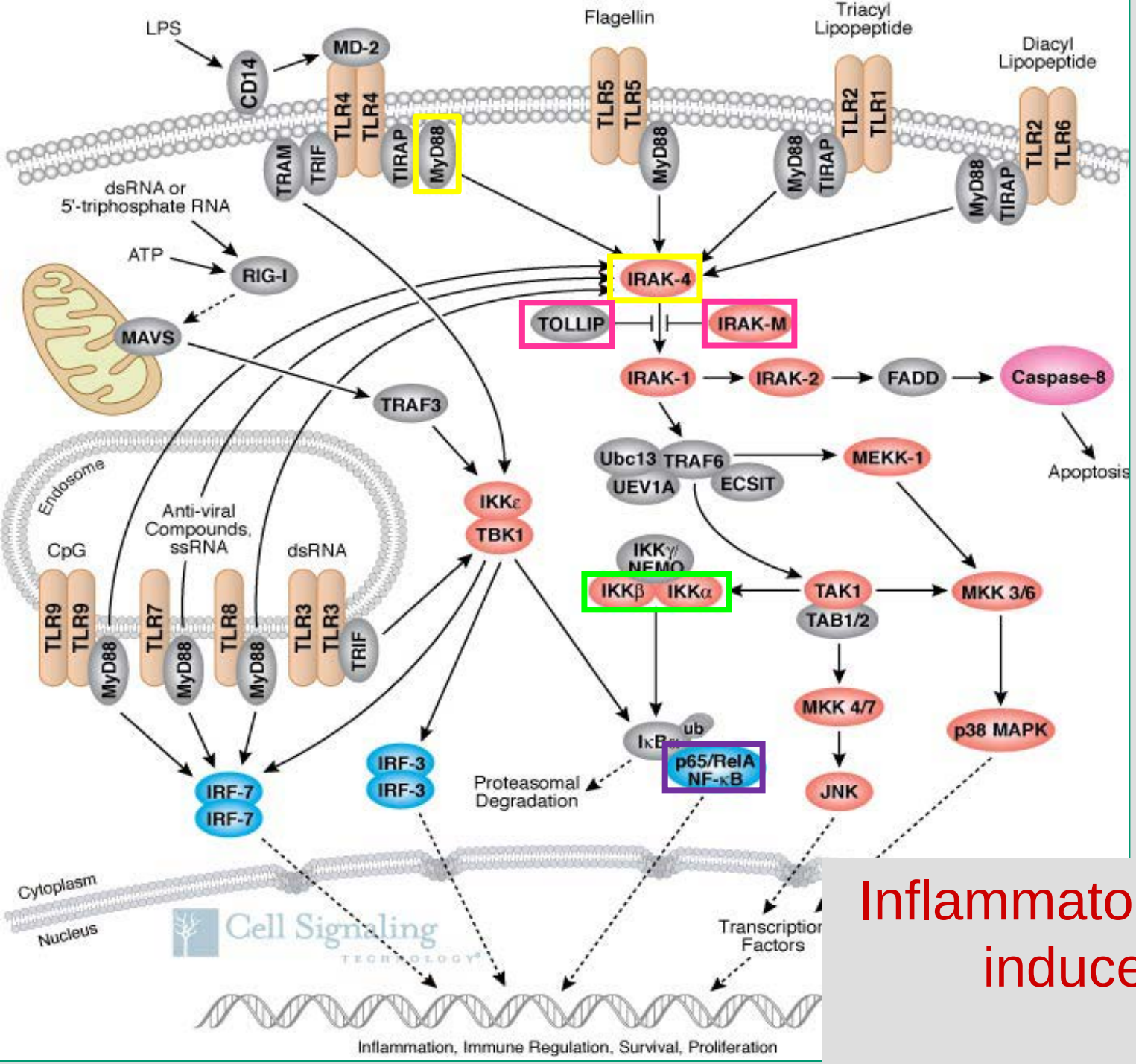


No effect of *L. sobrius* on the pro-inflammatory response

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

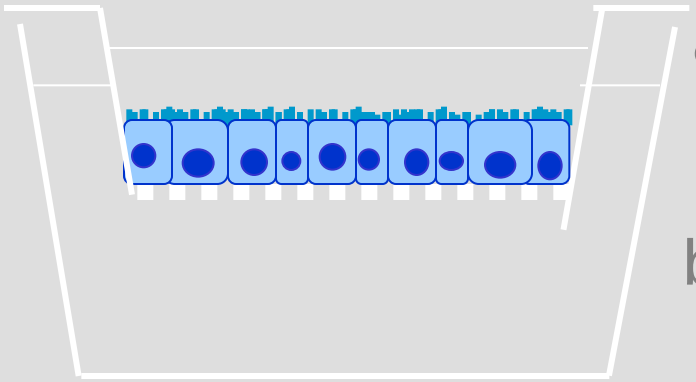
- To evaluate whether mucosal inflammation induced by enterotoxigenic E.coli K88 was mediated by activation of toll-like receptor (TLR)-4 signaling leading to NFkB activation and therefore to inflammatory cytokine expression.

- To evaluate whether *Lactobacillus amylovorus* DSM 16698 could inhibit this signaling



Inflammatory pathway induced by TLR4 activation

In vitro model: Transwell™ System

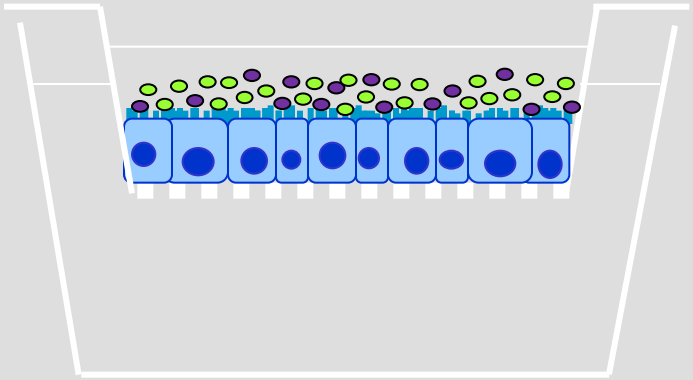


apical:

IPEC-1 or IPEC-J2 or Caco-2

basolateral:

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



apical:
Caco-2

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

ETEC K88 ($5x10^6$/ml)

