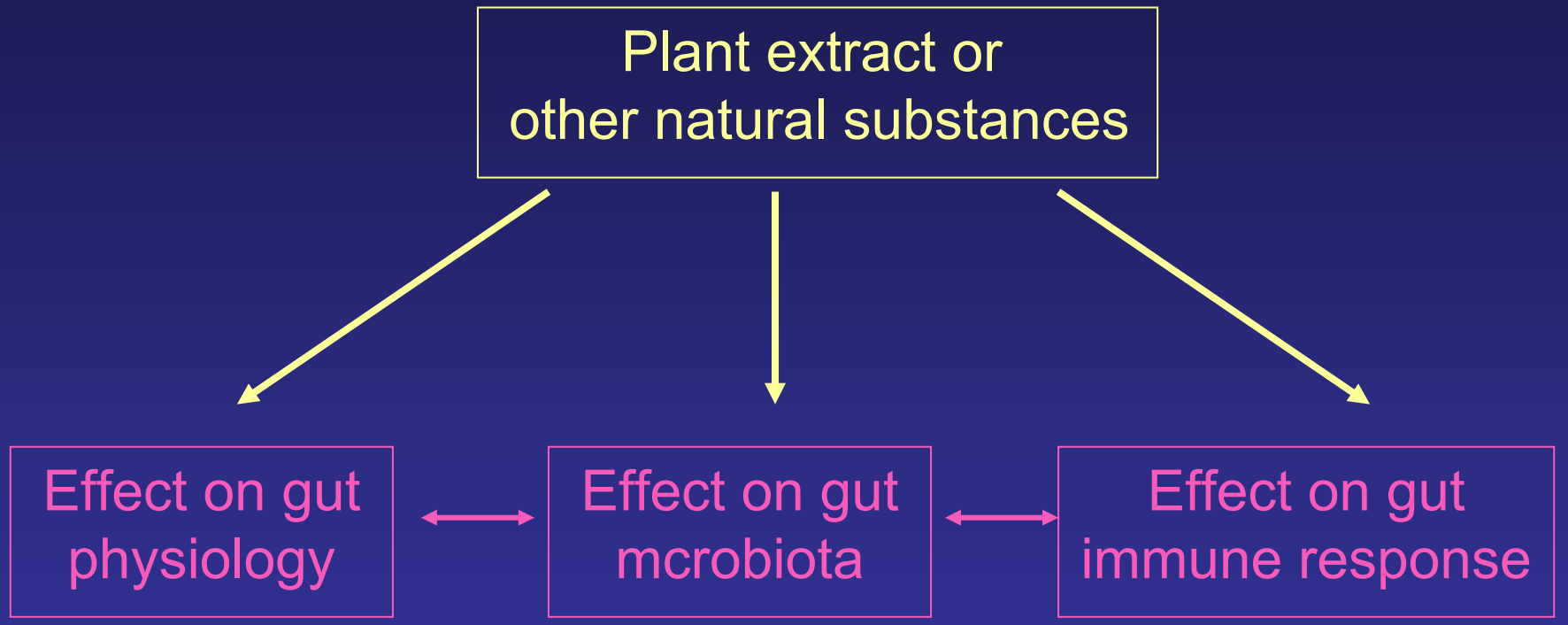
Gut immunology- or what keeps the outside world out of the organism

Isabelle P. OSWALD, Herman-Joseph ROTHKOETTER, Mick BAILEY and Christopher R. STOKES

INRA, Toulouse, France; Otto-von-Guericke University, Magdeburg, Germany; University of Bristol, United Kingdom

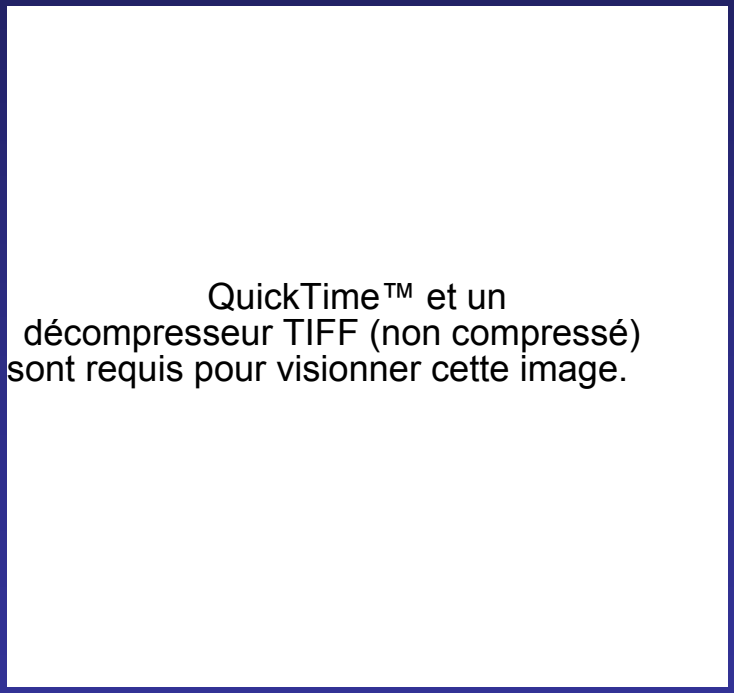
The Feed for Pig Health european project



FPH Feed for Pig Health

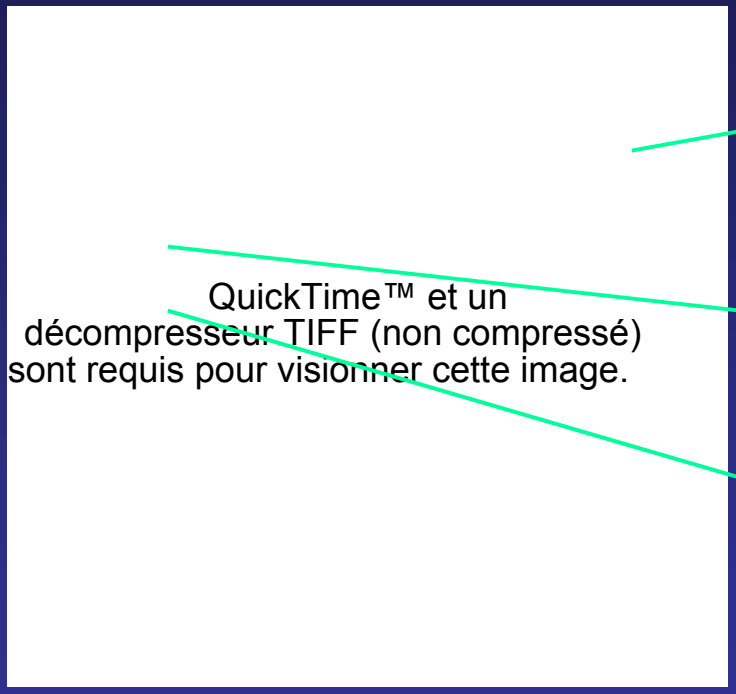
The pig intestinal immune system

The immune function of the intestinal mucosa results in either defense against pathogens or in tolerance to food and commensal bacteria



Various mechanisms involving several cell types are responsible for these complementary functions

The pig intestinal immune system : cells type involved



Intestinal epithelial cells

Dendritic cells

Lymphocytes

Feed for Pig Health

Porcine intestinal epithelial cell lines

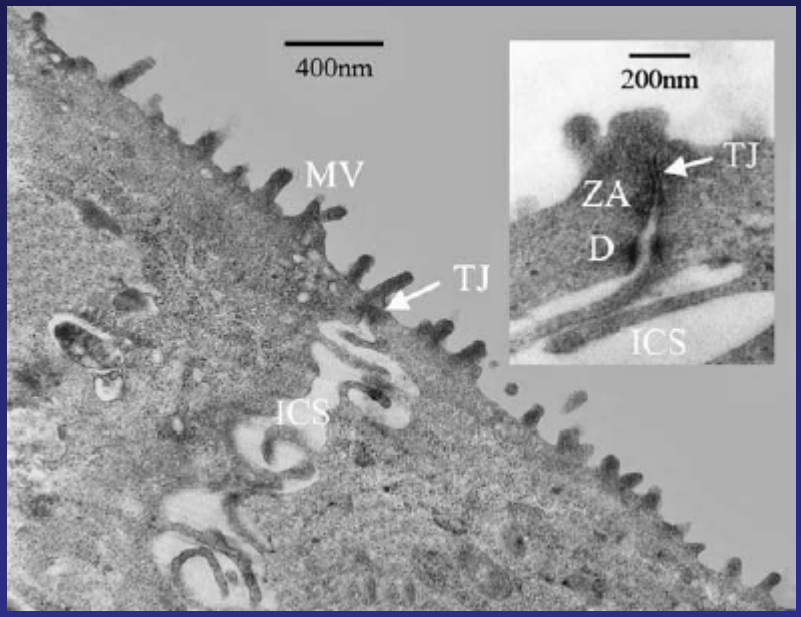
Developed by H. Berschneider, North Carolina, USA

