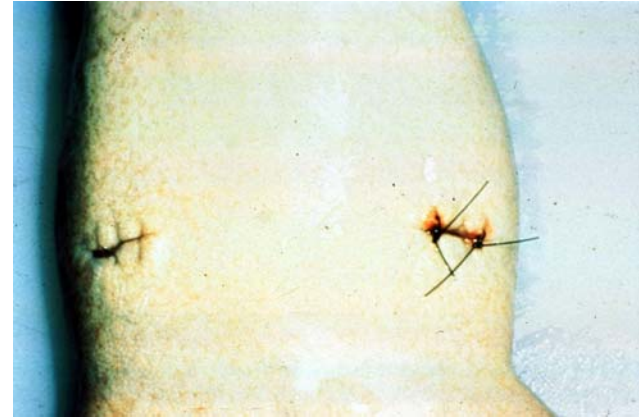
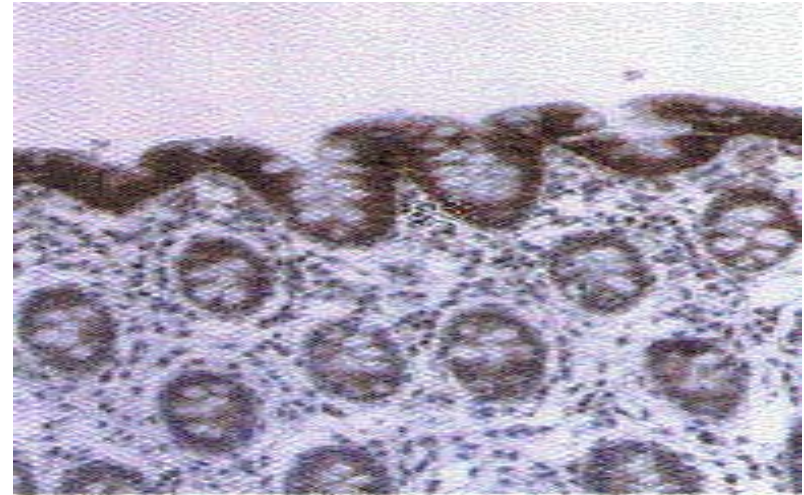
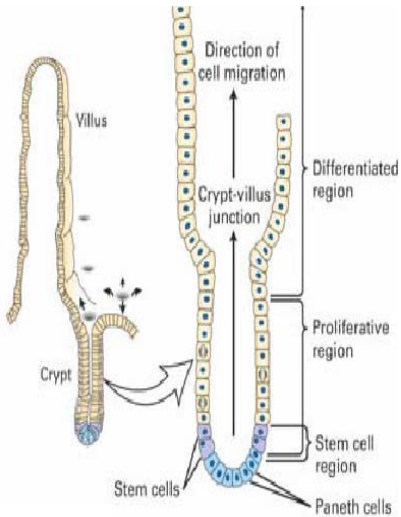
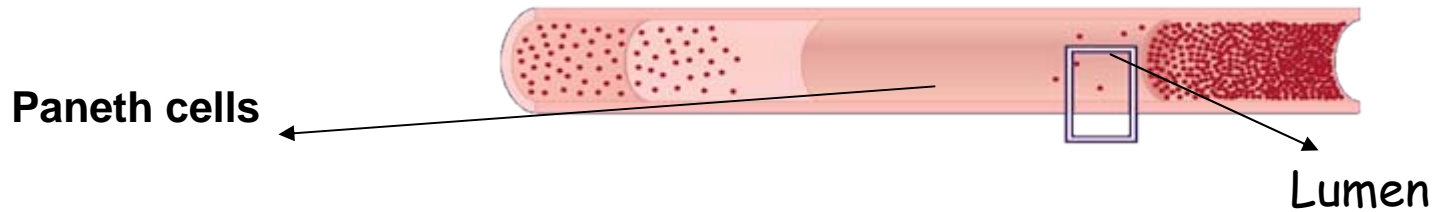


# Discovery of Magainin Peptides



# Antimicrobial peptides protect the bowel wall and affect microbial growth



## *Alpha-defensins*

Secreted into lumen; Limits microbial growth; Protect stem cells; Regulate composition of commensals

