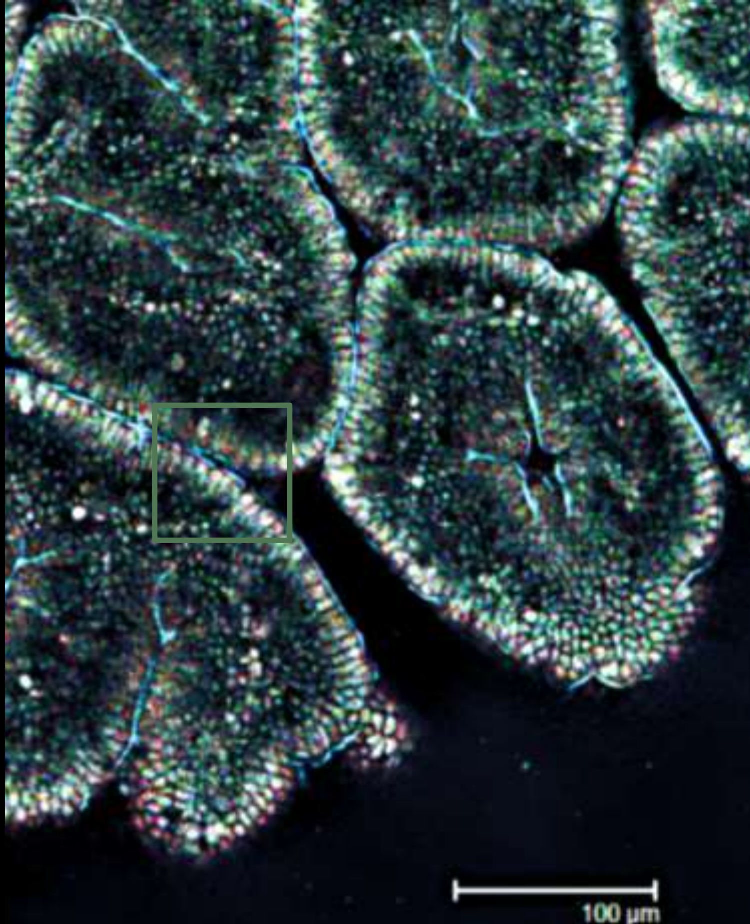
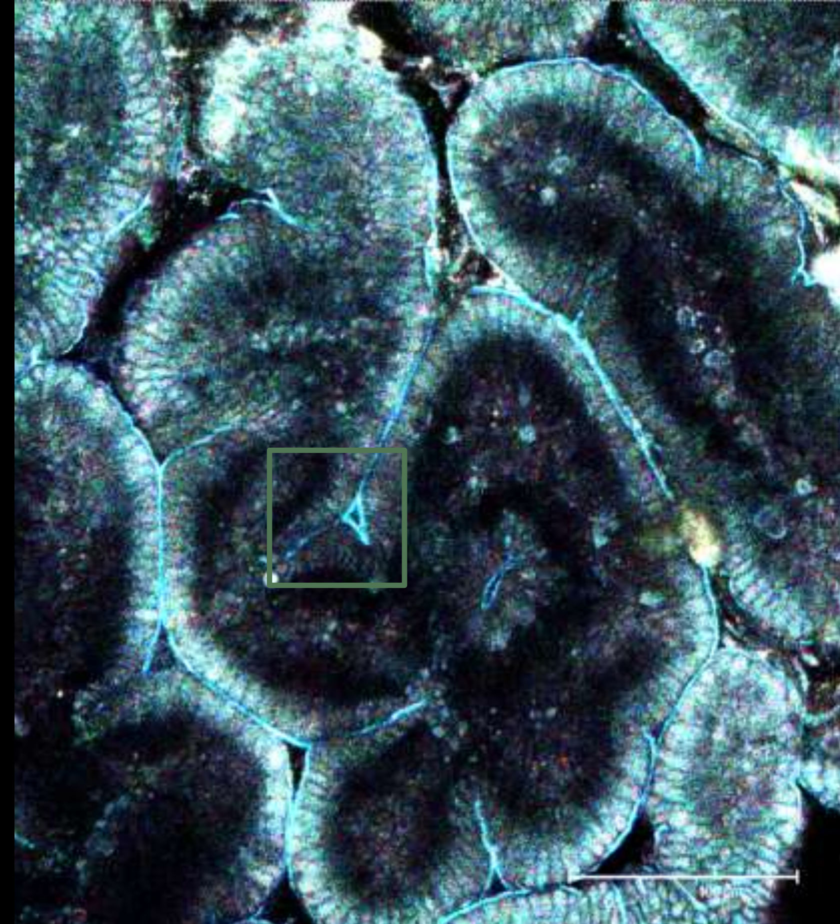
