

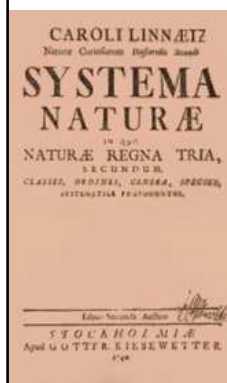
***Mycobacteria and Crohn's Disease:
The endless story***

By

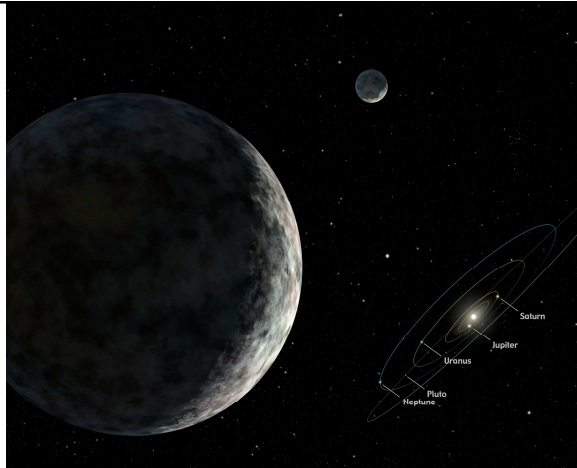
Cosimo Prantera

Roma, Novembre 2011

*Uno dei meccanismi principali della
conoscenza e' quello di raggruppare cose,
animali, piante, fatti perche' simili fra loro.
Questo processo nel campo medico ha
permesso tassonomie, cioe' classificazioni in
gruppi di malattie .*

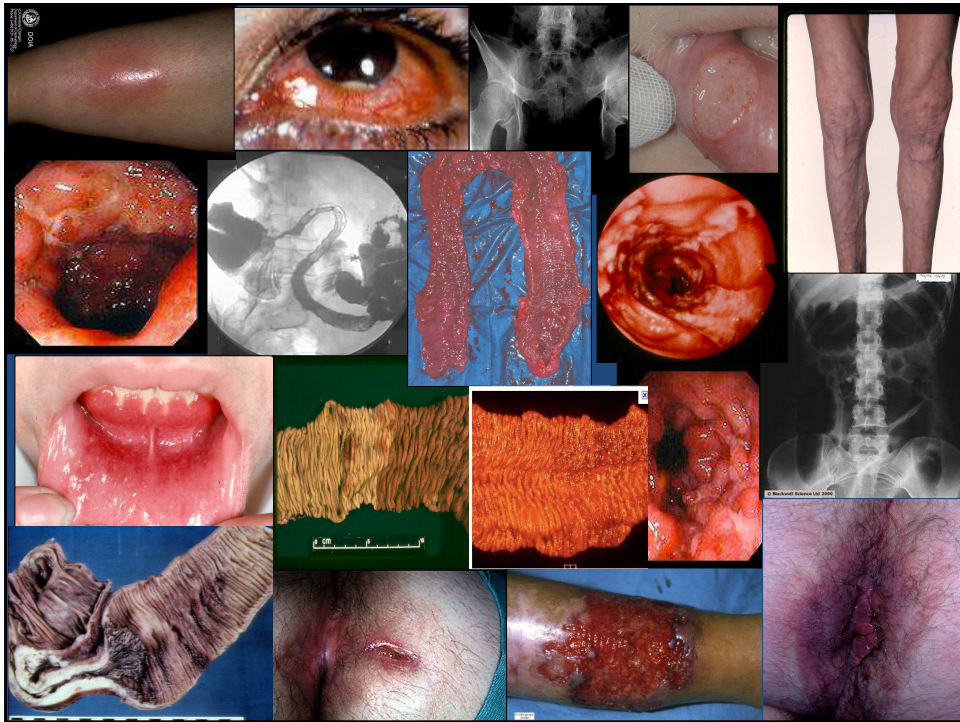


Differentemente da altre Scienze, come ad esempio l'astronomia, la scienza medica deve rispondere alle richieste del paziente in breve tempo. Questa e' una delle principali ragioni, per le quali la medicina deve sempre raggruppare pazienti in categorie definite come malattie.



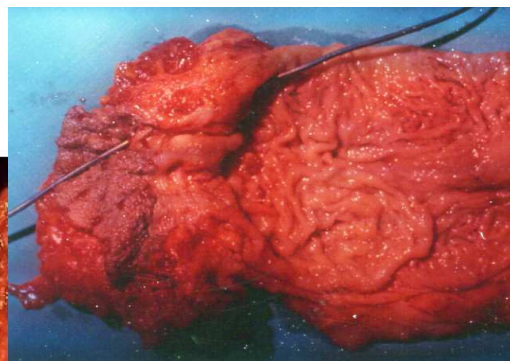
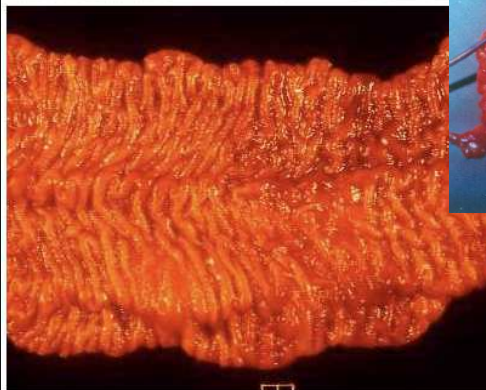
Le Malattie Infiammatorie Croniche Intestinali (IBD) (MICI) comprendono un gruppo molto eterogeneo di processi infiammatori dell'apparato digerente che condividono, in parte, sintomi ed aspetti patologici. Attualmente questa categoria comprende la Malattia di Crohn, la Colite Ulcerosa e la Colite non classificabile (IBDU)



