

Intestinal Barrier and Probiotics

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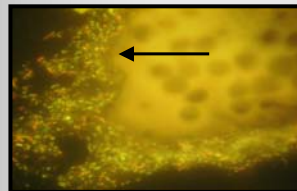
IBD: a barrier defect

Normal



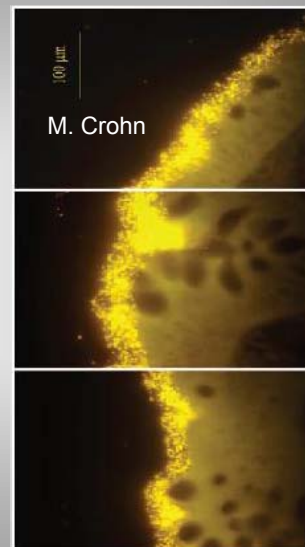
Intestinal Lumen
 10^{14} (an)aerobic bacteria

Outer mucus layer (700 μm)
mucins⁽⁻⁾ and
antibacterial peptides⁽⁺⁾
(defensins, cathelicidines)



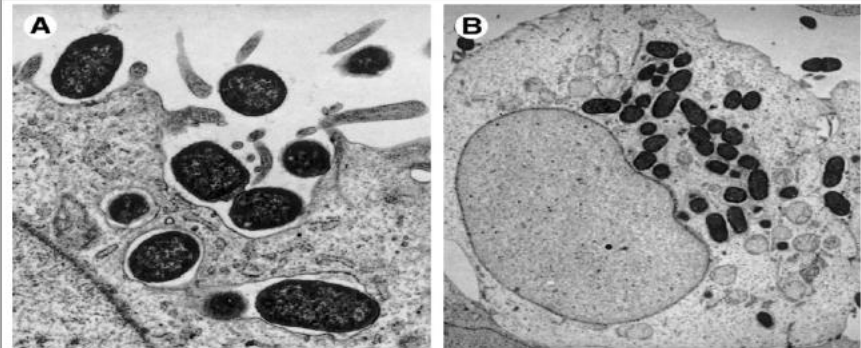
Inner mucus layer (100 μm)
adherent, rich in
antibacterial peptides

Crypts (200 μm)
epithelial cell barrier
secretion of mucus
and defensins etc.



A. Swidsinski

Adherent-invasive E. coli



AIEC in 21.7% of CD patients versus 6.2% of controls (ileal samples)

AIEC in 3.7% of CD patients versus 1.9% of controls (colonic samples)

Darfeuille-Michaud et al., Gastro 2004

No bacteria - no inflammation

- Diversion of the fecal stream is effective in ameliorating CD distally, luminal contents trigger inflammation

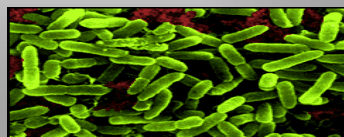
Harper et al. Gut 1983, Rutgeerts et al., Lancet 1991
D'Haens et al., Gastroenterology 1998

- T-cell response against the autologous bacterial flora in Crohn's disease

Duchmann et al., Gut 1999

- Serological response to bacteria/fungal antigens (ANCA, ASCA, anti-flagellin)

Mow et al. Gastroenterology 2004)
Lunardi et al. J Intern Med 2008)



Normal mucosa/mucus is rather sterile and protected by epithelial antibacterial peptides:

