Microbiome, Immune function, and murine pulmonary disease

CURES Symposium:

Addressing the Asthma and Allergy Epidemics

October 7, 2015

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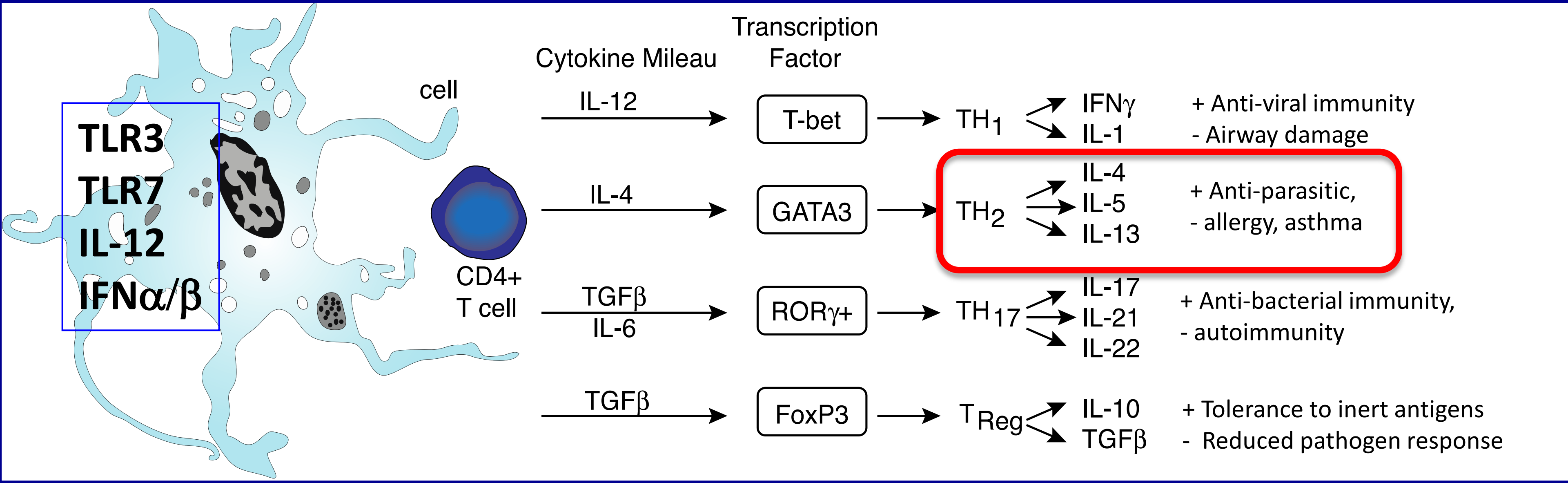
T cell maturation and differentiation depends upon Immune environments



TLR3
TLR7
IL-12
IFNα/β

cell

CD4+
T cell

Cytokine Mileau
Transcription Factor

IL-12 → T-bet → TH$_1$ → IFNγ, IL-1 — + Anti-viral immunity - Airway damage

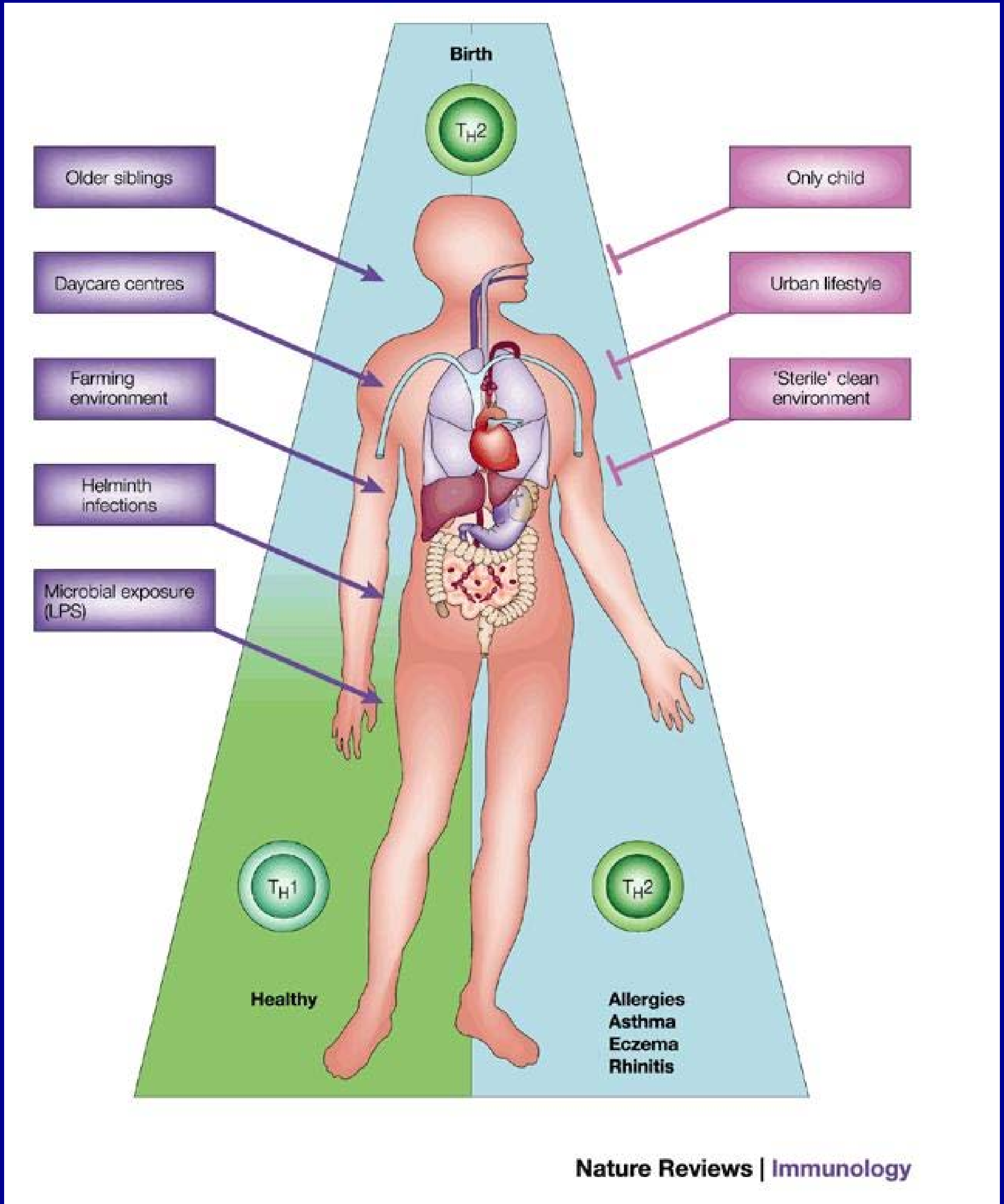
IL-4 → GATA3 → TH$_2$ → IL-4, IL-5, IL-13 — + Anti-parasitic, - allergy, asthma

TGFβ, IL-6 → RORγ+ → TH$_{17}$ → IL-17, IL-21, IL-22 — + Anti-bacterial immunity, - autoimmunity

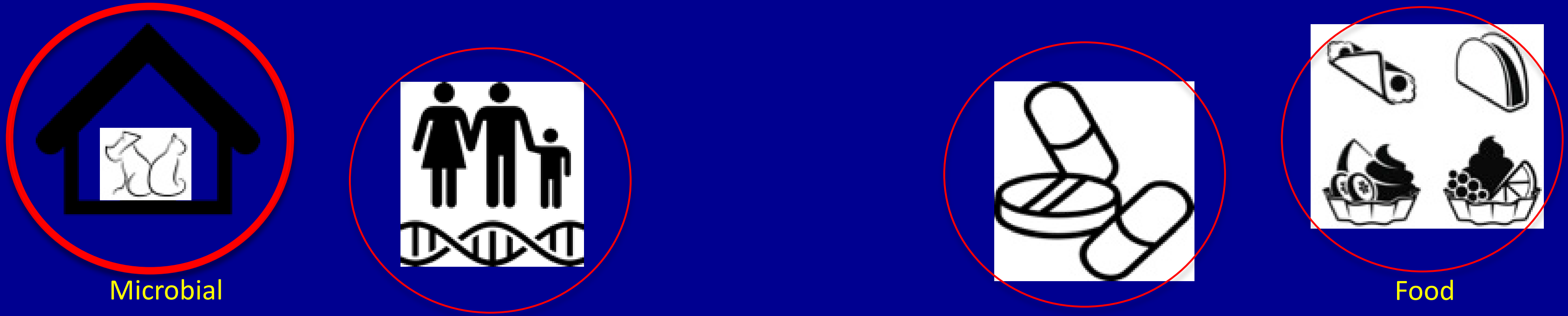
TGFβ → FoxP3 → T$_{Reg}$ → IL-10, TGFβ — + Tolerance to inert antigens - Reduced pathogen response

IL-4- IgE → Mast cell activation
IL-5- Eosinophilia → airway damage and fibrosis
IL-13- Goblet cell metaplasia → mucus and airway obstruction

