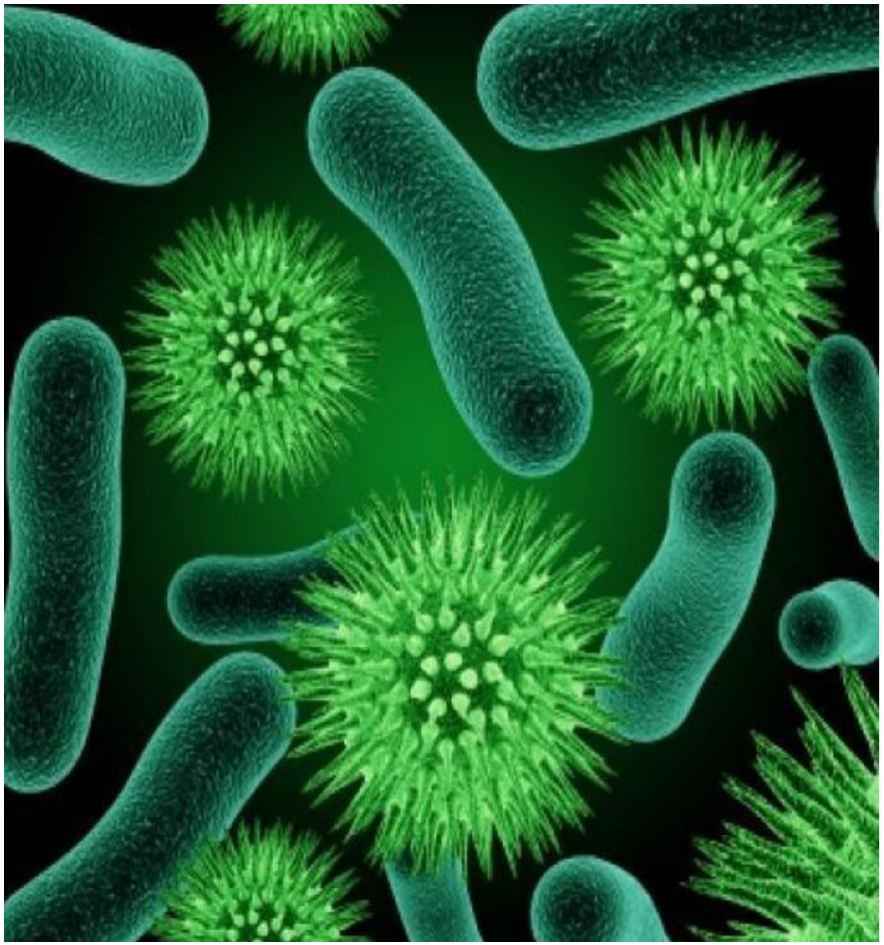
X Workshop Nazionale
Innovazione e Ricerca per la Pratica Clinica
TERAPIE INNOVATIVE DELLE EPATITI CRONICHE VIRALI
Firenze, 13-14 gennaio 2020
Centro Congressi Hotel Londra



Epatiti e Microbioma intestinale

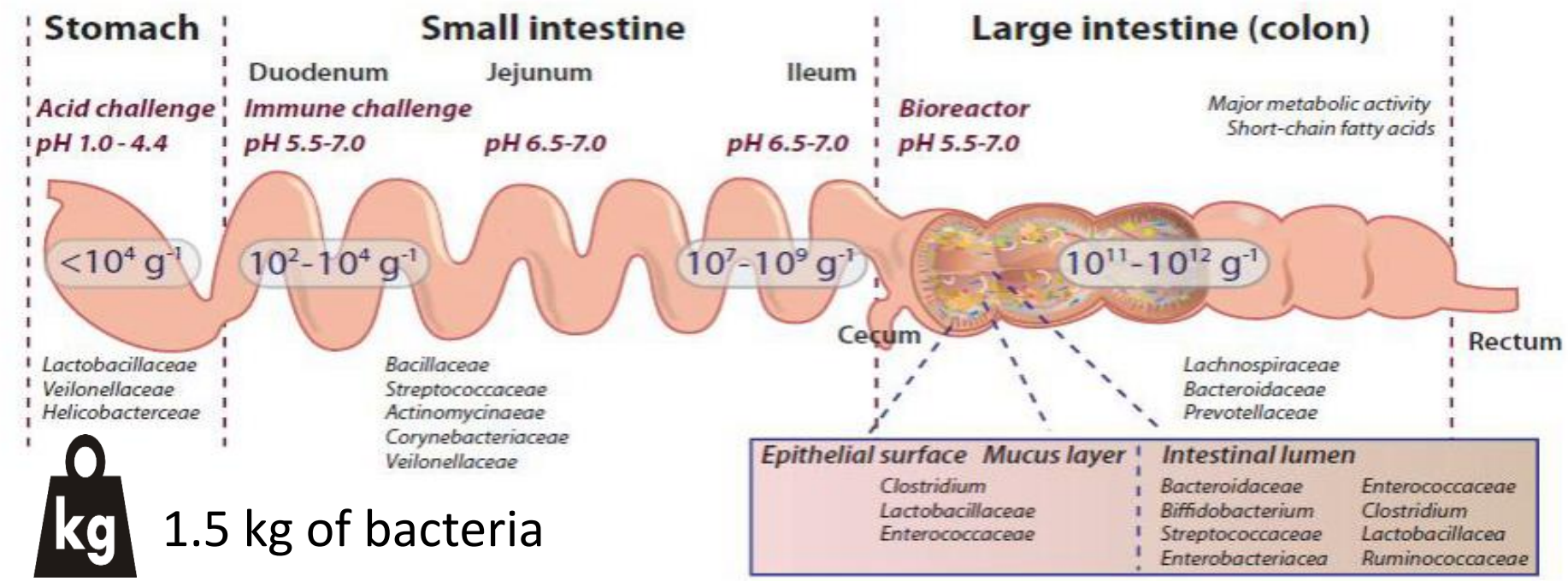
Gaetano Scifo

Siracusa

Conceptual origin of commensal organisms is old
" Animal Parasites and Messmates " , Pierre Van Beneden , 1875

" Ecological community of commensal , symbiotic and pathogenic microorganisms within a body space or other environment " Lederberg & Mc Gray , 2001

Microbial diversity (Microbioma , Virioma , Micobioma)

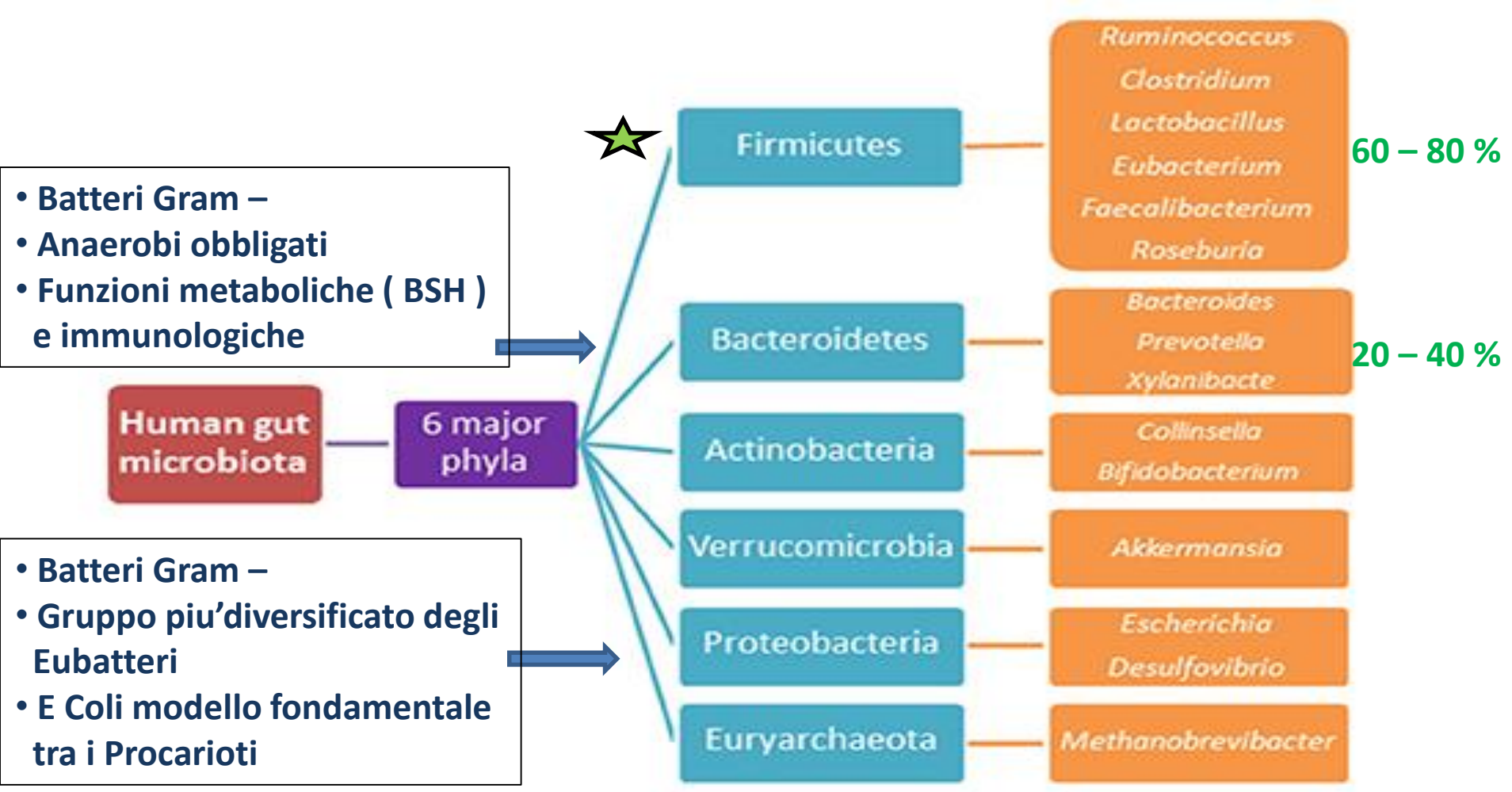


kg 1.5 kg of bacteria

- 10^13 bacterial cells in the gut (>10x total ensemble of human cells)
- 3-4 million genes (150x human genome)
- >1,000 bacterial species ,395 phylotypes , 8 bacteria phyla
- 1-3 % total body mass
- Generally non pathogenic & symbiotic with host

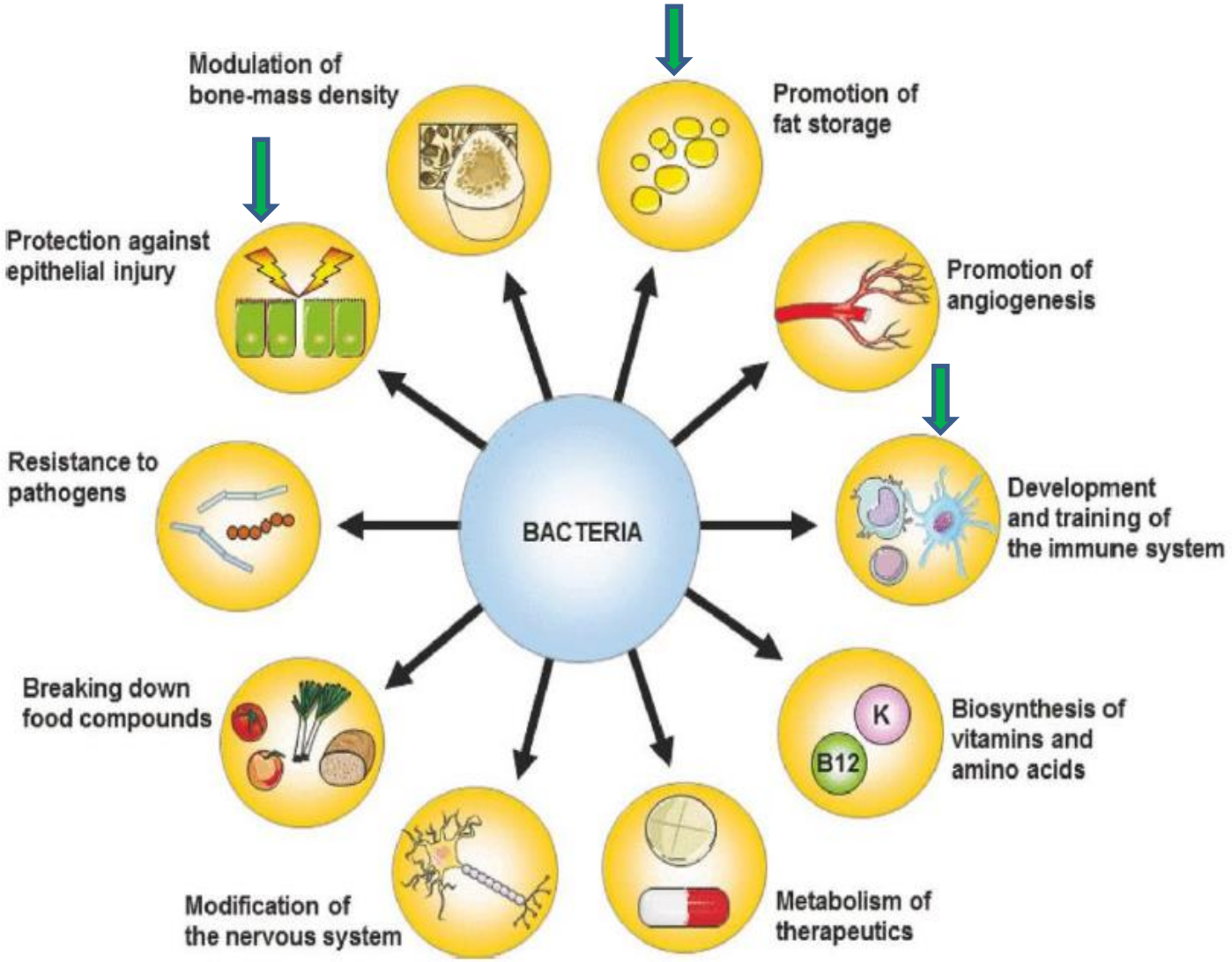
Backhed, Science 2005, Kovatcheva et al., The Prokaryotes

The 6 major phyla of human microbiota and their predominant species



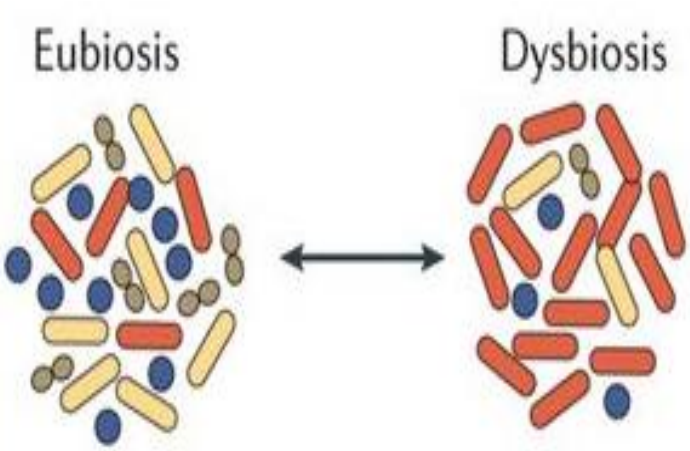
Bliss E. , Whiteside E. , Front Physiol , 2018

Multiple functions



Eubiosis & Dysbiosis

Healthy microbiota
- Diverse
- Rich in anaerobic commensals
- ↑ 3-Indoxyl sulfate
- Pathobionts are suppressed



Injured microbiota
- ↓ Diversity
- ↓ Anaerobic commensals
- Pathobionts expand:
  - ↑ Proteobacteria
  - ↑ *Enterococcus* spp.
  - ↑ *Streptococcus* spp.

Eubiosis

„Good" coexistance of host and microflora - Symbiosis

- **Protection** of the intestinal mucous membrane against invading microorganisms
- **Antagonistic effect** on undesired microorganisms
- Contribution to **maturation** and stimulation of host´s **immune system**
- **Nutrient digestion**
- **Vitamin** syntheses
- **Protein** syntheses

Dysbiosis

„Bad" coexistance of host and microflora

- **Damage** to the intestinal epithelium. Gut wall thickening > reduced resorption of nutrients
- **Toxic metabolic substances** (NH_3, biogene amines, toxins)
- Decomposition, **increased gas production** (CH_4, H_2S, CO_2)
- **Weakening** of immune system.
- **Immune reaction** >increased need of energy
- Acceleration of cell turnover > increased need of **energy**

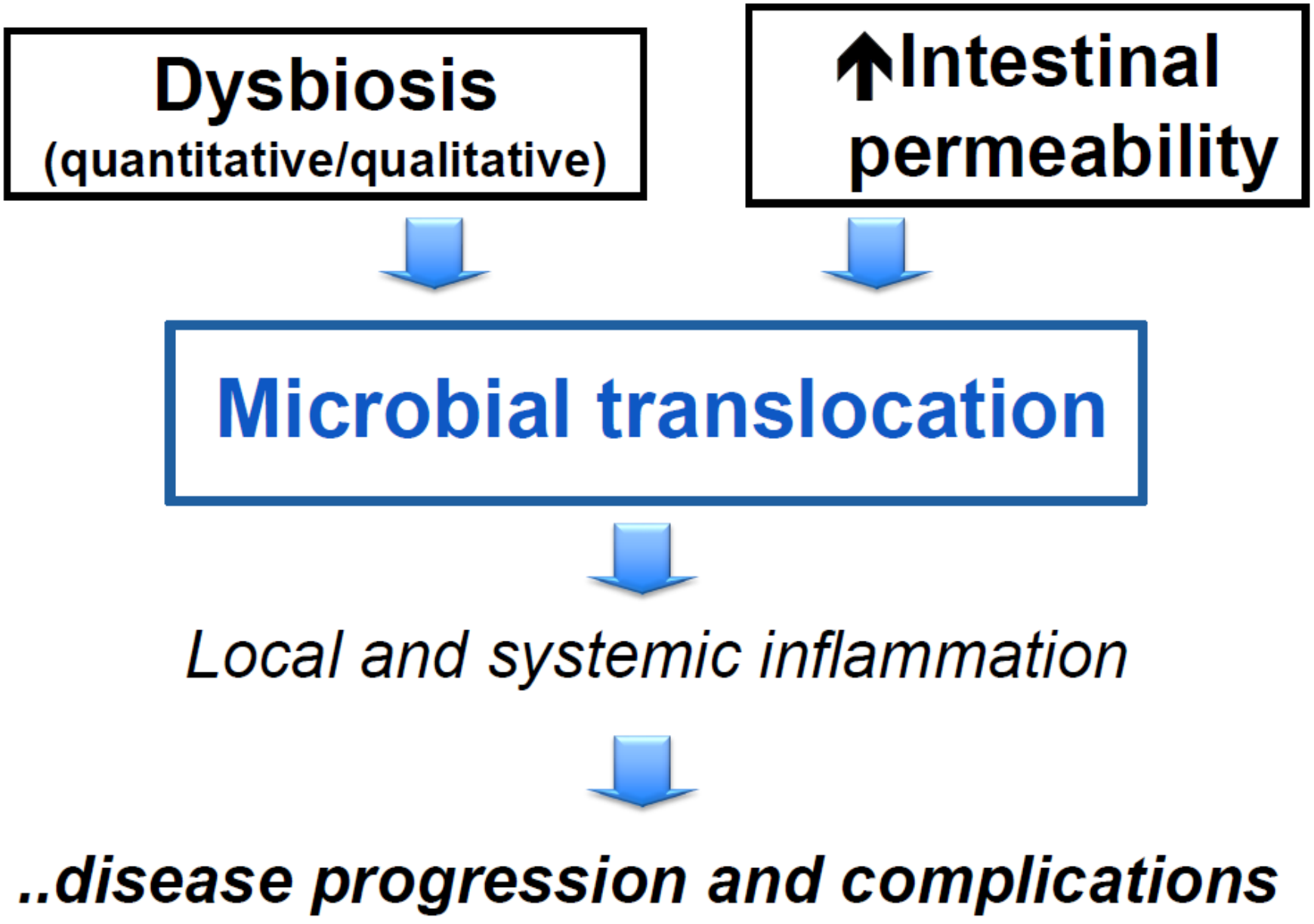
Background :
liver diseases inherently linked to microbiome

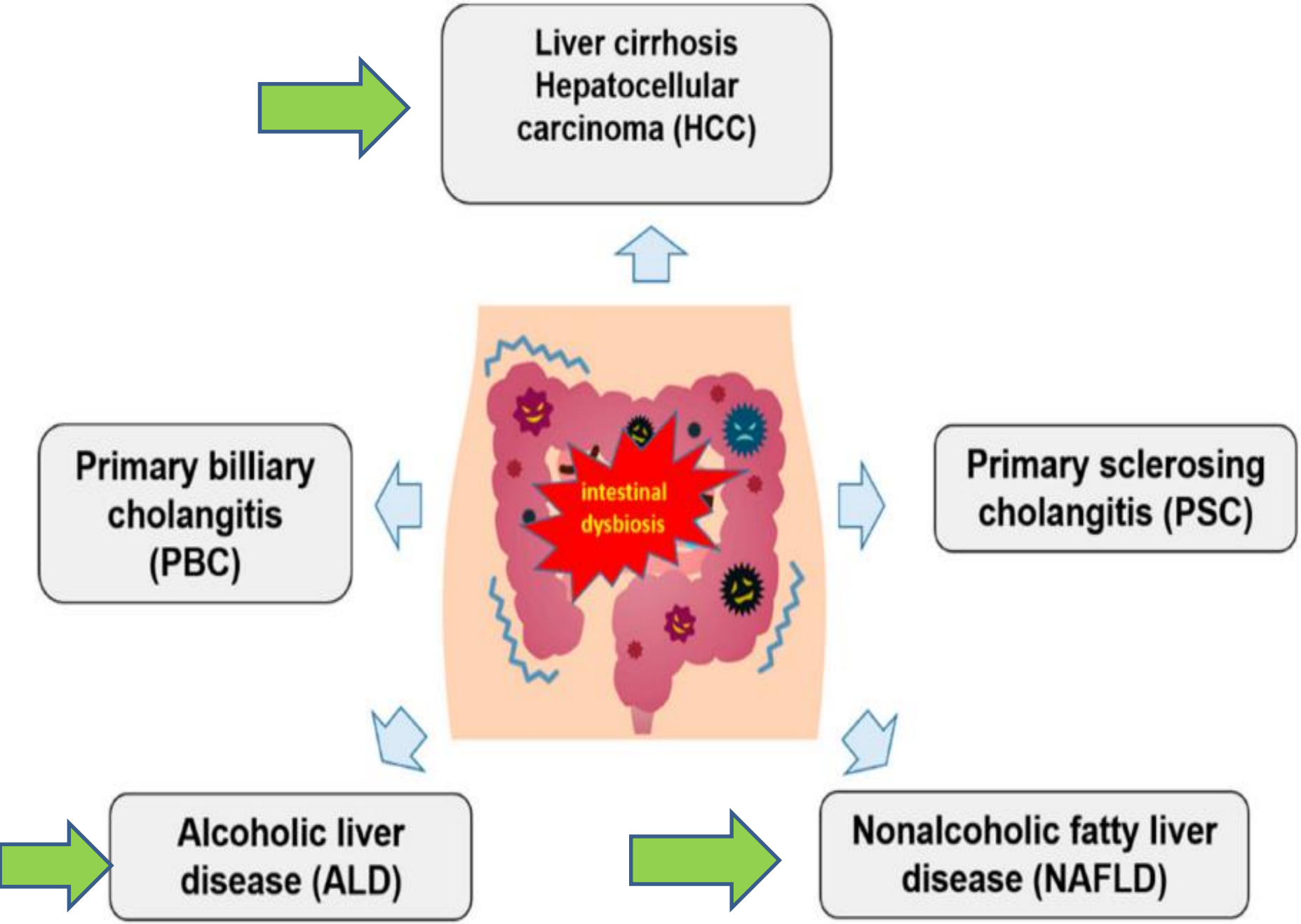
- Patients with cirrhosis have increased bacteremia , blood LPS levels and intestinal permeability
- Patients with cirrhosis have bacterial overgrowth in the small intestine
- Selective intestinale decontamination with antibiotics is beneficial for patients with decompensated cirrhosis :

  * Empiric ceftriaxone in patients presenting with suspected variceal bleeding
  * PBS clinical evidence of bacterial translocation leading to increased mortality
  * Floroquinolones for secondary and then primary prevention of PBS
  * Rifaximin for the treatment of hepatic encephalopathy
  * Infections and sepsis are the leading cause of mortality in liver failure

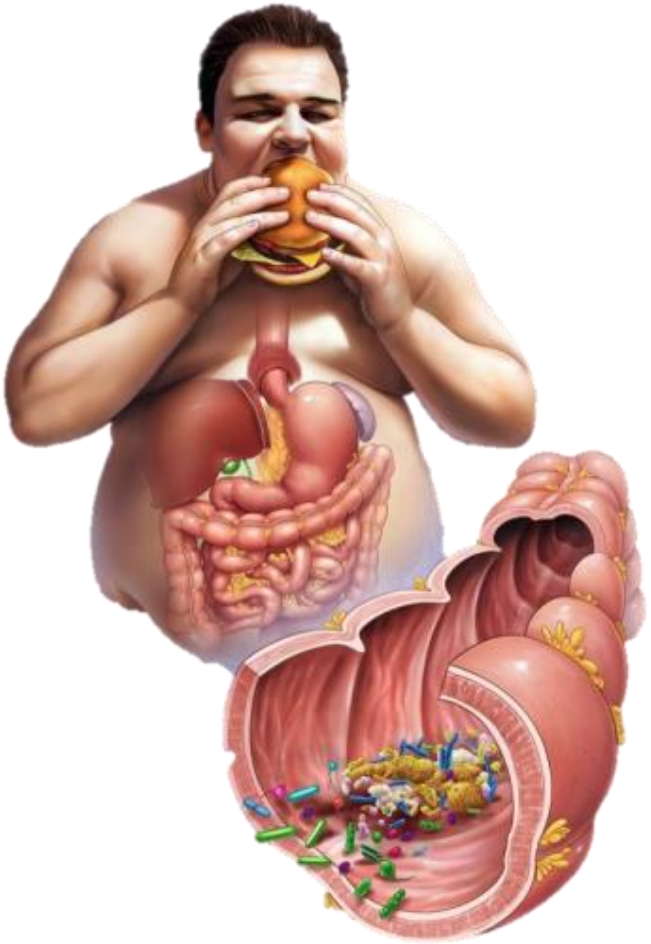
- TLRs for bacterial ligands are present on liver cell
- In experimental models,several TLRs make mice sensitive to liver fibrosis

Liver disease is a Gut Microbiota disease




Liver cirrhosis
Hepatocellular carcinoma (HCC)
Primary billiary cholangitis (PBC)
intestinal dysbiosis
Primary sclerosing cholangitis (PSC)
Alcoholic liver disease (ALD)
Nonalcoholic fatty liver disease (NAFLD)

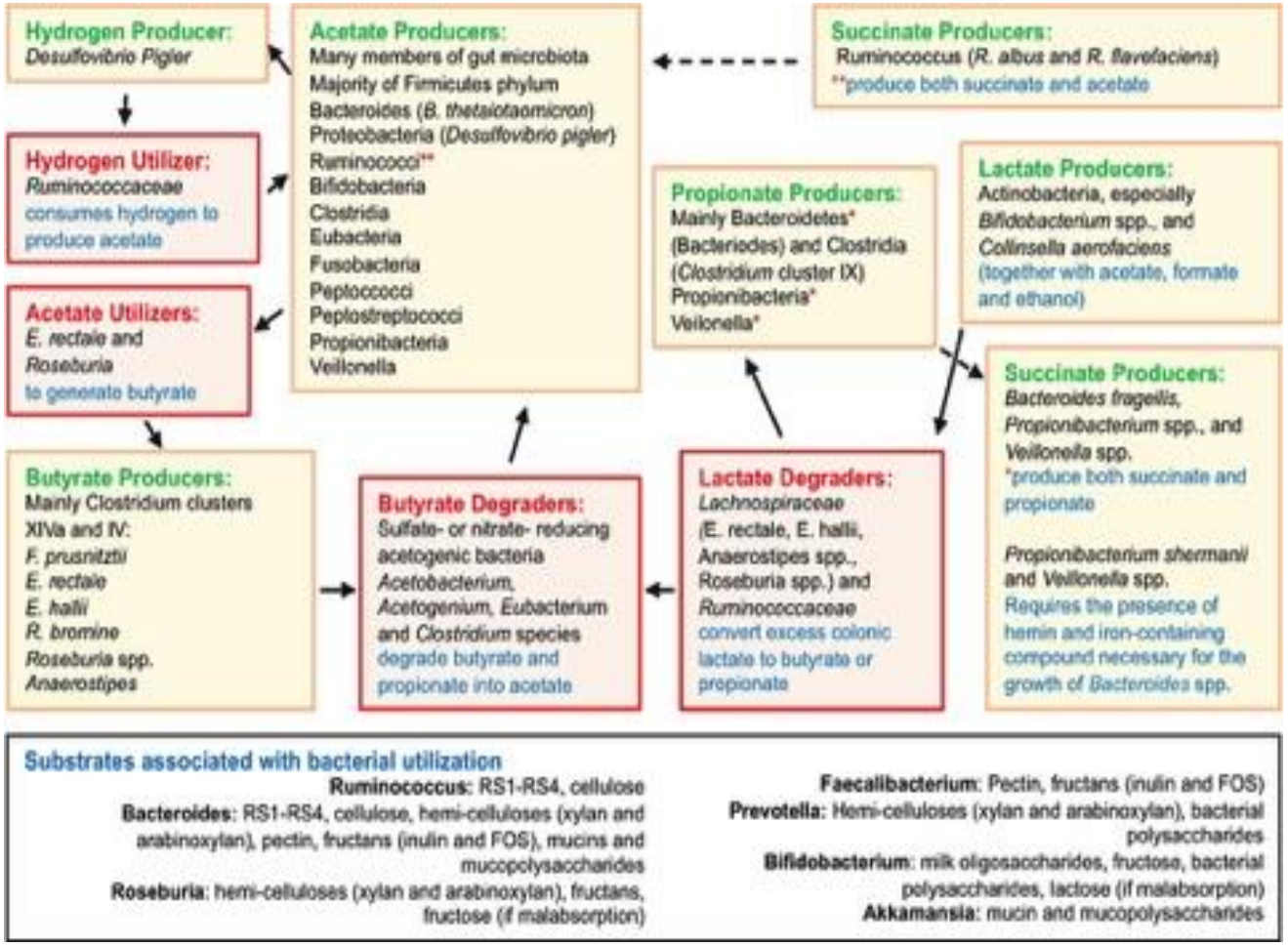
The Microbial Road to NAFLD



- Diet
- FXR
- Translocation
- Ethanol

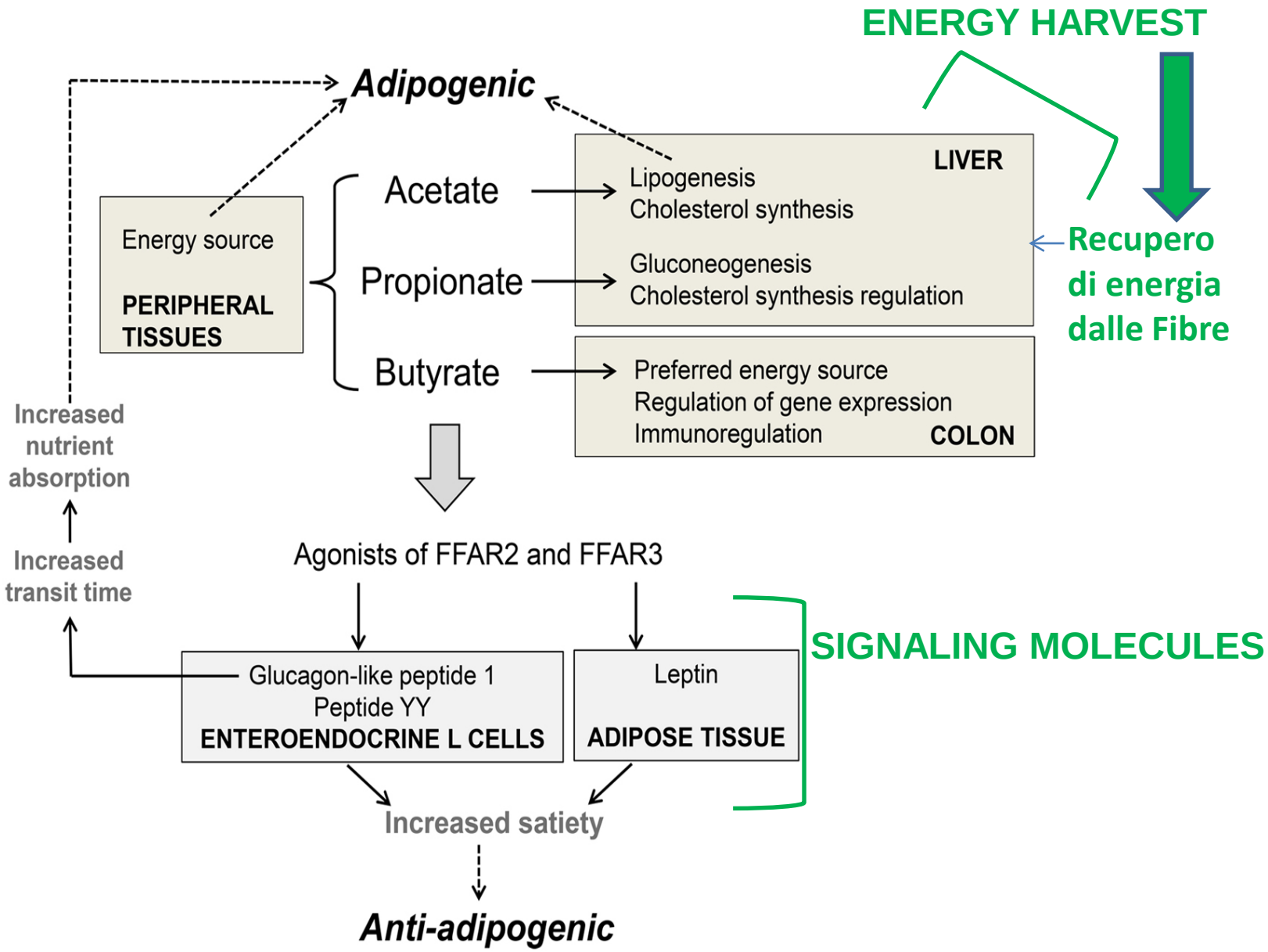
SCFAs & NAFLD

SCFAs, such as acetic, propionic and butyric acid, are the major products of carbohydrate fermentation by gut microorganisms.
SCFAs synthesized in the gut are changed by the amount of carbohydrate consumed and by dysbiosis.



