Impact de l'alimentation et du microbiote intestinal sur le développement de lésions biliaires : modèle expérimental

.

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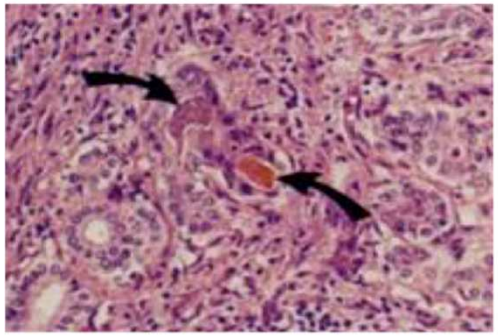
Mucoviscidose et atteinte hépatique

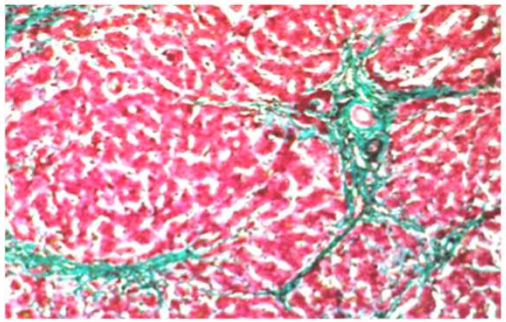
Fréquence et sévérité variables

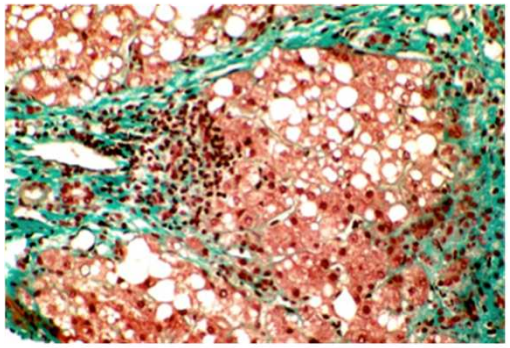
- Fibrose biliaire focale: 30 – 70%
- Cirrhose et hypertension portale : < 10%
- Stéatose: 25 - 75%
- Anomalies de la vésicule biliaire: atrophie; dysfonction de la vidange; lithiase : 30 - 90%

