



UNICUSANO
Università degli Studi Niccolò Cusano - Telematica Roma

Il Microbiota. L'organo Sconosciuto

Roma, 12 Novembre 2016

Il Ruolo del Microbiota nelle

Malattie Reumatiche infiammatorie ed autoimmuni

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Day Hospital Reumatologia
Ospedale Belcolle ASL Viterbo**

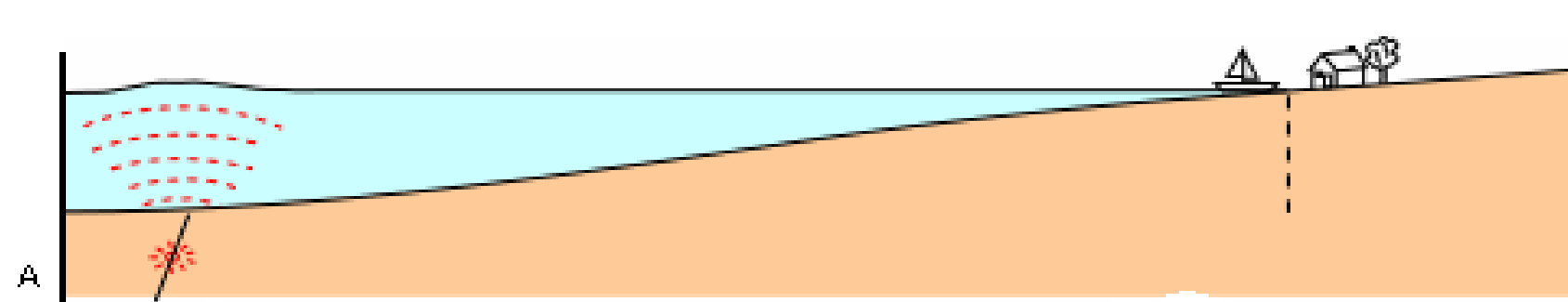




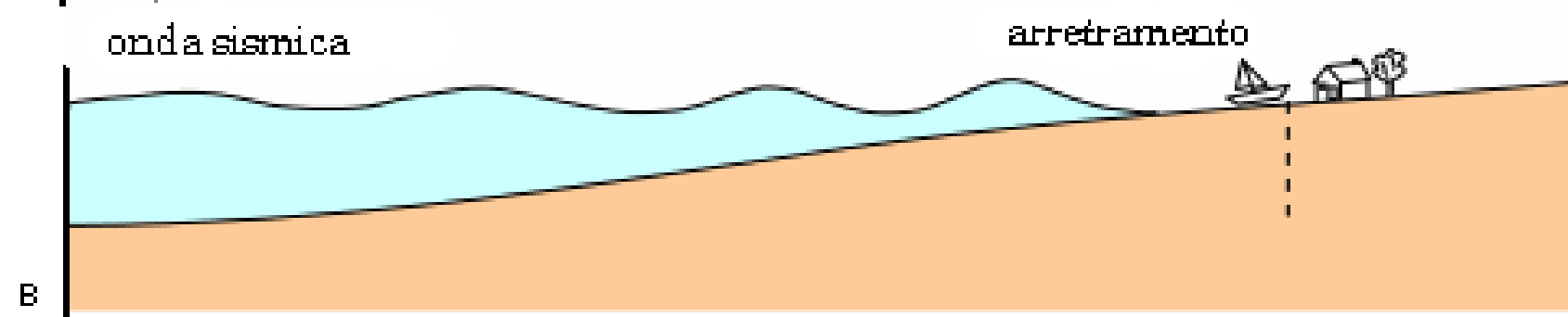
* s. f. [comp. del gr. ῥεῦμα -ματος «reuma» e -logia] **rèuma** s. m. [dal lat. rheuma -ātis, gr. ῥεῦμα -ματος, der. di ῥέω «scorrere», propr. «flusso», in quanto questi dolori sono variabili e vaganti]

dal Dizionario Treccani online

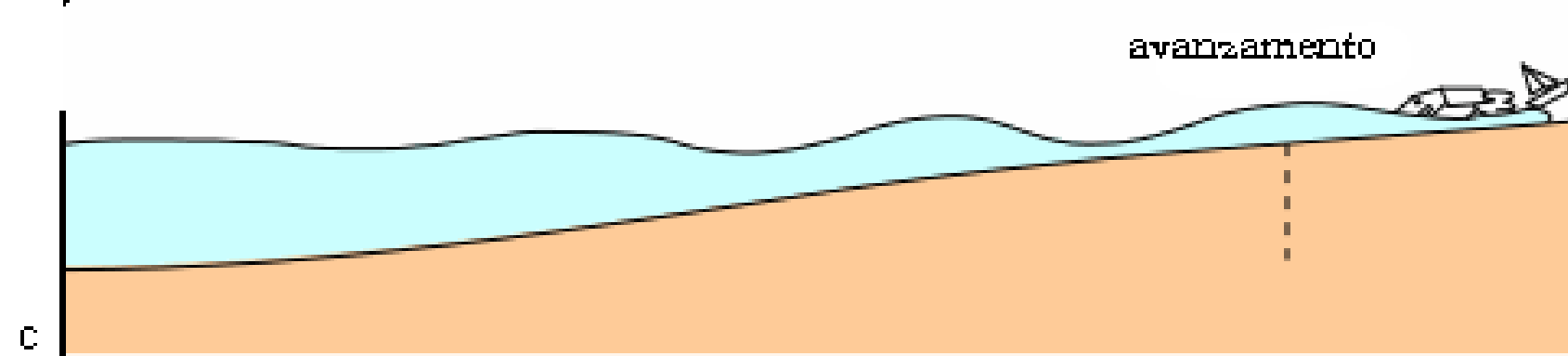
EARLY



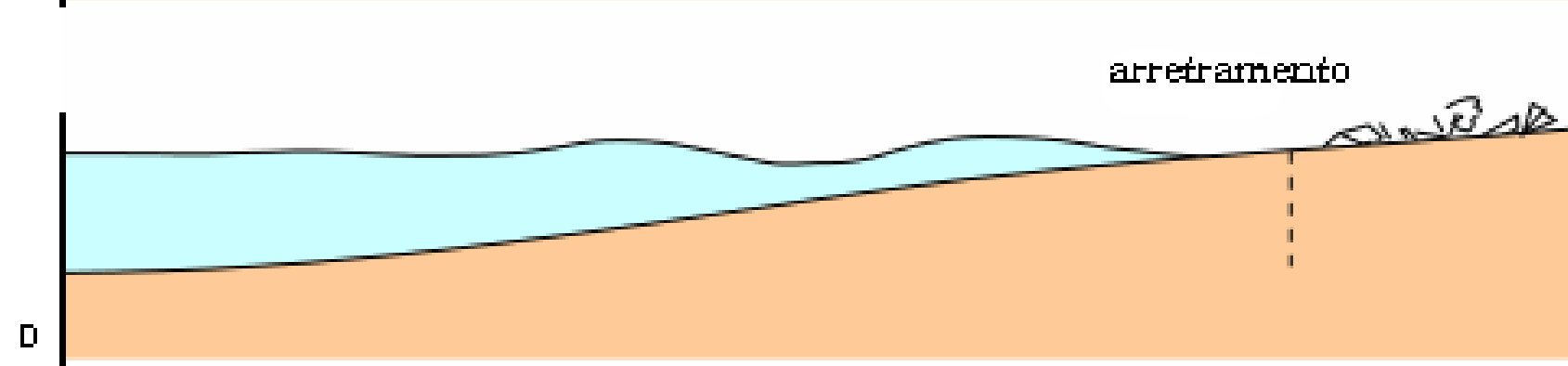
Malattia sub-clinica



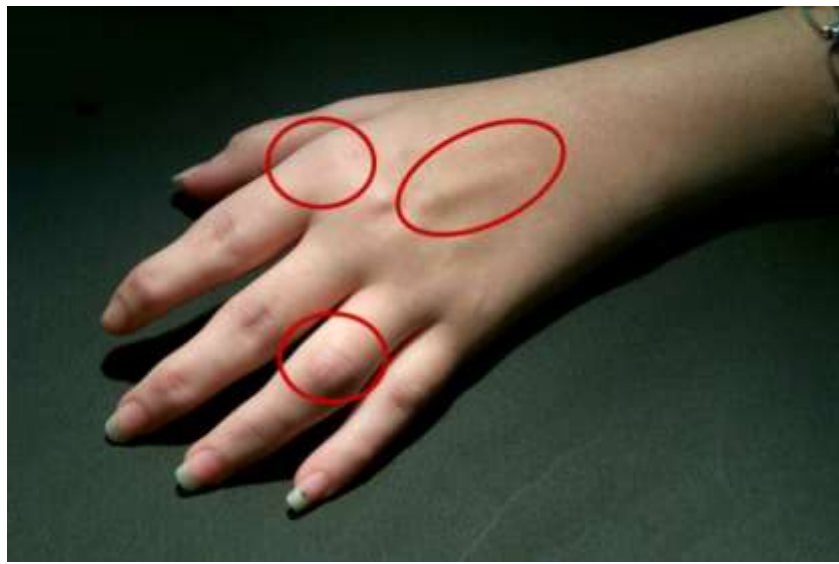
Malattia clinica
"Attività di malattia"



LATE



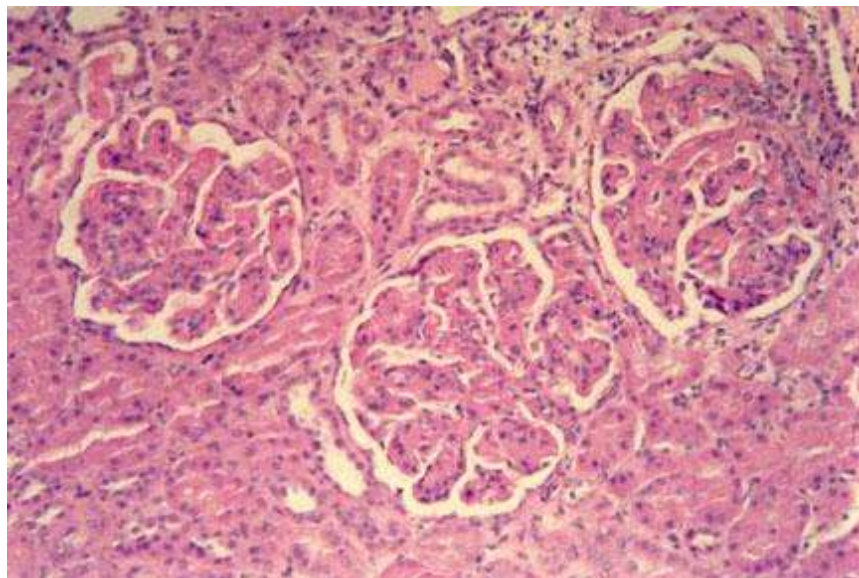
Esiti di malattia
"Danno d'organo"



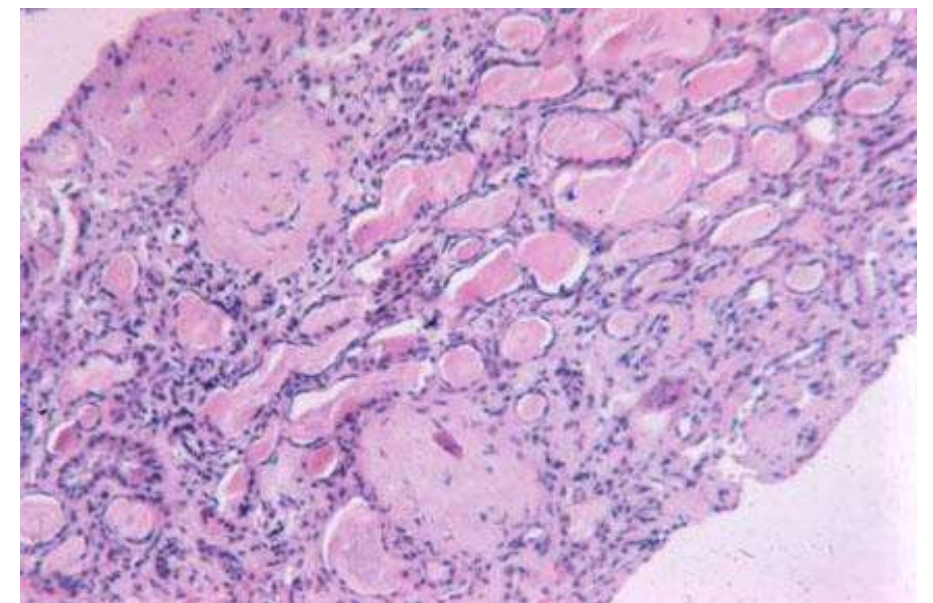
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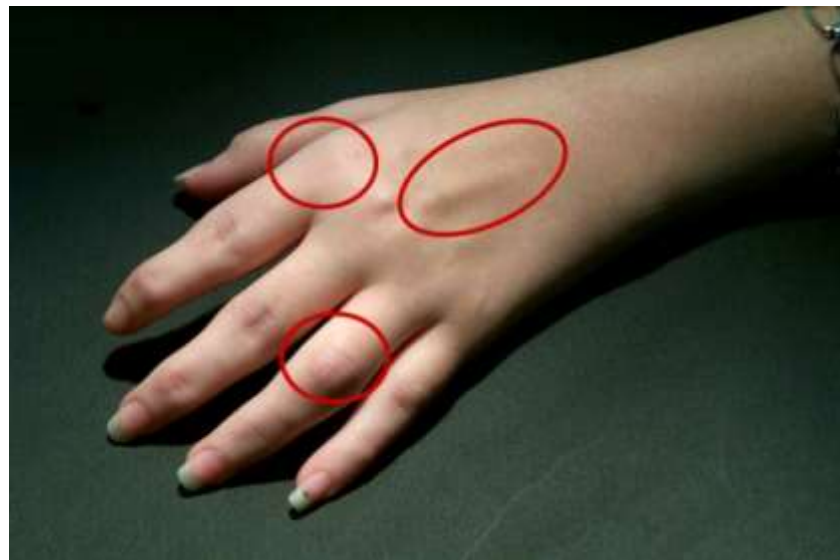
SSc



SLE

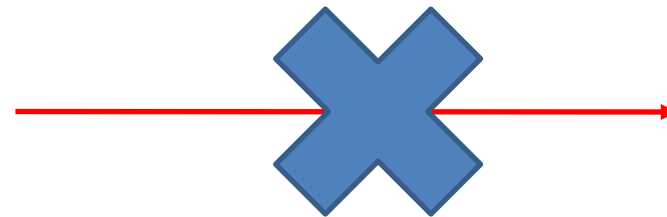


Il trattamento dell'Artrite Reumatoide



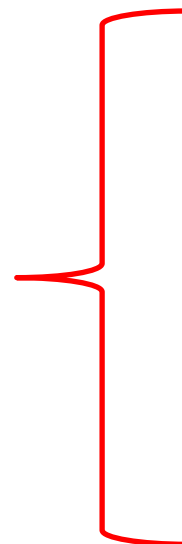
EARLY

WINDOW OF OPPORTUNITY



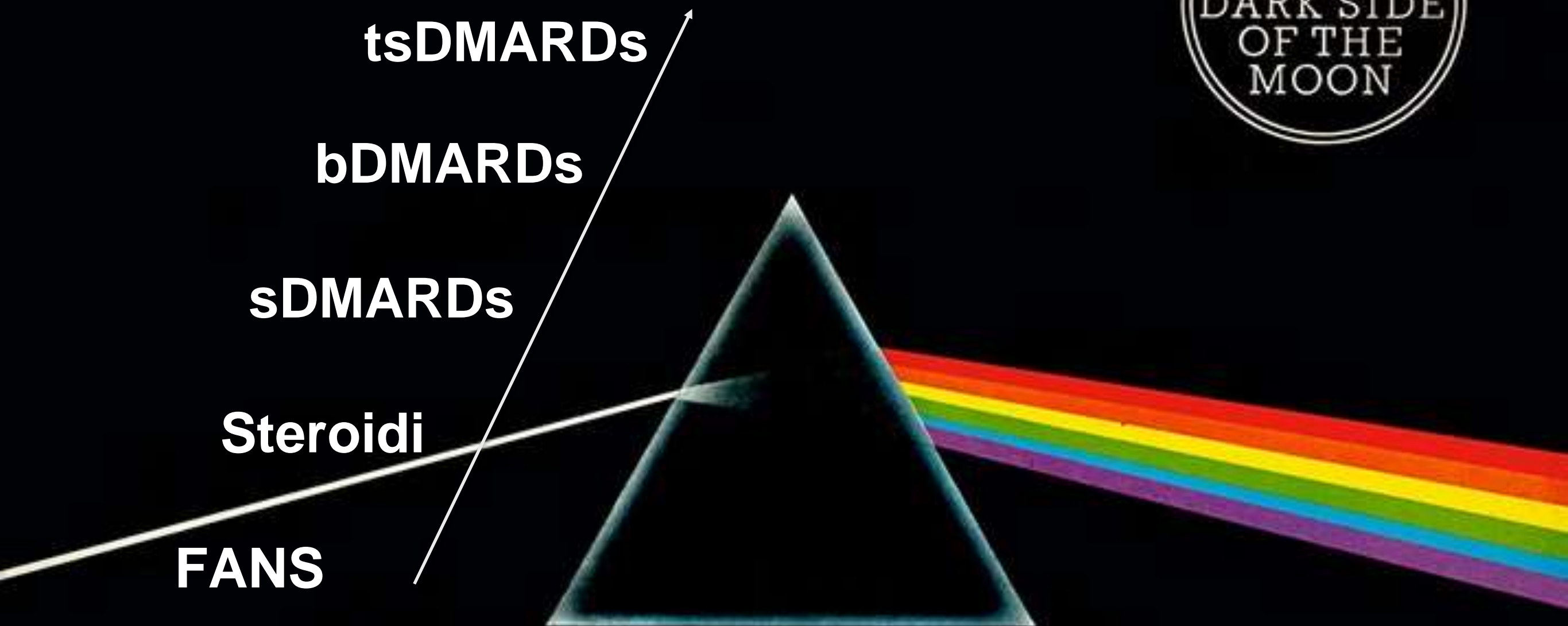
LATE

EARLY TREATMENT
T2T
CDC



La piramide della terapia dell'AR

UNMET NEEDS





"I could not sufficiently wonder at the intrepidity of these diminutive mortals, who durst venture to mount and walk upon my body [...] without trembling at the very sight of so prodigious a creature as I must appear to them."