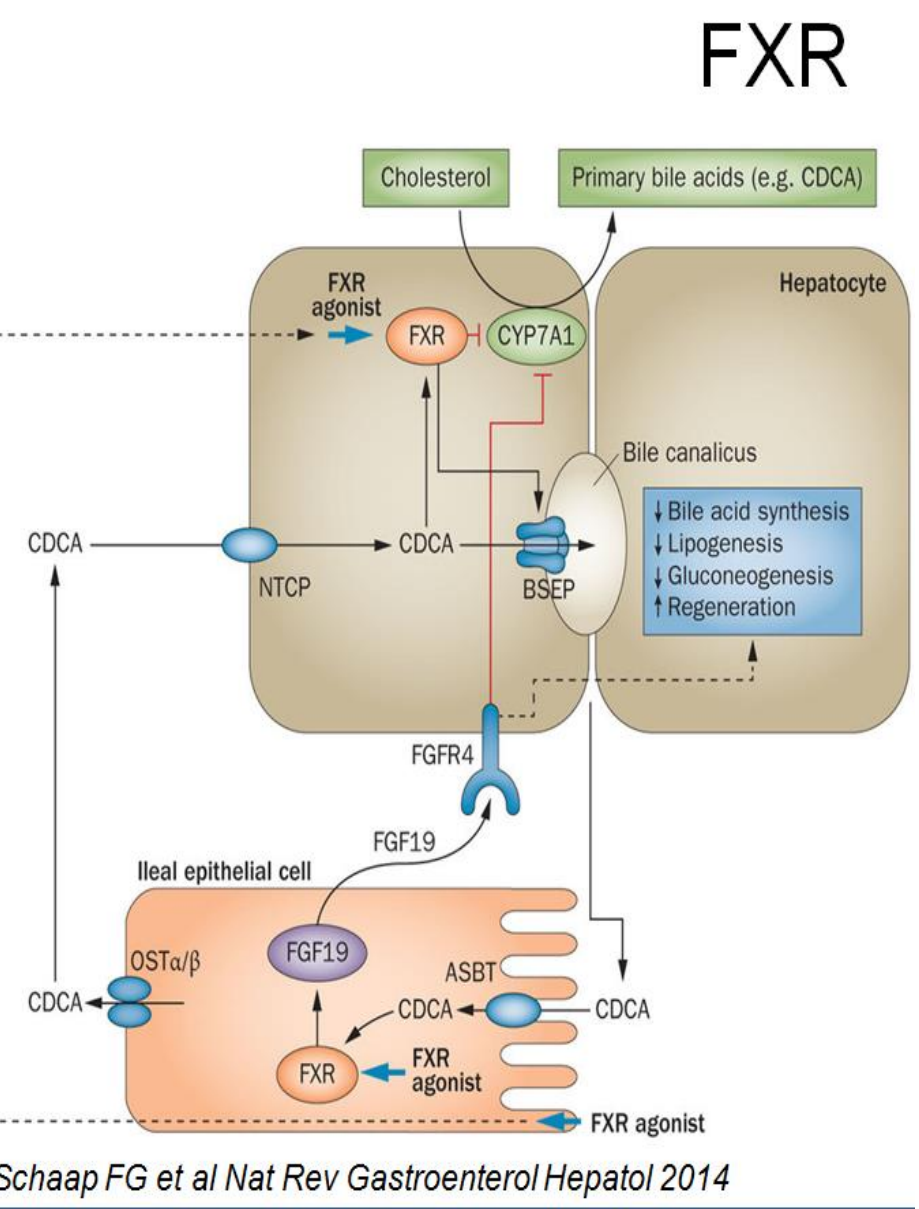
Arslan N et al. W J Gastroenterol 2014

SCFAs production



Blaut, Proceedings of the Nutrition Society 2015

FXR and BA
(common language of communication along the gut liver axis)



Schaap FG et al Nat Rev Gastroenterol Hepatol 2014

- B.A. end products of cholesterol catabolism in the liver
- B.A. control bacterial overgrowth and protect intestinal barrier
- Dysbiosis alter coniugated/secondary B.A. ratio (via reduction secondary B.A.
- Secondary B.A. reduction lowers activation of FXR and FG5 in the ileum
- B.A. linked to metabolix entoxinemia , I.R. , cytokines release
- B.A. regulate their synthesis through the activation of FXR
- In the intestine, FXR binds B.A. , resulting in the activation of its target gene fibroblast grown factor 19 (FGF19).
- FGF19 inhibits theexpression of hepatic cholesterol 7-a-hydroxylase (Cyp7a1), a rate-limiting enzyme in bile acid biosynthesis.
- In the liver, B.A bind to FXR leading to: suppression of bile acids synthesis, composition and pool size; liver regeneration; reduced accumulation of fat, and improved glucose and cholesterol metabolism
- FXR induces hepatic genes involved in lipoprotein clearance: the HDL receptor Scrab1, VLDL receptor, and ApoCII, a cofactor of lipoprotein lipase
- FXR also decreases hepatic SREBP-1c.

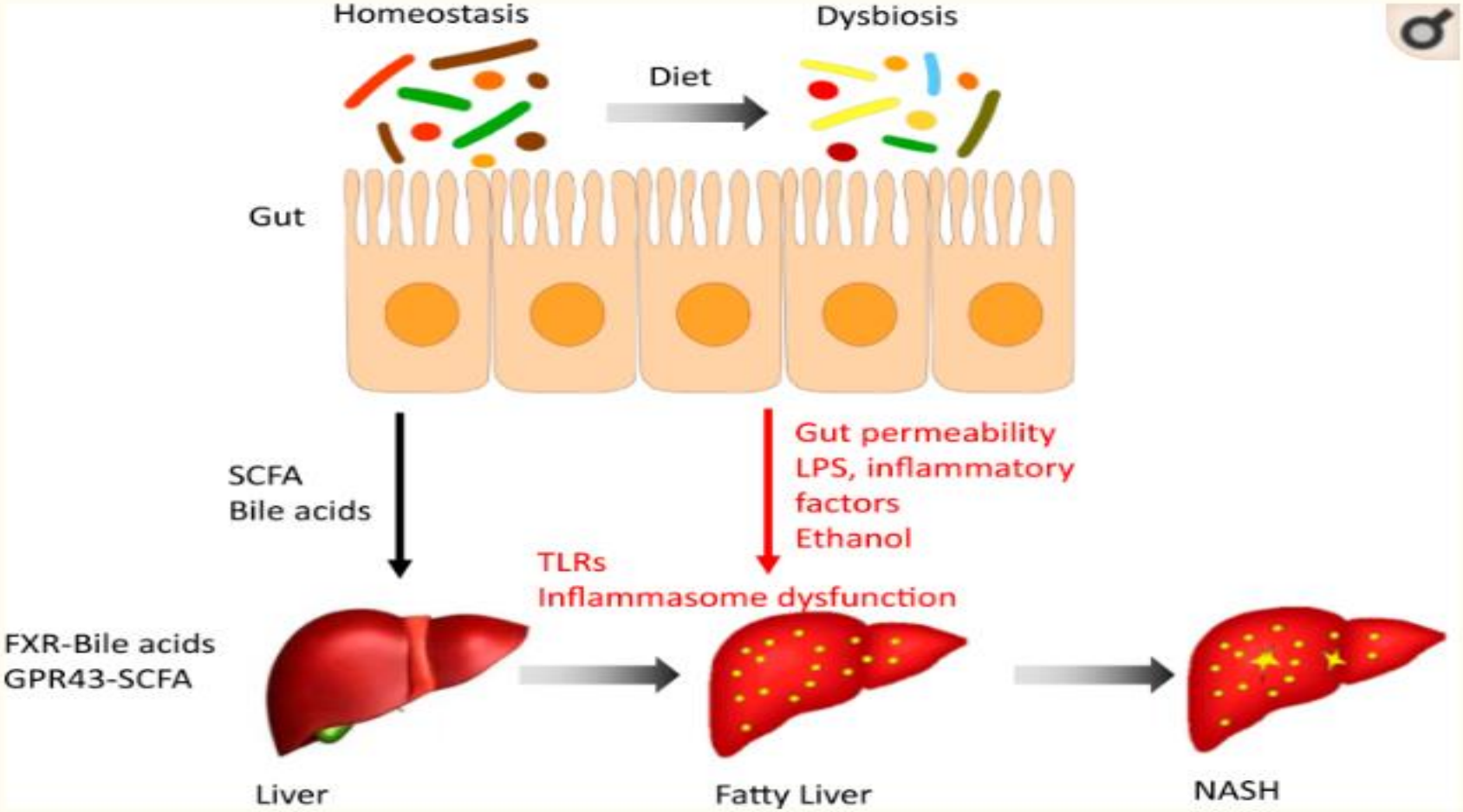


Figure 1

Suggested mechanisms for the effect of gut microbiome in NAFLD development and progression to NASH. Bacterial metabolites such as SCFA and bile acids may be potently involved in normal liver function and reduced liver lipogenesis and inflammation. Aberrations in commensal microbiome composition, diversity, and function may lead to increased gut permeability, production of LPS and other inflammatory factor, recued diversity of bile acids, and production of ethanol. All these metabolites and factors in combination with lipids derived from the diet can cause liver steatosis, inflammation and damage, which may lead to hepatic fibrosis, scarring, and NASH development.

EASL 2019

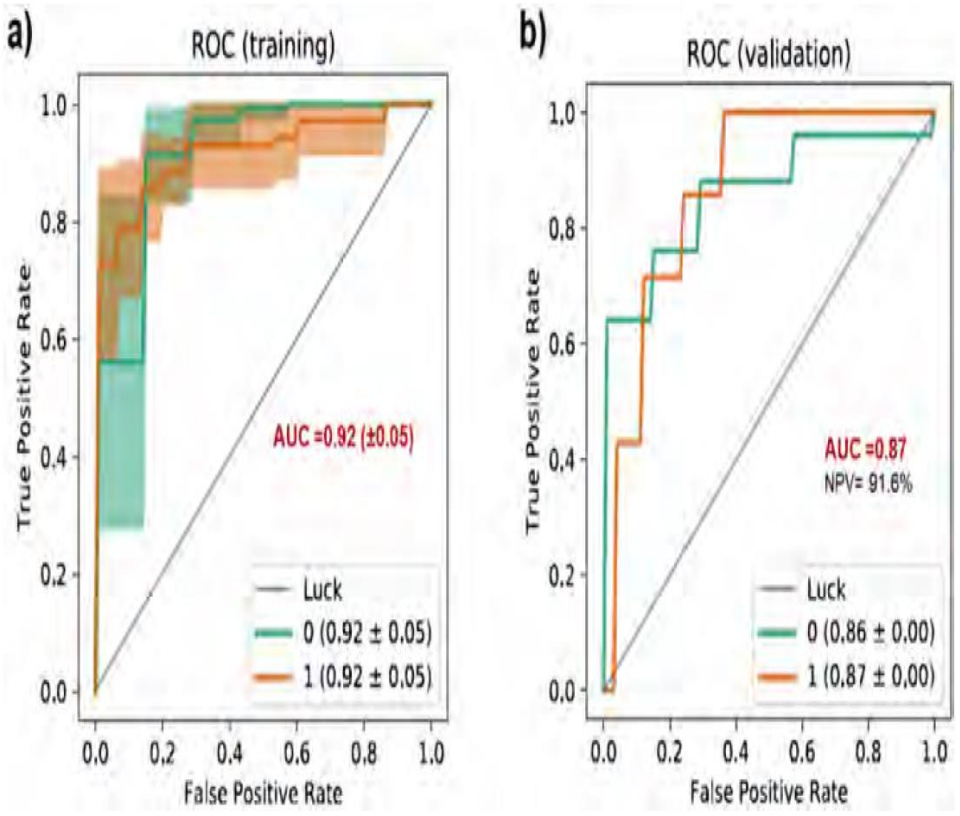
Diagnostic accuracy of gut-microbiome signatures for detecting NAFLD cirrhosis

Gut-Liver Axis

PS-157

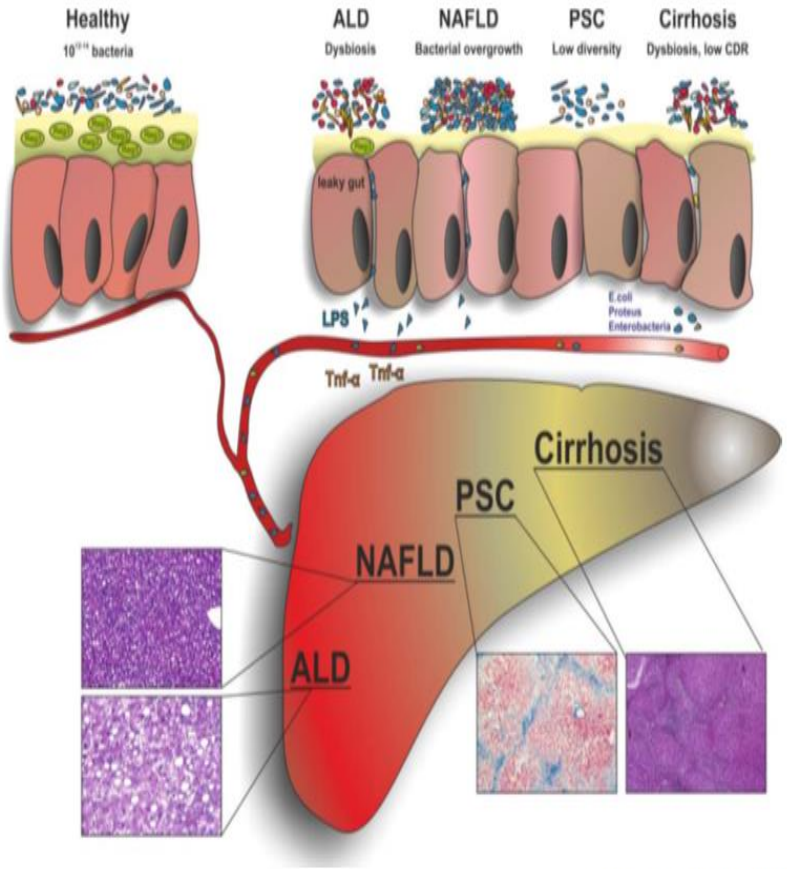
Gut microbiome signature for cirrhosis due to non-alcoholoic fatty liver disease: A prospective twin and family study

Cyrielle Caussy[1,2], Anupriya Tripathi[3,4,5,6], Greg Humphrey[3,4], Shirin Bassirian[1], Seema Singh[1], Claire Faulkner[1], Ricki Bettencourt[1], Emily Rizo[1], Lisa Richards[1], Zhenjiang Xu[4], Micheal Downes[7], Ronald E. Evans[7], David A. Brenner[1,8], Claude B. Sirlin[9], Rob Knight[3,4,10,11], Rohit Loomba[1,8,12]. [1]*University of California at San Diego, NAFLD Research Center, La Jolla, United States;* [2]*University Lyon 1, Hospices Civils de Lyon, Lyon, France;* [3]*University of California at San Diego, Center for Microbiome Innovation, La Jolla, United States;* [4]*University of California at San Diego, Department of Pediatrics, La Jolla, United States;* [5]*University of California at San Diego, Division of Biological Sciences, La Jolla, United States;* [6]*University of California at San Diego, Skaggs School of Pharmacy and Pharmaceutical Sciences, La Jolla, United States;* [7]*Salk Institute for Biological Studies, Gene Expression*



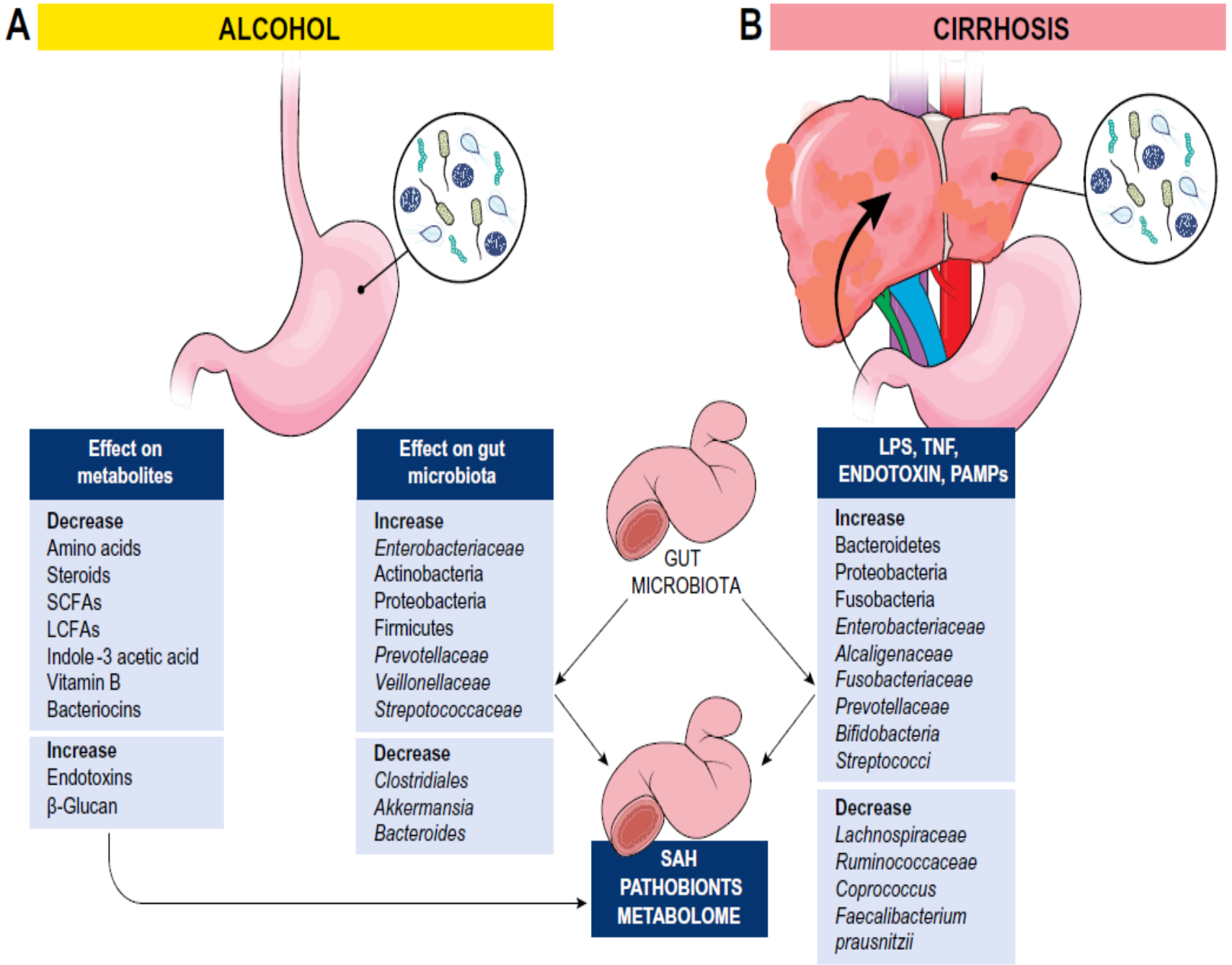
Random Forest Classifier , panel of 30 features including :
27 bacterial features , Age , Sex and BMI

Human ALD is characterized by a profound dysbiosis



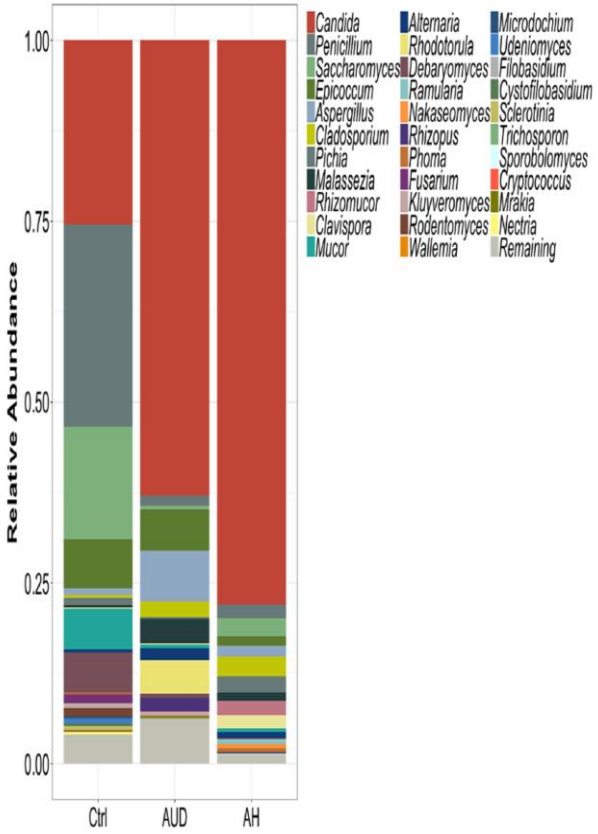
Tilg et al. Clin Liver Dis 2016

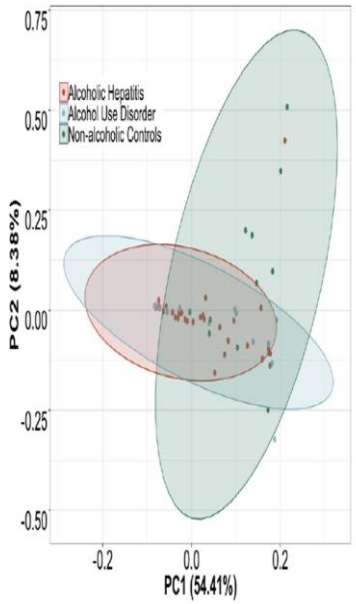
- Impaired intestinal epithelial barrier is early event in many experimental models of alcoholic liver injury even preceding intestinal dysbiosis
- A profoundly altered intestinal microbiome has been reported in several human ALD studies
- Disease transfer via FMT from patients with severe AH to mice has been reported
- The Gut's microbiota reflects a major driving force in ALD


A
ALCOHOL
B
CIRRHOSIS
Effect on metabolites
Decrease
Amino acids
Steroids
SCFAs
LCFAs
Indole-3 acetic acid
Vitamin B
Bacteriocins
Increase
Endotoxins
β-Glucan
Effect on gut microbiota
Increase
Enterobacteriaceae
Actinobacteria
Proteobacteria
Firmicutes
Prevotellaceae
Veillonellaceae
Strepotococcaceae
Decrease
Clostridiales
Akkermansia
Bacteroides
GUT MICROBIOTA
LPS, TNF, ENDOTOXIN, PAMPs
Increase
Bacteroidetes
Proteobacteria
Fusobacteria
Enterobacteriaceae
Alcaligenaceae
Fusobacteriaceae
Prevotellaceae
Bifidobacteria
Streptococci
Decrease
Lachnospiraceae
Ruminococcaceae
Coprococcus
Faecalibacterium prausnitzii
SAH PATHOBIONTS METABOLOME

Fungal & bacterial dysbiosis in ALD

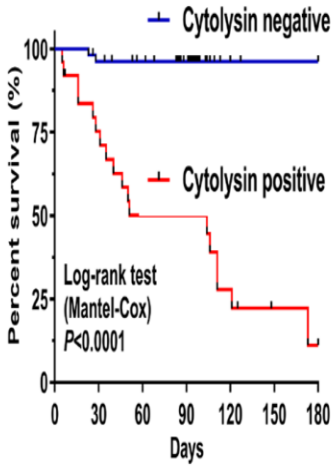
Fungal dysbiosis in patients with alcohol abuse





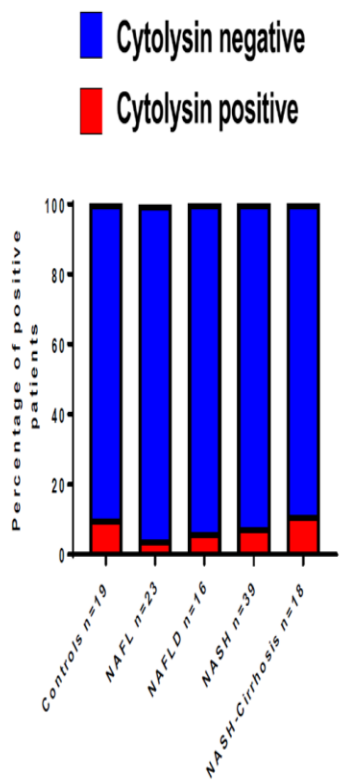
Yang A, Schnabl B, J Clin Invest 2017 ; Lang S, Schnabl B, Hepatology 2019

Enterecoccus faecalis cytolisin associates with mortality in patients with alcoholic hepatitis , but not in patients with NAFLD



Multivariate Cox Regression			
Variables	HR	95% CI	*P* value
Cytolysin	14.07	2.83-69.85	0.001
MELD	1.09	1.01-1.16	0.022

Duan Y, Llorente C, Schnabl B, 2019

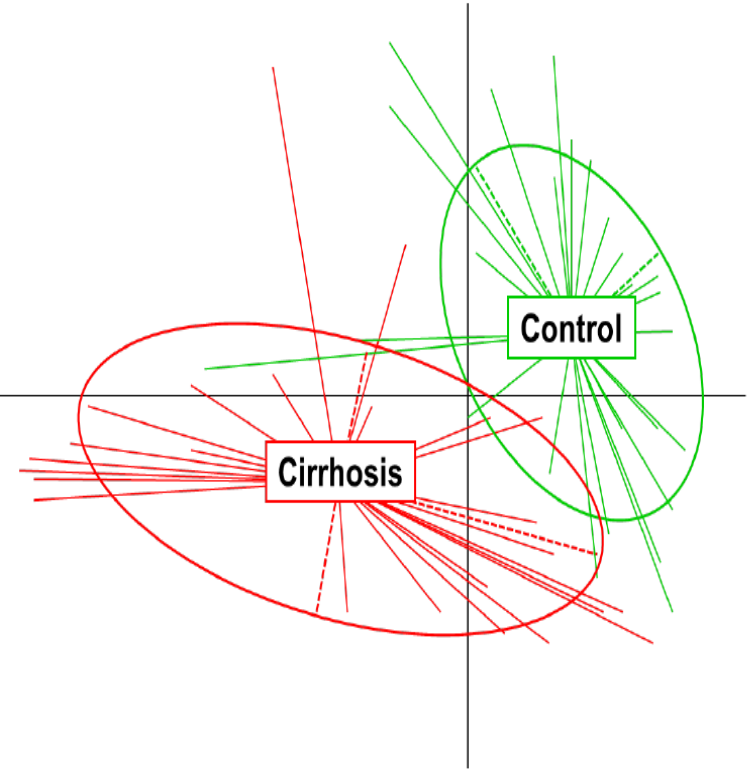


Lang S, Schnabl B, unpublished

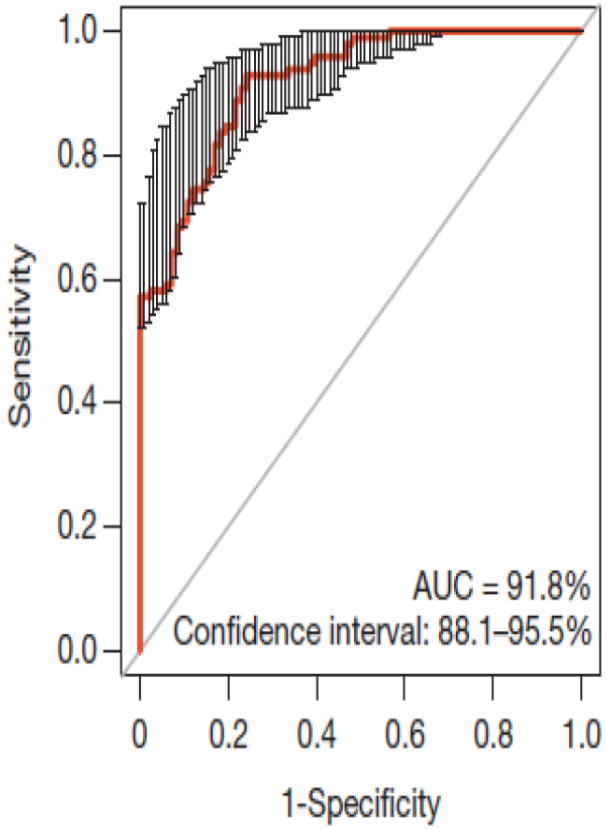
Microbiota nella cirrosi epatica

- La cirrosi epatica è associata a una profonda alterazione del microbiota (disbiosi)
- Bacteroidetes sono ridotti , Proteobacteriaceae sono altamente aumentati
- Batteri del cavo orale sono altamente rappresentati nell'intestino
- La cirrosi e il successivo scompenso sono caratterizzati : dal peggioramento della disbiosi , dalla disfunzione della barriera intestinale , dalla aumentata permeabilità intestinale , dalla traslocazione di batteri , metaboliti e mediatori microbici , da infiammazione locale e sistemica
- La progressione della cirrosi , la sarcopenia , le infezioni e gli eventi di scompenso (da EPS ad ACLF) sono " Gut-Liver-Brain Axis Driven "
- L'alterazione di Gut-Liver-Brain Axis costituisce un nuovo " target terapeutico " nella cirrosi epatica

Cirrhotic patients have a different fecal microbiota composition



Qin, Nature 2014



Cirrhosis dysbiosis ratio (CDR)

	CDR
Controls	2.05
Compensated Outpatients	0.89
Decompensated Outpatients	0.66
Inpatients	0.32

"Good" bacteria / "Bad" bacteria

Bajaj J Hepatol 2014

ARTICLE

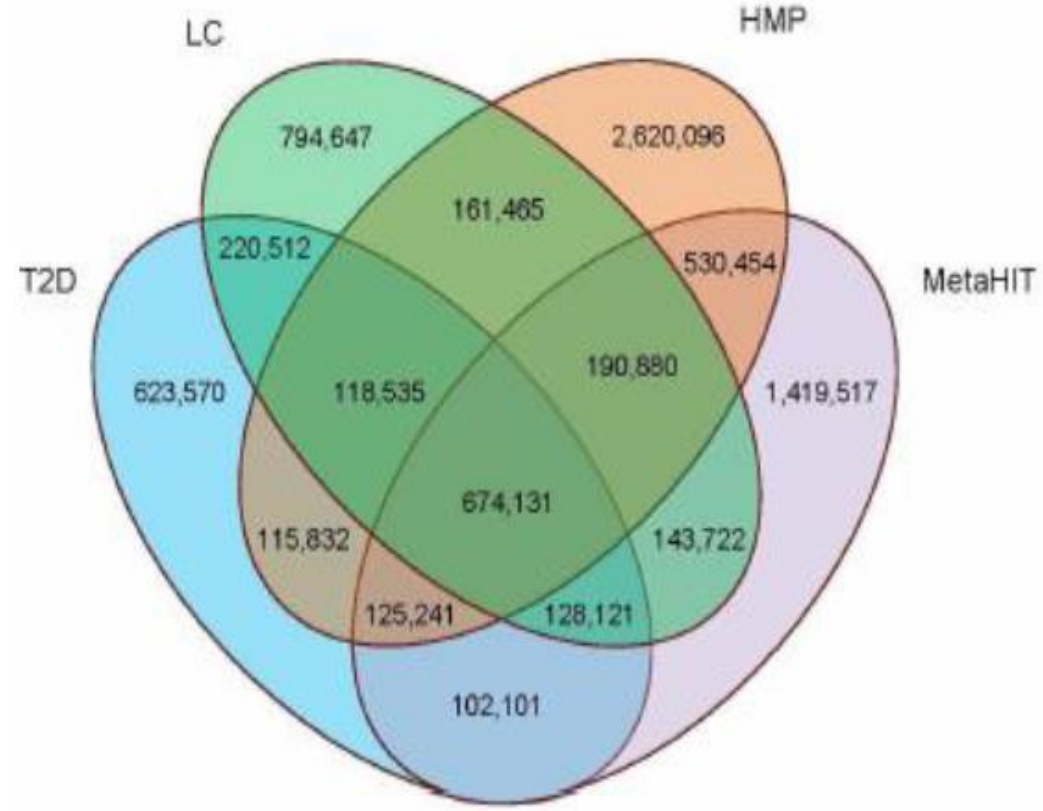
Dysbiosis in liver cirrhosis

A Chinese Study

doi:10.1038/nature13568

Alterations of the human gut microbiome in liver cirrhosis

Nan Qin[1,2]*, Fengling Yang[1]*, Ang Li[1]*, Edi Prifti[3]*, Yanfei Chen[1]*, Li Shao[1,2]*, Jing Guo[1], Emmanuelle Le Chatelier[3], Jian Yao[1,2], Lingjiao Wu[1], Jiawei Zhou[1], Shujun Ni[1], Lin Liu[1], Nicolas Pons[3], Jean Michel Batto[3], Sean P. Kennedy[3], Pierre Leonard[3], Chunhui Yuan[1], Wenchao Ding[1], Yuanting Chen[1], Xinjun Hu[1], Beiwen Zheng[1,2], Guirong Qian[1], Wei Xu[1], S. Dusko Ehrlich[3,4], Shusen Zheng[2,5] & Lanjuan Li[1,2]