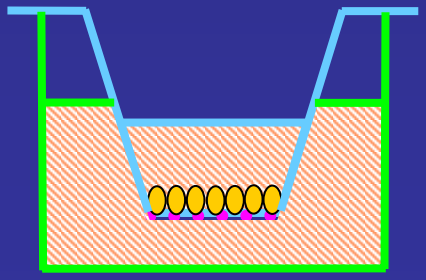
- IPEC-1:

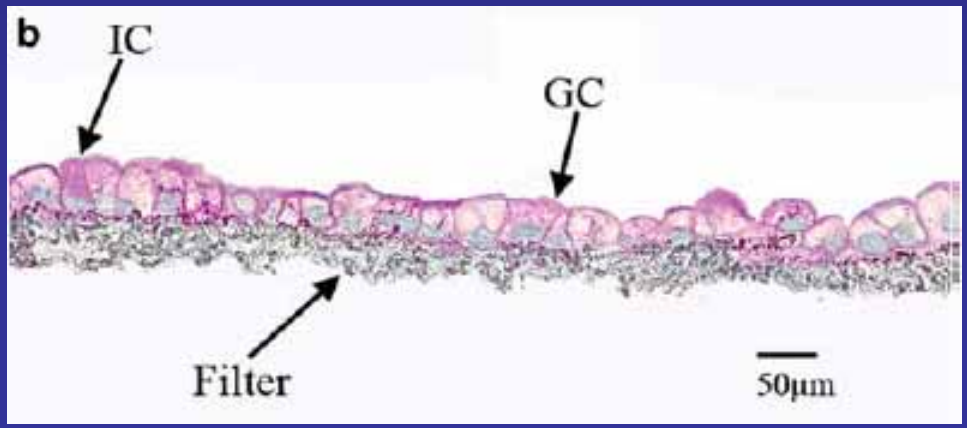
  - jejunal and ileal epithelial cells
  - polarized cells with apical microvilli

- IPEC-J2:

  - jejunal epithelial cells
  - polarized cells with apical microvilli
  - thin extracellular mucus layer (possibly MUC3)

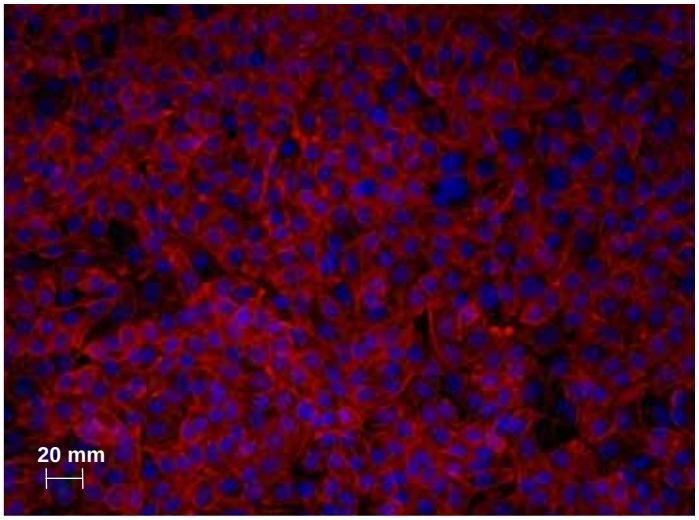






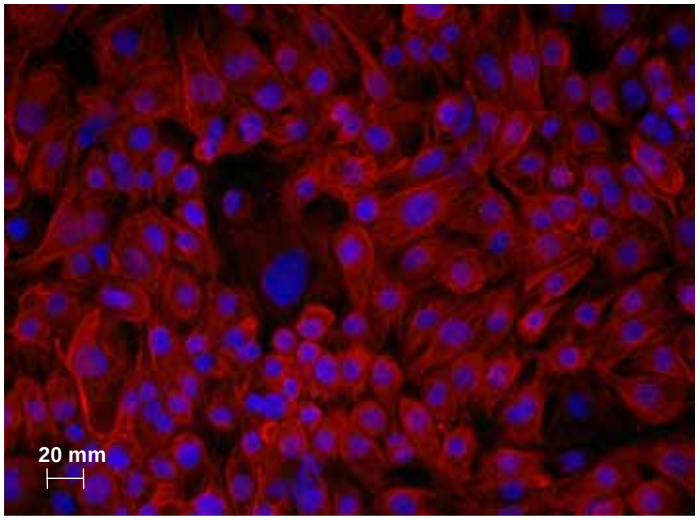
Characterization of IPEC cells

IPEC-1



red: cytokeratin-18 (Cy-90)