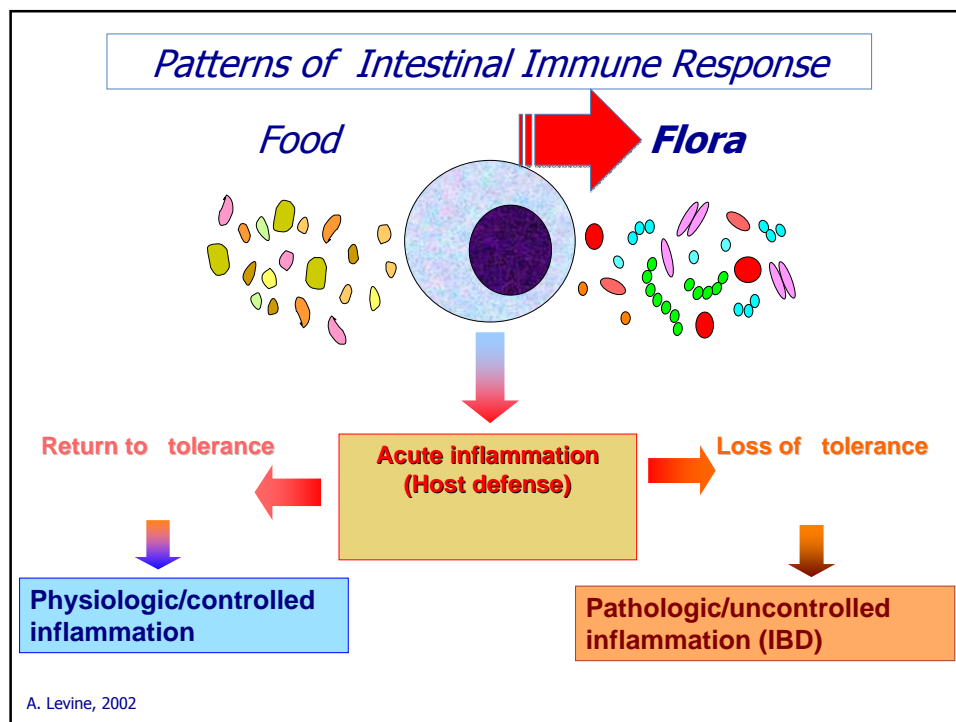
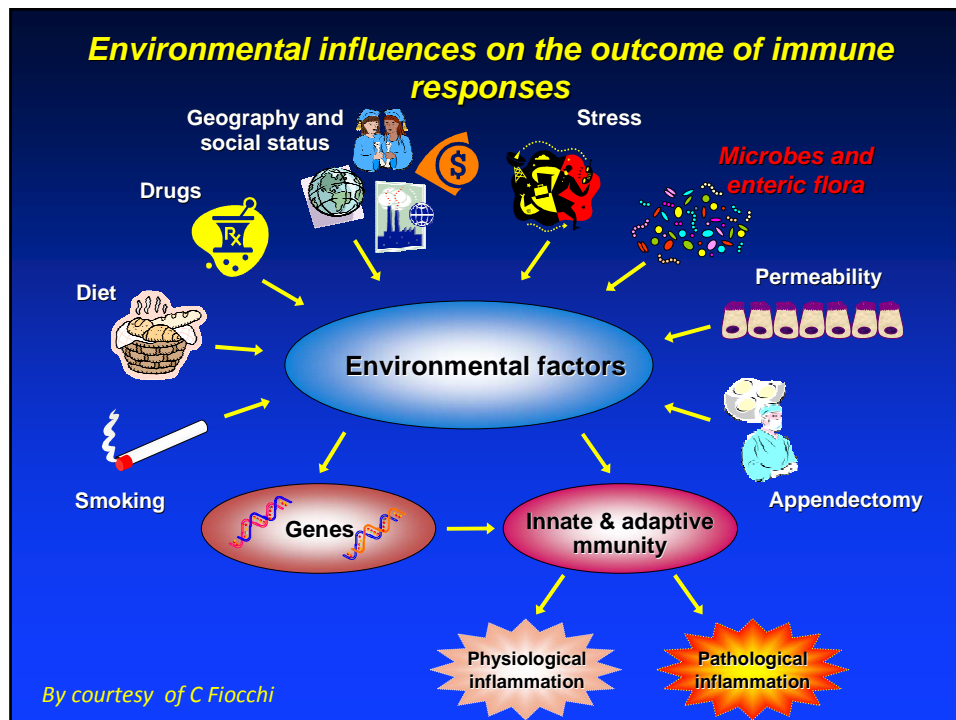