Constitutive HBD-1

Permanent war at host interface

Defensins
Cathelicidins
Elafin / Antiproteases
BPI
Lysozyme

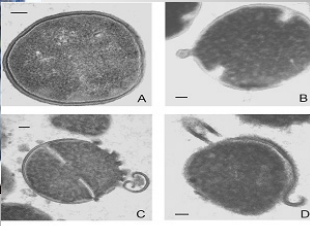
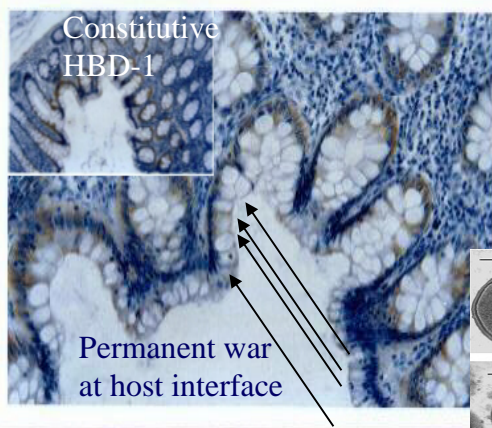
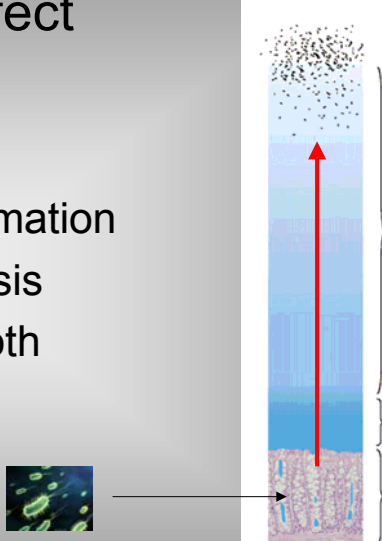


Figure 4

Harder et al. JBC 2003

Some probiotics affect the barrier

- Inducible defensin formation
- Mucin (Muc 1) synthesis
- The combination of both



Defensins

No eukaryotic organism without antimicrobial peptides, defensins are the major group

Small, cationic antimicrobial peptides, β - sheet structure

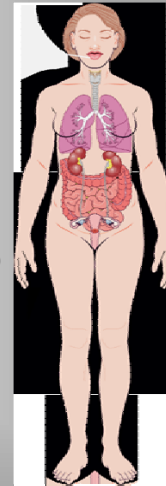
→ Disulfide bonds between cystein residues

→ Function via insertion in microbial membranes

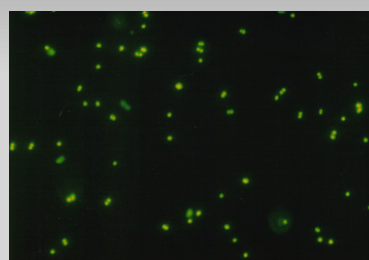
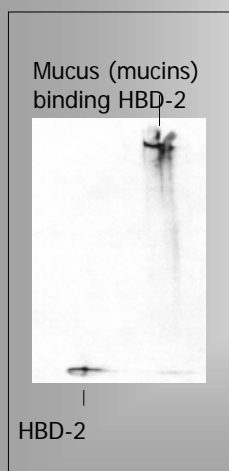
→ Production either constitutively or on demand

α - Defensins are produced in Paneth cells (HD5 and HD6)
+ granulocytes (HNP1-4)

β - Defensins are expressed in most surfaces (skin,
gastrointestinal mucosa, genitourinary tract, lung):
HBD1 (constitutive)
HBD2, 3 and 4 (inducible during inflammation)

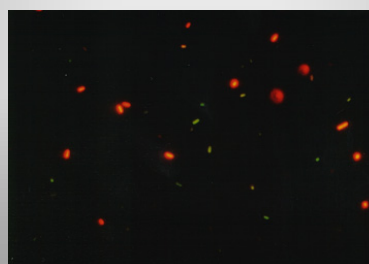


Binding of antimicrobial peptides to mucins in mucus while retaining functional activity



Staining with SYTO 9®
(living bacteria green) and
Propidium iodide (dead
bacteria red)

E. coli ATCC 29522
Untreated control



E. coli ATCC 29522
Incubated with
colonic mucus

Dr. Sabine Nuding

RBK Probiotic bacteria: mechanisms of action?