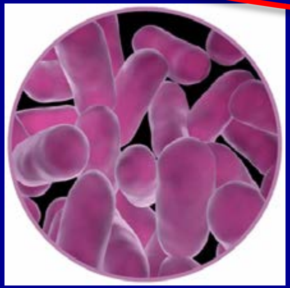
The hygiene hypothesis



Nature Reviews | Immunology



Factors that affect the gut microbiome

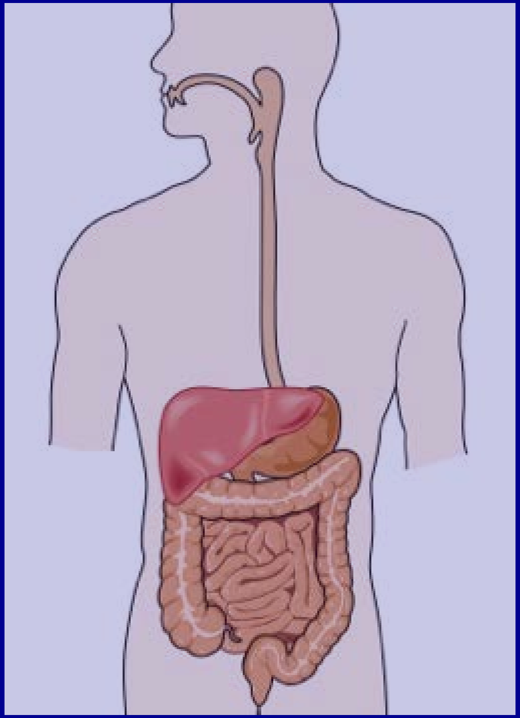


Commensal bacteria

Protection
- Mucosal barrier function
- Treg cell development

Development and modulation of the host immune responses

Metabolism



Pathobionts

The resulting overgrowth of the pathobiont may cause inflammation and bleeding of the lining of the colon.

Obesity
Cancer

Adapted from Environ Health Perspect ; DOI:10.1289

Infant's Environment shapes the Microbiome

- Natural vs. Cesarean section birth
- Bottle vs. Breast feeding
- Timing and type of solid food introduction
- Antibiotic use
- Vitamin and nutrition
- Household exposure- high % of early life

Man's best friend? The effect of pet ownership on house dust microbial communities.

-Increase in bacterial diversity and a decrease in fungal species

Fujimura KE, Johnson CC, Ownby DR, Cox MJ, Brodie EL, Havstad SL, Zoratti EM, Woodcroft KJ, Bobbitt KR, Wegienka G, Boushey HA, Lynch SV. JACI 126:410.