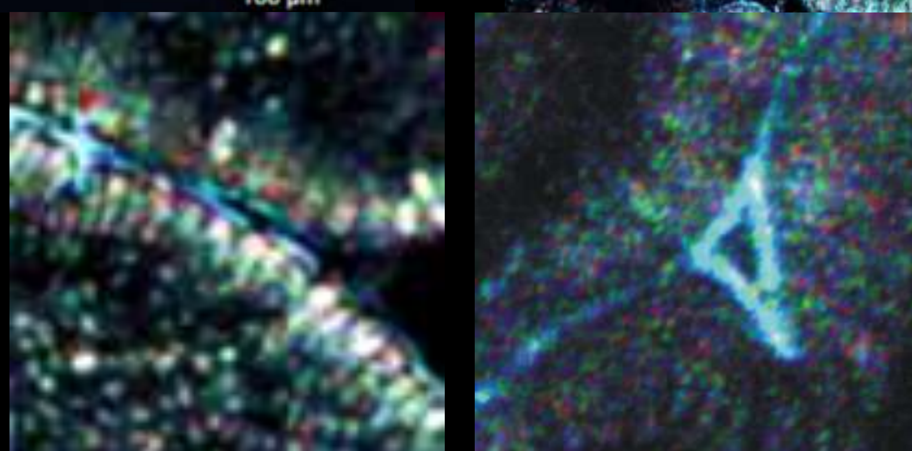


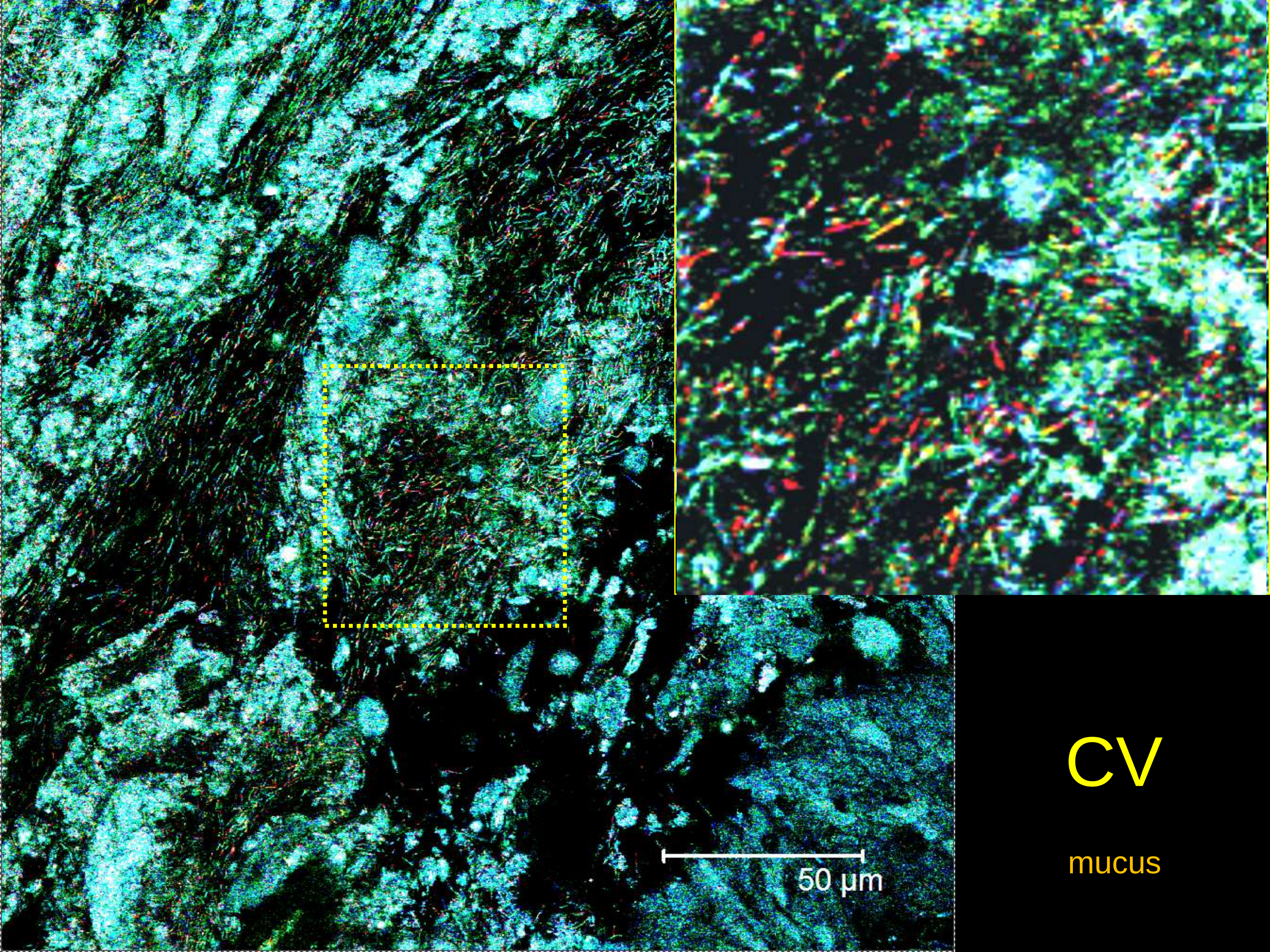


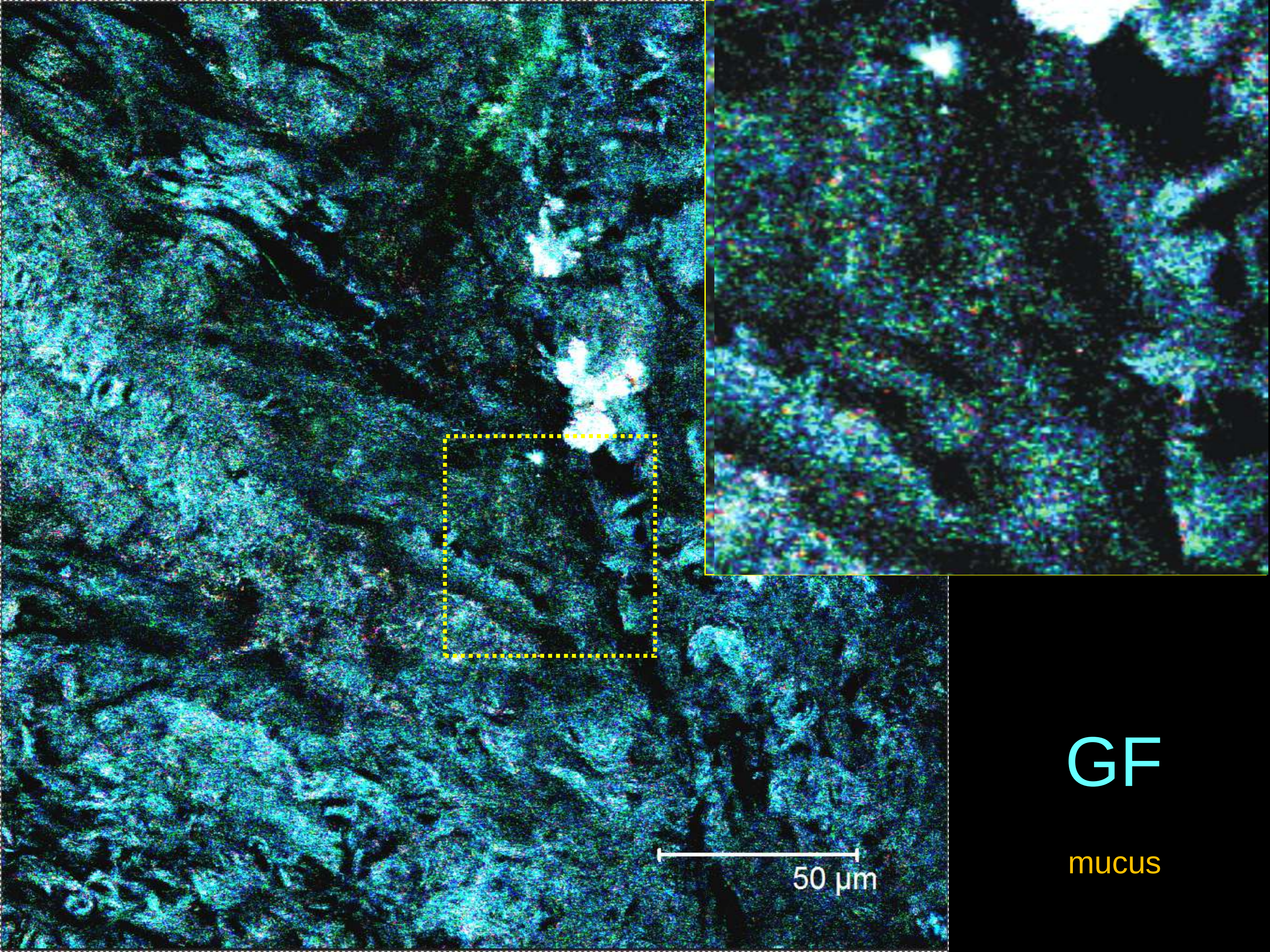