4 currently available major human microbiome gene sets

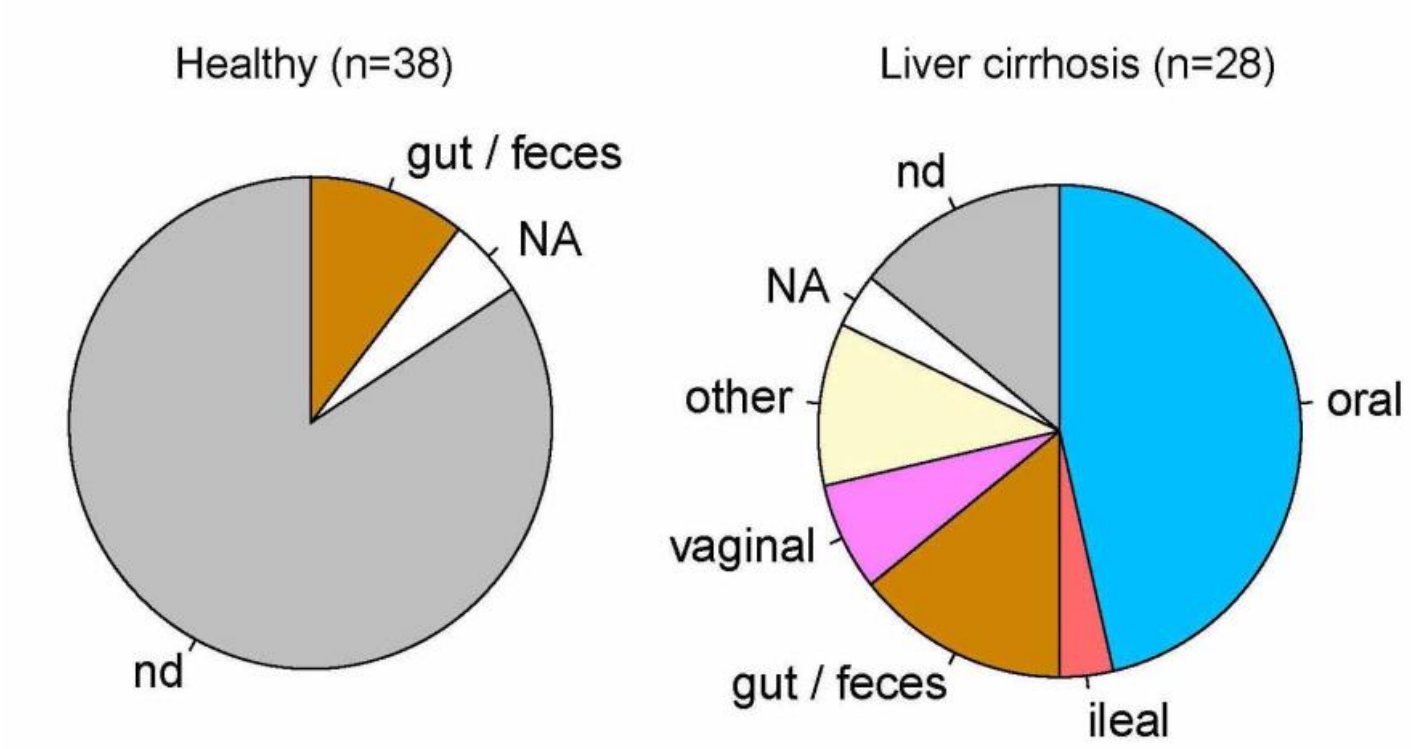
- ✓ **A novel gut gene catalogue (98 patients with liver cirrhosis and 83 controls)**
- ✓ **Comparison with MetaHIT, Human Microbiome Project, T2D catalogues**
- ✓ **2.69 million genes, 36.1% are novel: SEVERE DYSBIOSIS**

Qin, Nature 2014

The fingerprint of gut microbiota in liver cirrhosis

Invasion of the Gut from the Mouth in cirrhosis

Unbalance between potentially pathogenic and beneficial bacteria



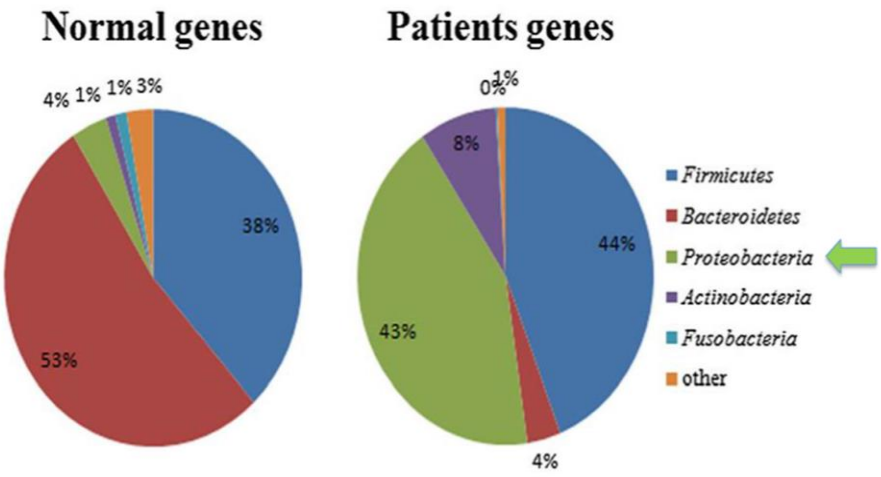
Species enriched in cirrhotics' fecal samples belong to *Veillonella* or *Streptococcus* taxa, which usually derive from the mouth or the small intestine

Qin N et al. Nature 2014; 513: 59-64

The fingerprint of gut microbiota in liver cirrhosis

Unbalance between potentially pathogenic and beneficial bacteria

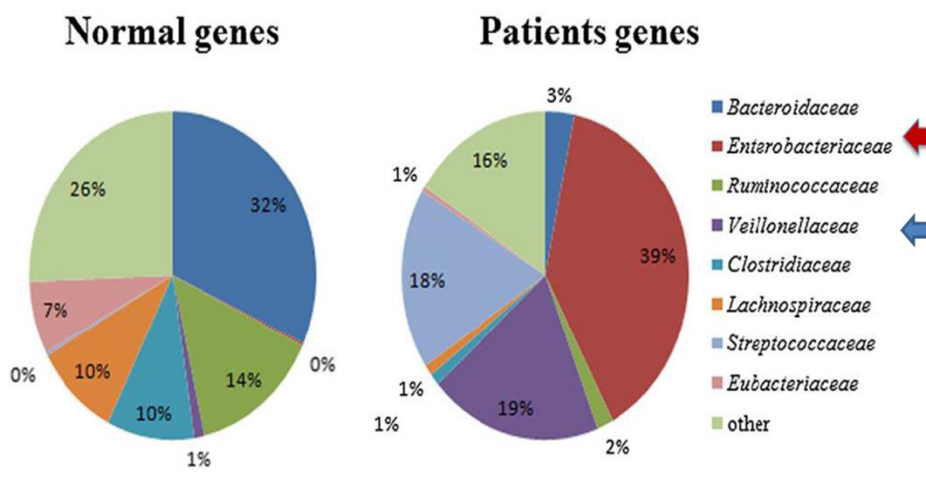
PHYLUM LEVEL



Lu et al. Microb Ecol 201
Chen et al. Hepatology 201
Wei X et al BMC Gastroenterology 201

Unbalance between potentially pathogenic and beneficial bacteria

FAMILIA LEVEL

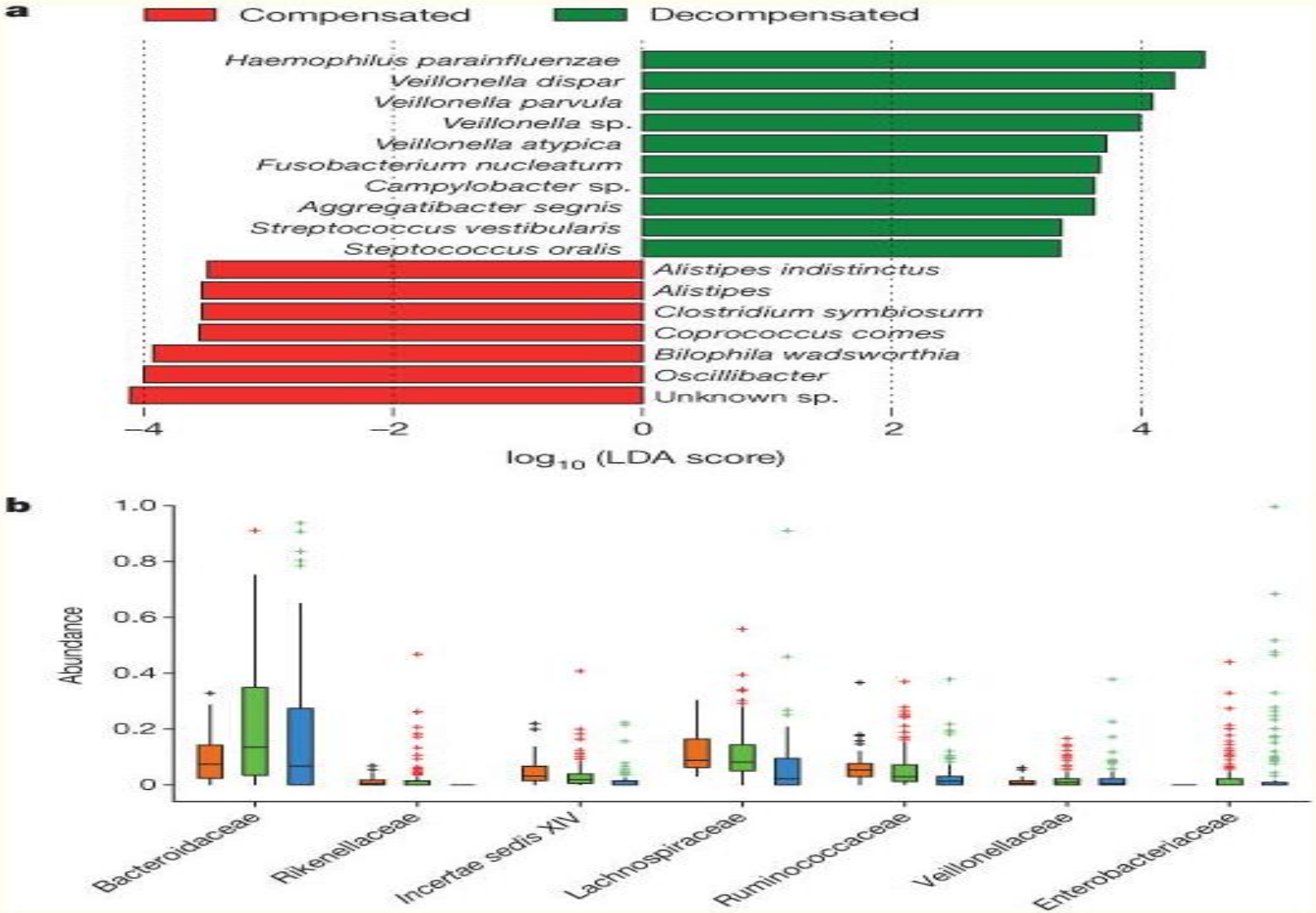


Lu et al. Microb Ecol 201
Chen et al. Hepatology 201
Wei X et al BMC Gastroenterology 201

Nature. Author manuscript; available in PMC 2016 Mar 17.
Published in final edited form as:
Nature. 2015 Sep 17; 525(7569): E1–E2.
doi: [10.1038/nature14851]

Decompensated cirrhosis and microbiome interpretation

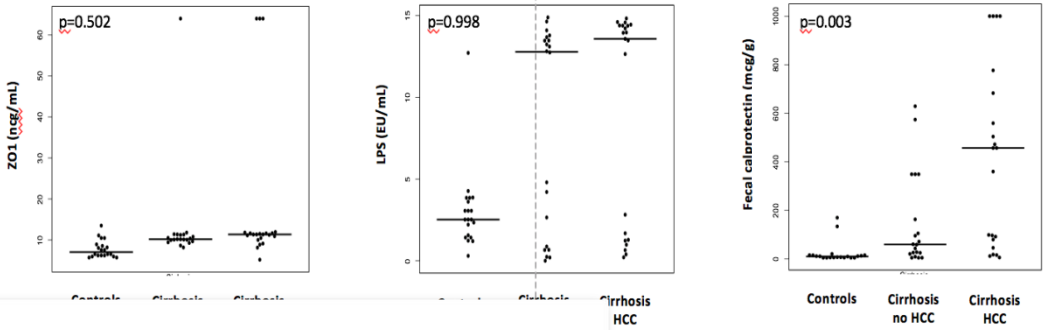
Jasmohan S. Bajaj,[1] Naga S. Betrapally,[2] and Patrick M. Gillevet[2]

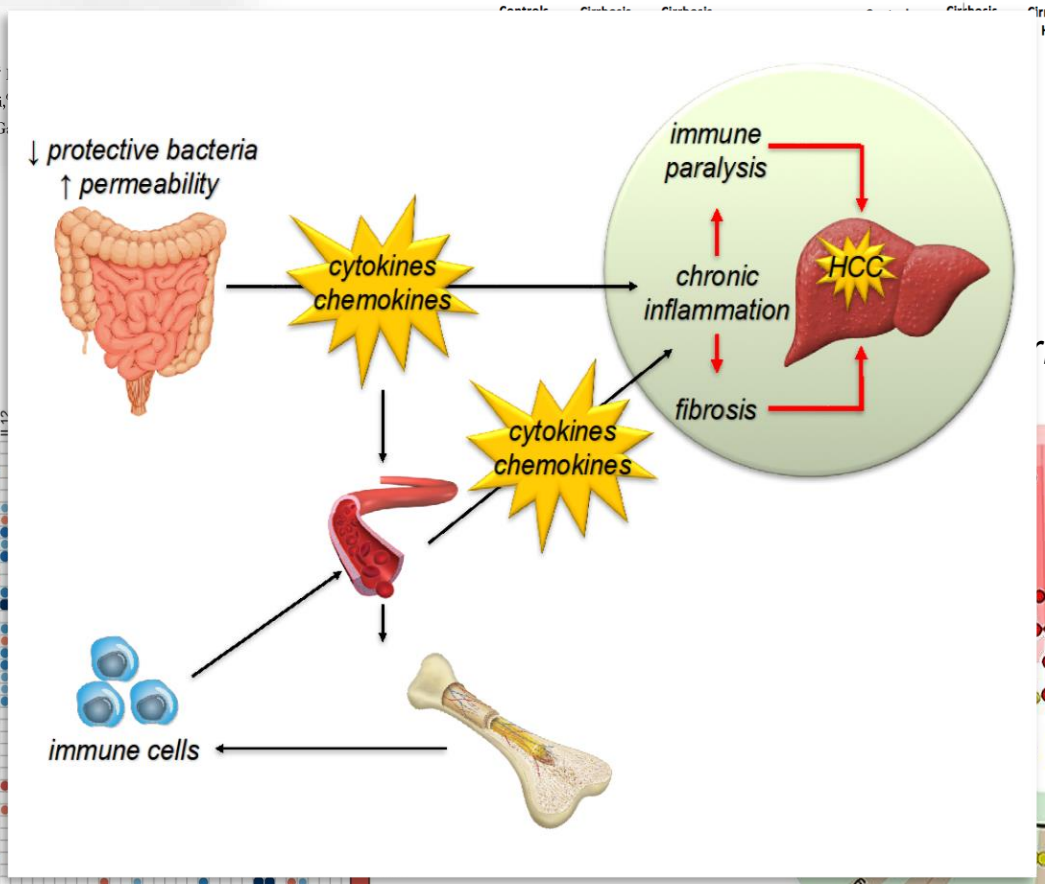


HEPATOLOGY
AASLD
HEPATOLOGY, VOL. 00, NO. 00, 2018

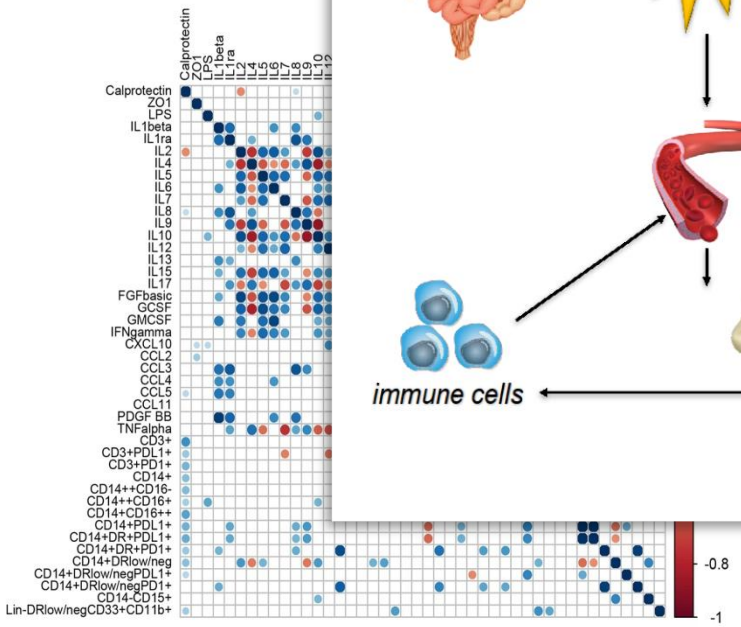
Hepatocellular Carcinoma Is Associated With Gut Microbiota Profile and Inflammation in Nonalcoholic Fatty Liver Disease

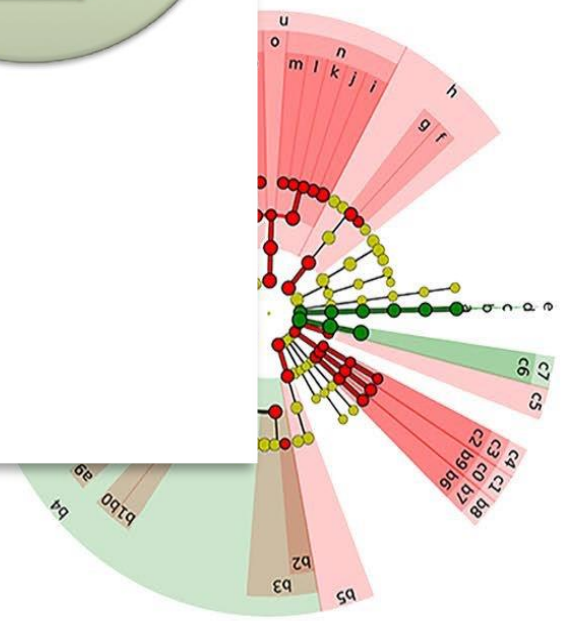
Francesca Romana Ponziani,[1] Sherrie Bhoori,[2] Chiara Castelli,[3]
Maurizio Sanguinetti,[6] Daniele Morelli,[7] Francesco Paroni Sterbini,
Chiara Camisaschi,[3] Anna Picca,[8] Alessandra Tuccitto,[3] Antonio G





ium & Akkermansia





GUT Microbiome & HCC

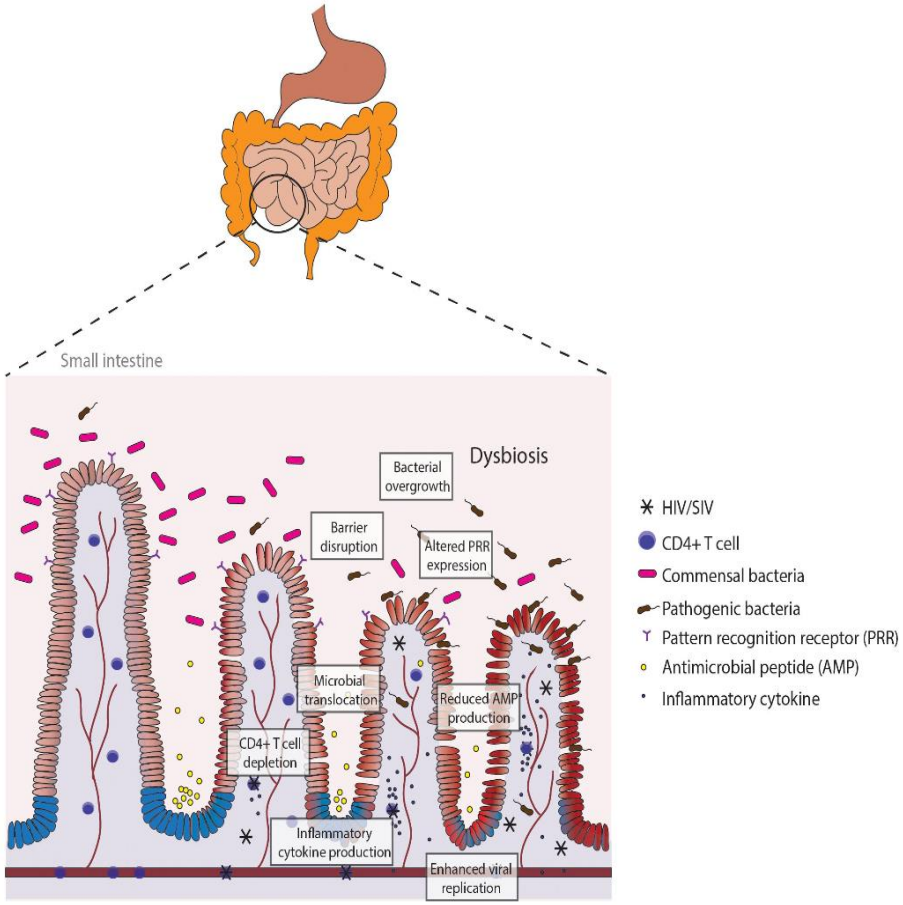
Gut microbiome analysis as a tool towards targeted non-invasive biomarkers for early hepatocellular carcinoma

What are the new findings?

- Faecal microbial diversity was decreased from healthy controls to cirrhosis, but it was increased from cirrhosis to early HCC with cirrhosis.
- Butyrate-producing bacterial genera were decreased, while genera producing-lipopolysaccharide were increased in early HCC versus healthy controls.

Ren Z et al. Gut 2018

Gut Microbiome in acute or chronic non cirrhotic hepatitis B/C



- HBV acute and chronic
- HCV chronic
- ACLF

HBV infection & Gut Microbiota

ORIGINAL RESEARCH ARTICLE

Front. Cell. Infect. Microbiol., 05 November 2019 | https://doi.org/10.3389/fcimb.2019.00377

Hepatitis B Virus Infection Alters Gut Microbiota Composition in Mice

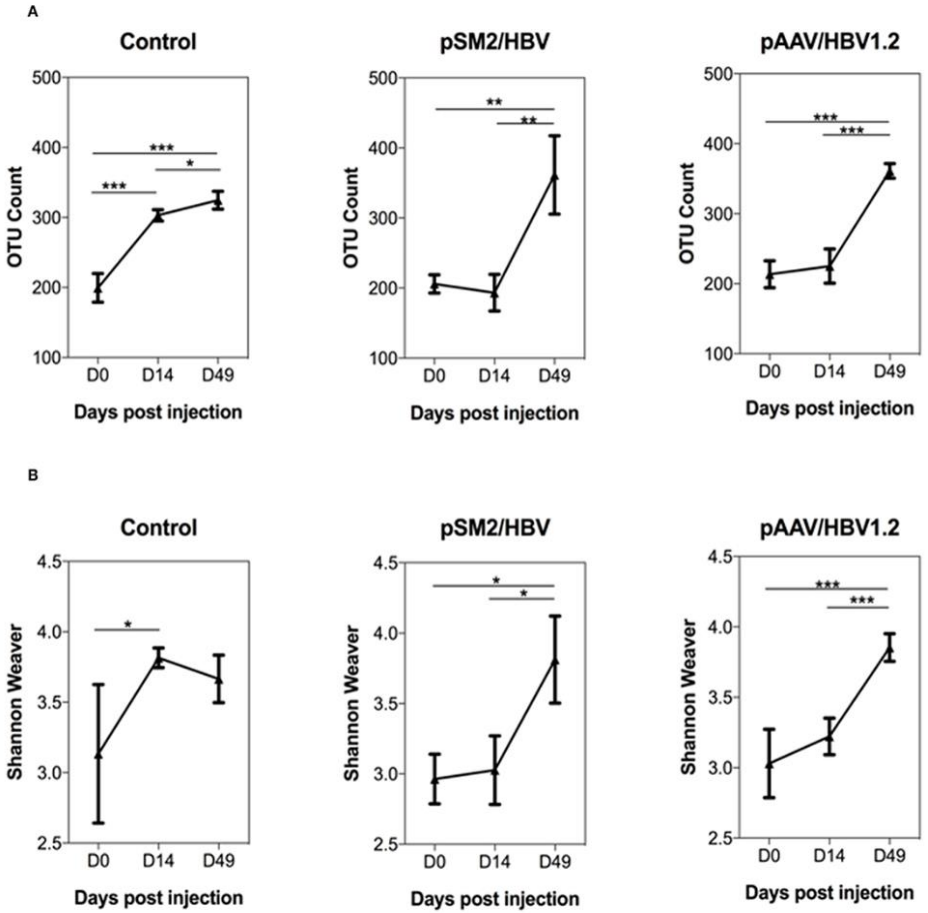
Qingfeng Zhu, Panpan Xia, Xin Zhou, Xiaoran Li, Weina Guo, Bin Zhu, Xin Zheng, Baoju Wang, Dongliang Yang* and Junzhong Wang*

Department of Infectious Diseases, Tongji Medical College, Union Hospital, Huazhong University of Science and Technology, Wuhan, China

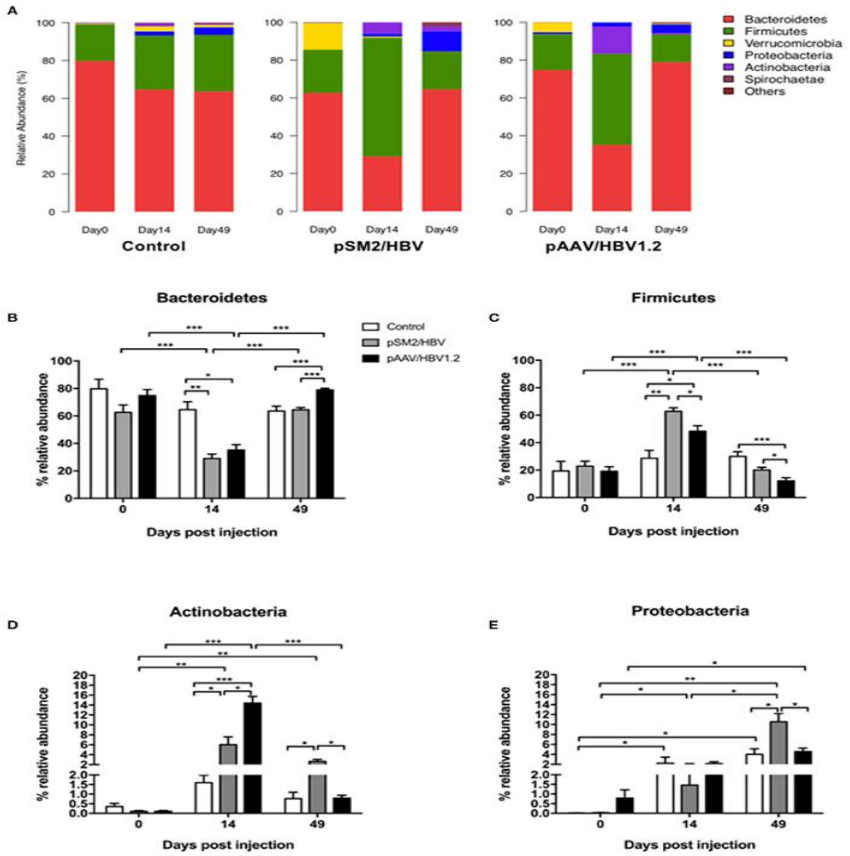
- Analysis of dynamics alterations of gut microbiota following HBV infection
- Hydrodynamic injection mouse model, mimicking acute or chronic HBV infection in humans with comparable virological and immunological features was used
- The composition of gut microbiota in the control mice and mice with acute or chronic infection was analyzed at different time points using the Illumina MiSeq platform
- Expression of immune molecules in the colon was detected by real-time PCR

HBV infection & Gut Microbiota
Results

Dynamic changes in the overall abundance and diversity of gut microbiota

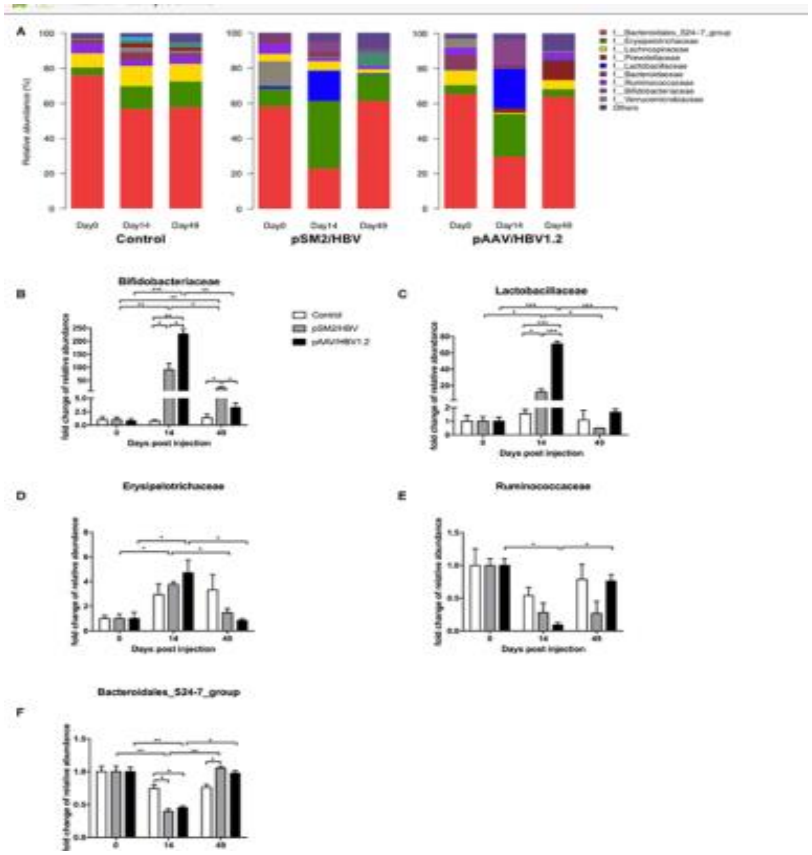


Phylum level dynamic changes in gut microbiota

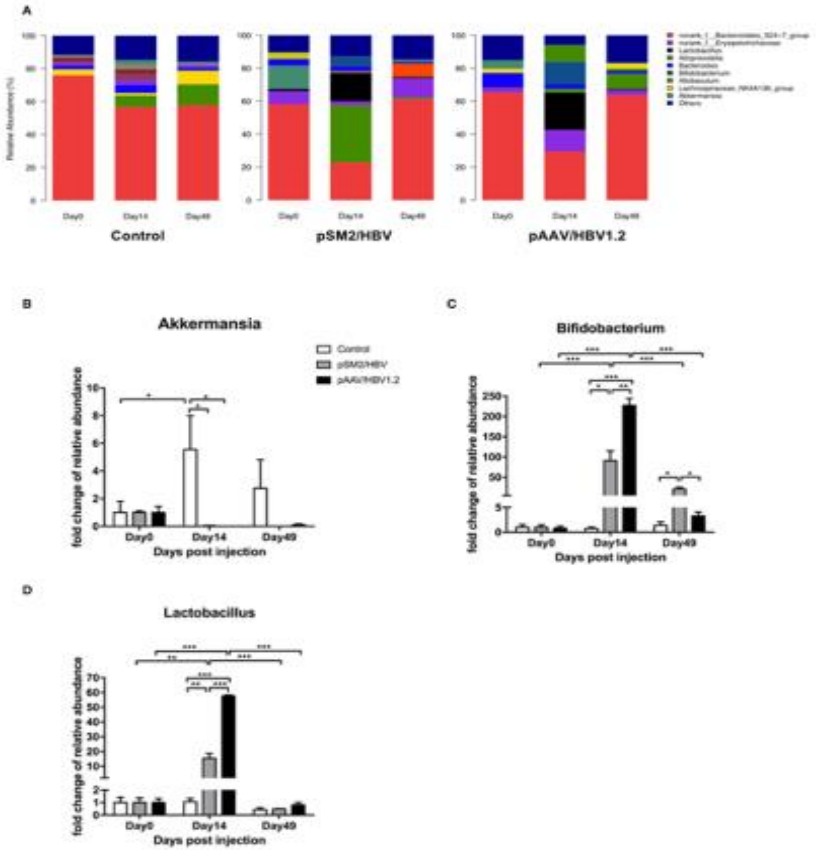


HBV infection & Gut Microbiota
Results

Family level dynamic changes

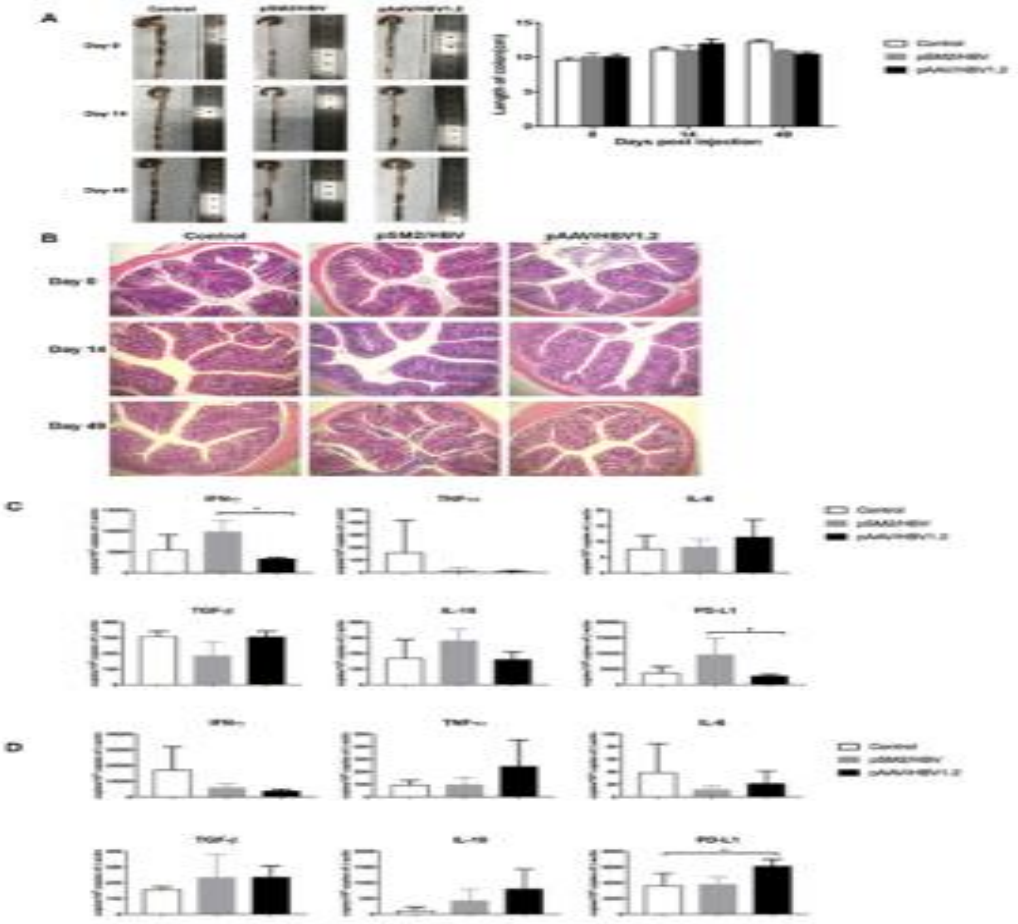


Genus level dynamic changes



HBV infection & Gut Microbiota
Results

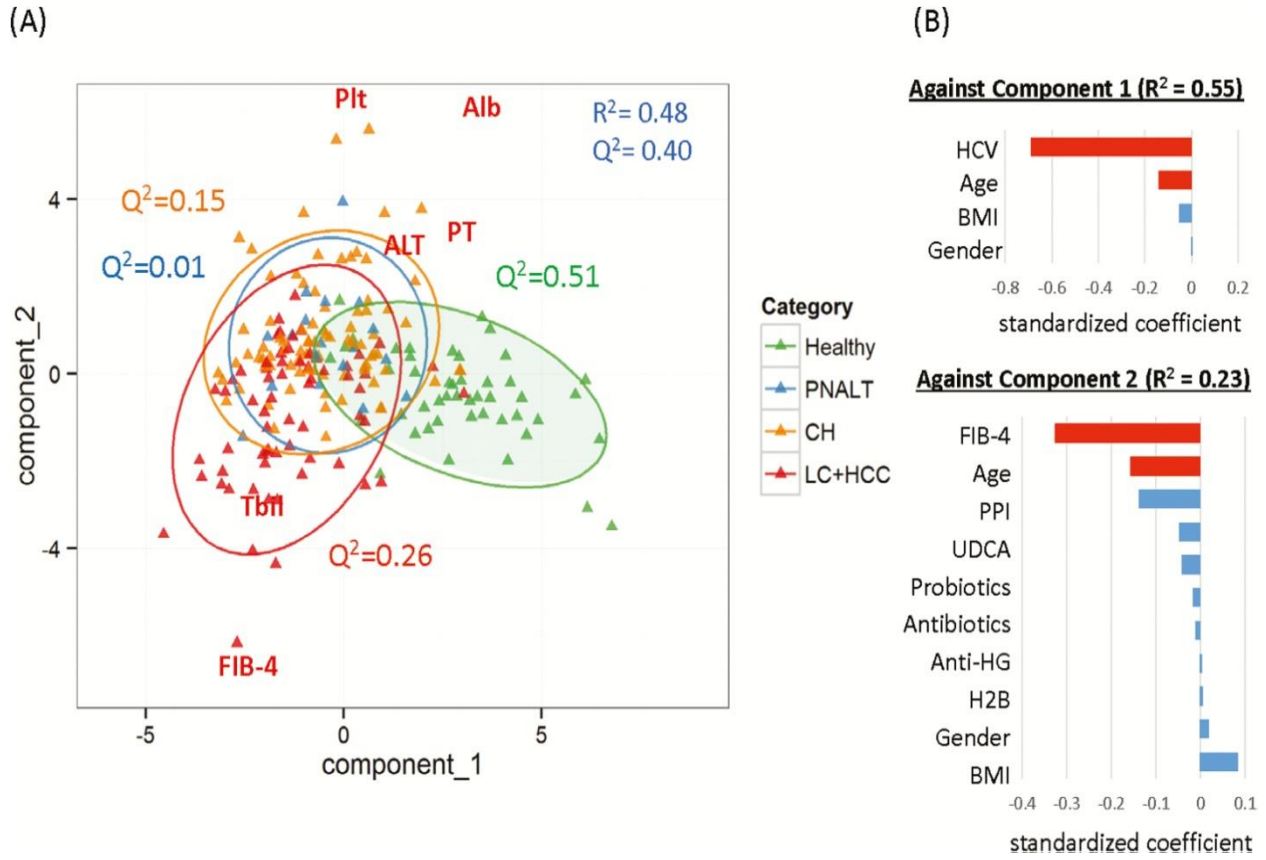
Pathological and immunological changes in the colon