Jonathan Swift, Gulliver's Travels

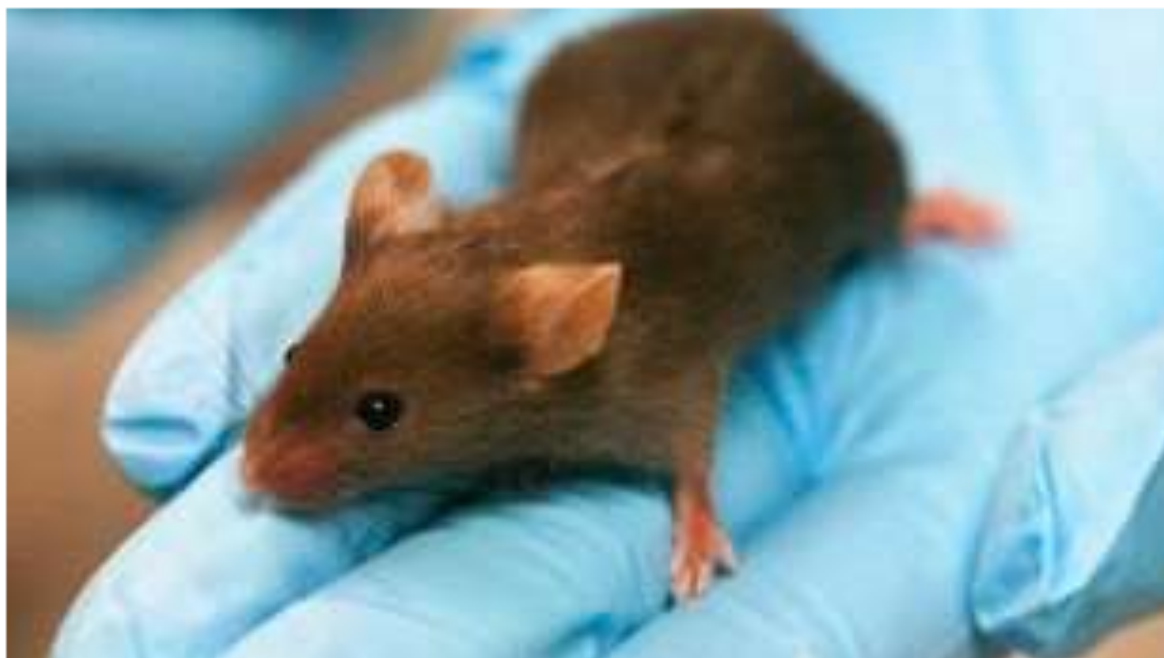


*“..animalcules ... were in such enormous numbers [and] seemed to be **alive**.”*

Antony van Leeuwenhoek (1632-1723)

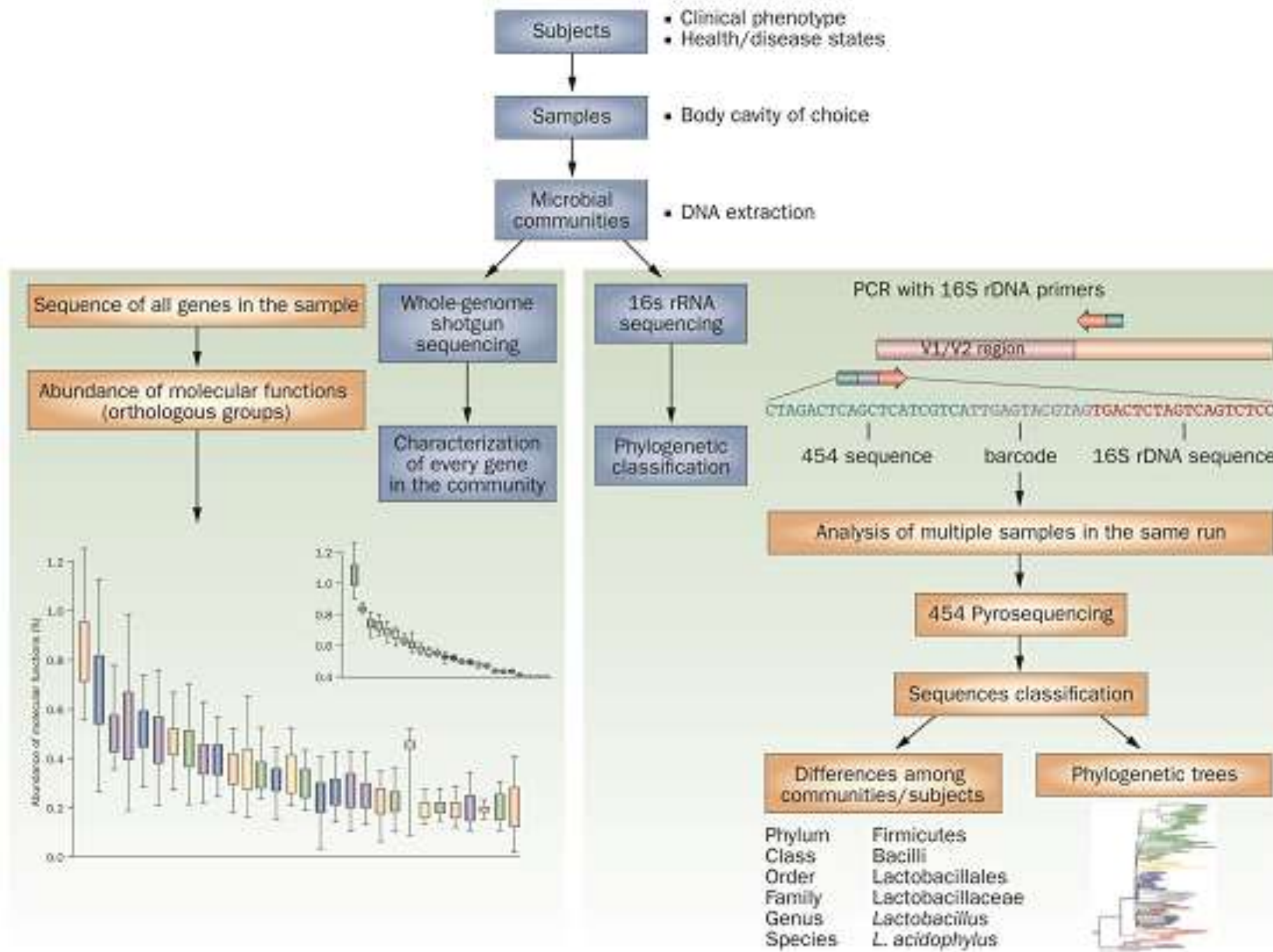
Nuovi modelli sperimentali

- Animali **gnotobiotici**
- Modelli murini di malattie infiammatorie
- Approcci coltura-indipendenti (*massive parallel sequencing technology*)



germ-free (GF) mouse

single pathogen free (SPF) mouse



“What are they doing?”

“Who are they?”

Scher JU et al, Nat Rev Rheum 2011

Il ruolo del sistema immunitario



Sistema Immunitario

Difendere l'organismo da agenti patogeni dall'esterno e dallo sviluppo di tumori dall'interno

Sistema Immunitario

Difendere l'organismo da agenti patogeni
dall'esterno e dallo sviluppo di tumori
dall'interno



Immunità innata



Immunità adattativa

Macrofagi
Cellule NK
Cellule dendritiche
Complemento

Linfociti T
CD4+ (Th1, Th2, Th17, Treg)
CD8+
Linfociti B -> Plasmacellule

GALT 70% del sistema immunitario umano

Il più grande e complesso compartimento del sistema immunitario

Pericoloso

Harmful
(immunity)

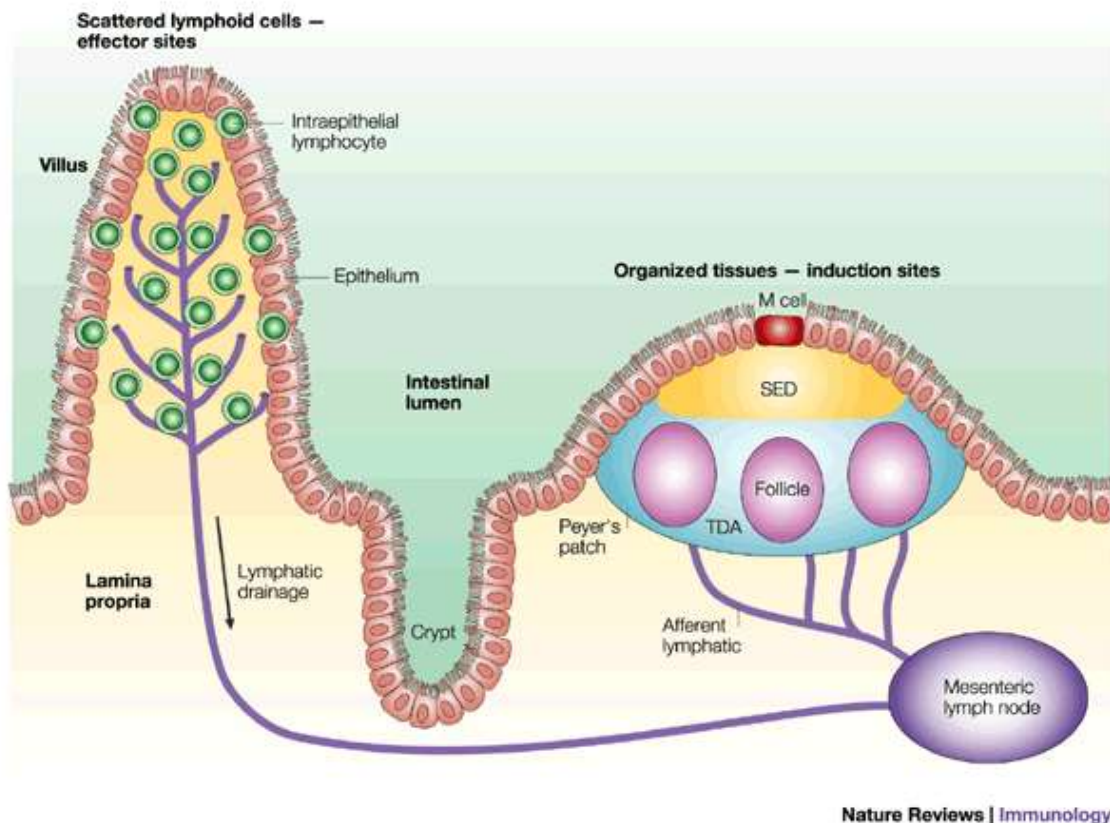


Innocuo

Harmless
(tolerance)

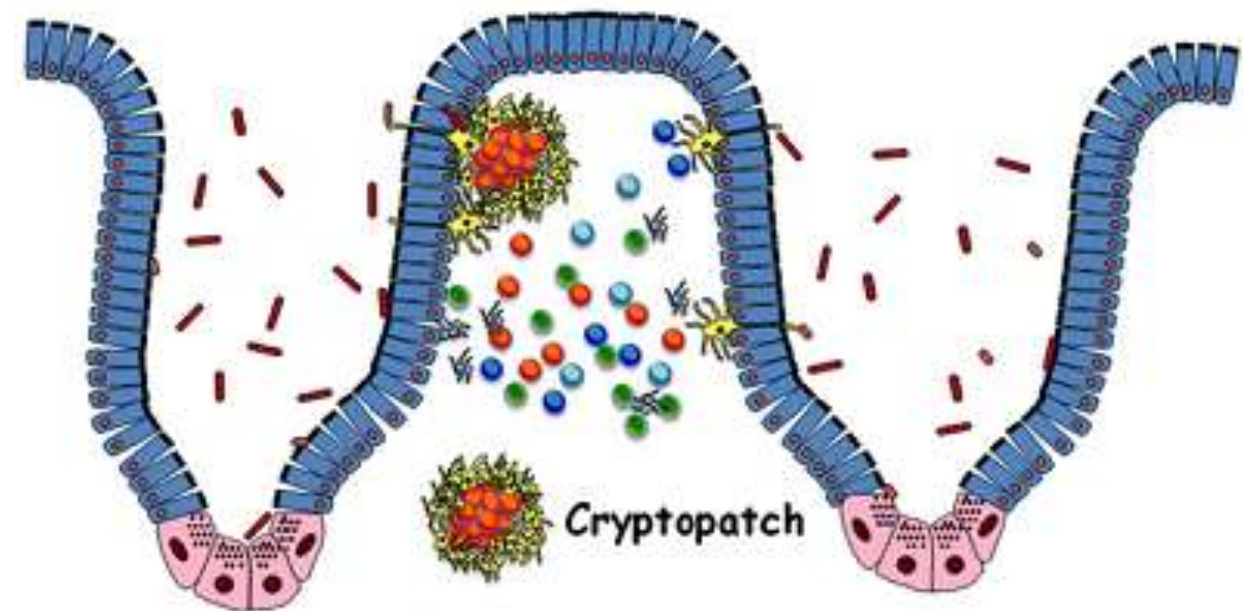
Anatomia Sistema immunitario intestinale (GALT)

Organi linfoidi *secondari*



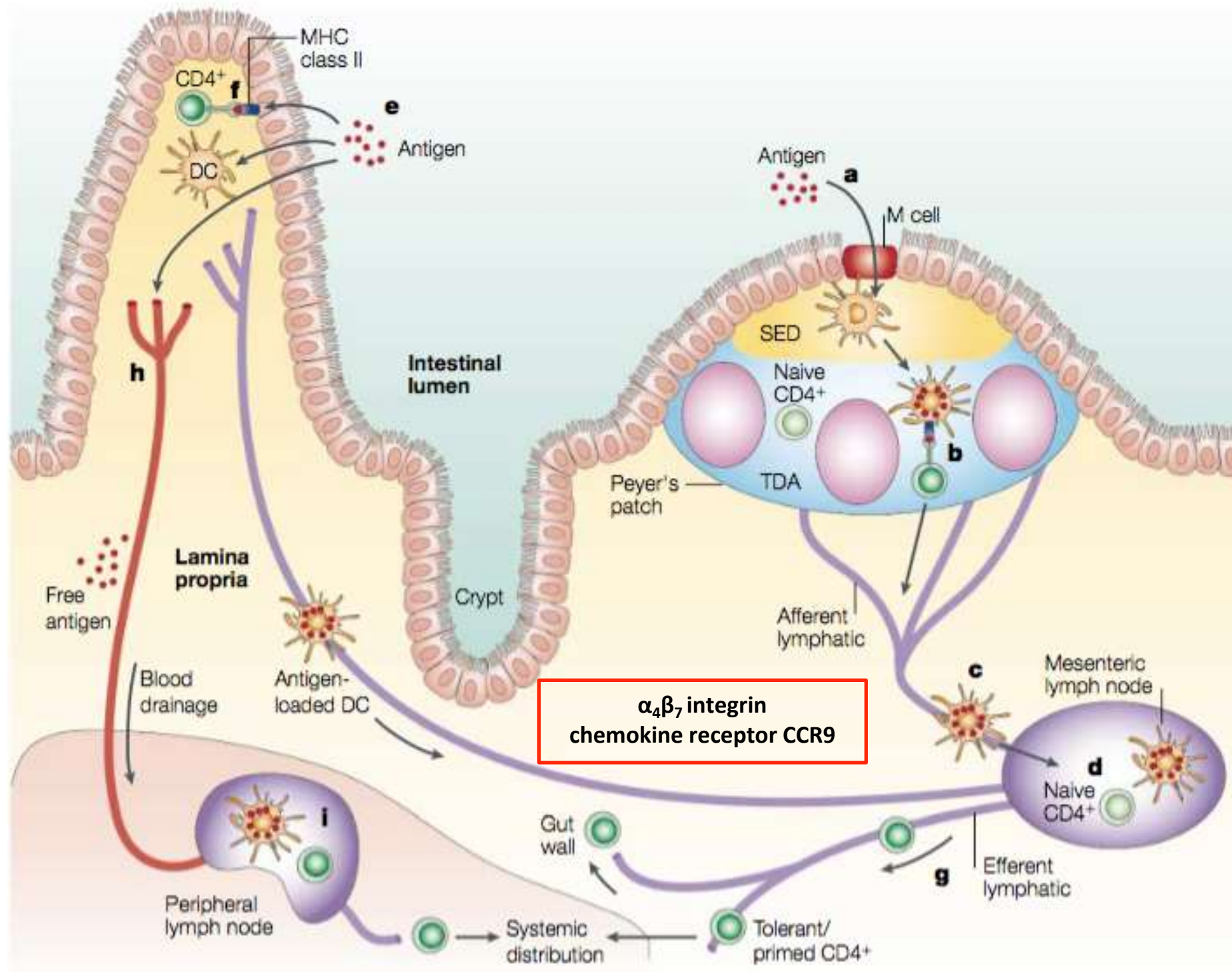
Placche di Peyer, linfonodi
mesenterici

Organi linfoidi *terziari*



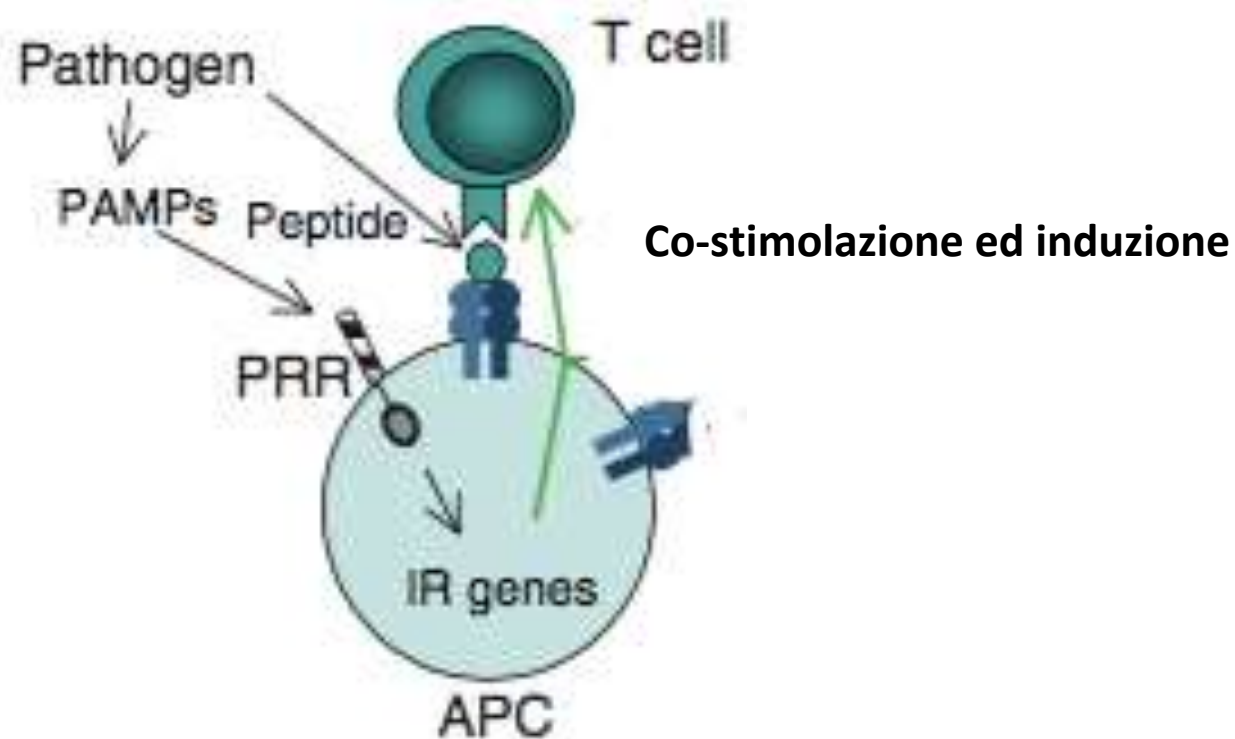
Cryptopatch del piccolo e grosso
intestino

Processazione dell'antigene



Immunità innata

La discriminazione del Self-Non Self va oltre la selezione clonale



PAMPs (pathogen associated molecular patterns)

PRR (non clonal pattern recognition receptor) -> *Toll-like-receptort (TLRs), C-type lectins, RNA helicase RIG-I-like receptors (RLRs), and nucleotide-binding oligomerization domain–like receptors (NLRs)*