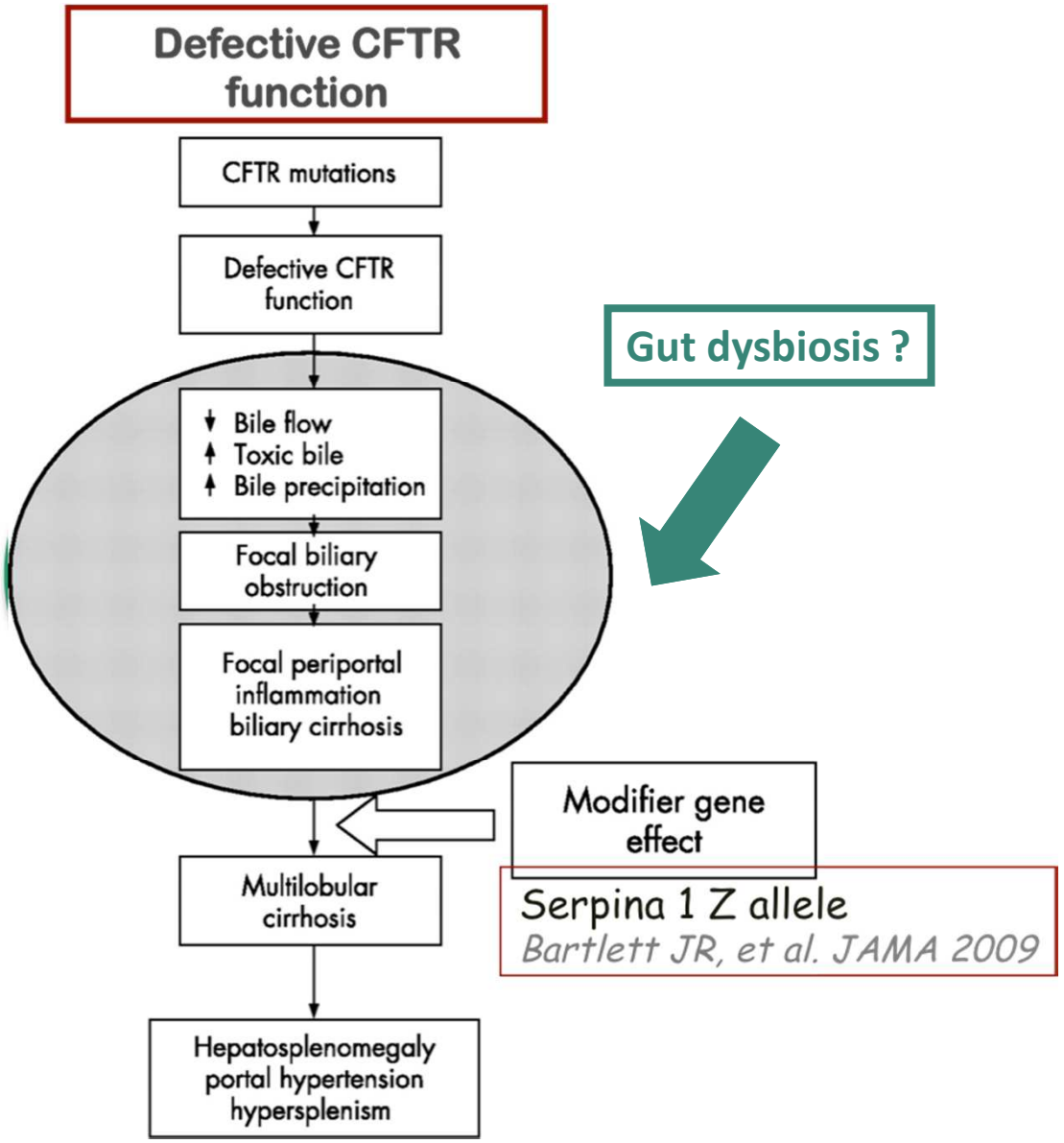
Atteinte hépato-biliaire

Hypothèses physiopathologiques

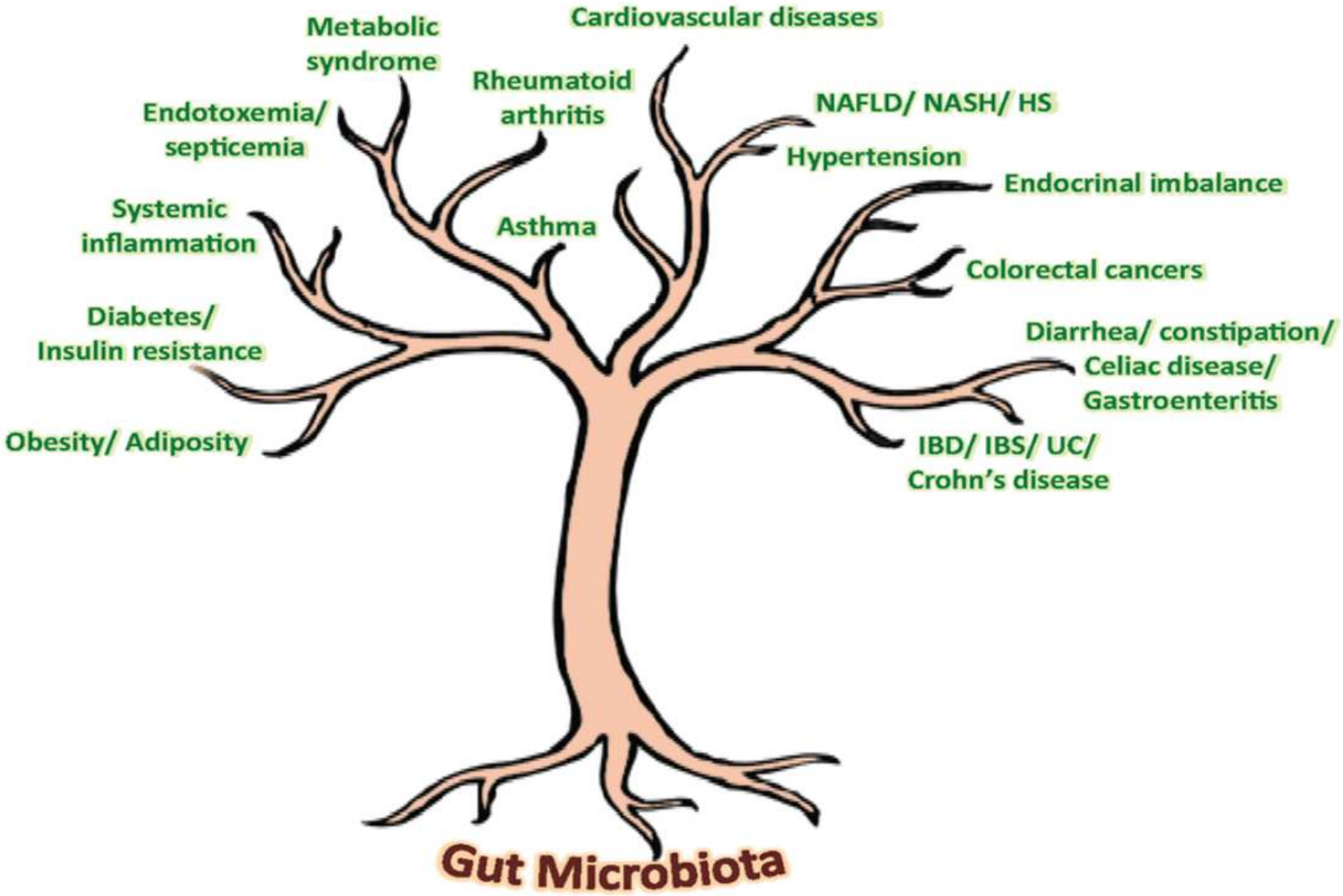




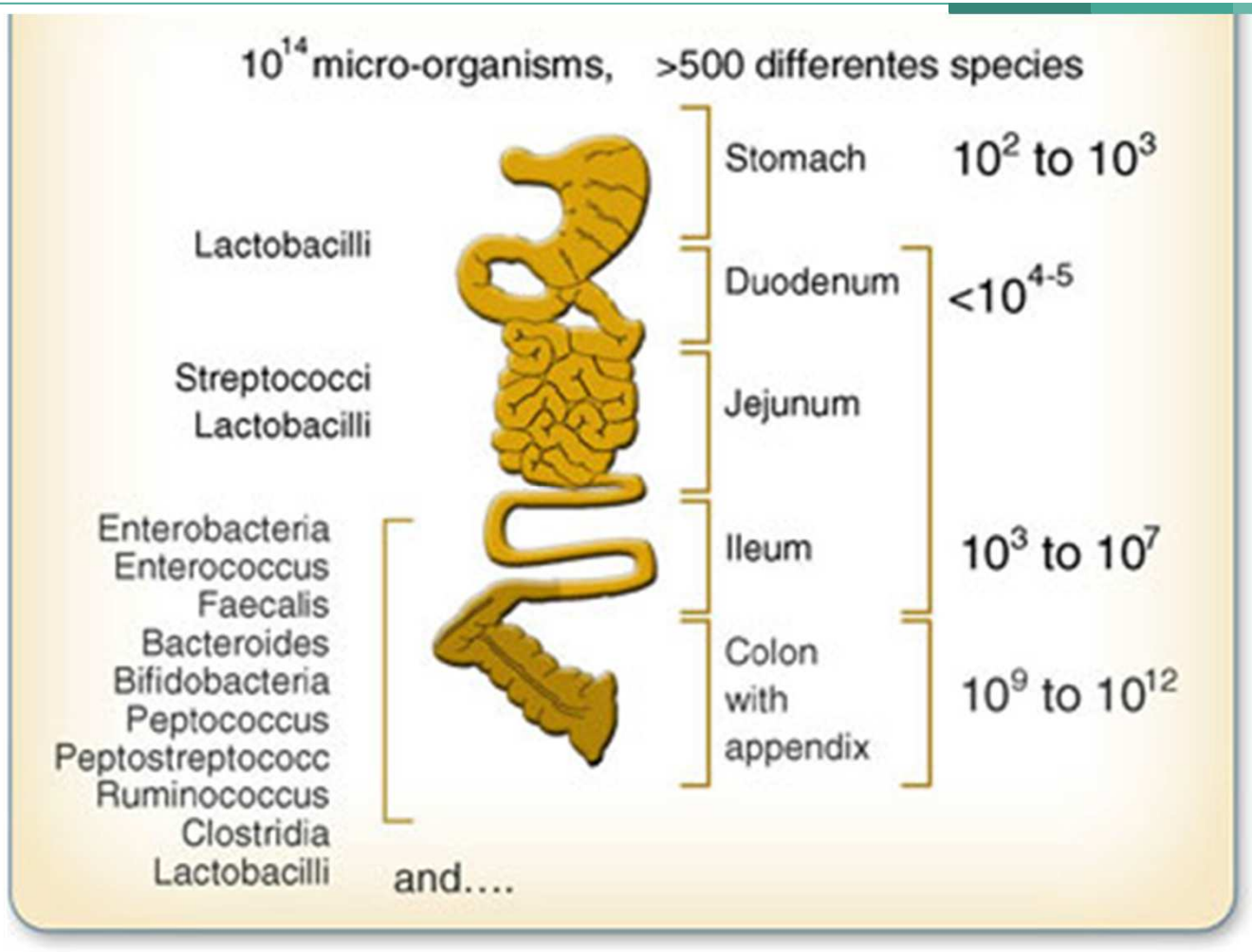




Dysbiose intestinale et maladies



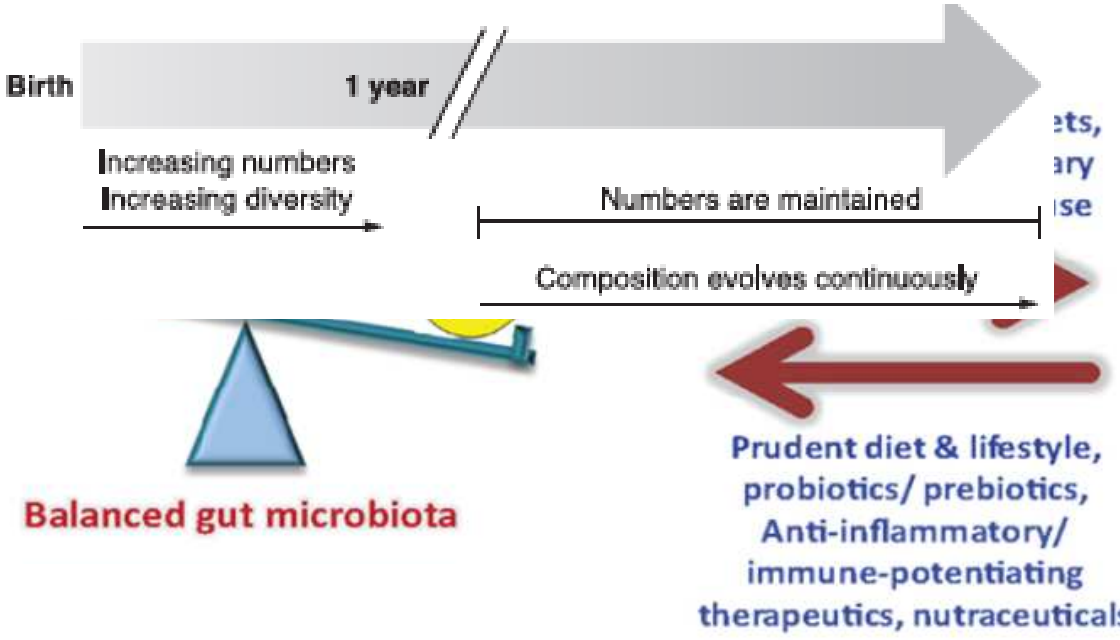
Microbiote intestinal

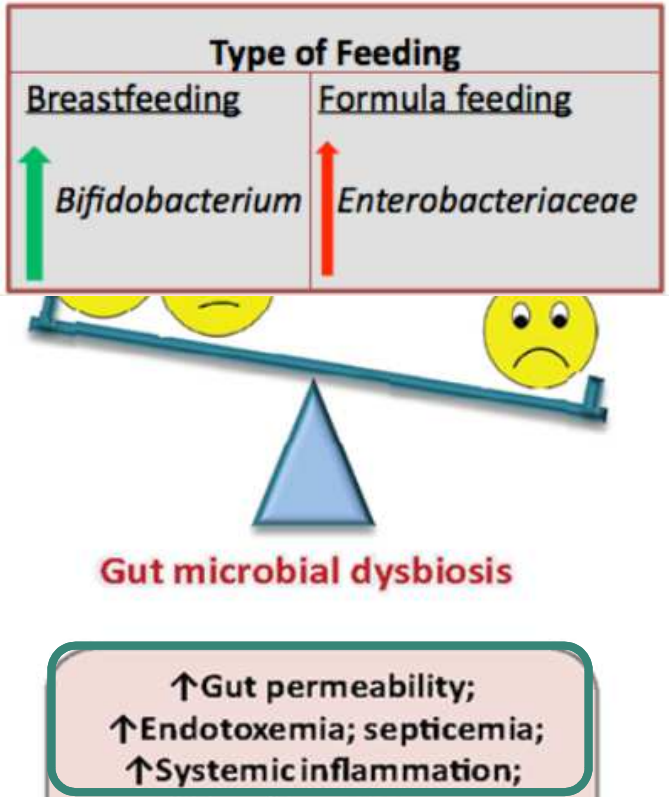


Modifications de la flore intestinale

Bifidobacterium

Microbial **biodiversity**
- **Bacteroidetes**
- **Firmicutes**

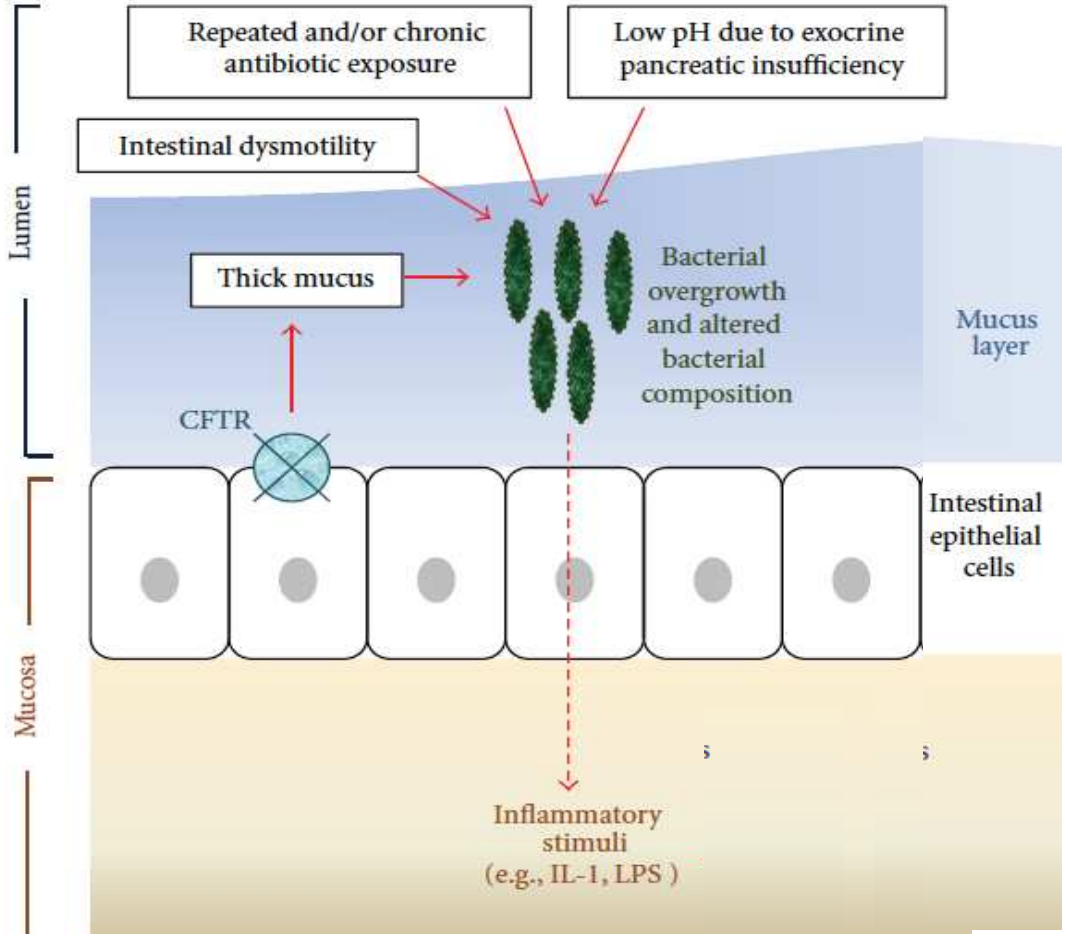




↑Gut permeability;
↑Endotoxemia; septicemia;
↑Systemic inflammation;

Mucoviscidose, Dysbiose et inflammation intestinale

Hypothèses physiopathologiques



Lee JM et al. Mediators of inflammation 2012.

Mucoviscidose et Dysbiose intestinale

- There is increasing evidence for:
  - Gut dysbiosis
  - Intestinal inflammation , particularly in CF patients with PI

Cross-Sectional and Longitudinal Comparisons of the Predominant Fecal Microbiota Compositions of a Group of Pediatric Patients with Cystic Fibrosis and Their Healthy Siblings[▽]†

Gwen Duytschaever,[1]* Geert Huys,[1,2] Maarten Bekaert,[3] Linda Boulanger,[4] Kris De Boeck,[4] and Peter Vandamme[1]

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 2011, p. 8015–8024 Vol. 77, No. 22

Disrupted Intestinal Microbiota and Intestinal Inflammation in Children with Cystic Fibrosis and Its Restoration with Lactobacillus GG: A Randomised Clinical Trial

PLOS ONE February 2014 | Volume 9 | Issue 2 | e87796

Eugenia Bruzzese[1], Maria Luisa Callegari[2], Valeria Raia[1], Sara Viscovo[1], Riccardo Scotto[1], Susanna Ferrari[2], Lorenzo Morelli[2], Vittoria Buccigrossi[1], Andrea Lo Vecchio[1], Eliana Ruberto[1], Alfredo Guarino[1]*