CV



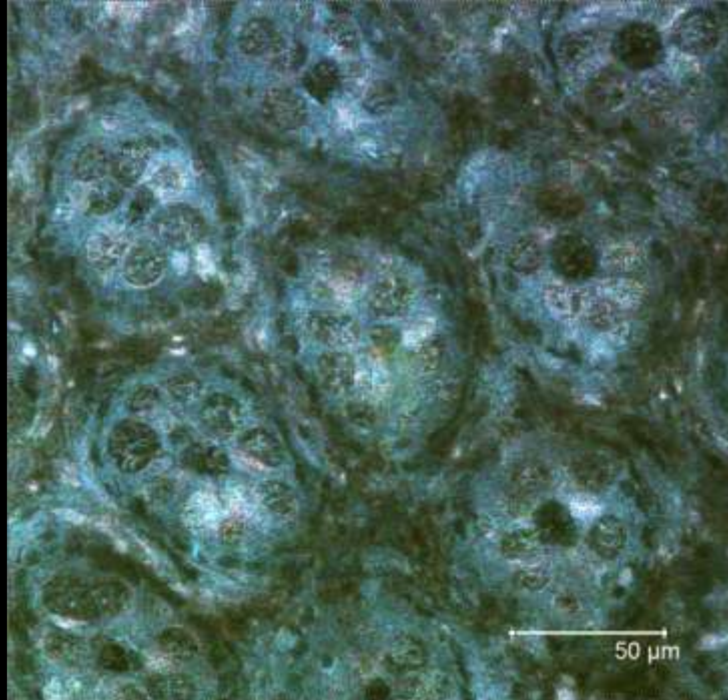
GF

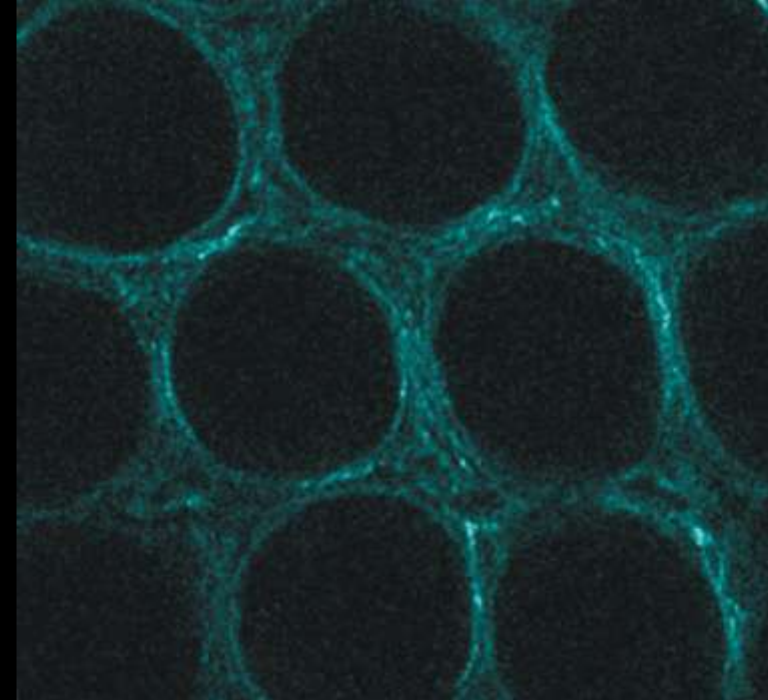