Il primo contatto

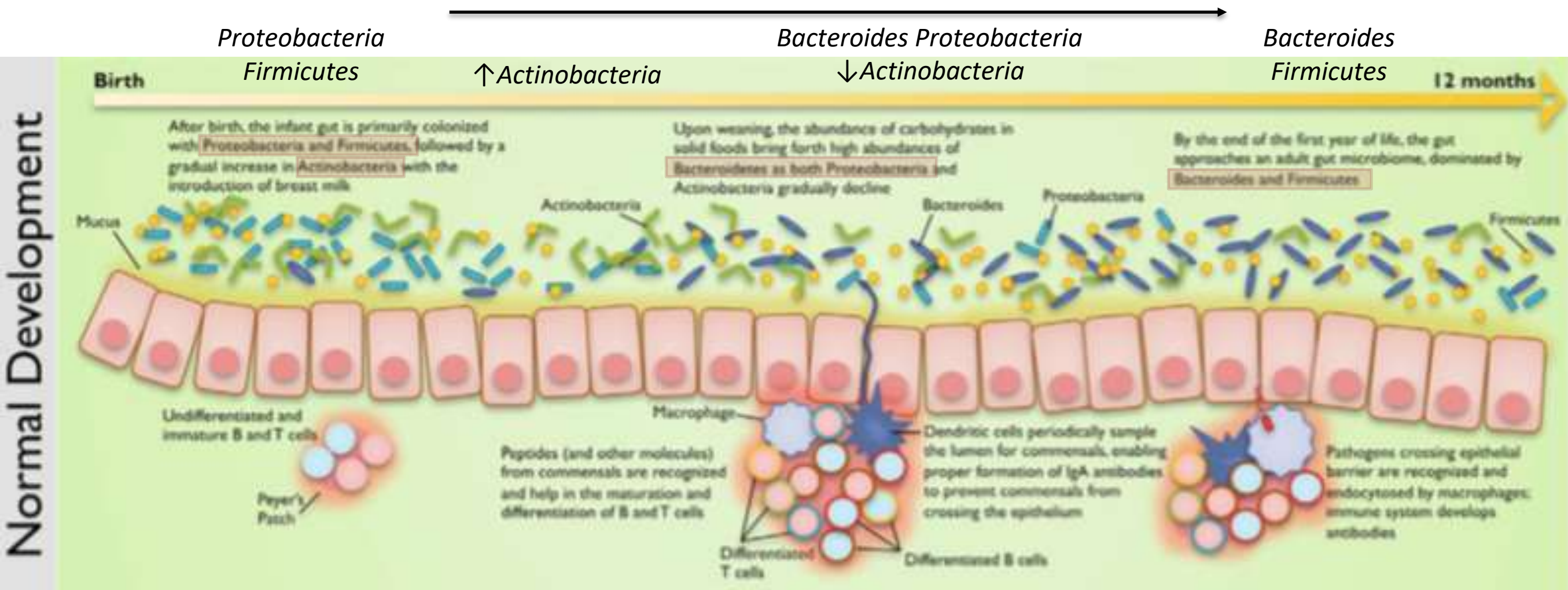


- **Immaturità** del sistema immunitario del neonato -> “*ambiente tollerogenico*”
- **Latte materno** -> *microbioma, prebiotici (oligosaccaridi), anti microbici (IgA, cellule immunitarie, citochine, lattoferrina, lisozima)*

Tono dell'immunità sistemica e mucosale
a lungo termine

Sviluppo del microbiota nel neonato

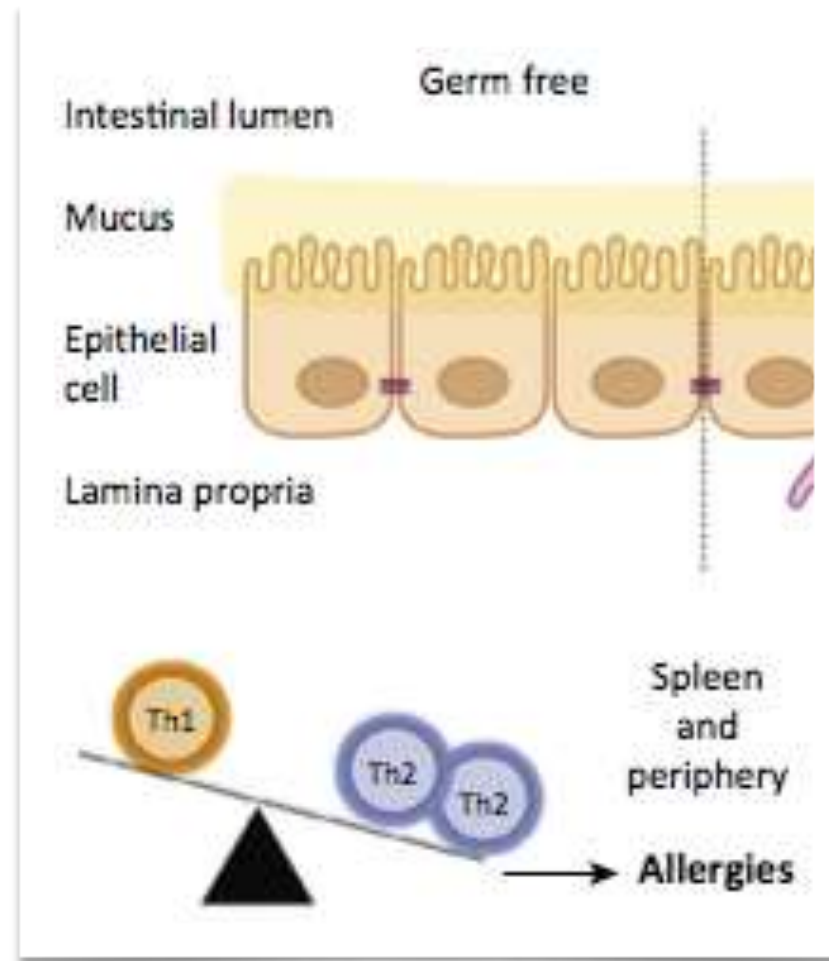
Sviluppo del microbiota intestinale



Sviluppo del sistema immunitario

Vangay P et al, *Cell Host Microb* 2015

Microbiota e sviluppo del sistema immunitario



Intestinal organ development	Site	Phenotype in Germfree mice
Small Intestine	Peyers Patches	fewer, less cellular
	Lamina propria	thinner, less cellular
	Germinal centers	fewer plasma cells
	Isolated lymphoid follicles	smaller, less cellular
Mesenteric Lymph nodes	Germinal centers	smaller, less cellular
		fewer plasma cells

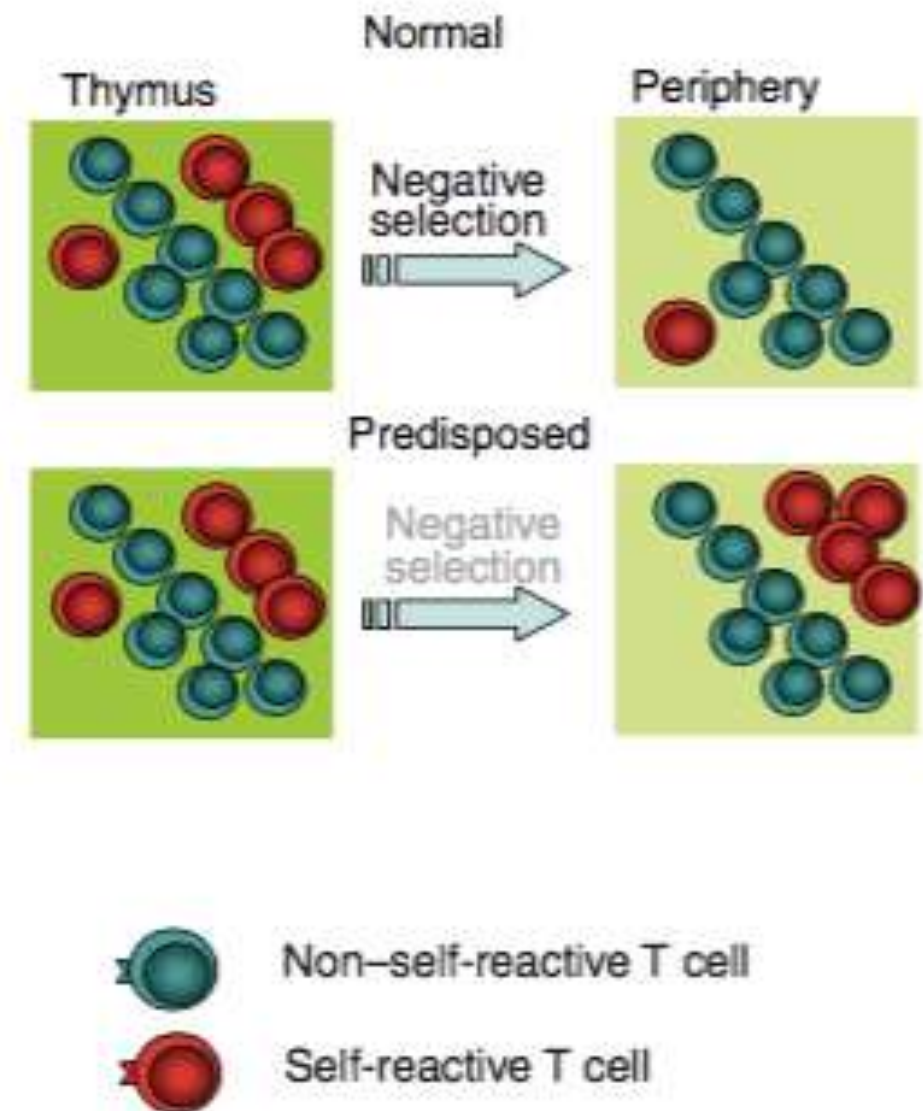
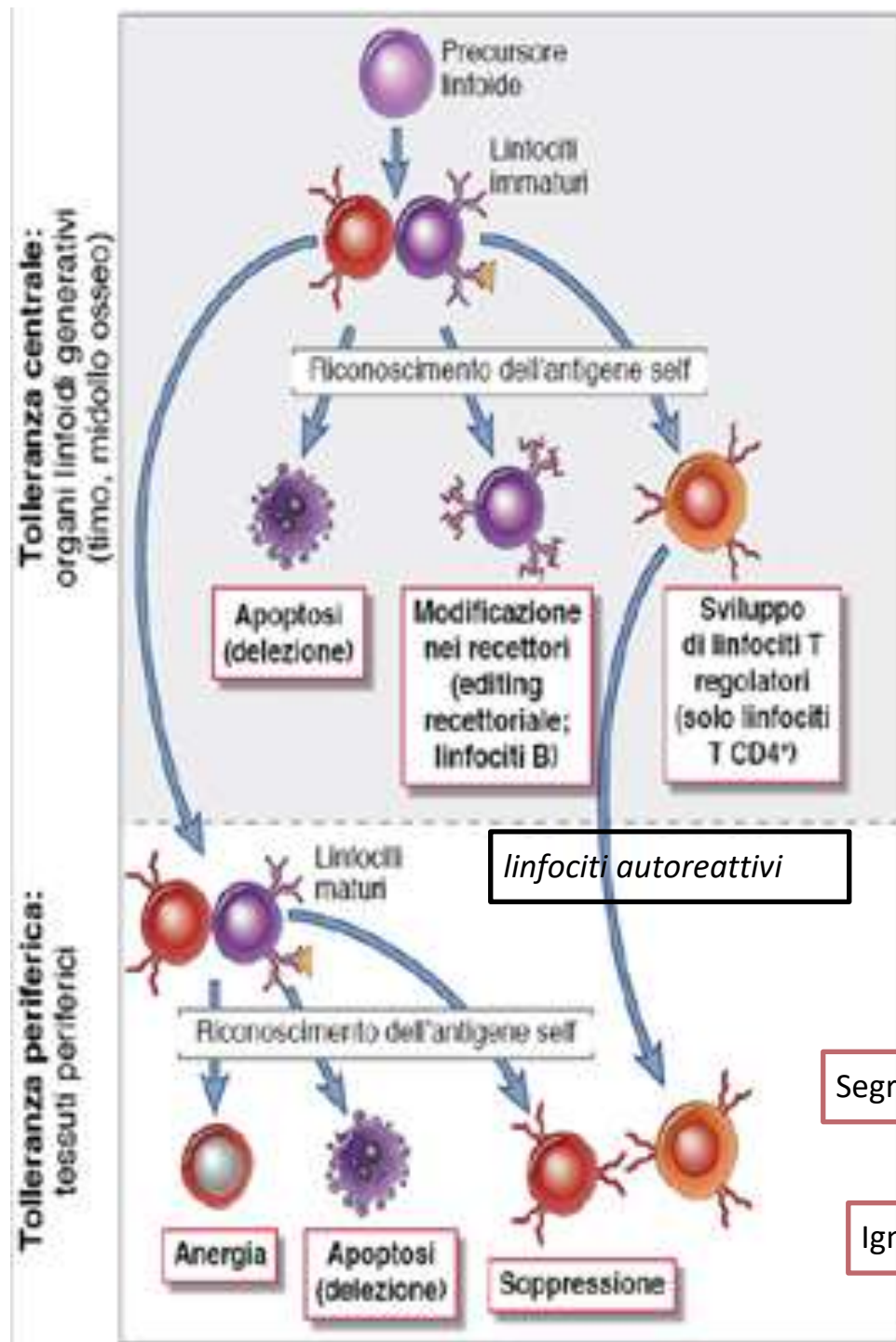
- Difetto nei compartimenti T, B, e dell'immunità innata nella mucosa
- Riduzione dei linfociti CD4⁺ T in tutti gli organi linfoidi periferici
- Sbilanciamento sistemico verso il fenotipo Th-2
- Ridotti livelli di IgG e IgA (Abs)

Round JL et al, *Nat Rev Imm* 2009

Mathis D et al, *Cell Host* 2011

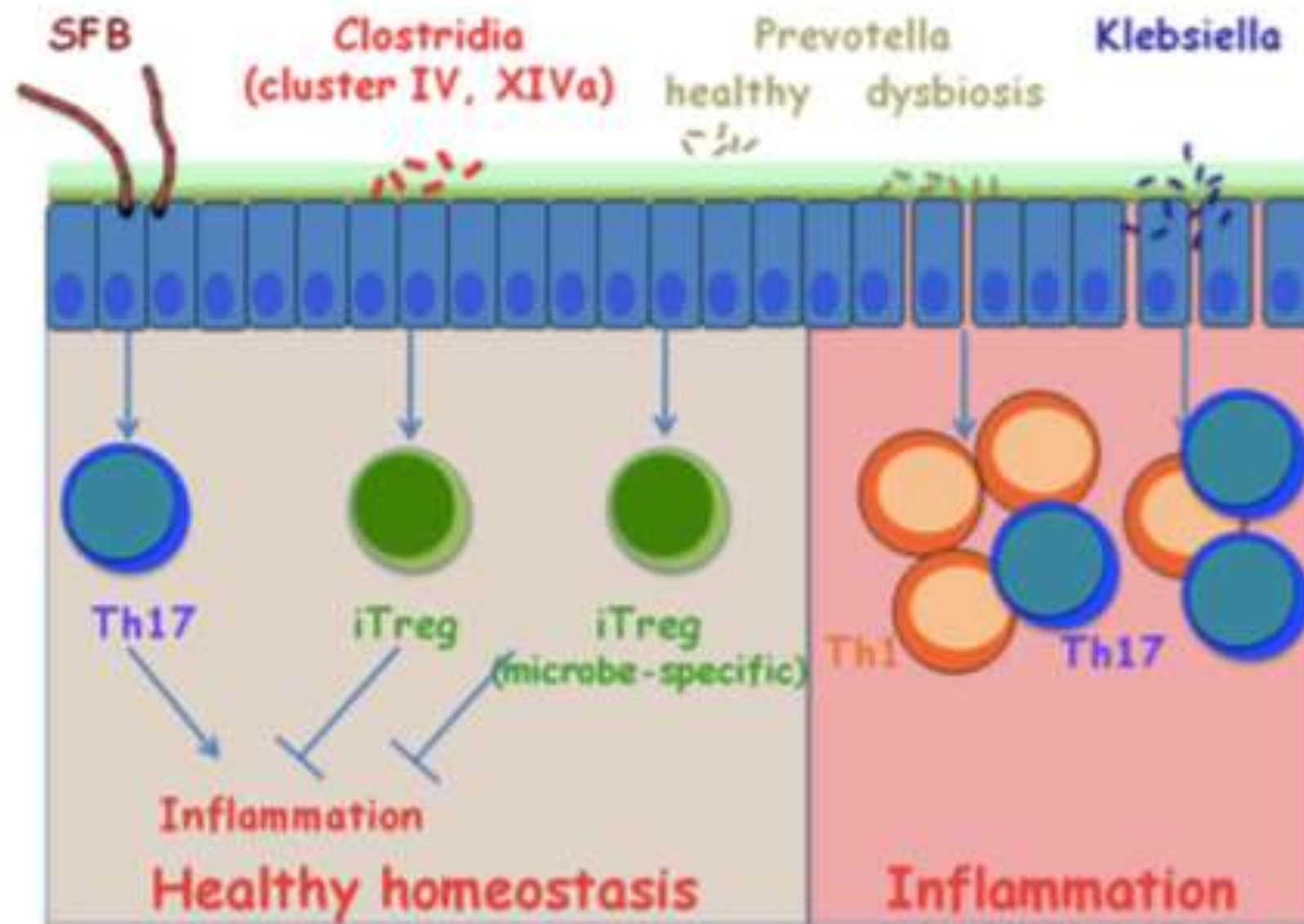
Ruff WE et al, *Trends Molec Med* 2015

Tolleranza immunologica



Abbas AK, *Immunologia cellulare e molecolare VIII* ed
Chervonsky Av, *Nat Rev Imm* 2009

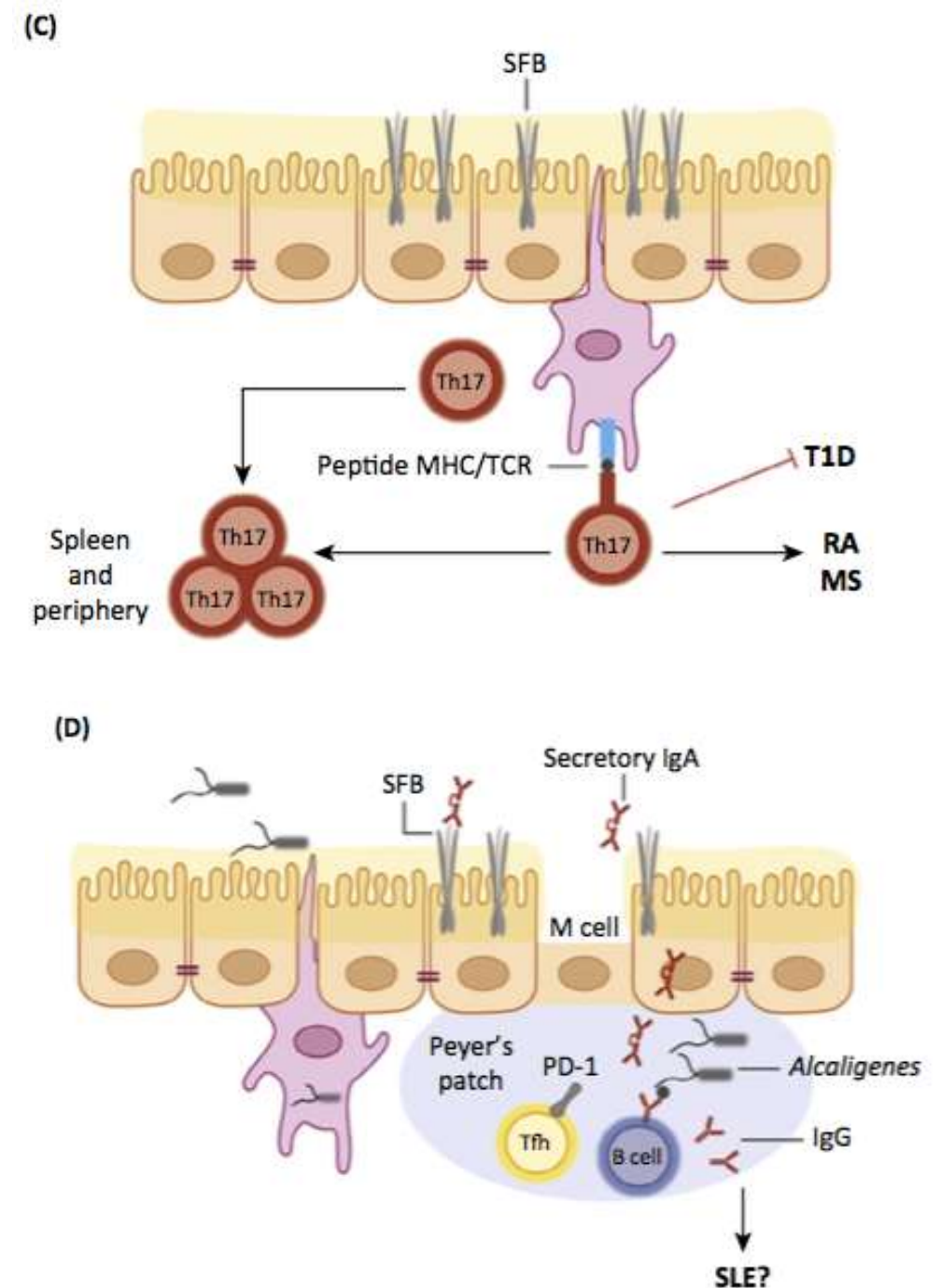
Specie batteriche chiave



Littman DR, *Cell Host & Microbe* 2011

Immunità ed Infiammazione regolata

- **Segmented Filamentous Bacteria (SFB)** -> induzione Th17 e Th1, produzione IgA nel piccolo intestino
- **Alcaligenes** -> secrezione IgA
- **Flagellina** -> TLR-5 e **LPS** > TLR-4 —> CD103+ DC -> IL-23 -> ILCs -> IL-22 -> produzione di peptidi antimicrobici (**es. RegIIIγ**) -> **potenziamento della funzione barriera**
- Produzione di **pro-IL-1β** dai macrofagi intestinali residenti (rapida attivazione cellulare attraverso la conversione di pro-IL-1β in IL1β attiva)
- **CpG** nel DNA procariotico commensale -> **TLR9** adiuvante locale

