

International Human Microbiome Congress



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GUT MICROBIOTA STUDIES IN ULCERATIVE COLITIS

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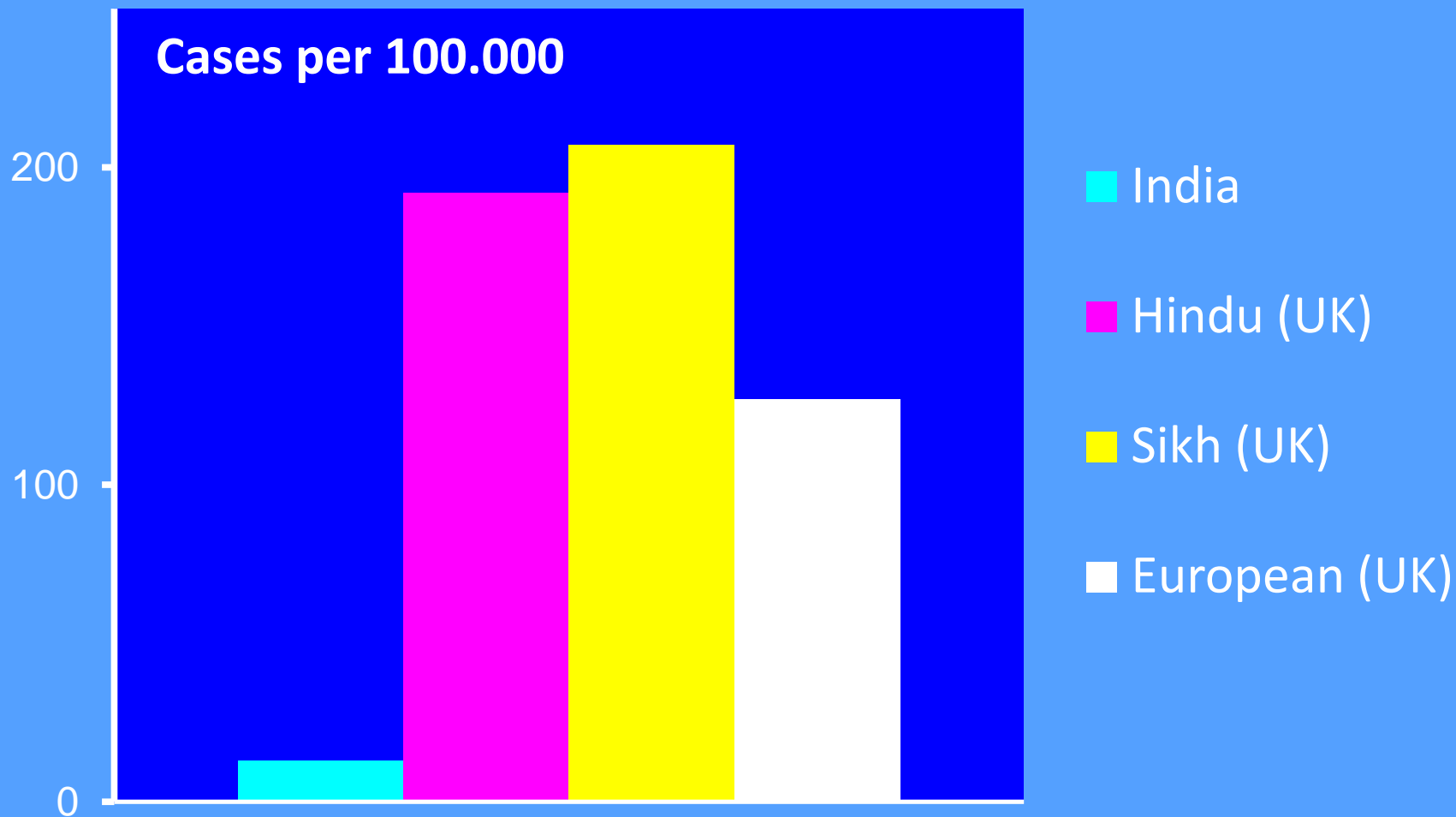
ULCERATIVE COLITIS

- Chronic inflammatory bleeding ulcers in the colonic mucosa of unknown etiology.
- No pathogen identified, contagious transmission never documented.
- Low rate of spontaneous remission.
- Lack of eradication therapy.
- Prevalence rates of 0.3% in Europe and North America.

GWAS STUDIES IN ULCERATIVE COLITIS

- Meta-analysis of GWA datasets (6,687 UC cases and 19,718 controls) have identified 47 susceptibility loci.
- 28 loci shared between Crohn's disease and UC.
- Candidate genes within associated loci include IL1R2, IL8RA-IL8RB, IL7R, IL12B, DAP, PRDM1, JAK2, IRF5, GNA12 and LSP1

PREVALENCE OF ULCERTIVE COLITIS



PATHOGENESIS OF IBD

Genetic Susceptibility

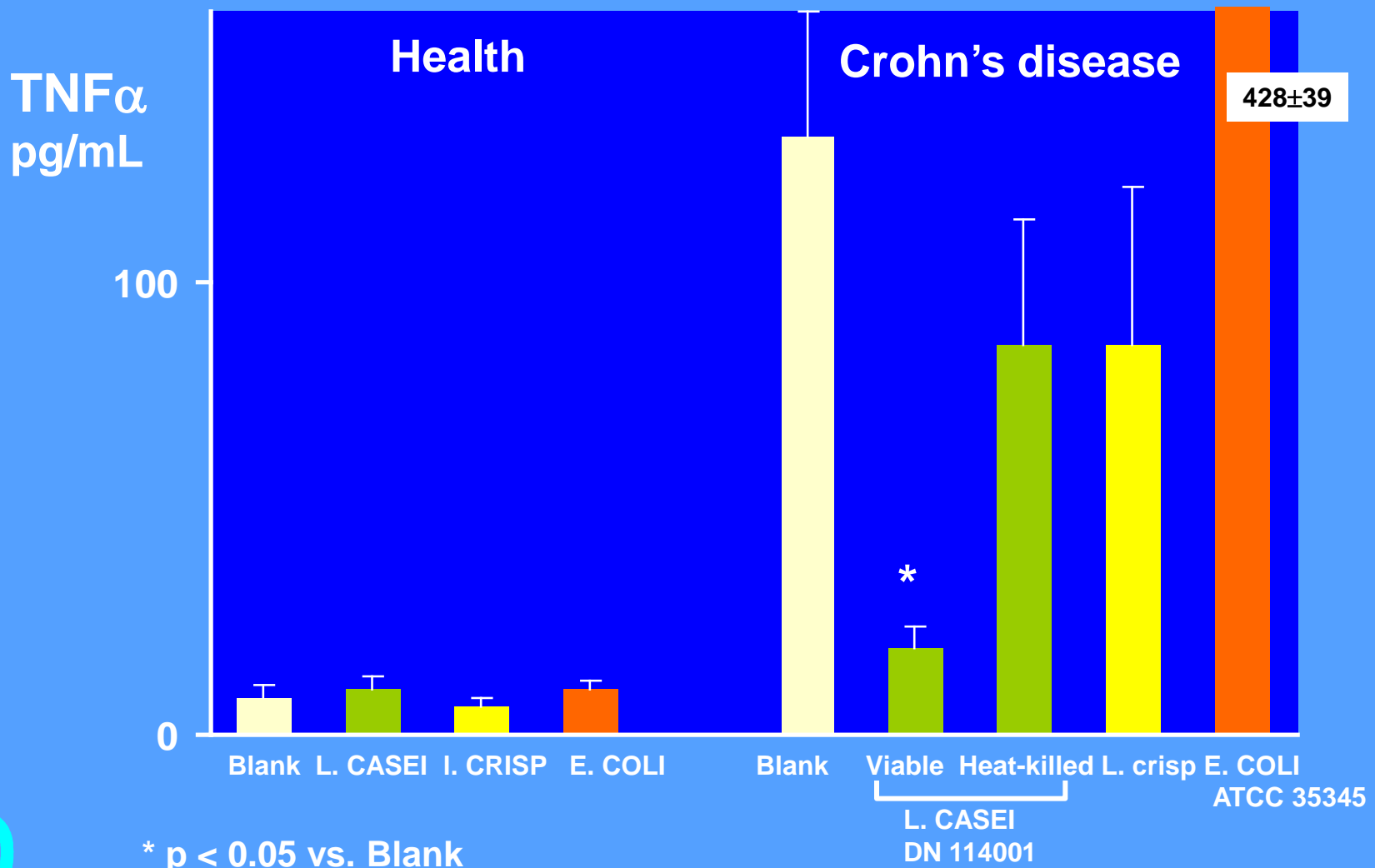
Dysregulated Immune Response



Commensal Gut Microbes

Xavier & Podolsky, Nature 2007
Strober et al, J Clin Invest 2007
Asquith & Powrie, J Exp Med 2010