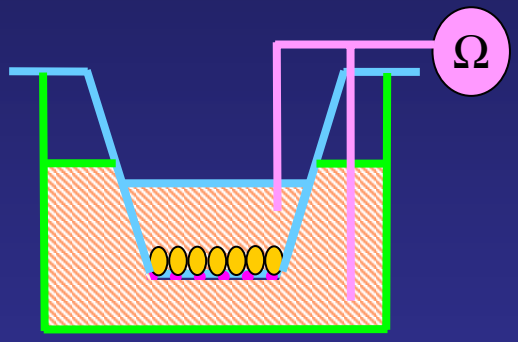
IPEC-J2

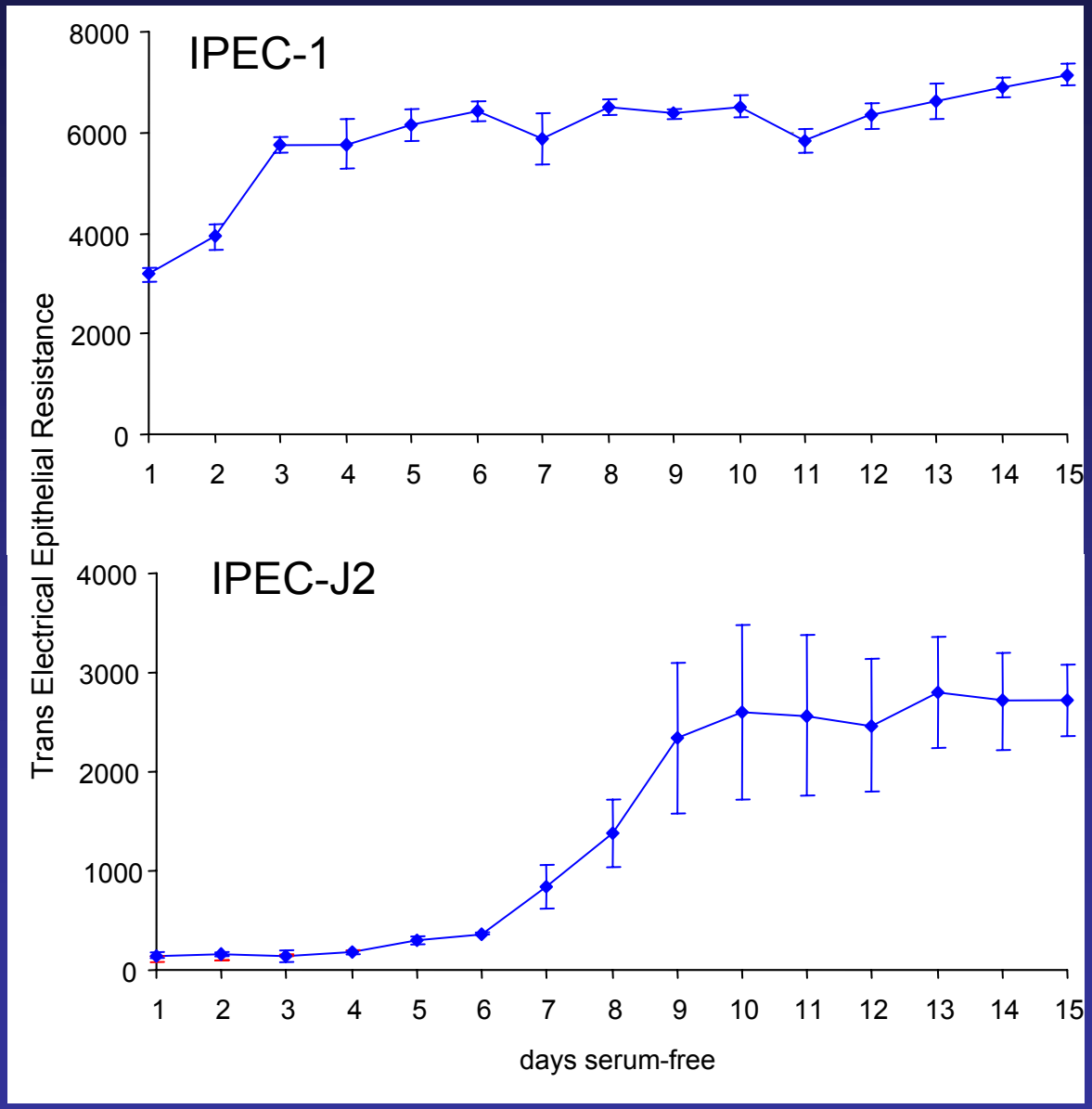


blue: DAPI nuclear staining

-> Cytokeratin-18 expression by IPEC cells
-> cell nuclei diameter: IPEC-1 versus IPEC-J2

TEER development of differentiating IPEC cells





Non-toxic concentration of PENS on IPEC-1

PENS	Highest non-toxic concentration
Yeast extract	5 %
Yeast nucleotides	1.3 % [a]
Unsaturated oligo-mannuronic acid	0.5 %
Ulvan	0.5 %
Bromelain	10 µg/ml
Bovine colostrum (BC)	5 %
Daidzein	0.25 mM [b]
Chlorella extract	10 %
Allicin	0.25 mM [b]
Cinnamaldehyde	5 mM
Carvacrol	5 mM

[a] Corresponding to the amount of nucleotides present in 5% yeast extract.
[b] Higher concentrations were not tested, since these amounts were already able to protect the cells against ETEC infection.

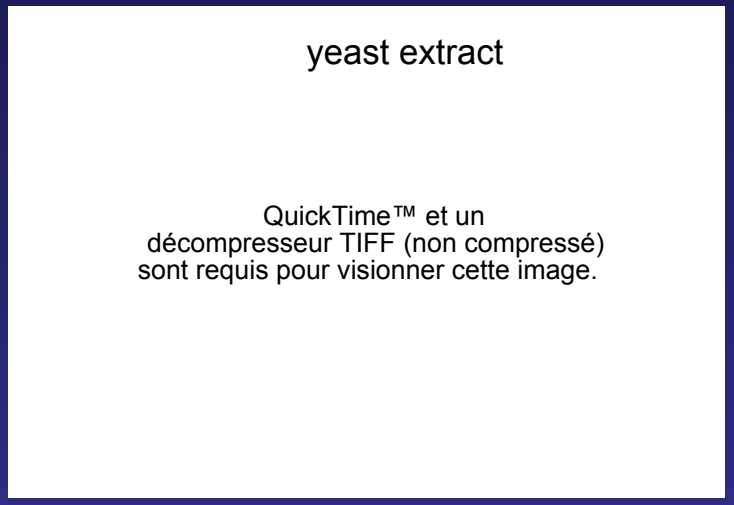
Roselli *et al.*, 2007

Protective effect of PENS

Tested against increased Trans Epithelial Electrical Resistance caused by enterotoxigenic *Escherichia coli* (K88)

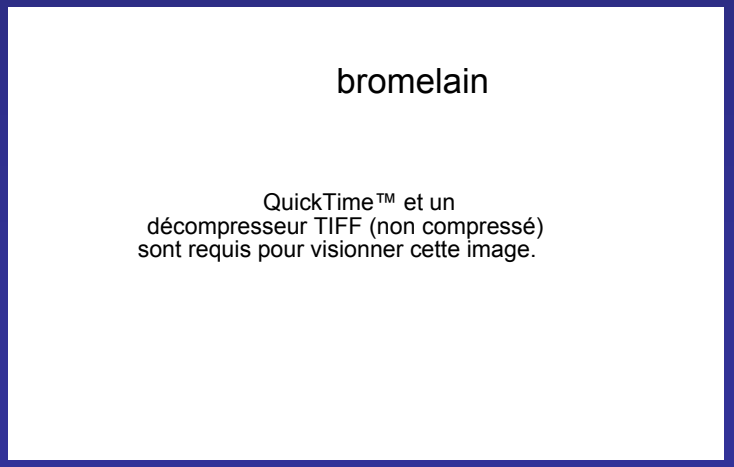
daidzein

yeast extract



allicin

bromelain



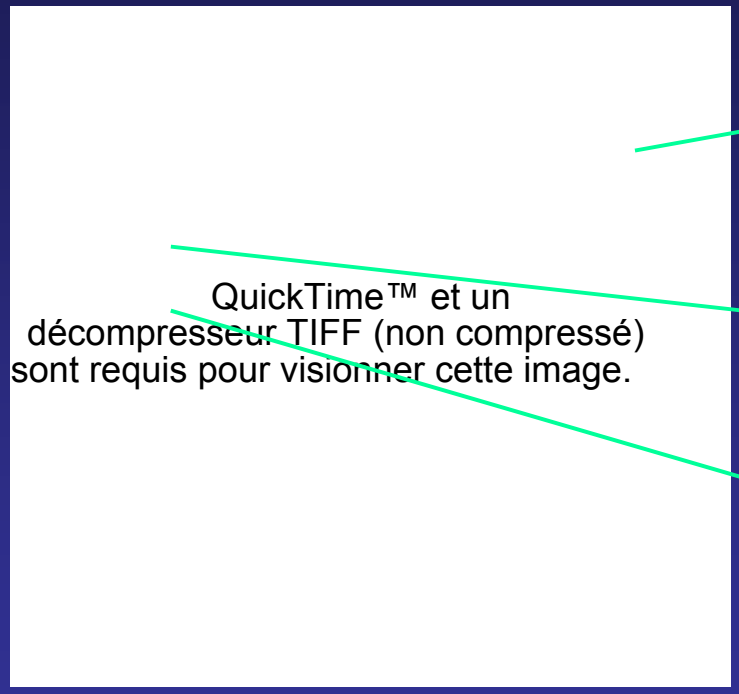
Roselli *et al.*, 2007

Protective effect of PENS

Tested against increased trans-epithelial passage of phenol red caused by enterotoxigenic *Escherichia coli* (K88)