- Probiotic derived antibacterial substances (microcins)
- Competitive inhibition of pathogens
- Modulation of inflammatory responses:
 - Increasing antiinflammatory cytokine production
 - Suppressing proinflammatory cytokine production
 - Modulation of TLR's
- Induction of barrier effector molecules

RBK Induction of the human β -defensin-2 in intestinal epithelial cells by *E. coli* Nissle **IKP STUTTGART**

relative transcript level HBD-2


25
20
15
10
5
0

unstimulated
E. coli Nissle 1917
EPEC
K12 DSM498
UPEC
PZ 865
PZ 860
PZ 862
PZ 866
PZ 915
PZ 861
PZ 864
PZ 868
PZ 870
PZ 863
PZ 867
PZ 835
PZ 830


30 more *E. coli* isolates tested: all negative.

***E. coli* Nissle is quite unique among *E. coli*'s**

Wehkamp et al. *Infection and Immunity* 2004

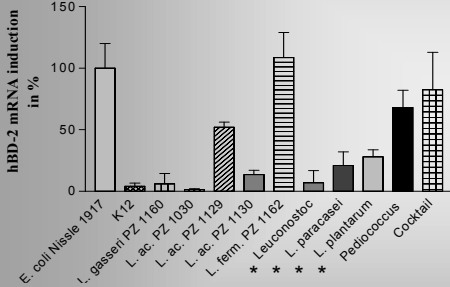


Induction of defensins: what about other probiotics?



Clinical and Experimental Immunology ORIGINAL ARTICLE
doi:10.1111/j.1365-2249.2007.0358

Probiotic lactobacilli and VSL#3 induce enterocyte β -defensin 2



Wehkamp et al. *Infection and Immunity* 2004

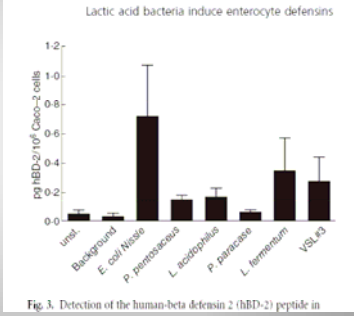
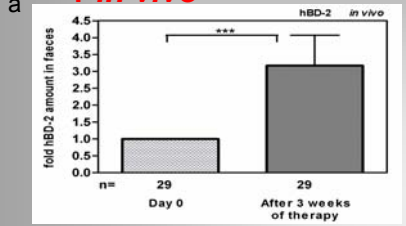


Fig. 3. Detection of the human-beta defensin 2 (hBD-2) peptide in
Schlee et al. *Clin and Exp. Immun* 2008

The effect is not specific to *E. coli* Nissle but can be found in other probiotic bacteria

Fecal HBD-2 peptide concentration in volunteers

1 in vivo



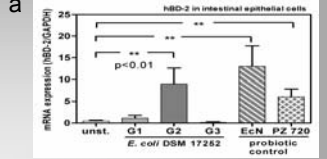
n= 29 Day 0 29 After 3 weeks of therapy

hBD-2 in vivo

fold HBD-2 amount in faeces

HBD-2 expression in cell culture

2 in vitro

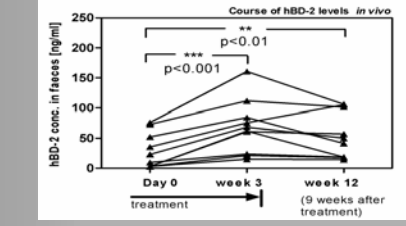


mRNA expression (HBD-2/HPH)

p<0.01

**

hBD-2 in intestinal epithelial cells

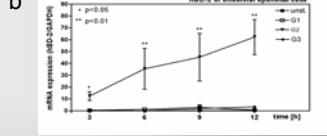


Course of hBD-2 levels in vivo

HBD-2 conc. in faeces [ng/ml]

p<0.001 p<0.01

Day 0 week 3 week 12
(9 weeks after treatment)