HBV & Gut Microbiota : Conclusions

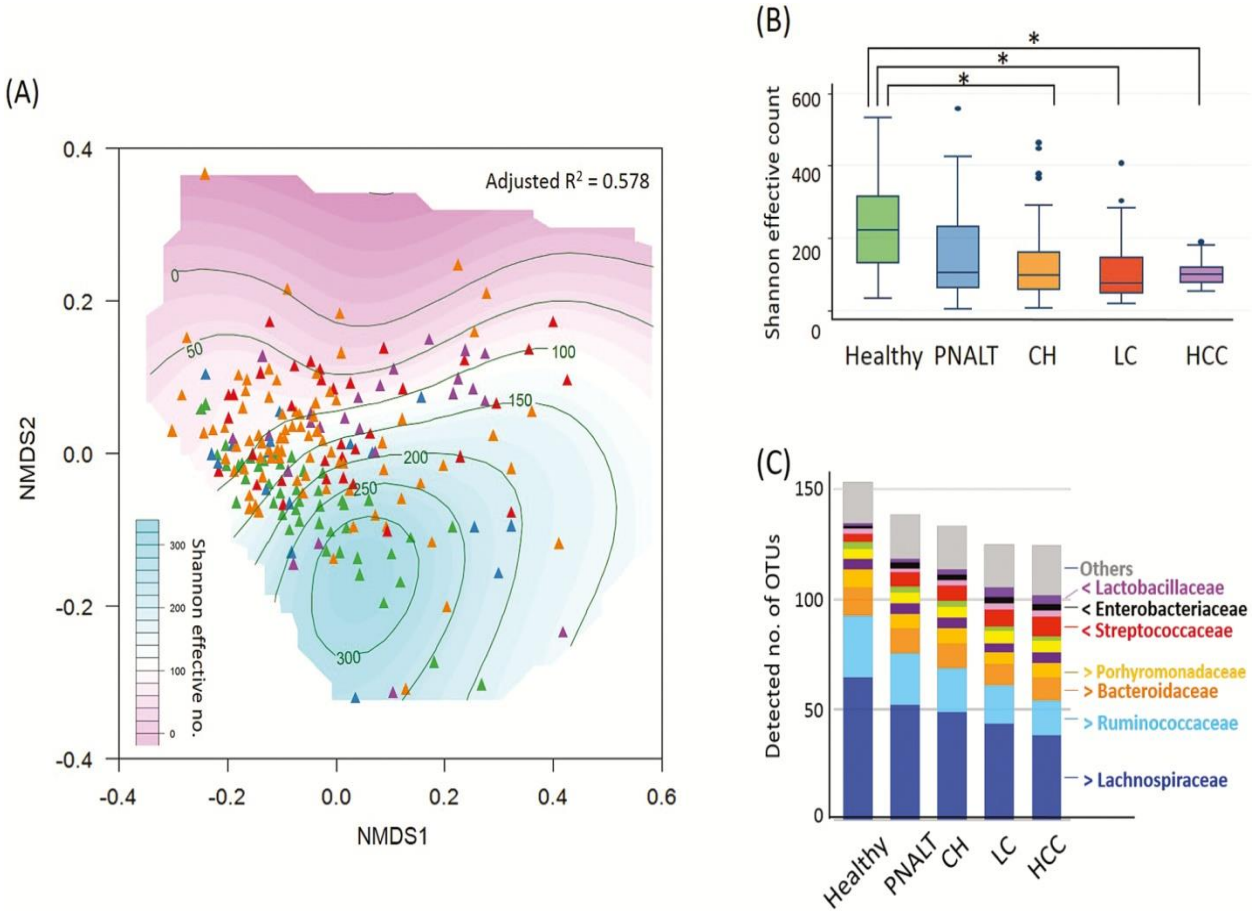
- Gut microbiota in the control mice changed remarkably by day 14 based on total observed OTU and Shannon-Weaver Index , while this was delayed in mice with HBV infection
- Abundance of Bacteroidetes decreased and that of Firmicutes increased by day 14 in mice with acute or chronic infection
- Bacteroidetes is critical for production of Butyrate an SCF inducing extrathymic T-reg differentiation
- Firmicutes can absorb and consume more calories and HBV specific immune response requires increasing energy consumption
- Firmicutes/Bacteroidetes ratio help to shape the HBV immune specific response via fatty acids metabolism and energy consumption
- Firmicutes / Bacteroidetes ratio decrease after 49 day
- The dynamic changing pattern in the abundance of Lactobacillus and Bifidobacterium differs in mice with acute vs chronic infection and is related to IFN–γ mediated immune response
- The expression of IFN-γ and PDL1 in the colon was up-regulated in mice with acute HBV infection vs chronic at day 14 and PD-L1 was up-regulated in mice with HBV chronic infection vs control

GUT Bacterial Community in Healthy Individuals and Chronic Hepatitis C



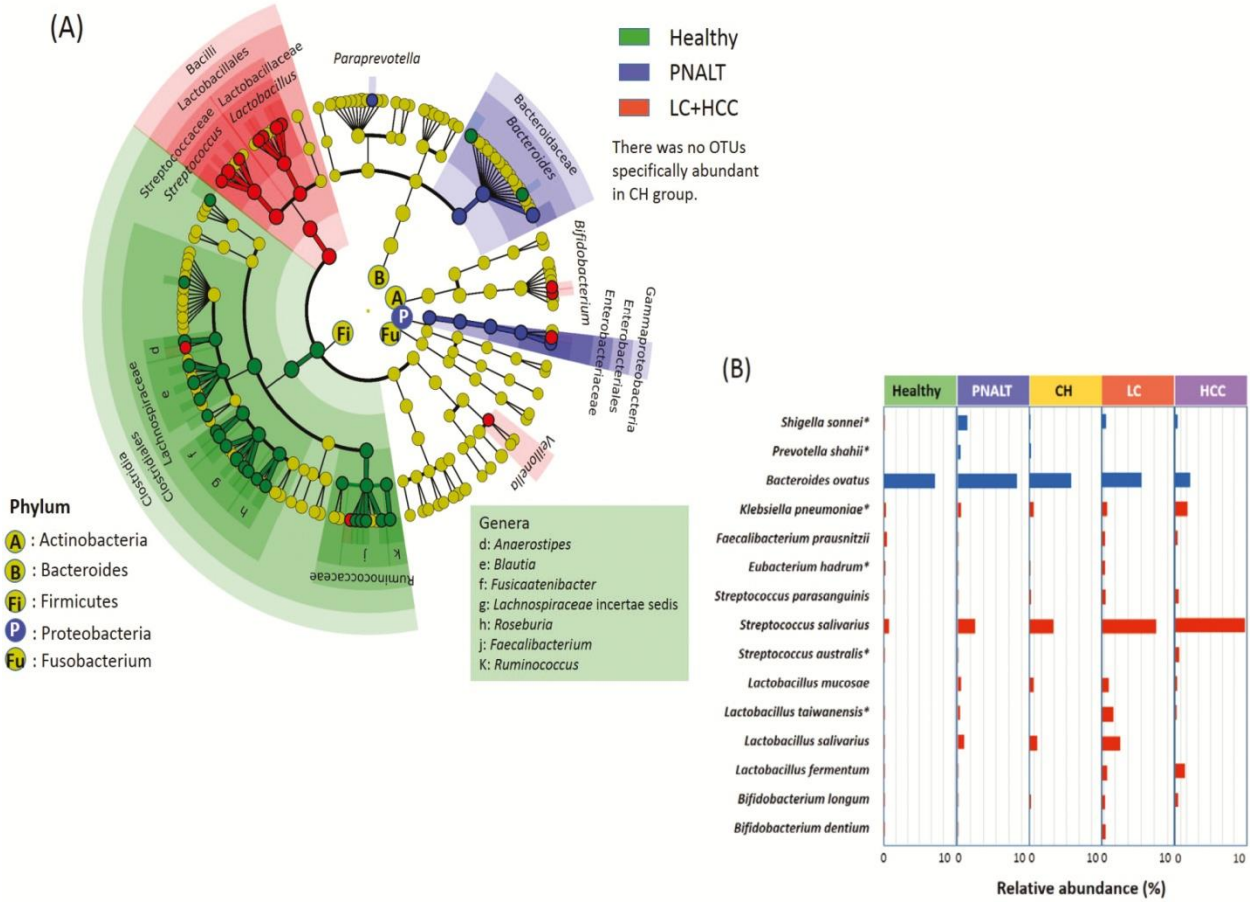
Clinical Infectious Diseases, Volume 67, Issue 6, 15 September 2018, Pages 869–877, https://doi.org/10.1093/cid/ciy205

Decreased Microbial Diversity in Progression of HCV Chronic Disease



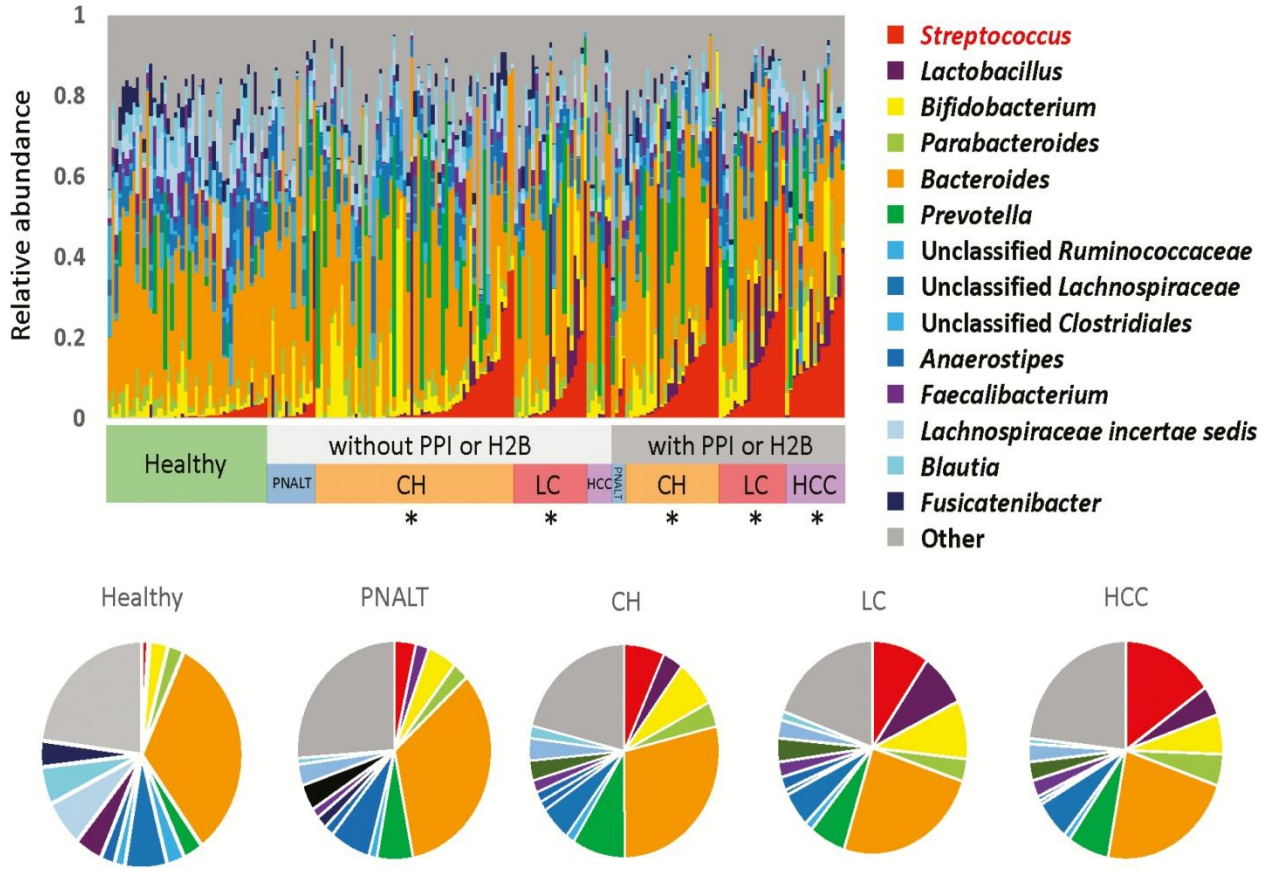
Clinical Infectious Diseases, Volume 67, Issue 6, 15 September 2018, Pages 869–877, https://doi.org/10.1093/cid/ciy205

Phylogenetic Skew in Gut Bacterial Community Associated with Chronic Hepatitis C



Clinical Infectious Diseases, Volume 67, Issue 6, 15 September 2018, Pages 869–877, https://doi.org/10.1093/cid/ciy205

Genus composition of fecal samples from healthy individuals and chronic hepatitis C patients

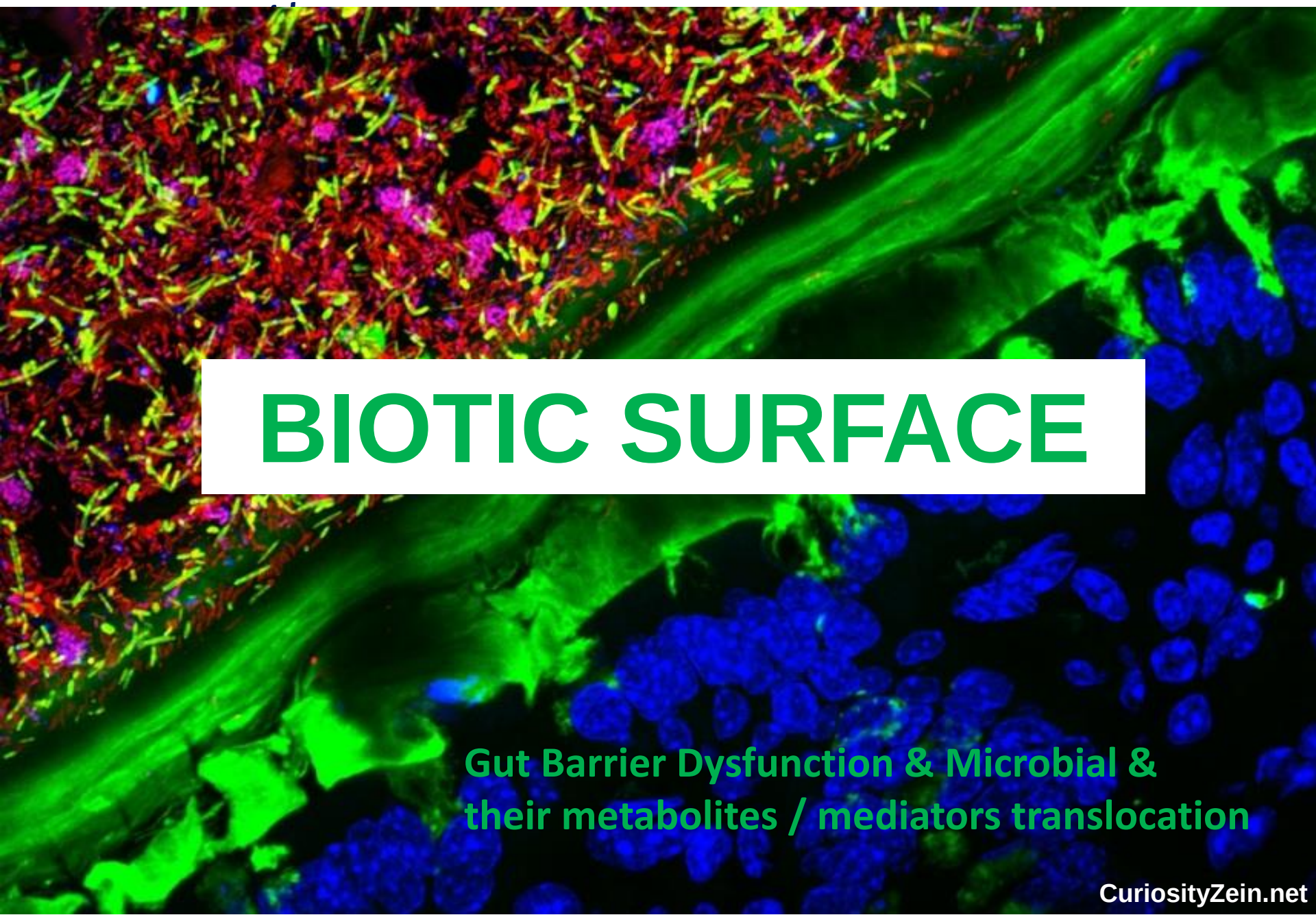


Clinical Infectious Diseases, Volume 67, Issue 6, 15 September 2018, Pages 869–877, https://doi.org/10.1093/cid/ciy205

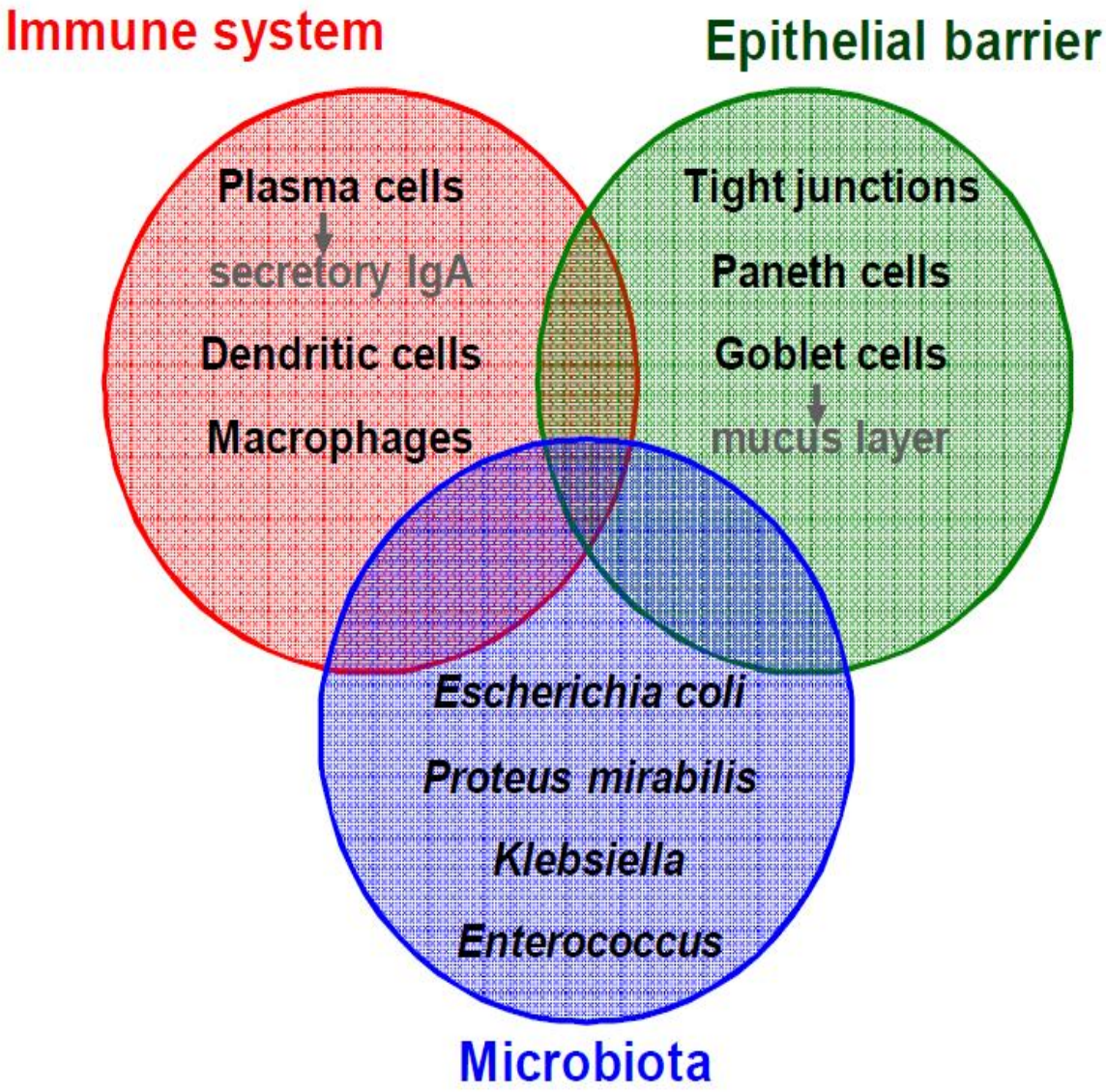
The Role of SCFA : common in viral infections

- SCFA decreases bacterial traslocation in cell models and modify expression of the tight junction proteins claudin-1 and claudin-2
- SCFA released by microbiota are absorbed in the colon and filtered intrahepatically were modulate the immune response, via the promotion of survival of CD8+T cells
- SCFAs modulate oncogenesis and are involved in epigenetic of liver pathogens through HDAC inhibition and transcriptional changes
- As HDAC inhibitors , SCFAs decrease HBV replication , mediate HCV replication , impede liver necrosis and prevent HCC
- Antibiotic-treated mice experienced an impaired adaptive immunity against HBV
- Only mice with maturation of gut microbiota can stimulate immunity and can have a rapid HBV- Clearance
- Recently butyrate was shown regulate the expression of tumor suppressive miRNAs through the bile acid nuclear receptor FXR while also promote liver apoptosis

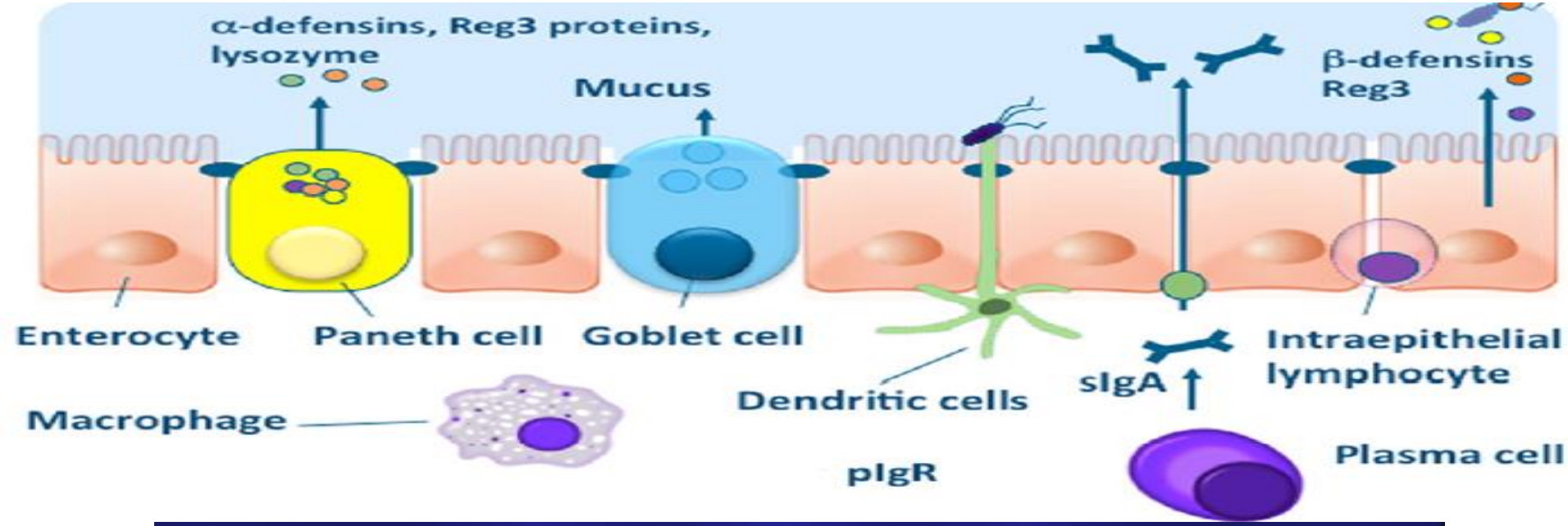
THE ANATOMO-MICROBIOLOGICAL GUT BARRIER

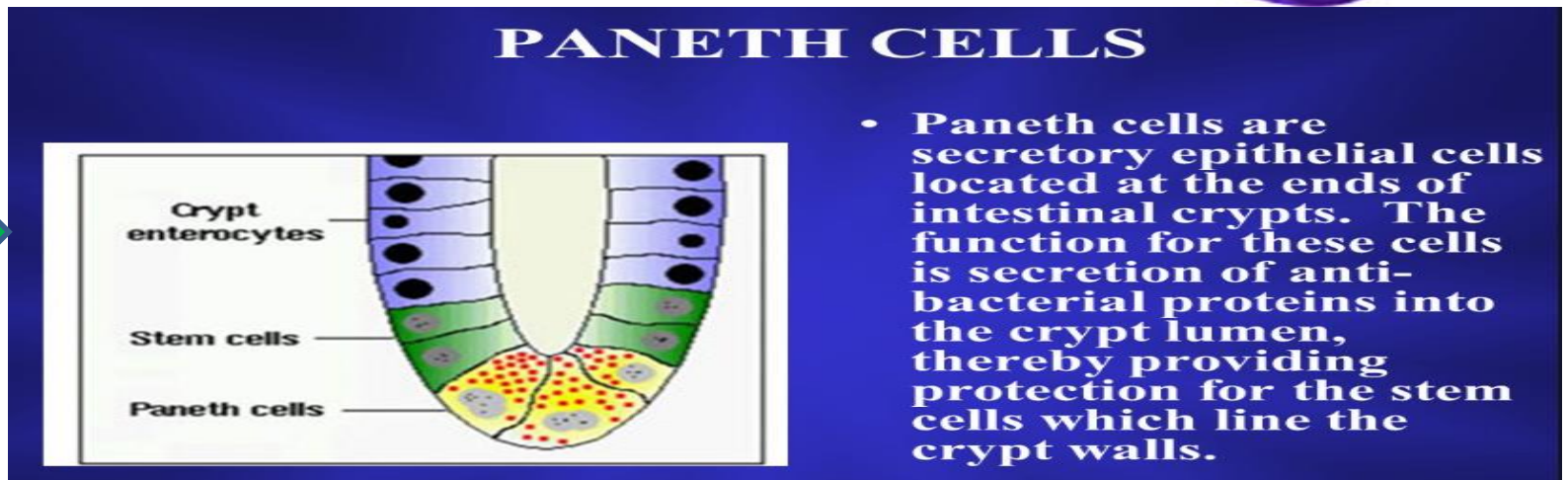


Mechanisms modulating bacterial translocation

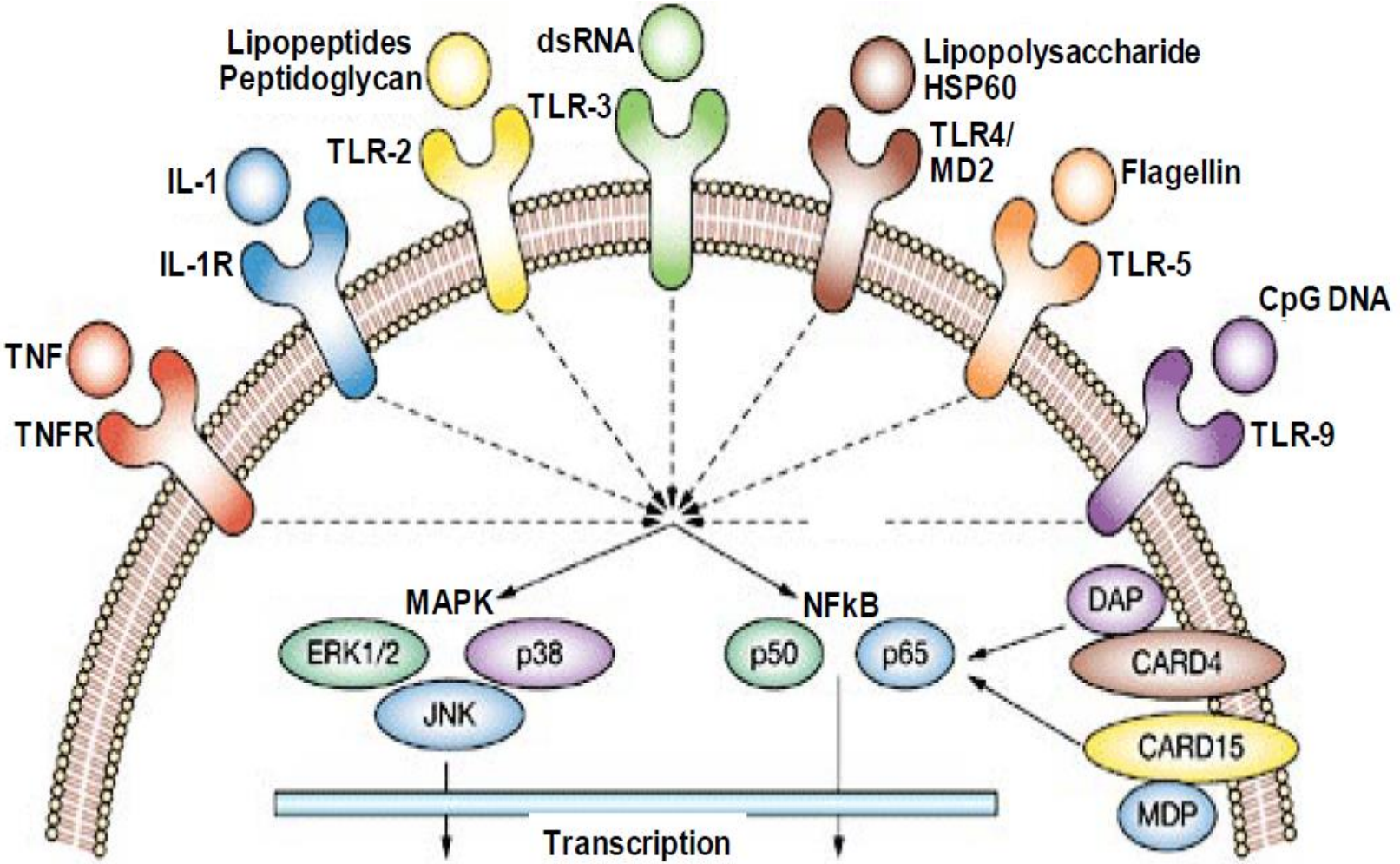


Chemical and physical intestinal barrier : first line of defense





PRRs (Pattern Recognition Receptors) recognise pathogens associated molecular patterns (PAMPS) on microbes and leads to microbial killing