Dust-exposed mice have a modified response in cockroach allergen (CRA) model

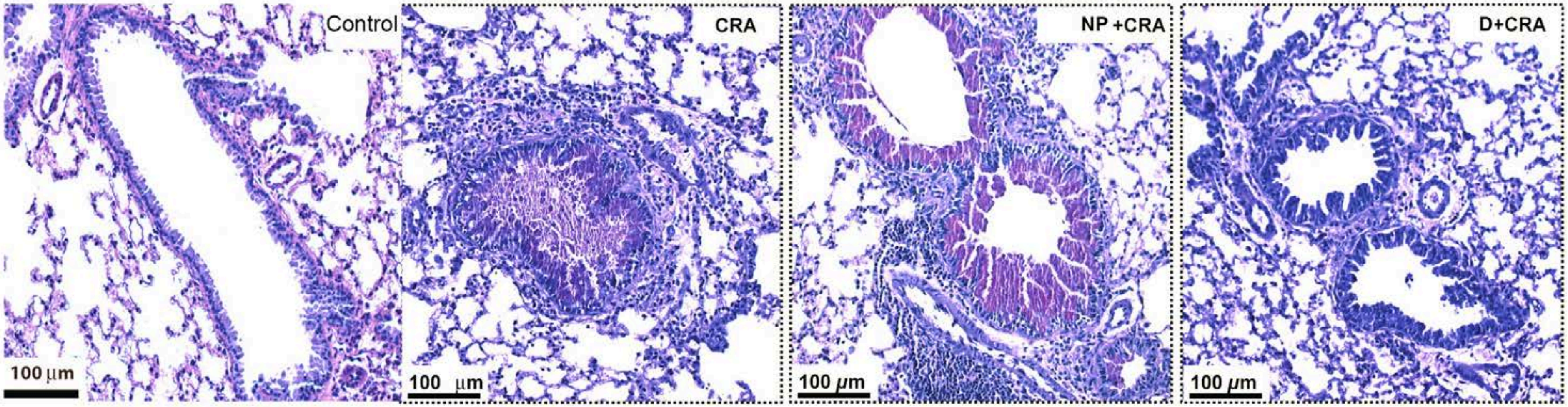
gavage w/dust

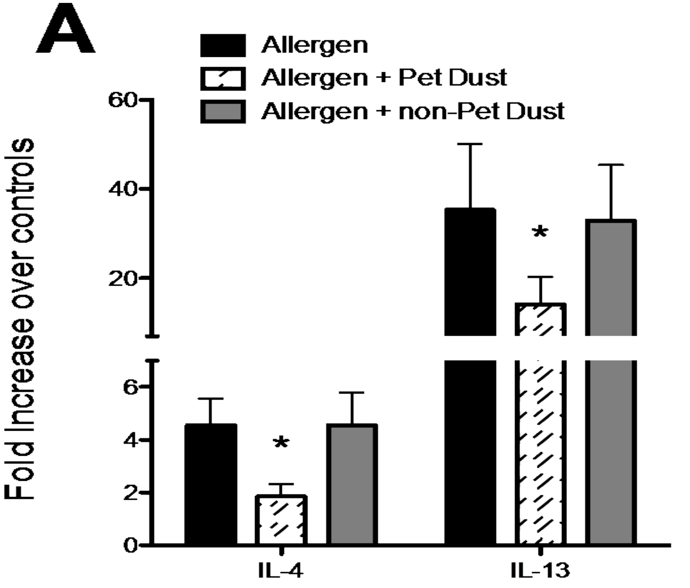
I.T. CRA

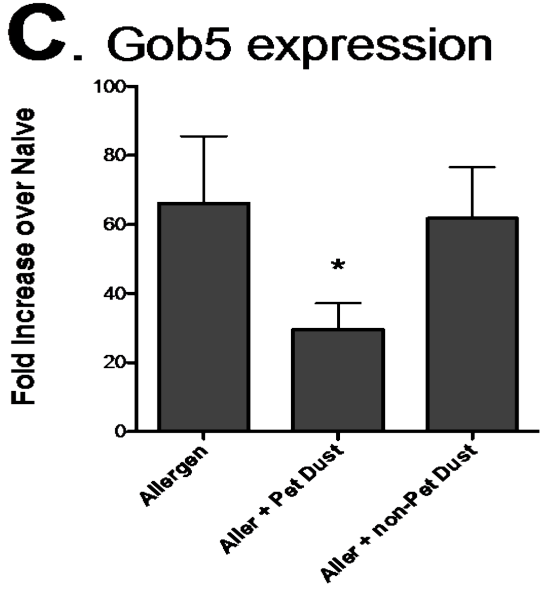
I.T. CRA

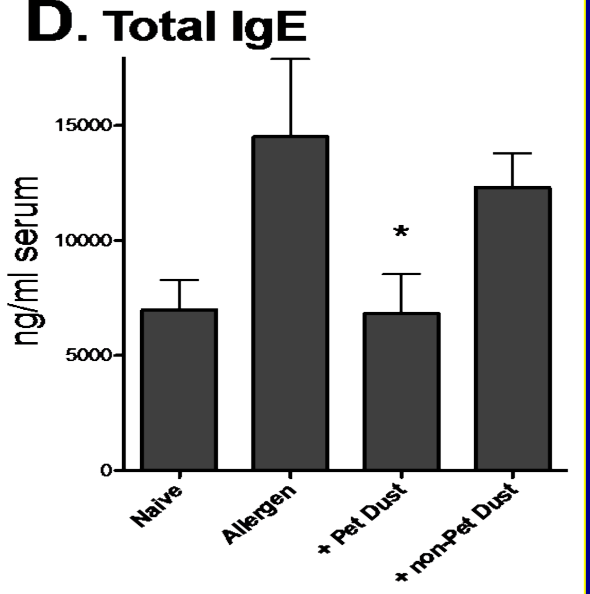
I.T. CRA

ER

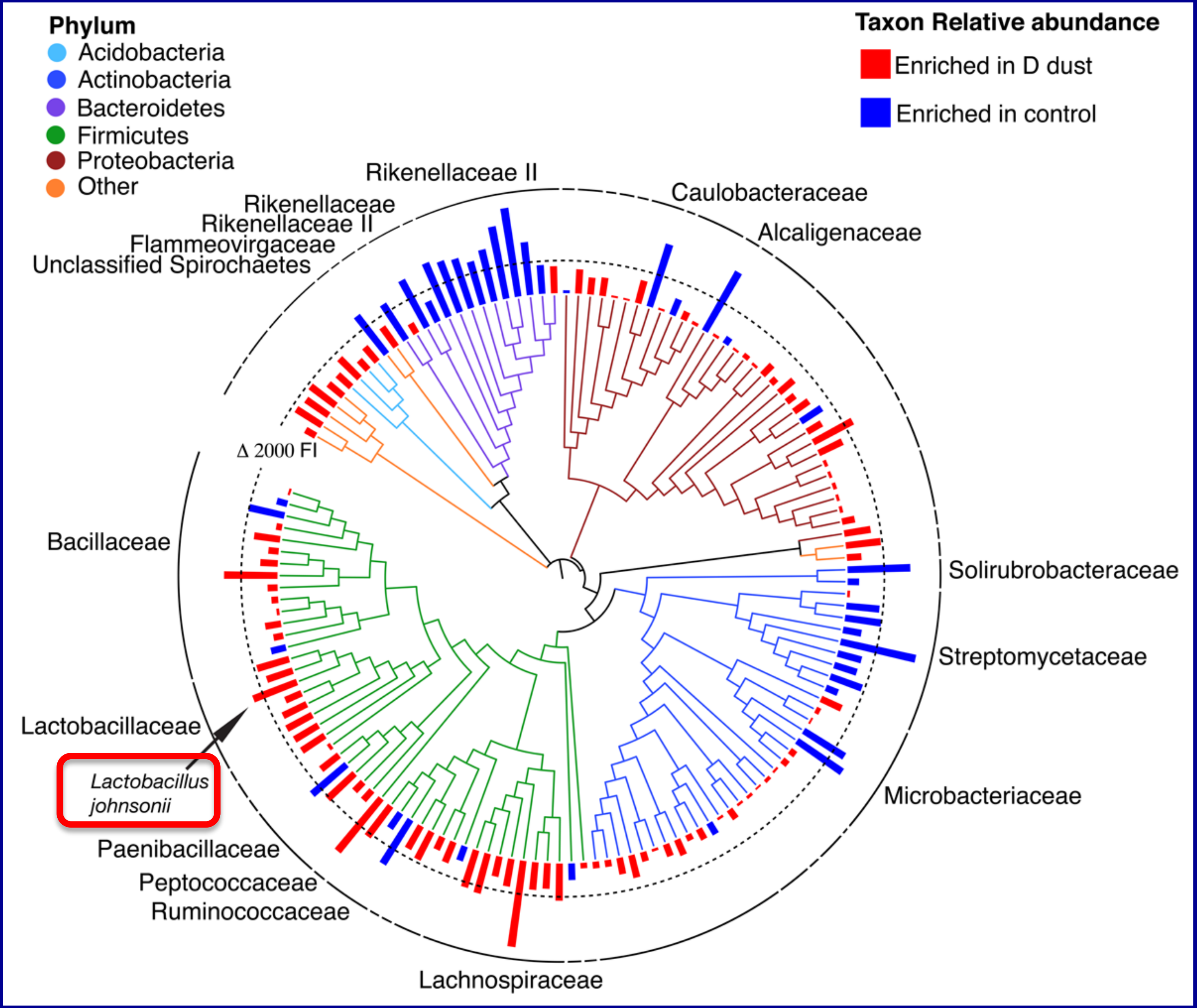








Bacterial diversity in Pet dust supplemented animals

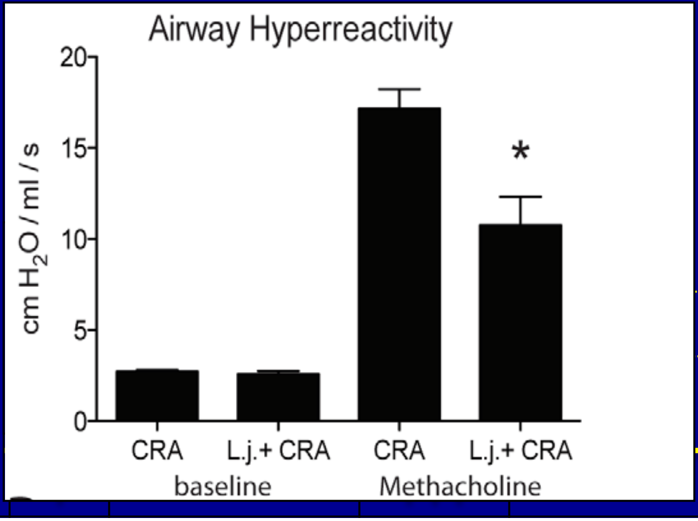


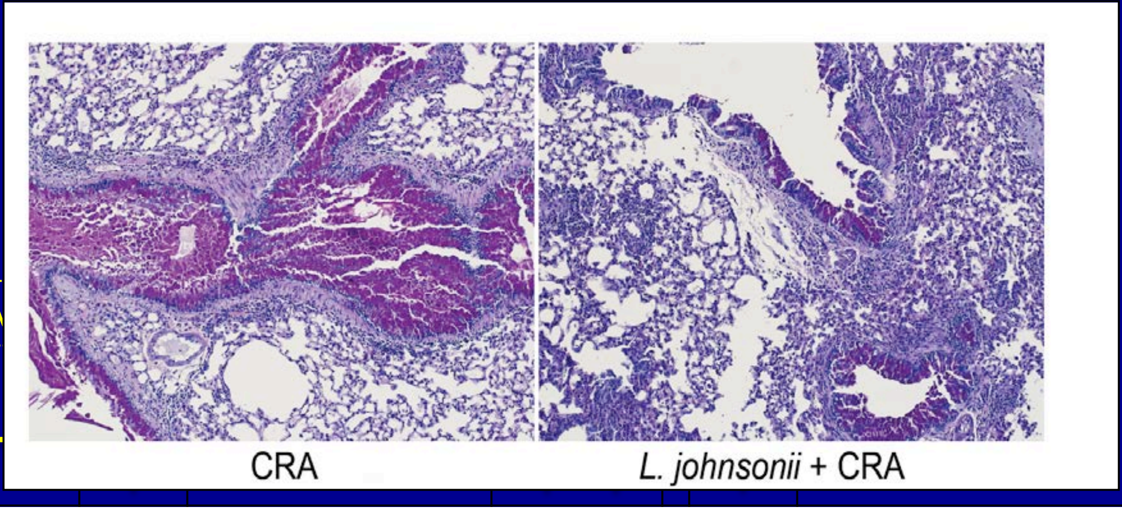
PNAS 111:805-810

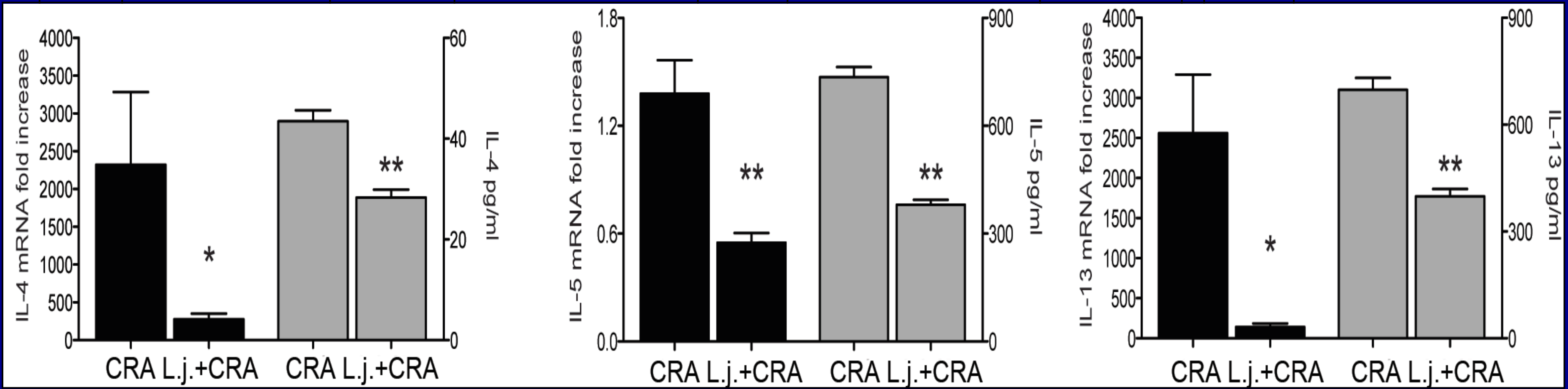
Lactobacillus Supplementation

- Colonic contents of 4 mice
- *Lactobacillus* isolation media
- Sequenced 6 isolates per mouse
- Twenty one isolates yielded high quality full length 16S rRNA sequence– **All were *L. johnsonii***
- 99% coverage and 99% homology to expected *Lactobacillus* species
- Batch culture
- Standardized (1 x 10^7 CFU) supplements in glycerol