Roselli *et al.*, 2007

The pig intestinal immune system : cells type involved



Intestinal epithelial cells

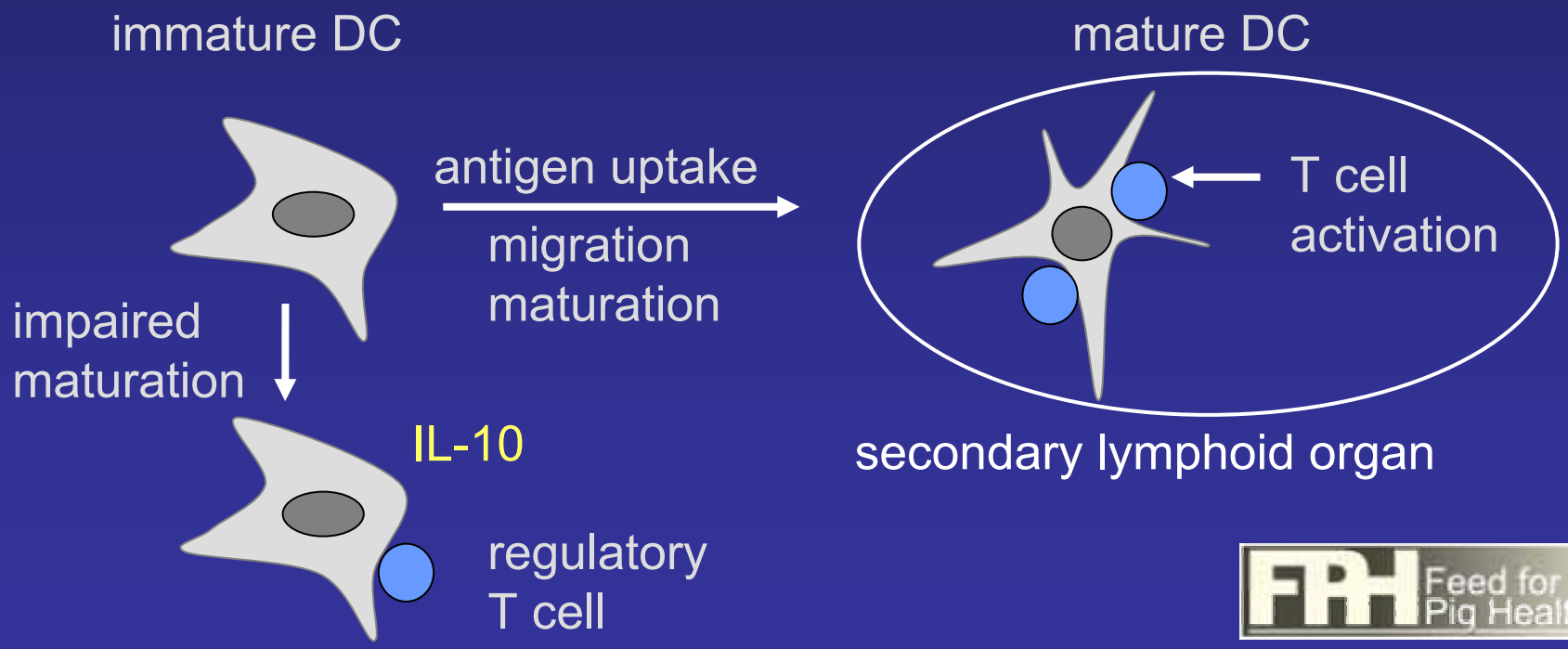
Dendritic cells

Lymphocytes

Feed for Pig Health

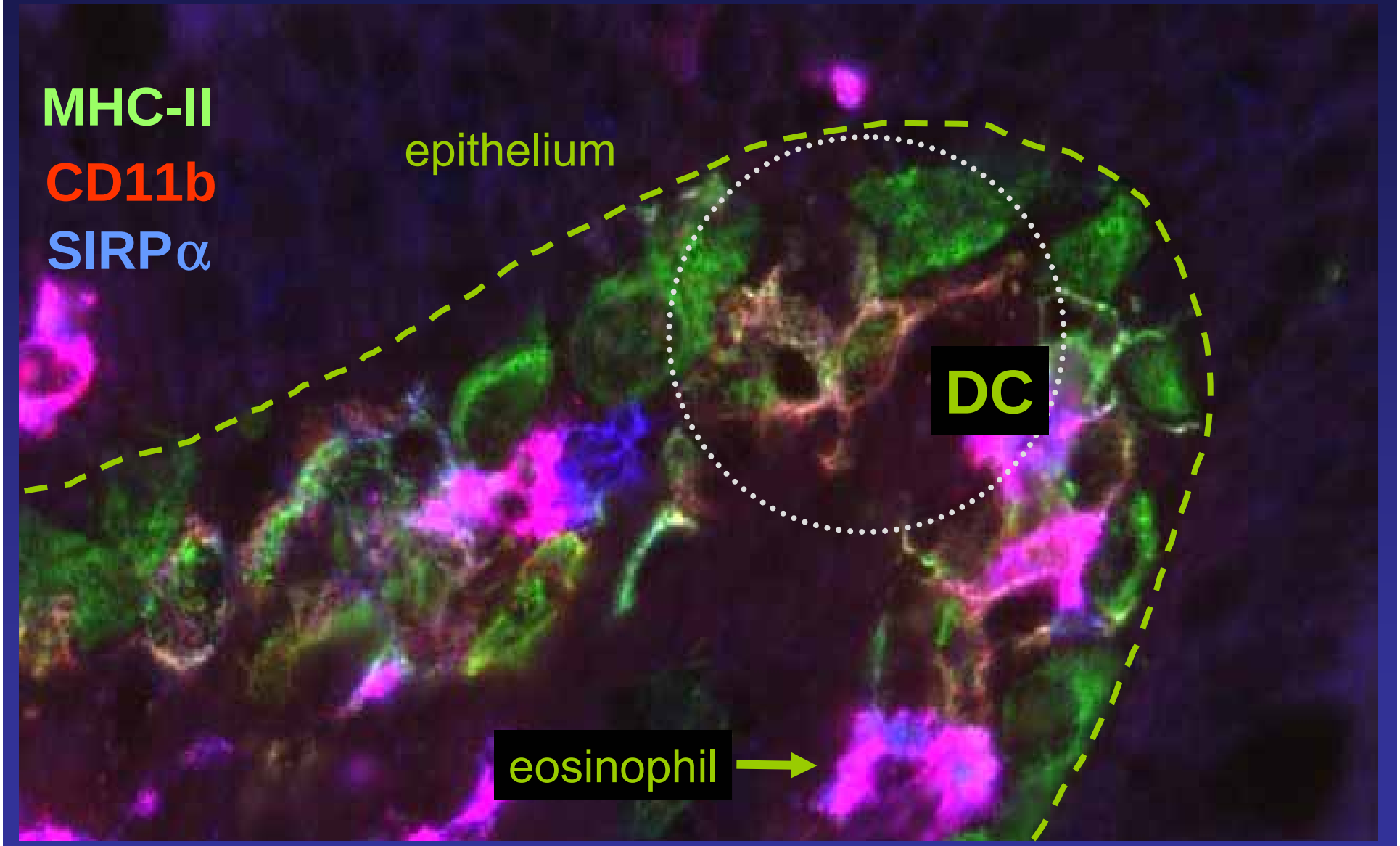
What are dendritic cells (DC)?

- professional antigen-presenting cells
- antigen uptake in tissues (immature DC)
- T cell activation (mature DC)



Feed for Pig Health

DC in intestinal lamina propria: CD11R1^{+} /SIRPα^{+}



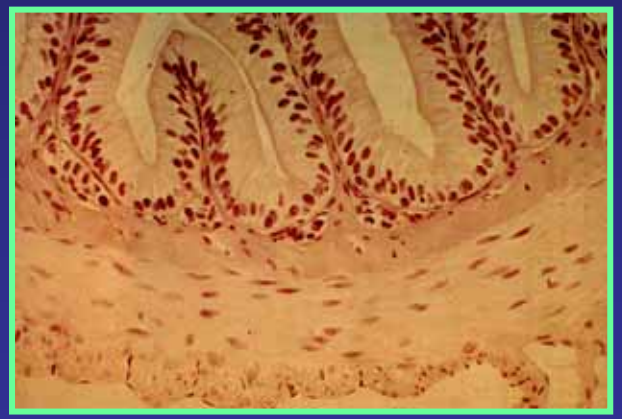
Dendritic cell subset distribution in the porcine intestinal tract

	CD11R1^{+} SIRPα^{+}	CD11R1^{+} SIRPα^{-}	CD11R1^{-} SIRPα^{+}	CD11R1^{-} SIRPα^{-}
Lamina propria, villus	+++	+	+	n.d.
PP, subepithelial	+	+	+++	n.d.
PP, interfollicular	+	+	+	+++
Intestinal lymph	++	+++	–	–
Mesenteric lymph node	+	+++	+	+