Recettori Circolanti : PTX3

Microbial killing

Recettori Citosolici : Proteine Nods / RLRs

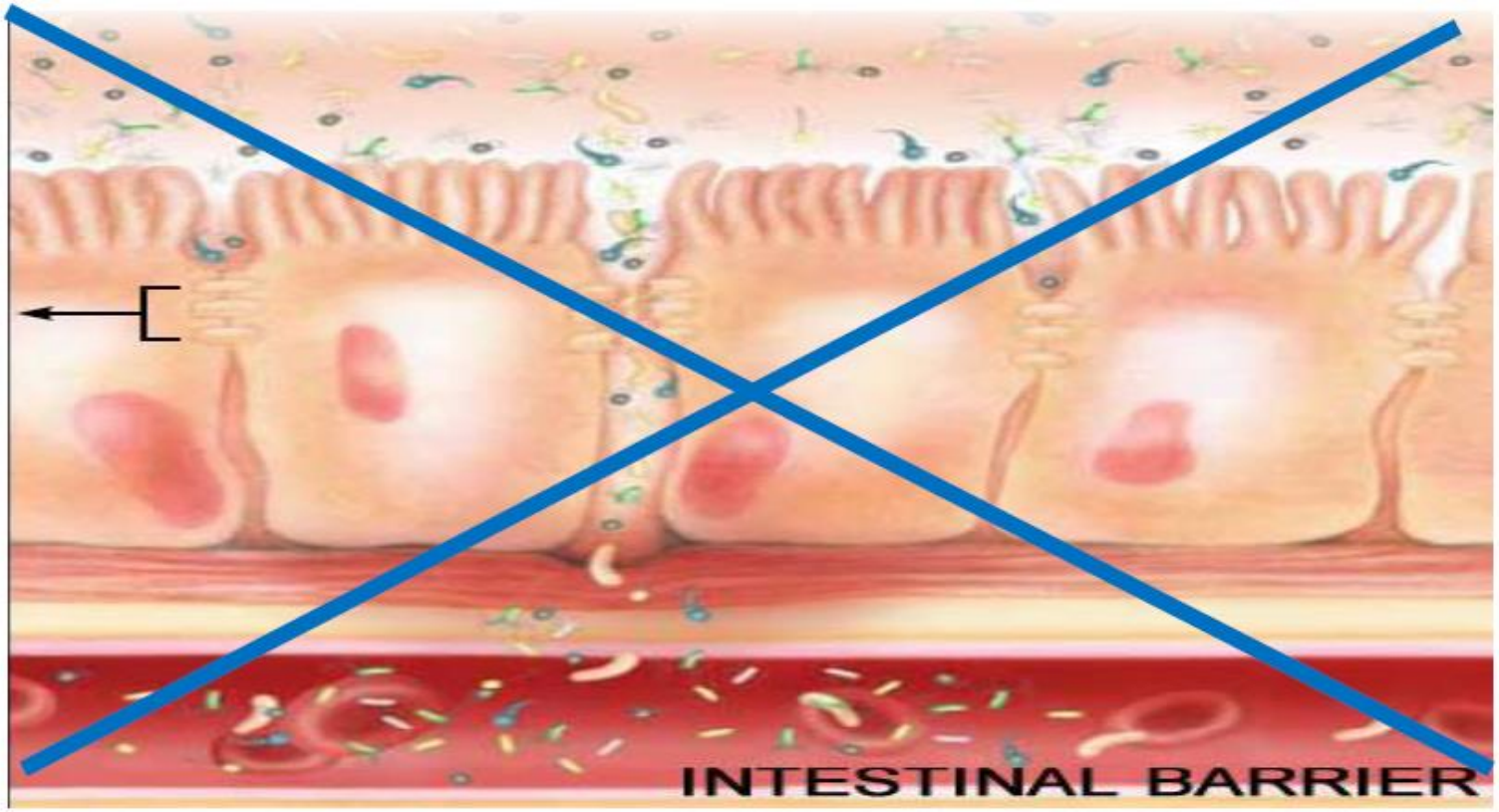
Disruption of intestinal barrier & consequences of Dysbiosis

Disruptors of intestinal epithelial barrier

- Dietary factors (alcohol or overdosed nutrients)
- Mucosal inflammation of any aetiology (↑ TNF)
- Drugs/medication (es. FANS , PPI)
- Infections & Toxins (direct damage : V. Cholerae , E. Coli , Salmonella , Clostridia , H. Pilori)
- Impaired production of factors modulating epithelial integrity (IL-22)
- Increased enterocyte apoptosis (↑ MMPS,↓ TIMPS)
- Hypoperfusion

Dysbiosis increase in the gut

- Ammonia
- Endotoxin
- Inflammatory cytokines
- Bacterial DNA
- Bacterial PAMPS (Pathogen Associated Molecular Patterns)

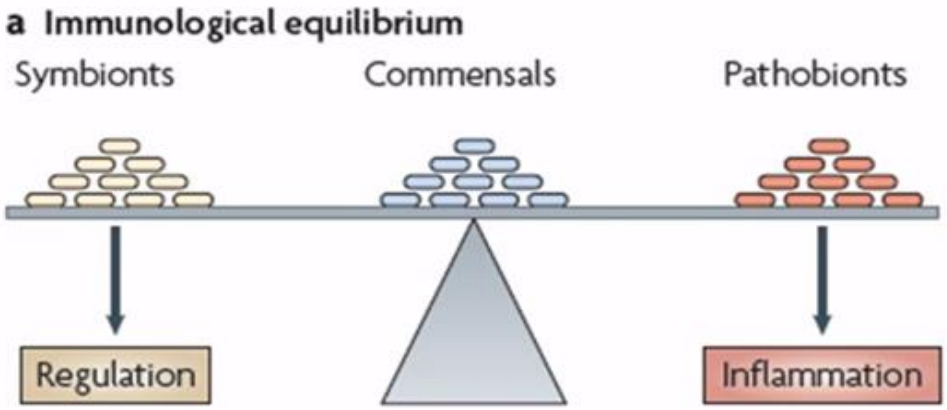


Gut Barrier disfunction

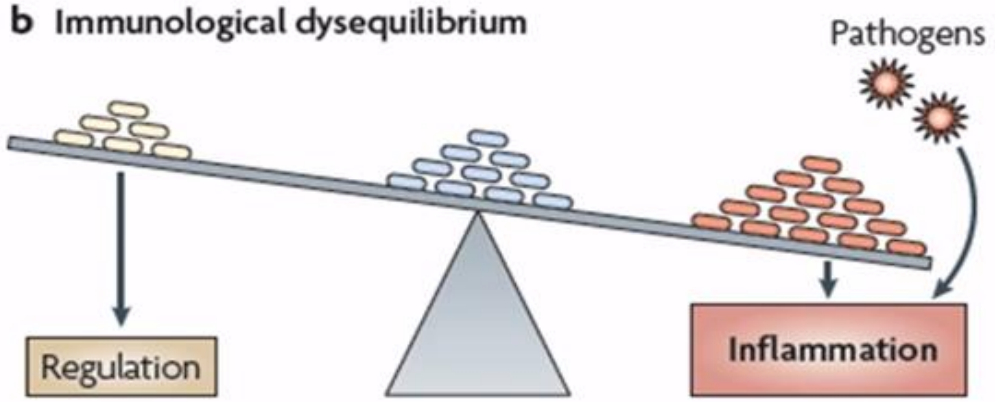
↑ Intestinal permeability: Leaky gut

Dysbiosis of microbiota associated with immunological dysregulaion

Eubiosis



Dysbiosis



(Round JL, Mazmanian SK. Nat Rev Immunol. 2009)

Hepatology Snapshot:

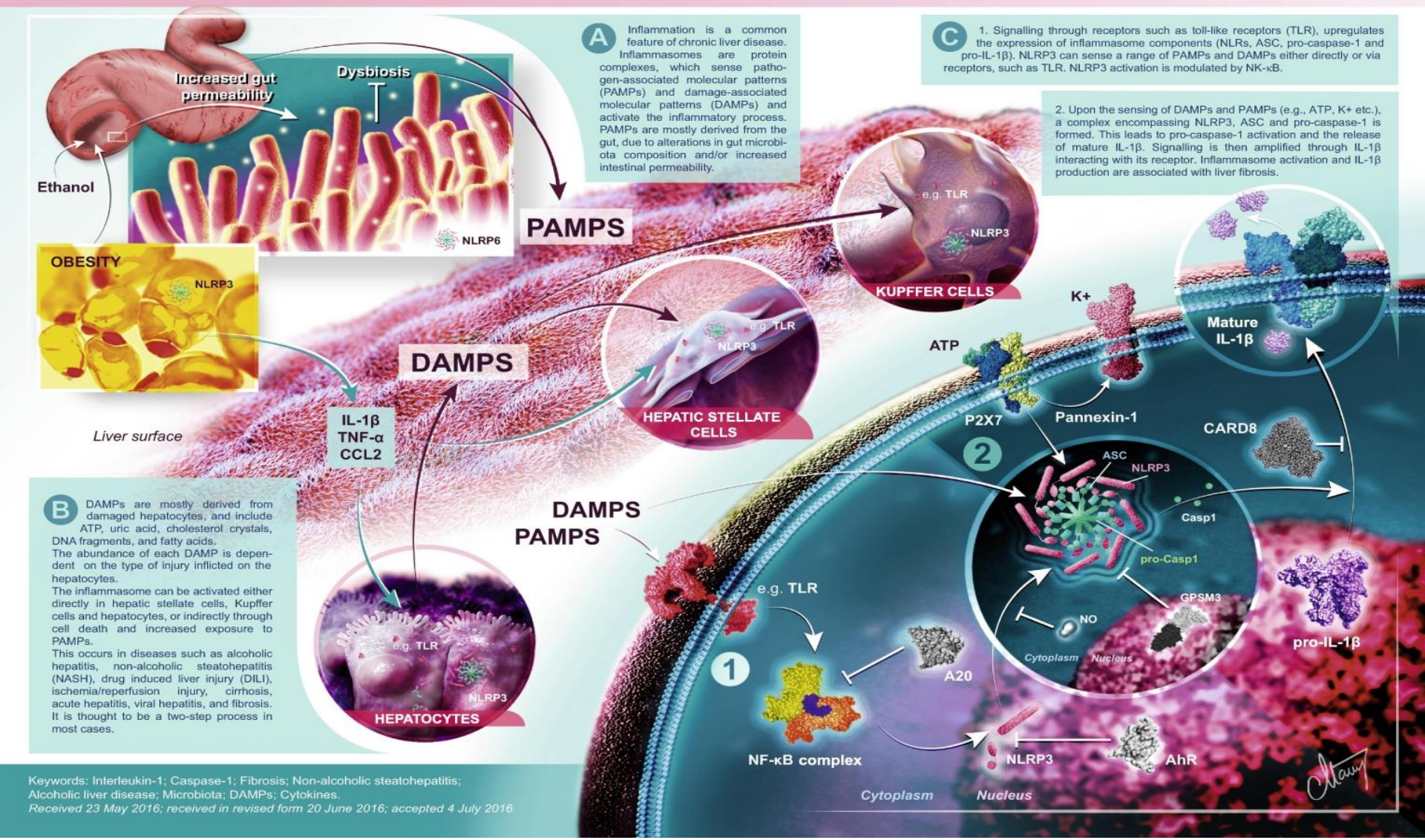
The inflammasome in liver disease

Alexander Wree[1,2,*], **Fabio Marra**[3,4,*]

[1]Department of Internal Medicine III, University Hospital, RWTH Aachen, Germany; [2]Department of Pediatric Gastroenterology, University of California San Diego (UCSD), and Rady Children's Hospital, San Diego, California, United States; [3]Dipartimento di Medicina Sperimentale e Clinica, University of Florence, Italy; [4]Research Center DENOTHE, University of Florence, Italy

EASL | JOURNAL OF HEPATOLOGY

Journal of Hepatology **2016** vol. 65 | 1055–1056



A Inflammation is a common feature of chronic liver disease. Inflammasomes are protein complexes, which sense pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) and activate the inflammatory process. PAMPs are mostly derived from the gut, due to alterations in gut microbiota composition and/or increased intestinal permeability.

B DAMPs are mostly derived from damaged hepatocytes, and include ATP, uric acid, cholesterol crystals, DNA fragments, and fatty acids.
The abundance of each DAMP is dependent on the type of injury inflicted on the hepatocytes.
The inflammasome can be activated either directly in hepatic stellate cells, Kupffer cells and hepatocytes, or indirectly through cell death and increased exposure to PAMPs.
This occurs in diseases such as alcoholic hepatitis, non-alcoholic steatohepatitis (NASH), drug induced liver injury (DILI), ischemia/reperfusion injury, cirrhosis, acute hepatitis, viral hepatitis, and fibrosis. It is thought to be a two-step process in most cases.

C 1. Signalling through receptors such as toll-like receptors (TLR), upregulates the expression of inflammasome components (NLRs, ASC, pro-caspase-1 and pro-IL-1β). NLRP3 can sense a range of PAMPs and DAMPs either directly or via receptors, such as TLR. NLRP3 activation is modulated by NK-κB.

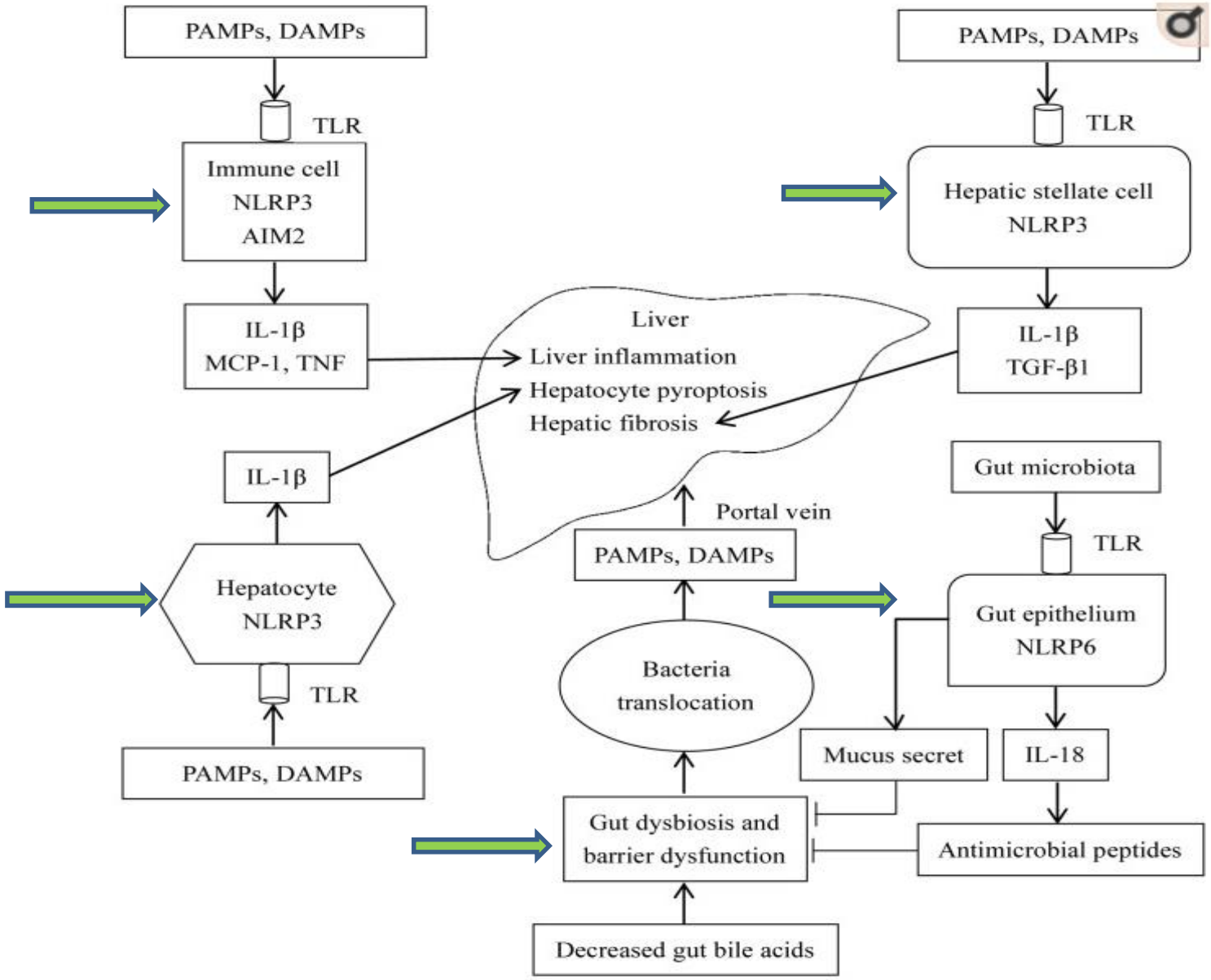
2. Upon the sensing of DAMPs and PAMPs (e.g., ATP, K+ etc.), a complex encompassing NLRP3, ASC and pro-caspase-1 is formed. This leads to pro-caspase-1 activation and the release of mature IL-1β. Signalling is then amplified through IL-1β interacting with its receptor. Inflammasome activation and IL-1β production are associated with liver fibrosis.

Keywords: Interleukin-1; Caspase-1; Fibrosis; Non-alcoholic steatohepatitis; Alcoholic liver disease; Microbiota; DAMPs; Cytokines.
Received 23 May 2016; received in revised form 20 June 2016; accepted 4 July 2016

ELSEVIER

EASL | JOURNAL OF HEPATOLOGY

Inflammasome in gut liver axis



Gut microbiome is profoundly altered in acute-on-chronic liver failure as evaluated by quantitative metagenomics

Aim: Investigation of gut microbiome alterations across the spectrum of disease (outpatients, AD, ACLF)

Methods: Microbial genes from stool DNA grouped into metagenomic species (MGS) based on abundance, and analyzed using non-parametric tests* and Spearman's correlation

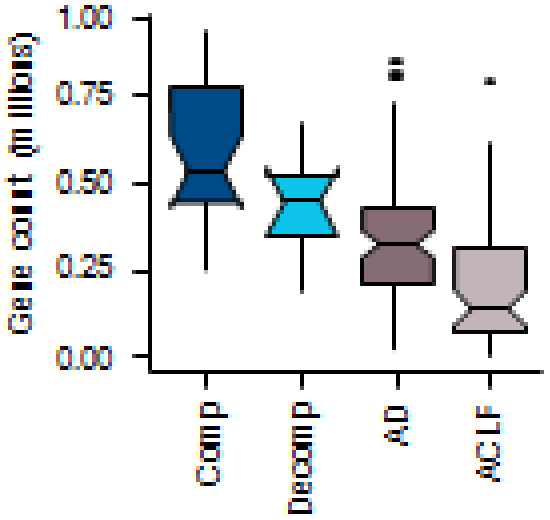
Results:

- 290/1,158 MGS contrasted between ≥1 disease stage. 44 contrasted between AD and ACLF
- Disease severity and organ failure correlated with human DNA in stools

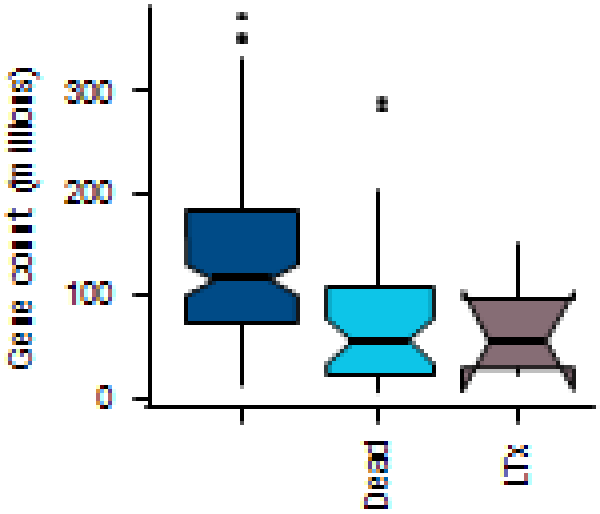
Conclusions

- Increasing disease severity is characterized by marked reductions in gene richness, especially in ACLF

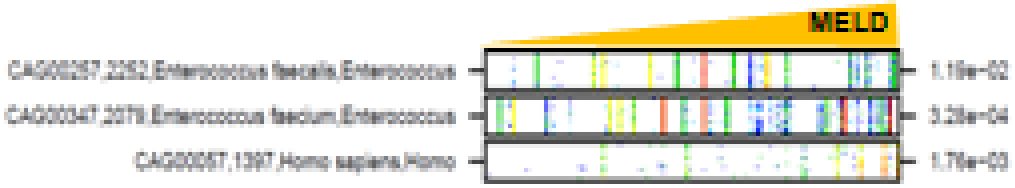
Disease stage correlates with reduced gene richness



Patient status at 3 months



Number of organ failures, and MELD and Child–Pugh scores, correlate with three *Enterococcus* MGS



*Kruskal–Wallis or Dunn tests Spearman's correlations

Bolle C, et al. ILC 2018, PS-015

Intestinal permeability in ACLF

Significant Increase in Intestinal Permeability in Acute-On-Chronic Liver Failure (ACLF) is Reversed After Slow Albumin Furosemide Infusion With or Without Terlipressin

V.P. Krishna, Gaurav Pandey*, Dinesh Kumar, Umesh Kumar, Samir Mohindra, Manjunath Hatti, Alok, Prabat N. Sharma, Vivek Saraswat

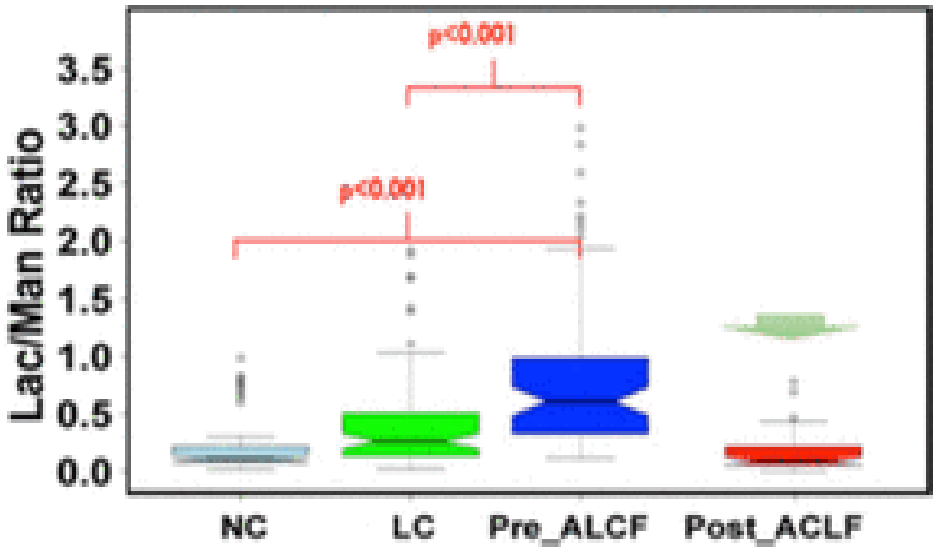
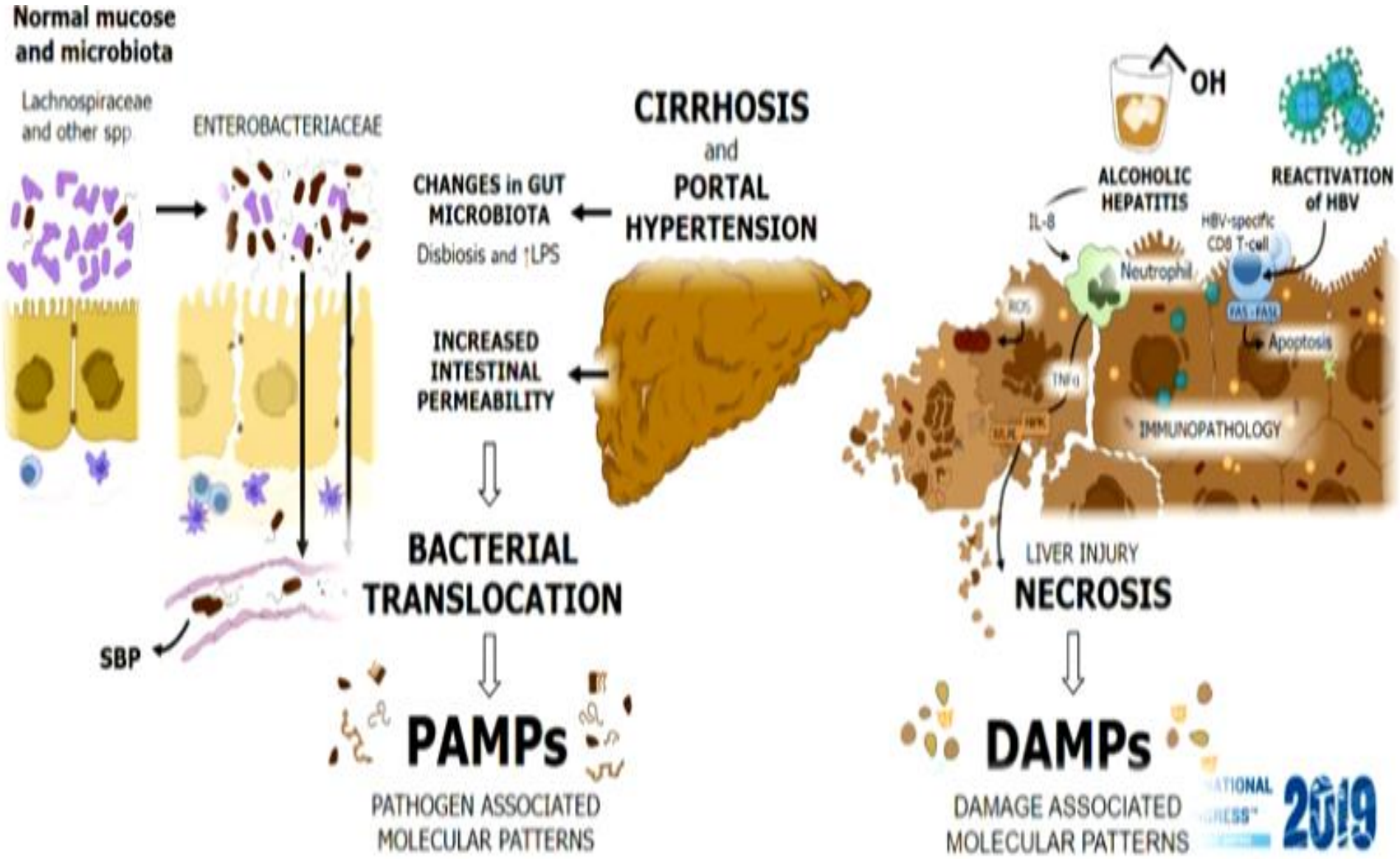
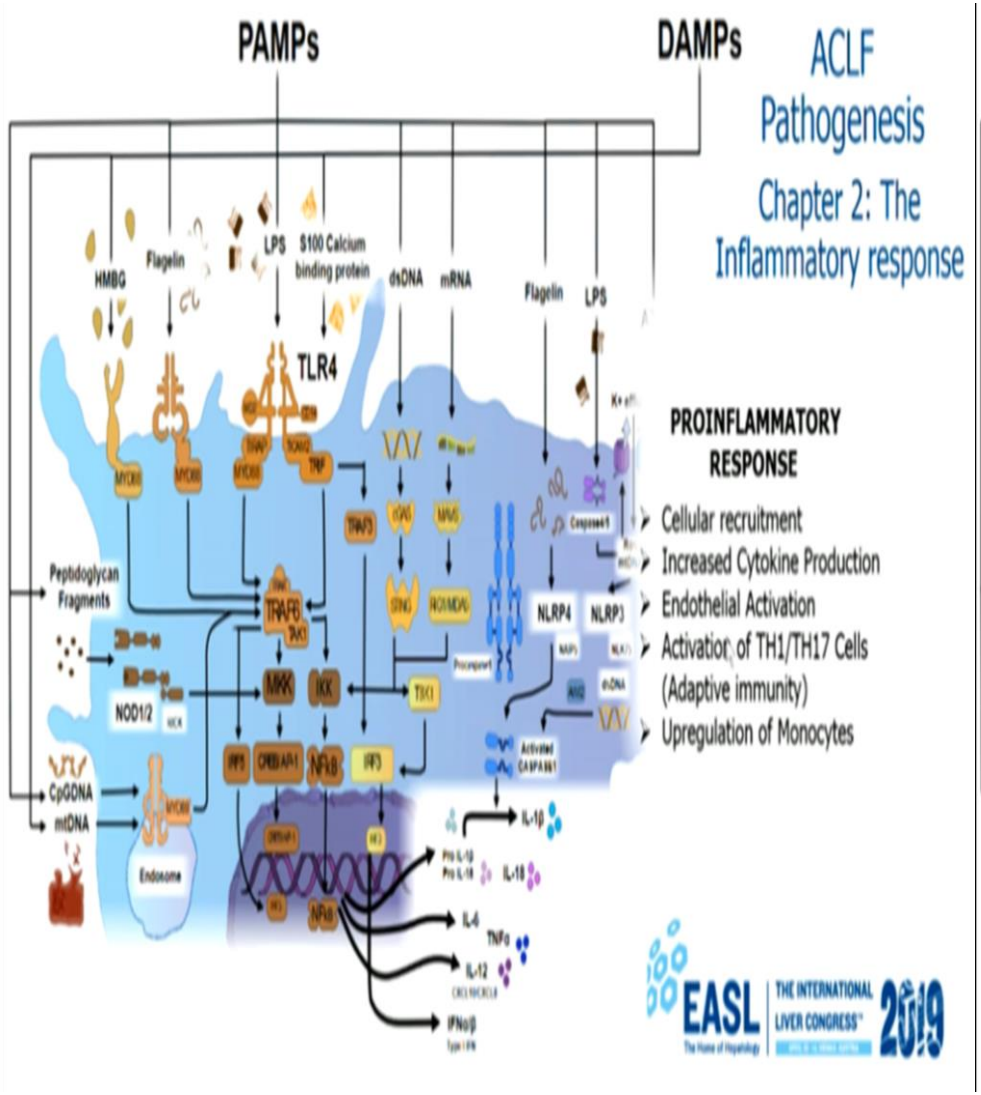


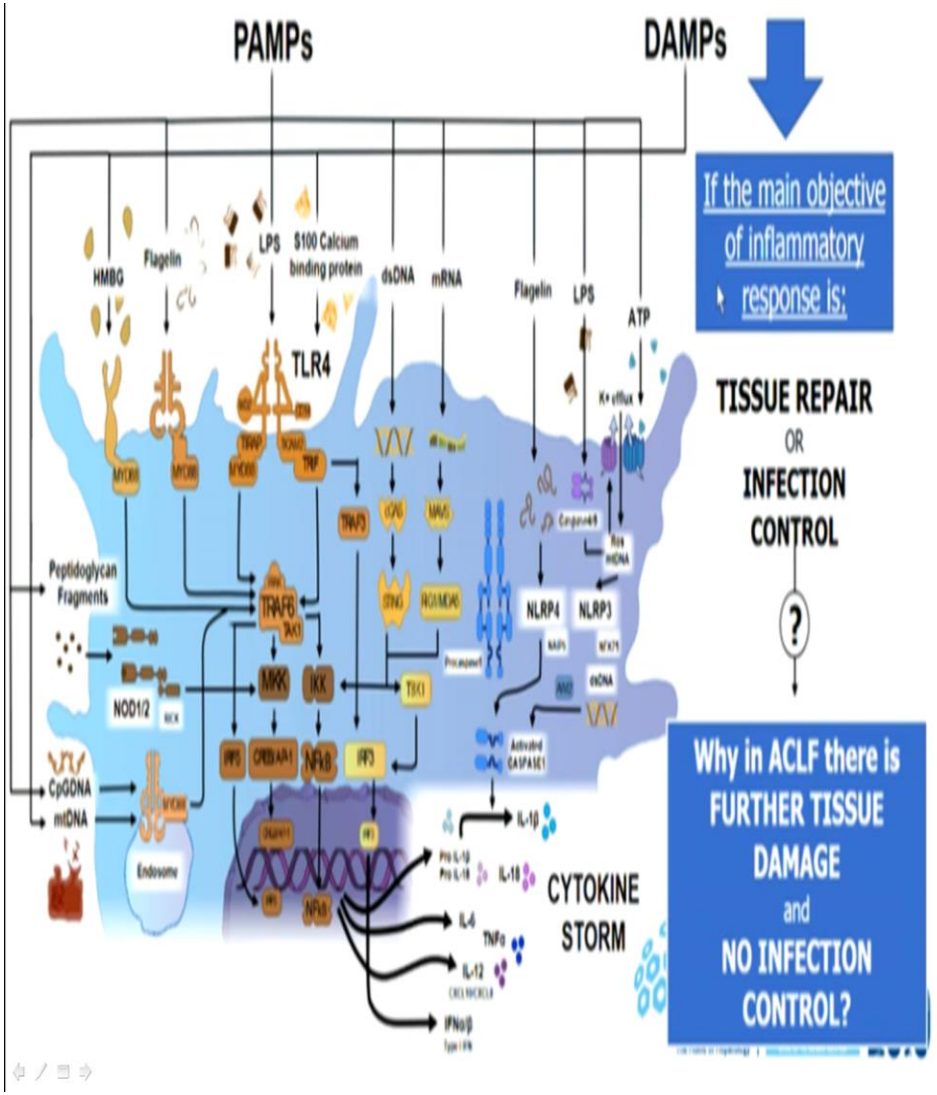
Figure 1. Mean LMR ratios among various groups – NC: normal controls, LC: decomp liver cirrhosis, and ACLF pre and post therapy.