Lactobacillus johnsonii supplementation protects asthmatic mice



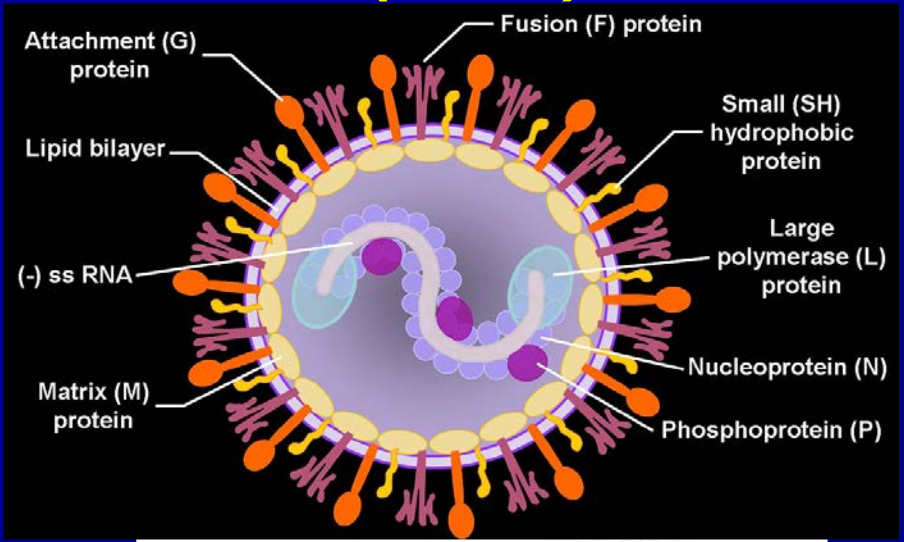




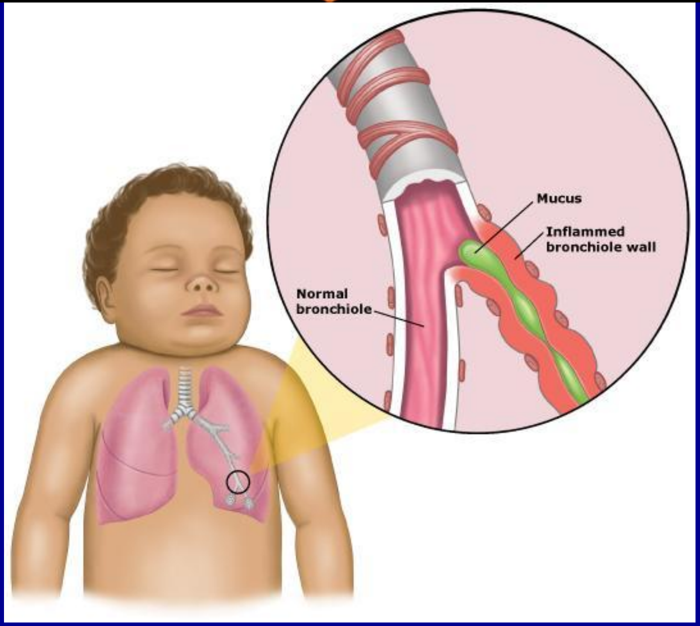
PNAS 111:805-810

Viral infections during infancy -→ Asthma?

Respiratory Syncytial Virus (RSV)



- Leading cause of respiratory illness and hospitalization in infants
- Airway epithelial damage
- Leads to long-term Respiratory disease
- Goblet cell hypertrophy, mucus hypersecretion;
- Th2 and Th17 cytokine production;
- Associated with increased Asthma
- During RSV infection-Tregs control the magnitude of cellular immune responses. *(Brincks EL, J. Immunology, 2013)*



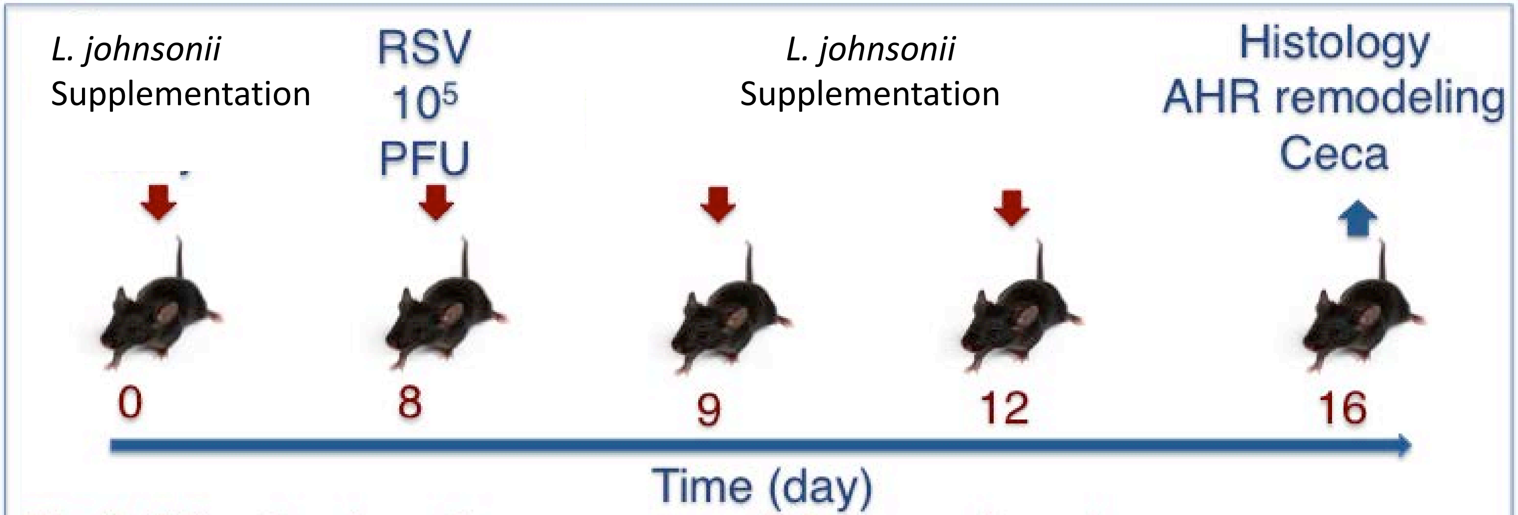


Fig 1. Mice treatment over course of the experiment

-Viable vs heat killed bacteria

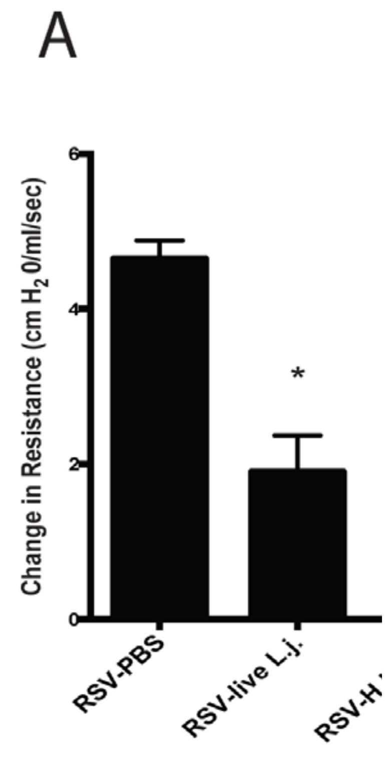
  1 x 10^7 CFU daily supplement – 7 days

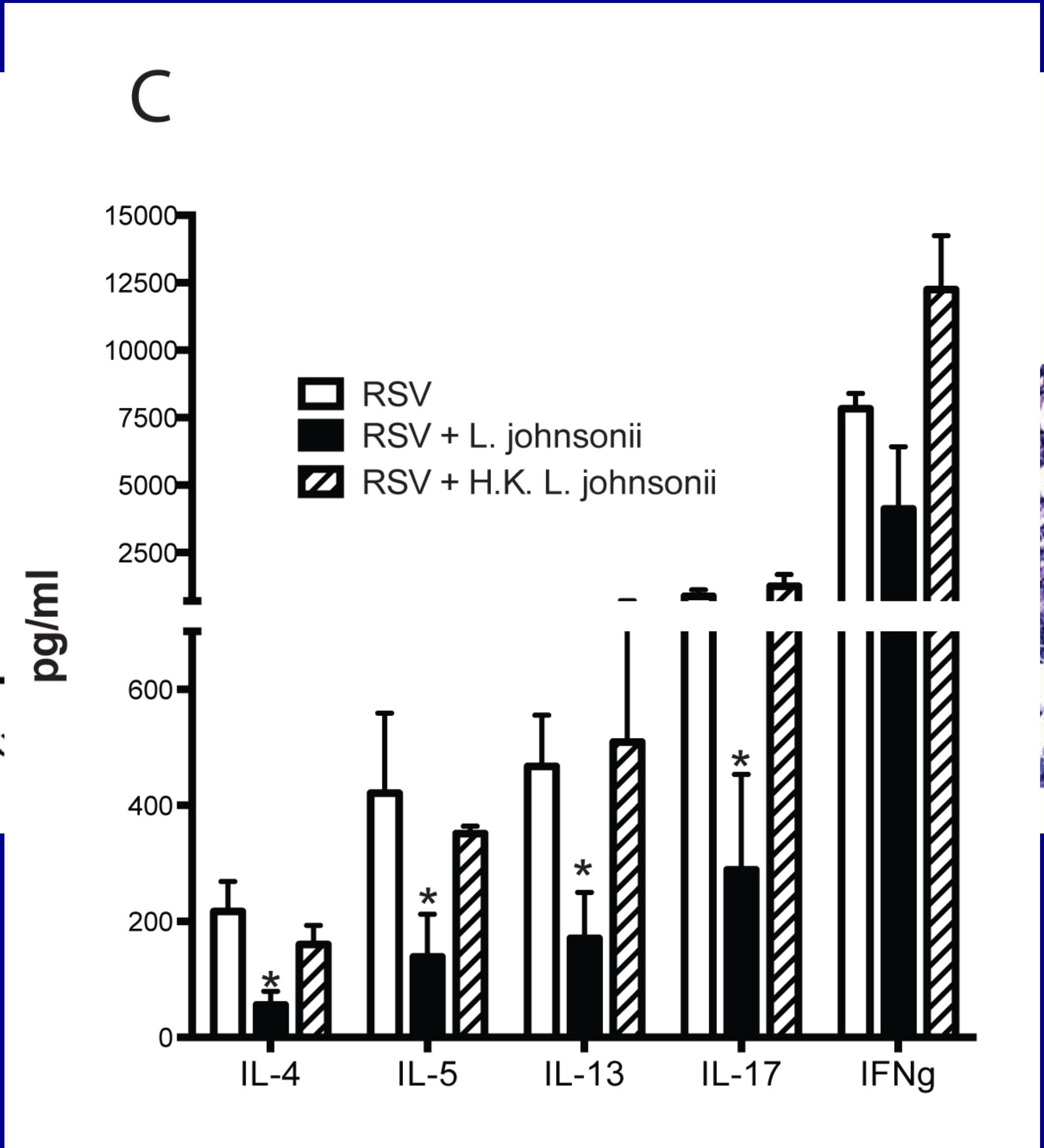
-RSV (line 19) infection on day 8 of treatment protocol