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.*

Activators of TLR4 signaling

In Caco-2 cells and pig intestinal explants

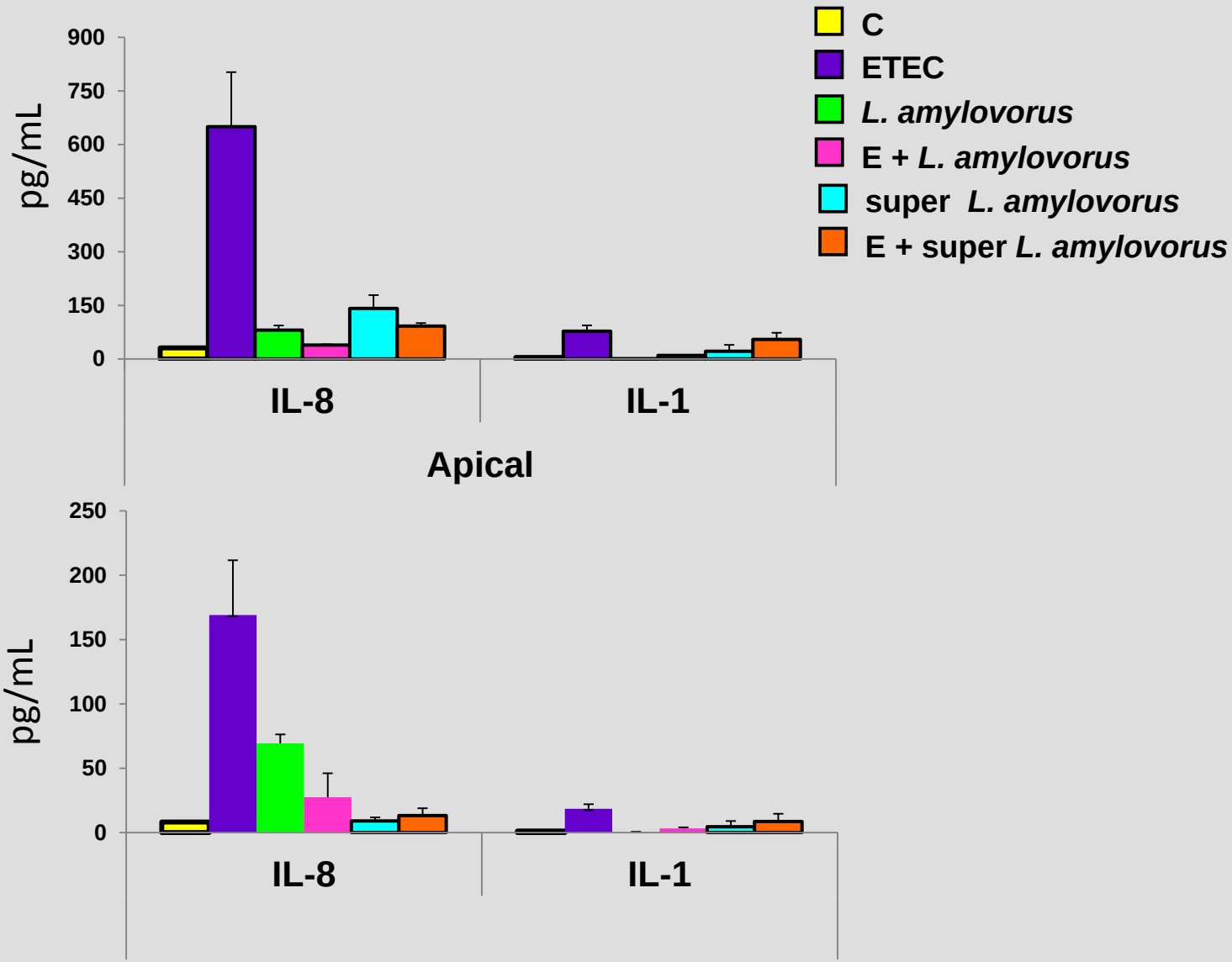
	ETEC K88	*L. amylovorus*
TLR4	↑	No increase
MyD88	↑	No increase
IRAK-4	↑	No increase
P-IKKα/IKKα	↑	No increase
P-IKKβ/IKKβ	↑	No increase
P-IkBα/IkBα	↑	No increase
P-p65/NFkB	↑	No increase