Chronic Inflammatory Diseases

Johne's Disease

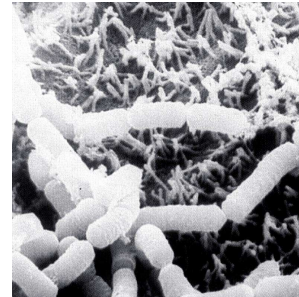


Crohn's Disease



Role of bacteria in Crohn's disease pathogenesis

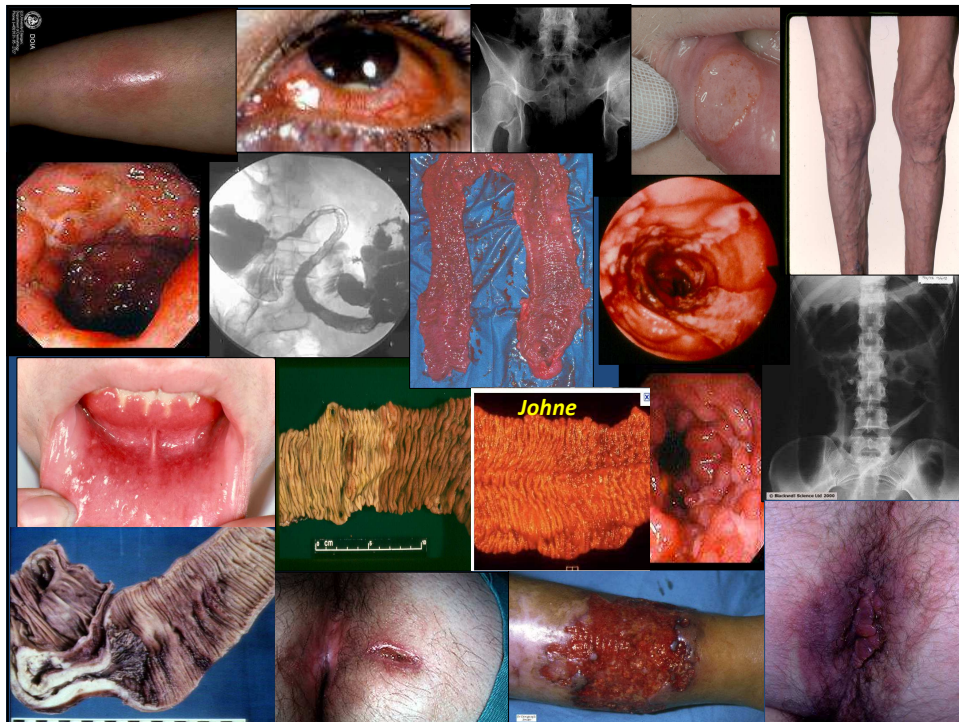
- *Lesions occur in gut areas with high bacterial concentration*
- *Bacteria are the cause of animal and human intestinal diseases similar to IBD*
- *Efficacy of diversion of fecal stream*
- *Recurrent lesions on restoration of fecal stream*



Role of bacteria in IBD: hypotheses

- *Unidentified persistent pathogen?*
- *Abnormally permeable mucosal barrier leading to excessive bacterial translocation?*
- *Immune system abnormality of effector cells activation of insufficient activity of Treg cells in response to bacteria?*
- *Break in the balance between protective versus harmful bacteria? Dysbiosis*

*Is an unidentified persistent
pathogen the cause of CD?*



Is a Mycobacterium the unidentified bacterium involved in CD?

- (a) the presence of MAP in CD patients*
- (b) the epidemiological connection*
- (c) the treatment of CD by immunosuppressors*
- d) the response to anti-mycobacterial agents.*

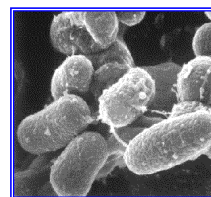
Mycobacterium avium ssp paratuberculosis (MAP)

Member of M. avium complex (MAC)

Small mycobacterium ~0.5 μm x 1-2 μm

Obligate intracellular pathogen

*Cultured from humans
months to years
Some non-culturable*



MAP & Crohn's disease

*Exposure to MAP is universal
MAP can survive in the body
MAP is pathogenic (JD)*



- Innocent bystander?*
- Disease in the susceptible?*

Map & CD: Pros-Cons

The presence of MAP in CD patients

-Studies:

- a) presence of Bacteria in the tissues through culture or DNA analysis*
- b) presence of antibodies*

-Some studies with positive results, but negative research in a large study on 305 patients with IBD

-Pros: MAP is a slow growing bacterium

-Cons: MAP is ubiquitous –Is it pathogenic or an innocent bystander?

Map &CD:

The presence of MAP in CD patients

- Pros: Atypical Mycobacteria can be pathogenic in immunosuppressed patients (such as AIDS)*
- Pros: Presence of anti-MAP antibodies*
- Cons: Also presence of antibodies against other different bacteria*

Map &CD:

Cons: the epidemiological connection

- Incidence and prevalence of Crohn's disease higher in developed countries*
- Non increased prevalence among colleagues and spouses of CD patients*
- Concordance of 44% in monozygotic twins in comparison of 3.9% in dizygotic twins*

*Map & CD: **Cons:**
the treatment of CD by Immunosuppressors*

- Anti-TNF treatment is highly effective in inducing remission and healing of CD lesions in the ileum and colon.*
- TNF play a key role in the host response to Mycobacteria and granuloma formation.*
- Anti-TNF drugs have caused some severe cases of tuberculosis in the disseminate form.*

*Map & CD: **Pros:**
Immune suppression will not induce
overwhelming infection*

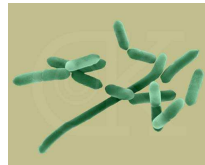
- Paucimicrobial 'infection' – low rate of replication*
- Immune suppression
easier for bacteria (eg, Myco. tb) to replicate*
- But, immune control not so important for MAP*

Map & CD: Classification of leprosy

- *Lepromatous leprosy*
 - *↑Humoral immunity*
 - *Multibacillary (skin smears or biopsy)*
 - *Serology positive/ high titers*

Johne's Disease

- *Tuberculoid leprosy*
 - *↑Cellular immunity (more effective)*
 - *Paucibacillary*