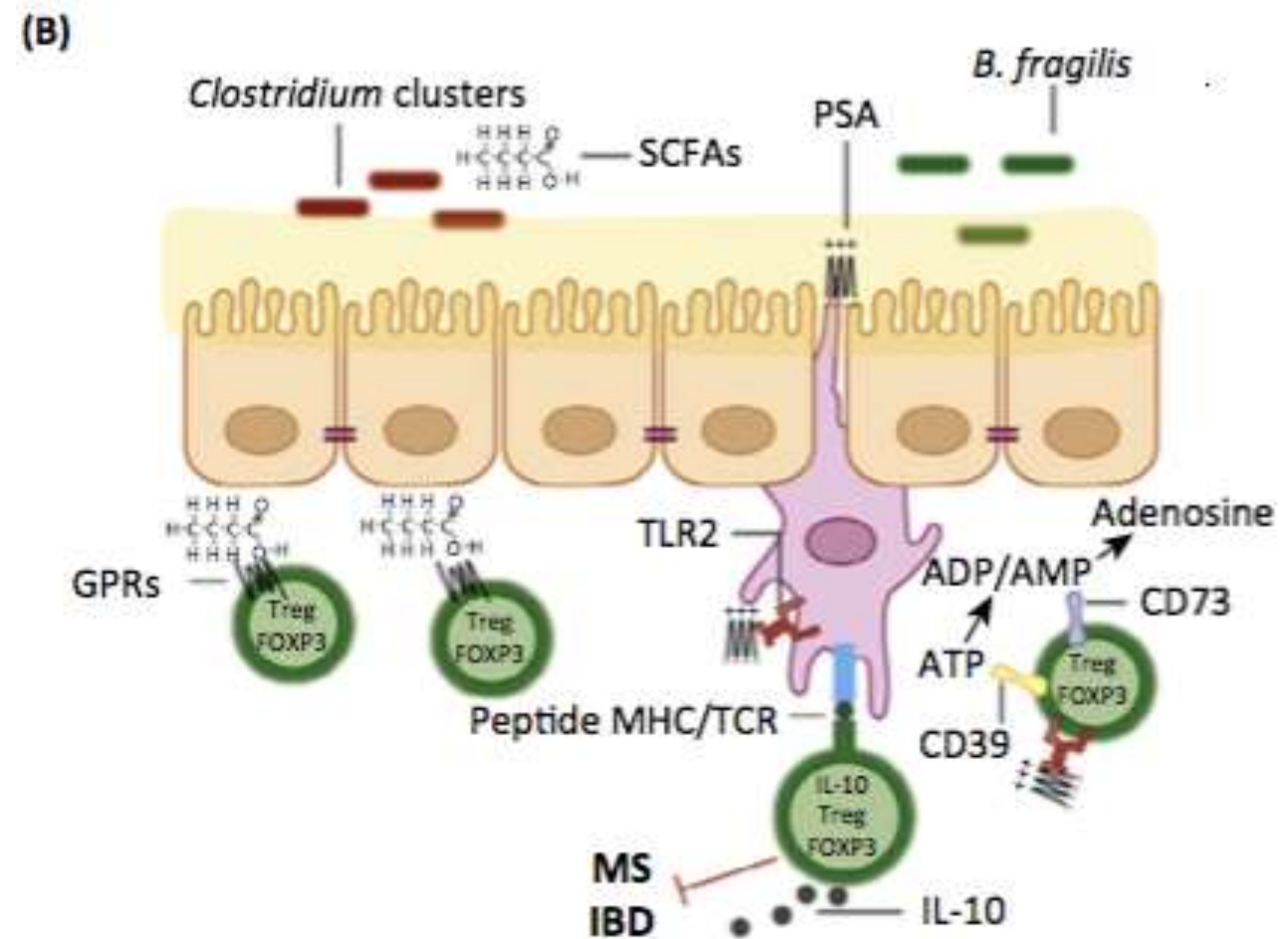


Belkaid Y et al, *Cell* 2014

Ruff WE et al, *Trends Molec Med* 2015

Tolleranza

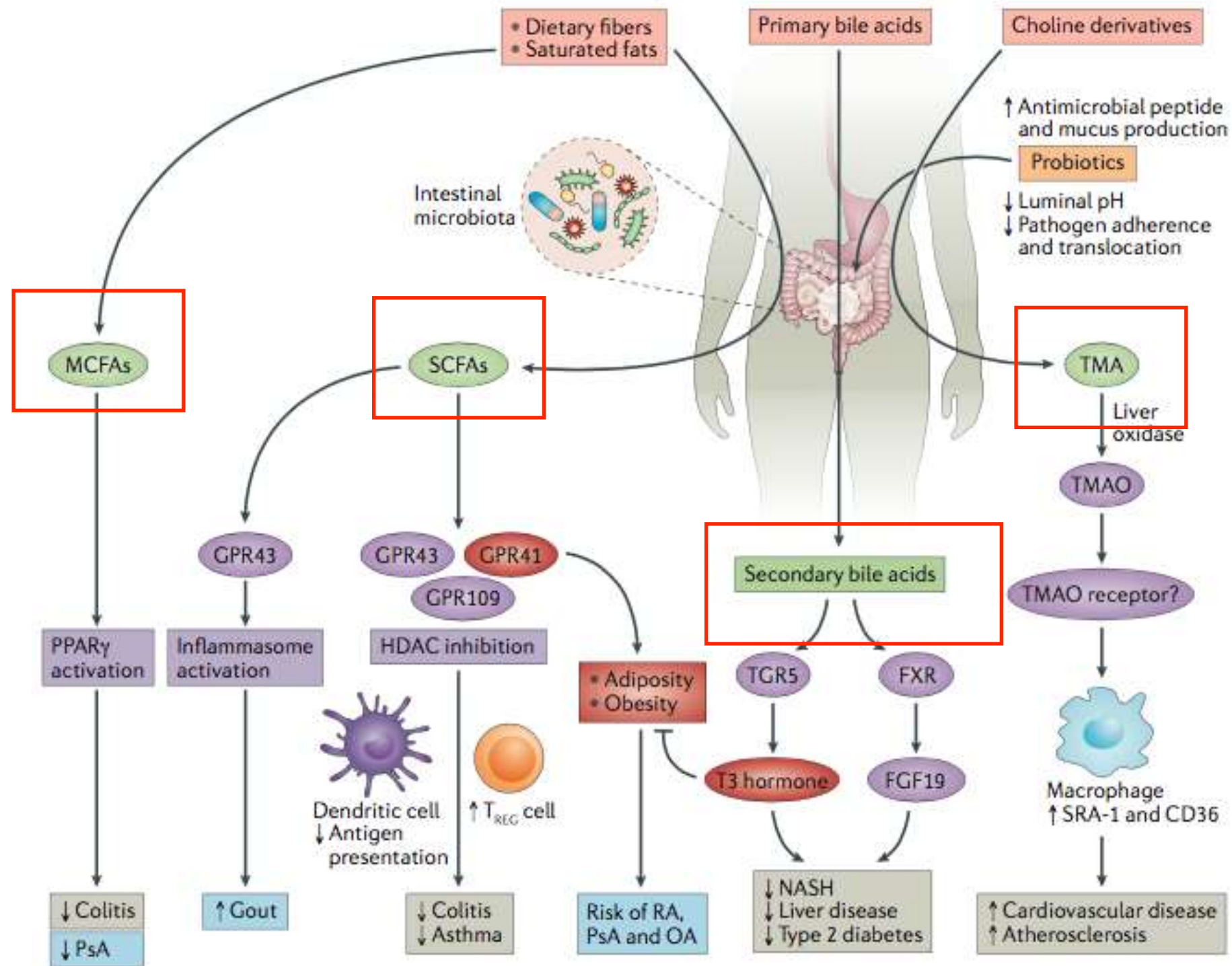
- **Bacteroides Fragilis** -> polisaccaride A (PSA) -> TLR2 T-cell -> differenziazione in iTreg
- **Comunità di Clostridi** -> ambiente ricco di TGF- β -> iTreg
- **SCFA (short chain fatty acids)**, in particolare il butirrato -> azione epigenetica sulle esone deacetilasi (HDACs) -> induzione delle Treg nel colon
- Ruolo protettivo dei commensali durante il danno acuto della mucosa (**limitazione del danno**)
- Molte comunità di batteri, virus e funghi induttrici di tolleranza (es. funghi commensali con **C type lectin receptor Dectin 1**)