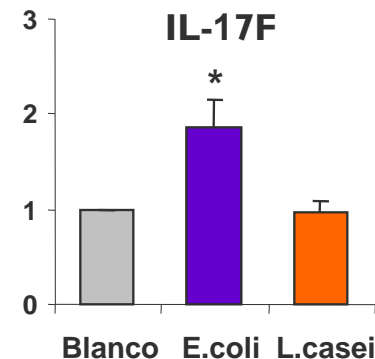
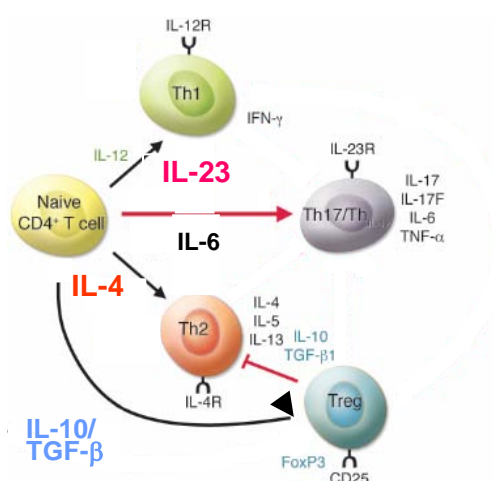
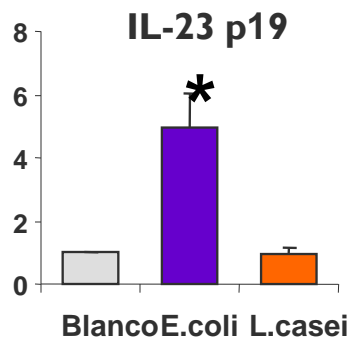
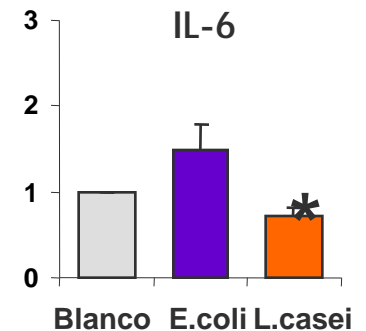
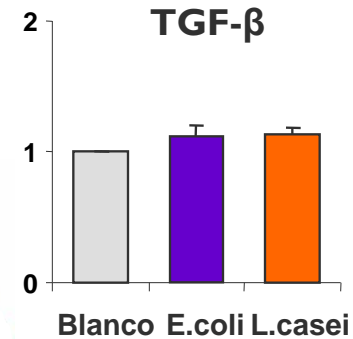
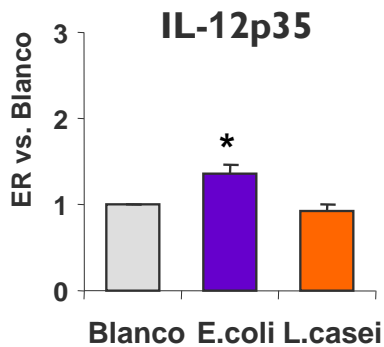
INTERACTION OF NONPATHOGENIC BACTERIA WITH HUMAN INTESTINAL MUCOSA



Cytokine/Chemokine expression by human intestinal mucosa in organ culture

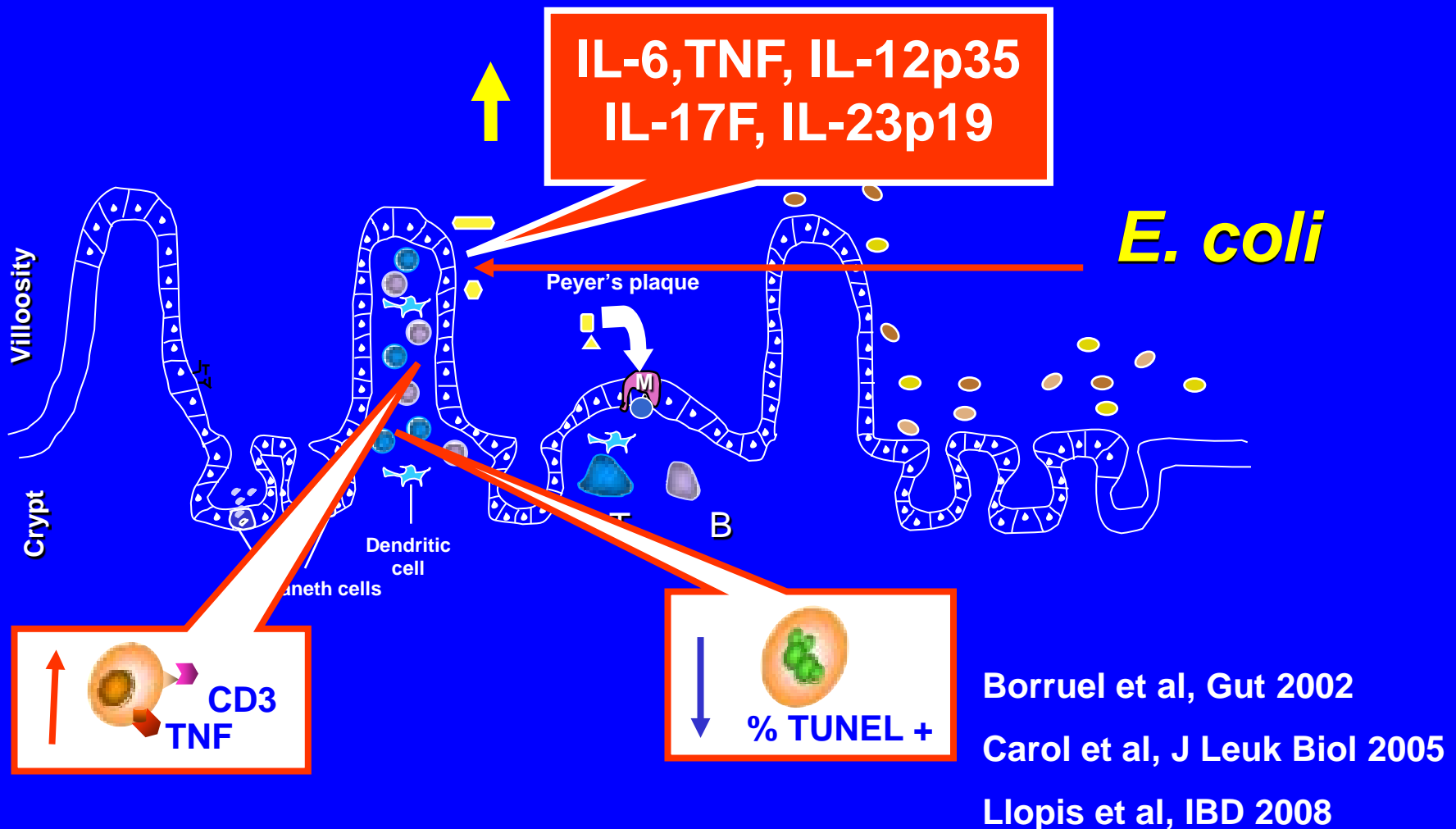
	IL-6	TGF- β	IL-23p19	IL-12p35	IL-17F	IL-8	CXCL1	CXCL2
Inflamed vs. Health	31,0 \pm 5,9	1,3 \pm 0,2	5,1 \pm 1,5	1,6 \pm 0,1	10,6 \pm 6,8	20,1 \pm 6,9	4,7 \pm 1,2	5,0 \pm 0,8

