Intestinal Barrier and Probiotics

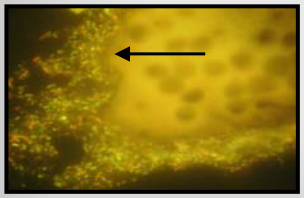
Eduard F. Stange

IBD: a barrier defect

Normal

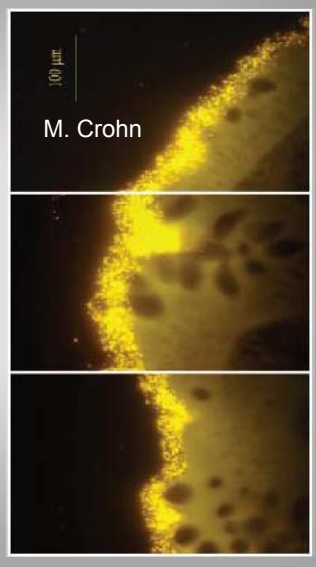
Intestinal Lumen
10^{14} (an)aerobic bacteria

Outer mucus layer (700 um)
mucins $^{(-)}$ and
antibacterial peptides $^{(+)}$
(defensins, cathelicidines)



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

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



A. Swidsinski

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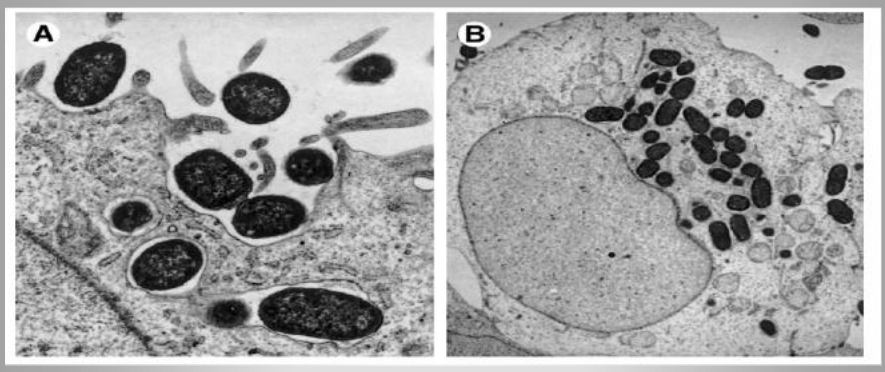
Adherent-invasive E. coli



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

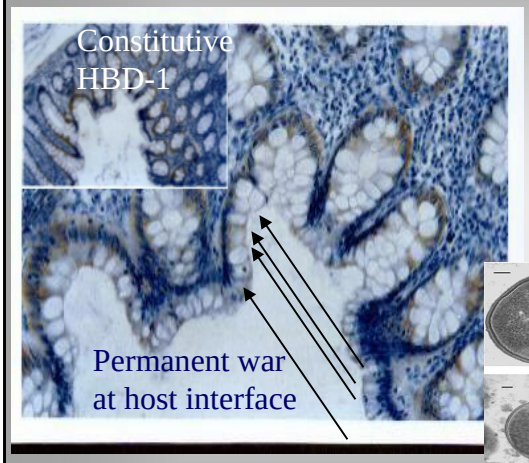
- T-cell response against the autologous bacterial flora in Crohn´s disease

  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:



Defensins
Cathelicidins
Elafin / Antiproteases
BPI
Lysozyme

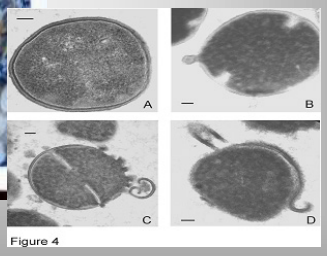
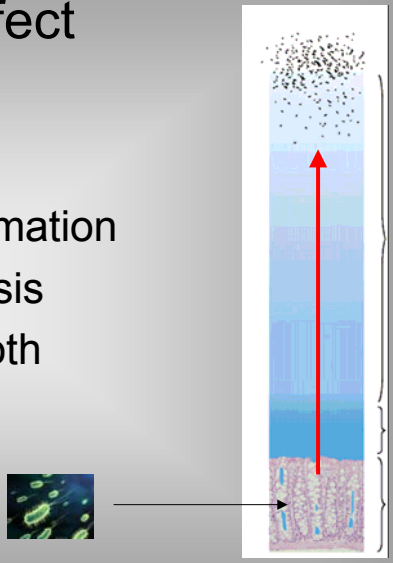