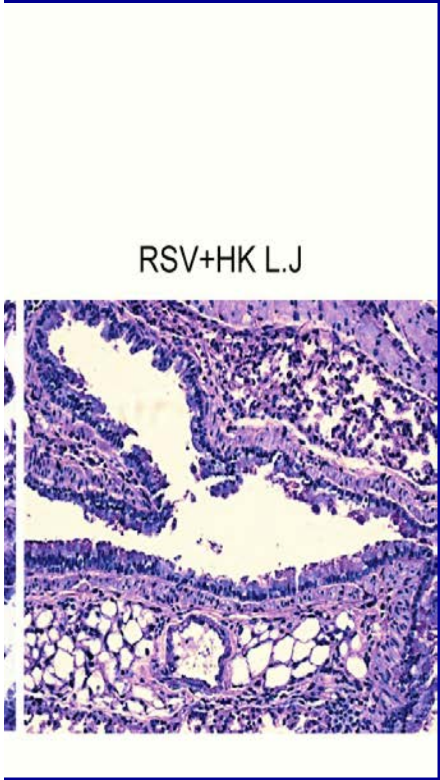
- Outcome measurements

  1. Airway responses – histology
  2. Immune responses – Th2, IFNg, muc5AC Gob5

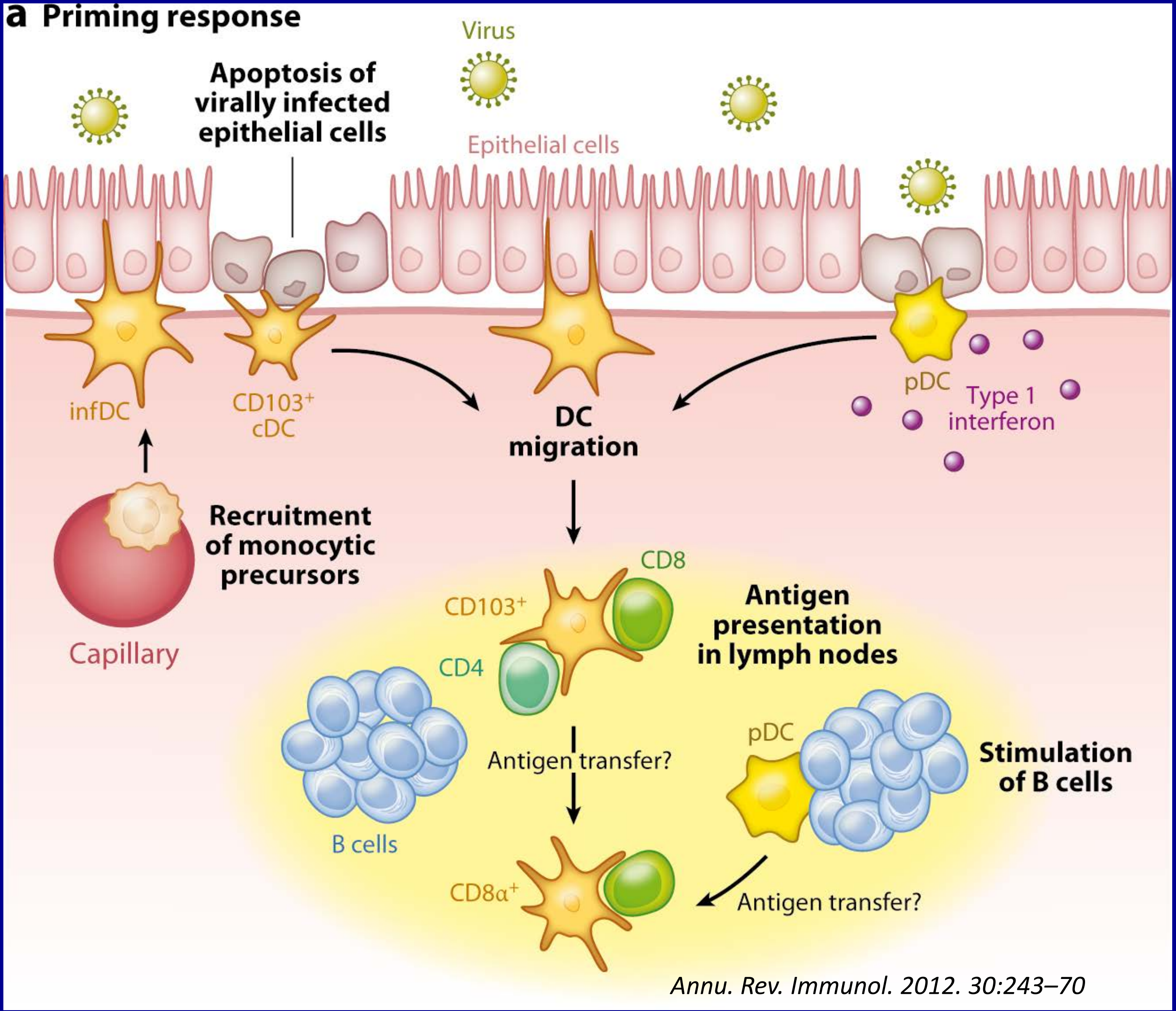
L. Johnsonii supplementation alters RSV-induced pathophysiology