ACLF Pathogenesis : a still uncompleted history
Inducers of inflammation

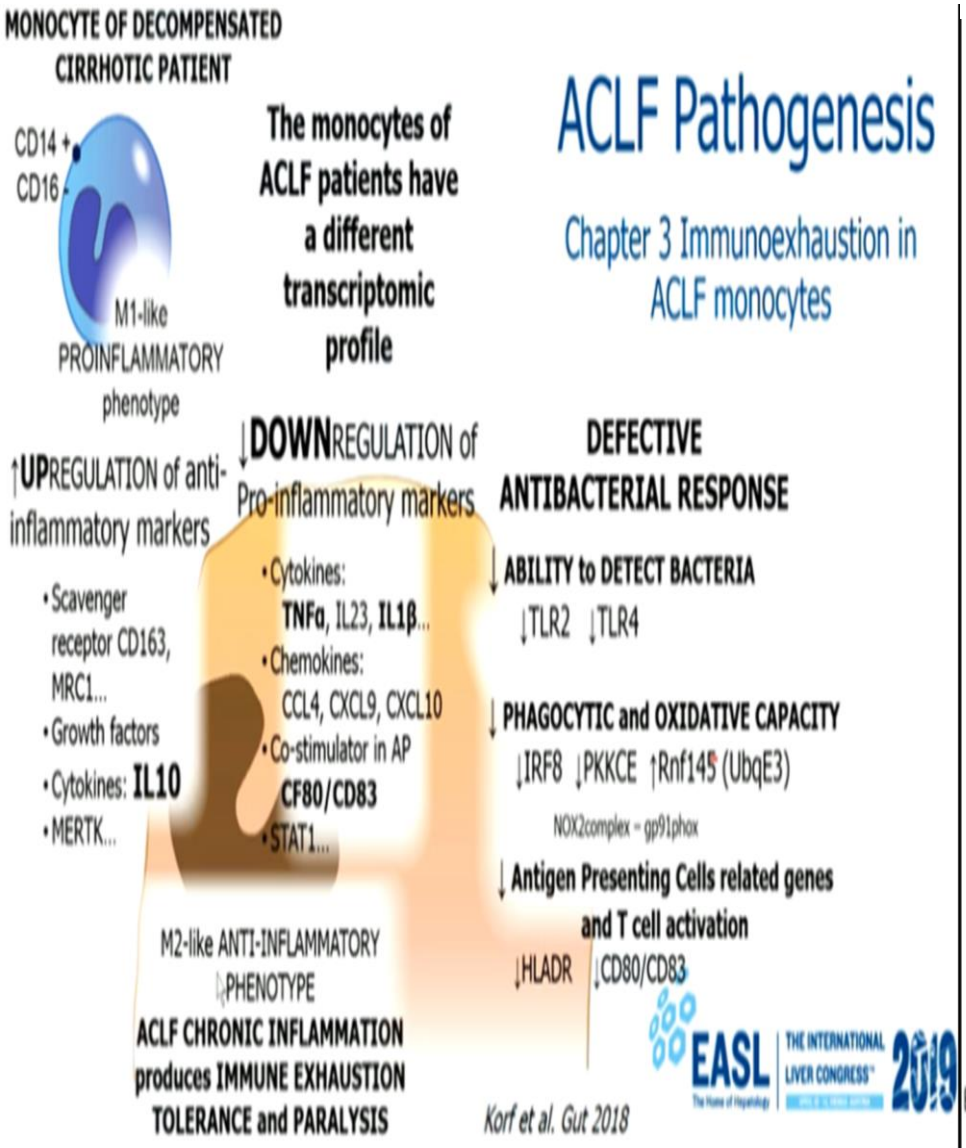


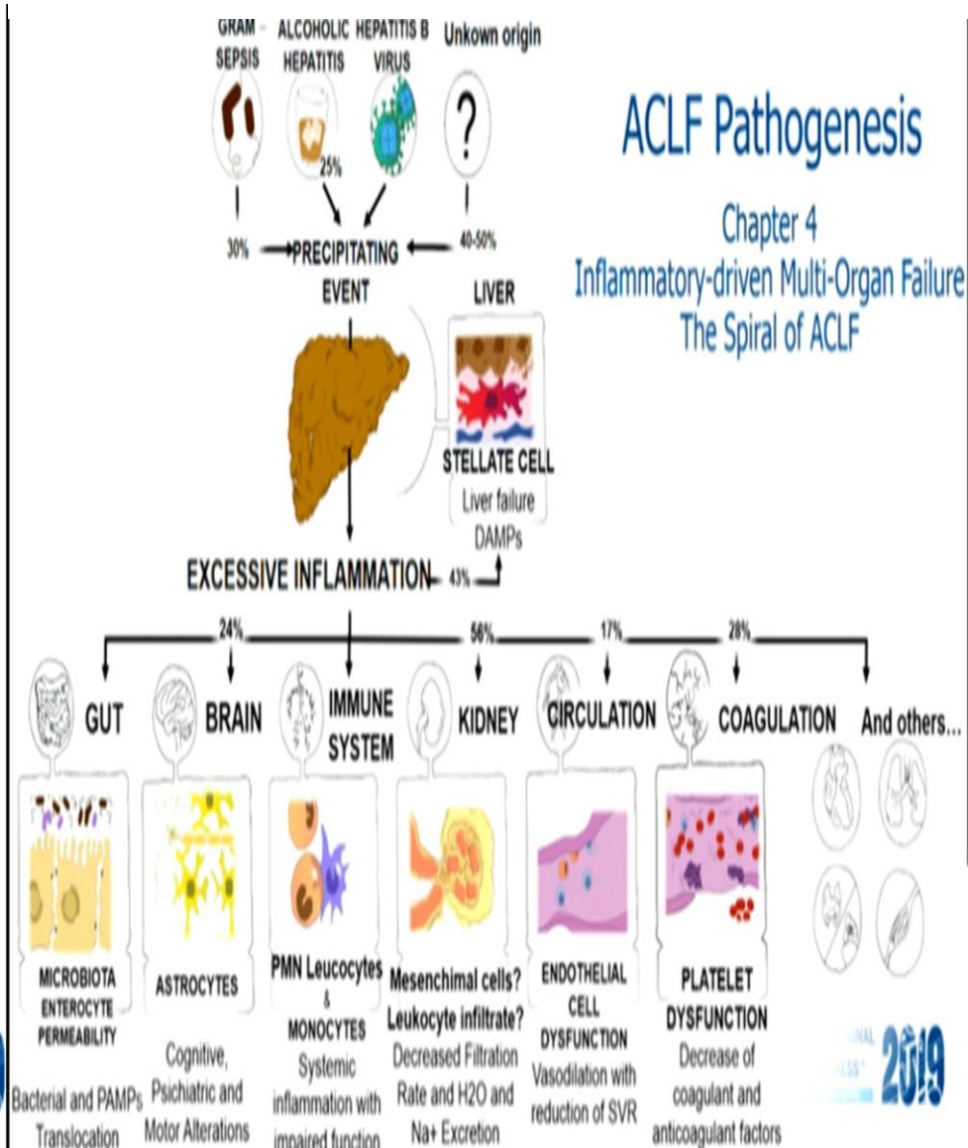
ACLF : Inflammatory response



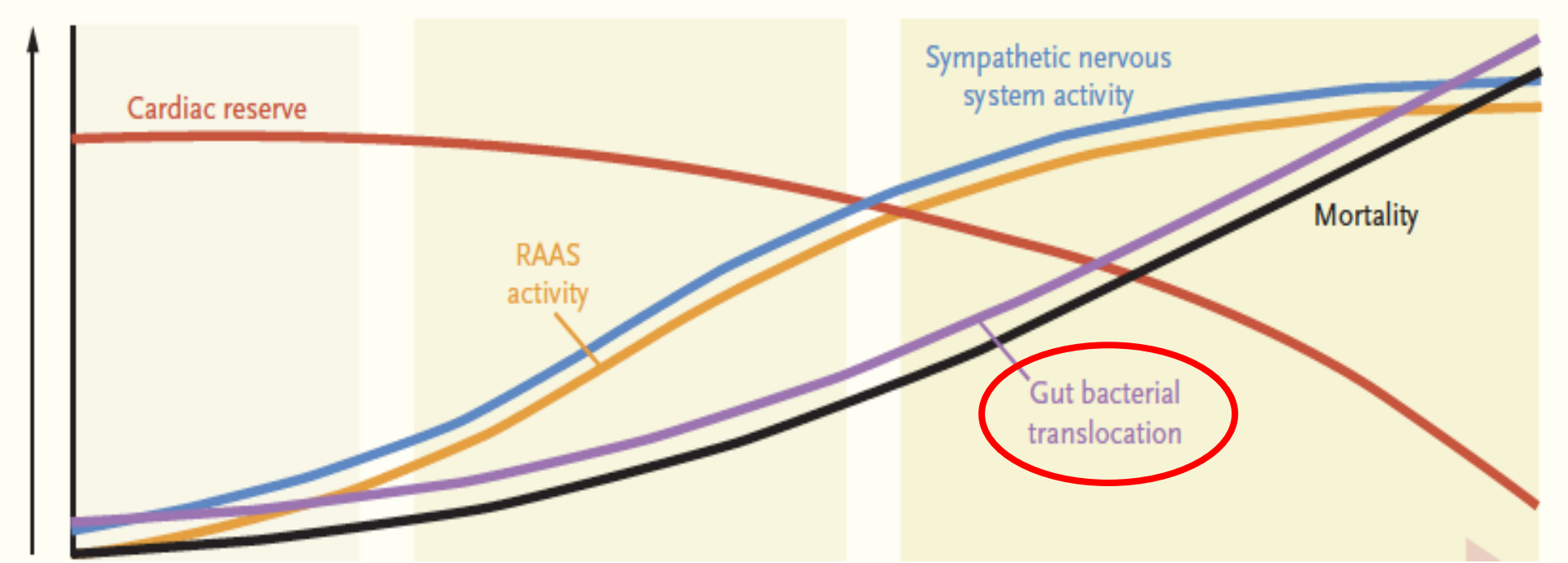


ACLF Pathogenesis





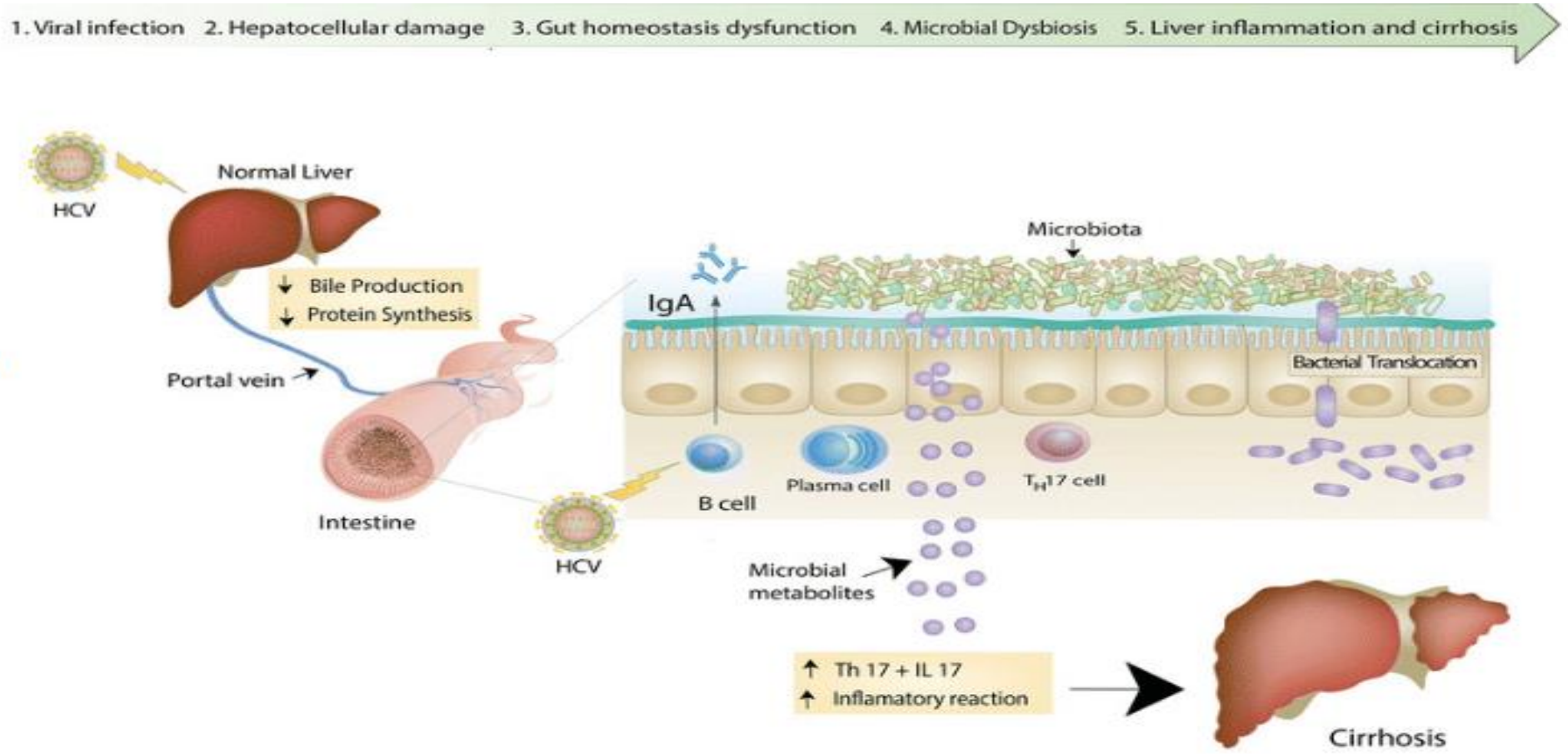
TODAY…
GUT MICROBIOTA HAS A PROGNOSTIC VALUE IN LIVER CIRRHOSIS



DISEASE PROGRESSION → **DISEASE PROGRESSION** → **DISEASE PROGRESSION**

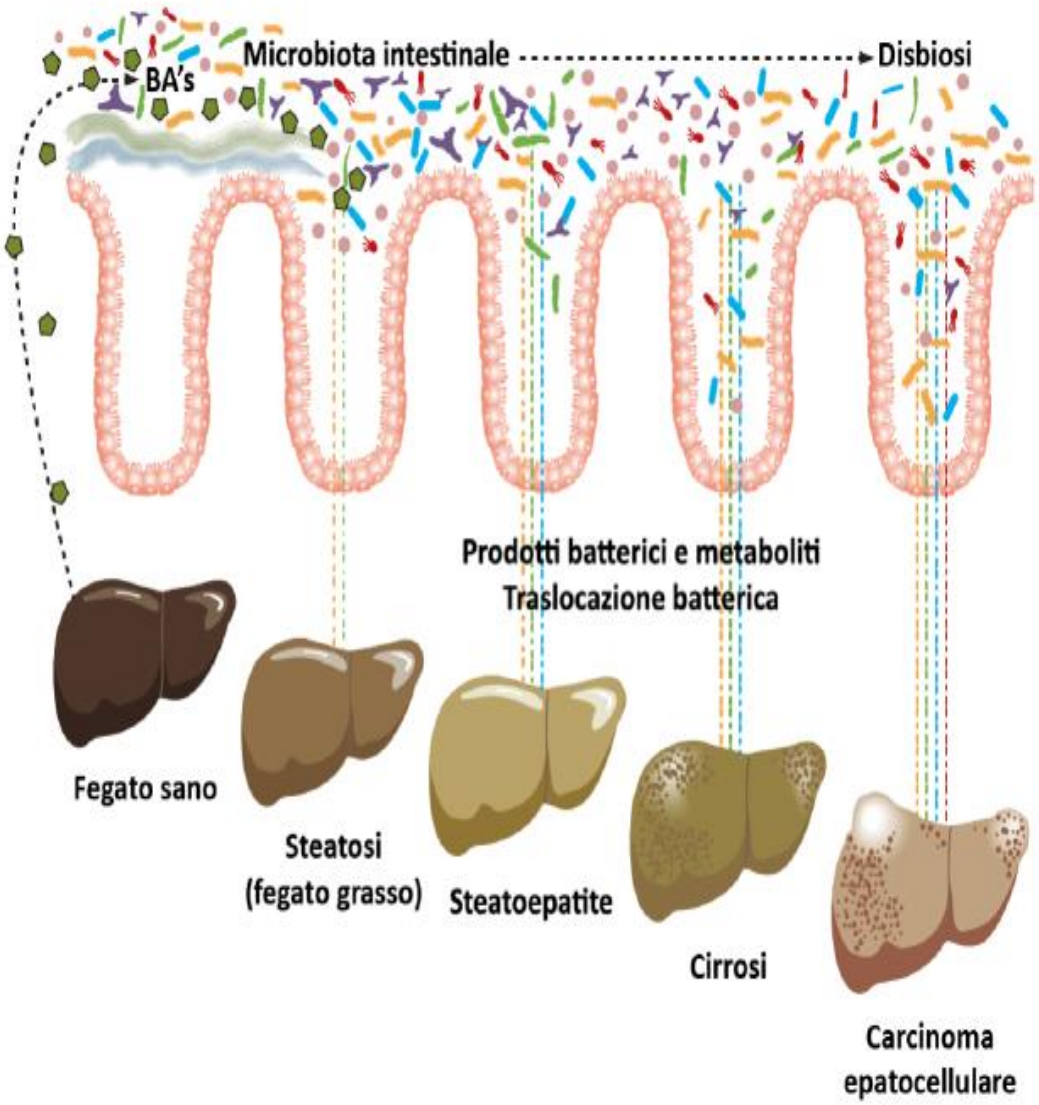
Ge PS et al. NEJM 2016

Consequences of acute HCV infection



Suggested gut-liver axis model for chronic HCV infection. HCV starts to invade hepatocytes with massive destruction of the hepatocyte architecture and structure (1), which leads to their loss of functions, such as bile salt production and protein synthesis (2). Consequently, such damage leads to disruption of the gut homeostasis and environmental changes (3), which can affect and change the microbiota abundance and composition (possible dysbiosis, 4). The overabundance of microbes, such as Prevotella, may produce signaling microbial metabolites that may influence and initiate the inflammatory mediators leading to liver inflammation and cirrhosis (5). This model is based on an integration of the findings of this study with literature [19, 20, 35–38, 42, 43, 45]

Disbiosi : sviluppo e progressione della malattia epatica

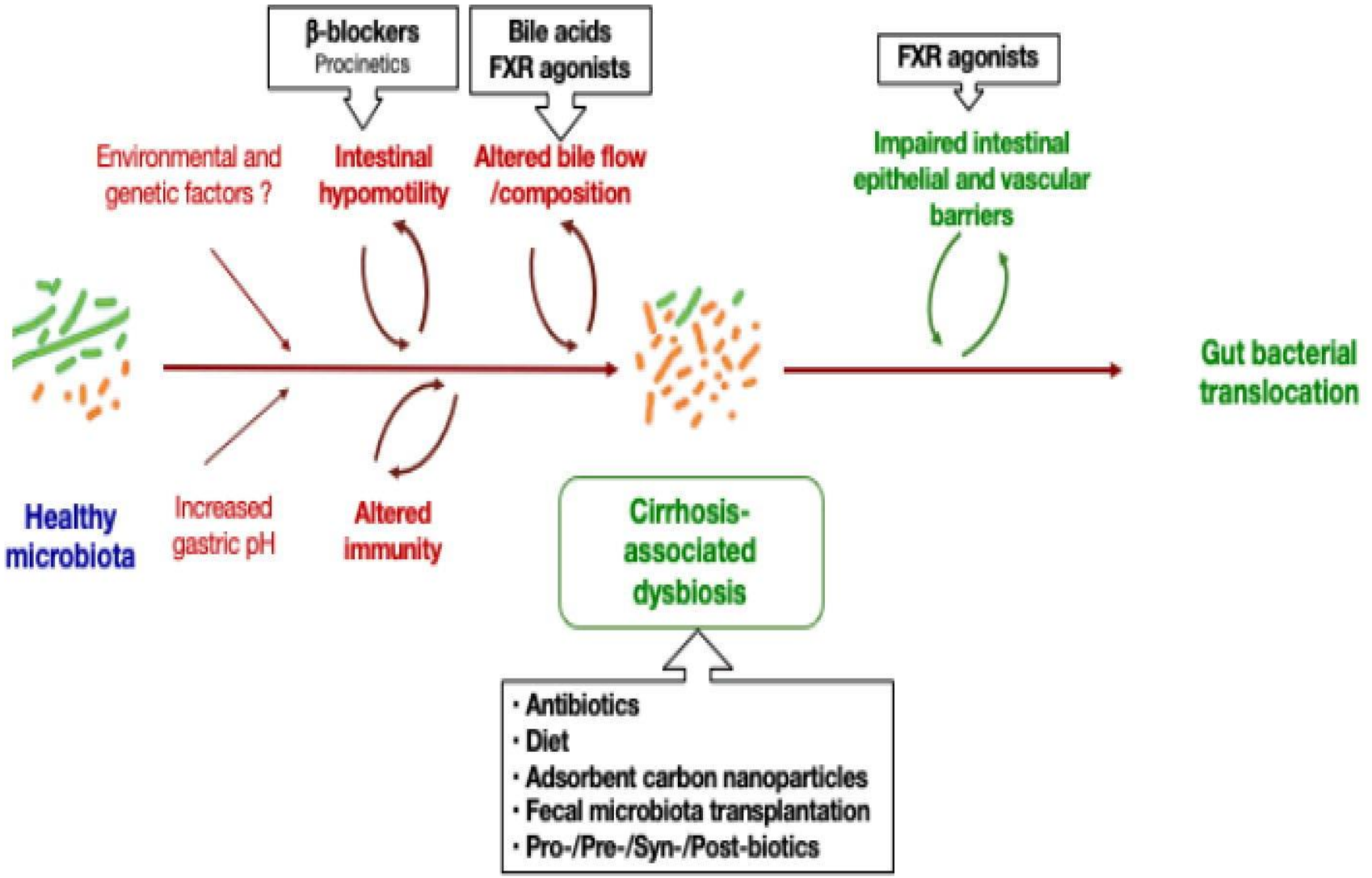


Infezione virale
Disordini metabolici
Alcol
Disordini autoimmuni
Disturbi coleastatici

DISFUNZIONE
DELLA BARRIERA
INTESTINALE

PERMEABILITÀ
DI MEMBRANA

TARGETING THE GUT LIVER AXIS INTERVENTIONS

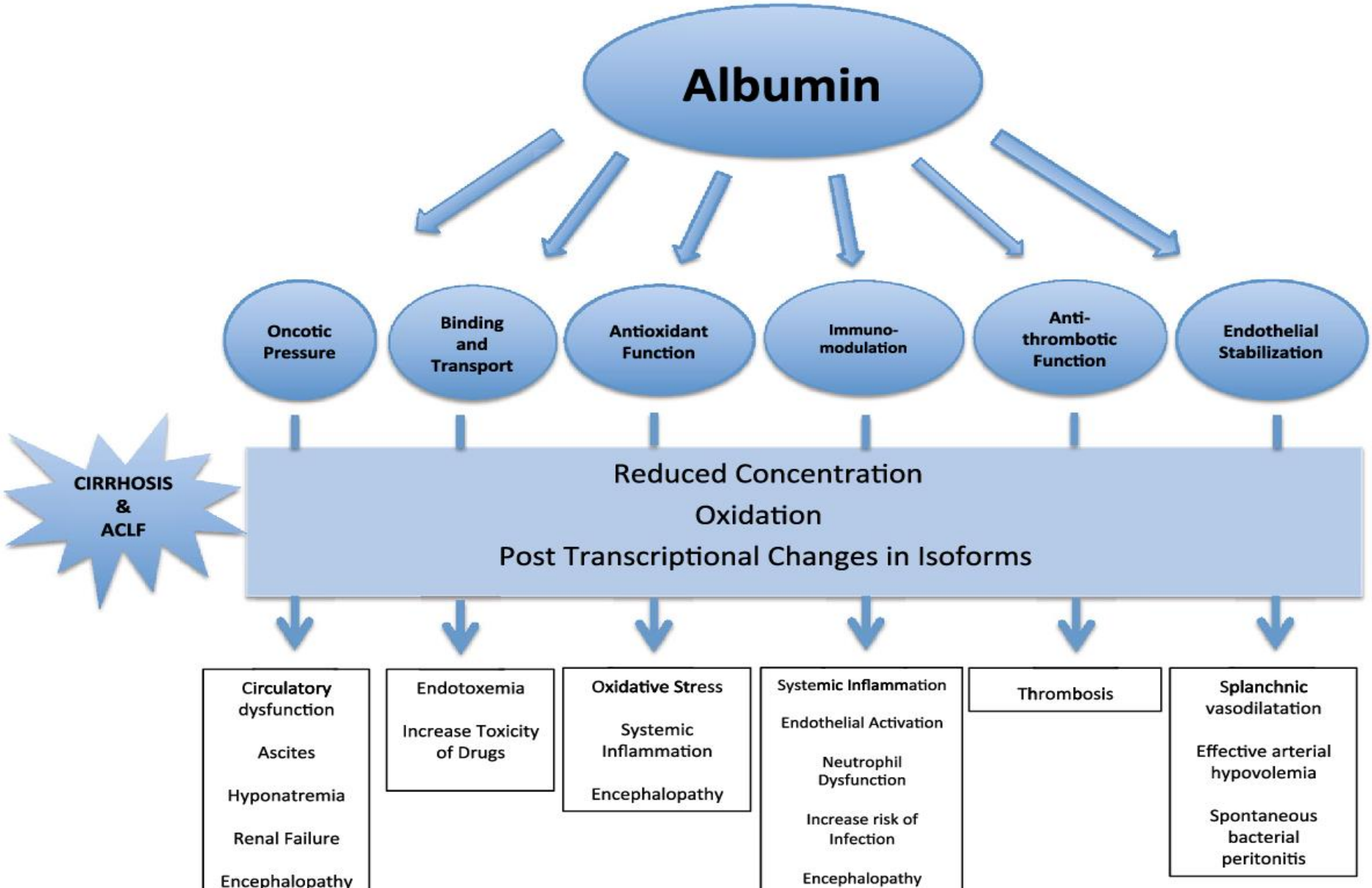


Albillos A, et al J Hepatol , 2019

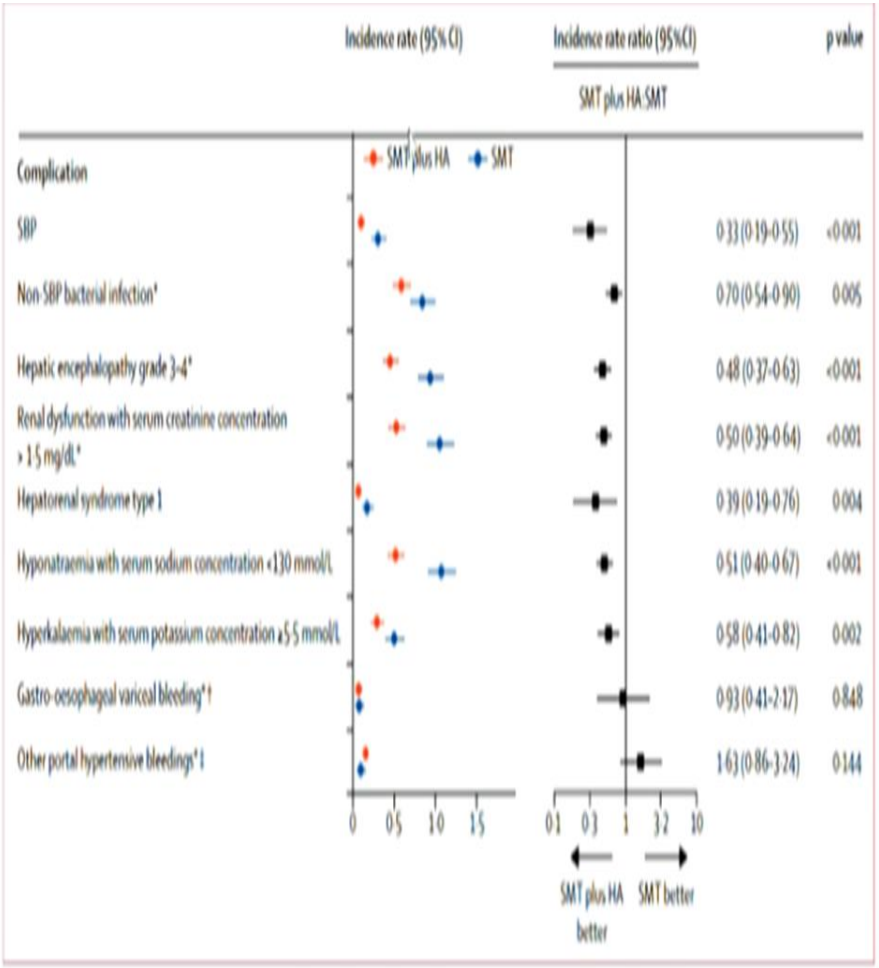
Agenda Gut – Liver – Axis Interventions

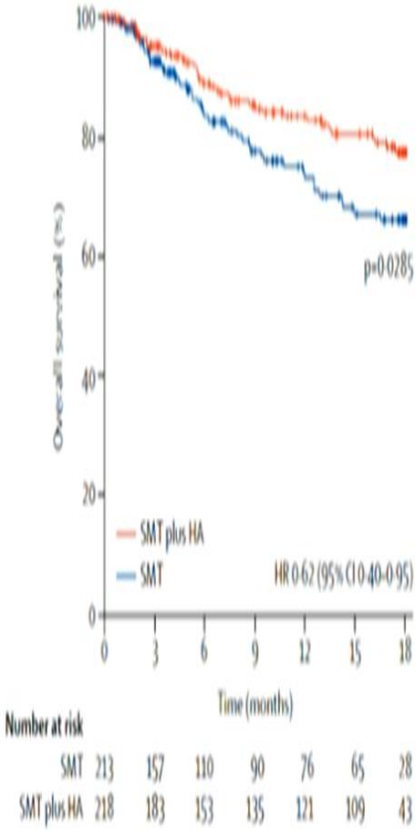
- Avoid PPI
- Prokinetic agent & NSBB
- Diet composition
- Intervention on immunity
- Poorly absorbable antibiotics
- Pre – Pro – Synbiotics
- FMT (Fecal Microbiota Transplantation)
- Bile Acids and FXR
- Absorbents
- GLP-2 agonist

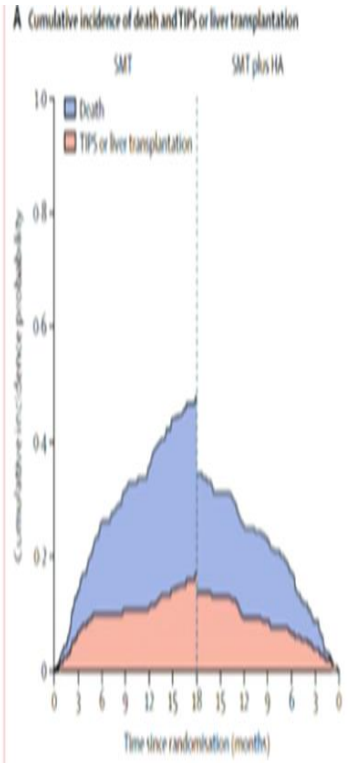
Functions of human serum albumin

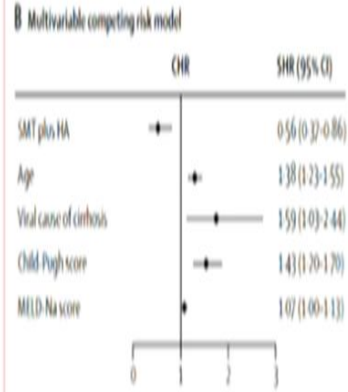


The Answer study : summary of results







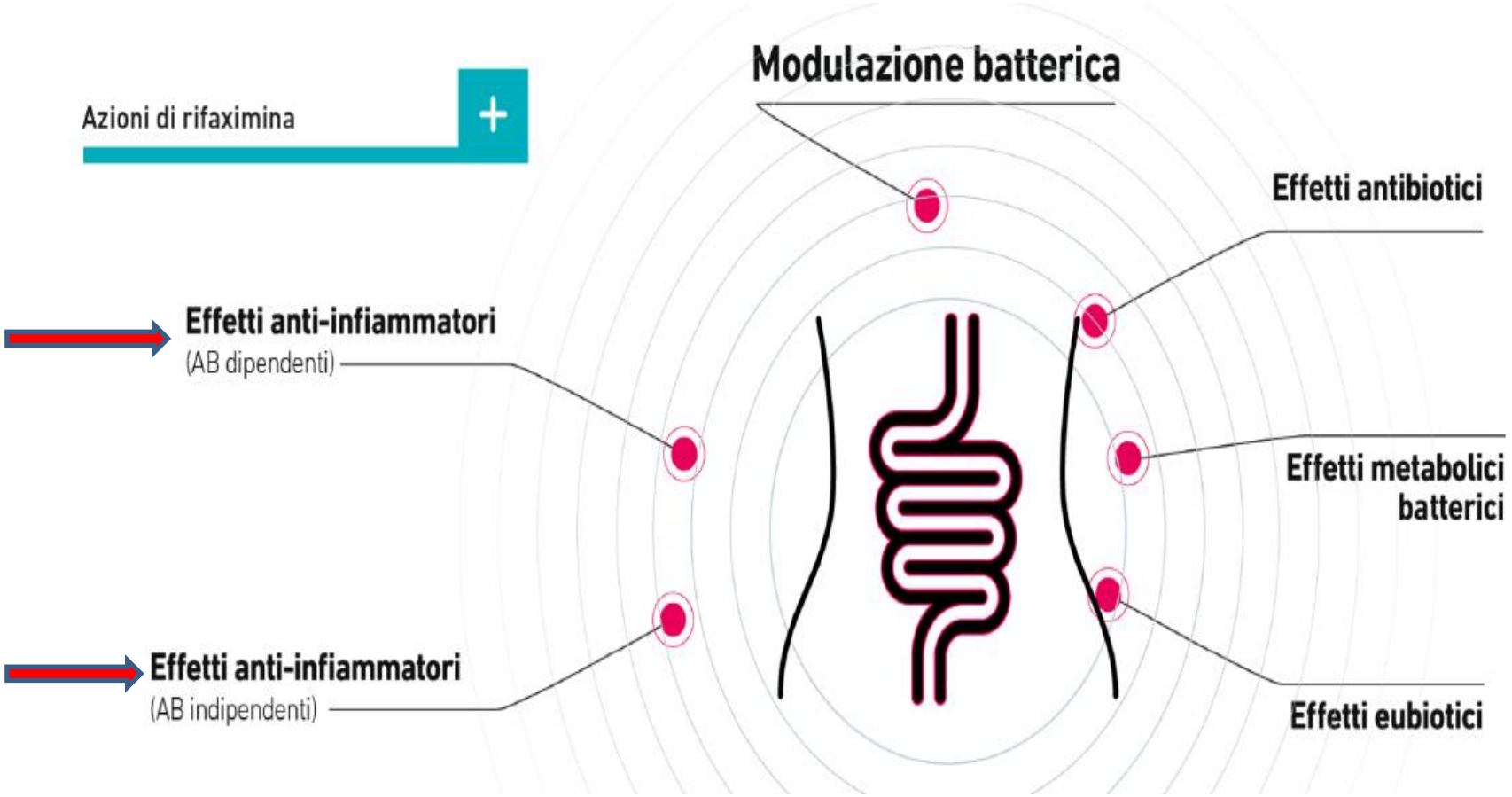


The chronic use of albumin decreased the incidence of the majority of complications of cirrhosis