Crohn's Disease

Anti-Mycobacterial therapy

Antimycobacterial agents

Author	Drug(s)	N	Duration (months)	Remission
Elliot et al 1982	Sulphadoxine+Pyrimethamin	51	12	33% vs 41.6%
Shaffer et al 1984	Rifampicin +Ethambutol	27	12	13 withdrawals
Basilisco et al 1989	Rifabutin	24	6	42% vs 50%
Afdhal et al 1991	Clofazimine	49	12	48% vs 25%
Swift et al 1994	Rifampicin+ Ethambutol +INH	12	24	NS
Prantera et al 1994	E+C+D+R*	40	9	50% vs 22.2% no healing

*Ethambutol, clofazimine, dapsone, rifampicin

Therapy administered

- **APT therapy**
 - Rifabutin 450 mg/d
 - Clarithromycin 750 mg/d
 - Clofazimine 50 mg/d
 - Dose escalation over 4 weeks
 - Continued for 104 weeks
- **Prednisolone**
 - 40 mg/d tapering over 16 weeks initially and for relapse
- **Stable Crohn's medication continued**



Australian Trial: W Selby et Al 2007

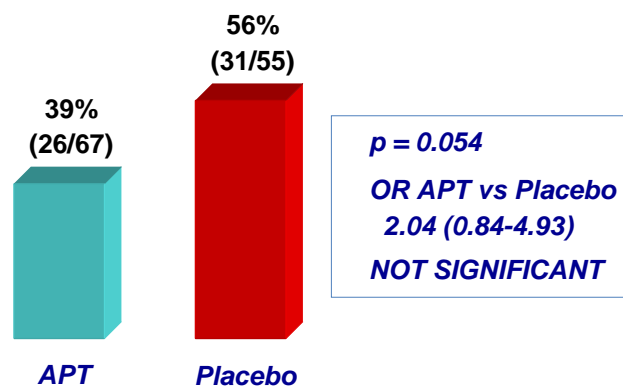
Subjects

	<i>APT</i>	<i>Placebo</i>
<i>No.</i>	102	111
<i>M:F</i>	51:51	50:61
<i>Age</i>	36.5 ± 11.3	34.8 ± 10.0
<i>Smokers</i>	33%	43%
<i>Duration of disease</i>	8.1 ± 7.2 yrs	8.7 ± 7.3 yrs
<i>Site of disease – ileum</i>	30	34
<i>colon</i>	41	36
<i>ileocolonic</i>	31	38*
<i>Azathioprine/6-MP</i>	35	32
<i>CDAI</i>	291 ± 72.5	282 ± 75.0

Australian Trial: W Selby et Al 2007

Relapse rates

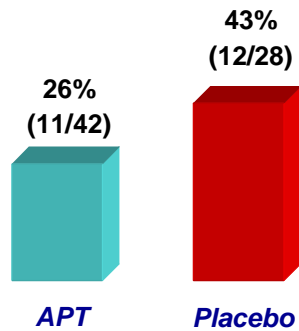
52 weeks (1 year treatment)



*Australian Trial: W Selby et Al
2007*

Relapse rates

104 weeks (2 years treatment)



$p = 0.14$

APT vs Placebo

OR: 2.22 (0.62-7.96)

NOT SIGNIFICANT

Australian Trial: W Selby et Al 2007

Secondary end-points

No differences in:

number of relapses

time to relapse

endoscopic score (CDEIS)

– remission at 156 weeks

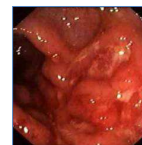
» 9/23 APT

» 4/15 Placebo

surgery (11)

– 6 APT

– 5 Placebo



Australian Trial: W Selby et Al 2007

Adverse events

significant increase in APT group in:

abnormal LFTs (2.3% weeks 0-16)

vaginal candidiasis

urine discoloration

arthralgia

tooth discoloration



withdrawals for AE

APT 9

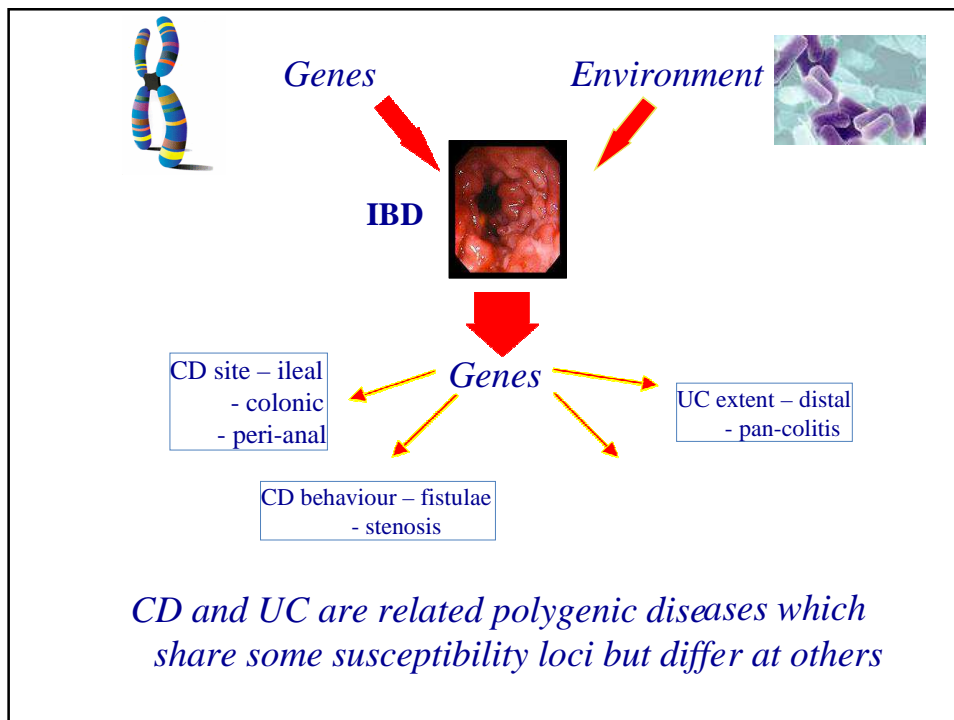
Placebo 7

Australian Trial: W Selby et Al 2007

MAP & CD- Conclusion

The link between MAP and CD cannot be confirmed mainly because of:

- inefficacy of anti-MAP treatment*
- epidemiological implausibility*
- absence of the healing of lesions after a specific treatment*



Different commensal bacterial species selectively initiate intestinal inflammation with distinct anatomic distribution in Interleukin 10 deficient mice

Enterococcus faecalis causes distal colon and duodenum involvement;

Kim S et al. (2005)

It causes obstruction



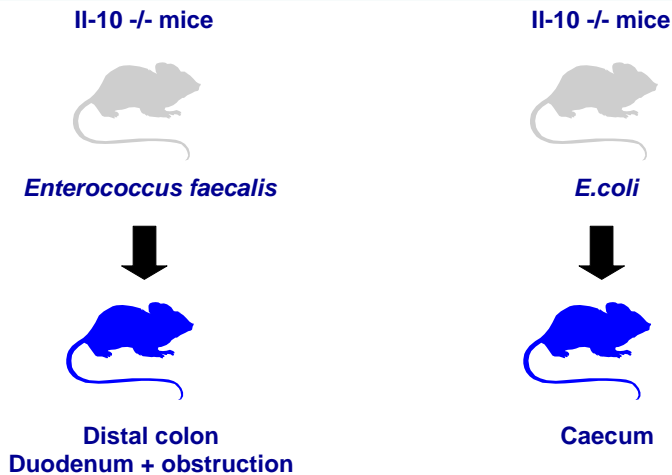
E.coli is the cause of cecum involvement

*Metronidazole is effective in the inflammation of the colon.
Vancomycin-Imipemen treat ileal and colonic locations.*

Hoentjen F et al. (2003)

Ciprofloxacin is most effective in the treatment of caecal inflammation.

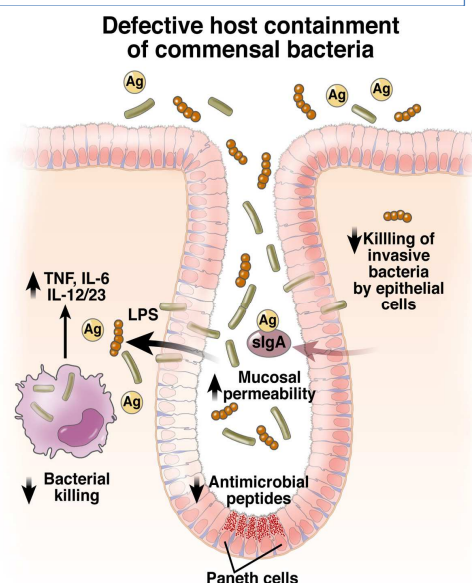
Different commensal bacterial species selectively initiate intestinal inflammation with distinct anatomic distribution in the same host



Kim S et al. Gastroenterology 2005

Genes associated with Crohn's disease that affect bacterial killing

- **Nod2** -defective α defensin production, clearance of intracellular bacteria
- **ATG 16L1** - autophagy, killing and processing of phagocytosed bacteria
- **NCF4** - NADPH- killing of phagocytosed bacteria
- **RGMI** - IFN γ induced killing of phagocytosed bacteria



“Hit and Run” Hypothesis

Intestinal infection



Genetic determination

No IBD mutations

Limited acute inflammatory damage

Complete repair

IBD mutations

Acute inflammatory damage
Triggering the overshooting reaction against “normal antigens”
Continuous cell recruiting
Changes in cell cycle
“Permanent” damage

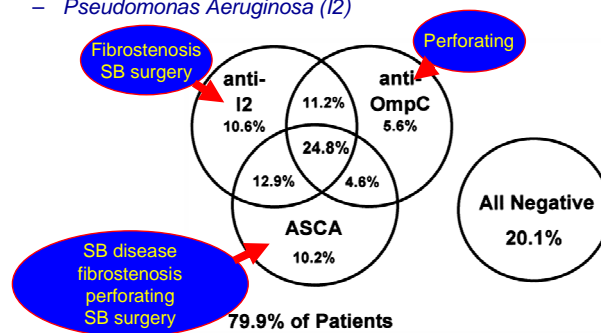
Sartor B et al, (1992)



Role of bacteria in IBD

Serologic anti-microbial responses in CD

- *Saccharomyces cerevisiae* (ASCA)
- *E.Coli* outer membrane porin C (Omp-C)
- Flagellin (cBir1)
- *Pseudomonas Aeruginosa* (I2)



Mow S et al, Gastroenterology 2004; Targan SR et al, Gastroenterology 2005

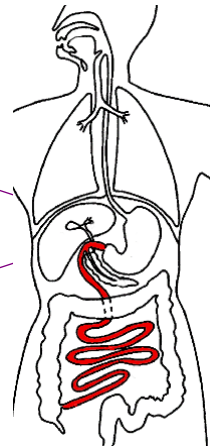
Bacteria & CD

- Are there commensal bacteria that become pathogenic?*
- Is the immunological response to commensal bacteria pathological?*