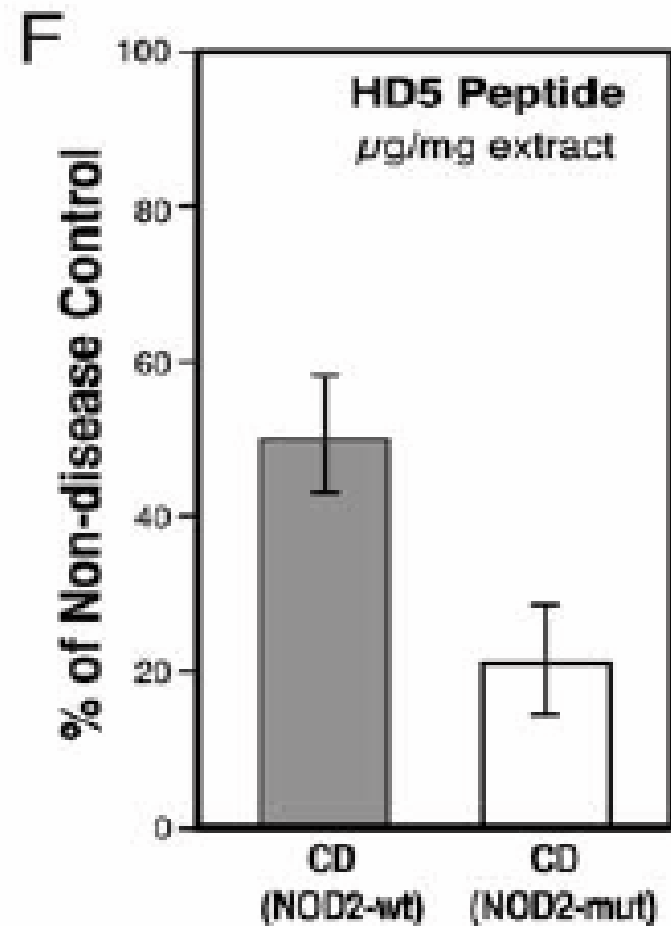
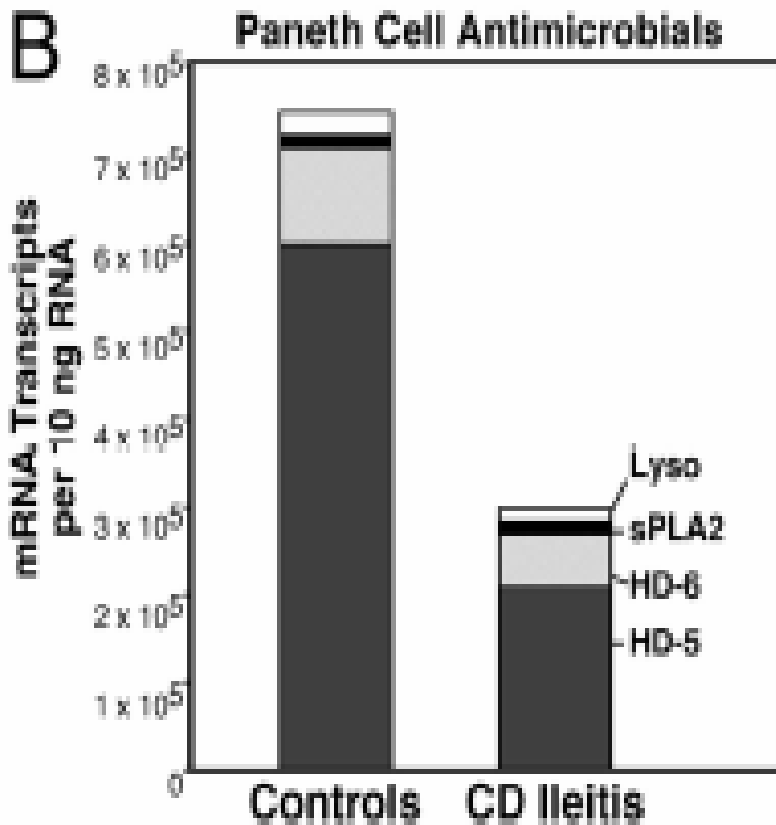
## *Beta-defensins; LL-37*

Secreted into mucous biofilm; limit microbial access to epithelial layer (chemical shield)