Bimczok *et al.*, 2005

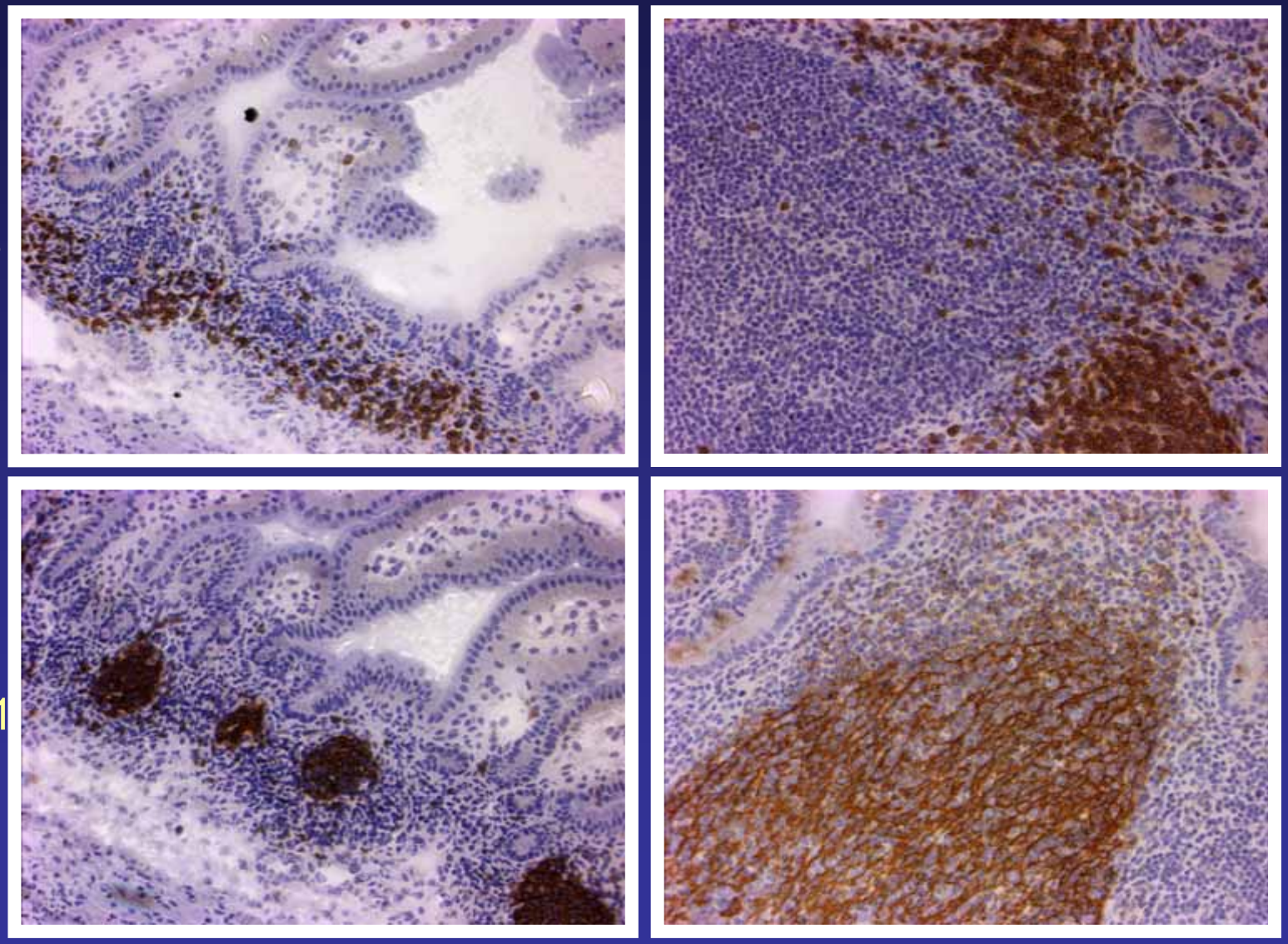
The pig intestinal immune system

QuickTime™ et un
décompresseur TIFF (non compressé)
sont requis pour visionner cette image.



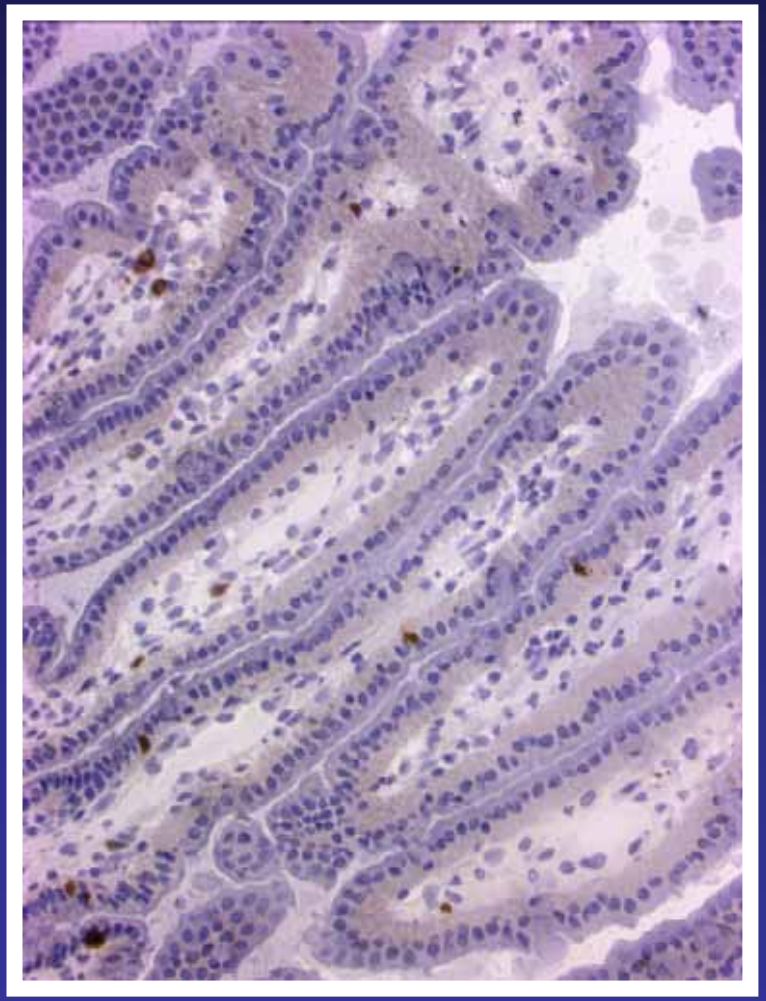
The piglet is profoundly immunodeficient at birth and is only able to generate limited T and B cell responses.