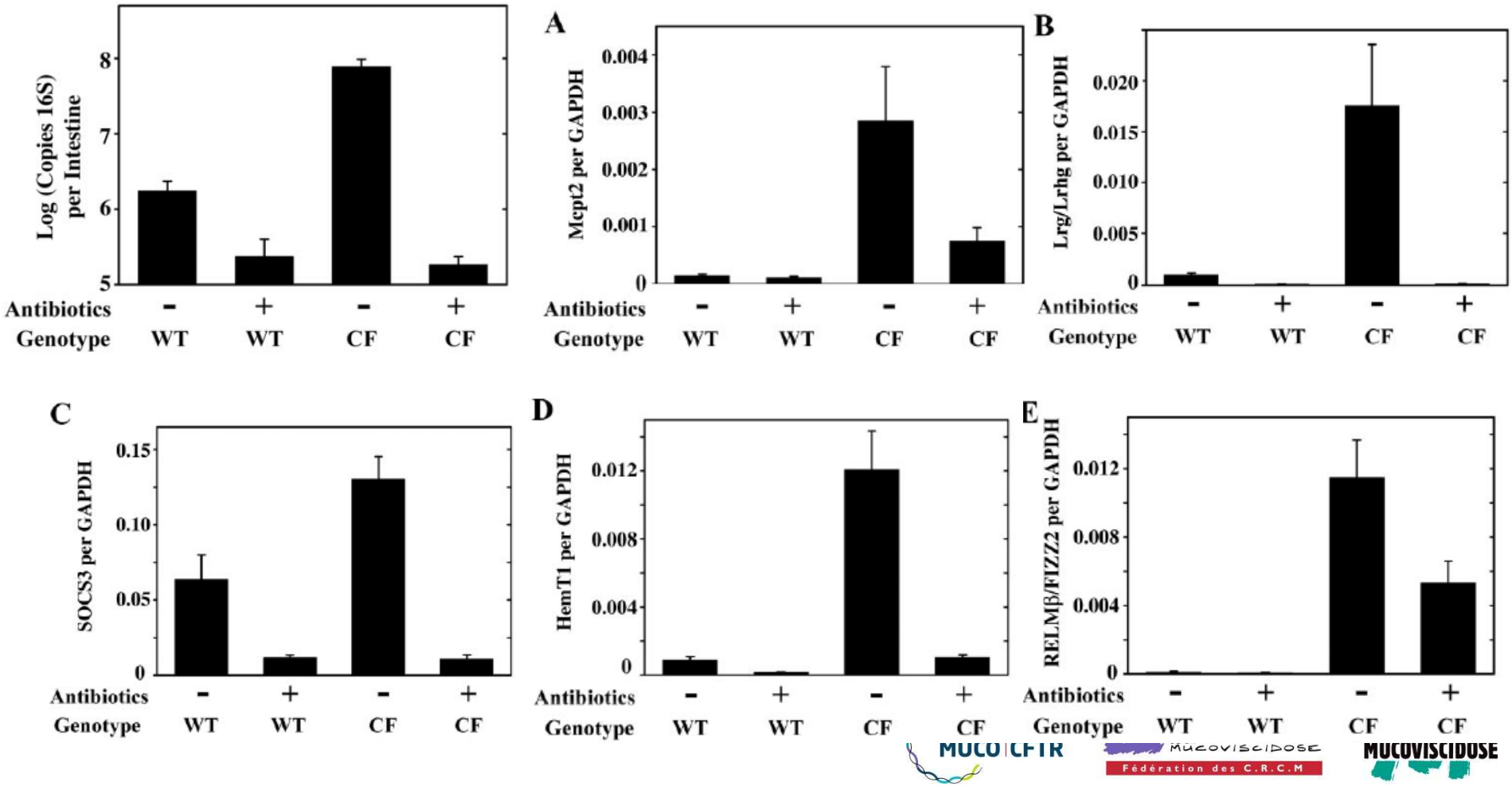
Mucoviscidose et Dysbiose intestinale

Qu’avons nous appris des modèles murins ?

Bacterial overgrowth in CF mice small intestine

Effect of antibiotics on SIBO

Effect of antibiotics on inflammatory gene expression



Norkina O, et al. Infection and Immunity, 2004

Intestinal phenotype in CF mice

Cystic fibrosis transmembrane conductance regulator knockout mice exhibit aberrant gastrointestinal microbiota

Susan V. Lynch,[1,*] Katherine C. Goldfarb,[2] Yvette K. Wild,[3] Weidong Kong,[1] Robert C. De Lisle[4] and Eoin L. Brodie[2]

Gut Microbes 4:1, 41–45; January/February 2013

Disrupted tight junctions in the small intestine of cystic fibrosis mice

Robert C. De Lisle *Cell Tissue Res*. 2014 January ; 355(1): 131–142.

Atteinte hépatique
Souris invalidée (KO) pour cftr

Atteinte hépatique varie selon le fond génétique et le type d'alimentation:

✧ ***Cftr* KO** (Cftrtm1Unc) **, C57BL6 (87,5%)/129 SvJ (fond mixte) , alimentation normale (AO3) + PEG (Forlax) : histologie hépatique normale**

- **interruption du cycle entérohépatique des AB; défaut de vidange de la vésicule biliaire et shunt cholécystohépatique des ABs**

(Debray D et al. Gastroenterology 2012)

✧***Cftr* KO** (Cftrtm1Unc) , **C57BL6/129SvJ (fond mixte) , alimentation riche en lipides (Peptamen (Nestle)): stéatose**

(Cottart CH et al. Pediatr Res 2007)

✧***Cftr* KO** (Cftrtm1Unc), **C57BL6 (100%) congénique , Peptamen (Nestle): fibrose biliaire focale**

(Durie P et al. Am J Pathol 2004)

Quid de l'impact de l'alimentation et de la dysbiose intestinale sur le développement des lésions biliaires ?

Methods

- **Mice:** males, *Cftr tm1Unc* (-/-), WT (+/+) littermates
- **Background :** Congenic: C57BL6 (100%)
  or Mixed: C57BL6 (87.5%), 129SvJ (12.5%)
- **Diet until investigations at 3 mo of age :**
  - AO3 Chow + PEG
  - or Liquid diet
    (Peptamen®, Nestle)

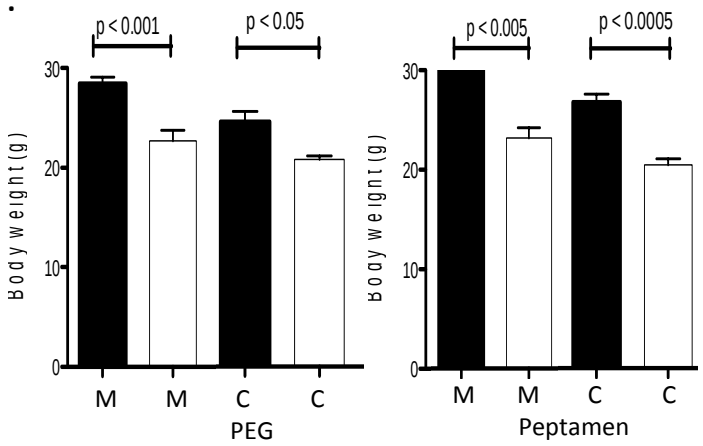
	peptamen	AO3 Chow (Safe®)
Prot (g/100 kCal)	4	6,7
Glucides (g/100 kCal)	12,7	16,2
Lipides (g/100 kCal)	16,2	1,6
MC/MC+LC Ratio	70%	<5%

- **Experimental design** (7 à 10 mice/group)

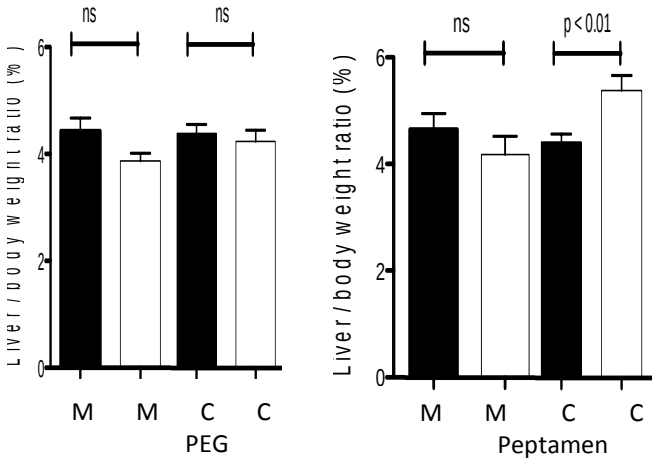
PEG				Peptamen			
Mixed B6;129		Congenic B6		Mixed B6;129		Congenic B6	
+/+	–/–	+/+	–/–	+/+	–/–	+/+	–/–