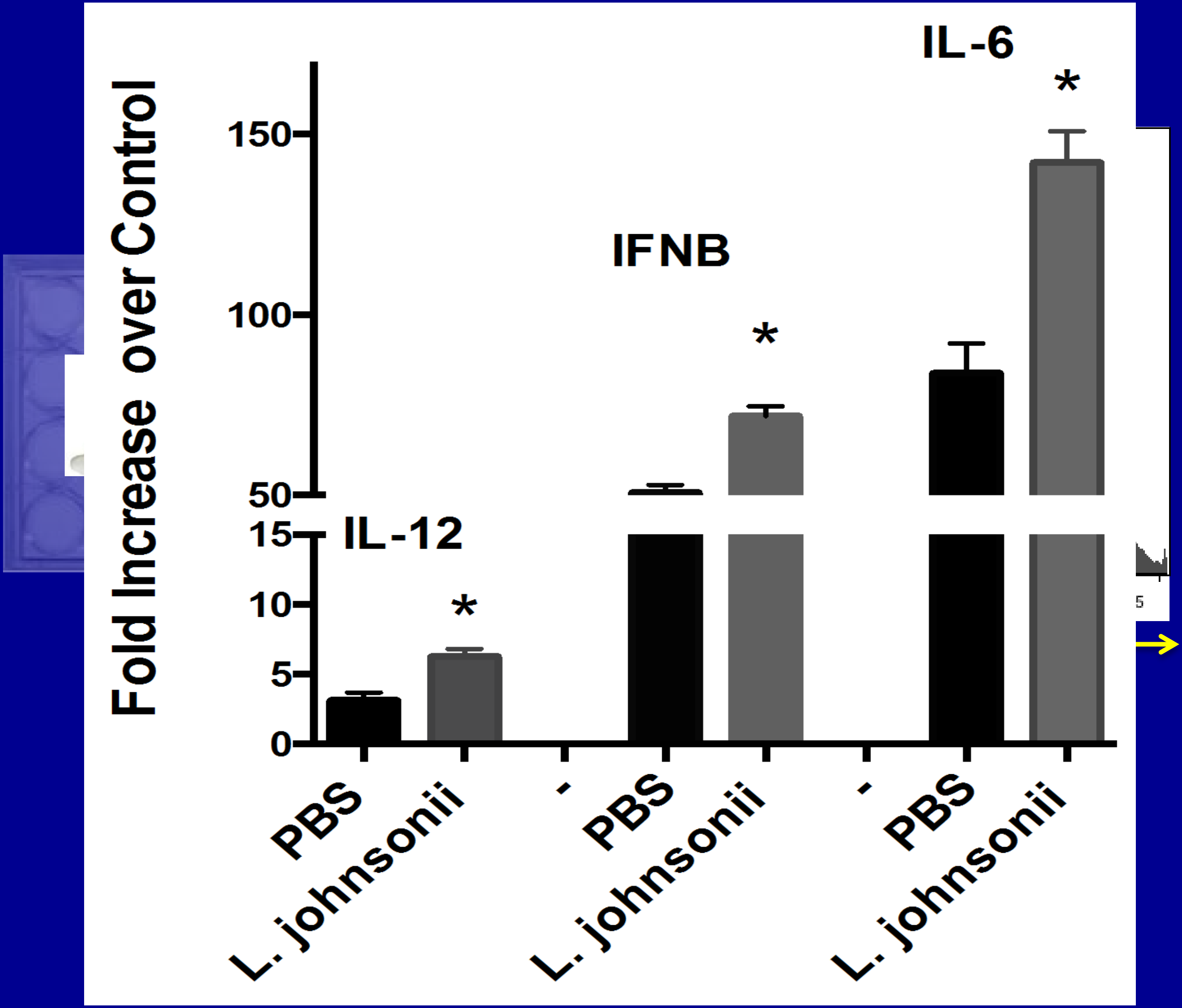


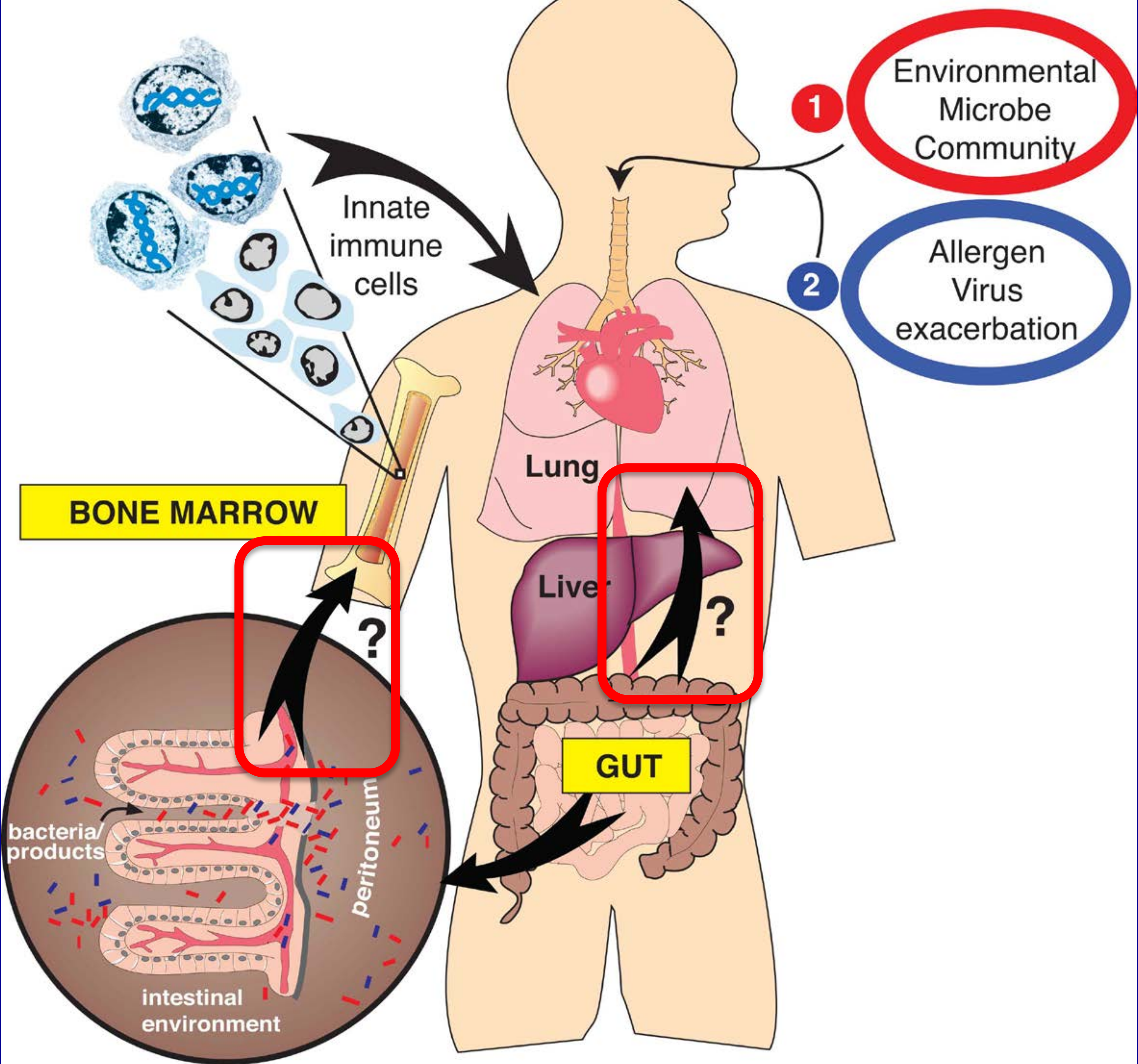
Dendritic Cells (DC) prime the adaptive immune response



