The Human Gut Microbiome in HIV infection

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I CONGRÉS NACIONAL DE SEGURETAT I CONTROL ALIMENTARI: PARASITOLOGIA, PROBIÒTICS, AL·LÈRGIES



The human microbiome

The human microbiota and microbiome





Delsuc et al. 2005 Nat Rev Gen

The **human microbiota** is the collection of all the microorganisms living in association with the human body, including eukaryotes, archaea, bacteria and viruses

The human microbiome refers to their genomes

The Human Microbiome Project



2008 Characterization of the microorganisms associated to healthy and diseased humans



Graphic Sciencie. Perrin Ireland. www.wagsrevue.com/thewag/

Samples from different parts of the human body

Unprecedented information about the complexity of human microbial communities

3,000 microbial genome sequences



Gut microbiota



"All diseases begin in the gut"

(Epidemias) Hippocrates 460 – 370 BC

<u>The Phisiology of defecation</u> The examination of the **stool** (alvine discharge) yields the most information about the nature, temperament and overall quality of the initial **First Digestion** of food and drink in the **gastrointestinal tract**



Anton Van Leeuwenhoek &

Robert Hooke 1665-1683 Microscopy, first description of bacteria



First published depiction of a microorganism (R. Hooke, Micrographia, 1665)

Leeuwenhoek's microscope

The human gut microbiota

"Organ" of ~10¹⁴ microorganisms ~2kg responsible for multiple physiological functions





Intestinal microbiota variation is generally stratified in three **clusters** or **enterotypes**, driven by genus composition



Bacteroides sp.

Bacteria that drive the different enterotypes tend to be mutually excluding

Recovery ?



The pathogenesis of intestinal disorders including inflammatory bowel disease, irritable bowel disease (IBD) and coeliac disease, as well as extra-intestinal disorders such as allergy, asthma or obesity, is associated with gut dysbiosis

HIV infection and gut damage



Brenchley et al. 2004 J Exp Med



Brenchley et al. 2004 J Exp Med

Influence of the gut microbiota on <u>HIV-1 infection</u>

Chronic infection

HIV-1 specific gut dysbiosis?







^{1.049.116} codes







01100-51215 (c) Kandrina Laboratorius

HIV-1 specific gut dysbiosis?



16S rRNA sequencing



Noguera-Julian et al. 2016 eBioMedicine

The MetaHIV Project

The Barcelona MetaHIV Project

Barcelona: Test dataset 156 subjects

- 27 HIV negative, mostly MSM
- 129 HIV positive with ≠ phenotypes
 100 (64%) MSM, 41 (26%) HTS, 15 (10%) PWID



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Phenotype	N	T CD4+ count (c/mm³)	Viral load (c/mL)	ART
Late Presenter	11	< 200	-	No
Immune Discordant	18	< 300	< 50	Yes
Immune Concordant	53	> 500	< 50	Yes
Early Treated	13	> 500	< 50	Yes
Naïve	15	> 500	> 2,000	No
Viremic Controller	11	> 500	50 – 2,000	No
Elite Controller	8	> 500	< 50	No
HIV Negative	27	-	-	-

Microbial diversity and richness



MSM individuals had the highest microbial diversity and richness compared to HTS and PWID in both cohorts



After stratifying for sexual preference HIV-1 infection consistently associated with significant reduction in microbial richness

Microbial diversity and richness



The lowest microbial richness is observed in subjects with a virological-immune discordant response to ART





MSM microbiota of individuals from Bcn and Stk has an enrichment of *Prevotella* and nonMSM an enrichment of *Bacteroides*

Diet influence

Short term recall questionnaire

Nutrients



1: Prevotella	18: Paraprevotella
2: Faecalibacterium	19: Ruminococcaceae_Incertae_Sedis
3: Bacteroides	20: Bacteroidales_unclassified
4: Lachnospiraceae_unclassified	21: Victivallis
5: Succinivibrio	22: Sutterella
6: Alloprevotella	23: vadinBB60_unclassified
7: Ruminococcaceae_unclassified	24: Elusimicrobium
8: Blautia	25: Christensenellaceae_unclassified
9: Lachnospira	26: Intestinimonas
10: RC9_gut_group	27: Butyricimonas
11: Alistipes	28: Saturated fat
12: Barnesiella	29: Water
13: Bacteria_unclassified	30: Ethanol
14: Coprococcus	31: Iron
15: Dialister	32: Total Protein
16: Clostridium_sensu_stricto_1	33: Plant protein
17: Ruminococcus	34: Digestible sugars

Long-term dietary patterns have been linked to alternative enterotype states, but **limited effect of diet on the** composition of the microbiota was found in our setting Take-home message Part I

- Factors related with sexual preference might also affect the gut microbiota composition by unknown mechanisms
- HIV-1 infection is consistently associated with reduced bacterial richness (hallmark of HIV-1 infection) independently of sexual orientation
- Early ART initiation might help to preserve gut microbial richness