Liver Phenotype according to diet

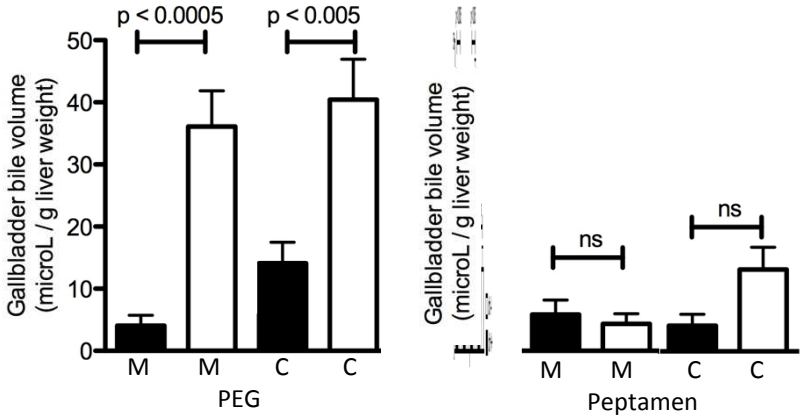
Poids



Poids du Foie

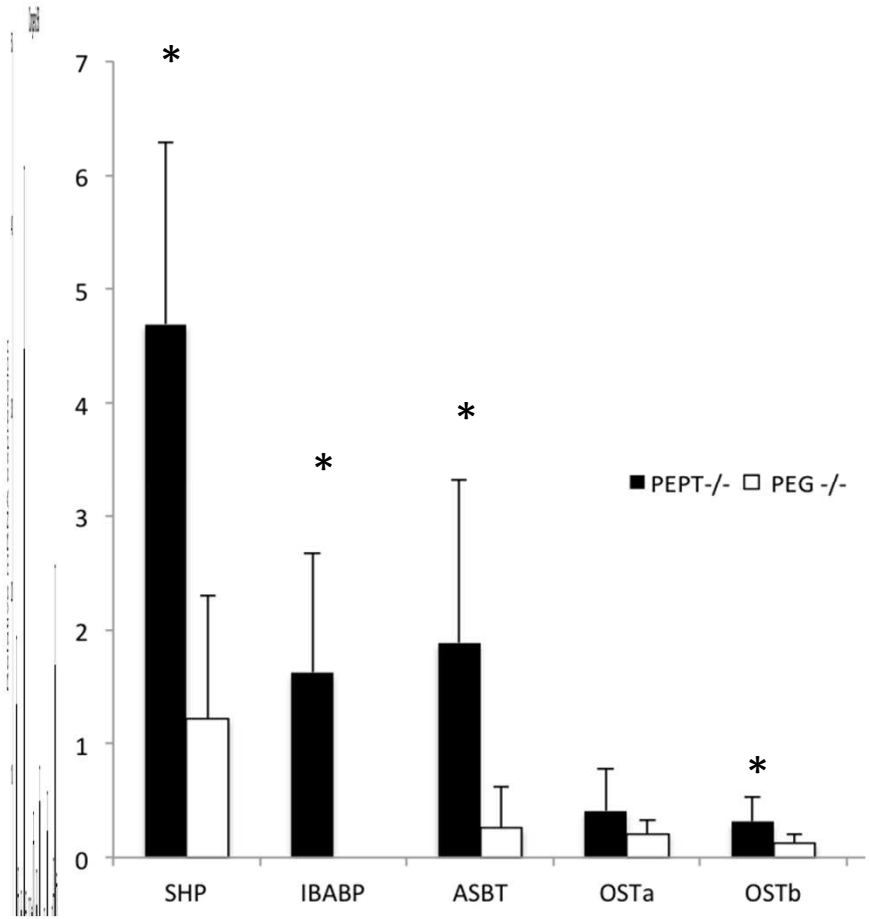


Volume de la bile de la VB

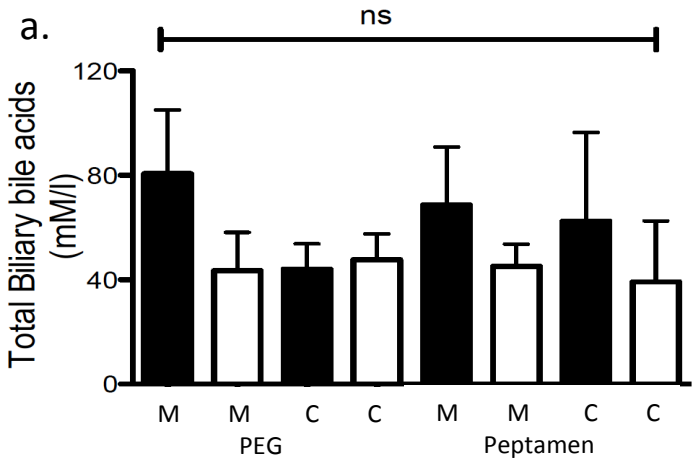


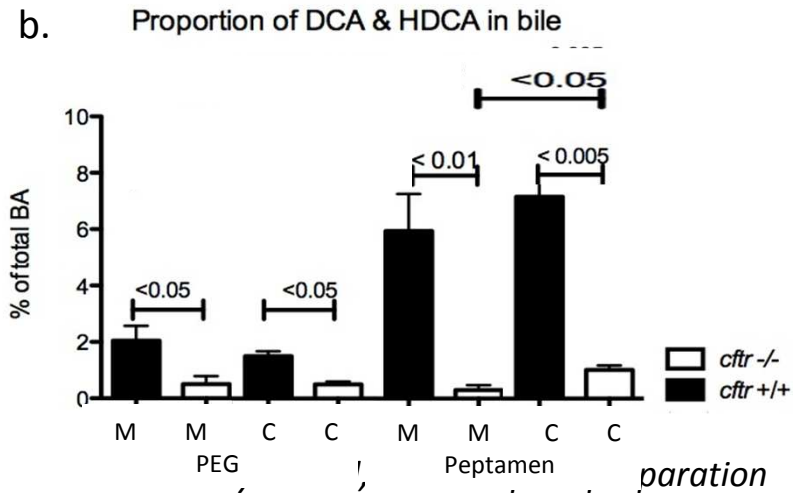
Bile acid metabolism in CF mice

Ileal expresssion of bile acid regulated genes



Acides biliaires totaux dans la bile

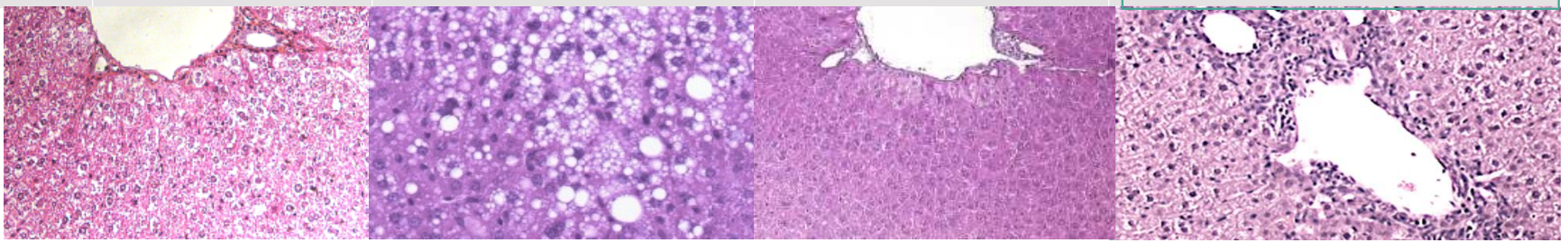




paration

Liver Phenotype according to diet (2)

	Mixed B6; 129		Congenic B6	
	PEG	Peptamen	PEG	Peptamen
+/+	Normal (100%)	Steatosis (70%)	Normal (100%)	Steatosis (30%)
–/–	Normal (100%)	Steatosis (90%)	Normal (100%)	Ductular reaction and mild fibrosis (40%) But no steatosis