EASL THE INTERNATIONAL LIVER CONGRESS™

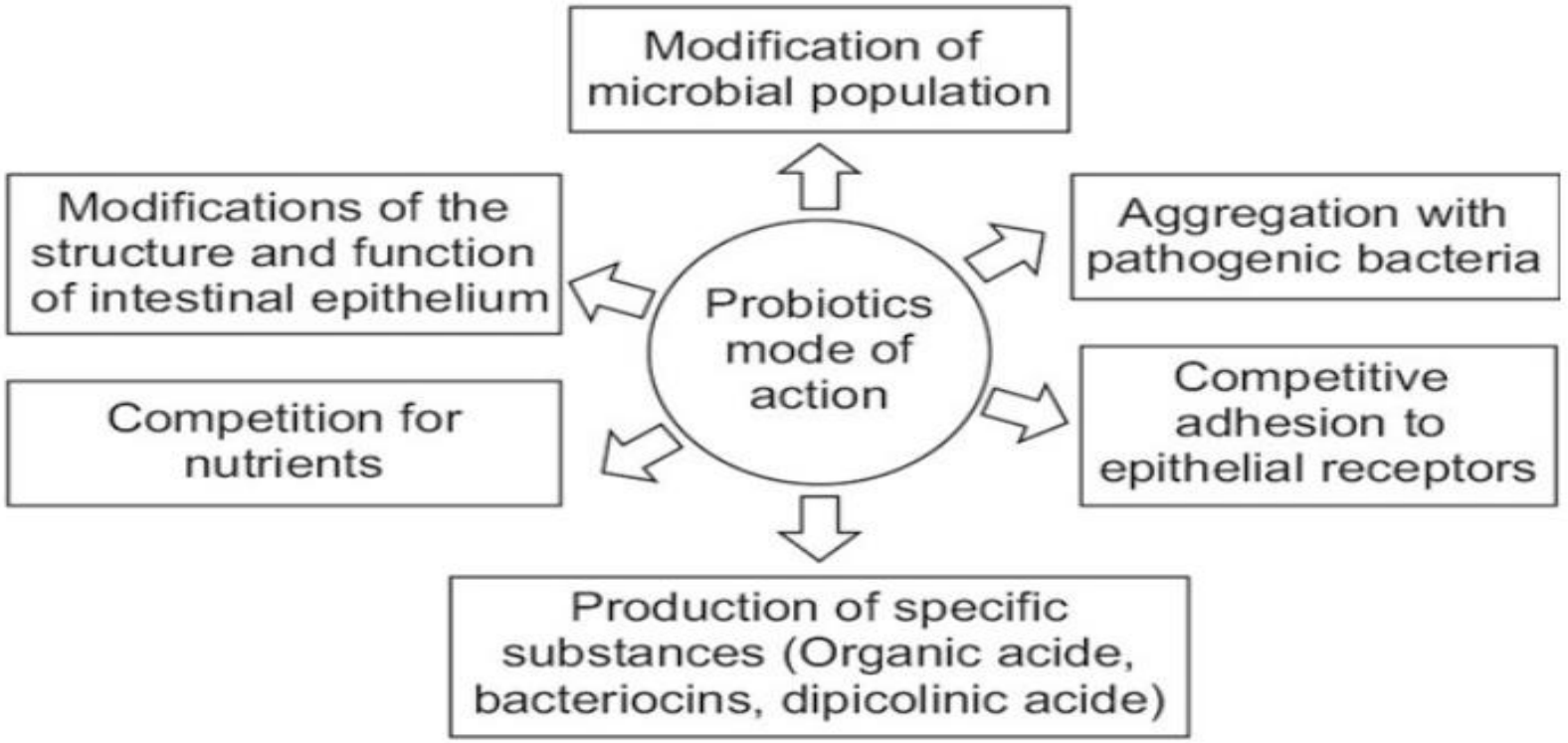
The chronic use of albumin increased survival

Rifaximin is broad-spectrum compound wich exerts endotoxin-lowering and anti-inflammatory effects rather than changing the composition of microbiota



RIFAXIMIN IS MORE THAN AN ANTIBIOTIC !

Probiotics : Mode of Action

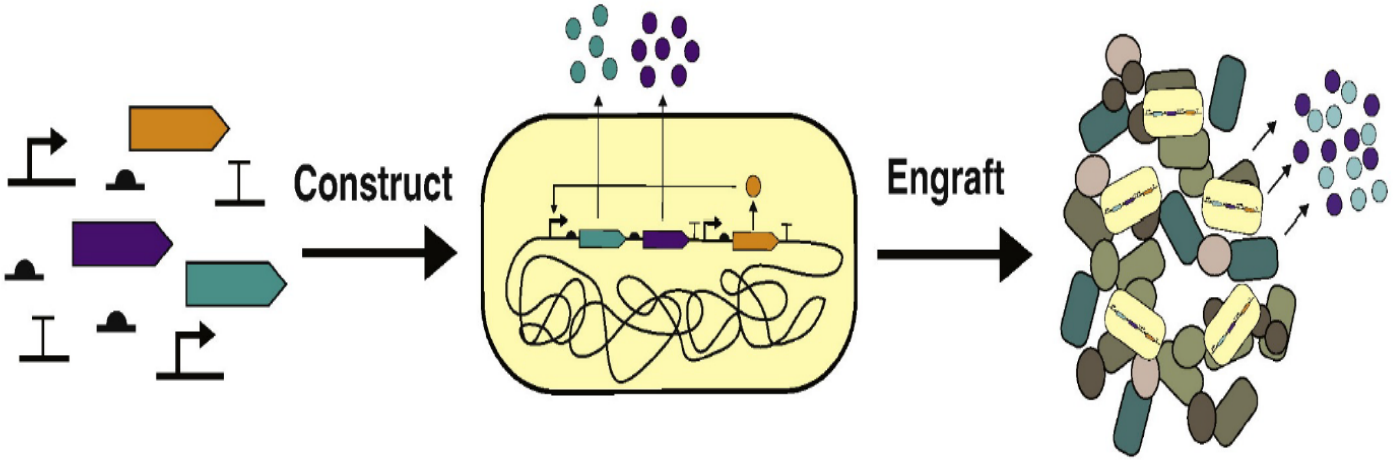


Tiwari et al, 2012

Prebiotics

Some prebiotic food products , like pectin , have been shown to prevent liver injury in " rodent models " by restoring the levels of Bacteroidetes

Precision Medecine
Engineered microbes to restore microbial ecology



Mimee M, Adv Drug Deliv Rev 2016

Engineered bacteria to reduce intestinal ammonia

Shen TC, J Clin Invest 2015; Kurtz CB, Sci Transl Med 2019

Synlogic, Aug 20, 2019: Interim analysis of a randomized, double-blind, placebo-controlled Phase 1b/2a study with SYNB1020 in 23 patients with cirrhosis and elevated blood ammonia: no evidence of blood ammonia lowering or changes in other exploratory endpoints relative to placebo

Lactobacillus Reuteri bio-engineered

Hepatology
Original article

PDF

Bacteria engineered to produce IL-22 in intestine induce expression of REG3G to reduce ethanol-induced liver disease in mice

Tim Hendrikx[1], Yi Duan[1, 2], Yanhan Wang[1, 2], Jee-Hwan Oh[3], Laura M Alexander[3], Wendy Huang[4], Peter Stärkel[5], Samuel B Ho[1, 2], Bei Gao[6], Oliver Fiehn[6], Patrick Emond[7, 8], Harry Sokol[9, 10, 11], Jan-Peter van Pijkeren[3], Bernd Schnabl[1, 2]

Author affiliations

Abstract

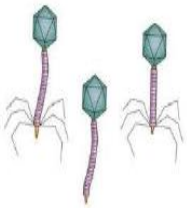
Objective Antimicrobial C-type lectin regenerating islet-derived 3 gamma (REG3G) is suppressed in the small intestine during chronic ethanol feeding. Our aim was to determine the mechanism that underlies REG3G suppression during experimental alcoholic liver disease.

Design Interleukin 22 (IL-22) regulates expression of REG3G. Therefore, we investigated the role of IL-22 in mice subjected to chronic-binge ethanol feeding (NIAAA model).

Results In a mouse model of alcoholic liver disease, we found that type 3 innate lymphoid cells produce lower levels of IL-22. Reduced IL-22 production was the result of ethanol-induced dysbiosis and lower intestinal levels of indole-3-acetic acid (IAA), a microbiota-derived ligand of the aryl hydrocarbon receptor (AHR), which regulates expression of IL-22. Importantly, faecal levels of IAA were also found to be lower in patients with alcoholic hepatitis compared with healthy controls. Supplementation to restore intestinal levels of IAA protected mice from ethanol-induced steatohepatitis by inducing intestinal expression of IL-22 and REG3G, which prevented translocation of bacteria to liver. We engineered *Lactobacillus reuteri* to produce IL-22 (*L. reuteri*/IL-22) and fed them to mice along with the ethanol diet; these mice had reduced liver damage, inflammation and bacterial translocation to the liver compared with mice fed an isogenic control strain and upregulated expression of REG3G in intestine. However, *L. reuteri*/IL-22 did not reduce ethanol-induced liver disease in *Reg3g*$^{-/-}$ mice.

Conclusion Ethanol-associated dysbiosis reduces levels of IAA and activation of the AHR to decrease expression of IL-22 in the intestine, leading to reduced expression of REG3G; this results in bacterial translocation to the liver and steatohepatitis. Bacteria engineered to produce IL-22 induce expression of REG3G to reduce ethanol-induced steatohepatitis.

Bacteriophages to selectively eliminate deleterious microbes



Bacteriophage

Bacteriocins

Antibiotics

Use of a bacteriophage cocktail for eradication of *Klebsiella pneumoniae* in primary sclerosing cholangitis

Objectives:

Klebsiella pneumoniae (KP) has been shown to be a PSC pathobiont. The present study aims to (1) validate KP as a disease-associated species and (2) initiate development of a bacteriophage-based therapy for KP eradication.

Methods:

Stool from patients was collected from several geographic regions and was assessed for the presence of KP by metagenomic sequencing and culturing on selective-media plates. For phage discovery, environmental samples were screened on lawns of PSC derived KP and natural phage isolated from plaques.

Main Findings:

- Cohort analyses of fecal samples from multiple geographies revealed that KP abundance was higher in PSC patients than in healthy controls and was associated with disease severity.
- Phage hunting efforts for the pathobiont KPs yielded over 20 phages from which >100 combinations (cocktails) were designed and tested for their ability to reduce KP bacterial burden in vitro (Figure 1).

Conclusions:

These results support the role of KP as a PSC pathobiont and establish the first steps in development of a rationally designed phage cocktail targeting KP, which may provide a novel treatment approach for PSC patients.

Kredo-Russo S, et al., Abstract 26

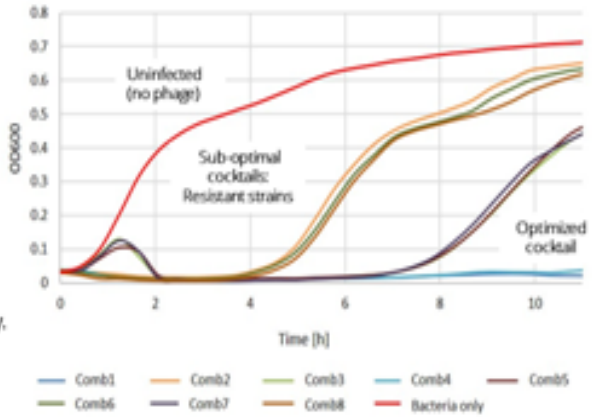


Figure 1. KP strains were grown over night at 37 °C with agitation to OD600=1.5, diluted 1:1000 in BHIS and grown to the desired OD. Different phage combinations were added at 10^6 PFU each per well. NPC, negative control without phages (red). Several combinations are non-optimal for eradication as growth resumes at 4 and 7 hours (green and brown), while other combinations are optimal for eradication (blue).

Mimee M, Adv Drug Deliv Rev 2016

FMT for seroconversion HBeAg / antiHBe ?

HEPATOLOGY

AASLD

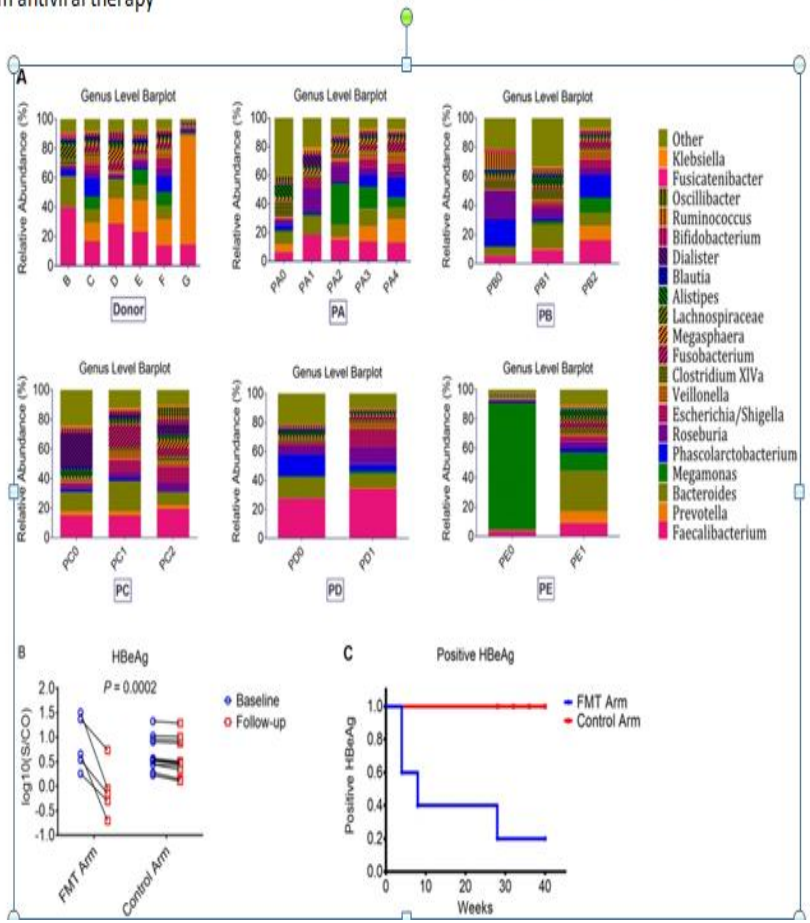
Clinical Observations in Hepatology | Free Access

Fecal microbiota transplantation induces hepatitis B virus e-antigen (HBeAg) clearance in patients with positive HBeAg after long-term antiviral therapy

Yan-Dan Ren, Zhen-Shi Ye, Liu-Zhu Yang, Li-Xin Jin, Wen-Jun Wei, Yong-Yue Deng ... See all authors

First published: 27 December 2016 | https://doi.org/10.1002/hep.29008 | Citations: 27

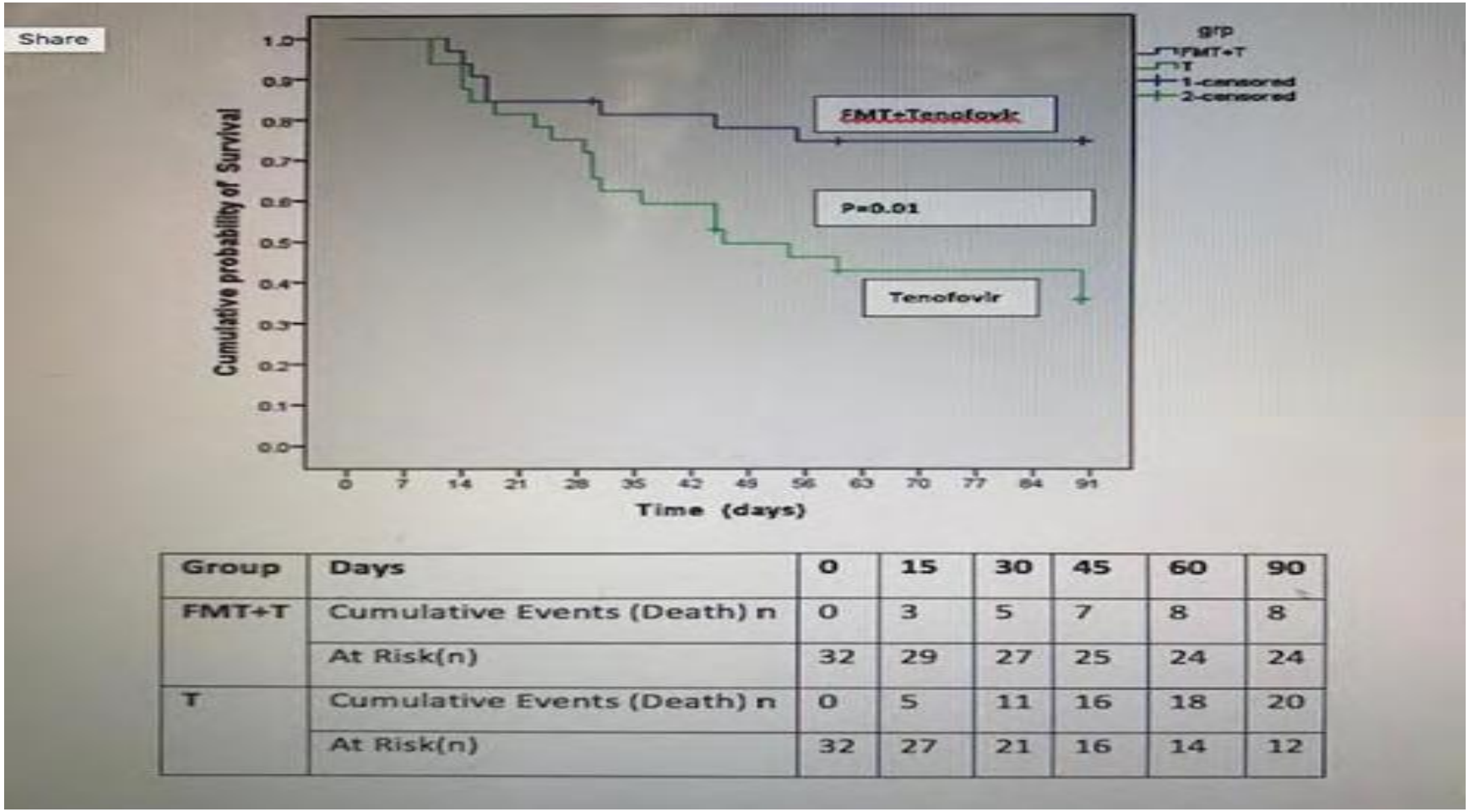
Fecal microbiota transplantation induces hepatitis B virus e-antigen (HBeAg) clearance in patients with positive HBeAg after long-term antiviral therapy



EASL 2019

Fecal microbiota transplantation with Tenofovir is superior to Tenofovir alone in improving clinical outcomes in ACLF due to HBV reactivation. An open label randomized controlled trial

Juned Ahmal et al .



Group	Days	0	15	30	45	60	90
FMT+T	Cumulative Events (Death) n	0	3	5	7	8	8
	At Risk(n)	32	29	27	25	24	24
T	Cumulative Events (Death) n	0	5	11	16	18	20
	At Risk(n)	32	27	21	16	14	12

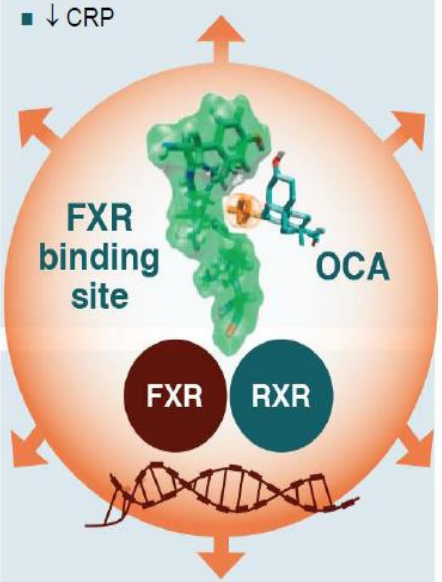
Bile acid receptor Farnesoid X (FXR) is central to a multitude of pathways

FIBROSIS

- ↓Stellate cell activation (α-SMA)
- ↑Stellate cell apoptosis (TIMP-1)
- ↓Fibrogenesis (TGF-β1)
- ↑Matrix degradation (MMP-2)

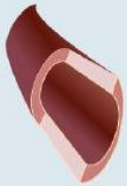
INFLAMMATION

- ↓ NF-κB
- ↓ TNFα, IL-1β, IL-17, IFN-γ
- ↓ CRP



GLUCOSE METABOLISM

- ↑ Insulin signaling (FGF19)
- ↑ Insulin sensitivity (IRS-1, IRS-2)
- ↑ Insulin production (KLF11, GLUT-2)
- ↓ Hepatic gluconeogenesis (PEPCK)



ATHEROSCLEROSIS

- ↑Vasodilation (eNOS)
- ↓Inflammation (COX-2, iNOS)
- ↓Calcification (JNK)
- ↓Smooth muscle cell migration

LIPID METABOLISM

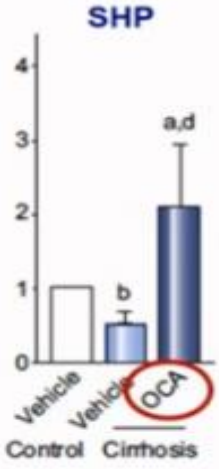
- ↓Triglyceride synthesis (SREBP-1c)
- ↑Triglyceride clearance (apoC-III)
- ↓HDL-C (SR-B1, CETP)
- ↑LDL-C (CETP)

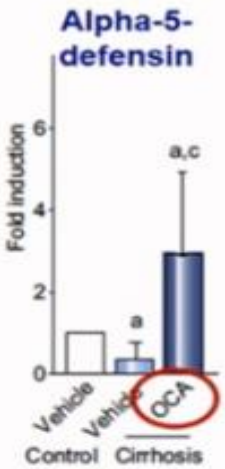
BILE ACID HOMEOSTASIS

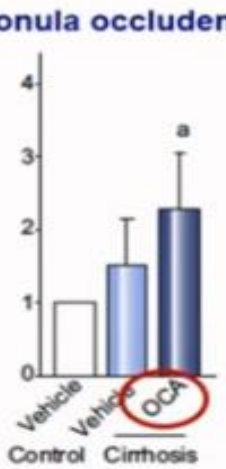
- ↓ Bile acid synthesis (CYP7A1)
- ↓ Bile acid uptake (NTCP)
- ↑ Bile acid secretion (BSEP)
- ↓Bile acid absorption (ASBT)

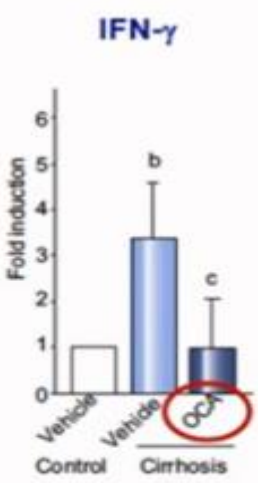
Obeticholic acid reduces gut bacterial translocation , modifies dysbiosis , improves innate defense and reduce inflammation in the ileum of CCl4- cirrhotic rats

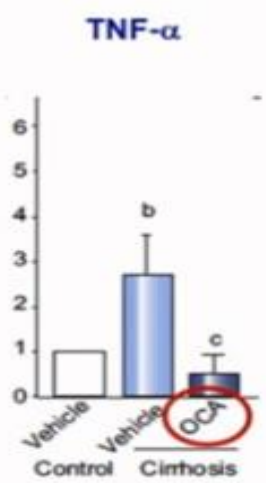
Obeticholic acid 5 mg/kg.d, 2 wk / vehicle
CCl_4-cirrhotic rats with ascites or controls

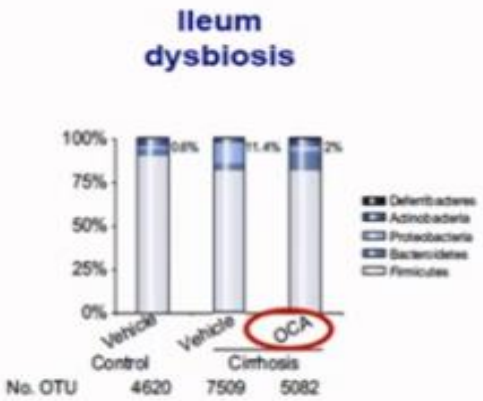


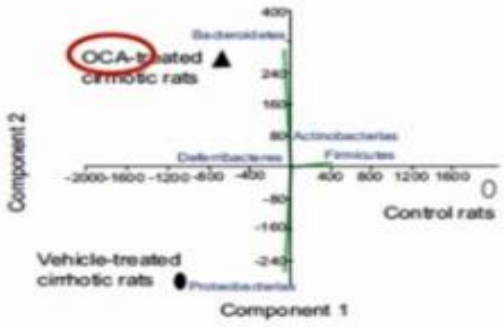


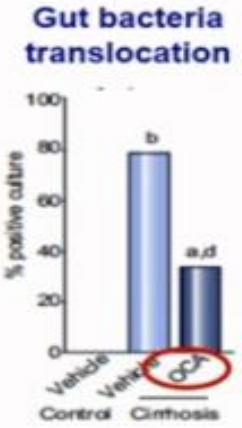










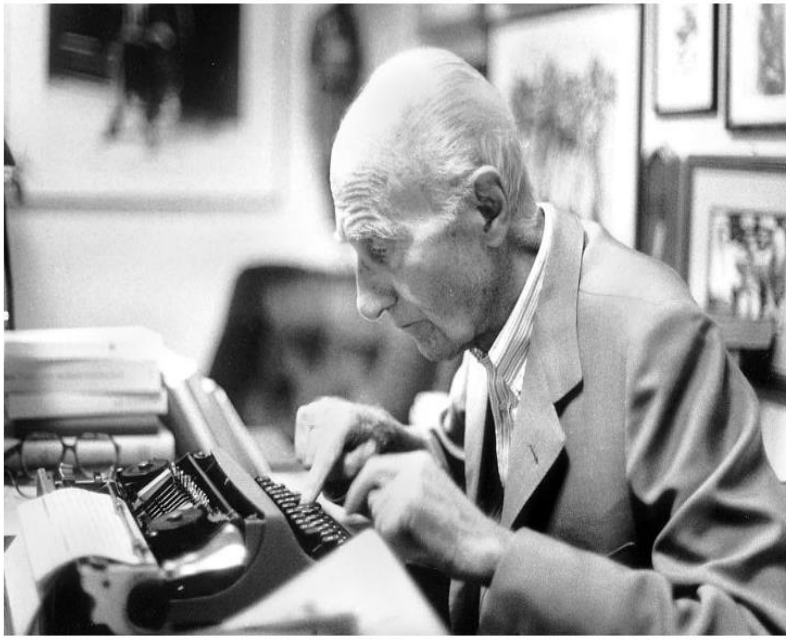


M Úbeda et al.
JHEP 2016

L’ Italia e gli italiani

Moglie malata Sla, ipoteca casa per finanziare ricerca

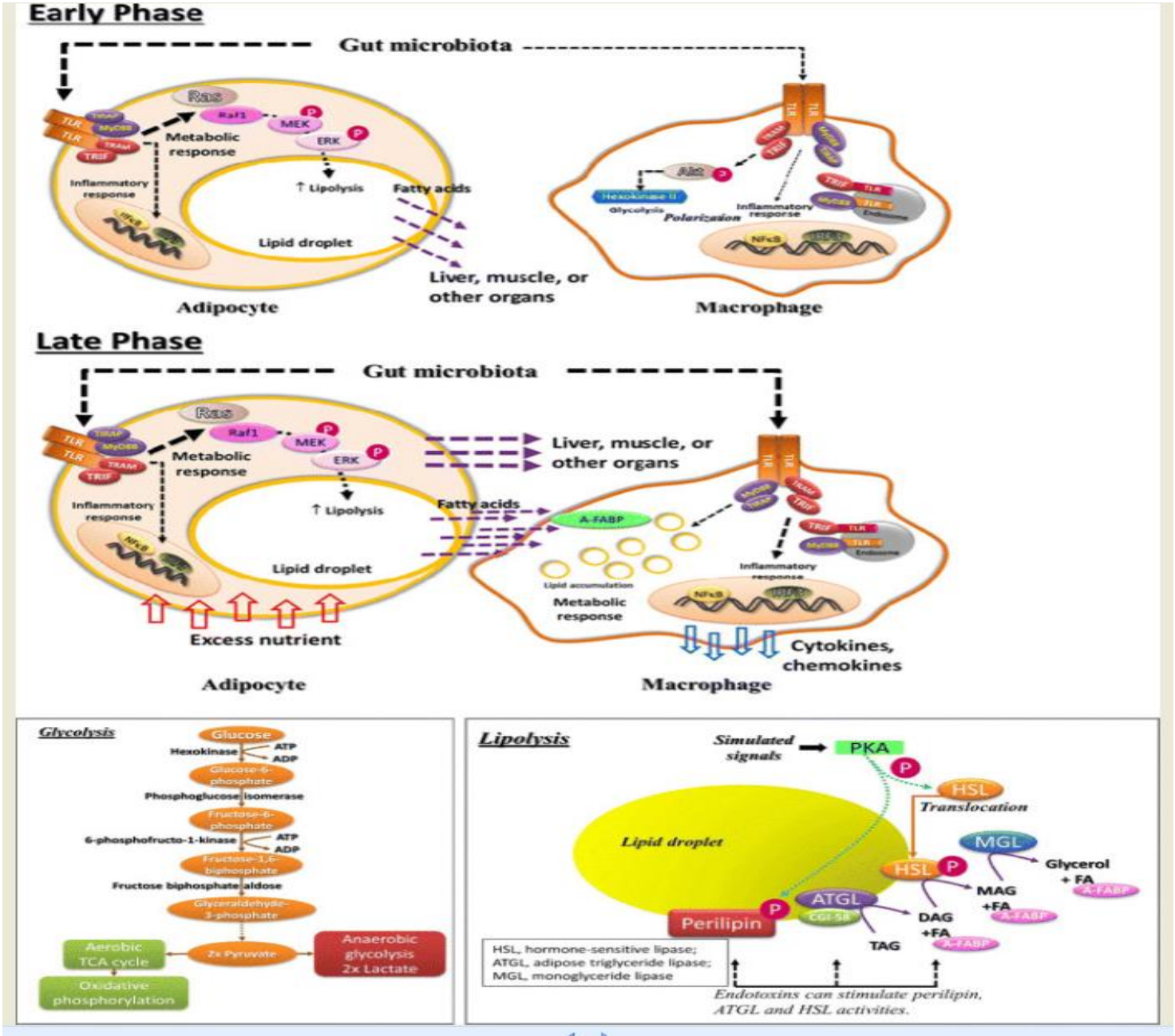
Commercialista Benevento contribuisce a uno studio di un Centro di Novara