# Reduced paneth cell $\alpha$ -defensins in ileal Crohn's disease

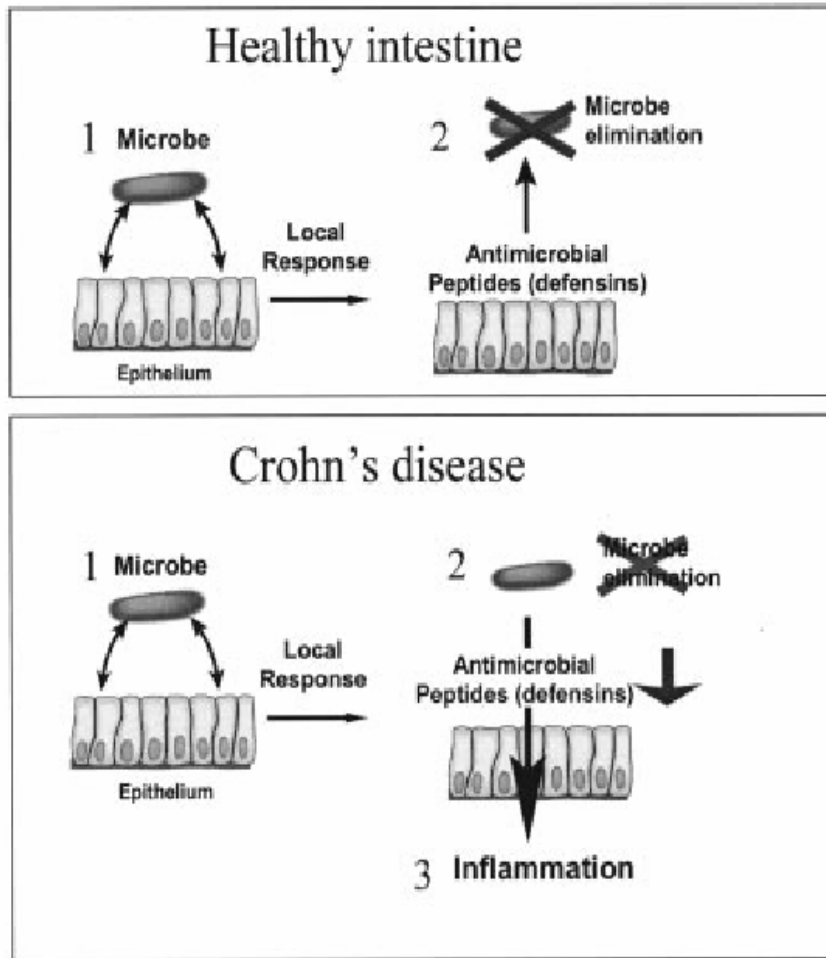
PNAS, Feb 06

Jan Wehkamp\*, Nita H. Salzman<sup>†</sup>, Edith Porter<sup>‡§</sup>, Sabine Nuding<sup>¶||</sup>, Michael Weichenthal<sup>\*\*</sup>, Robert E. Petras<sup>††</sup>, Bo Shen<sup>‡‡</sup>, Elke Schaeffeler<sup>||</sup>, Matthias Schwab<sup>||</sup>, Rose Linzmeier<sup>§</sup>, Ryan W. Feathers\*, Hiutung Chu\*, Heriberto Lima, Jr.<sup>‡</sup>, Klaus Fellermann<sup>¶||</sup>, Tomas Ganz<sup>§</sup>, Eduard F. Stange<sup>¶||§§</sup>, and Charles L. Bevins<sup>\*§§</sup>