Serum biochemical tests in *cftr*$^{-/-}$ mice and *cftr*$^{+/+}$ littermates

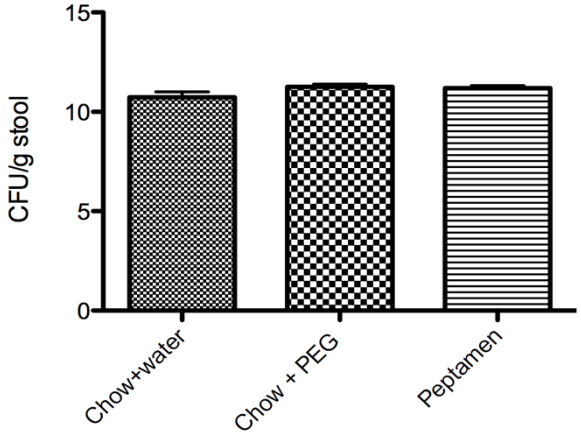
Diet	*Standard chow + PEG*				*Liquid diet (Peptamen)*			
Genetic background	*M*	*M*	*C*	*C*	*M*	*M*	*C*	*C*
Genotype	*Cftr*$^{+/+}$	*Cftr* $^{-/-}$	*Cftr*$^{+/+}$	*Cftr* $^{-/-}$	*Cftr*$^{+/+}$	*Cftr* $^{-/-}$	*Cftr* $^{+/+}$	*Cftr* $^{-/-}$
Serum tests (n = 5)								
Lipase (IU/l)	74.5 ± 9.6	77.8 ± 10	51 ± 6.6	63.6 ± 11	49.6 ± 5	67.5 ± 9	43 ± 2	59.2 ± 5
Cholesterol (mmol/l)	2.48 ± 0.4	2.77 ± 0.3	1.85 ± 0.14	1.8 ± 0.07	3.65 ± 0.3	3.25 ± 0.3	2.62 ± 0.1	2.39 ± 0.16
Triglycerides (mmol/l)	1.2 ± 0.3	1.15 ± 0.3	0.54 ± 0.05	0.5 ± 0.04	1.8 ± 0.6	0.63 ± 0.9	0.79 ± 0.1	0.63 ± 0.1
Total bilirubin (µmol/l)	2.08 ± 0.2	1.77 ± 0.3	2.12 ± 0.4	1.5 ± 0.2	1.52 ± 0.3	1.77 ± 0.3	1.71 ± 0.2	1.46 ± 0.2
Alanine aminotransferase (IU/l)	12.8 ± 2	16.3 ± 6	22.8 ± 6	21.2 ± 3	16.4 ± 6	8.8 ± 1	8.2 ± 3	6.2 ± 1
Alkaline phosphatase (IU/l)	51.5 ± 3	66.3 ± 7	56.7 ± 1	88.4 ± 18	63.2 ± 7	66.3± 7	53.2 ± 6	64.3 ± 13

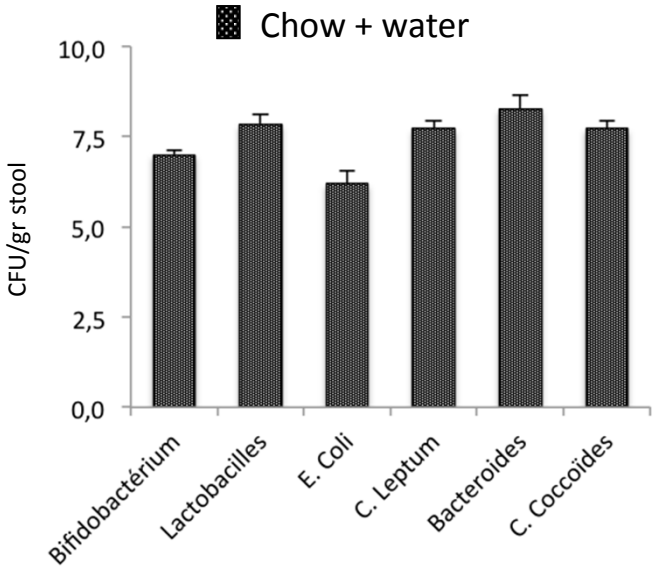
In summary : Liver phenotype in CF mice

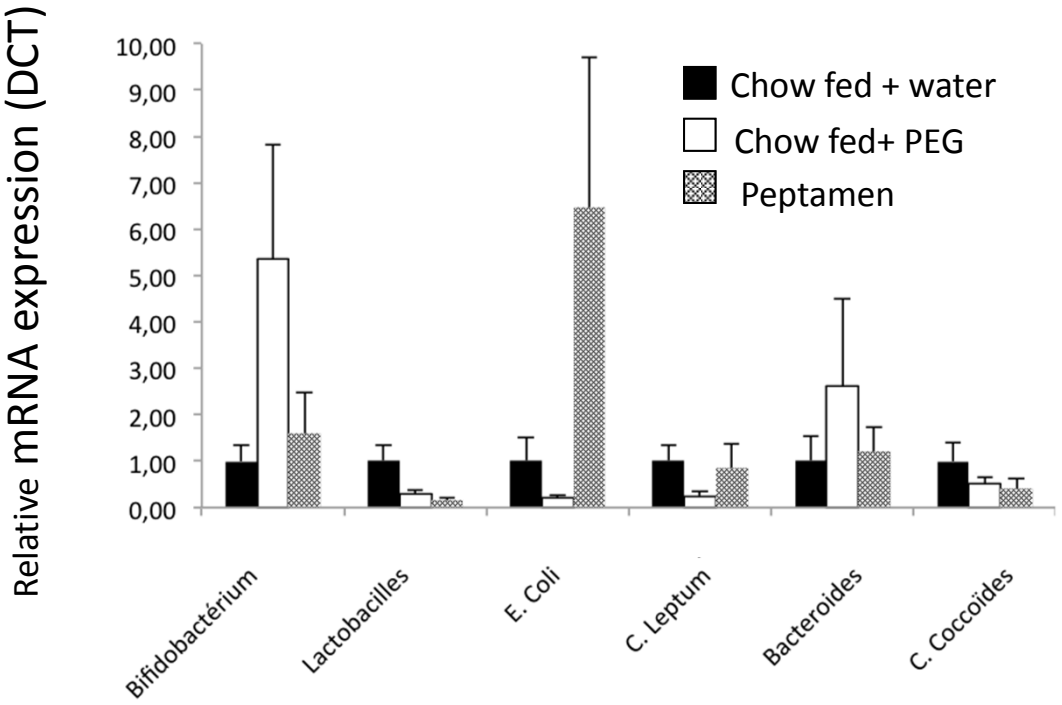
Diet	PEG				Peptamen			
Background	Mixed		Congenic		Mixed		Congenic	
Genotype	+/+	-/-	+/ +	-/-	+/+	-/-	+/+	-/-
Hepatomegaly	0	0	0	0	0	0	0	+
Defect in GB emptying	0	+	0	+	0	0	0	0
Secondary BA in bile (%)	2	0.5	1.5	0.6	6	0.3	7.8	1*
Steatosis (% mice studied)	0	0	0	0	+	+	+	0
Biliary lesions	0	0	0	0	0	0	0	+