Right colon (fresh samples, unstained, RM, x20)


50 µm
CV
mucus

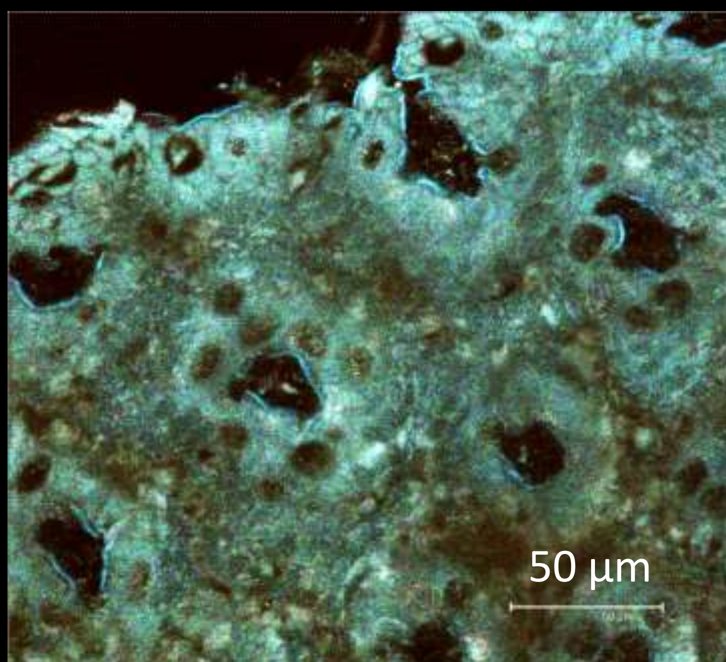

50 µm
GF
mucus

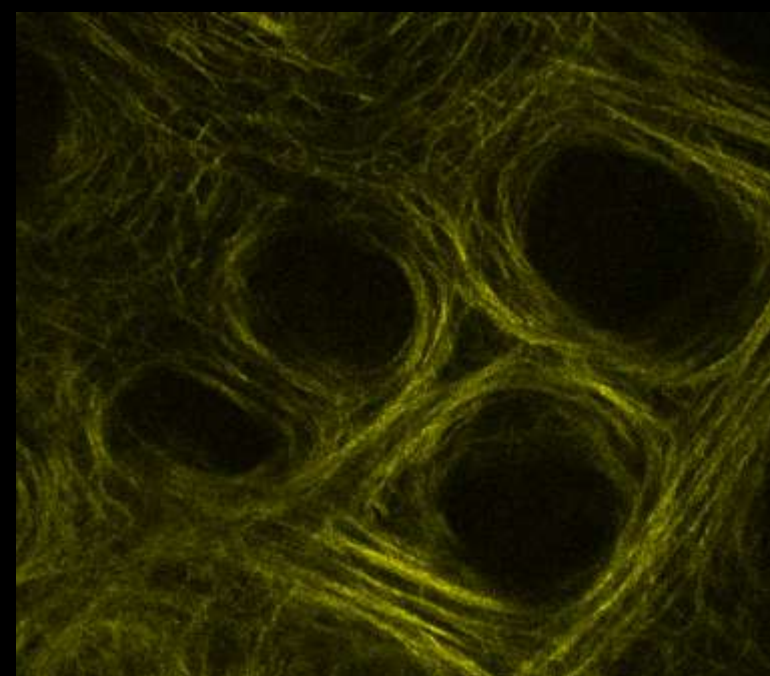
CV