Gut Microbiota Linked to Sexual Preference and HIV Infection Marc Noguera-Julian ^{a,b,c,1} , Muntsa Rocafort ^{a,c,1} , Yolanda Guillén ^{a,c} , Javier Rivera ^{a,b} , Maria Casadellà ^{a,c} , Piotr Nowak ^d , Falk Hildebrand ^e , Georg Zeller ^e , Mariona Parera ^a , Rocío Bellido ^a , Cristina Rodríguez ^a , Jorge Carrillo ^{a,c,g} , Beatriz Mothe ^{a,b,c,f} , Josep Coll ^{a,f} , Isabel Bravo ^f , Carla Estany ^f , Cristina Herrero ^f , Jorge Saz ^h , Guillem Sirera ^f , Ariadna Torrela ⁱ , Jordi Navarro ⁱ , Manel Crespo ⁱ , Christian Brander ^{a,b,c,j} , Eugènia Negredo ^{b,c,f} , Julià Blanco ^{a,b,c,} , Francisco Guarner ^k , Maria Luz Calle ^b , Peer Bork ^{e,l,m} , Anders Sönnerborg ^d , Bonaventura Clotet ^{a,b,c,f} , Roger Paredes ^{a,b,c,f,*}
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HIV-1 specific gut dysbiosis?







Gene count rank

- Downsampling 1M → 70,865 313,572
- Downsampling 10M → 163,558 904,589
- All sequences → 226,219 1,373,562



Two populations according to gene richness

С Gene richness, all subjects χ^2 p-value = 0.001 HIV-1 negative 3e-06-Density 2e-06-HIV-1 positive 1e-06-0e+00-250000 500000 750000 Gene counts (10 M) LGC HGC HIV + 92 37 HIV -11 16

- Chi-square p < 0.001
- Other factors linked to LGC: HIV risk group, older age, female gender, caucasian ethnicity, HIV phenotype (viremic controller and HIV-negative higher in HGC), Nadir CD4+, creatinin, FA, fibrinogen and platelets

• Chi-square p < 0.055

Gene richness by nadir CD4+ T-cell counts χ^2 p-value = 0.002 100-18 18 34 21 11 75 50 25 16 16 15 0 100,200 200,500 1200 7500 HIV-I negative CD4+ T cells / mm³

Subjects with LGC were gradually more abundant in lower nadir CD4+ T-cell count categories

Dose-effect relationship between nadir CD4+ T-cell counts and microbial gene richness in multivariate regression model

Table 2. Factors associated with low-microbial gene counts ^a							
		Univariate			Multivariate		
		OR	95% CI	p Value	OR	95% Cl	p Value
Age	Per each additional year	1.03	[1-1.07]	0.074	-	-	-
Gender	Female	1					
	Male	0.32	[0.10-0.82]	0.028	-	-	_
	Transgender woman	-	-	-			
Ethnic Group	Caucasian	1			1		
	Hispanic-Latin	0.27	[0.11-0.64]	0.003	0.26	[0.10-0.67]	0.006
	Asiatic and others ^b	-	-	-	-	-	_
HIV-1 risk group	Non-MSM	1			1		
	MSM	0.17	[0.06-0.39]	<0.001	0.20	[0.07-0.51]	0.002
HIV-1 status	Negative	1					
	Positive	3.68	[1.57-8.89]	0.003	-	-	-
Nadir CD4+ T-cell count, cells/mm ³	HIV-1 negative	1			1		
	>500	2.04	[0.75-5.74]	0.169	2.13	[0.73-6.45]	0.173
	200-500	3.09	[1.19-8.37]	0.006	2.92	[1.03-8.62]	0.047
	100-200	6.55	[1.86-27.71]	0.023	5.55	[1.40-26.15]	0.020
	<100	26.18	[4.40-506.6]	0.003	14.00	[2.02-288.71]	0.023

^aFull dataset analysis, n = 156 subjects

^bAnalysis does not apply because all subjects are included in the same response group

MSM men-who-have-sex-with-men

Increased risk of being LGC when nadir CD4+ T cell were < 500

P = 0.030

R = 0.192

Iron

750000

P = 0.007

R = 0.237

Diet questionnaire Moderate influence of diet on gene richness

Positive correlation between gene richness and intake of monounsaturated fats, carotenoids, iron, fiber and Vitamin A

(10)

Dirichlet multinomial regression
Association between iron intake
and <i>P. copri, E. eligens</i> and
Ruminococcus spp.

Microbial species and gene richness

-0.50 -0.25 0.00 0.25

a

Specialists

Methanogenesis Basal transcription Machinery (Archaea) Production of butyrate Transport and metabolism of free fatty acids Microbial growth, replication and protein production

43 metabolic pathways correlated with gene richness

Generalists

Synthesis of sphingolipids LPS biosynthesis (Gram -) Synthesis of biotin Complex carbohydrates enzymes

IONNSW

HIV, Inflammation and Oxidative Stress

Reactive Oxygen Species (ROS) production during the host inflammatory response

Antioxidants, such as glutathione peroxidase and catalase could catalyze the decomposition of ROS into non-toxic compounds such as oxygen and water.