Belkaid Y et al, *Cell* 2014

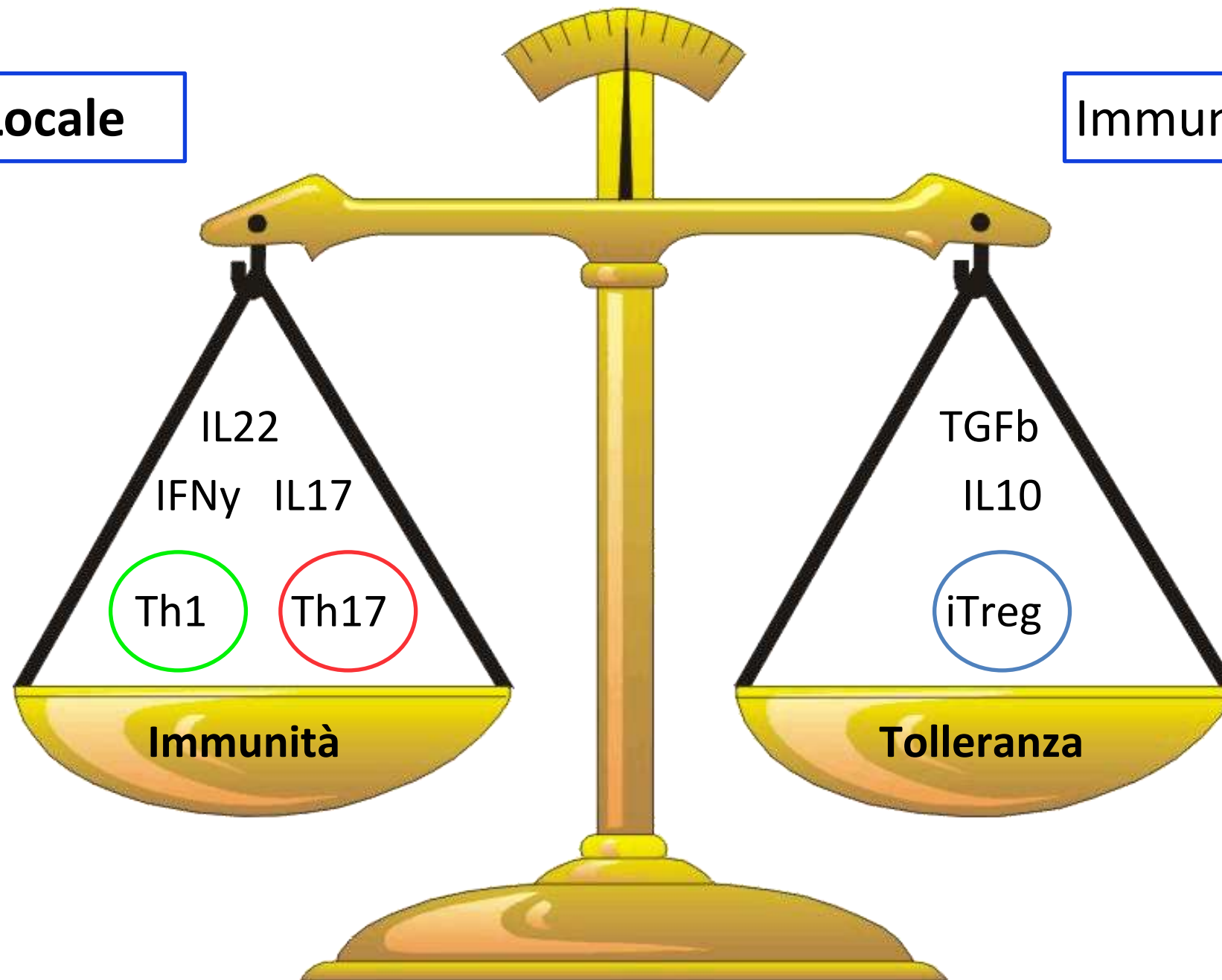
Ruff WE et al, *Trends Molec Med* 2015

I metaboliti del Microbiota intestinale



Immunità Locale

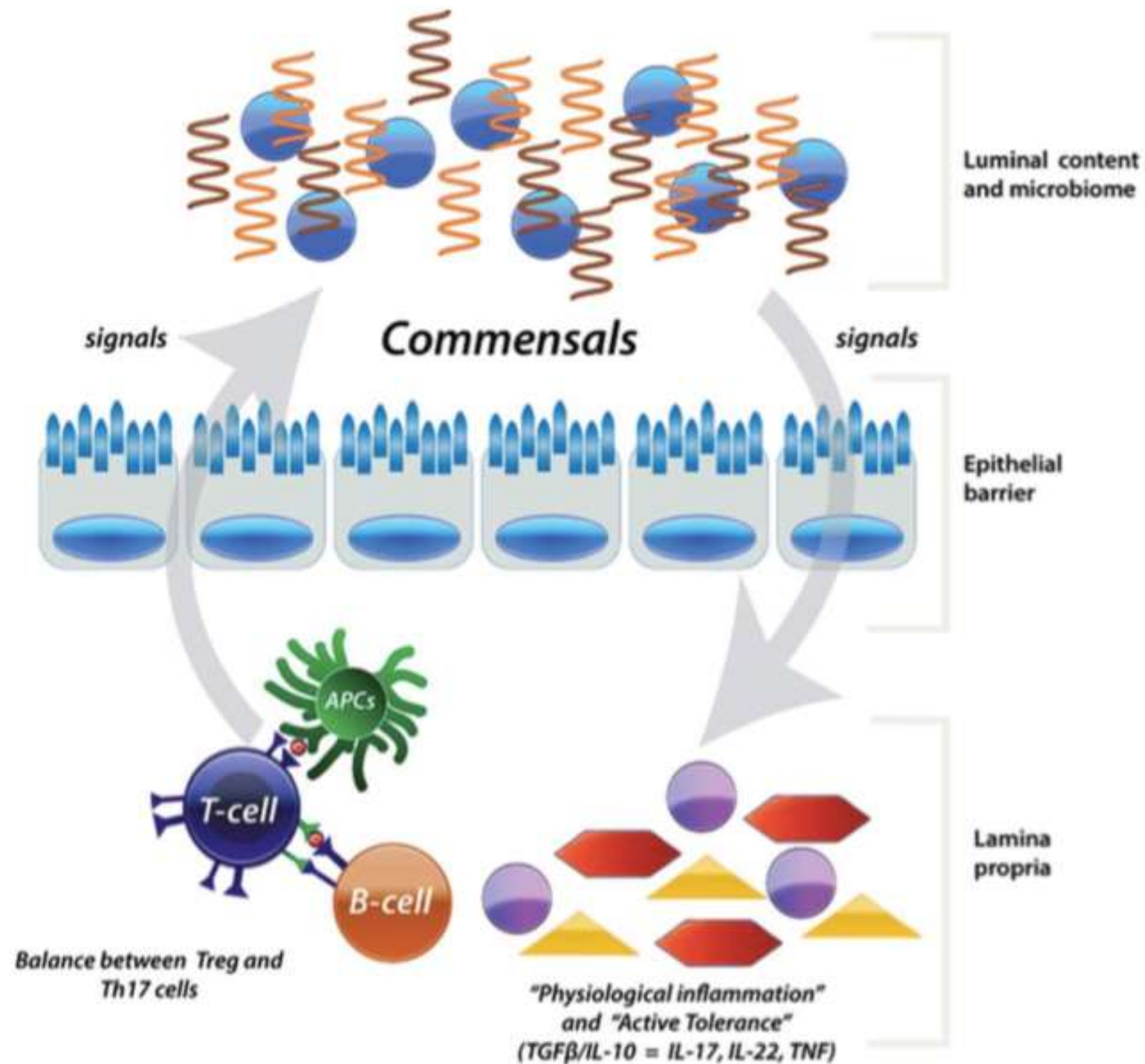
Immunità Sistemica



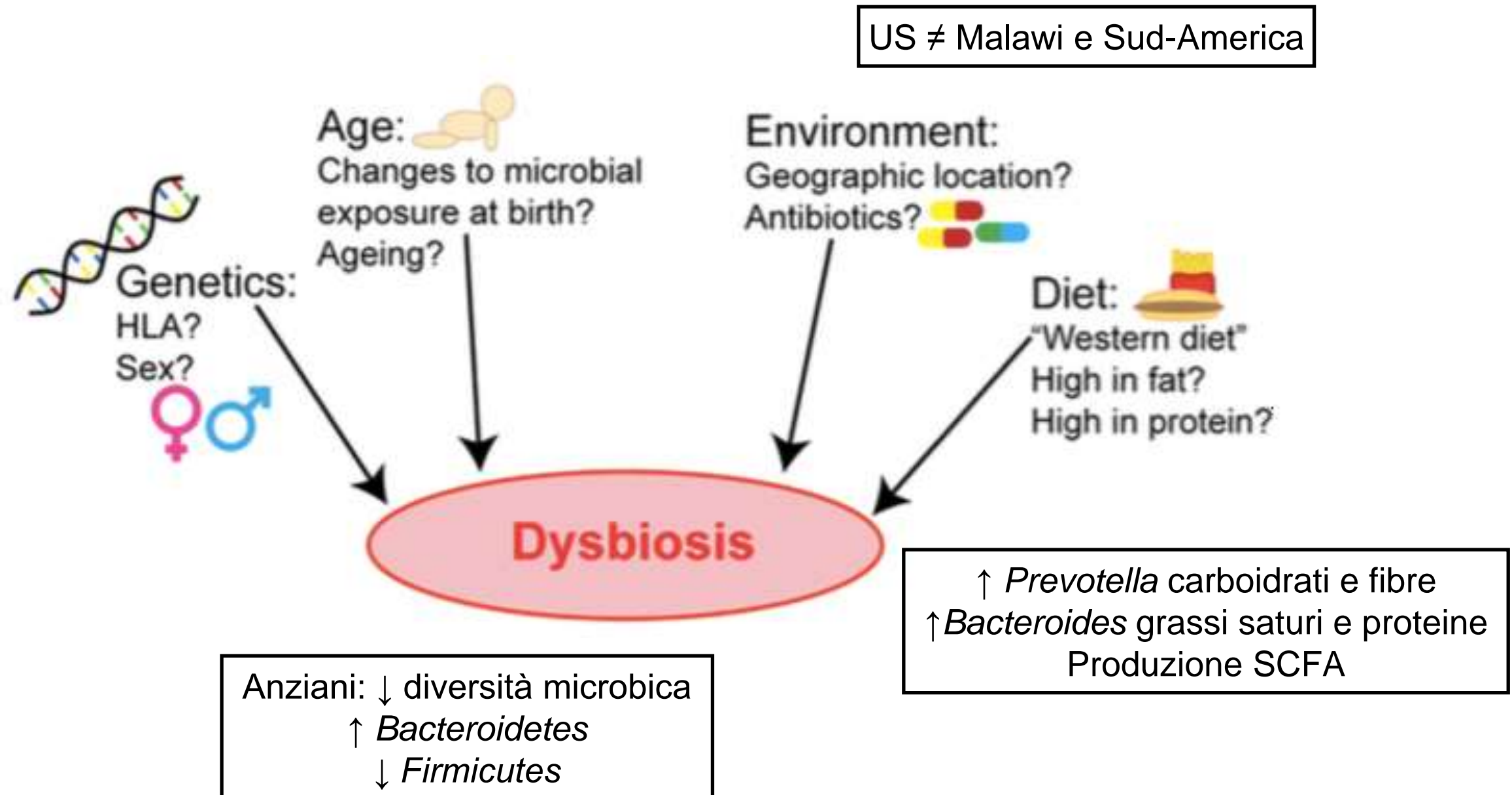
Sito dell'intestino

Composizione microbiota

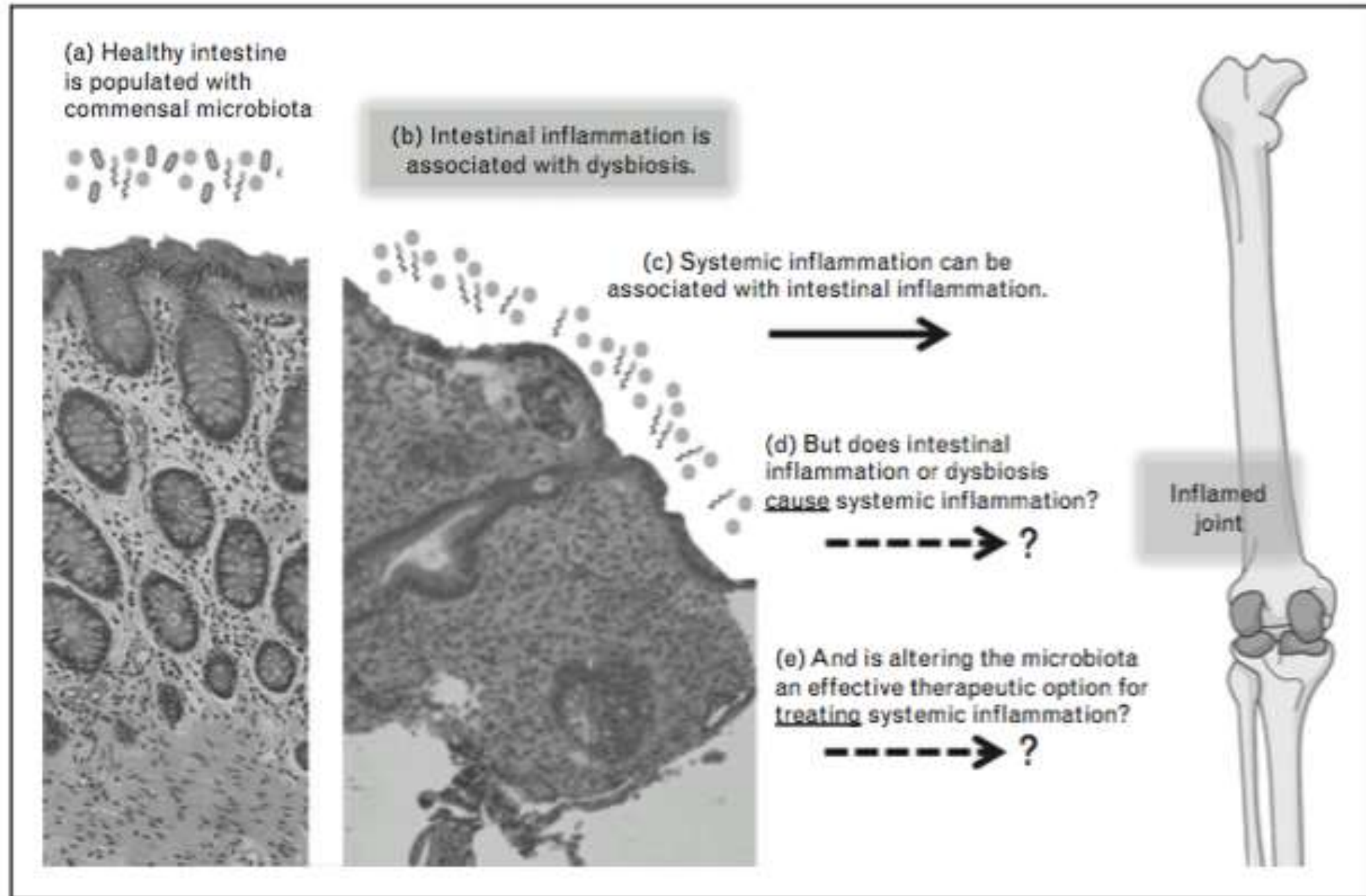
Ruolo regolatorio del microbiota



Fattori che influenzano il microbiota



Esiste una relazione tra disbiosi, infiammazione intestinale ed infiammazione/autoimmunità sistemica?



Vecchi trattamenti per nuove ipotesi



L'ipotesi della sepsi focale



Il fattore tossiemico

THE TOXEMIC FACTOR IN RHEUMATOID ARTHRITIS.*

By CARL C. WARDEN, M. D., Los Angeles.

stimulated many observations on the question. Metschnikoff contends that albuminous putrefaction is frequently due to the pathological abundance in the intestinal canal of anaerobic, alkali producing organisms, which condition alters the normal acidity of bowel contents, banishes aerobic flora from the intestine and causes an abnormal splitting of nitrogenous molecules into toxic radicals which, once absorbed, lead to systemic toxemia. It is this toxemia which we seize upon as an etiologic factor in rheumatoid arthritis. Metschnikoff and his followers commended as a therapeutic measure the reduction of nitrogenous food in the dietary together with the correction of alkalinity and anaerobic invasion by administering lactic acid producing organisms which in suitable media will generate nascent lactic acid in the intestinal canal and assist in rendering the digestive tract uninhabitable by these noxious bacteria. With this end in view he gives

Intestino ed infiammazione periferica

Esempi dalla clinica

- Artrite secondaria a chirurgia di bypass digiuno-ileale
- Spondiloartriti associate a malattie infiammatorie intestinali
- Artrite reattiva ad infezioni gastrointestinali
- Malattia di Whipple
- Utilizzo della *Salazopirina* (sulfonamide+salicilato)



Modelli animali sperimentali

Animal	Manipulation	Phenotype	Germ-free effect	Taxa/molecule involved	Ref.
Rat	Adjuvant-induced arthritis	RA-like synovitis	Arthritis	<i>Escherichia coli</i> , <i>Bacteroides</i> species	42, 43
Rat and mouse	Collagen-induced arthritis	RA-like synovitis	Arthritis	Type II collagen, LPS	44, 45
Rat	SCW-induced arthritis	RA-like synovitis	Arthritis	<i>Streptococcus pyogenes</i> cell wall	46
Mouse	IL-1 α ^{-/-}	RA-like synovitis	No arthritis	<i>Lactobacillus bifidus</i>	47
Mouse	K/BxN transgenic	RA-like synovitis	No arthritis	Segmented filamentous bacteria	48
Rat	HLA-B27/human β_2 -microglobulin transgenic	Colitis, psoriasis, and arthritis	No disease	<i>Bacteroides</i> species	62, 63
Mouse	HLA-B27/ankylosing enthesopathy	Enthesitis and ankylosis	No disease	<i>Bacteroides</i> / <i>Enterococcus</i> species, <i>Veillonella</i> / <i>Staphylococcus</i> species (LPS suppresses enthesitis)	68
Mouse	SKG (ZAP-70 single-point mutation)	Arthritis, psoriasiform lesions, and colitis	No disease	β -glucan, gut commensal microbiota	69, 70

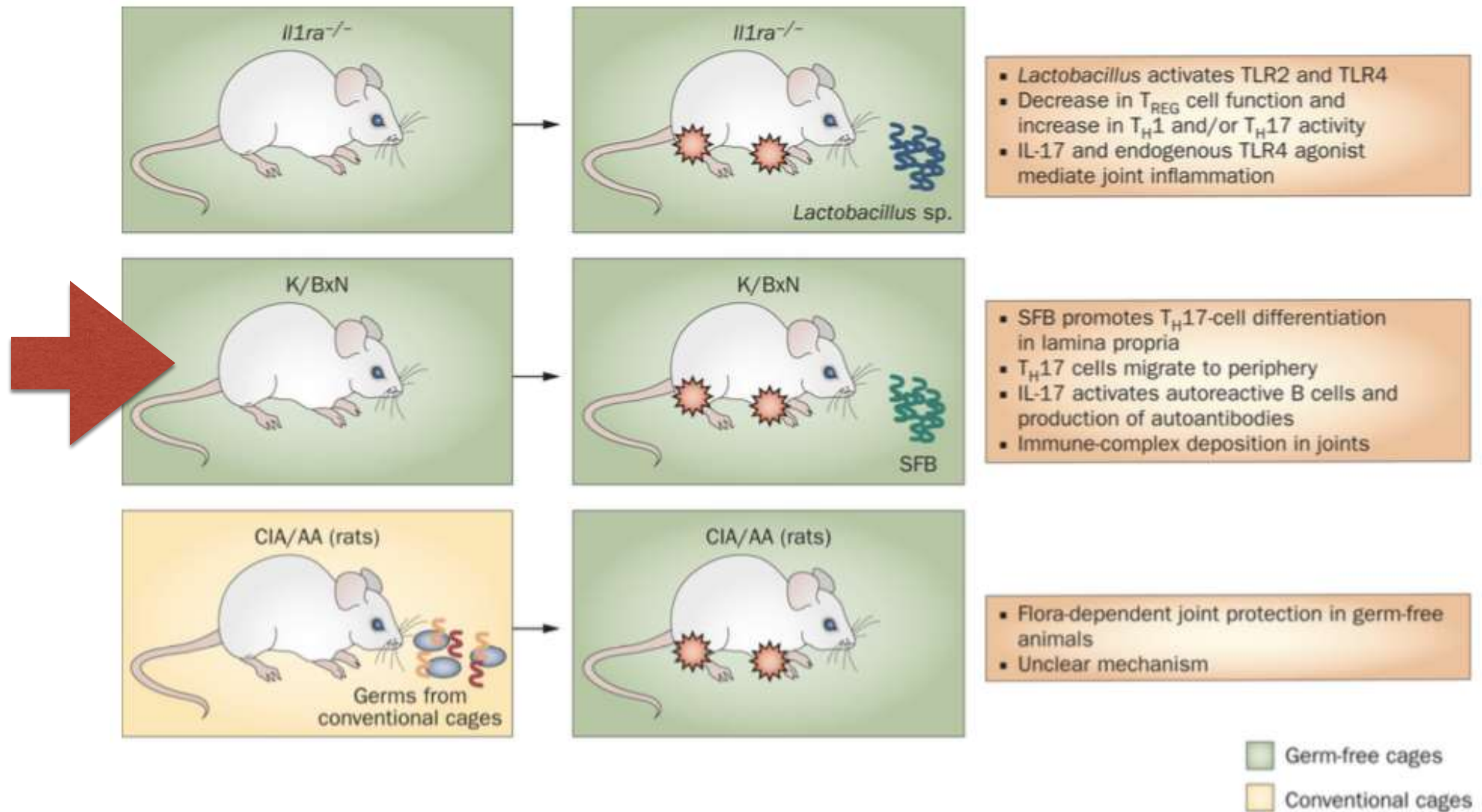
* RA = rheumatoid arthritis; LPS = lipopolysaccharide; SCW = streptococcal cell wall; IL-1 α ^{-/-} = interleukin-1 receptor antagonist knockout.

Table 1. Mouse Models OF Autoimmune Disease

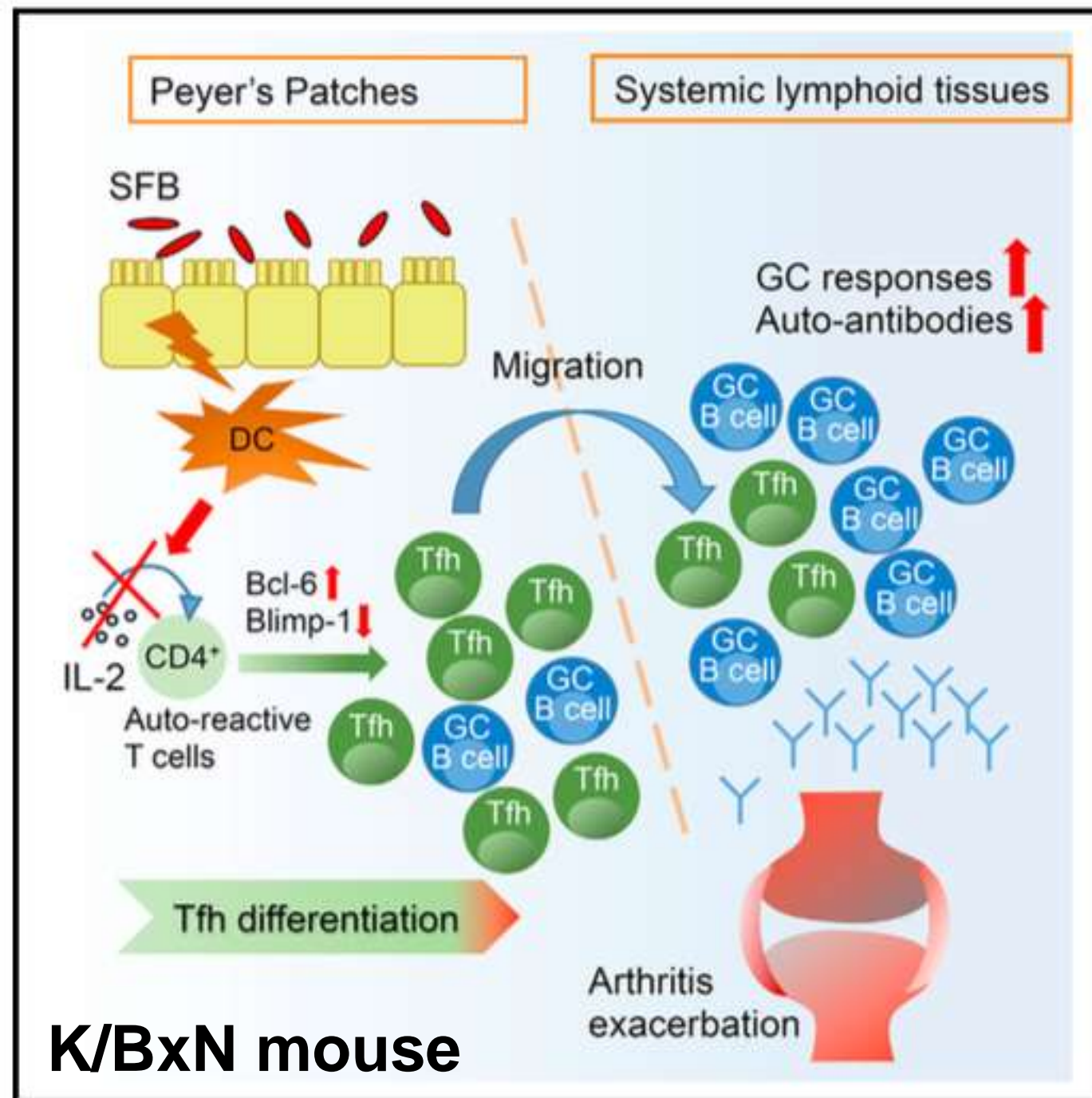
Human disease	Mouse Model	Reference	Primary Immunological Mechanism(s)	Effect of Introducing Microbiota
Multiple Sclerosis	Experimental autoimmune encephalomyelitis (EAE) (mouse)	(Lee et al, 2010)	T-cell-mediated, though multiple other cell-types play a role. Adjuvant-induced.	Full complement (GF vs. SPF): enhanced disease. SFB: enhanced disease
Autoimmune polyglandular syndrome	<i>Aire</i> ^{-/-} (knockout mouse line)	(Gray et al, 2007)	T-cell-mediated. Defective central tolerance of T cells.	Full complement (GF vs. SPF): no effect
Type-1 diabetes	Nonobese diabetic (NOD) (genetically selected inbred mouse strain)	(King and Sarvetnick, 2011; Kriegel et al, 2011)	T-cell-mediated, though multiple other immune cells impact. Multigenic.	Full complement (GF vs SPF): varies in different colonies. SFB: protects females

Conclusione: la perturbazione del microbiota intestinale può essere sufficiente per lo sviluppo dell'artrite

Modelli animali in AR



Cellule Tfh: link tra intestino ed artrite



Teng F et al, Immunity 2016

Block KE et al, J Immunol 2016

Onuora S, Nature 2016

Healthy State



Psoriasis



Psoriatic Arthritis



Rheumatoid Arthritis

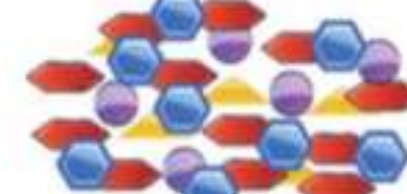
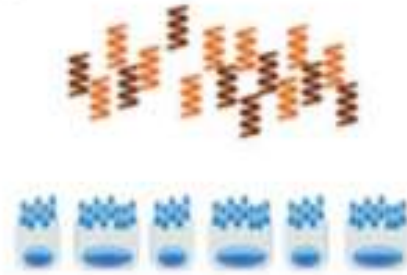
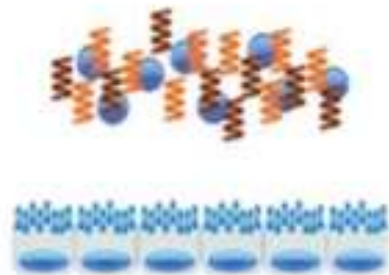


Ankylosing Spondylitis



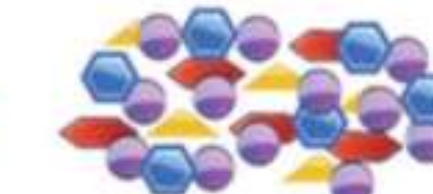
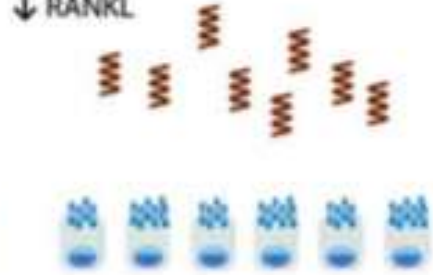
Homeostasis of gut microbiota

↓ Coprococcus/Coriobacteriaceae
↓ MCFAs (Hexanoate/Heptanoate)
~ sIgA
↑ RANKL



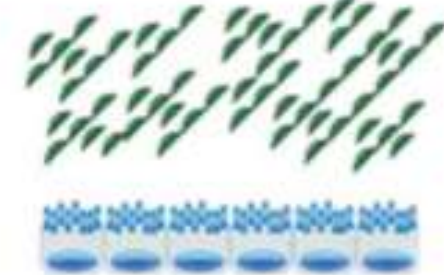
↑ IL-12/23 → TH17 cells → IL-17
↑ TNFα
↑ S100 (serum)

↓ Coprococcus/Coriobacteriaceae
↓ Ruminococcus/Akkermansia
↓ MCFAs (Hexanoate/Heptanoate)
↑ sIgA
↓ RANKL