Figure 4

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

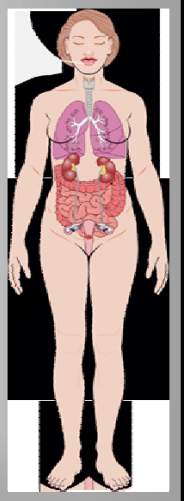
→Disulfide bonds between cystein residues

→Function via insertion in microbial membranes

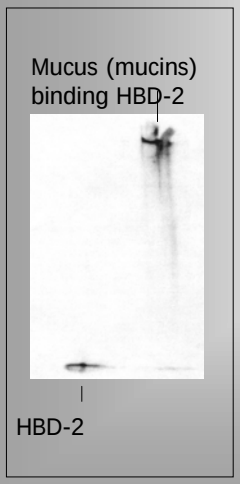
→Production either constitutively or on demand

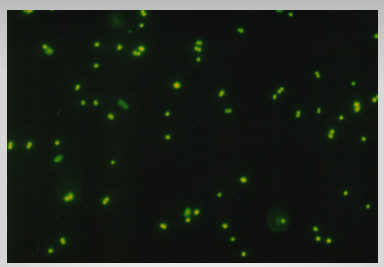
α- Defensins are produced in Paneth cells (HD5 and HD6) + granulocytes (HNP1-4)

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

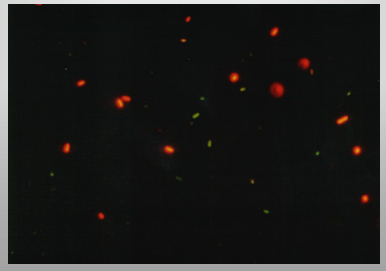


Binding of antimicrobial peptides to mucins in mucus while retaining functional activity





E. coli ATCC 29522
Untreated control



E. coli ATCC 29522
Incubated with colonic mucus

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

Dr. Sabine Nuding

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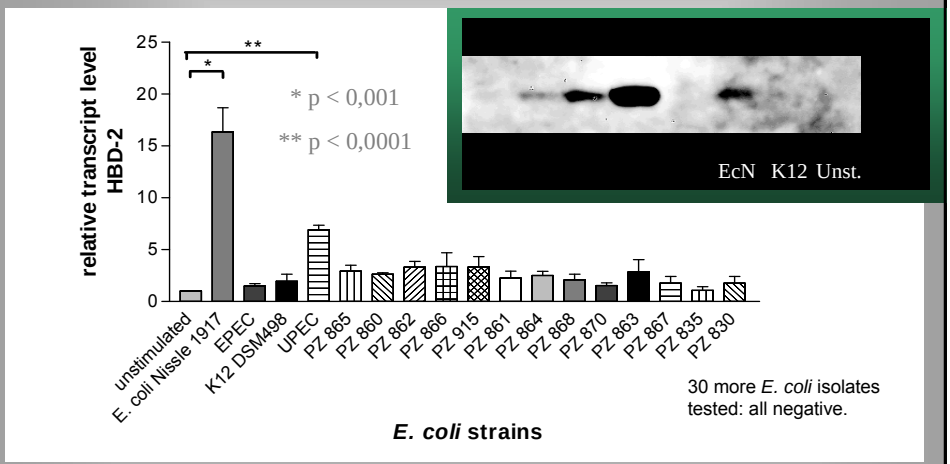
Probiotic bacteria: mechanisms of action?

- 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

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



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

Wehkamp et al. *Infection and Immunity* 2004

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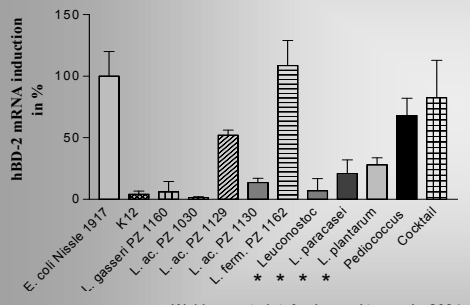
Induction of defensins: what about other probiotics?