Wittmann et al. 2012. Advances in Hematology

ROS Metabolism enzymes enriched in LGC

Gene copies were significantly more abundant as nadir CD4 decreased

ROS Metabolism enzymes enriched in LGC

Fecal short chain fatty acid (SCFA) levels

Spearman's rho

Bacterial virulence factors and antimicrobial resistance

Enzymatic toxins enriched in LGC

Bifidobacterium longum Bacteroides eggerthii Bacteroides thetaiotaom Prevotella copri Erysipelotrichaceae noname biforn Ruminococcus 5 1 398FAA Bacteroides uniformis Subdoligranulum unclassifie Alistipes finegoldii Bacteroides faecis Prevotella stercorea Eubacterium eligens Alistipes onderdonkii Eubactorium halli Sutterella wadsworthensis Bacteroides finegoldii Pactomidos vulnatus Acidaminococcus unclassif Alistipes putredinis **Bantomidos franilis** Partemides wlanisolvens Butyrivibrio crossotus Eubacterium siraeum Other taxa(151) Bacteroidales noname ph8 Bacteroides massiliensis Barnesiella intestinihominis Cateribacterium mitsuokai Faecalibacterium prausnitzi Bacteroides caccar Bacteroides ovatus Odoribacter splanchnicus Clostridium L2 50 Phascolarctobacterium succinatuters Parabacternidos distasonis Bacternides clarus Bacteroides plebeius Coprococcus comes Rosphuria hominis Dialister invisus Roseburia intestinalis Bactemides controla Bacteroides salversiae Parahacternides merdae Parabacteroides unclassified Dorea longicatena Roseburia inulinivorans Bacteroides dorei Bacteroides stercoris

Microbial shifts associated with immune deficiency implied increases in bacterial virulence factors and changes in the gut microbial resistome.

Take-home message Part II

- HIV-1-induced immune deficiency is strongly linked to reduced microbial richness and results in significant shifts in the composition and function of the gut microbiome.
- Gut microbiome shifts observed in HIV-1 are not necessarily unique to the infection, but instead, share important characteristics with those seen in other diseases featuring gut inflammation

MucosalImmunology

ARTICLE

Low nadir CD4+ T-cell counts predict gut dysbiosis in HIV-1 infection

Yolanda Guillén^{1,2}, Marc Noguera-Julian^{1,2,3}, Javier Rivera^{1,3}, Maria Casadellà^{1,2}, Alexander S. Zevin⁴, Muntsa Rocafort^{1,2}, Mariona Parera¹, Cristina Rodríguez¹, Marçal Arumí¹, Jorge Carrillo^{1,2}, Beatriz Mothe^{1,3,5}, Carla Estany⁵, Josep Coll^{1,5}, Isabel Bravo⁵, Cristina Herrero⁵, Jorge Saz⁶, Guillem Sirera⁵, Ariadna Torrella⁷, Jordi Navarro^{2,7}, Manuel Crespo⁸, Eugènia Negredo^{2,3,5}, Christian Brander^{1,2,3,9}, Julià Blanco^{1,2,3}, Maria Luz Calle³, Nichole R. Klatt⁴, Bonaventura Clotet^{1,2,3,5} and Roger Paredes^{1,2,3,5}

www.nature.com/mi

Influence of the gut microbiota on <u>HIV-1 eradication</u>

Why should we care about microbiota in HIV eradication?

- 1. Microbiota priming of CD4 T and B cell repertories. Cross reactivity of HIV-1 vaccine responses (HVTN 505)
- 2. "Good bacteria" help fight cancer in mice. Antibiotic consumption associated with poor response to immunotherapeutic PD-1 blockade. Oral supplementation of Akkermansia muciniphila restores tumor-specific CTL responses.
- 3. Epigenetic modulation of the reservoir and virus production. Butyrate is and HDAC inhibitor.

Williams et al. Curr Op HIV 2017

Matson et al. Science 2018

Cases of post-treatment control and cure

Deeks et al. lat Med. International aids Society: Global Scientific Strategy Towards an HIV Cure 2016

Deeks et al. lat Med. International aids Society: Global Scientific Strategy Towards an HIV Cure 2016

What is viral latency?

- Virus is able to persist by integrating its genome into the host cell DNA. It remains hidden from immune responses.
- 2. Virus is present but **not active in a cell.**
- 3. **Reservoirs** are cells where HIV is able to persist in the latent phase, **even while on antiretroviral therapy (ART).**
- 4. Cellular reservoirs are widely dispersed throughout the body.

Avettland-Fènoël et al. Clin Micr Rev 2016

"Kick and kill" and gut microbiota

- 1. Asses the influence of the gut microbiota in a "kick-&-kill" based strategy by characterizing:
- a. Microbial composition
- b. Fecal metabolites
- c. Immune response
- d. Viral reservoir

2. Assess the effect on the microbial composition through the assay of:

- a. Romidepsin and vaccination.
- b. ART stop and restart.
- *c.* Potential probiotics for future interventions targeting HIV eradication.