# Crohn's Disease: A disease of innate immunity

**Nod2**, an MDP receptor (enterocytes, macrophages, and Paneth cells) is defective in 30% of CD.



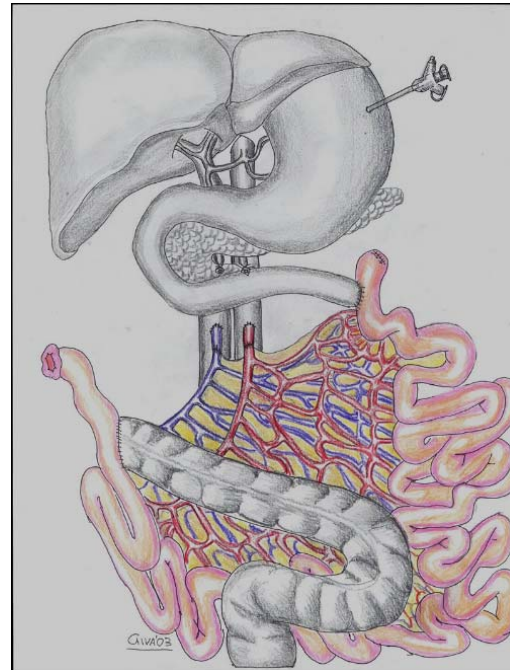
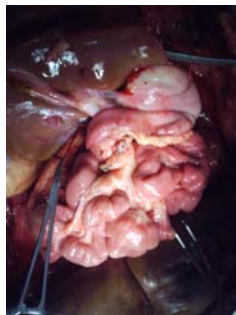
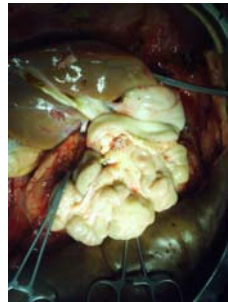
# A defective enterocyte microbial sensing system can lead to ...

- Impaired amp production
- Impaired enterocyte responses involved in repair and maintenance of barrier functions
- Impaired enterocyte communication with the adaptive immune system

How does the innate immune system of a human bowel accommodate the “stresses” of transplantation?



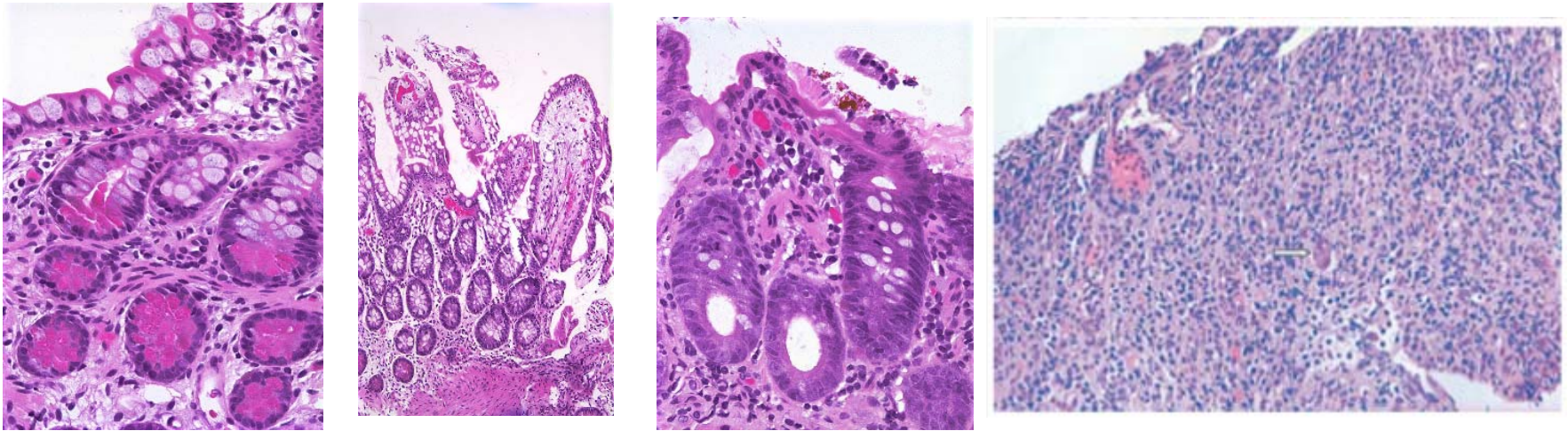
The surgical details of small bowel transplantation



## Small intestinal transplantation provides a unique experimental setting

- Repeat tissue sampling and endoscopy routine
- Bowel sterilized, followed by oral feeding
- Limited antibiotic use
- Nervous system isolated
- Bowel functions "normally" by 3 months

## Small intestine "rejection" resembles inflammatory bowel disease



"Normal" → Mild → Moderate → Severe

- Most develop some degree of rejection, with 35% severe
- The crypts (and Paneth cells) appear to be a target
- Inflammatory picture resembles what is seen in Crohn's disease
- Severe disease can recover a normal bowel lining, following appropriate therapy