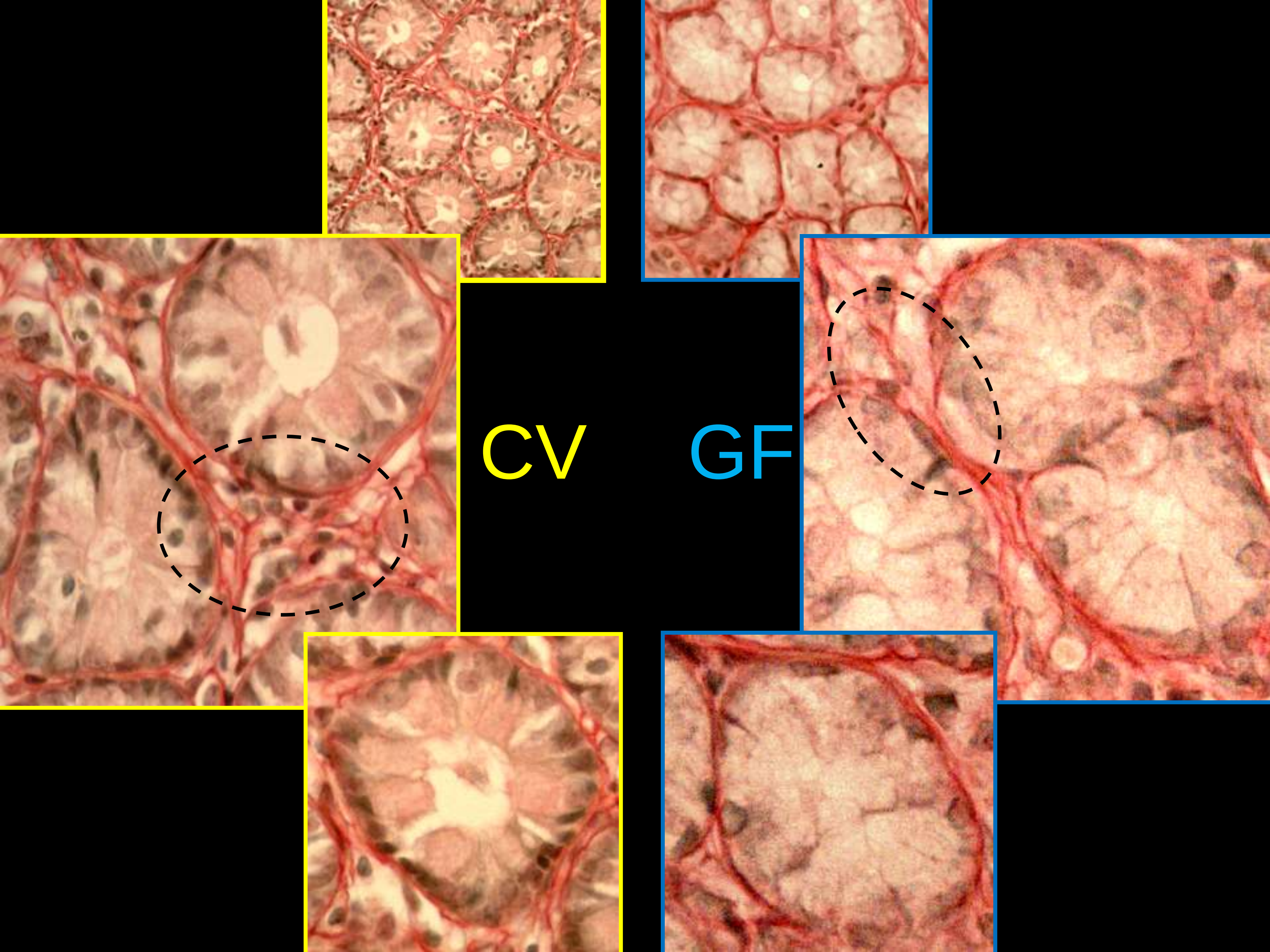


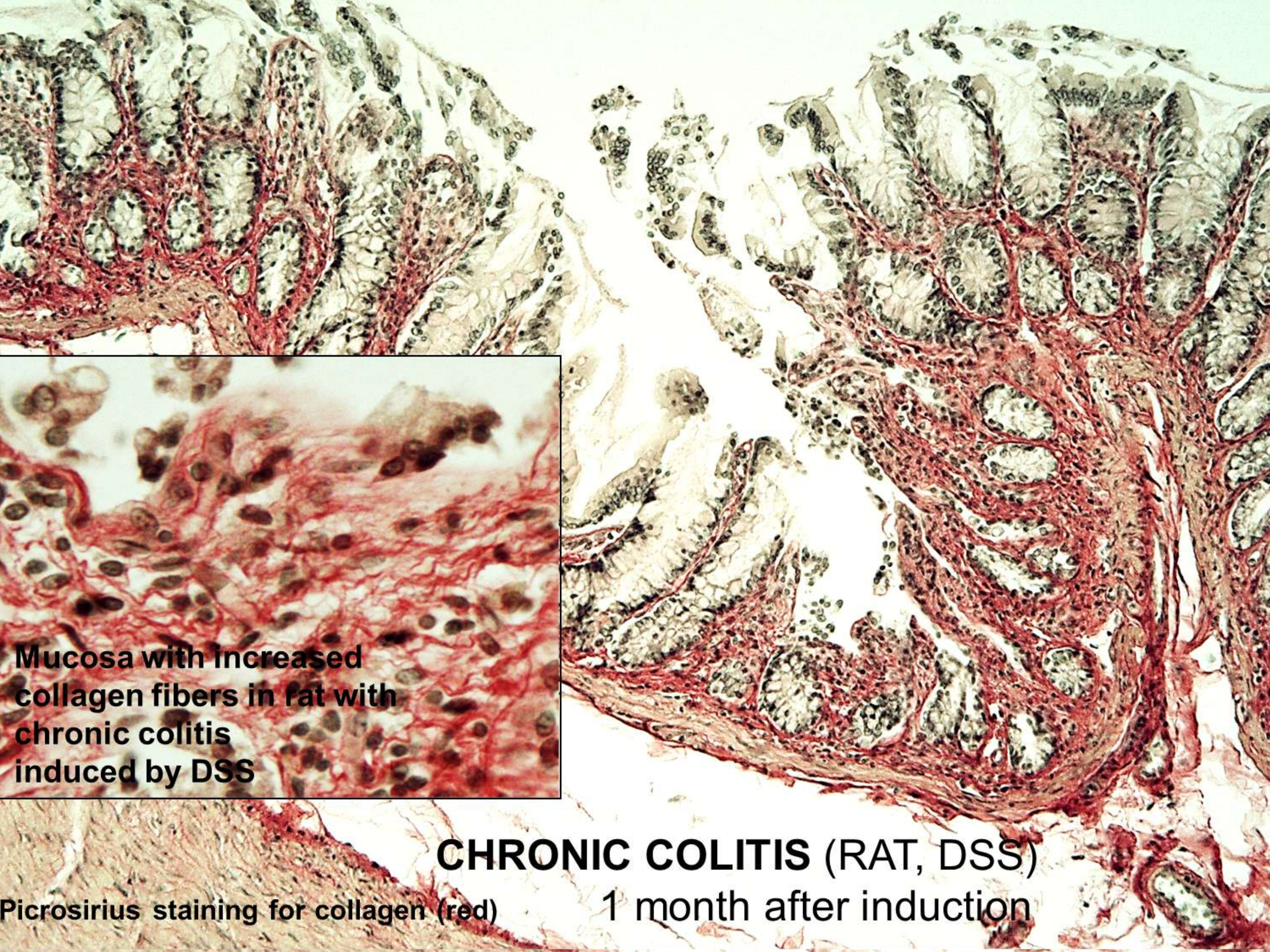
GF