In vitro cytokine release by inflamed intestinal mucosa



Llopis et al,
IBD 2009

INTERACTION OF NONPATHOGENIC BACTERIA WITH INFLAMED INTESTINAL MUCOSA

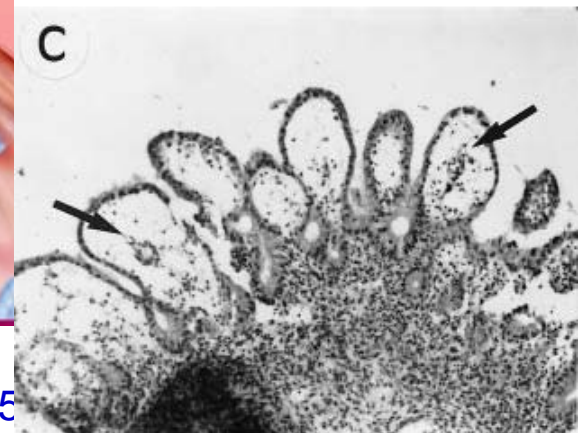
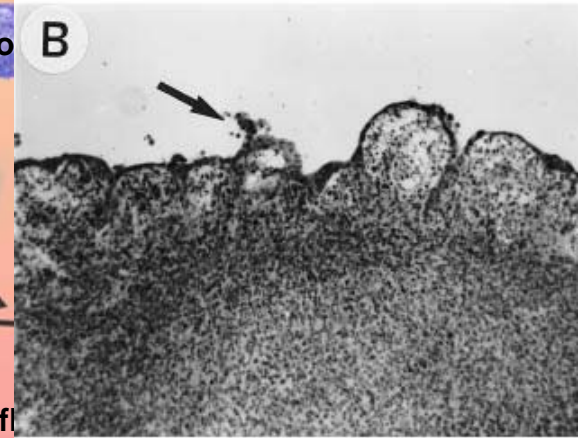
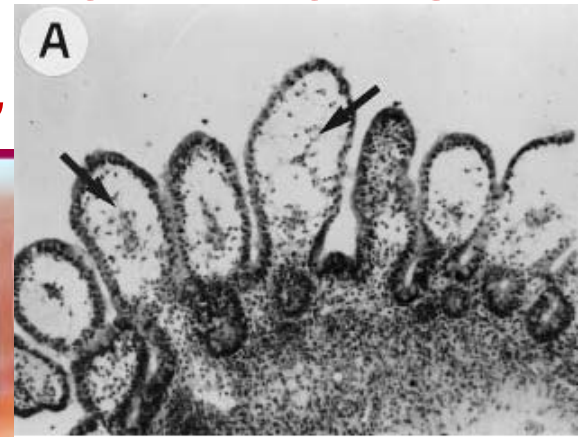
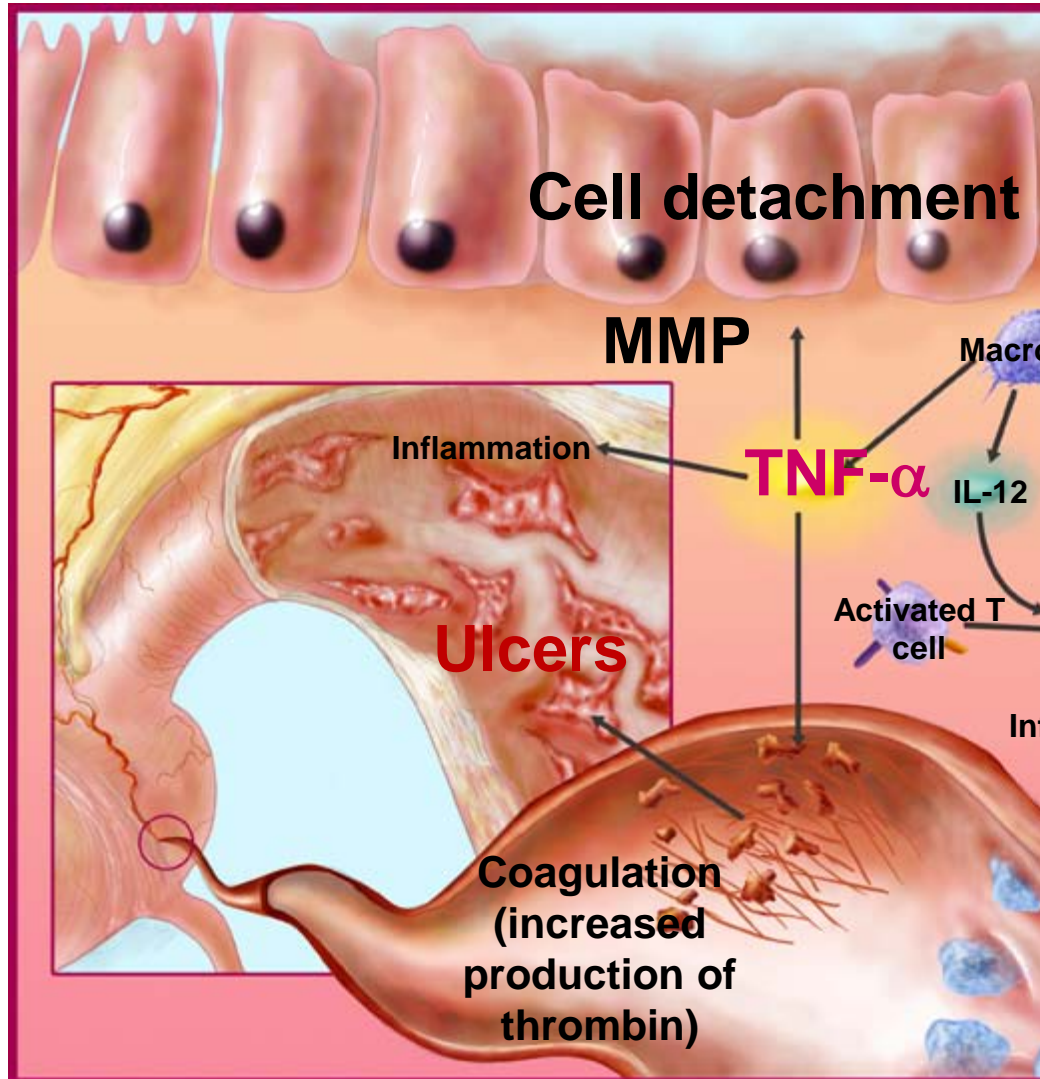


IBD: CURRENT DRUG THERAPY

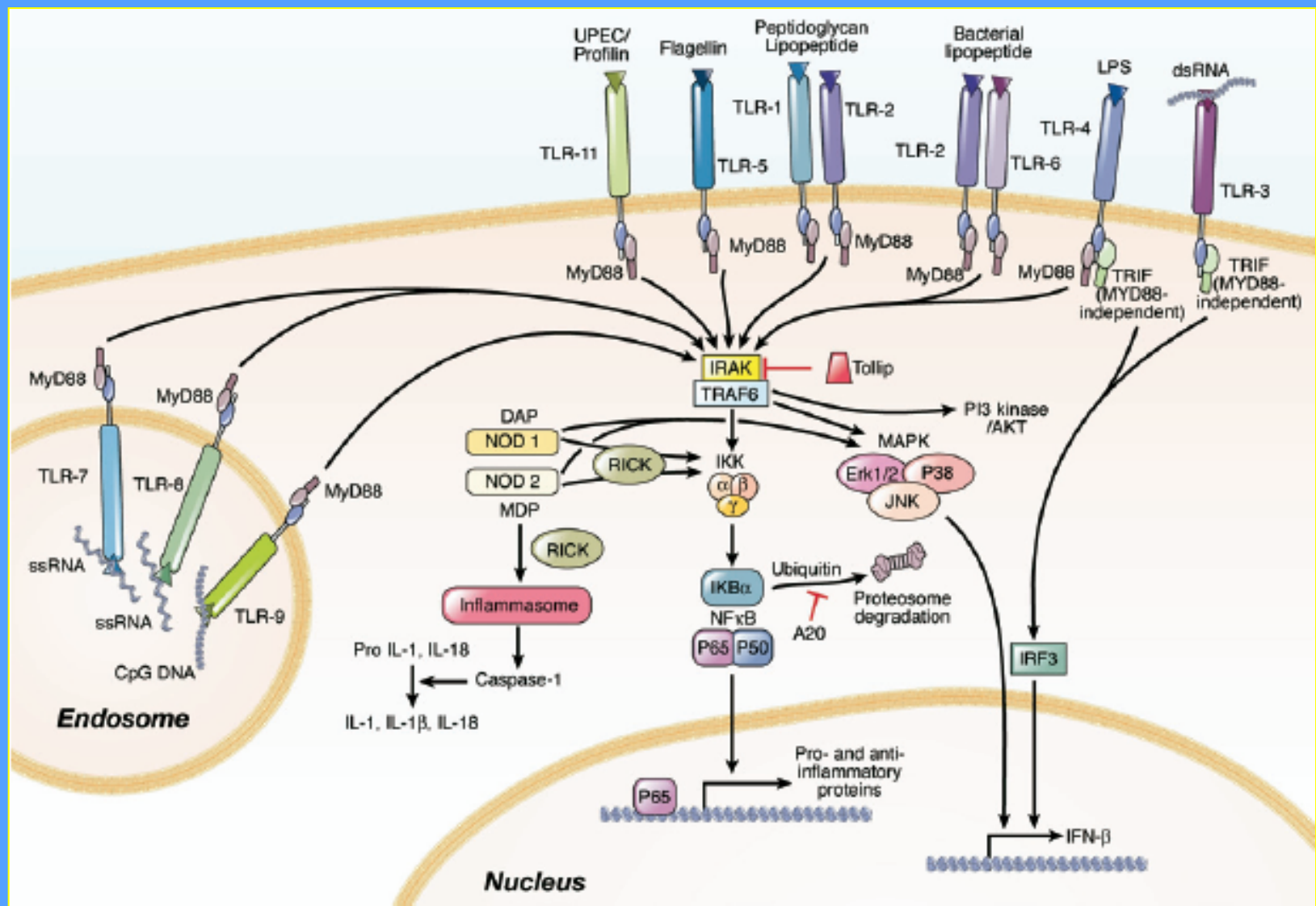
Immuno-suppression	Drug	Mucosal healing
Strong	Anti-TNF Anti-integrins	High
Mild	Aminosalicylate Budesonide	Low

Mucosal lesions are caused by an aggressive immune response against gut bacteria in genetically susceptible individuals.