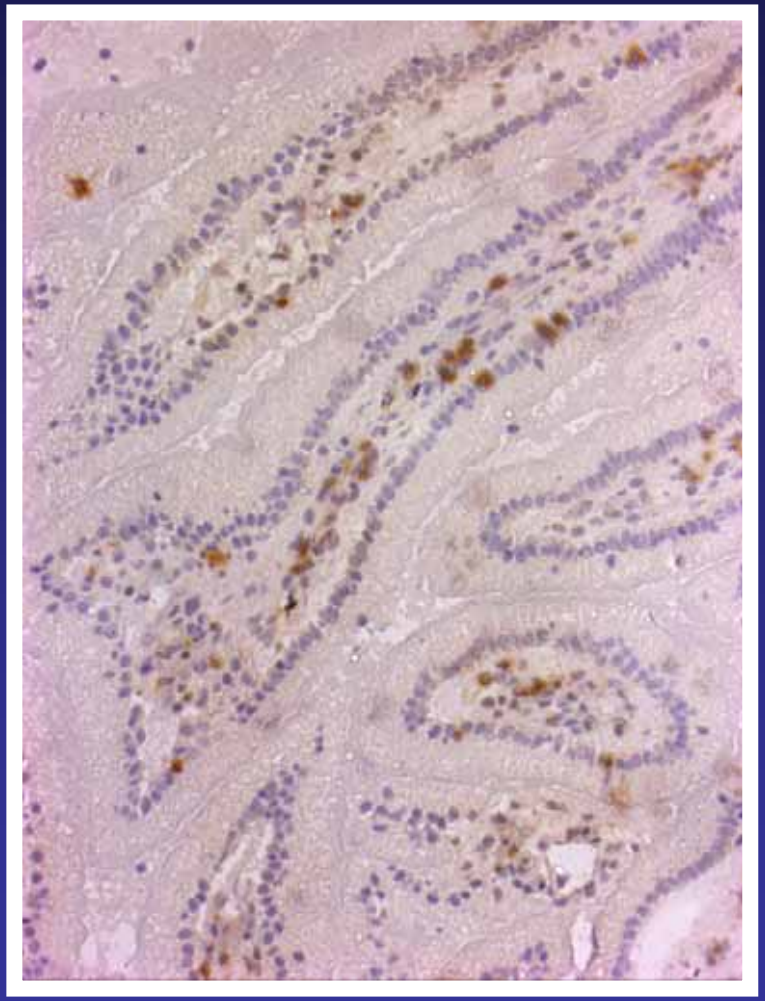
Maturation of Peyer's patches



Maturation of Lamina propria T cells

CD3





Newborn

10 days

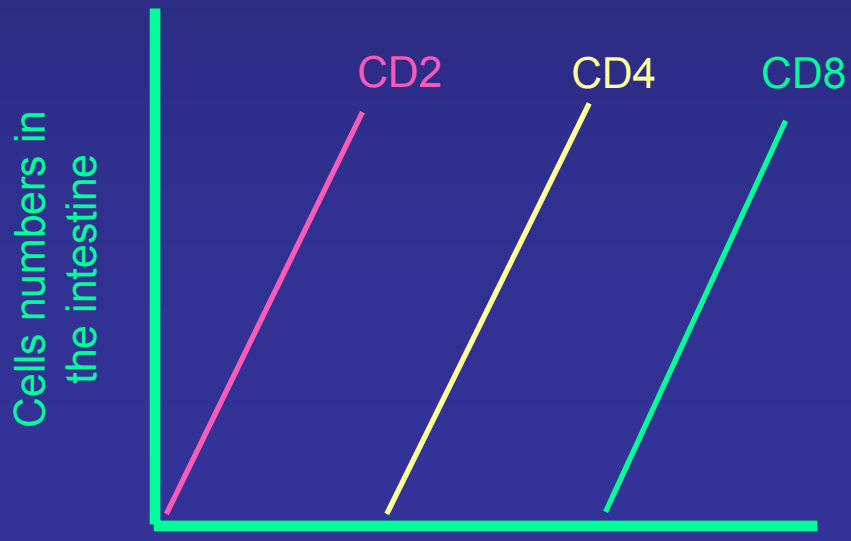
Phenotypic stages in the development of the mucosal immune system of the pig

1. Rudimentary Peyers patches, essentially no mucosal T cells. The newborn pig.

2. Expansion of Peyers patches, appearance of CD2+ cells, influx of MHCII+ cells. 1 days to 2 weeks.

3. Appearance of CD4+ T cells. 2 weeks to 4 weeks.

4. Appearance of CD8+ T cells. 4 weeks to 6 weeks.



WORKING HYPOTHESIS

- The piglet is profoundly immunodeficient at birth and is only able to generate limited T and B cell responses.
- The stages in the development of the mucosal immune system can impact on the piglets ability to mount appropriate responses following weaning.
- Various “immune-stimulants” can influence the rate of development of the mucosal immune system.

FPH Feed for Pig Health

Weaning is also a critical period

Weaning

→ Modification of the “environment”
Modification of the feed
Modification of the intestinal flora

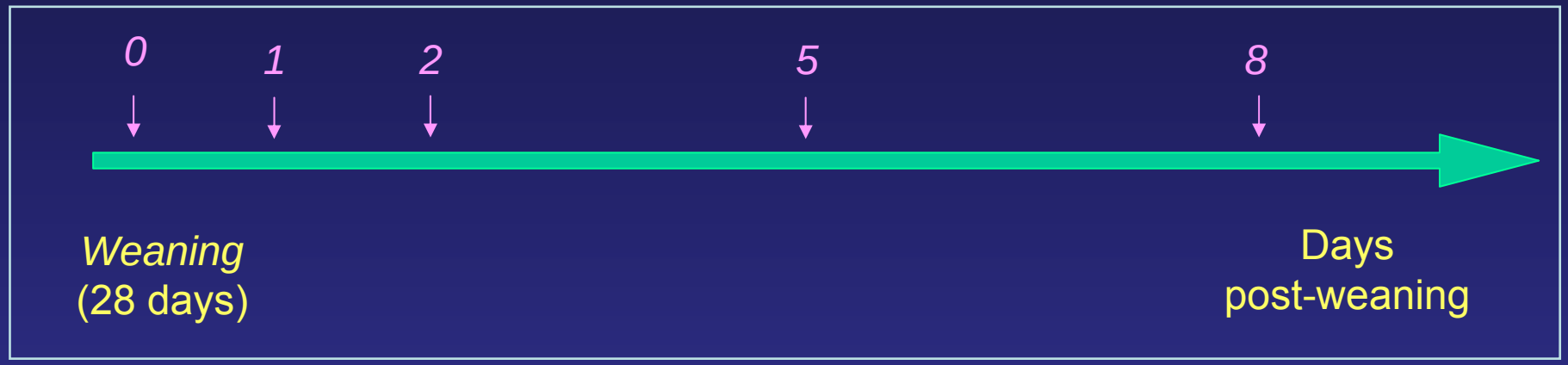
↓

Perturbation of the gut

→ Morphological changes
Functional changes

What is the impact of PENS on gut immune response, especially cytokine expression profile ?

Weaning is associated with a transient inflammatory response : experimental protocol



Samples

Small intestine :
Duodenum, jejunum, ileum

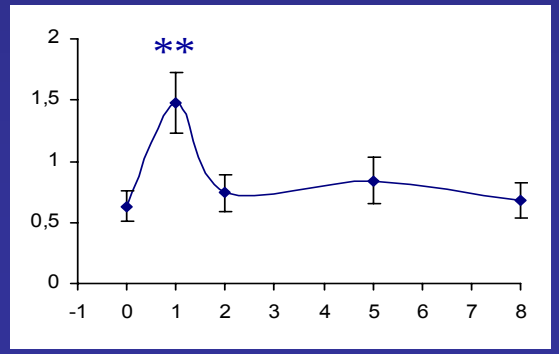
Large intestine :
Colon

Inflammatory cytokines

IL-1β, IL-6, TNF-α
IL-8, IL-12, IL-18

8 to 10 piglets/ time point

Conventional weaning



Jejunum, IL-6

Weaning is associated with a transient inflammatory response

(Pié *et al.* 2004)

	Early response (D0-2 post-weaning)						Late response (D2-8 post-weaning)					
	IL-1b	IL-6	TNF-a	IL-8	IL-12	IL-18	IL-1b	IL-6	TNF-a	IL-8	IL-12	IL-18
Duodenum	↗	↗	–	–	–	–	–	–	↘	–	↘	–
Jejunum	↗	↗	↗	–	–	–	–	–	–	↘	–	↘
Ileum	↗	–	↗	–	–	–	–	–	↗	–	↘	–
Colon	↗	↗	–	–	–	–	–	–	↗	↗	↘	–

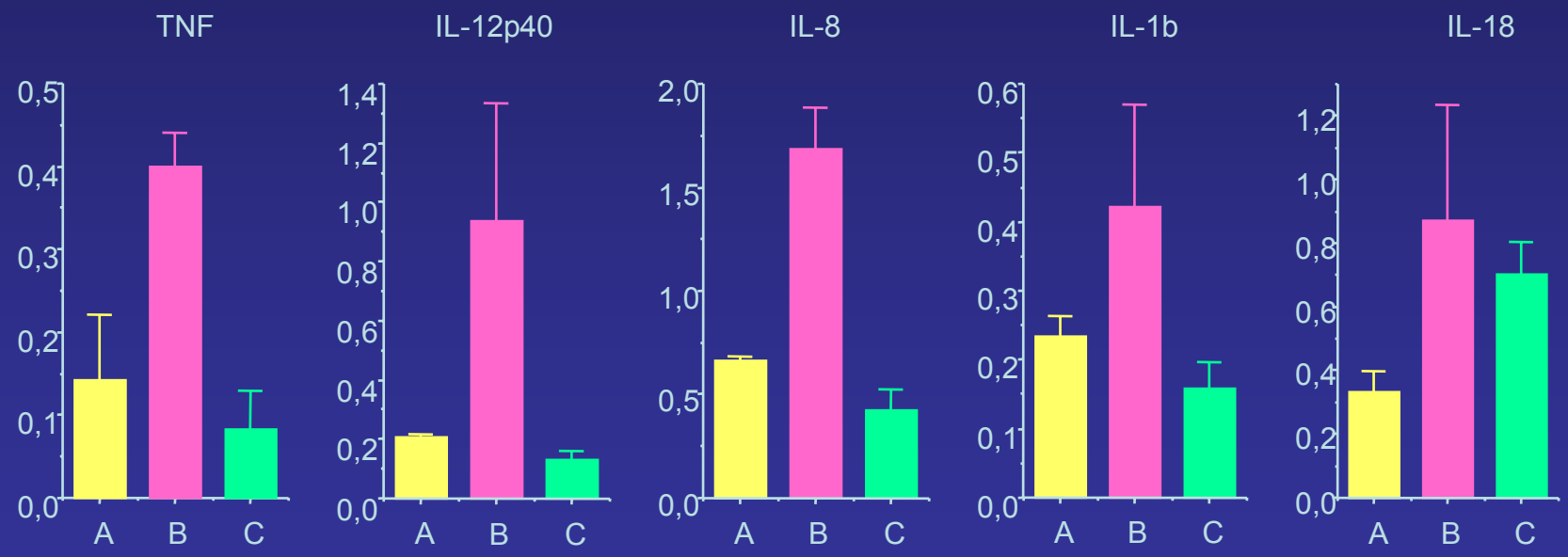
Weaning is a complex phenomena : stress, feeding changes, fasting ...