Inhibitors of TLR4 signaling

In Caco-2 cells and pig intestinal explants

	ETEC K88	*L. amylovorus*
TOLLIP	↓	↑
IRAK-M	↓	No decrease

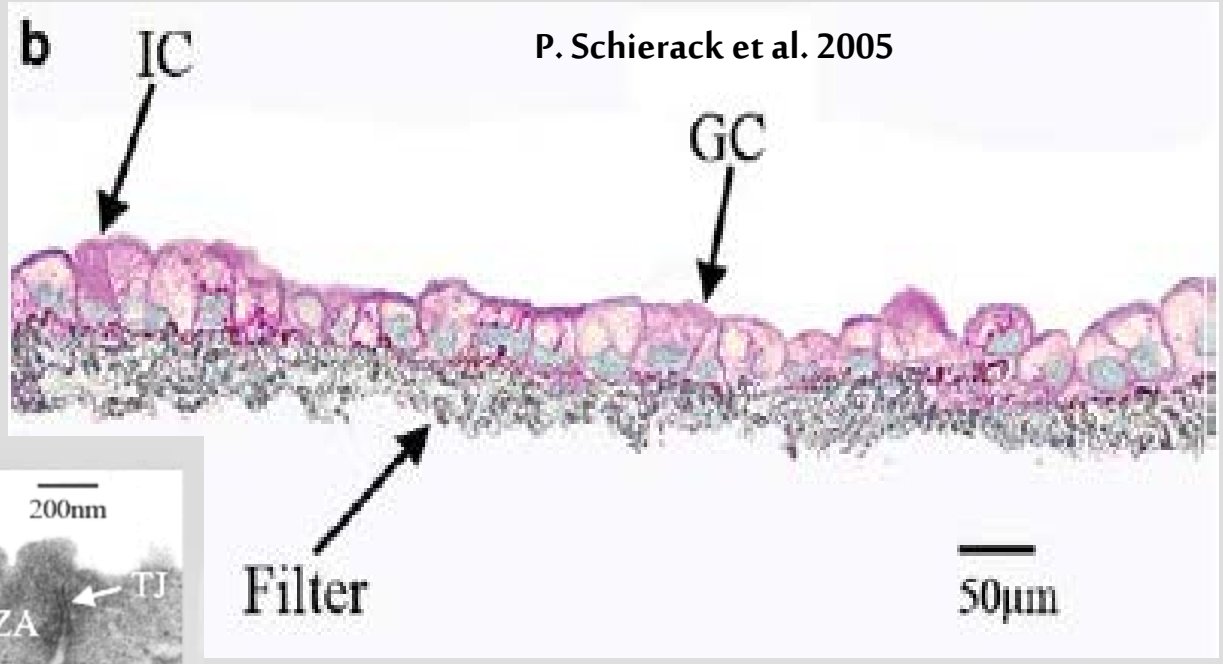
Cytokine secretion by Caco-2 cell

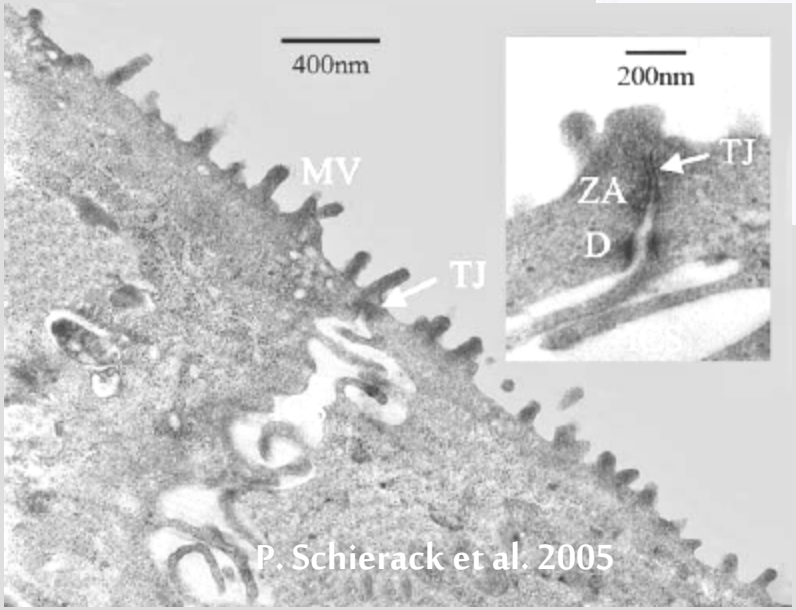


CONCLUSIONS TLR4 signaling

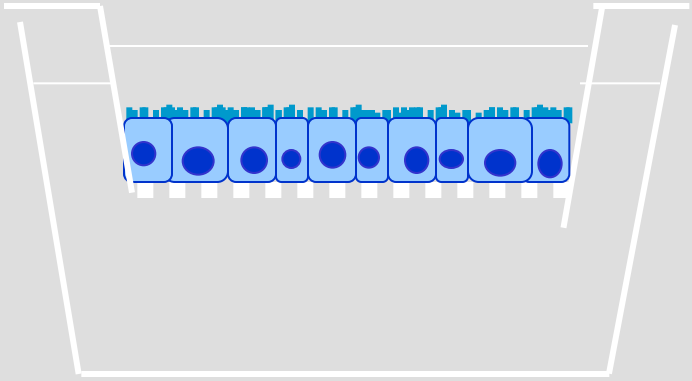
- ETEC activates the TLR4 signaling
- *L. amylovorus* is able to inhibit the TLR4 inflammatory cascade induced by ETEC likely through activation of the inhibitor TOLLIP
- Similar results were obtained in intestinal explants from piglets