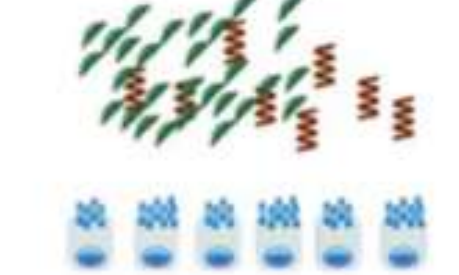
↑ IL-12/23 → TH17 cells → IL-17
↑ TNFα
↑ RANKL (serum)

↑ Prevotella copri
↓ Bacteroides



↑ TNFα, IL-1, IL-6
↑ T-cells (Th1)
↑ B-cells → Plasma cells & ACPAs
↑ RANKL

↑ Lachnospiraceae/Prevotellaceae
↓ Ruminococcaceae/Rikenellaceae



↑ IL-23 → Th17 and other IL-17 producing cells (NKs and IELs)
↑ IL-22
↑ TNFα

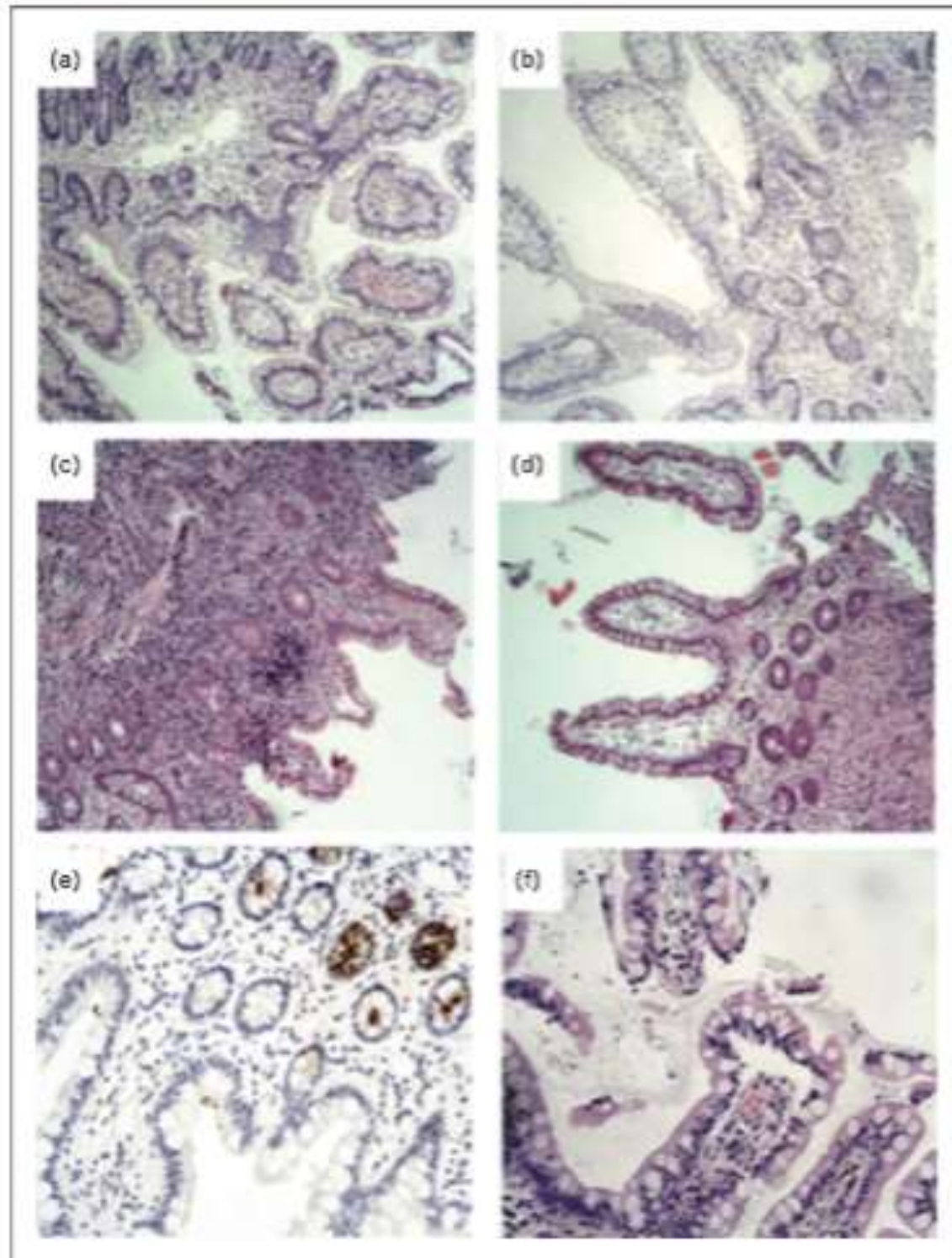


Diversità batterica e riduzione di batteri con azione anti-infiammatoria (Fi

Scher JU, A&R 2016

Breban M, Joint Bone Spine 2016

Spondilite Anchilosante



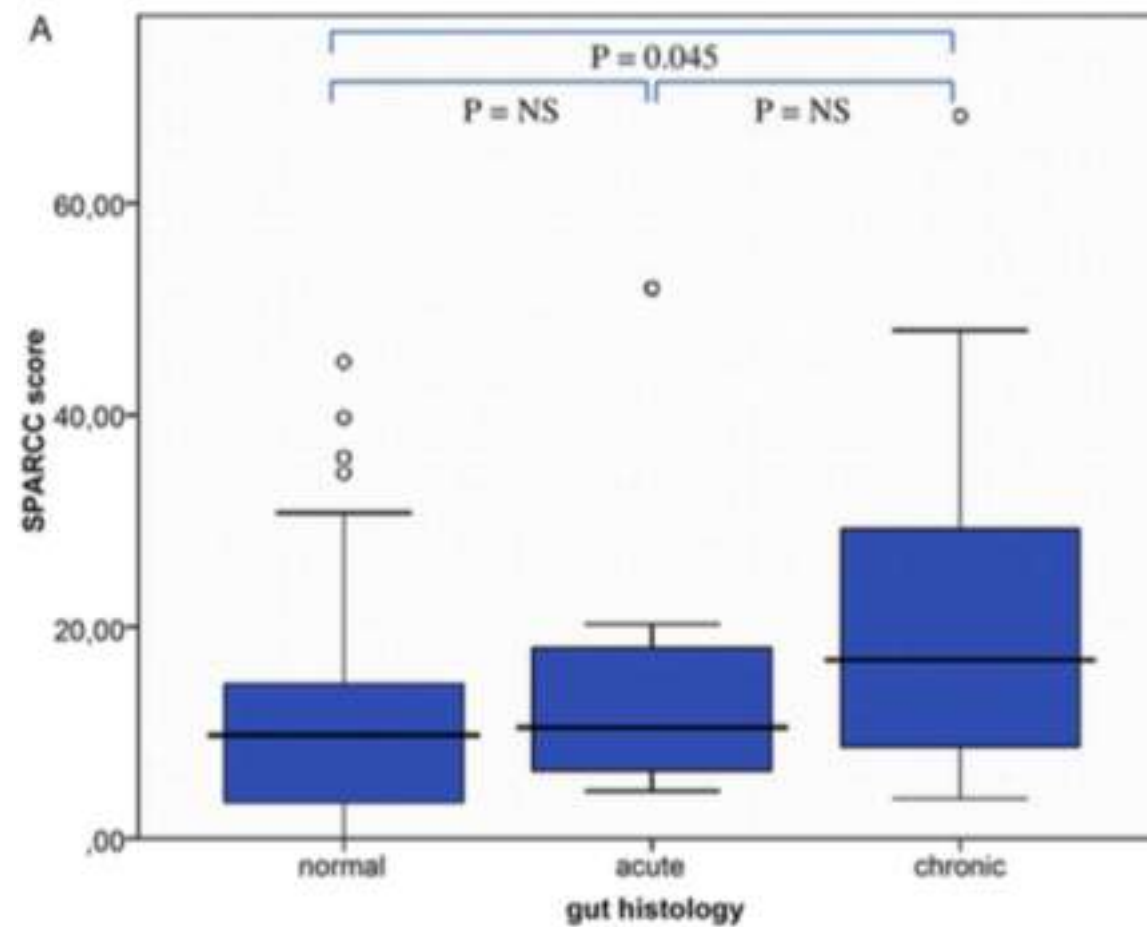
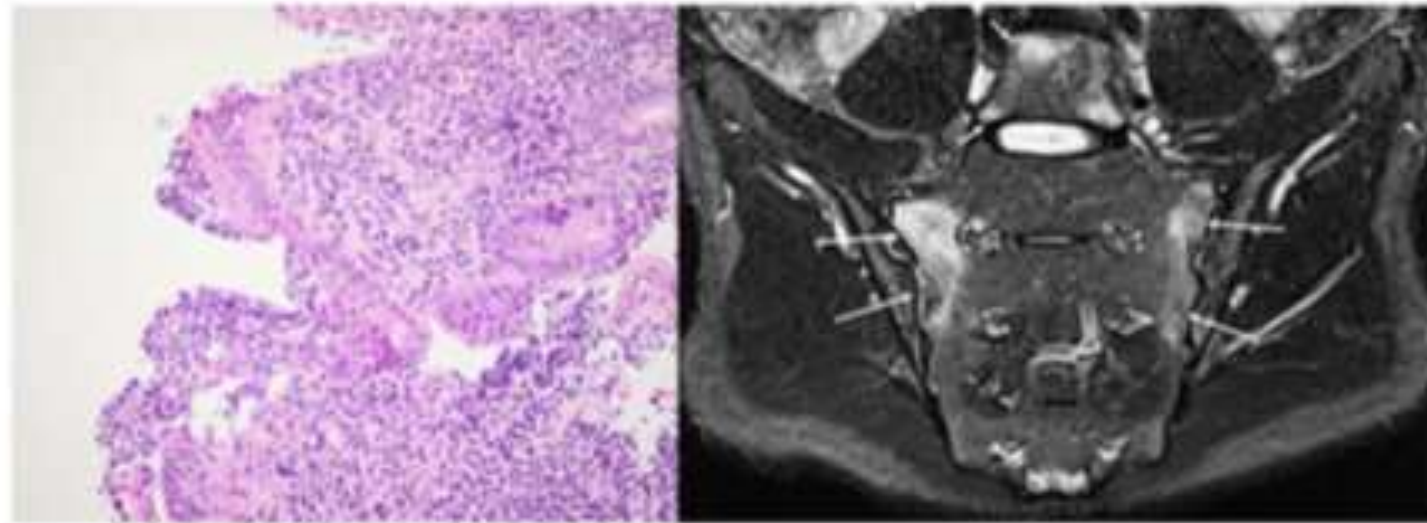
Infiammazione intestinale subclinica -> 60% dei pazienti con SpA

fino al 10% può evolvere in una malattia di Crohn

Caratteristiche istologiche:

- Infiammazione acuta (tipo enterocolite batterica acuta)
- Infiammazione cronica (forte infiltrazione di cellule mononucleate, eventualmente aggregate in follicoli linfoidi, tipo ileocolite di Crohn)
- Iperplasia delle goblet cells con aumentata produzione di mucina
- Attivazione delle cellule di paneth
- Scollamento delle cellule epiteliali dalla membrana basale
- Lesioni vasculitiche (intenso stravasamento emorragico nel contesto della lamina propria)

Infiammazione intestinale ed attività di malattia



Permeabilità intestinale e SA

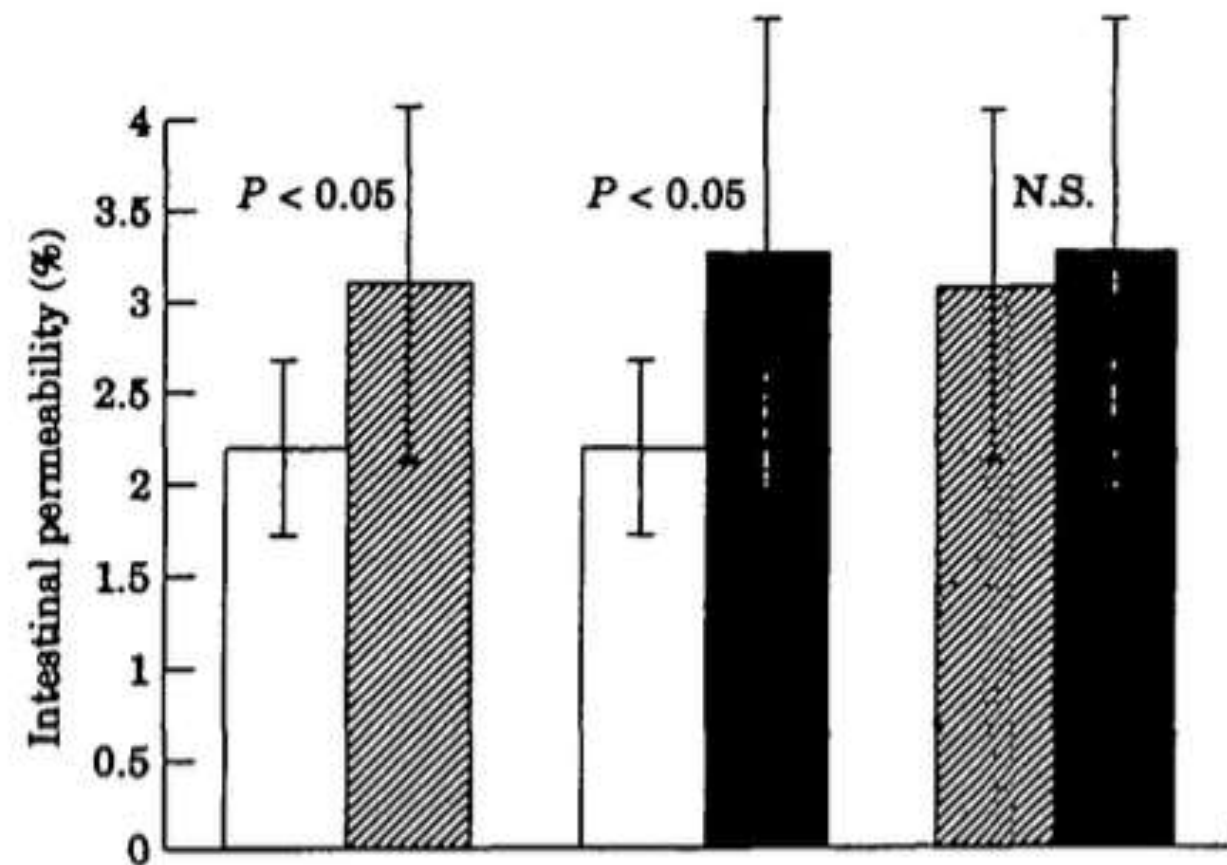


FIG. 1.—Comparison of intestinal permeability values (%) in patients with AS (▨), their relatives (■) and controls (□).

No difference in gut permeability was found between patients and relatives **regardless of** whether they had the **HLA B27 antigen or not**. The increased intestinal permeability in the patients had **no relation to the disease activity, to the presence of peripheral arthritis or to the intake of NS AIDs**. Gut permeability was shown to bear **no relation to IgA levels, ESR or CRP**