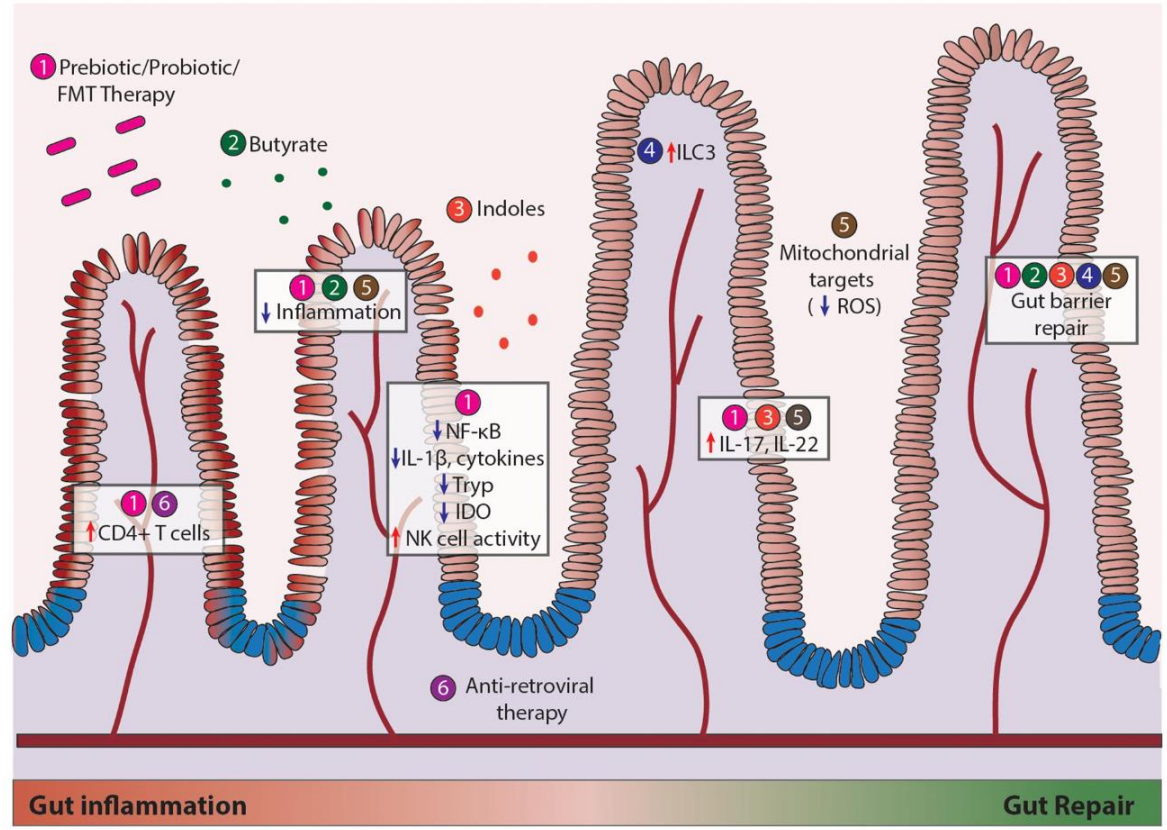


Indro Montanelli: il futuro dell’Italia

“ Per l’Italia non vedo un futuro .
Per gli Italiani ne vedo uno brillante “

Hypothesis of intercellular energy relocation triggered by Gut microbiota




1 Prebiotic/Probiotic/ FMT Therapy
2 Butyrate
3 Indoles
4 ↑ILC3
5 Mitochondrial targets (↓ ROS)
1 2 5 ↓Inflammation
1 ↓NF-κB ↓IL-1β, cytokines ↓Tryp ↓IDO ↑NK cell activity
1 6 ↑CD4+ T cells
1 3 5 ↑IL-17, IL-22
1 2 3 4 5 Gut barrier repair
6 Anti-retroviral therapy
Gut inflammation
Gut Repair

Transmissibility of alcohol-related liver disease via FMT

Intestinal microbiota contributes to individual susceptibility to alcoholic liver disease

M Llopis[1, 2, 3, 4], A M Cassard[1, 2*], L Wrzosek[1, 2], L Boschat[3, 4], A Bruneau[3, 4], G Ferrere[1, 2], V Puchois[1, 2], J C Martin[5, 6], P Lepage[3, 4], T Le Roy[3, 4], L Lefèvre[7], B Langelier[3, 4], F Cailleux[1, 2], A M González-Castro[8], S Rabot[3, 4], F Gaudin[9], H Agostini[10], S Prévot[2, 11], D Berrebi[1, 12], D Ciocan[1, 2, 13], C Jousse[14], S Naveau[1, 2, 13], P Gérard[3, 4], G Perlemuter[1, 2, 13*]

Author affiliations

Abstract

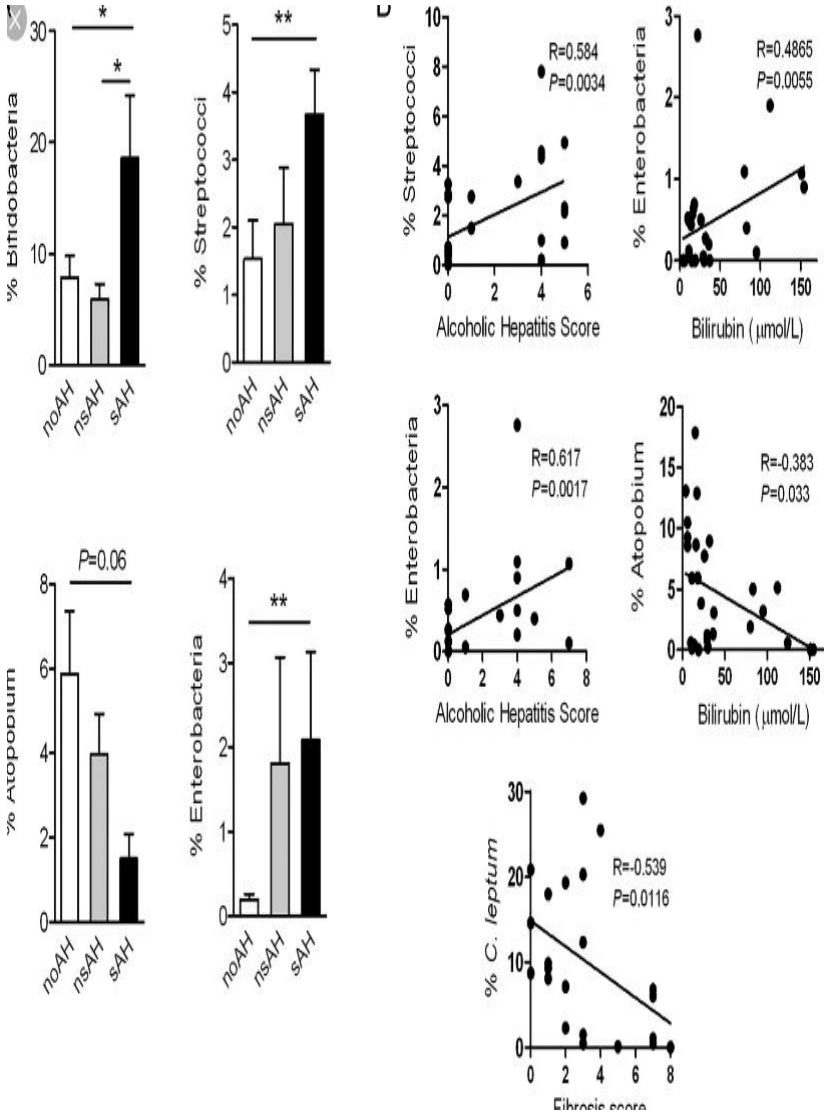
Objective There is substantial inter-individual diversity in the susceptibility of alcoholics to liver injury. Alterations of intestinal microbiota (IM) have been reported in alcoholic liver disease (ALD), but the extent to which they are merely a consequence or a cause is unknown. We aimed to demonstrate that a specific dysbiosis contributes to the development of alcoholic hepatitis (AH).

Design We humanised germ-free and conventional mice using human IM transplant from alcoholic patients with or without AH. The consequences on alcohol-fed recipient mice were studied.

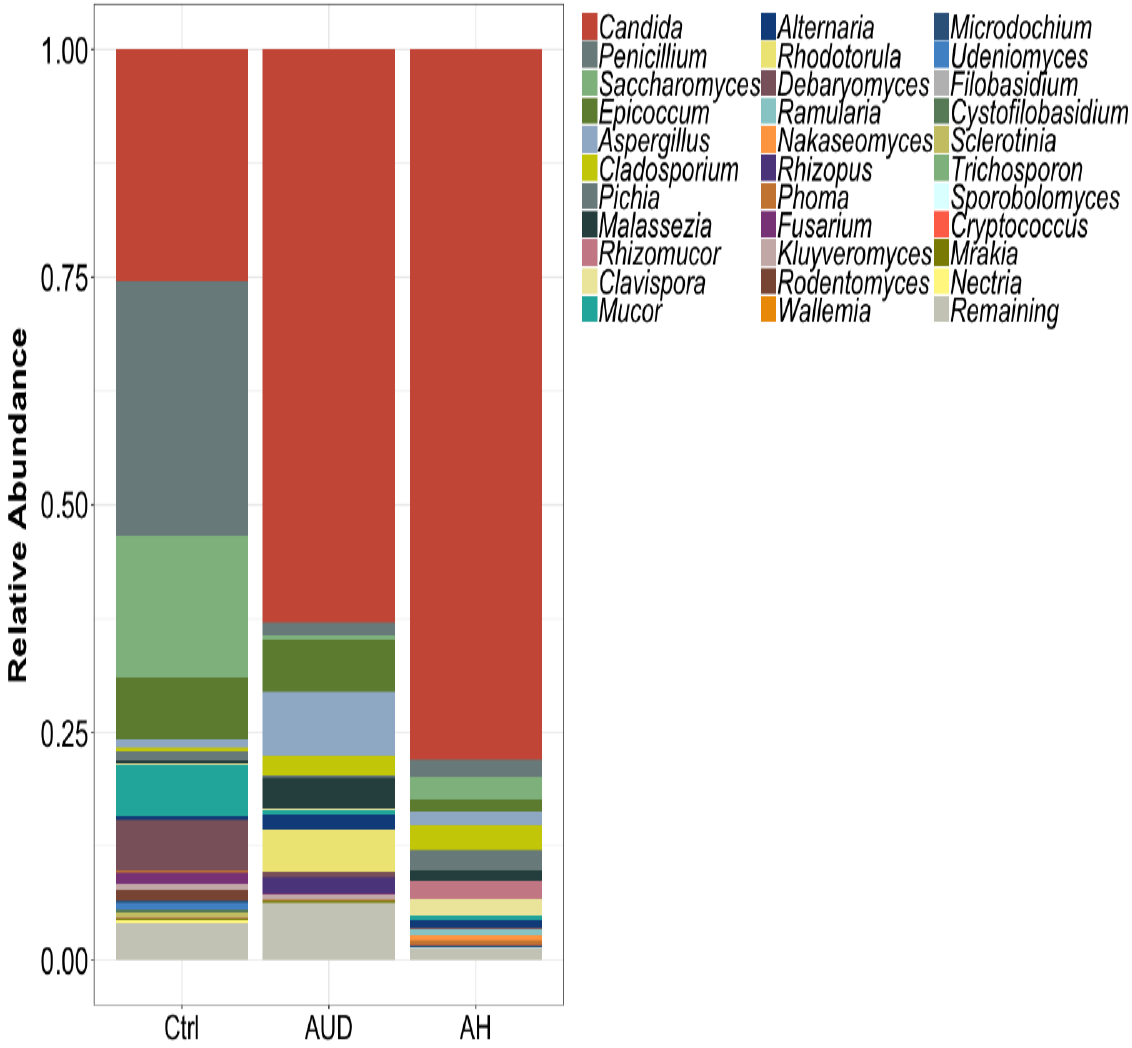
Results A specific dysbiosis was associated with ALD severity in patients. Mice harbouring the IM from a patient with severe AH (sAH) developed more severe liver inflammation with an increased number of liver T lymphocyte subsets and Natural Killer T (NKT) lymphocytes, higher liver necrosis, greater intestinal permeability and higher translocation of bacteria than mice harbouring the IM from an alcoholic patient without AH (noAH). Similarly, $CD45^+$ lymphocyte subsets were increased in visceral adipose tissue, and $CD4^+$T and NKT lymphocytes in mesenteric lymph nodes. The IM associated with sAH and noAH could be distinguished by differences in bacterial abundance and composition. Key deleterious species were associated with sAH while the *Faecalibacterium* genus was associated with noAH. Ursodeoxycholic acid was more abundant in faeces from noAH mice. Additionally, in conventional mice humanised with the IM from an sAH patient, a second subsequent transfer of IM from an noAH patient improved alcohol-induced liver lesions.

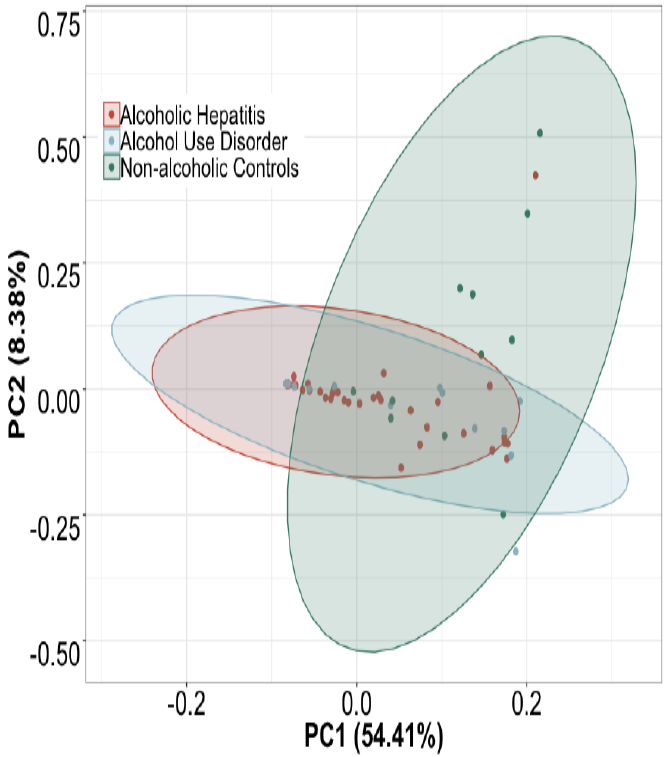
Conclusions Individual susceptibility to ALD is substantially driven by IM. It may, therefore, be possible to prevent and manage ALD by IM manipulation.

View Full Text



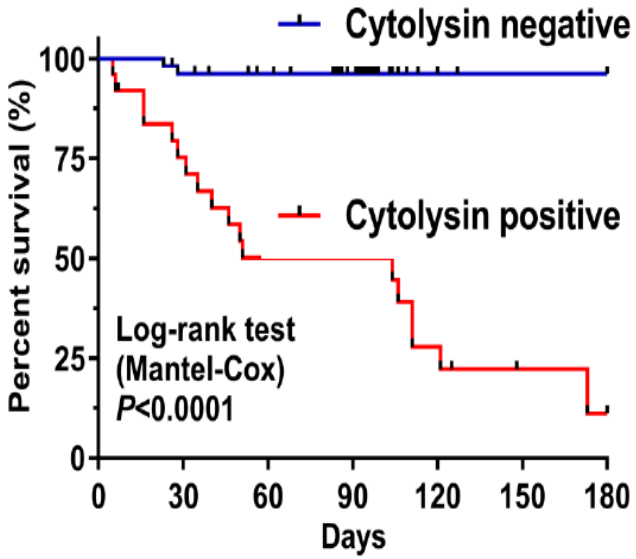
Fungal dysbiosis in patients with alcohol abuse





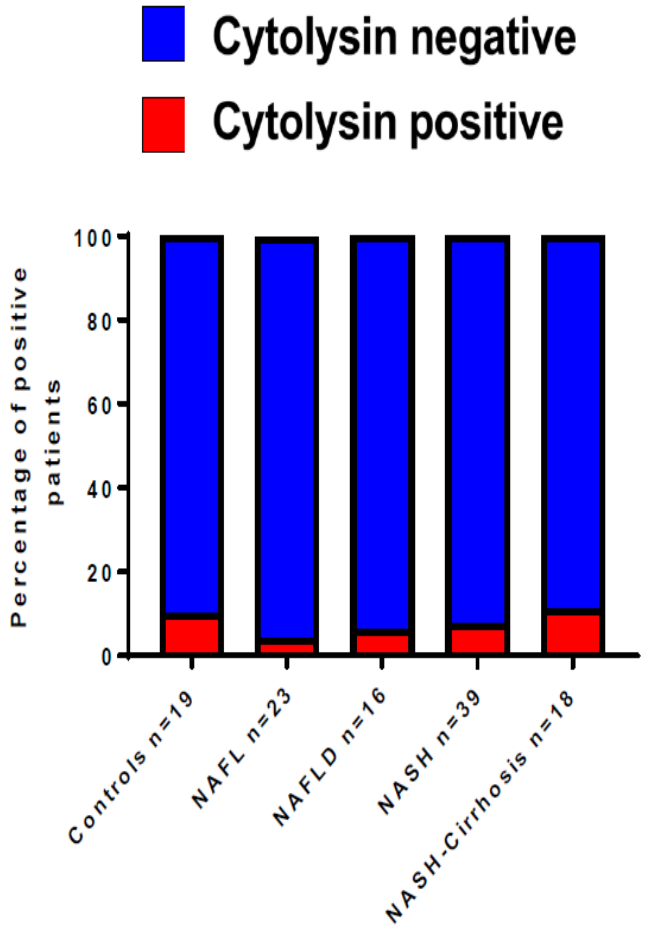
Yang A, Schnabl B, J Clin Invest 2017 ; Lang S, Schnabl B, Hepatology 2019

Enterecoccus faecalis cytolisin associates with mortality in patients with alcoholic hepatitis , but not in patients with NAFLD



	Multivariate Cox Regression		
Variables	HR	95% CI	*P* value
Cytolysin	14.07	2.83-69.85	0.001
MELD	1.09	1.01-1.16	0.022

Duan Y, Llorente C, Schnabl B, 2019



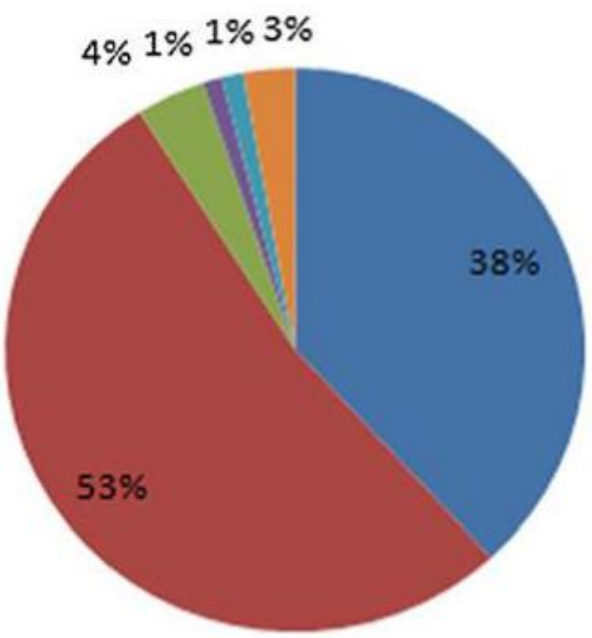
Lang S, Schnabl B, unpublished

The fingerprint of gut microbiota in liver cirrhosis

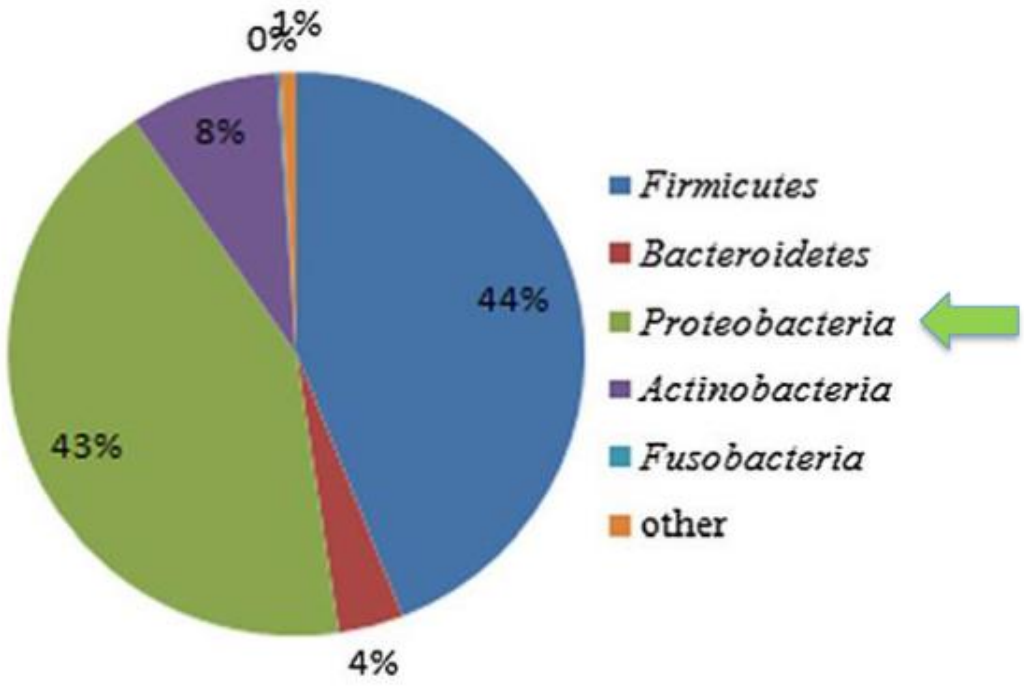
Unbalance between potentially pathogenic and beneficial bacteria

PHYLUM LEVEL

Normal genes



Patients genes



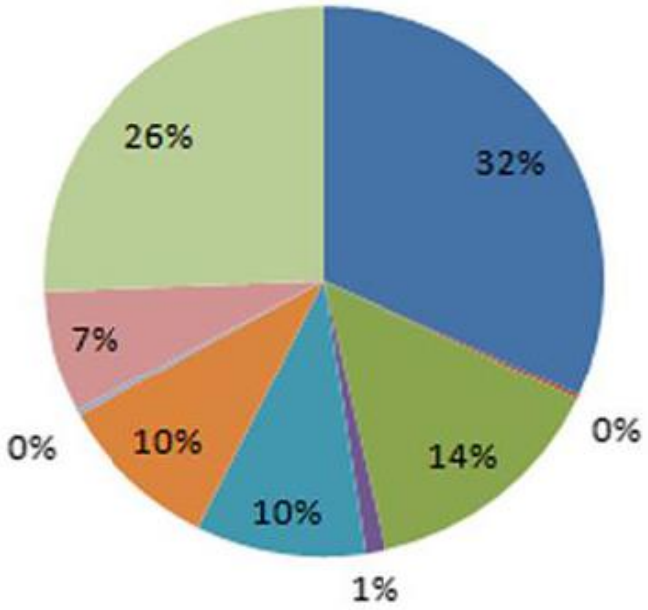
Lu et al. Microb Ecol 2011
Chen et al. Hepatology 2011
Wei X et al BMC Gastroenterology 2013

The fingerprint of gut microbiota in liver cirrhosis

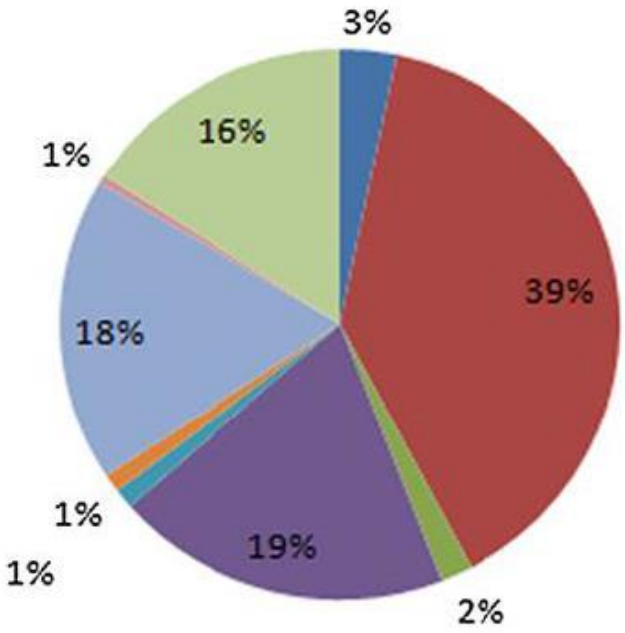
Unbalance between potentially pathogenic and beneficial bacteria

FAMILIA LEVEL

Normal genes



Patients genes



- *Bacteroidaceae*
- *Enterobacteriaceae*
- *Ruminococcaceae*
- *Veillonellaceae*
- *Clostridiaceae*
- *Lachnospiraceae*
- *Streptococcaceae*
- *Eubacteriaceae*
- other

Lu et al. Microb Ecol 2011
Chen et al. Hepatology 2011
Wei X et al BMC Gastroenterology 2013

GUT Microbiome in chronic HBV infection

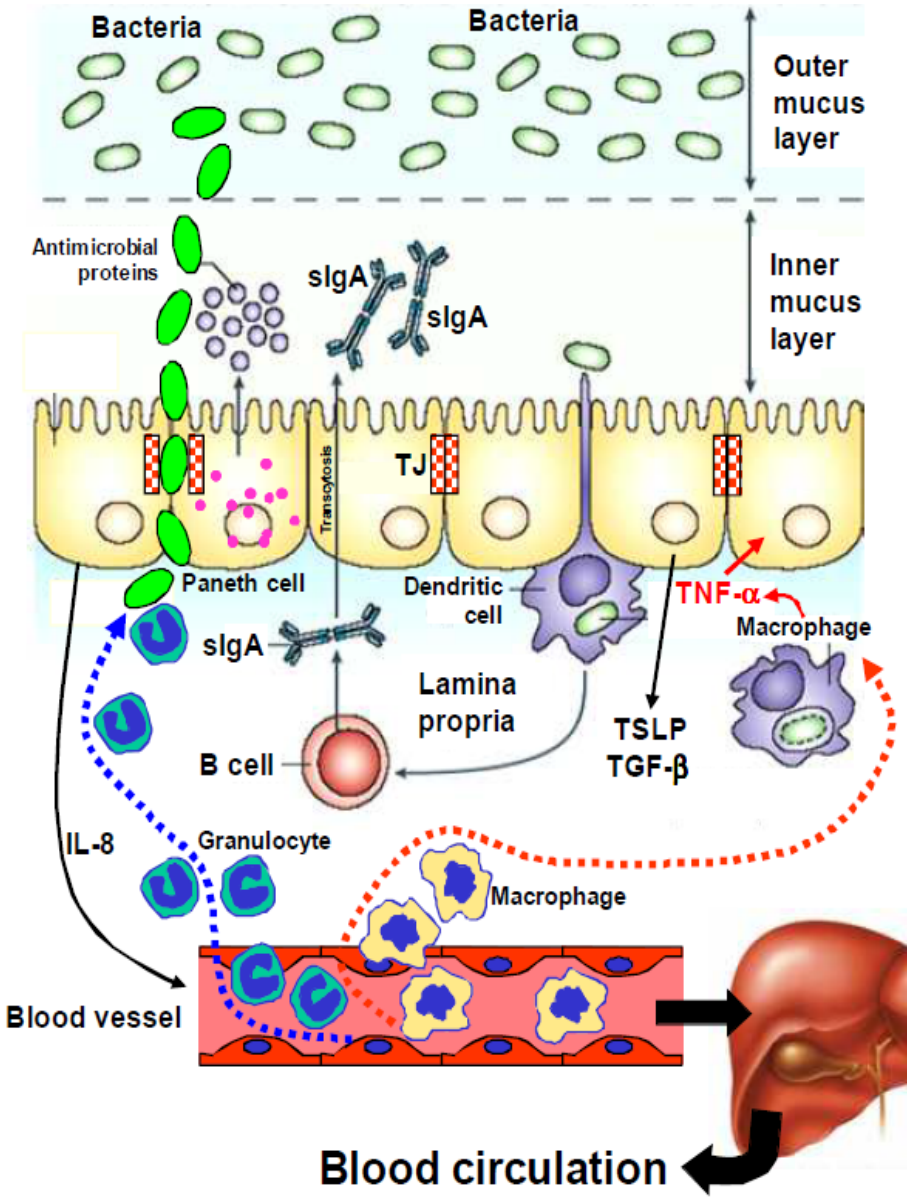
J Immunol Res. 2018; 2018: 2361963. PMCID: PMC6083645
Published online 2018 Jul 25. doi: 10.1155/2018/2361963 PMID: 30148173

The Immunologic Role of Gut Microbiota in Patients with Chronic HBV Infection

Ruilin Yang,[1] Yao Xu,[1] Zhifeng Dai,[1] Xuhong Lin,[✉1] and Huichao Wang[2]

- Gut-liver axis
- Progressive evolution of microecology

Mechanisms preventing bacterial translocation



1. MICROBIAL (NORMAL MICROBIOTA)

Contact inhibition

Colonization resistance

2. MECHANICAL

Mucus layer

Peristalsis

Epithelial barrier

Junctional complexes

3. IMMUNOLOGIC

Gut-associated lymphoid tissue

Secretory immunoglobulins

4. GUT/LIVER AXIS

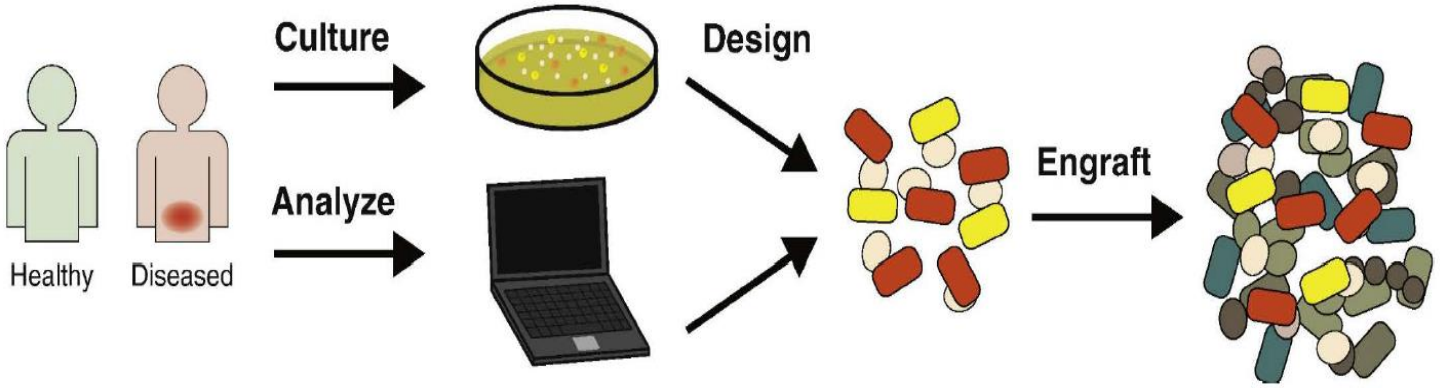
Bile salts

Reticuloendothelial function

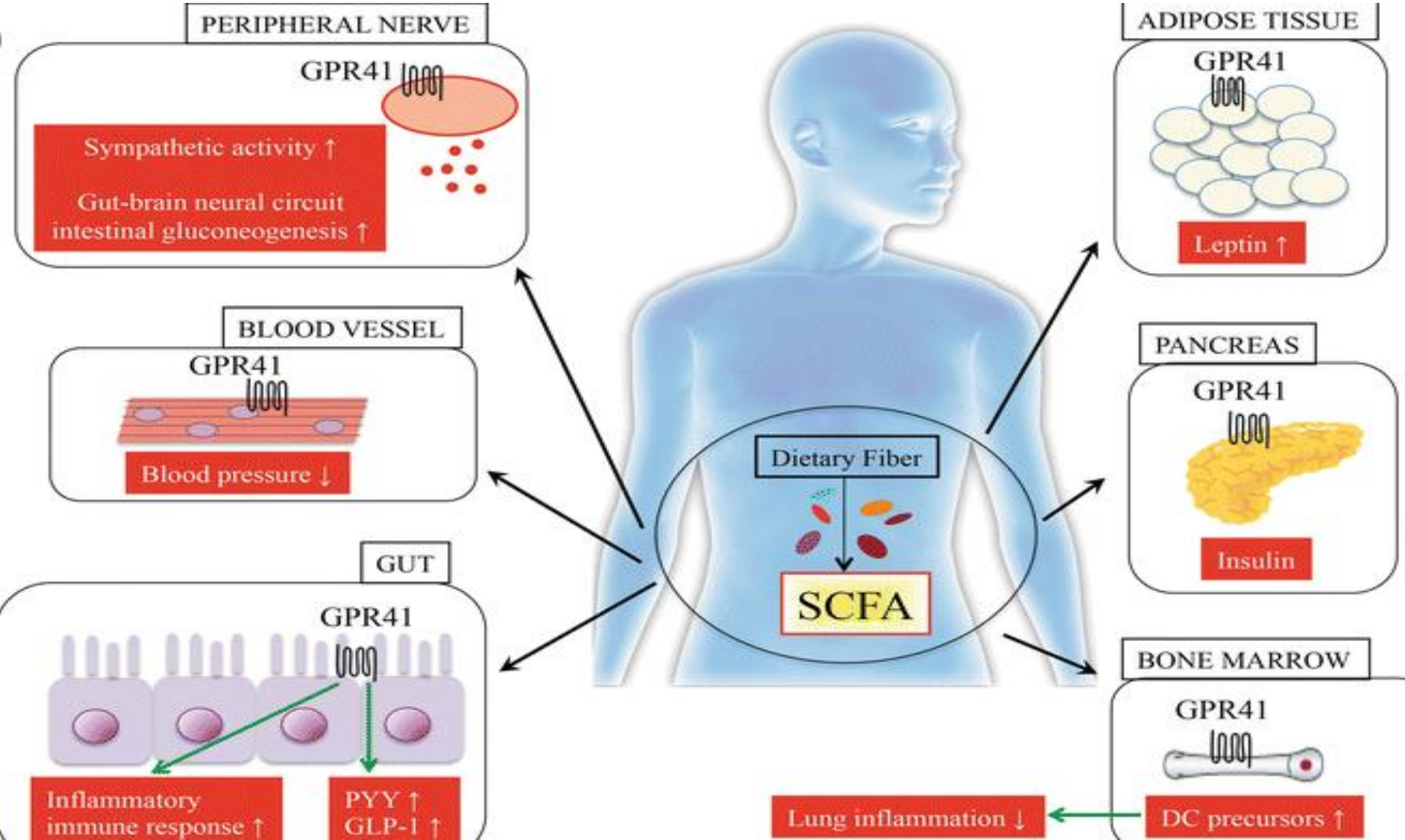
Precision Medecine :
Approaches to restore Host Microbiome Homeostasis

Bugs as Drugs

Defined mixture of microorganisms to reprogram the microbial ecology



Mimee M, Adv Drug Deliv Rev 2016


PERIPHERAL NERVE
GPR41
Sympathetic activity ↑
Gut-brain neural circuit
intestinal gluconeogenesis ↑
BLOOD VESSEL
GPR41
Blood pressure ↓
GUT
GPR41
Inflammatory
immune response ↑
PYY ↑
GLP-1 ↑
Dietary Fiber
SCFA
ADIPOSE TISSUE
GPR41
Leptin ↑
PANCREAS
GPR41
Insulin
BONE MARROW
GPR41
DC precursors ↑
Lung inflammation ↓