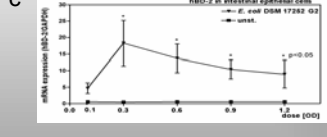


hBD-2 in intestinal epithelial cells

mRNA expression (HBD-2/HPH)

p<0.01 **

0 0.5 1 2
time [h]



hBD-2 in intestinal epithelial cells

mRNA expression (HBD-2/HPH)

p=0.05

0.0 0.1 0.5 0.9 1.2
dose [OD₆₀₀]

E. Coli probiotic (Symbioflor) treatment induces intestinal secretion of HBD-2 peptide in man

Antimicrobial Activity against Probiotics

A

vehicle control

hBD-2 (1 µg)

E. coli strain: K12, Nissle1917, G2

antimicrobial activity hBD-2

diameter inhibition zone (mm)

hBD-2 (µg): +, -

E. coli strain: K12, Nissle1917, G1, G2, G3

DSM 17252

B

vehicle control

hBD-3 (1 µg)

E. coli strain: K12, Nissle1917, G2

antimicrobial activity hBD-3

diameter inhibition zone (mm)

hBD-3 (µg): +, -

E. coli strain: K12, Nissle1917, G1, G2, G3

DSM 17252

C

vehicle control

lysozyme (1 µg)

E. coli strain: K12, Nissle1917, G2

antimicrobial activity lysozyme

diameter inhibition zone (mm)

lysozyme (µg): +, -

E. coli strain: K12, Nissle1917, G1, G2, G3

DSM 17252

- Suicidal effect of probiotic stimulation?
Explanation for need of daily delivery?

Defensin expression in IBD

Defensins = antimicrobial peptides
Spectrum: gram-positive and gram-negative bacteria, viruses

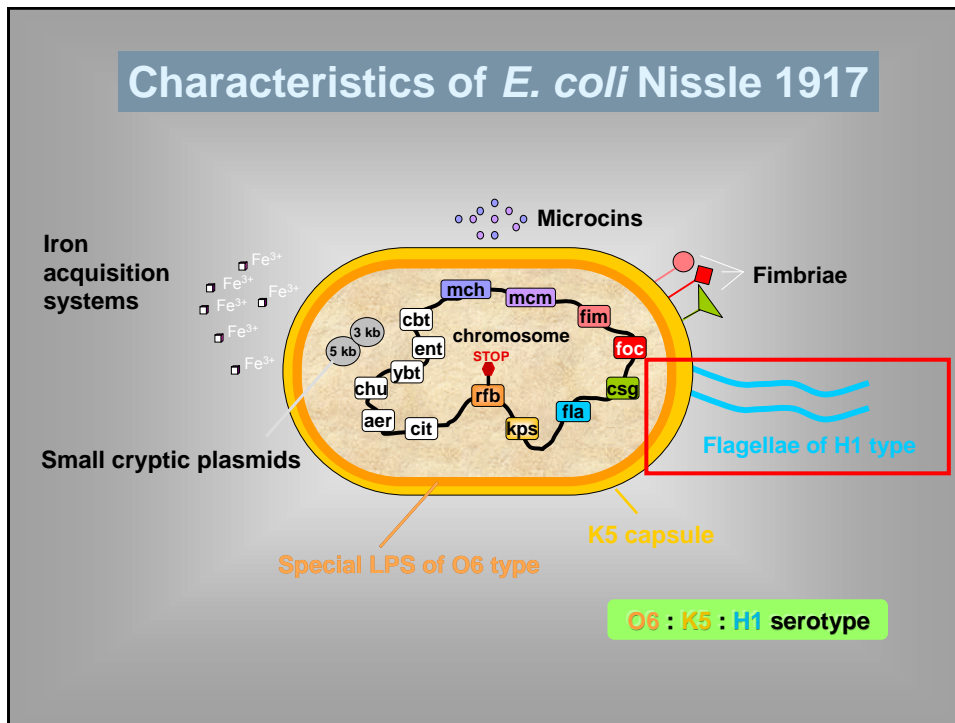
Lack of induction of the human beta defensin-2 (hBD-2) in inflamed Crohn's disease and in noninflamed UC