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

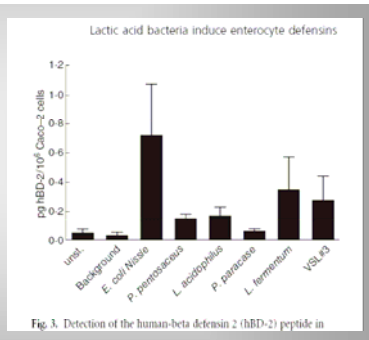


Fig. 3. Detection of the human-beta defensin 2 (hBD-2) peptide in

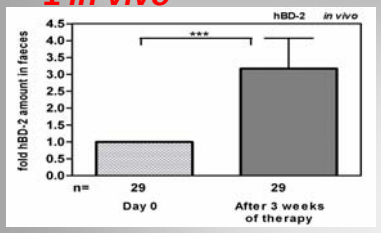
Schlee et al. Clin and Exp. Immun 2008

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

Fecal HBD-2 peptide concentration in volunteers

1 *in vivo*

a



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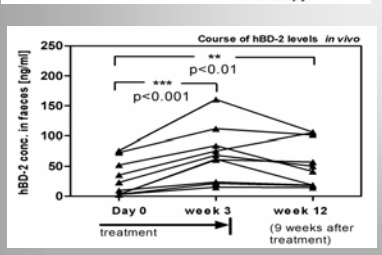
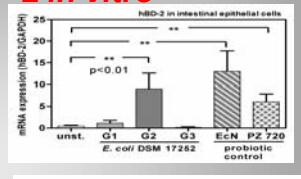


HBD-2 expression in cell culture

2 *in vitro*

a



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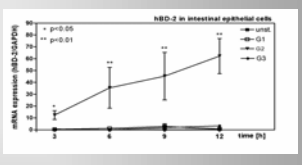

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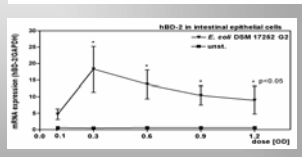

E. Coli probiotic (Symbioflor) treatment induces intestinal secretion of HBD-2 peptide *in man*

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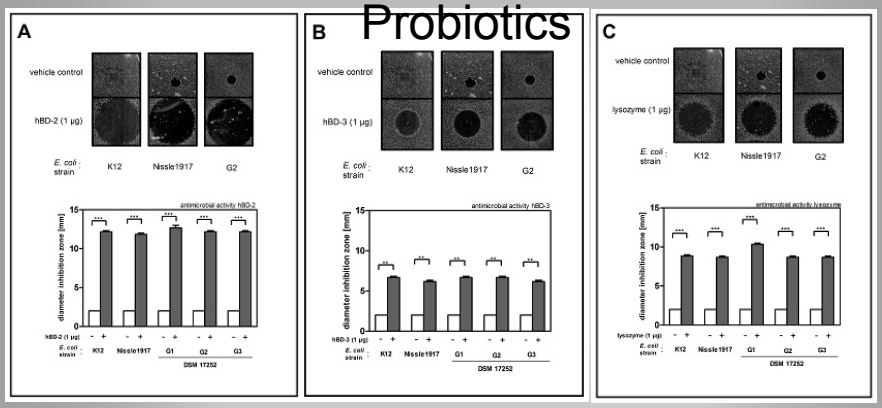
Antimicrobial Activity against Probiotics

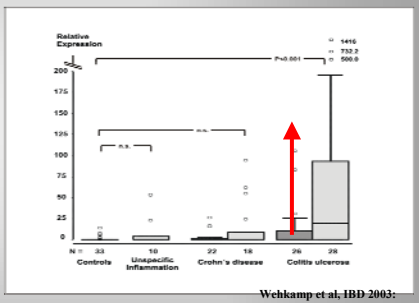


- Suicidal effect of probiotic stimulation?
  Explanation for need of daily delivery?

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



Wehkamp et al, IBD 2003:

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

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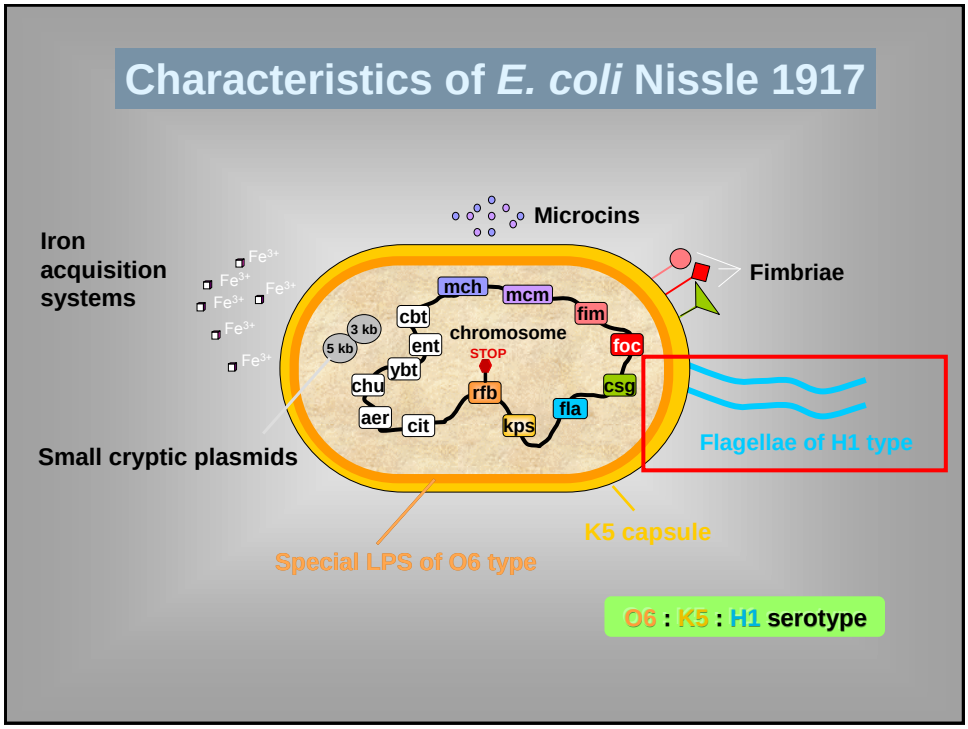

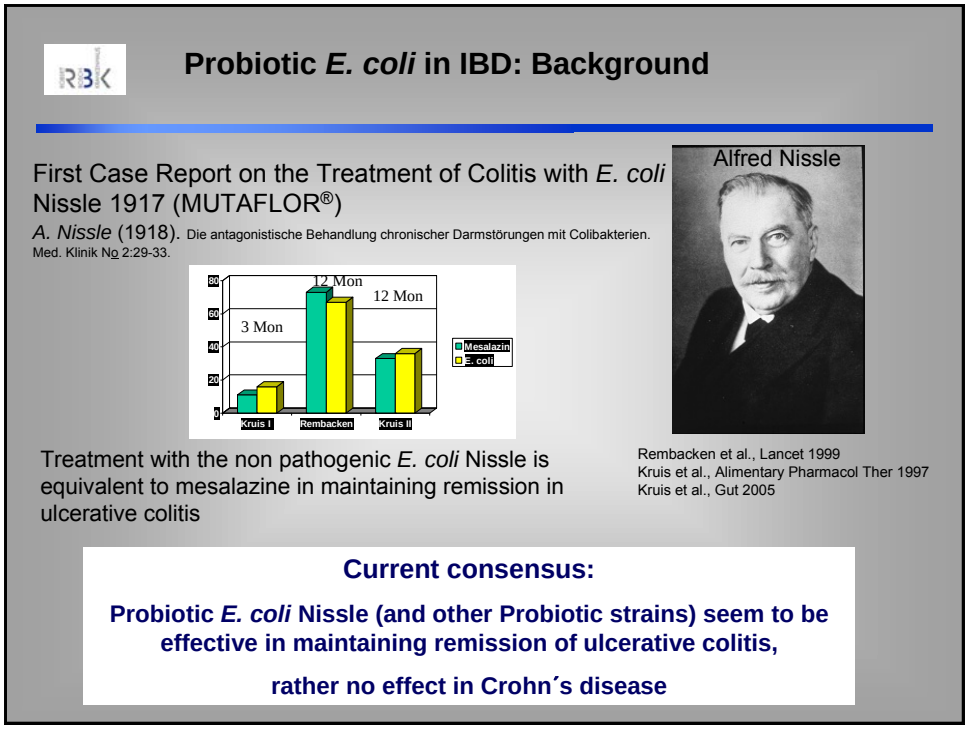

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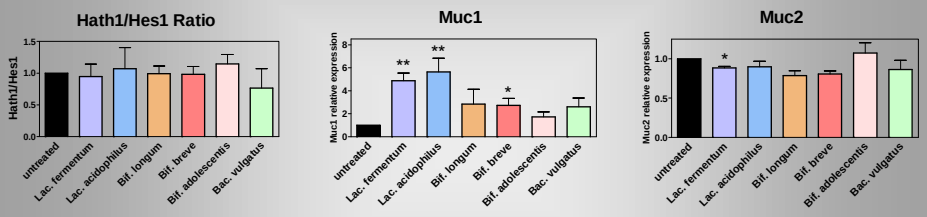
Goblet cell like colonic adenocarcinoma cells LS174T treated with probiotics and commensals



Some Lactobacteria and bifidum induce Muc1 independent of the Hath1/Hes1 ratio

S. Becker

Intestinal Barrier and Probiotics

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

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