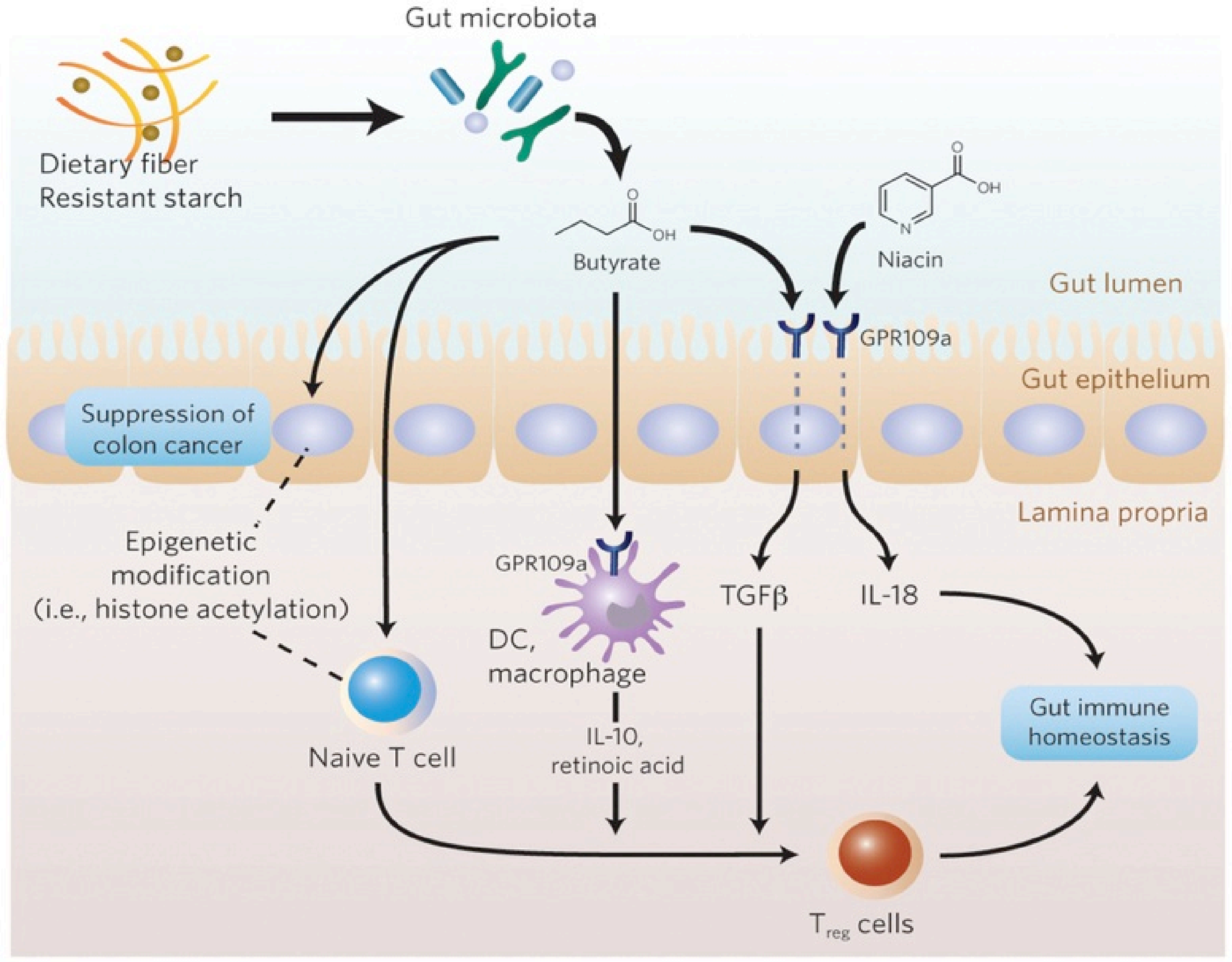
Annu. Rev. Immunol. 2012. 30:243–70

Bone marrow DC are altered in L. johnsonii exposed animals in Response to RSV



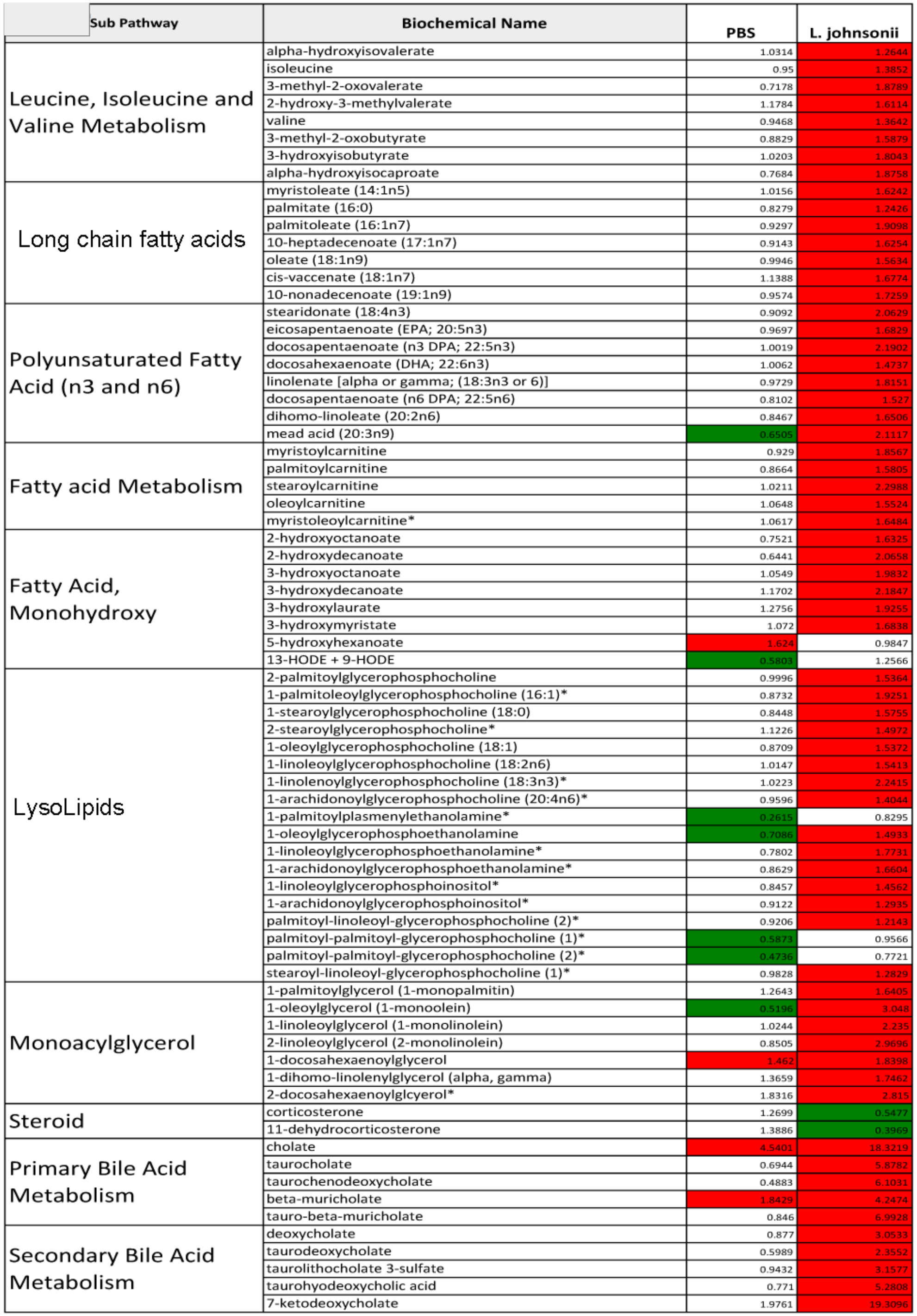

1
Environmental Microbe Community
2
Allergen Virus exacerbation
Innate immune cells
Lung
Liver
BONE MARROW
GUT
?
?
bacteria/ products
peritoneum
intestinal environment

Microbiome can determine metabolite production available to alter immune function



Won-Jae Lee & Koji Hase, 2014

Upregulation of plasma metabolites in animals supplemented with *L. johnsonii*



Sub Pathway	Biochemical Name	PBS	L. johnsonii
Leucine, Isoleucine and Valine Metabolism	alpha-hydroxyisovalerate	1.0314	1.2644
	isoleucine	0.95	1.3852
	3-methyl-2-oxovalerate	0.7178	1.8789
	2-hydroxy-3-methylvalerate	1.1784	1.6114
	valine	0.9468	1.3642
	3-methyl-2-oxobutyrate	0.8829	1.5879
	3-hydroxyisobutyrate	1.0203	1.8043
	alpha-hydroxyisocaproate	0.7684	1.8758
Long chain fatty acids	myristoleate (14:1n5)	1.0156	1.6242
	palmitate (16:0)	0.8279	1.2426
	palmitoleate (16:1n7)	0.9297	1.9098
	10-heptadecenoate (17:1n7)	0.9143	1.6254
	oleate (18:1n9)	0.9946	1.5634
	cis-vaccenate (18:1n7)	1.1388	1.6774
	10-nonadecenoate (19:1n9)	0.9574	1.7259
Polyunsaturated Fatty Acid (n3 and n6)	stearidonate (18:4n3)	0.9092	2.0629
	eicosapentaenoate (EPA; 20:5n3)	0.9697	1.6829
	docosapentaenoate (n3 DPA; 22:5n3)	1.0019	2.1902
	docosahexaenoate (DHA; 22:6n3)	1.0062	1.4737
	linolenate [alpha or gamma; (18:3n3 or 6)]	0.9729	1.8151
	docosapentaenoate (n6 DPA; 22:5n6)	0.8102	1.527
	dihomo-linoleate (20:2n6)	0.8467	1.6506
	mead acid (20:3n9)	0.6505	2.1117
Fatty acid Metabolism	myristoylcarnitine	0.929	1.8567
	palmitoylcarnitine	0.8664	1.5805
	stearoylcarnitine	1.0211	2.2988
	oleoylcarnitine	1.0648	1.5524
	myristoleoylcarnitine*	1.0617	1.6484
Fatty Acid, Monohydroxy	2-hydroxyoctanoate	0.7521	1.6325
	2-hydroxydecanoate	0.6441	2.0658
	3-hydroxyoctanoate	1.0549	1.9832
	3-hydroxydecanoate	1.1702	2.1847
	3-hydroxylaurate	1.2756	1.9255
	3-hydroxymyristate	1.072	1.6838
	5-hydroxyhexanoate	1.624	0.9847
	13-HODE + 9-HODE	0.5803	1.2566
LysoLipids	2-palmitoylglycerophosphocholine	0.9996	1.5364
	1-palmitoleoylglycerophosphocholine (16:1)*	0.8732	1.9251
	1-stearoylglycerophosphocholine (18:0)	0.8448	1.5755
	2-stearoylglycerophosphocholine*	1.1226	1.4972
	1-oleoylglycerophosphocholine (18:1)	0.8709	1.5372
	1-linoleoylglycerophosphocholine (18:2n6)	1.0147	1.5413
	1-linolenoylglycerophosphocholine (18:3n3)*	1.0223	2.2415
	1-arachidonoylglycerophosphocholine (20:4n6)*	0.9596	1.4044
	1-palmitoylplasmenylethanolamine*	0.2615	0.8295
	1-oleoylglycerophosphoethanolamine	0.7086	1.4933
	1-linoleoylglycerophosphoethanolamine*	0.7802	1.7731
	1-arachidonoylglycerophosphoethanolamine*	0.8629	1.6604
	1-linoleoylglycerophosphoinositol*	0.8457	1.4562
	1-arachidonoylglycerophosphoinositol*	0.9122	1.2935
	palmitoyl-linoleoyl-glycerophosphocholine (2)*	0.9206	1.2143
	palmitoyl-palmitoyl-glycerophosphocholine (1)*	0.5873	0.9566
	palmitoyl-palmitoyl-glycerophosphocholine (2)*	0.4736	0.7721
	stearoyl-linoleoyl-glycerophosphocholine (1)*	0.9828	1.2829
Monoacylglycerol	1-palmitoylglycerol (1-monopalmitin)	1.2643	1.6405
	1-oleoylglycerol (1-monoolein)	0.5196	3.048
	1-linoleoylglycerol (1-monolinolein)	1.0244	2.235
	2-linoleoylglycerol (2-monolinolein)	0.8505	2.9696
	1-docosahexaenoylglycerol	1.462	1.8398
	1-dihomo-linolenylglycerol (alpha, gamma)	1.3659	1.7462
	2-docosahexaenoylglcyerol*	1.8316	2.815
Steroid	corticosterone	1.2699	0.5477
	11-dehydrocorticosterone	1.3886	0.3969
Primary Bile Acid Metabolism	cholate	4.5401	18.3219
	taurocholate	0.6944	5.8782
	taurochenodeoxycholate	0.4883	6.1031
	beta-muricholate	1.8429	4.2474
	tauro-beta-muricholate	0.846	6.9928
Secondary Bile Acid Metabolism	deoxycholate	0.877	3.0533
	taurodeoxycholate	0.5989	2.3552
	taurolithocholate 3-sulfate	0.9432	3.1577
	taurohyodeoxycholic acid	0.771	5.2808
	7-ketodeoxycholate	1.9761	19.3096

- Animals were supplemented with 1 X 10^7 cfu of *L. johnsonii* by oral gavage for 7 days and plasma metabolite levels were assessed
- Supplemented animals were then infected with RSV. After 2 days of RSV infection plasma from sacrificed animals (5/group) was harvested and the metabolite levels compared to supplemented mice at day 0 prior to infection.