Martinez-Gonzalez O et al, *Rheum* 1994

Ab anti-CBir1 aumentati nei pazienti con SA

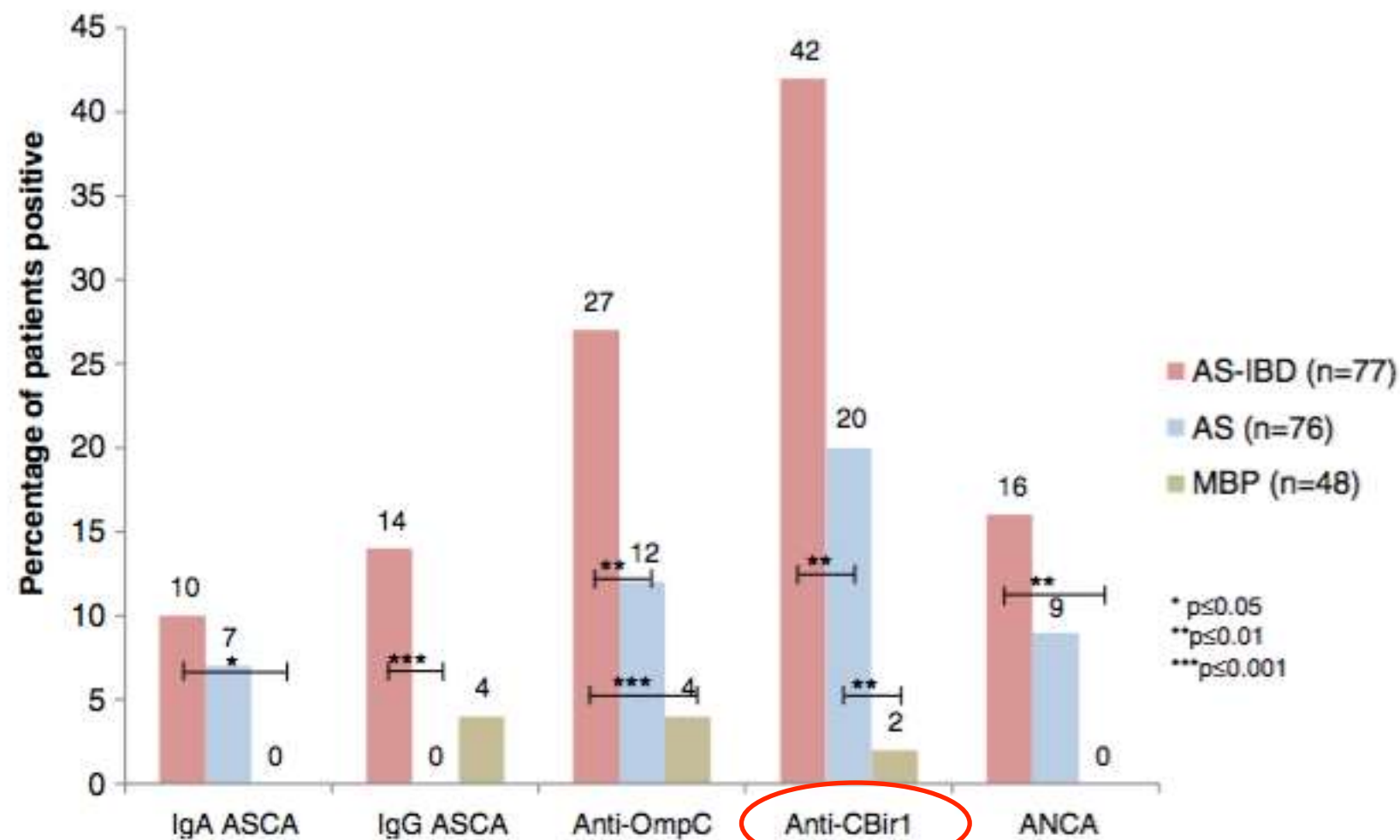
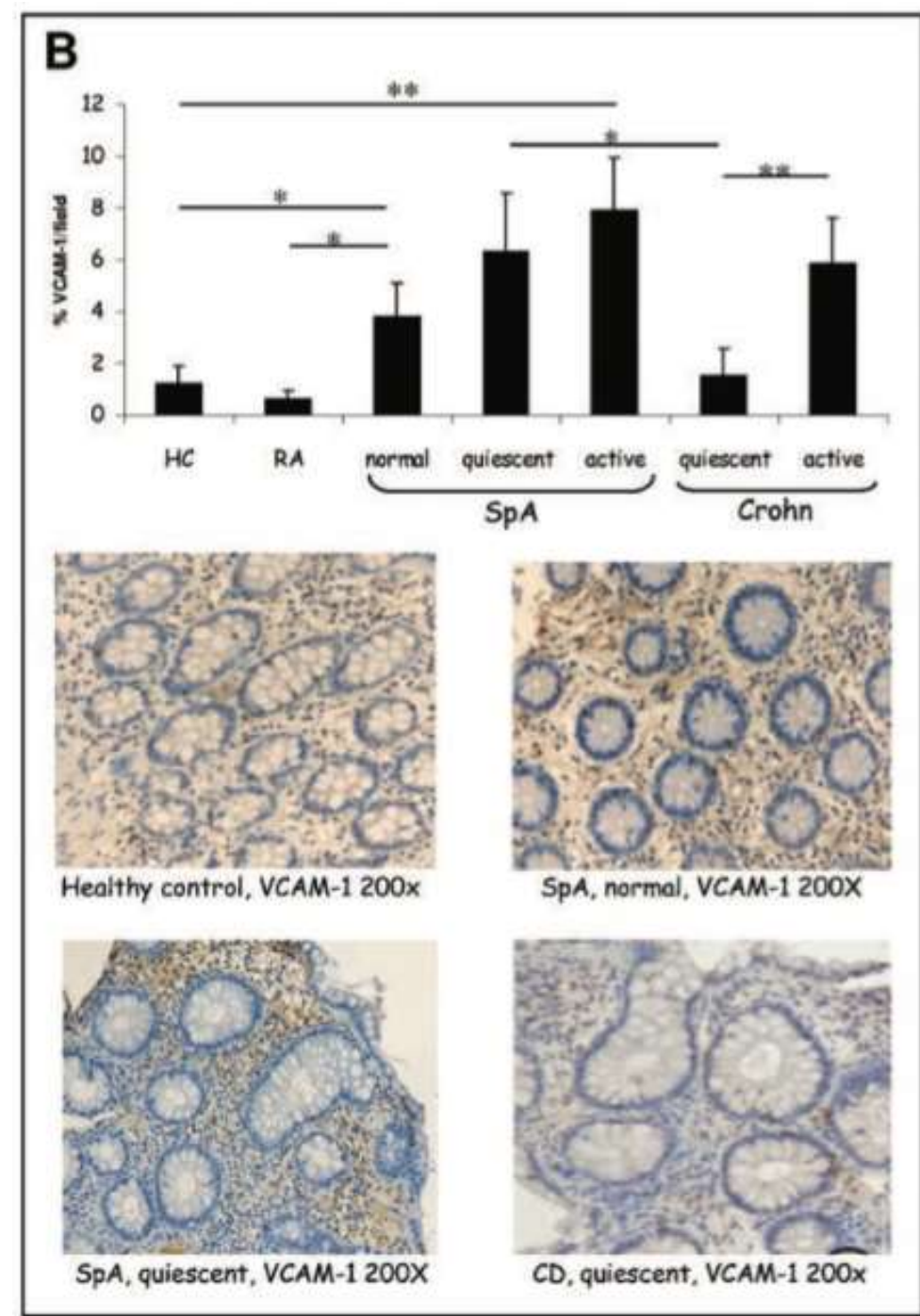
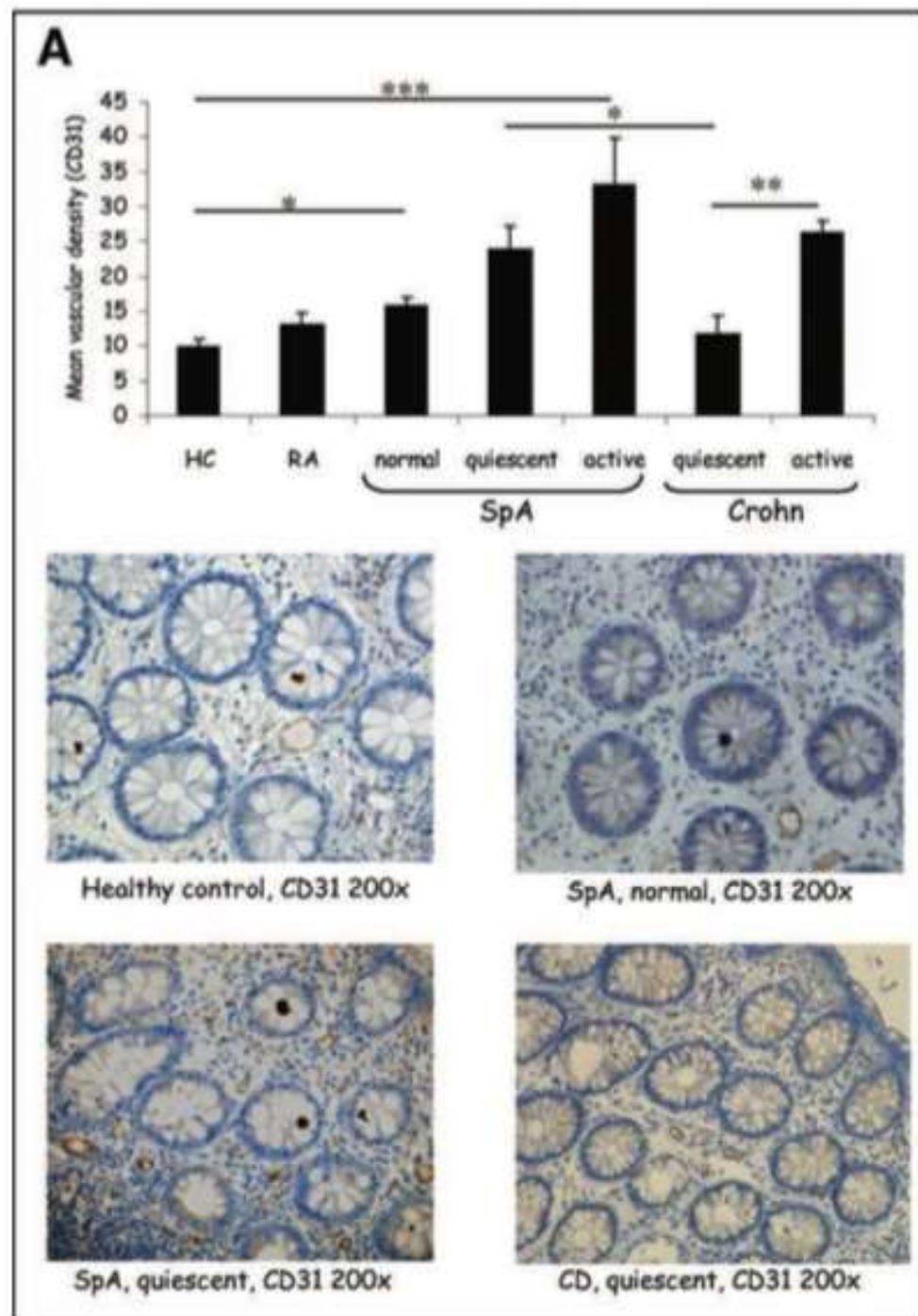


Figure 1 Positivity rates of all antibodies. AS-IBD, ankylosing spondylitis-inflammatory bowel disease; AS, ankylosing spondylitis; MBP, mechanical back pain; Ig, immunoglobulin; ASCA, anti-Saccharomyces cerevesiae antibody; Anti-OmpC, anti-outer membrane porin C; Anti-CBir-1, anti-flagellin; ANCA, anti-neutrophil cytoplasmic antibody.

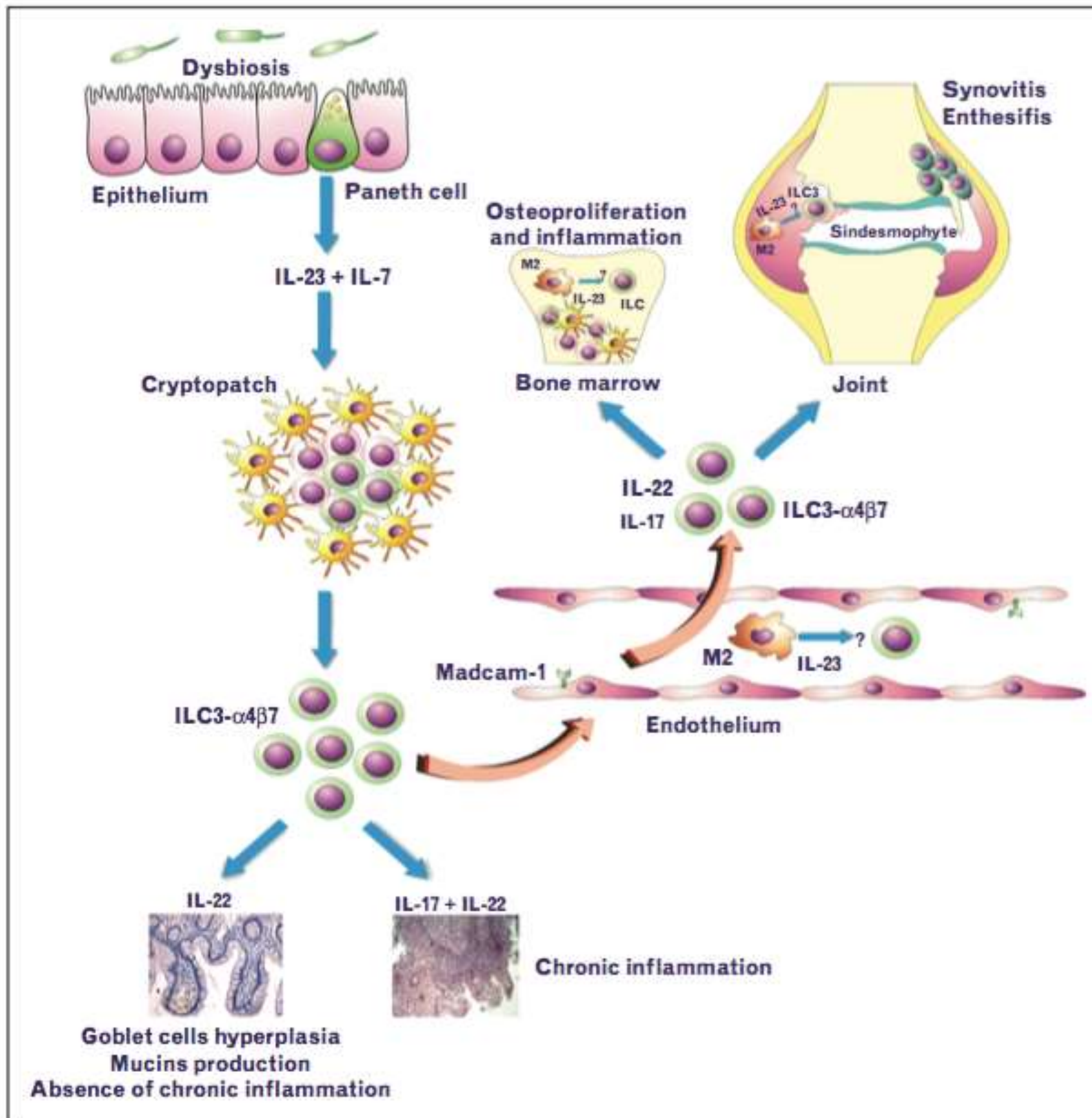
Aumentata espressione di VEGF-A, PlGF, VCAM-1, CD31 nei pazienti affetti da SpA con infiammazione intestinale subclinica



Type 3 innate lymphoid cells producing IL-17 and IL-22 are expanded in the gut, in the peripheral blood, synovial fluid and bone marrow of patients with ankylosing spondylitis

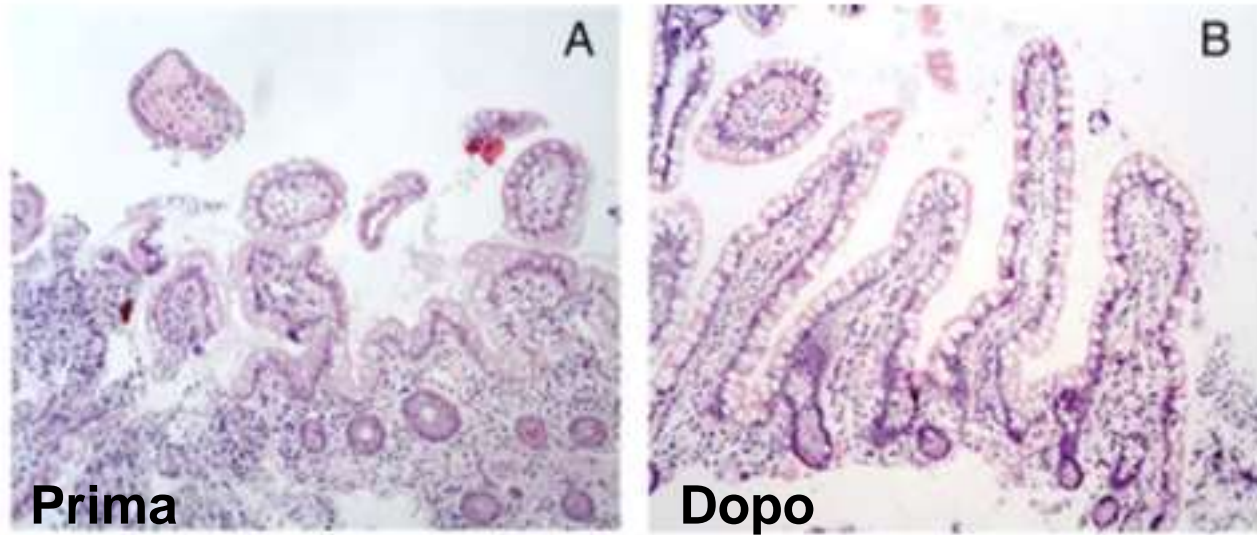
Francesco Ciccia,¹ Giuliana Guggino,¹ Aroldo Rizzo,² Laura Saieva,³ Sergio Peralta,⁴ AnnaRita Giardina,¹ Alessandra Cannizzaro,² Guido Sireci,³ Giacomo De Leo,³ Riccardo Alessandro,³ Giovanni Triolo¹

- **ILC3** cells were **significantly expanded in the gut, SF and BM of patients with AS** compared with controls, produced high levels of IL-17 and IL-22 and **expressed $\alpha 4\beta 7$**
- **MADcAM1** was overexpressed in BM and ileal high endothelial venules
- **IL-7** was significantly increased in AS gut, especially in the context of **Paneth cells**, and accompanied by the presence of aggregates of c-kit/IL- +7R cells (LTi). In in vitro experiments, epithelial cells from patients with AS actively induced differentiation of ILC3 from LTi.
- **TNFi efficacy** was accompanied by a significant decrease in the percentage of intestinal and circulating ILC3 and in the expression of MADCAM1



Clinical efficacy of $\alpha 4$ integrin block with natalizumab in ankylosing spondylitis

Pz 45 anni con AS e infiammazione intestinale cronica. Dopo trattamento con Adalimumab sviluppo di Sclerosi Multipla.



Microbiota e LES

Year	Experimental system	Findings	Ref
2014	MRL-lpr mice Readout: disease severity, analysis of stool by 16S rRNA sequencing	Faecal samples from female mice with severe lupus-like disease have an increase in <i>Lachnospiraceae</i> compared to samples from male mice with mild disease	[56]
* 2015	SNF₁ mice Readout: disease severity, analysis of stool by 16S rRNA sequencing	Feeding mice acidified water delays onset of lupus-like disease; delayed disease onset associated with decrease in β -diversity of faecal bacteria compared to control mice	[59]
2015	SLE patients Readout: analysis of stool by 16S rRNA sequencing	Lower Firmicutes/Bacteroidetes ratio in stool of SLE patients compared to healthy controls	[2]
2015	SLE patients; Readout: analysis of stool by liquid chromatography and mass spectrometry	Reduction in metabolites associated with purine, pyrimidine and amino acid metabolism in faecal samples from SLE patients compared to healthy controls.	[3]

- * female SNF₁ mice express much higher levels of pro-inflammatory mediators including IL-6, IL-9, IL-17, IL-22, IFN- α and IFN- β than male mice



Rosser EC et al, J Autoimm 2016



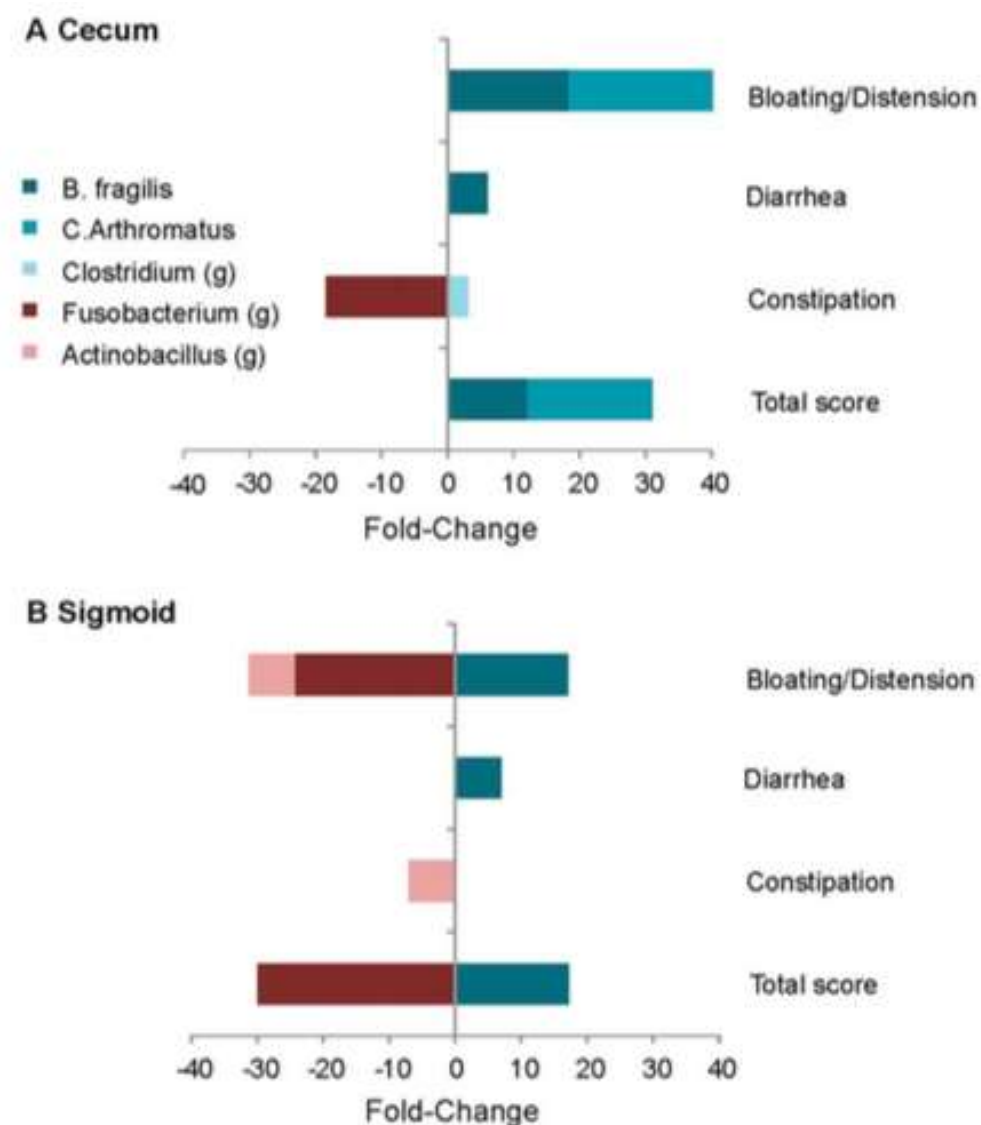
Sclerosi Sistemica

Unique colonic microbial consortium

Significant **increases** in *Fusobacterium*, *Prevotella*, and uncommon *gamma-Proteobacteria* (i.e., *Erwinia* and *Trabsulsiella*) genera

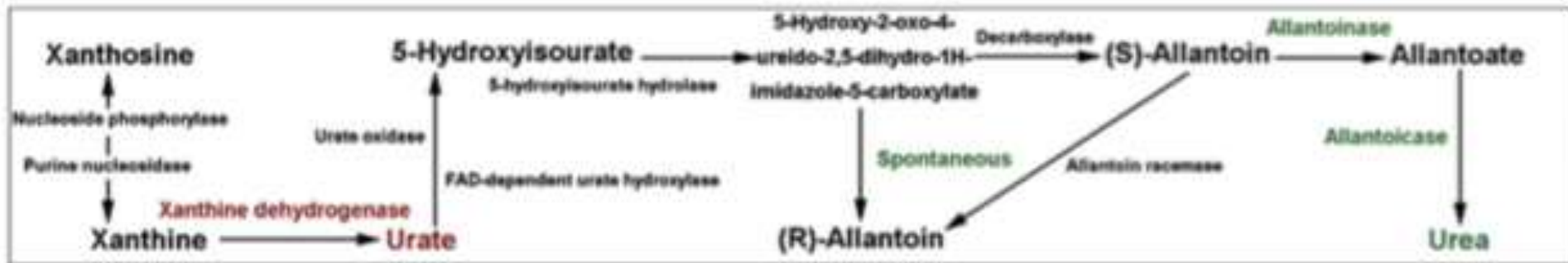
and significant **decreases** in *Faecalibacterium* and *Clostridium* genera, compared with age- and sex-matched healthy controls.