More bacteria than cells in the body

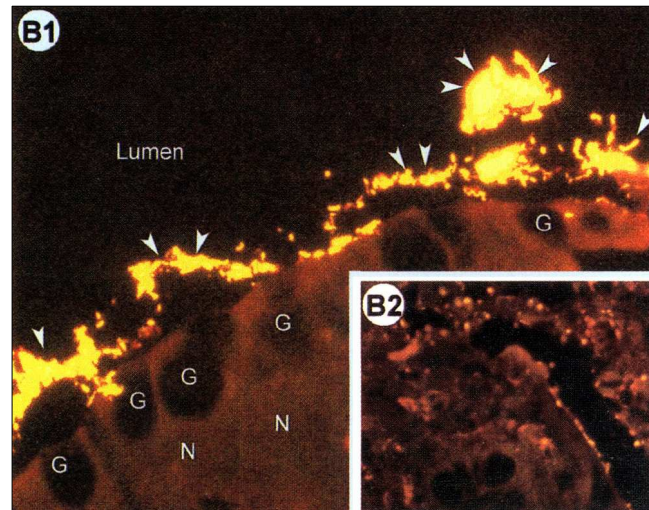
- More than people on the planet!*
- 1-2kg of bacteria in human gut*

**A belly
full of
bacteria**



High concentrations of mucosal bacteria in patients with CD

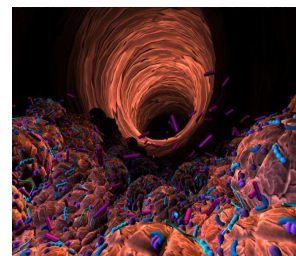
**40% of CD with
concentrations
> 10 000 cfu/ml**



Swidzinski et al. Gastroenterology 2002

Commensal flora of the gut: wolf in sheep's clothing?

*« ...there is now more and more
evidence that resident members
of the microflora, which are normally « commensal »
can initiate pathological mucosal inflammation
in a susceptible host... »*



Xavier R, Podolsky DK. 2005

What is dysbiosis?

*Disturbance of intestinal microflora
Breakdown in the balance between "protective" vs "harmful" intestinal bacteria*

- *Dysbiosis implicated in many chronic diseases associated with modern Western life style:*
 - *Irritable bowel syndrome*
 - *inflammatory bowel disease*
 - *rheumatoid arthritis*
 - *ankylosing spondylitis*

Intestinal inflammation vs. homeostasis depends on the relative balance of beneficial vs. detrimental bacteria

Bad Guys **Injurious Bastards**

Pro-inflammatory



*Bacteroides vulgatus, B. theta
Enterococcus faecalis
E. coli - enteroadherent / invasive
Klebsiella pneumoniae
Bifidobacterium animalis
Fusobacterium varium
Intestinal Helicobacter species*

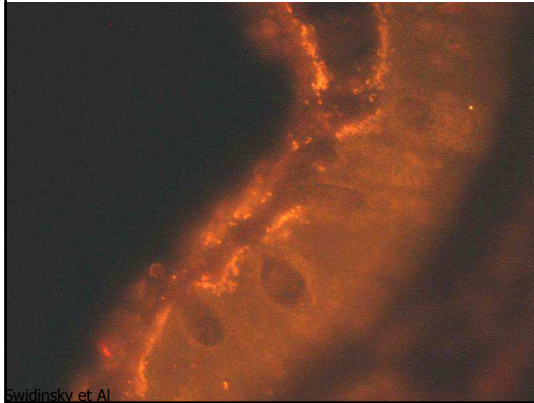
Good Guys **Protective**



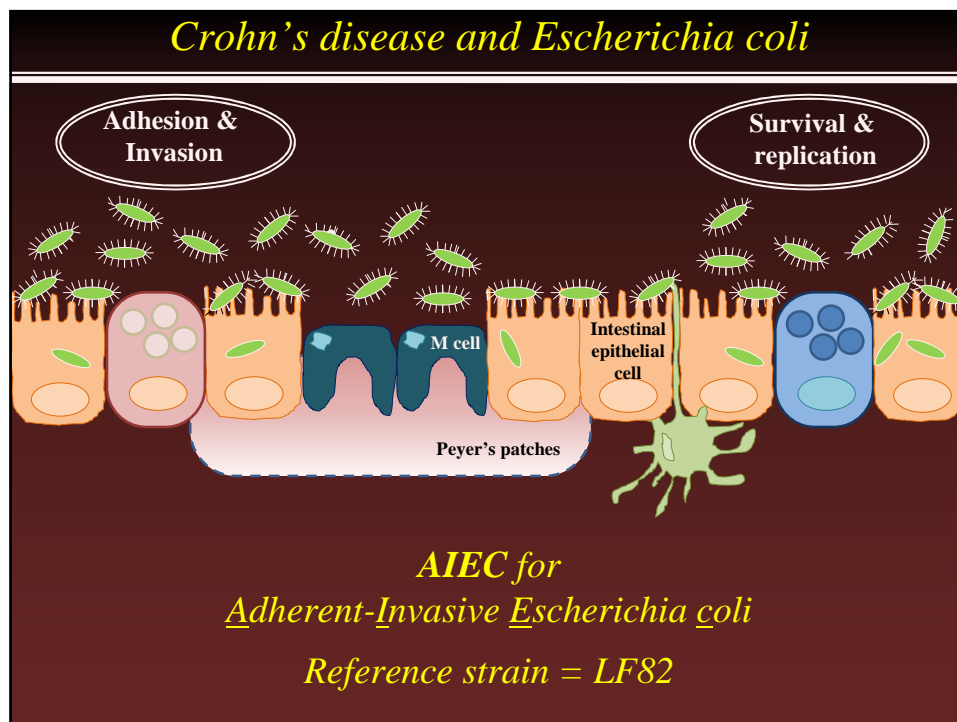
*Lactobacillus sp.
Bifidobacterium sp.
Non-pathogenic E. coli
Saccharomyces boulardii
Bacteroides thetaiotaomicron
Faecalibacterium prausnitzii*

-A mucous layer divides the lumen flora from the mucosa.