Relative Expression

Controls (N=33), Unspecific Inflammation (N=10), Crohn's disease (N=22), Colitis ulcerosa (N=28)

Webkamp et al. IBD 2003

Hypothesis:
The benefit from *E. coli* Nissle in maintenance of remission may be due to induction of defensins






Probiotic *E. coli* in IBD: Background

First Case Report on the Treatment of Colitis with *E. coli* Nissle 1917 (MUTAFLO[®])

A. Nissle (1918). Die antagonistische Behandlung chronischer Darmstörungen mit Colibakterien. Med. Klinik No. 2:29-33.



Alfred Nissle

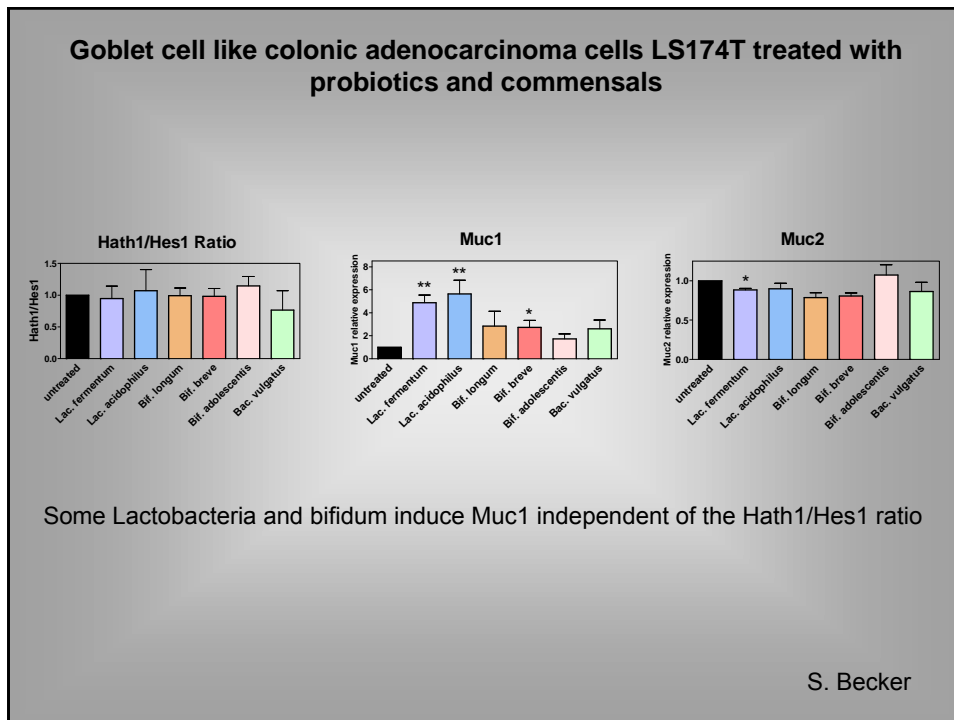
Study	Treatment	Duration	Remission Rate (%)
Krus I	Mesalazine	3 Mon	~10
	<i>E. coli</i>	3 Mon	~15
Rembacken	Mesalazine	12 Mon	~75
	<i>E. coli</i>	12 Mon	~70
Krus II	Mesalazine	12 Mon	~35
	<i>E. coli</i>	12 Mon	~35

Treatment with the non pathogenic *E. coli* Nissle is equivalent to mesalazine in maintaining remission in ulcerative colitis

Rembacken et al., Lancet 1999
Krus et al., Alimentary Pharmacol Ther 1997
Krus et al., Gut 2005

Current consensus:

Probiotic *E. coli* Nissle (and other Probiotic strains) seem to be effective in maintaining remission of ulcerative colitis, rather no effect in Crohn's disease



Intestinal Barrier and Probiotics

- The intestinal is the clue to understand health and IBD
- Probiotics directly enhance this barrier by inducing defensins and mucins