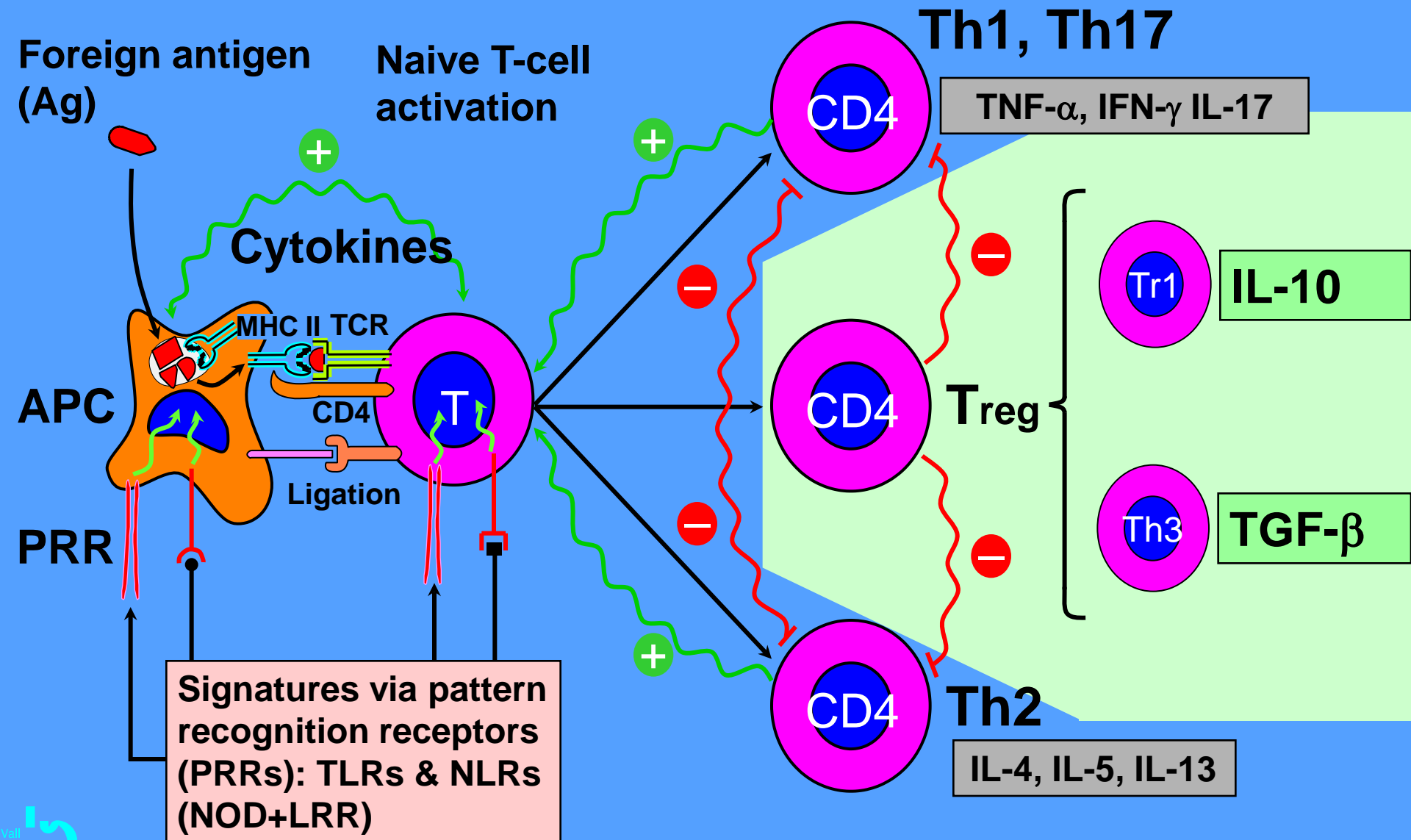
Severe tissue injury by inflammation: role of TNF- α



INTESTINAL HOMEOSTASIS

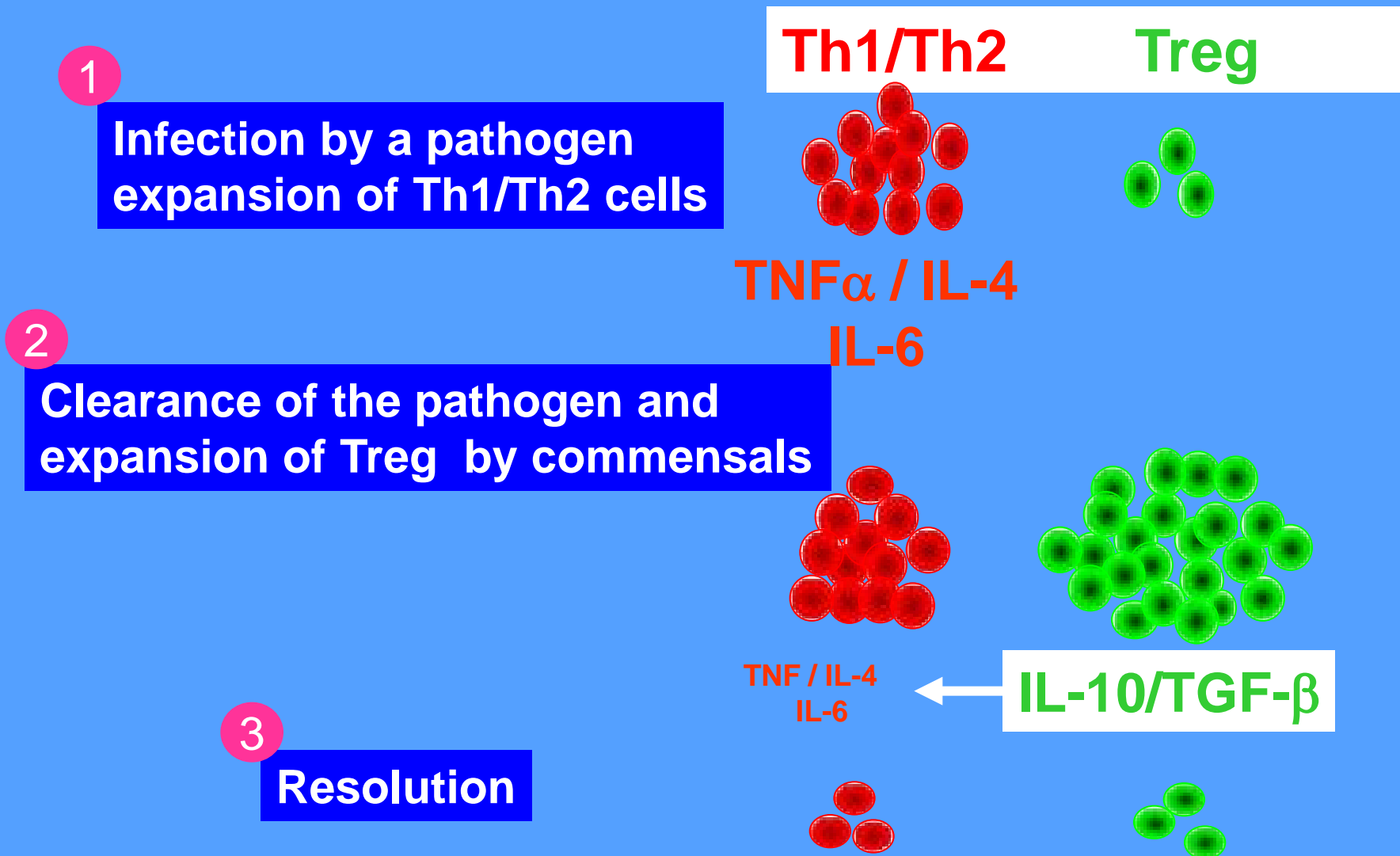


Decision making in the adaptive (acquired) immune system is instructed by the microbial impact on APCs and T cells