-Aerobic culture of colon biopsies after removal of the mucous layer is sterile in healthy individuals, while the CD colon contains increased bacterial number.



More than half of bacteria in the mucosa are E Coli (in the faeces E Coli is less than 1% of the bacterial content).



From an undecided bench to practical bed-side

Use of antibiotics in the trials and at the bedside

Antibiotic trials as primary therapy in acute flares

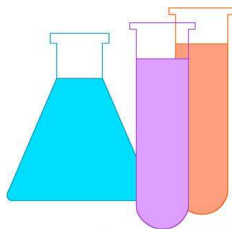
Author (year)	Pts (n°)	Antibiotic	Period (wks)	control	Remission active control
Blichfeldt(1978)	40	Metronidazole	16	placebo	n.s.
Ursing (1982) (CCDSS)	78	Metronidazole 800 mg/d	16	sulfasalazine	n.s. M:44% S:41%
Ambrose (1985)	72	Metronidazole 800 mg/d, Co-trimossazole	4	placebo	M: 44% P: 41% C: 62% M+C: 57%
Sutherland (1991)	99	Metronidazole 10 or 20 mg/kg	16	placebo	27% 25% 36%
Colombel (1999)	40	Ciprofloxacin 500 mg bid	6	Mesalamine 4 gr	56% 55%
Arnold (2002)	47	Ciprofloxacin 500 mg bid	26	placebo	P <0.001
Prantera (1996)	41	Ciprofloxacin 500 mg bid + metronidazole 500 mg bid	12	Methylprednisol one 0.7-1 mg/kg	45.5% 63%
Greenbloom (1998)	72	Ciprofloxacin 500 mg bid + metronidazole 250 mg tid	10	----	68% ----
Leiper (2000)	25	Clarithromycin 250 mg bid	4	----	48%
Leiper (2008)	41	Clarithromycin 1 gr od	12	placebo	26% 27%

Controlled trials of antibiotic therapy in prevention of postoperative recurrence of Crohn's

Author	Year	N° pts	Antibiotic	Duration of treatment	Endoscopic recurrence
Rutgeerts	1995	60	Metronidazole	12 wks	52%
			20 mg / kg Placebo		75%
Rutgeerts	1999	71	Ornidazole	52 wks	62%
			1g / day Placebo		94%



LABORATORY TESTS AND ANTIBIOTIC TREATMENT IN CROHN'S DISEASE



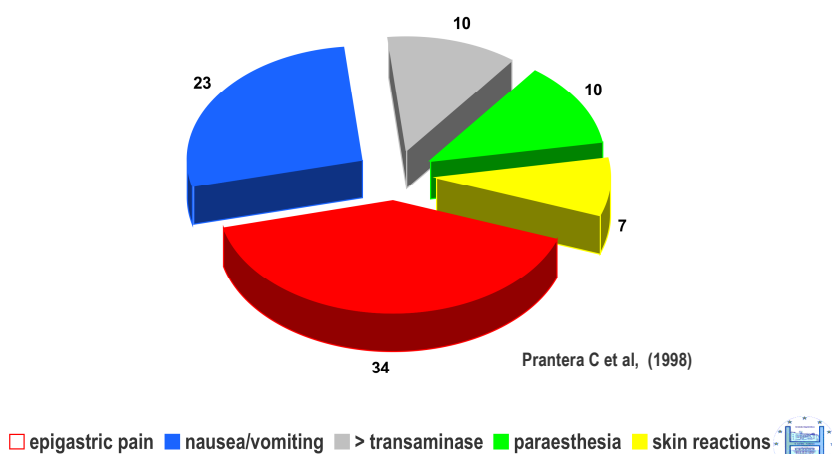
TEST	VALUE	
	<u>before treatment</u>	<u>after treatment</u>
CRP	27 ± 36	9 ± 11
<i>(Colombel, 1999 - Ciprofloxacin)</i>		
Ferritin	113 ± 13.4	37.1 ± 33.1
Serum Iron	38 ± 25.8	61 ± 37.6
ESR	40.8 ± 24.2	21.7 ± 13.4
Seromucoids	166 ± 71.9	120 ± 27.4
<i>(Prantera, 1996 - Metronidazole + Ciprofloxacin)</i>		



Author (year)	Patients (n°)	Antibiotic	Period (wks)	Withdrawn due to side effects %
Sutherland (1991)	99	Metronidazole 10 or 20 mg/kg	16	9.5%
Colombel (1999)	40	Ciprofloxacin 500 mg bid	6	11%
Ambrose (1981)	72	Metronidazole 400 mg bid/ Cotrimoxazole 360 mg bid/ Metronidazole +Cotrimoxazole	4	5%
Prantera (1994)	31	Ciprofloxacin 500 mg bid + metronidazole 500 mg bid	12	13%
Prantera (1996)	41	Ciprofloxacin 500 mg bid + metronidazole 500 mg bid	12	27%
Green bloom (1998)	72	Ciprofloxacin 500 mg bid + metronidazole 250 mg tid	10	7%
Steinhart (2002)	130	Ciprofloxacin 500 mg bid + Metronidazole 250 mg tid+ Budesonide 9 mg	4	20%
PERIANAL DISEASE				
Bernstein (1980)	21	Metronidazole 20 mg/kg	12	5%
Dejaco (2003)	52	Ciprofloxacin 500-1000 mg / day + metronidazole 1000-1500 mg / day+ AZA	8	0