in vitro-culture: recent improvements





In vitro model: IPEC cells



apical:
IPEC-1 or IPEC-J2

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

Histochem Cell Biol (2011) 136:103–115
DOI 10.1007/s00418-011-0826-y

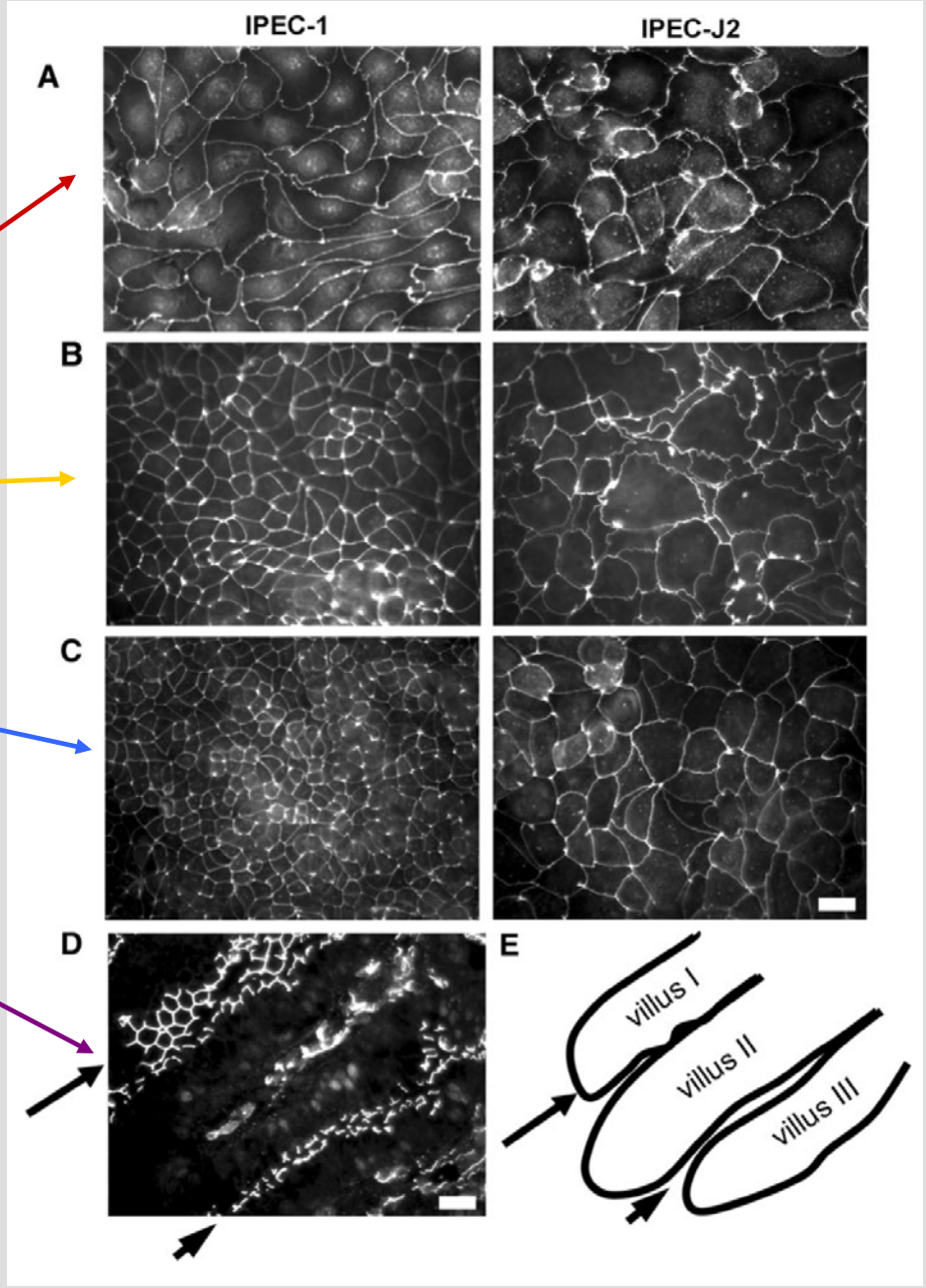
ORIGINAL PAPER

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

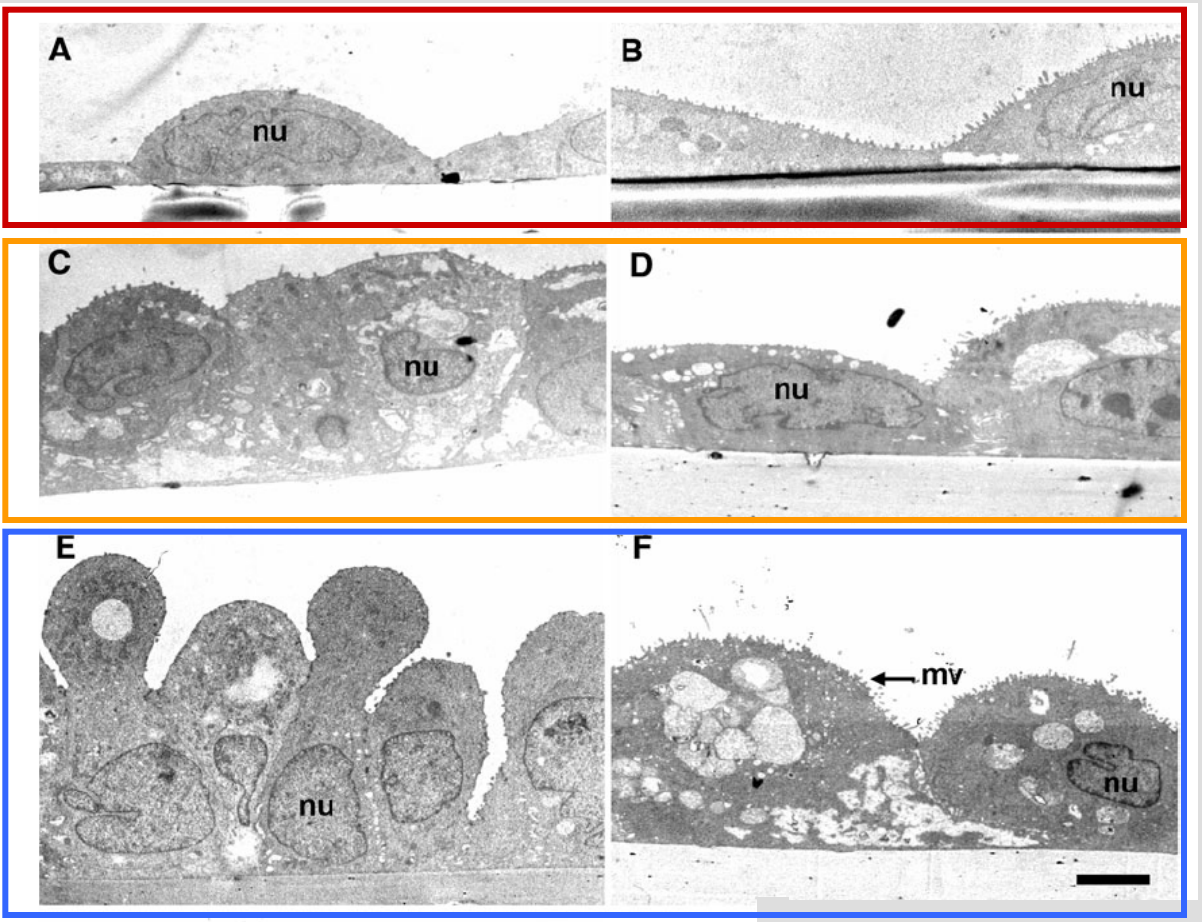
Constanze Nossol · A.-K. Diesing · N. Walk · H. Faber-Zuschratter · R. Hartig · A. Post · J. Kluess · H.-J. Rothkötter · S. Kahlert

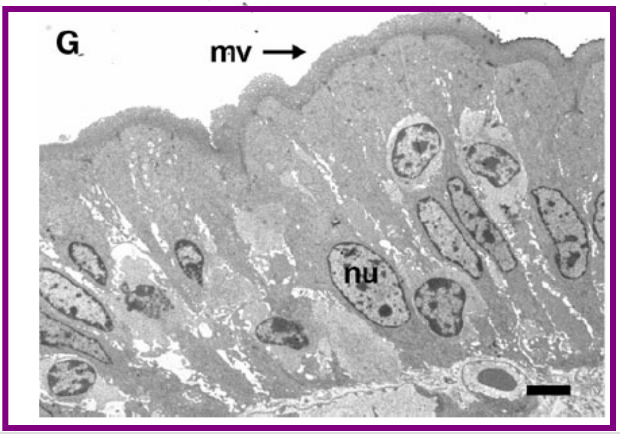
Nossol C. et al., Histochem. Cell Biol. 136 (2011) 103-115

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. Immunfluorescence 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



Nossol C. et al., Histochem. Cell Biol. 136 (2011) 103-115





Nossol C. et al., Histochem. Cell Biol. 136 (2011) 103-115

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-J2 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!