By Brandtzaeg in Guarner et al, Nature Clin Practice 2006

A MODEL FOR THE RESOLUTION OF INFLAMMATION AFTER INFECTION

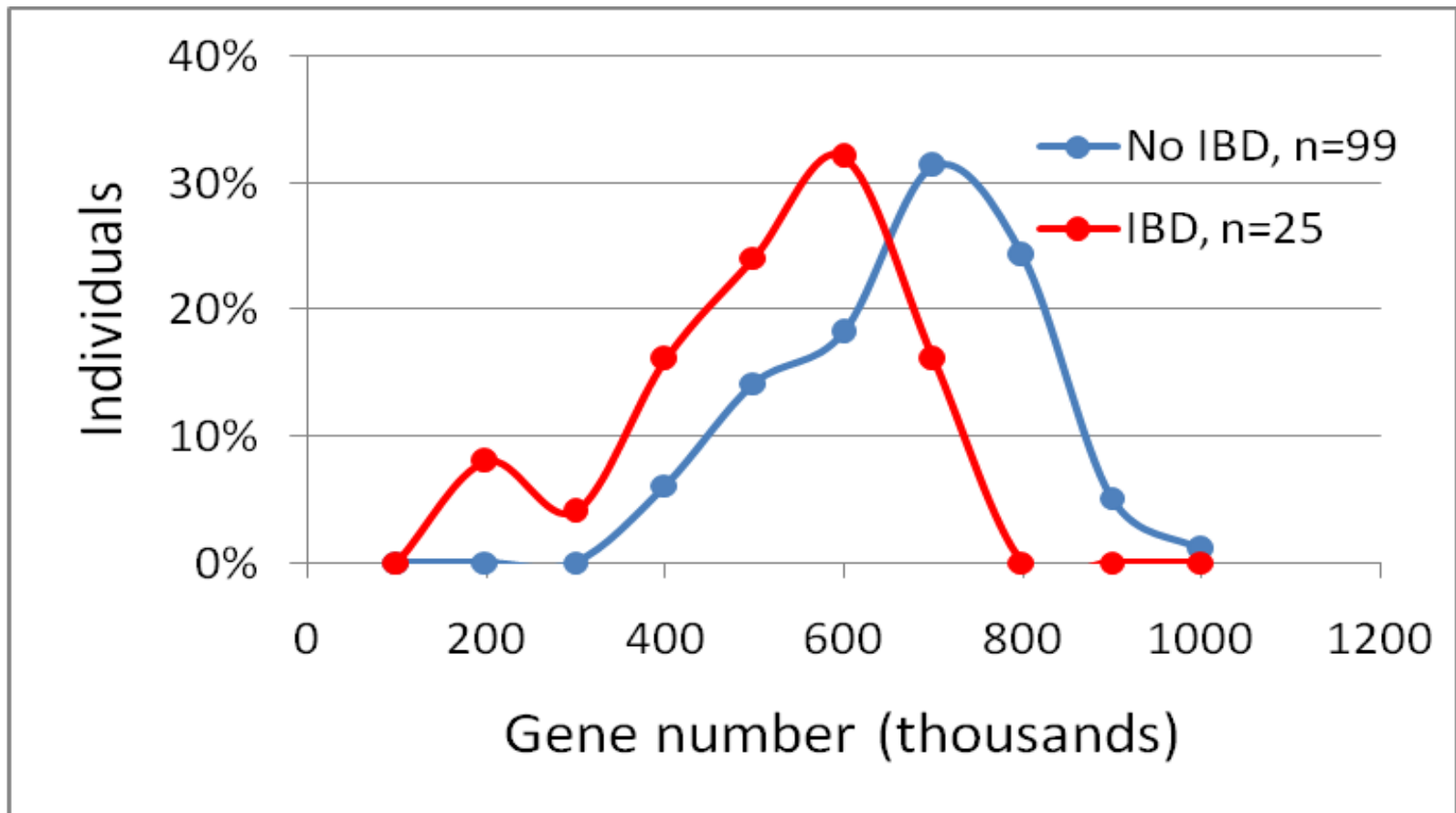


The Human Gut Metagenome

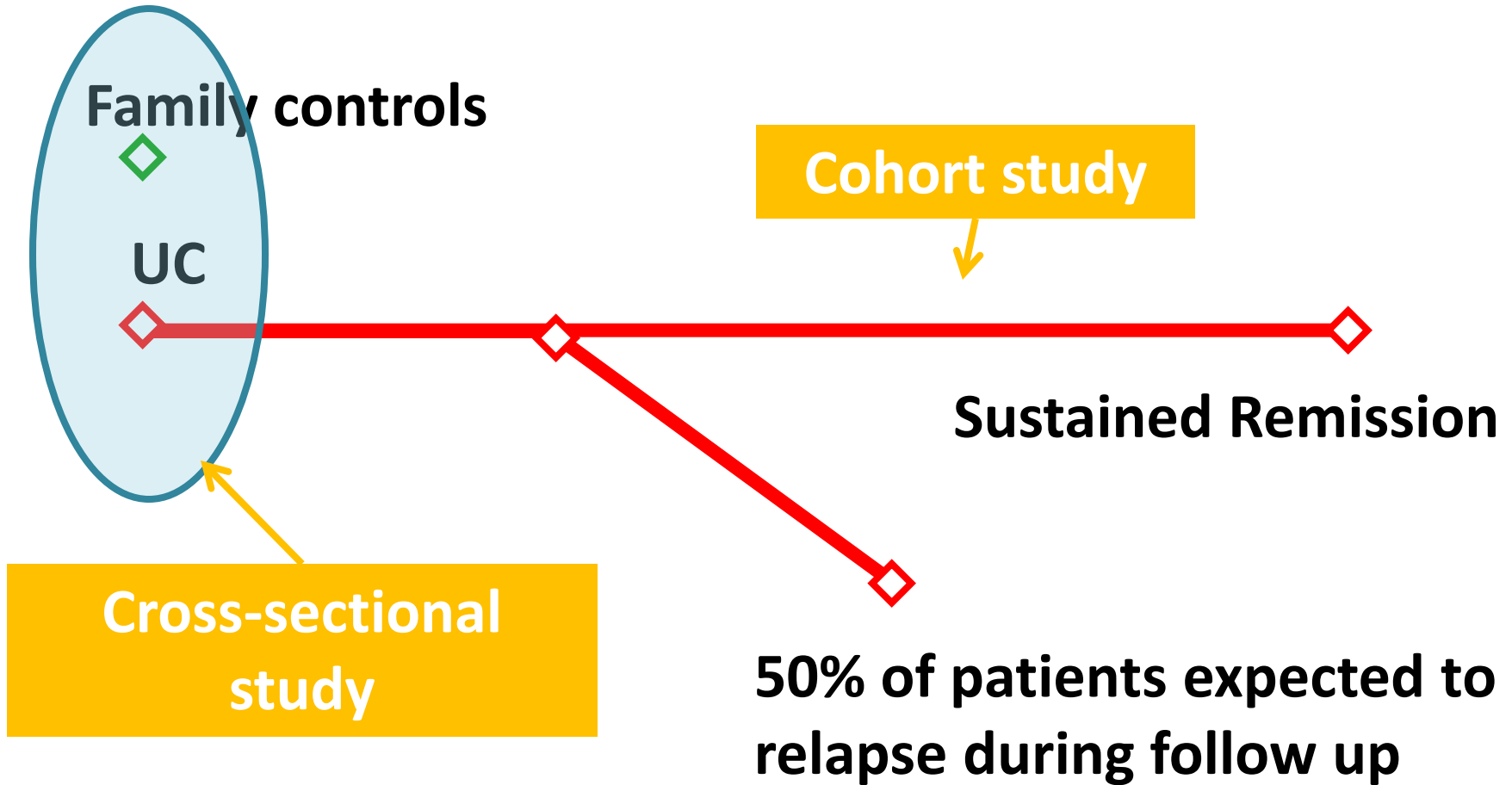
	# of genes
Non-redundant gene set	3,299,822
Average gene set per individual	590,384
Common (>50% of individuals)	294,110
Rare (<20% of individuals)	2,375,655

Qin et al, Nature 2010

Reduced Microbial Gene Diversity in IBD



ULCERATIVE COLITIS



 analysis of faecal microbiota


Ulcerative colitis related microbiome

- 26 UC patients in remission
- 32 healthy first degree relatives

Cross-sectional study

Gene frequency measured by Illumina sequencing

- 1) ~30 million paired-end reads/individual determined
- 2) relative gene frequency calculated
= (reads/kb)/ Σ sample reads

Genes different in frequency between patients & healthy searched by Ranksum analysis