Etude du microbiote intestinal

C57Bl6 mice

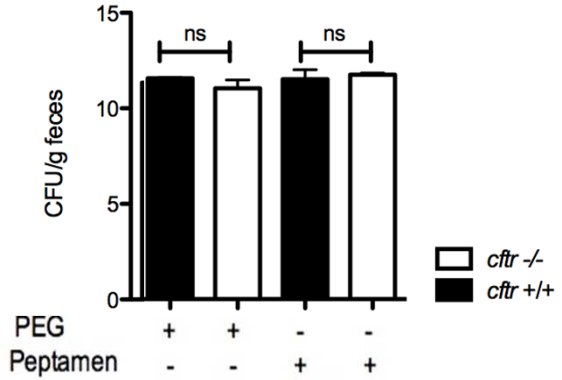


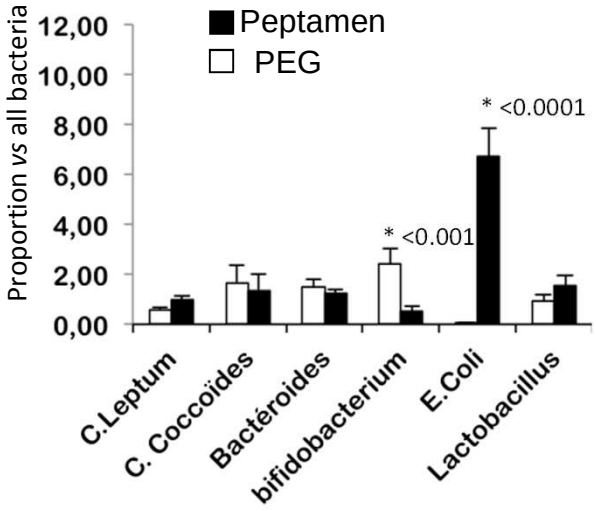


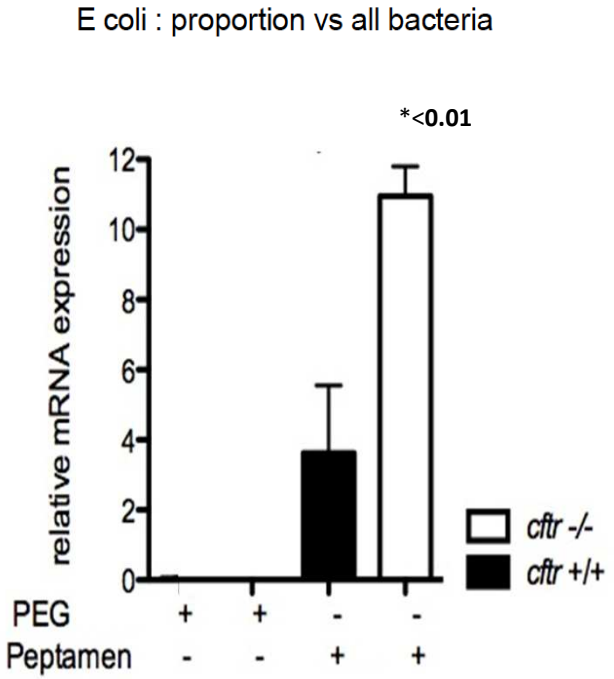


Etude du microbiote intestinal

◆ *Cftr -/- and Cftr +/+ congenic mice*

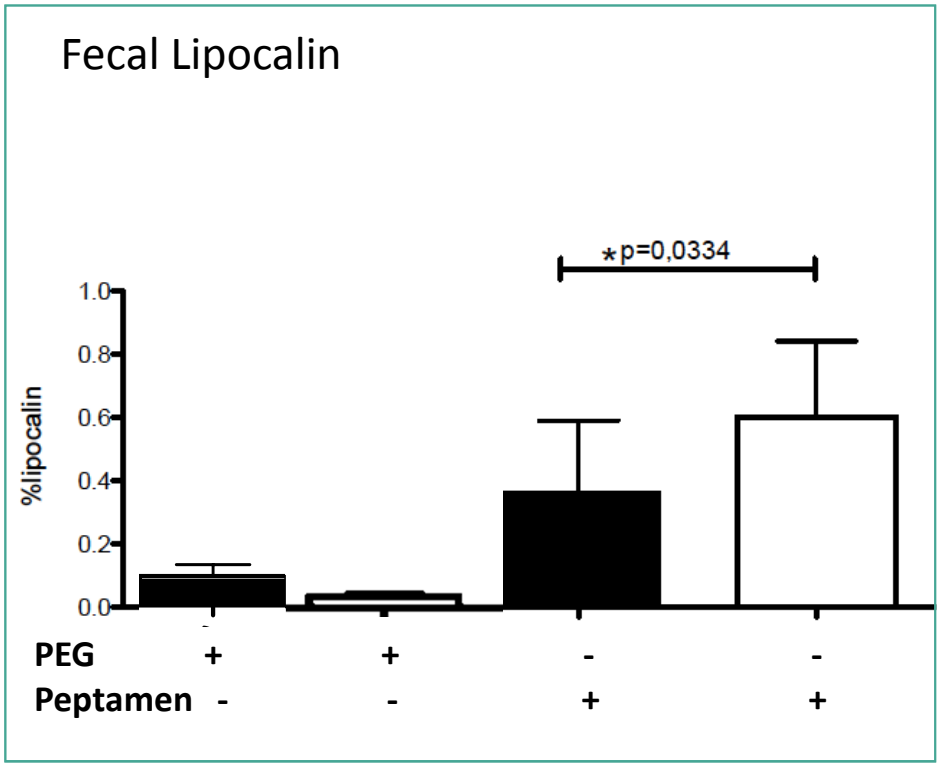


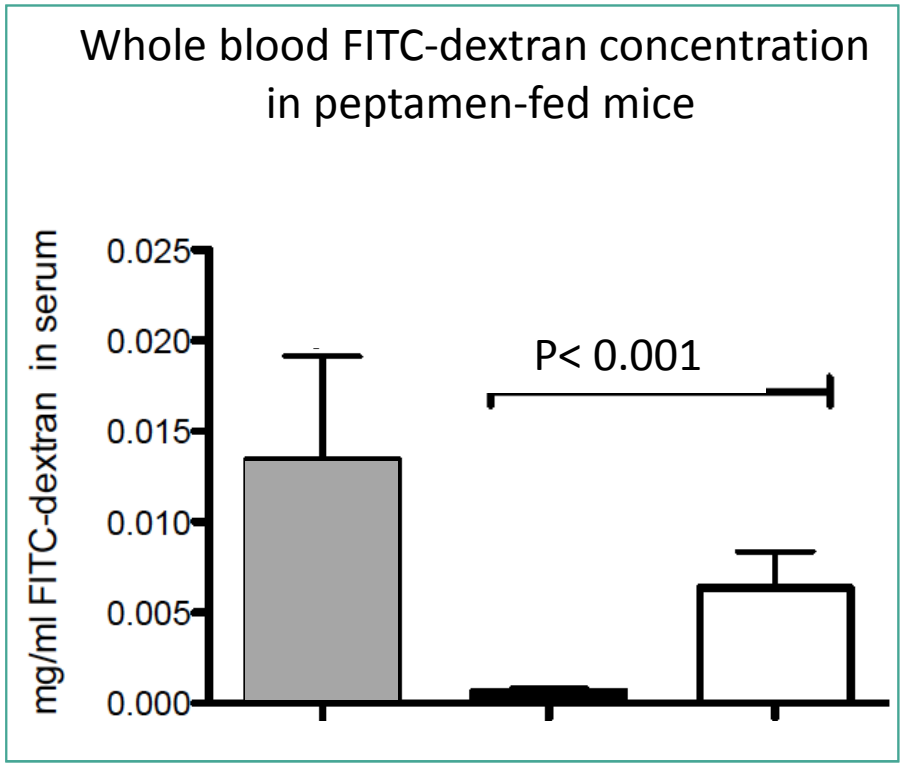




Inflammation intestinale et perméabilité intestinale

◆ *Cftr -/- and Cftr +/+ congenic mice*





cftr -/-

cftr +/+

C57Bl6 treated DSS

En résumé…

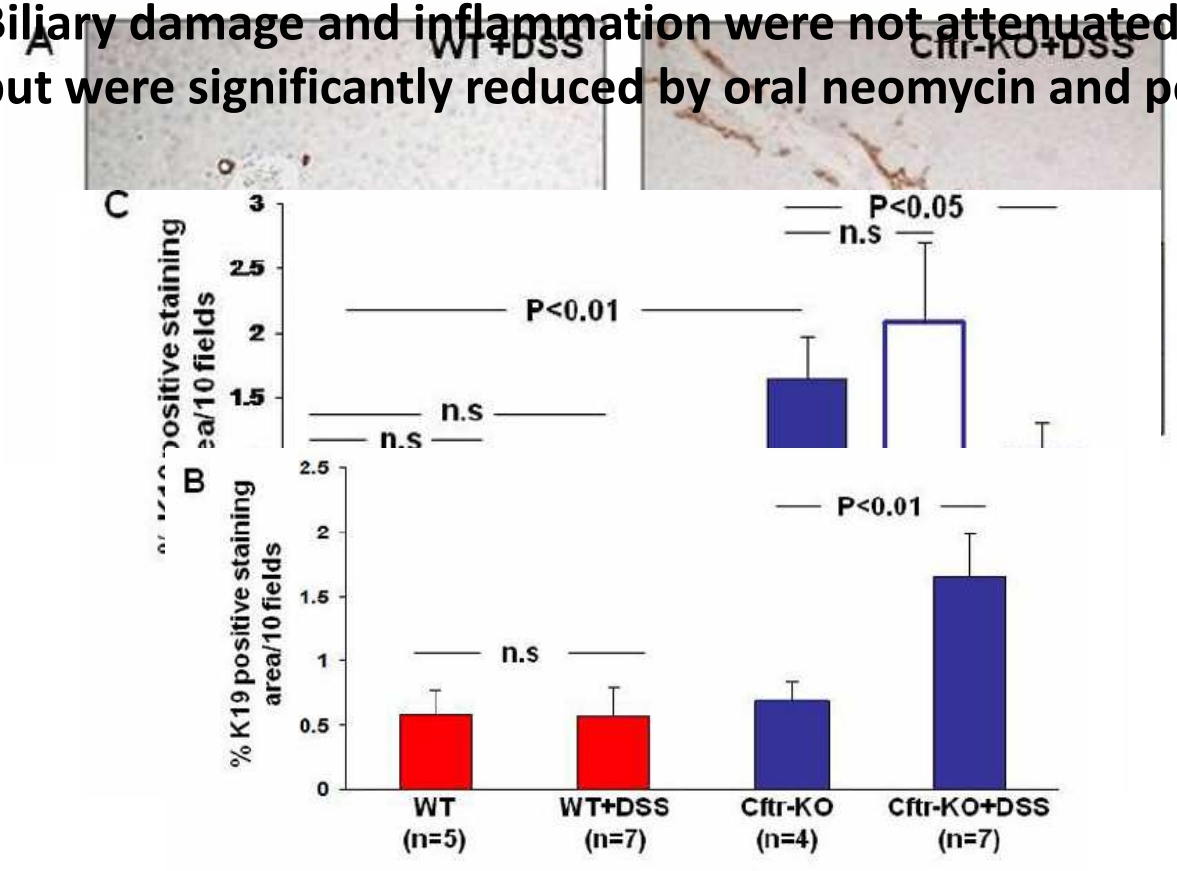
- Chez la souris, une alimentation riche en lipides (Peptamen), modifie la flore microbienne intestinale, caractérisée par une augmentation majeure de la proportion d'E.Coli.