# The hypothesis that has guided our study

- Small transplantation “fails” when the innate immune system can no longer protect the gut wall from bowel microbes
- AMP production from the Paneth cells and/or the enterocyte lining cells are inadequate
- Gut microbes lose the “normal” constraints on their numbers and diversity
- Severe inflammation ensues in an attempt to “protect” the bowel, resulting in tissue damage

# Methods

- Collection of tissue at specific times post transplant
- Collection of bowel contents from ileum for determination of microbe # and species
- Global gene expression profile from isolated crypts
- Paneth cell gene products by PCR
- Possible therapeutic intervention



# The results: "first peek"

21 Transplants at GUMC in 2003-2005

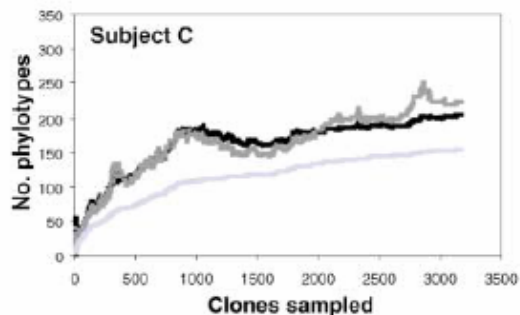
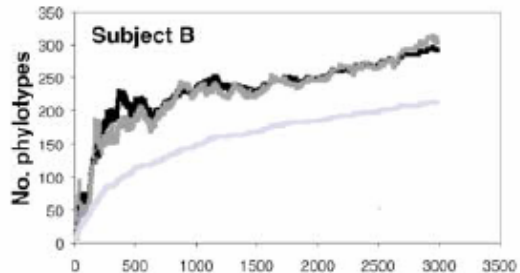
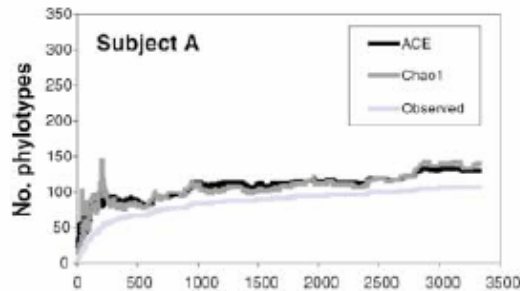
11 NOD2 Crohn's polymorphism in either donor and/or recipient

10 Patients were WT in both donor and recipient

<b>NOD2 status of recipient/donor</b> *	Crypt Apoptosis	Graft survival 1 year	Transplant loss: rejection	Death
wt/wt	mild	10/10	0/10	0/10
<b>NOD2 POLYMORPHISM</b>	severe	9/11	10/11	2/11

# The results: "first peek"

Patient with NOD2 CD genotype who received a bowel with NOD2 CD genotype harbored gut microbes of limited diversity



Relman, Science 05

Phylum	Class	Order	Family	GT41.tally Start Feeding	GT52.tally Diet ^	GT70.tally Diet ^	Total
FIRMICUTES				21	82	16	119
	CLOSTRIDIA			21	82	16	119
		CLOSTRIDIALES		21	82	16	119
			ACIDAMINOCOCCACEAE	0	1	1	2
			CLOSTRIDIACEAE	21	80	8	109
			EUBACTERIACEAE	0	0	1	1
			LACHNOSPIRACEAE	0	1	5	6
PROTEOBACTERIA				74	6	51	131
	GAMMAPROTEOBACTERIA			74	6	51	131
		ENTEROBACTERIALES		72	6	51	129
			ENTEROBACTERIACEAE	72	6	51	129
		PSEUDOMONADALES		2	0	0	2
			PSEUDOMONADACEAE	2	0	0	2
VERRUCOMICROBIA				0	0	2	2
	VERRUCOMICROBIAE			0	0	2	2
		VERRUCOMICROBIALES		0	0	2	2
			VERRUCOMICROBIACEAE	0	0	2	2
			Phylotypes	3	3	6	
			Clones sequenced	98	88	69	

# Implications of work for IBD

- **Rejection** involves NOD2 mediated mechanisms and Paneth cell dysfunction, similar to IBD.
- **Immediate impact on bowel transplantation:** Donor NOD2 genotyping--immediate impact on success of intestinal transplantation
- **Interventions to control transplant rejection might benefit individuals with IBD:**
  1. induce expression of gut antimicrobial peptide -- vitamin D, probiotic bacteria
  2. stem cells repopulation of the small intestine
  3. growth factors