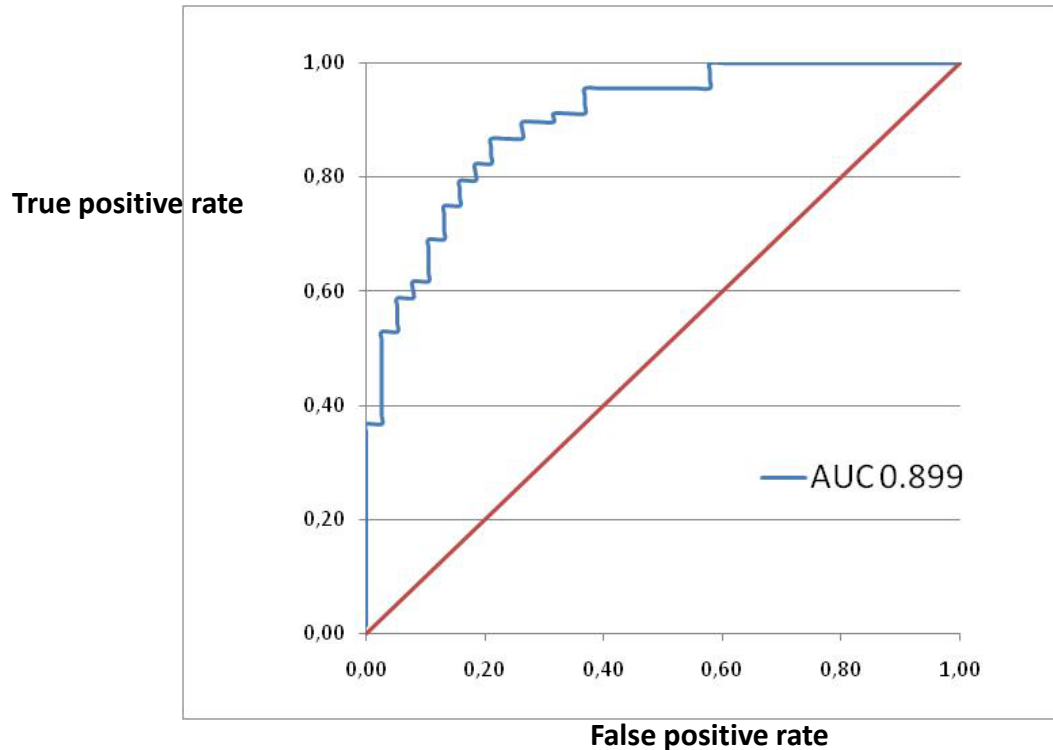
7770 genes found different at $p < 10^{-3}$

From UC-related genes to Meta-species by co-variance analysis

Hypothesis:

- Genes from the same species should have similar abundance in an individual
- The genes that co-vary accross individuals should belong to the same species
- ✓ 5800 of 7770 UC-associated genes assigned to 24 groups >10 genes by covariance analysis
- ✓ Other catalog genes belonging to the same groups retrieved by covariance analysis

Four meta-species only show a good discriminatory capacity between UC and health



Receiver operator characteristics (ROC) analysis

Linear additive model: $\sum(\text{genes of meta-species correlated with healthy}) - \sum(\text{genes of meta-species correlated with UC})$

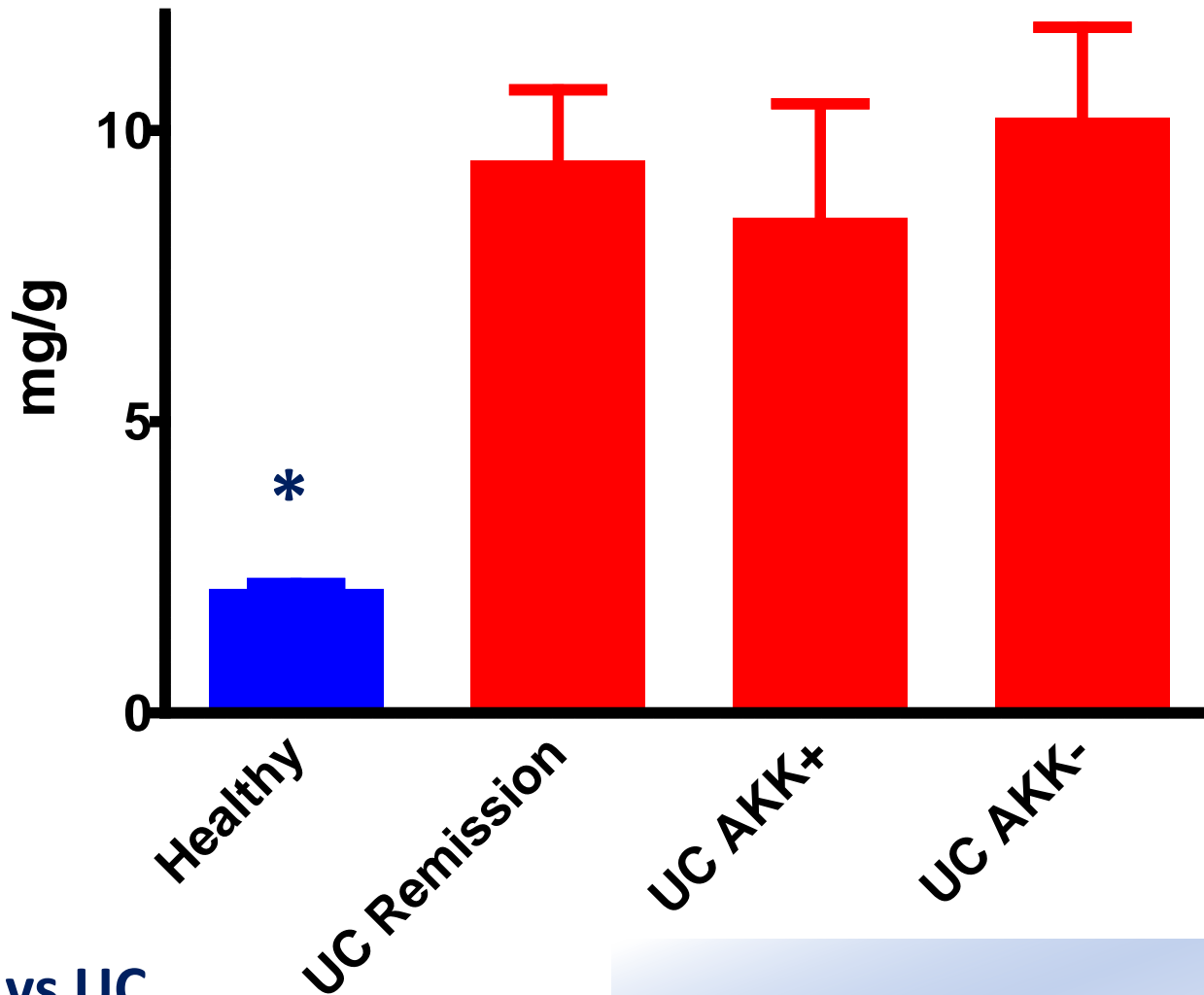
50 marker genes were used for each species

A small number of species
diagnostic for the UC patients

Bacterial species are associated to
chronic disease !!