Analyse the effect of fasting in the transient inflammation observed at weaning

Effect of fasting on the cytokine profile in the intestine

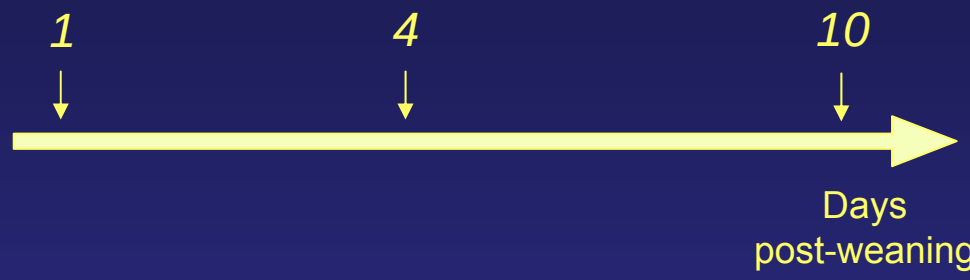
- Protocol : 3 groups of 5 piglets control = A,
  fasted for 36 hours just before autopsy = B
  starved for 36 hours two days before autopsy = C

- Résults : analysis of cytokine in the ileum by real time PCR



- Conclusion : fasting has a major influence on the transient inflammatory response observed at weaning.

Influence of fermentable carbohydrate on cytokine expression in the pig intestine

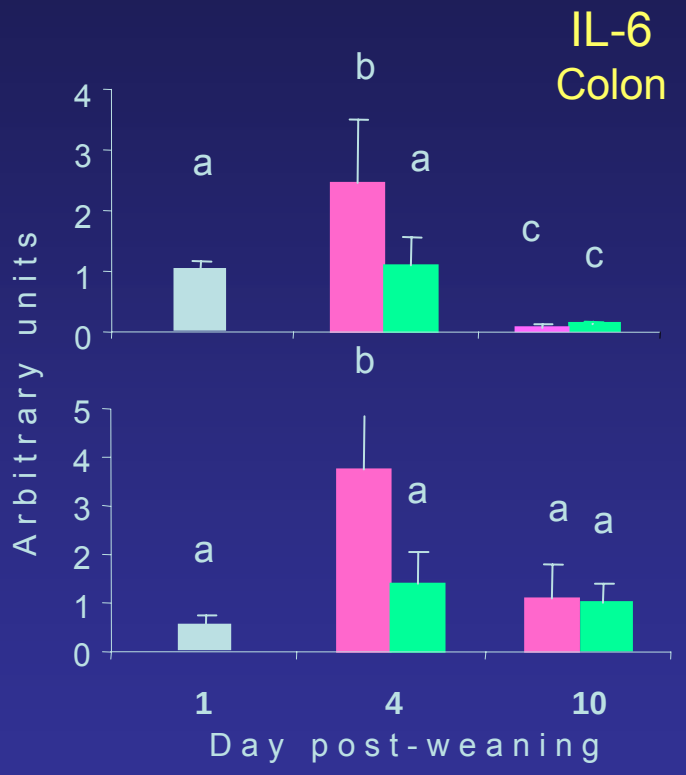


Diets

- Fermentable carbohydrate diet: lactulose, inulin, sugar beet pulp & wheat starch (CHO)
- Control diet (CONT)

Samples : Ileum & Colon

Inflammatory cytokines : IL-1β, IL-6, TNF-α, IL-8, IL-12, IL-18



(Pié *et al.* 2007)

Feed for Pig Health

Influence of sodium butyrate on cytokine expression in the pig intestine

32 piglets in 2 replications
4 treatments:
- Control / Control
- Control / Butyrate
- Butyrate / Control
- Butyrate / Butyrate

Supplementation with Na-butyrate:

Dose: 0.3% of the dry matter intake

Administration:

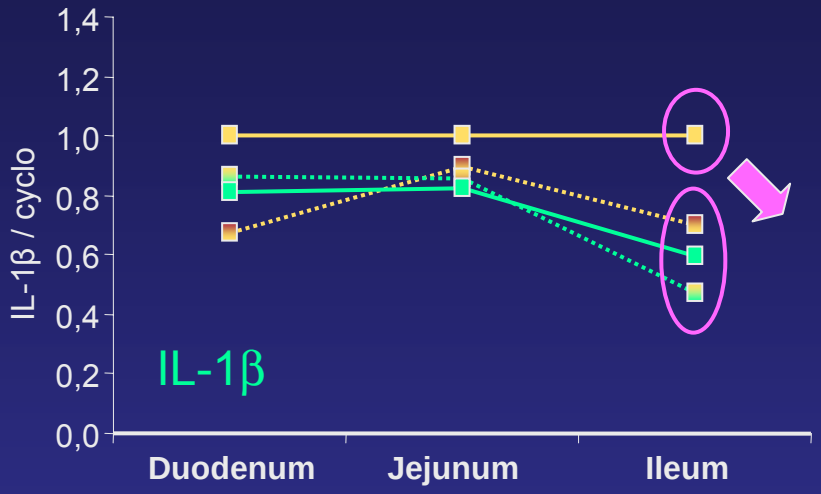
- Suckling period: œsophagal tube twice a day
- Post-weaning period: incorporated in diet, twice a day

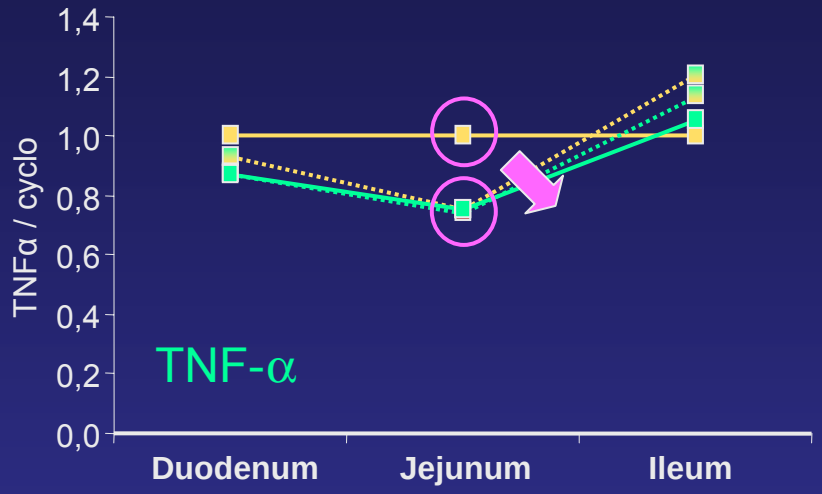
Samples : Duodenum, jejunum, Duodenum, jejunum

