

#### CarboHealth

- Exploring the impact of pre- and probiotics
- on intestinal barrier function
- SP2: Focus on immune system

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### **SP2: Aims**



- Study the bioactivity of pre-/probiotics/synbiotics and SCFA towards gut function (small intestine & colon), innate immune function of gut and
- crosstalk to accompanied/systemic immune cells
- > (Support) hypotheses on mechanism that underlie the effect of pre-/
- probiotics/synbiotics and SCFA
- > Leads for selection of compounds to support effectiveness of vaccine in pig
- trial

### **Experimental set up Caco2 incubations**



### **Caco-2 experiments within CCC3**

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Caco2 incubations with products from industrial partners:

Company	Product	Conc	TEER	ARRAY (21d)
Sensus	Inulins	0.5mg/ml	$\checkmark$	$\checkmark$
Winclove	W63, W37, W56 (6, 18h)	10 <sup>7</sup> , 10 <sup>8</sup>	$\checkmark$	$\checkmark$
	Prebiotics (18h)	0.5, 5mg/ml	$\checkmark$	
	Synbiotics (18h)	10 <sup>7</sup> , 5mg/ml	$\checkmark$	
Nutricia Research	GOS/FOS, FOS/FOS	0.5, 5mg/ml	~	$\checkmark$
Friesland Campina	GOS, GOSLL, lactose	0.5, 5mg/ml (0.2, 2mg/ml)	~	$\checkmark$
SCFA	Ac:Pr:Bu 60:25:15	5, 10, 20, 40, 80mM	✓	$\checkmark$

### **Overview results SP2**



Company	Product	TEER	Immune-related gene expression (arrays)
Sensus	Frutafit TEX, HD, CLR Frutalose OFP	CLR, OFP (21d; 1-6h) ↑	CLR: ameliorating intestinal inflammation OFP: inducing acute phase response
Winclove	Lactobacilli strains W63, W37, W56	W37 (7+21d; 6, 18h) ↑ W56 (7+21d;18h) ↑	W37 (6h): stimulating tight junction, lymphogenesis and differentiation FAE
Nutricia Research	GOS/FOS (9:1) FOS/FOS (9:1)	F/F (21d; 1-6h) ↑	F/F: Stimulating DC/T cell response and tight junctions
Friesland Campina	GOS Vivinal, GOSLL, lactose	GOS, GOSLL, lactose (21d; 1h) ↑	GOS: Antimicrobial effect (actin cytoskeleton signalling, adhesion, ECM remodelling, Ag presentation)
SCFA	Ac:Pr:Bu 60:25:15 (based on inulin fermentation -lit)	10nM no effect (other conc. osmolality effect)	Influence NK cell activation (Stimulation of TICE)

In general hardly any effect on TEER in colonic cells (7d Caco-2)

#### TEER: Caco2 incubations with inulins - SENSUS

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21 days differentiated Caco2 (small intestinal enterocyte-like)



This indicates that short chain inulins enhance epithelial barrier integrity

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#### Microarray data: 21 days-differentiated Caco2 with Inulins - Sensus

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### Frutafit CLR: Ameliorating intestinal inflammation (role in IBD?)



(partly) in accordance with Johnson-Henry et al. J Nutr. 2014, 144:1725-33 and Leenen et al. J Nutr. 2007,137:2572S-2575S

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#### TEER: 21 days-differentiated Caco2 with probiotics - Winclove



This indicates that especially strain W37 enhances epithelial barrier integrity

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#### Microarray data: 21 days-differentiated Caco2 with probiotics - Winclove



#### Microarray data: 21 days-differentiated Caco2 with probiotics - Winclove

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<u>L. acidophilus W37:</u> Differentiation into follicle associated epithelial cells (FAE) ↑



- FAE enterocytes contribute to antigen sampling by sensing luminal pathogens and
- their products and releasing cytokine and chemokine signals that attract and activate
- DCs (Marian R. Neutra & Pamela A. Kozlowski Nature Reviews Immunology 6, 148-158 (February 2006)

Enhanced immune stimulation?