Red – significantly upregulated

Green- significantly downregulated

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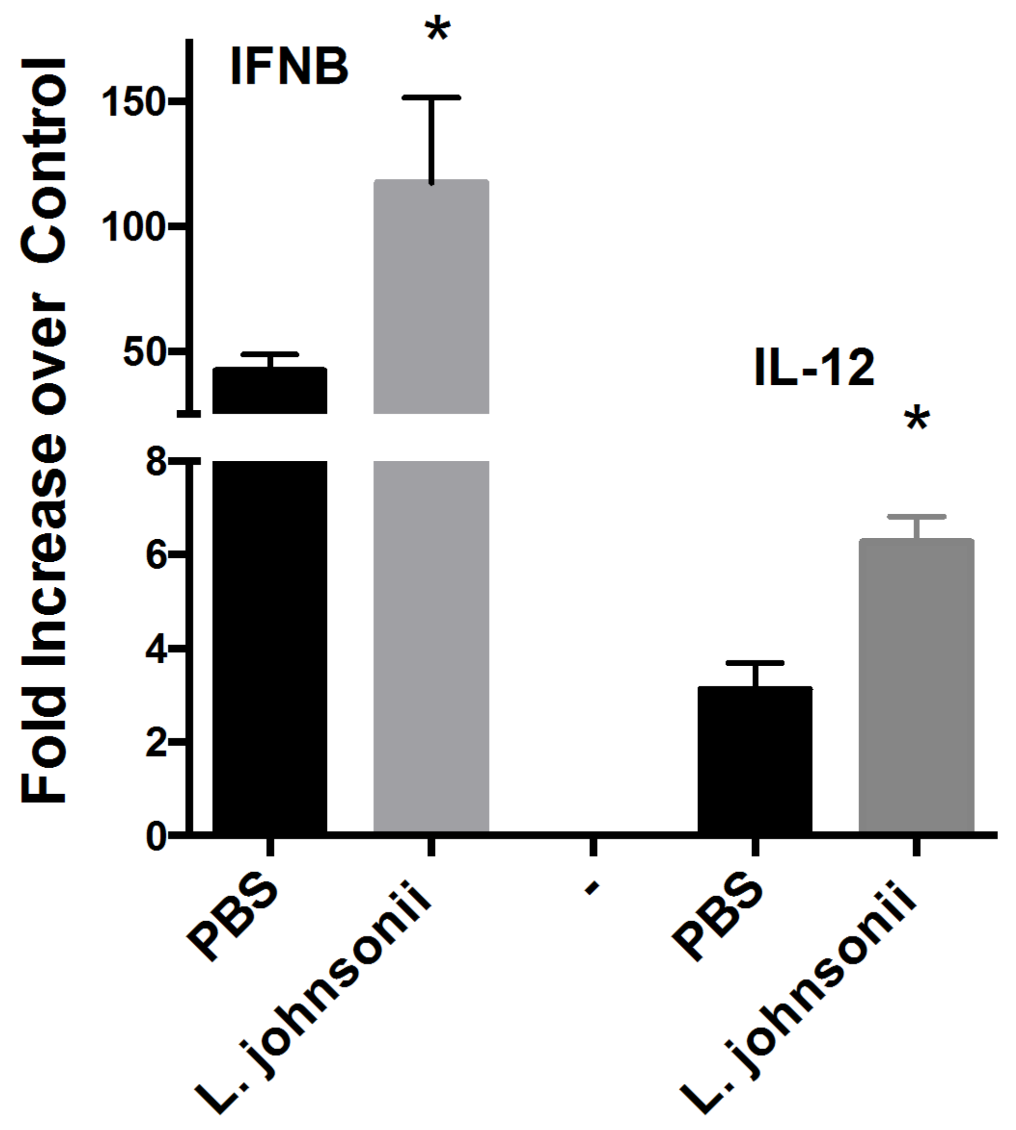
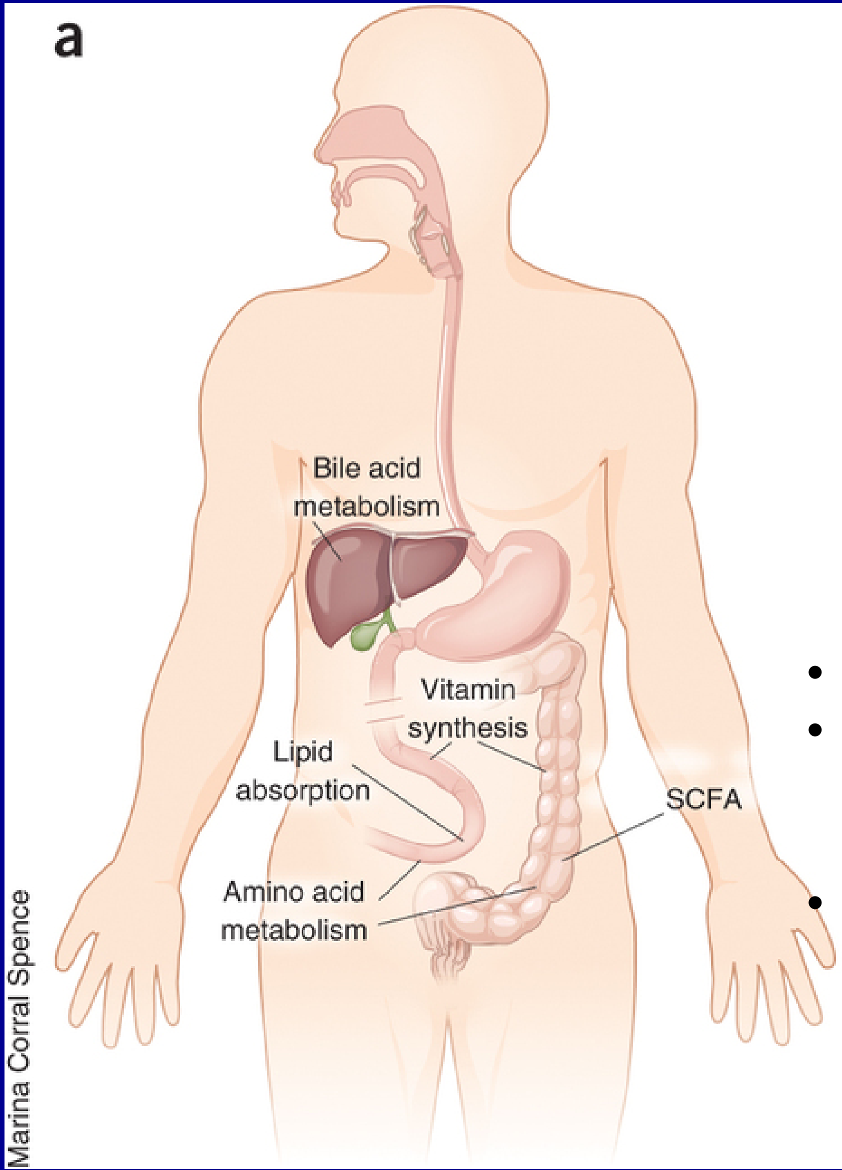
	3-hydroxymyristate	1.072	1.6838
	5-hydroxyhexanoate	1.624	0.9847

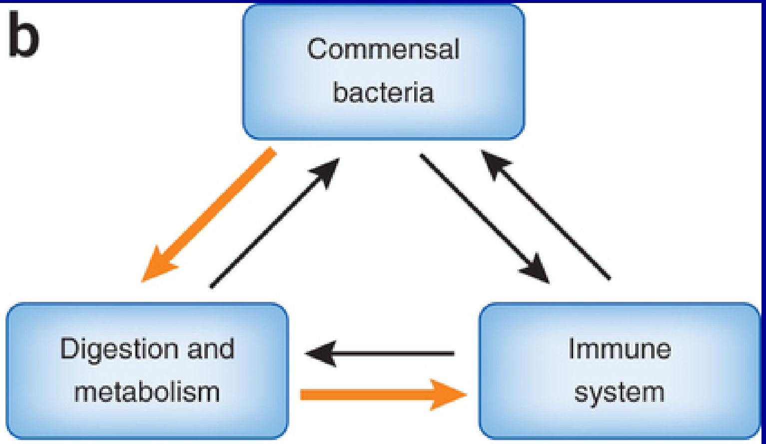
Alteration of RSV-induced DC activation by plasma from L. johnsonii supplemented mice



- BMDC were pre-incubated with **plasma** from supplemented animals at day 2 of RSV infection.
- DC were infected with RSV for 24 hrs and assessed for cytokine expression.
- Similar to the response of BMDC from supplemented mice, the plasma *from L. johnsonii* supplemented mice induced higher cytokine production.

Environment, Microbiome, Metabolic activity, and immunity





Susceptibility to chronic disease

- Environmental influence and exposure
- Early influence may be crucial for establishing the appropriate immune environment.
- Interface of genetics with the environment has a contributory role in disease susceptibility and severity.

Brestoff and Artis, Nat. Immun., 14:676, 2013

M.A.A.P.
Microbes, Allergy, Asthma, & Pets

MAAP Collaboration

NIH, NIAID- P01AI089473

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