- Cette dysbiose intestinale est associée à:
  - une inflammation intestinale plus marquée chez les souris KO
  - une augmentation de la perméabilité intestinale uniquement chez les souris KO

  - ➢ Endotoxinémie responsable des lésions biliaires observées chez les souris KO ?

Loss of CFTR Affects Biliary Epithelium Innate Immunity

- **DSS induced similar degree of colitis in *CF* (C57BL/6J-Cftrtm1Unc) and WT mice but caused biliary damage and portal inflammation only in the CF mice**
- **Biliary damage and inflammation were not attenuated by nor-UDCA , but were significantly reduced by oral neomycin and polymyxin B.**



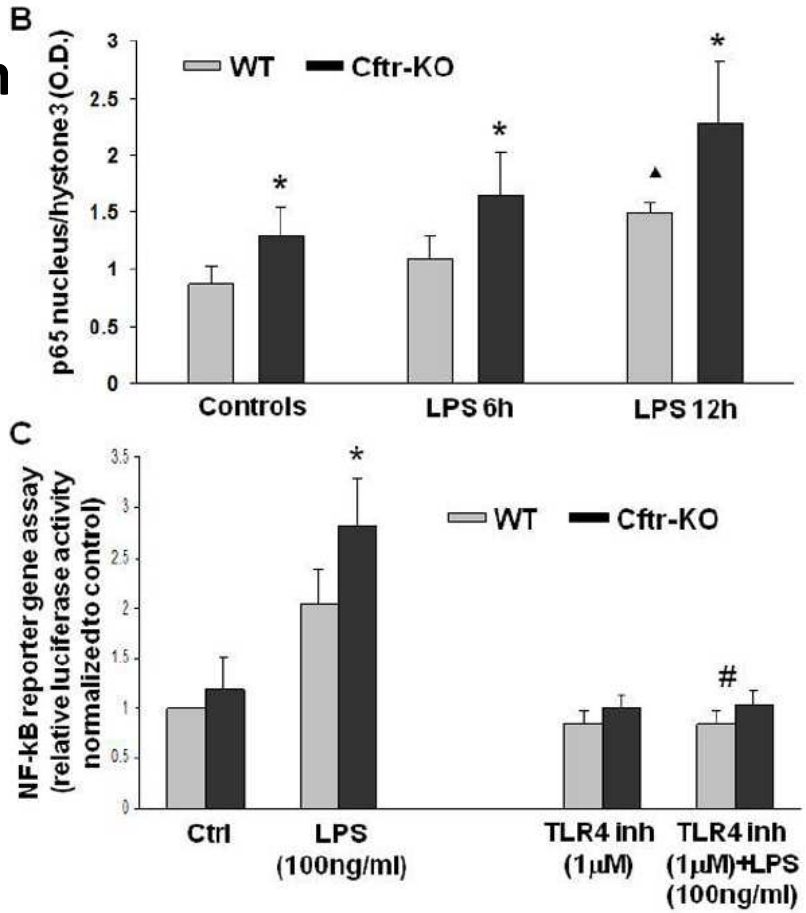
Fiorotto R et al. Gastroenterolgy 2011 ; 141 : 1498

Loss of CFTR Causes an increased TLR4–NF-κB–Mediated Inflammatory Response in Mice

- **CF cholangiocytes exposed to LPS :**

- **NF-κB activity significantly increased in CF, as compared to WT cholangiocytes.**

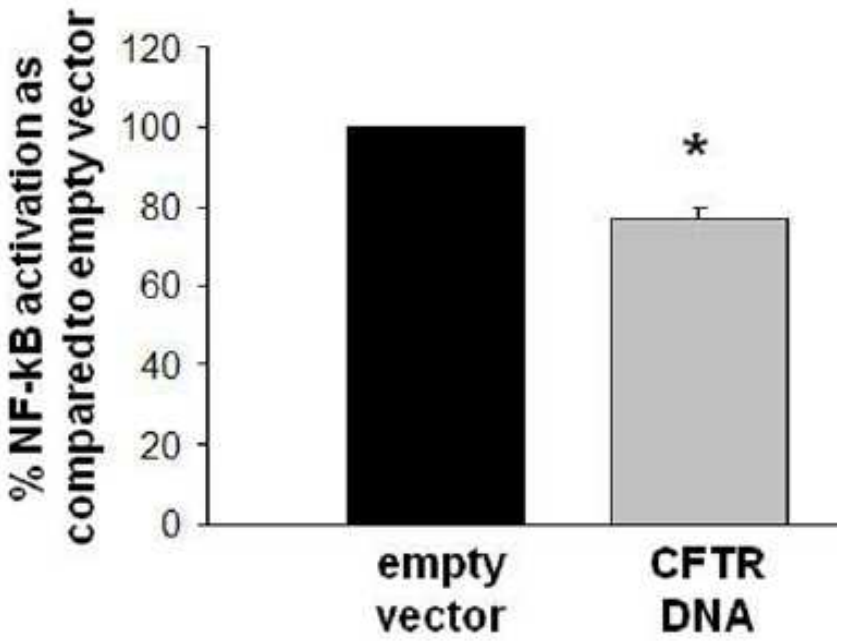
- **NF-κB activity induced by LPS was significantly inhibited by treatment with a TLR4 inhibitor.**



Fiorotto R et al. Gastroenterolgy 2011 ; 141 : 1498

Loss of CFTR Causes TLR4–NF-κB–Mediated Inflammatory Response in Mice

- **CFTR expression in epithelial biliary cells reduces the LPS induced NF-κB activation**



- **CFTR deficiency reduces tolerance of the biliary epithelium to endotoxin**

- **CFTR maintains a state of « endotoxin tolerance » of the biliary epithelium**

Fiorotto R et al. Gastroenterolgy 2011 ; 141 : 1498

Modifications of the gut microbiota and cirrhosis in CF patients

Intestinal Lesions Are Associated with Altered Intestinal Microbiome and Are More Frequent in Children and Young Adults with Cystic Fibrosis and Cirrhosis

Thomas Flass[1], Suhong Tong[3], Daniel N. Frank[5], Brandie D. Wagner[3,6], Charles E. Robertson[7], Cassandra Vogel Kotter[5], Ronald J. Sokol[1,4], Edith Zemanick[2], Frank Accurso[2], Edward J. Hoffenberg[1], Michael R. Narkewicz[1]*

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Filière MUCO CFTR

SOCIETE FRANCAISE DE LA MUCOVISCIDOSE
Fédération des C.R.C.M

VAINCRE LA MUCOVISCIDOSE

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Lydie Humbert
Loic Brot
Harry Sokol
Peter Durie

VAINCRE LA MUCOVISCIDOSE

Fonds CSP
Vaincre la Cholangite Sclérosante Primitive

Is Your Microbiome Happy?