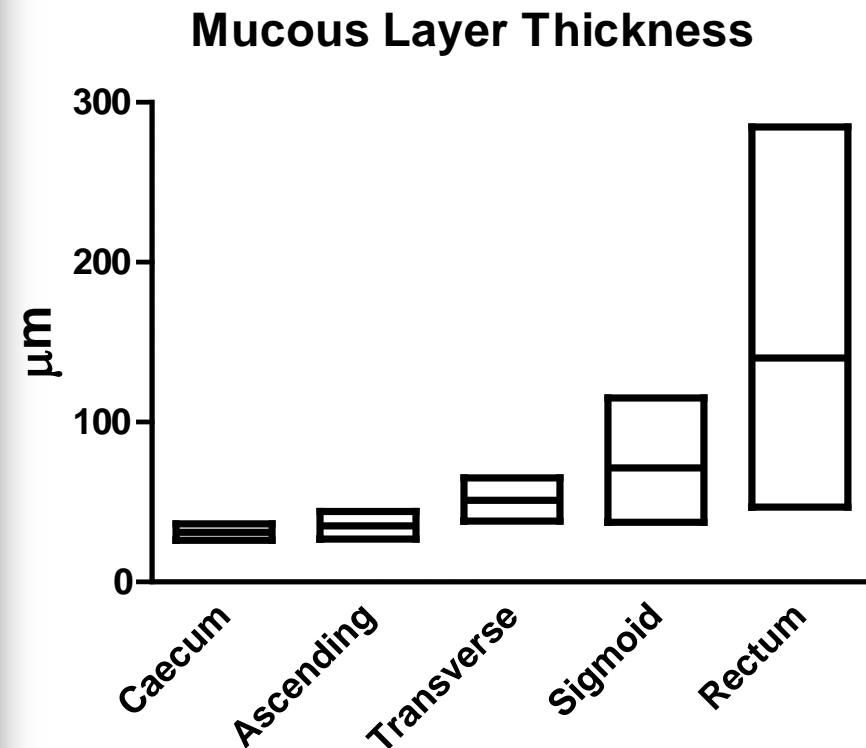
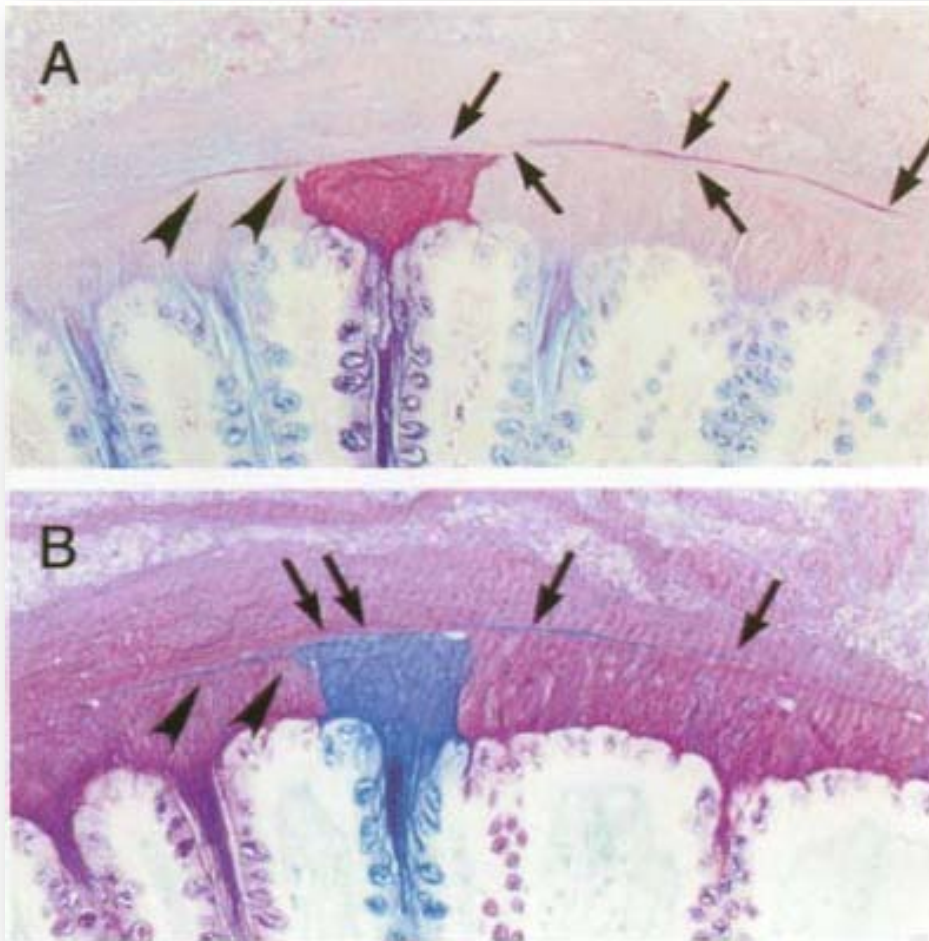
Causal agents, contributors,
consequence??

Faecal Mucin in UC



* $p < 0.01$ vs UC

The Mucous Gel Layer of the Human Colon



Matsuo et al, Gut 1997

Randomized, Double-blind, Placebo controlled Probiotic intervention

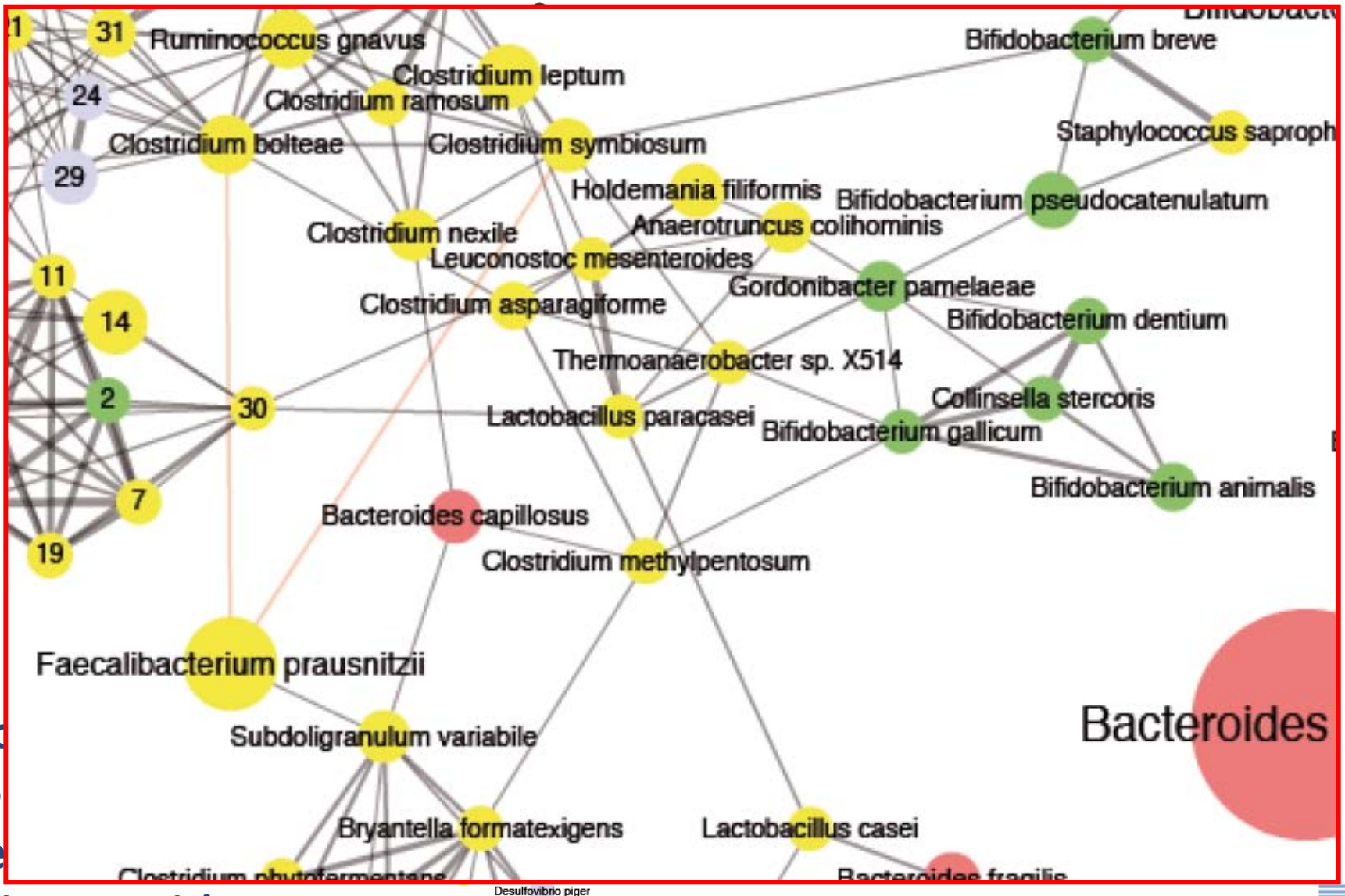
BACKGROUND

During remission, dominant gut microbial communities vary over time in UC patients. Temporal instability may be a consequence of low biodiversity, and enriching the ecosystem could favor stability.

AIM

To evaluate the effect of a fermented milk containing the probiotic *B. animalis ssp. lactis* DN-173 010 in the stability of the gut ecosystem in UC patients in clinical remission as assessed by metagenomic analysis of faecal samples.

Co-variation of species



Circ
mo
spe
variance, with $R \geq 0.4$

STUDY DESIGN



- ✓ UC Verum (n=24): stable drug therapy + **probiotic yogurt**
- ✓ UC Placebo (n=24): stable drug therapy + **acidified milk**
- ✓ Healthy controls (n=12): **no intervention**

◇ Clinical assessment, QoL, faecal sampling.