SIDE EFFECTS

metronidazole + ciprofloxacin 50/160 (31.2%)
 withdrawn 35/160 (21.8%)



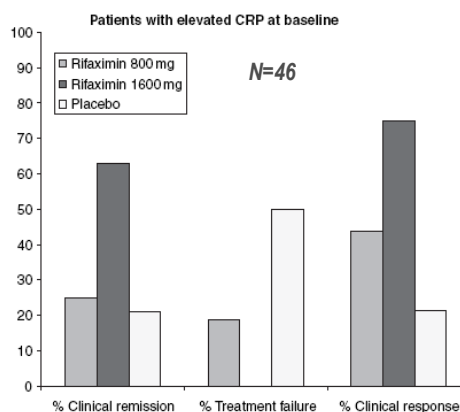
Antibiotic treatment of Crohn's disease: results of a multicentre, double blind, randomized, placebo-controlled trial with rifaximin

Aliment Pharmacol Ther 23, 1117–1125

83 patients
mild-to-moderate CD disease



three treatments for 12 weeks:
Group A (rifaximin 800 mg o.d.)
Group B (rifaximin 800 mg b.d.)
Group C (placebo b.d.).



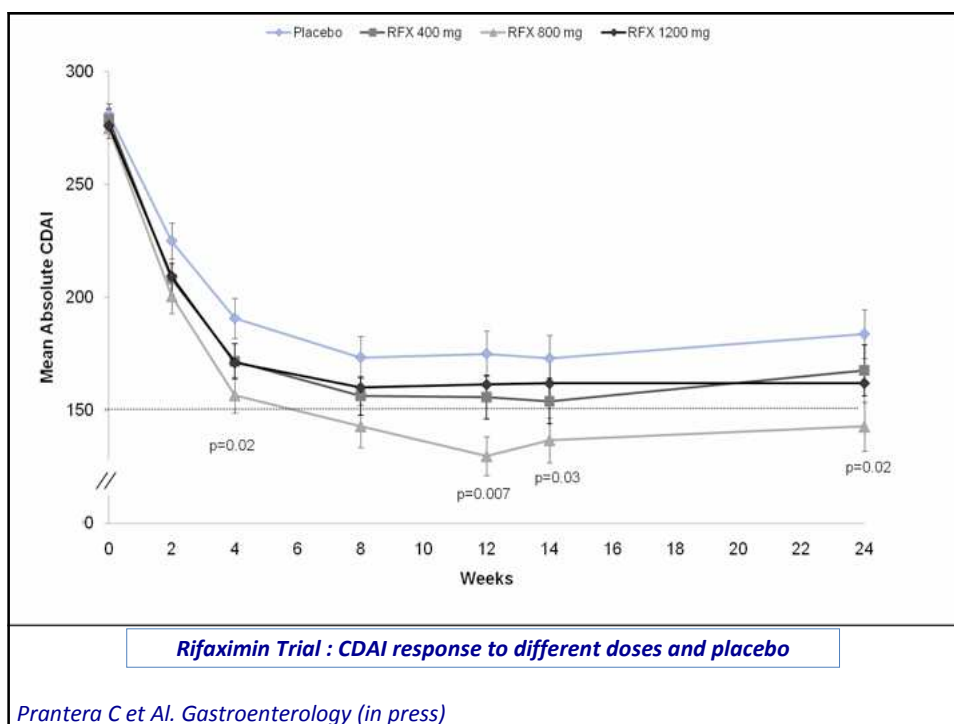
C Prantera et al. 2006

Primary end-point:

Clinical remission (CDAI≤150) at week 12 (V6) – All patients

	Placebo % (n)	400 mg bid % (n)	800 mg bid % (n)	1200 mg bid % (n)	All active doses % (n)
FA	42.6% (43/101)	53.8% (56/104)	62.2% (61/ 98)	47.5% (47/ 99)	54.5% (164/301)
<i>P value (vs placebo)</i>		0.106	0.005	0.486	0.038
mFA	44.8% (43/ 96)	53.8% (56/104)	63.5% (61/ 96)	50.5% (47/ 93)	56.0% (164/293)
<i>P value (vs placebo)</i>		0.201	0.009	0.429	0.057
PP	44.7% (42/ 94)	54.1% (53/ 98)	65.9% (58/ 88)	53.5% (46/ 86)	57.7% (157/272)
<i>P value (vs placebo)</i>		0.193	0.004	0.238	0.029

Prantera C et Al. *Gastroenterology* (in press)



Primary end-point:
Clinical remission (CDAI≤150) for patients with baseline CRP level of ≥ 5

	Placebo % (n)	400 mg bid % (n)	800 mg bid % (n)	1200 mg bid % (n)
FA	36.5% (19/52)	47.1% (24/ 51)	62.0% (31/ 50)	46.8% (22/ 47)
<i>P value (vs placebo)</i>		0.279	0.010	0.300

Prantera C et Al. Gastroenterology (in press)

Conclusion

- IBDs are multifactorial diseases both from the genetic and environmental points of view*
- Consequently is improbable that only one cause of disease exists*
- Bacteria and genes play the most important role in this game*
- However IBDs cannot be considered as typical infectious diseases; but bacteria, eliciting an immune response, could start the inflammatory process that continues also in their absence*
- The use of antibiotics is useful even if not fundamental for treating IBDs.*