Interestingly, the SSc microbial consortium was also **enriched** with *Lactobacillus* and *Bifidobacterium*

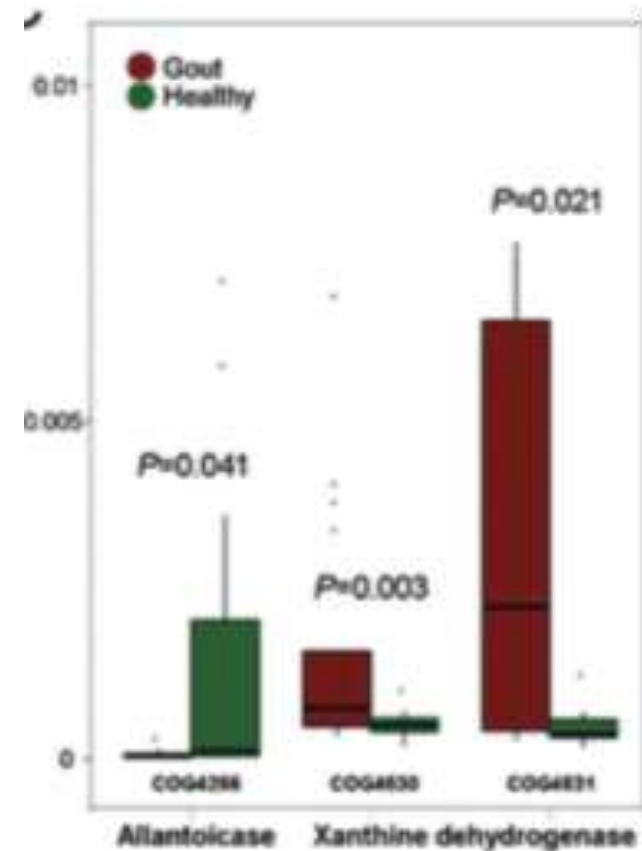


17 pazienti affetti da Ssc

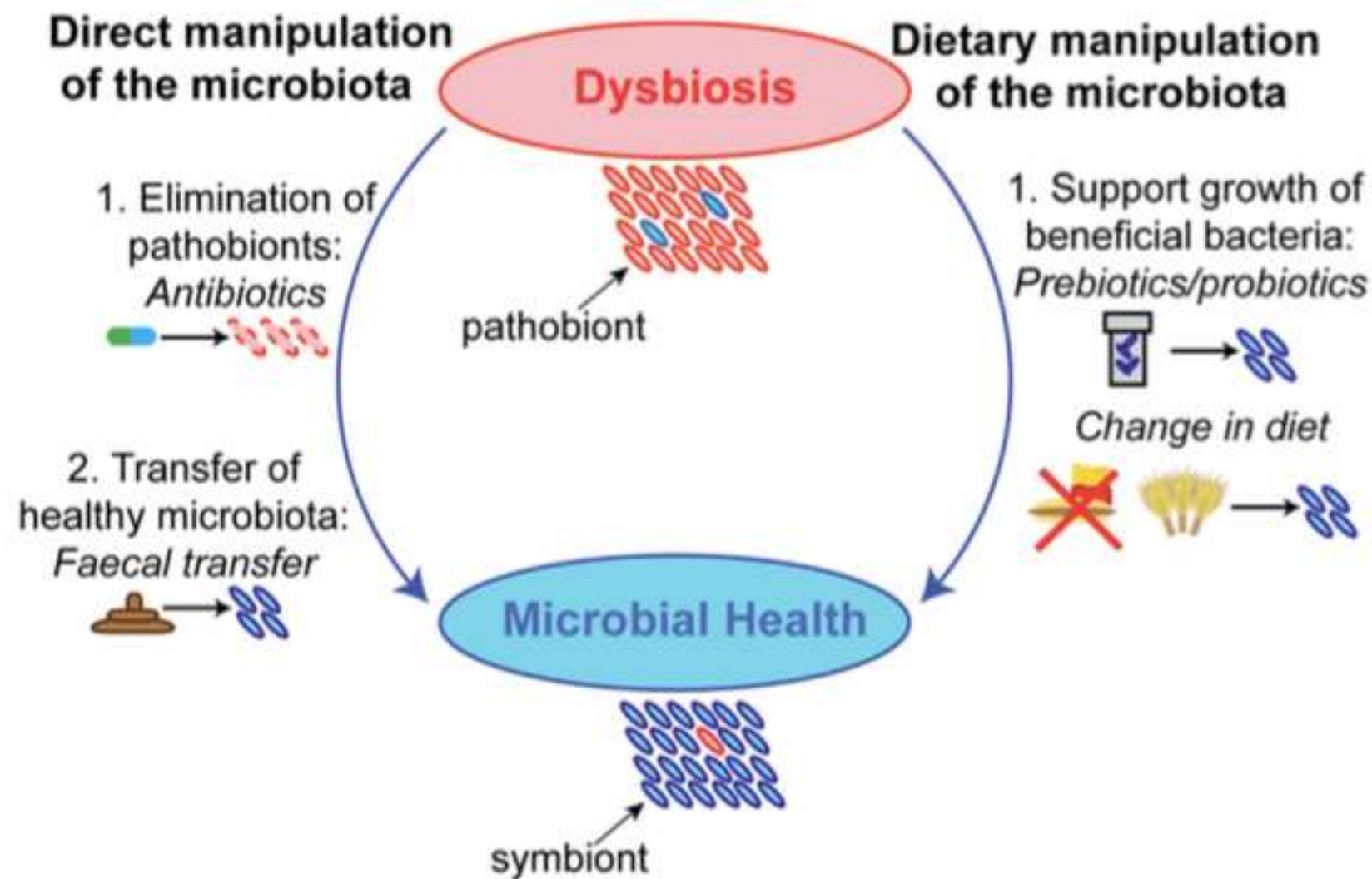
Gotta



- Ridotta diversità alfa
- ↓ *Faecalibacterium prausnitzii*, *Clostridium* butyrate-producing bacterium and *Bifidobacterium pseudocatenulatum*
- ↑ *Bacteroides caccae* and *Bacteroides xylanisolvens*



Il microbiota e l'intestino possono essere un bersaglio terapeutico nelle malattie reumatiche?

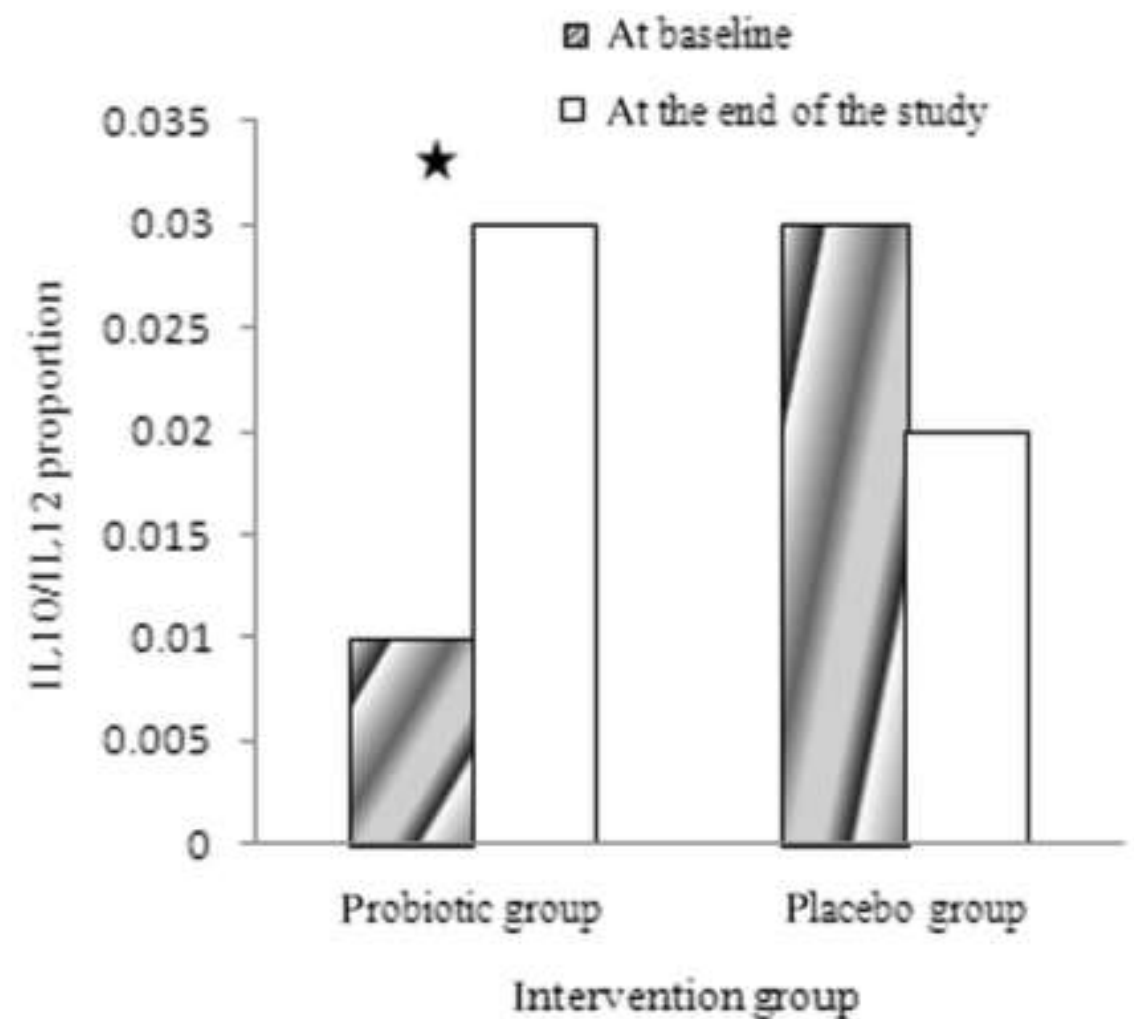
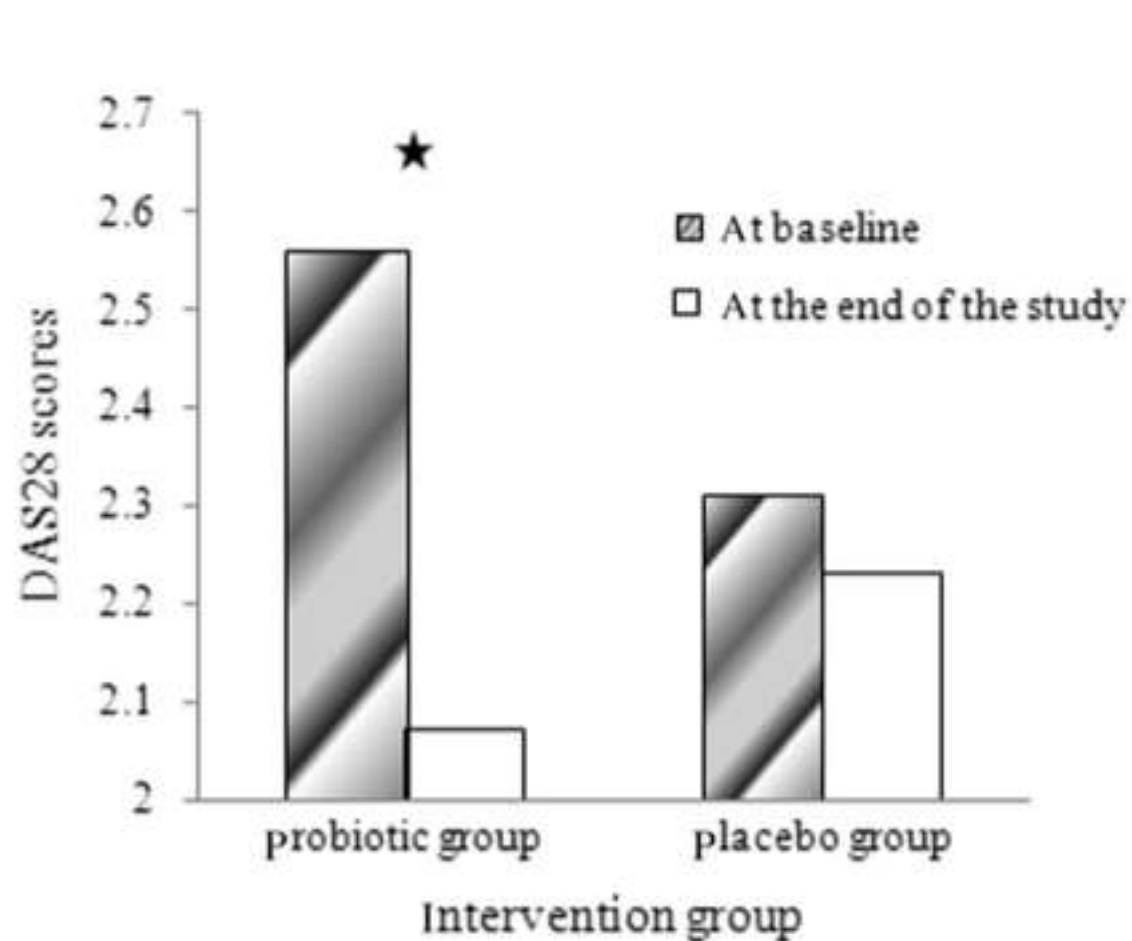


Probiotici e Artrite Reumatoide

Probiotic preparation	Study design	Sample size/duration of therapy	Results	References
<i>Lactobacillus rhamnosus</i> GR-1 and <i>Lactobacillus reuteri</i> RC-14	Randomized, double-blind, placebo-controlled	29/12 weeks	Probiotics did not clinically improve RA as measured by the ACR20, but there was functional improvement seen within the probiotic group compared to placebo	de Pineda et al. (2011)
<i>Lactobacillus rhamnosus</i> GG	Randomized, double-blind, placebo-controlled	21/52 weeks	No statistically significant differences in the clinical parameters, biochemical variables and HAQ index between the study groups	Hatakka et al. (2003)
<i>Lactobacillus casei</i> 01	Randomized, double-blind, placebo-controlled	46/12 weeks	More patients in the <i>Lactobacillus casei</i> 01 group had moderate response to the treatment at the end of the study, a significant difference was observed between the two groups for IL-10, IL12 and TNF- α changes through the study course, in favor of the probiotic group	Alipour et al. (2014)
<i>Lactobacillus casei</i> 01	Randomized, double-blind, placebo-controlled	46/8 weeks	Disease activity score was significantly decreased by the intervention, and there was a significant difference between the two groups at the end of the study, three of the assessed serum pro-inflammatory cytokines (TNF- α , IL-6, and IL-12) significantly decreased in the probiotic group	Vaghef-Mehrabany et al. (2014)
<i>Bacillus coagulans</i>	Randomized, double-blind, placebo-controlled	45/60 days	<i>Bacillus coagulans</i> treatment resulted in greater improvement in patient global assessment and self-assessed disability; reduction in CRP; as well as the ability to walk two miles, reach, and participate in daily activities	Mandel et al. (2010)

ACR20 American College of Rheumatology core set of disease activity measures for RA, HAQ Health Assessment Questionnaire, TNF- α tumor necrosis factor- α , IL interleukin

RCT 46 pz Artrite Reumatoide in terapia stabile *L.Casei 01* vs placebo per 8 wk



Non eventi avversi

Probiotici e Artrite Reumatoide

- La letteratura sull'uso dei probiotici è ancora limitata
- Non esistono studi head to head tra differenti specie di probiotici
- Simple size dei campioni spesso poco numeroso, diversi standard di riferimento (**ceppi**, **dose**, durata della terapia) e distinte condizioni patologiche
- Non evidenza di eventi avversi seri
- L'uso dei probiotici nel trattamento dell'artrite reumatoide può essere considerata un'opzione terapeutica sicura, efficace e conveniente

Dieta e Malattie Reumatiche

Disease	Study design	Dietary intervention	Outcome	Refs
RA	Population-based	Oily fish consumption	Modest decrease in RA incidence	68
RA	Dose-response meta-analysis	Fish consumption	Non-statistically significant inverse association between fish consumption and RA	57
RA	Population-based	Alcohol consumption	Moderate consumption was inversely correlated with RA incidence	58
RA	Dose-response meta-analysis	Alcohol consumption	Low to moderate consumption was inversely correlated with RA incidence	59
RA	Prospective	Alcohol consumption	Moderate consumption was inversely correlated with RA incidence in women	64
RA	Prospective	Mediterranean diet	No effect on RA incidence	63
RA	RCT	Mediterranean diet	Functional improvement and decreased disease activity	62
RA	RCT	Mediterranean diet	Decreased disease activity	61
PsA	Prospective	Weight loss (Mediterranean diet)	≥5% of weight loss predicted achievement of minimal disease activity in patients treated with TNF inhibitor	60

PsA, psoriatic arthritis; RA, rheumatoid arthritis; RCT, randomized controlled trial.

Olio pesce (PUFAs)
benefico nel
paziente con AR
stabile ed early in
terapia

Frutta e vegetali
associati ad una
ridotta incidenza
di AR. Rischio tra
consumo di
carne rossa e
AR non definito

Glutine ed Artrite Reumatoide

38 pazienti con AR in dieta vegana aglutinata vs
28 pazienti in dieta bilanciata per 1 anno

	Vegan diet			Non-vegan diet		
	3 months	6 months	12 months	3 months	6 months	12 months
Patients, ITT (<i>n</i>)	38	38	35	28	28	26
Patients, VCC (<i>n</i>)	22	22	22	25	25	25
Patients showing 20% improvement, VCC/ITT (<i>n</i>)	6/8	10/13	9/12	1/1	2/2	1/1
%, VCC/ITT	27.3/23.7	45.5/31.6	40.9/34.3	4/3.6	8/7.1	4/3.8

ACR20 40% vs 4%

IgG anti gliadina e anti beta-lattoglobulina ridotte
significativamente nel sottogruppo di pazienti trattati con
dieta vegana

Non differenze nella progressione radiografica

Farmacomicrobiomica

La manipolazione del microbiota intestinale può *migliorare l'efficacia* di un farmaco e *ridurre gli effetti avversi*



Meccanismo diretto: produzione di composti bioattivi, detossificazione, legame diretto allo xenobiotico

Meccanismo indiretto: ciclo entero-epatico, alterazione di espressione di specifici geni dell'ospite (es. CYP450), competizione con specifici siti di legame su enzimi dell'ospite

Prospettive future

- » Migliore comprensione dell'interazione ospite (genetica)-microbiota -> *direzionalità*
- » Ruolo del microbioma nel metabolismo dei farmaci (farmacomicrobiomica)
- » Ruolo del *micobioma* e dei virus
- » Ruolo del microbioma di altre nicchie dell'organismo (cute, bocca, vie aeree)
- » Timing della perturbazione del microbioma nelle varie fasi di malattia (preclinica, precoce, tardiva)
- » “Umanizzazione” dei modelli animali
- » Trials di conferma del ruolo dei probiotici e della dieta



Grazie!