Drop outs:

2 verum + 2 placebo for clinical relapse

3 placebo for patient decision

Demographic Data at Entry

	UC VERUM	UC PLACEBO	CONTROL
Gender	14 F/ 10 M	15 F/ 9 M	5 F/ 7 M
Age (y)	41 (25-68)	42 (24-64)	31 (20-44)
BMI(Kg/m ²)	24(18.7-30.5)	24.7(18.72-35.6)	23.1 (17.6-27.8)
Smoking habit	12 no/5 yes/7 ex	12 no/4 yes/8 ex	7 no/4 yes/1 ex
Montreal classification	2 A1/ 18 A2/ 4 A3 9 E1/8 E2/ 7 E3	1 A1/ 20 A2/ 3 A3 13E1/4 E2/ 7 E3	-
Disease duration (mo)	140.4 (15- 277)	103 (16- 418)	-
Treatment	15 ASA/7 AZA/2 no	15 ASA/7 AZA/2 no	-
CAI (remission <4)	Score 0 - 24 Score 1 - 0 Score 2 - 0	Score 0 - 18 Score 1 - 4 Score 2 - 2	-

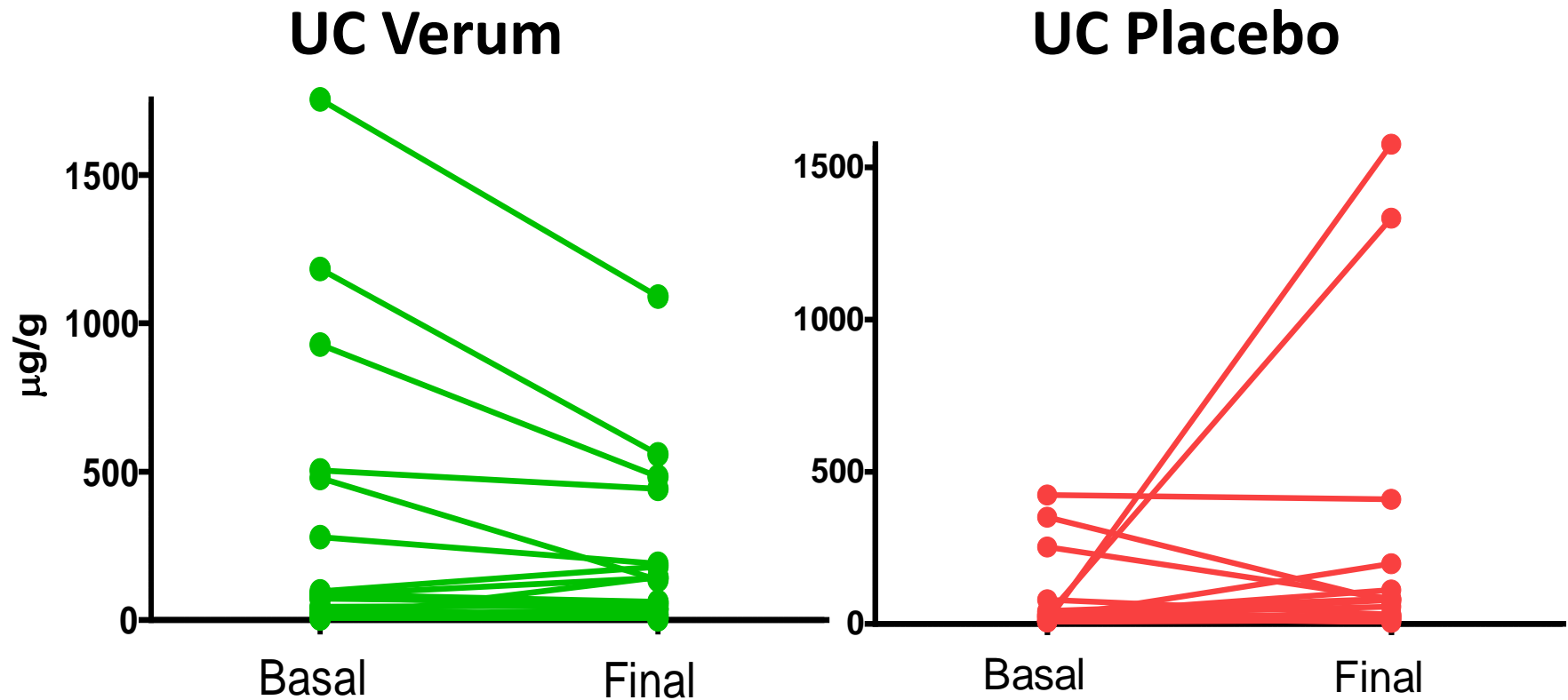
Bifidobacterium animalis* DN- 173 010

	Basal (run-in)	Final
UC Verum	9/21	20/21
UC Placebo	9/19	1/19**
Control	3/12	1/12**

* Marker of compliance assessed by qPCR of faecal samples: positive out of total assayed.

** Two sided p value <0.0001 vs. Final UC Verum (Fisher's exact test).

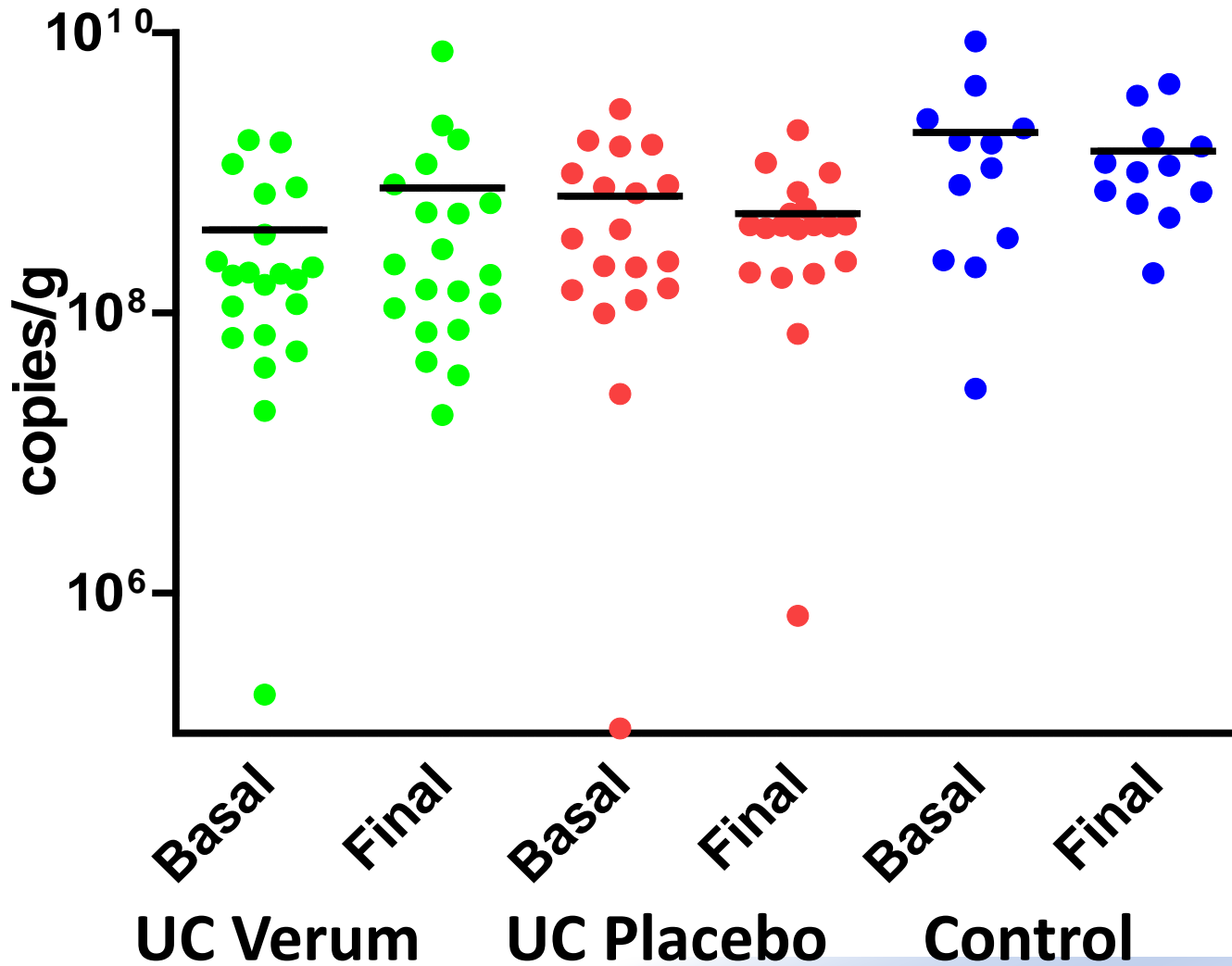
CALPROTECTIN*



* Biochemical marker of intestinal inflammation.

Values in healthy controls, median and range: 15 (5-70) µg/g.

Faecalibacterium prausnitzii



Acknowledgments

MetaHIT Consortium

INRA: E. Le Chatelier, M. Almeida, N. Pons, J.M. Batto, P. Renault.

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