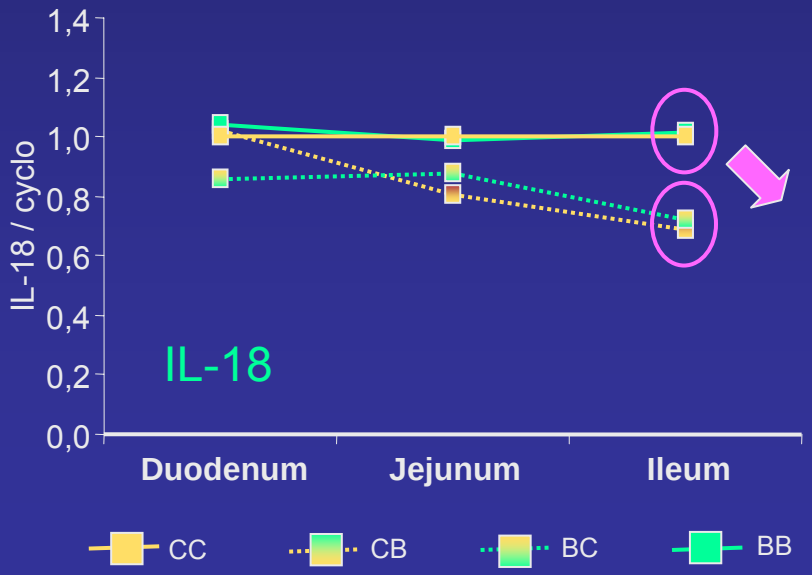
Cytokines : IL-1β, TNF-α, IL-18

FPH Feed for Pig Health

Influence of sodium butyrate on cytokine expression in the intestine



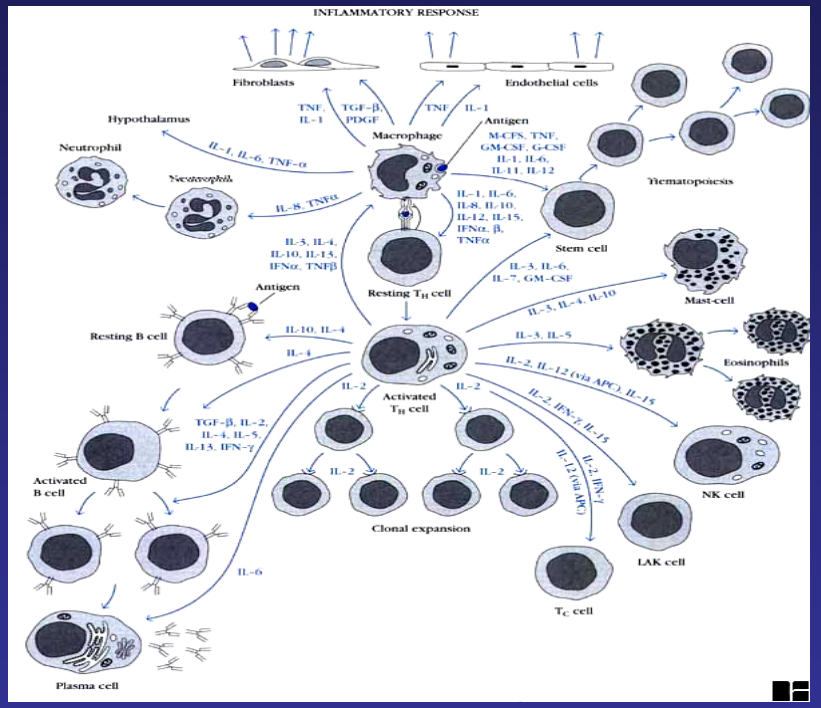




⇒ Expression levels of cytokines decreased or remained stable in Na-butyrate supplemented animals

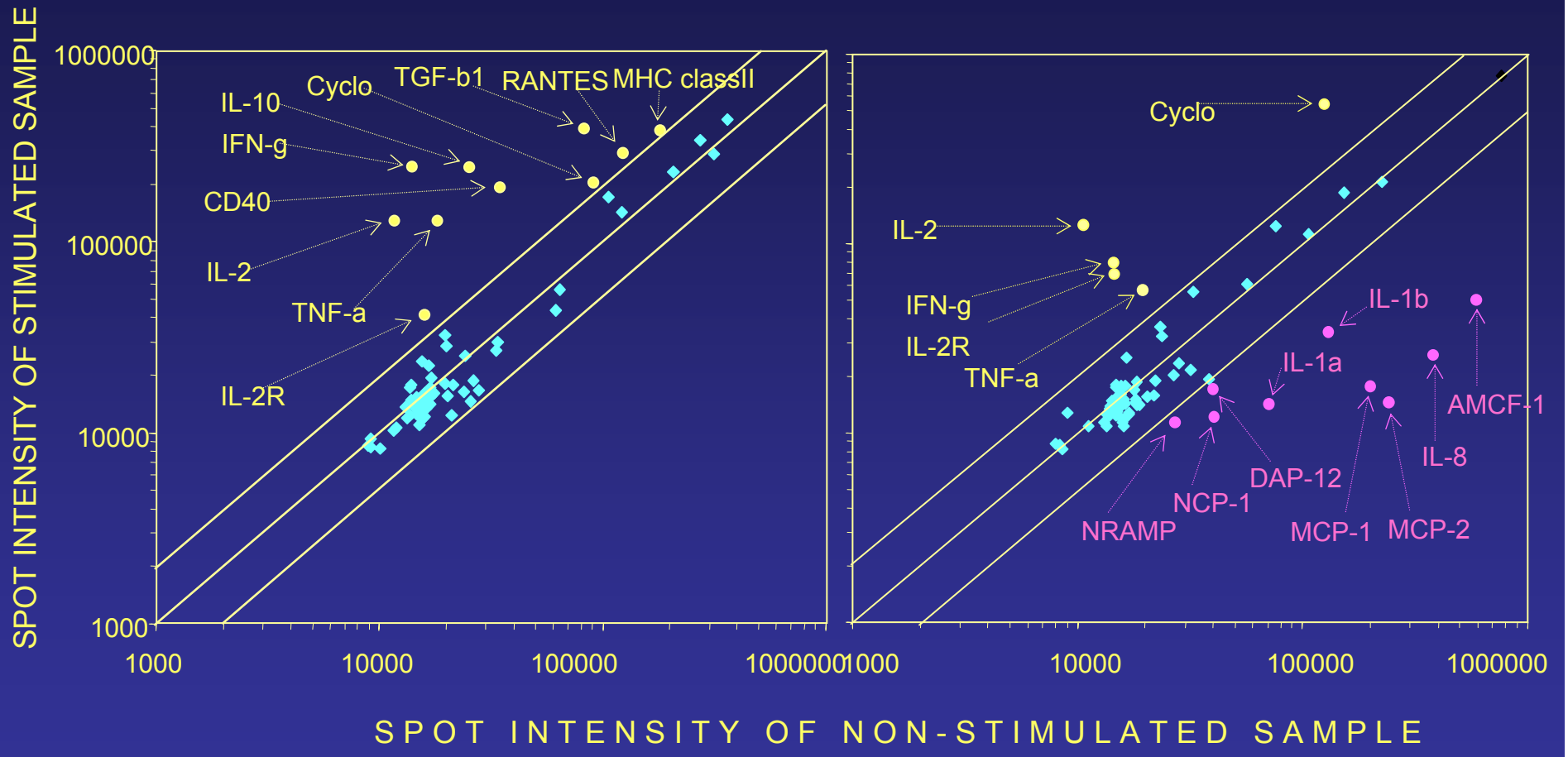
THE IMMUNE RESPONSE : a complex phenomena

- Involvement of many cell types
  - immune cells : lymphocytes, macrophages, neutrophils, dendritic cells ...
  - "non immune" cells : epithelial cells, endothelial cells, fibroblasts…
- Production of many mediators
  - antibodies
  - cytokines, chemokines, growth factors
  - reactive metabolite of oxygen and nitrogen : NO, H_2O_2
  - reactive metabolite of arachidonic acid …
- Many cellular modification during activation
  - synthesis of new mediators
  - expression of new surface molecules
  - proliferation, cell death



NEED OF AN INTEGRATIVE APPROACH

Differential expression of immune genes in unstimulated and stimulated porcine PBMC



We are currently producing a DNA array containing 3500 porcine immune genes

THANK YOU FOR YOUR ATTENTION

FPH Feed for Pig Health