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### TEER: Caco2 with GOS/GOSLL and lactose - Friesland Campina





- This indicates an early effect of GOS on epithelial barrier integrity, especially with higher concentrations
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#### Microarray data: Caco2 with GOS/GOSLL and lactose - Friesland Campina



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#### TEER:Caco2 with scGOS/lcFOS & scFOS/lcFOS (9:1) - Nutricia Research



- 21 days differentiated Caco2 (small intestinal enterocyte-like) 110 TEER (% compared to DMEM) \* # \* # \* # 105 100 → F/F 5 95 **—**F/F 0.5 90 5 0 2 3 6 1 4 hours \* (F/F 5), # (F/F 0.5) significantly different from DMEM (p<0.05)
  - This indicates that scFOS/lcFOS (9:1) enhance epithelial barrier integrity
  - FF profile resembles that of short chain insulins
  - GF profile resembles that of GOS

### Microarray data: Caco2 with scGOS/IcFOS & scFOS/IcFOS (9:1) - Nutricia Research

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- Stimulate DC / T cell response
  (attraction / NEKP activation)?
- (attraction/NFKB activation)?

Immune response / Inflammation



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### Microarray data: Caco2 with SCFA (10mM) - Ac:Pr:Bu 60:25:15 (6h)

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thereby support vaccination efficacy

# Potential compounds that stimulate vaccination efficacy



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### **Pig trial: synbiotic improves vaccination efficacy**



# **Pig trial: inulin enhances NK cell population?**



- NK cells 1, only 2 days before vaccination (day 23),
- mainly in contaminated animals



t1 NK cells CD56 dim

Non-vacc/Vaccinated Inulin Synbiotic

# Pig trial: qPCR + histology on ileum (punch last 2 cm)

**ø2mm** Image shown as reference. See description for actual punch size.

- No significant effects found by FAE-related gene expression analysed by qPCR (n=10)
- Histology (n=7, 6 pictures/pig)



Treatment	M cells	SEM
Non-Vaccinated	2.2679ª	0.0263
Vaccinated <sup>#</sup>	3.4167ª	0.0598
Prebiotic	4.0192ª	0.0683
Synbiotic*	3.3611ª	0.0755

- Only very small piece of tissue analysed
- Only samples at the end of the pig trial

### **Other array generated leads**



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# **Other array generated leads – literature support**

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Validation gave variable results.....

# **Overall conclusions SP2**



 In vitro experiments show and support an effect of pre-/probiotics/synbiotics and SCFA on intestinal barrier functioning, including immune regulation

- Array data can provide valuable leads for generating new hypotheses:
  - pre-/probiotics/synbiotics support of vaccination efficacy
  - (potential underlying mechanisms)
  - SCFA stimulate TICE
  - GOS might have an antimicrobial effect



# Thanks for your attention



















## **Other array generated leads - SCFA**



Hypothesis: SCFA can stimulate transintestinal cholesterol excretion (TICE)?

# Hypothesis validation in vivo: inulin intervention in mice



### **Other array generated leads**



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# **Other array generated leads - GOS**



- <u>ORM1 (orosomucoid 1) ↑</u>: immunomodulatory functions (on

- granulocytes, lymphocytes, monocytes and other cells involve in
- immune response); antiinflammatory molecule, direct protection against
- pathogens (LPS) secreted
- Ceciliana et al. Curr Protein Pept Sci. 2007 Feb;8(1):91-108
- <u>GBP1 (guanylate-binding protein 1) ↑</u>: protection against pathogens -
- cytoplasmic
- Vestal et al. J Interferon Cytokine Res. 2011; 31(1): 89–97
- <u>SLPI (secretory leukocyte peptidase inhibitor) ↑</u>: protecting epithelial
- surfaces from attack by endogenous proteolytic enzymes. This
- antimicrobial protein has antibacterial (inhibits bacterial growth),
- antifungal and antiviral activity secreted
- Si-Tahar et al. Gastroenterology. 2000 Jun;118(6):1061-71
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#### Effect of GOS Vivinal (5mg/ml) on antimicrobial gene expression (time course)



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### Detection ORM1/SLPI in apical/BL medium of Caco-2 cells (time course study)



- ORM1 and SLPI secretion most pronounced on apical site
- GOS (5mg/ml) stimulates production and secretion of ORM1 and SLPI
- Hard to reproduce these data
- > Also additional experiments like bacterial translocation, -adhesion assays
- gave variable, so no conclusive results (yet)