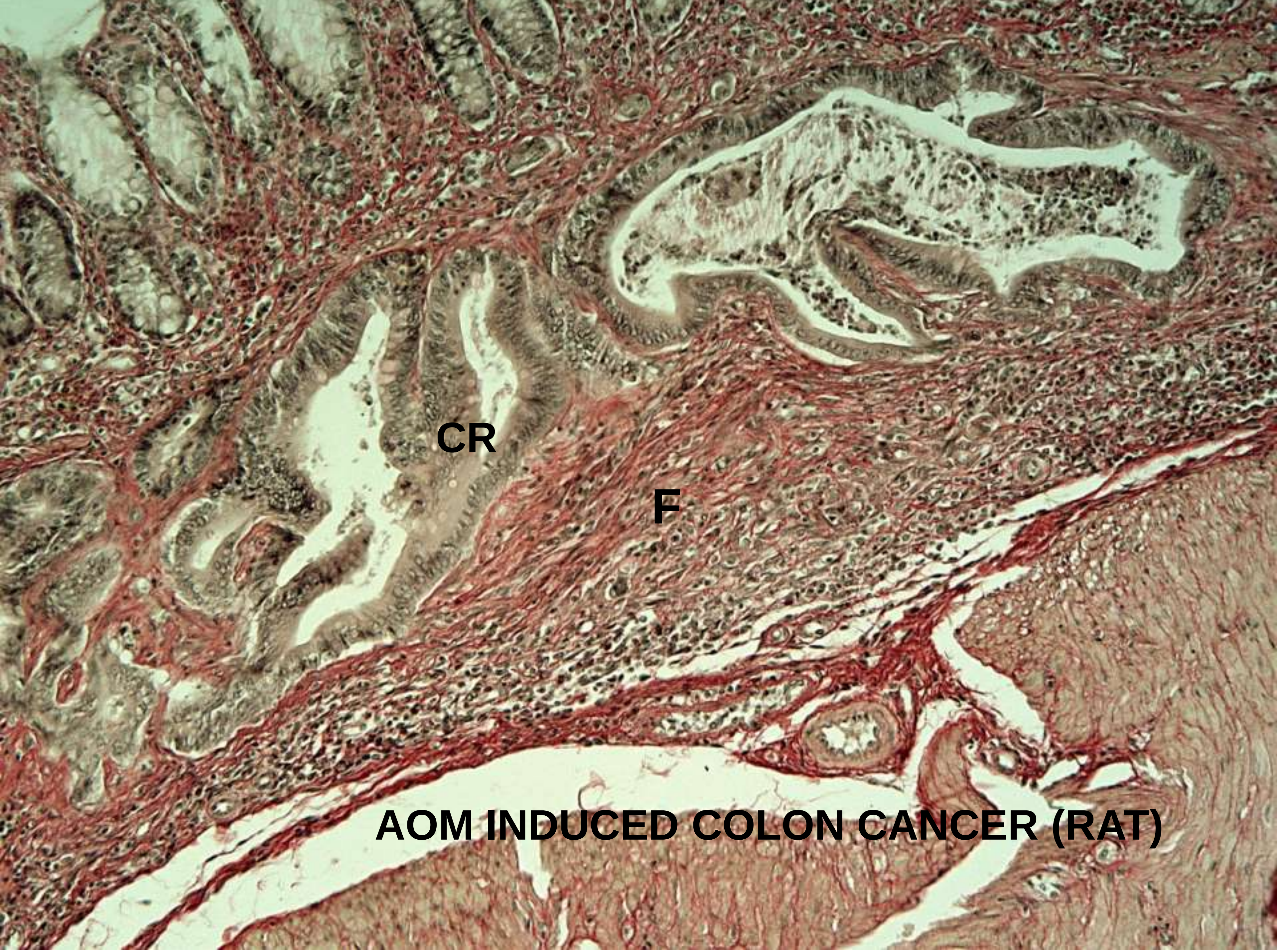


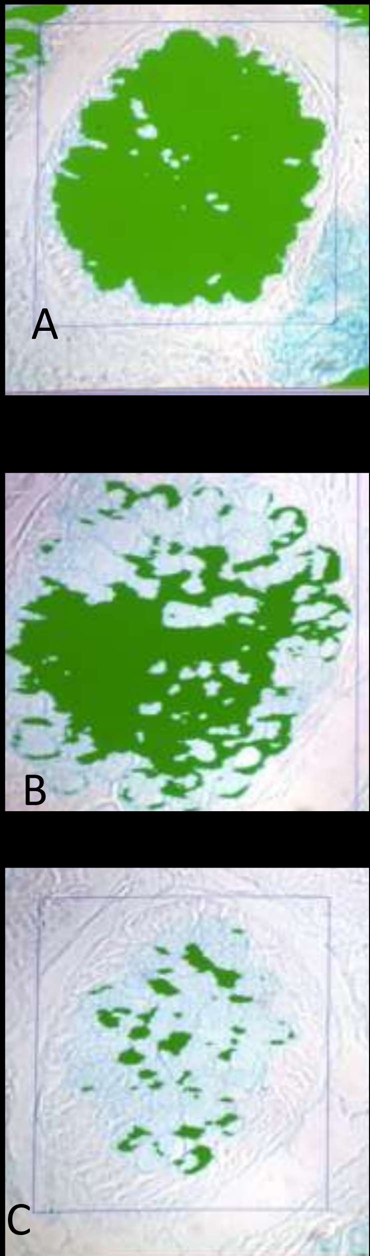


fresh samples directly evaluated by photon scanning microscopy in reflectance mode (three color filters) and SHG x20, x63

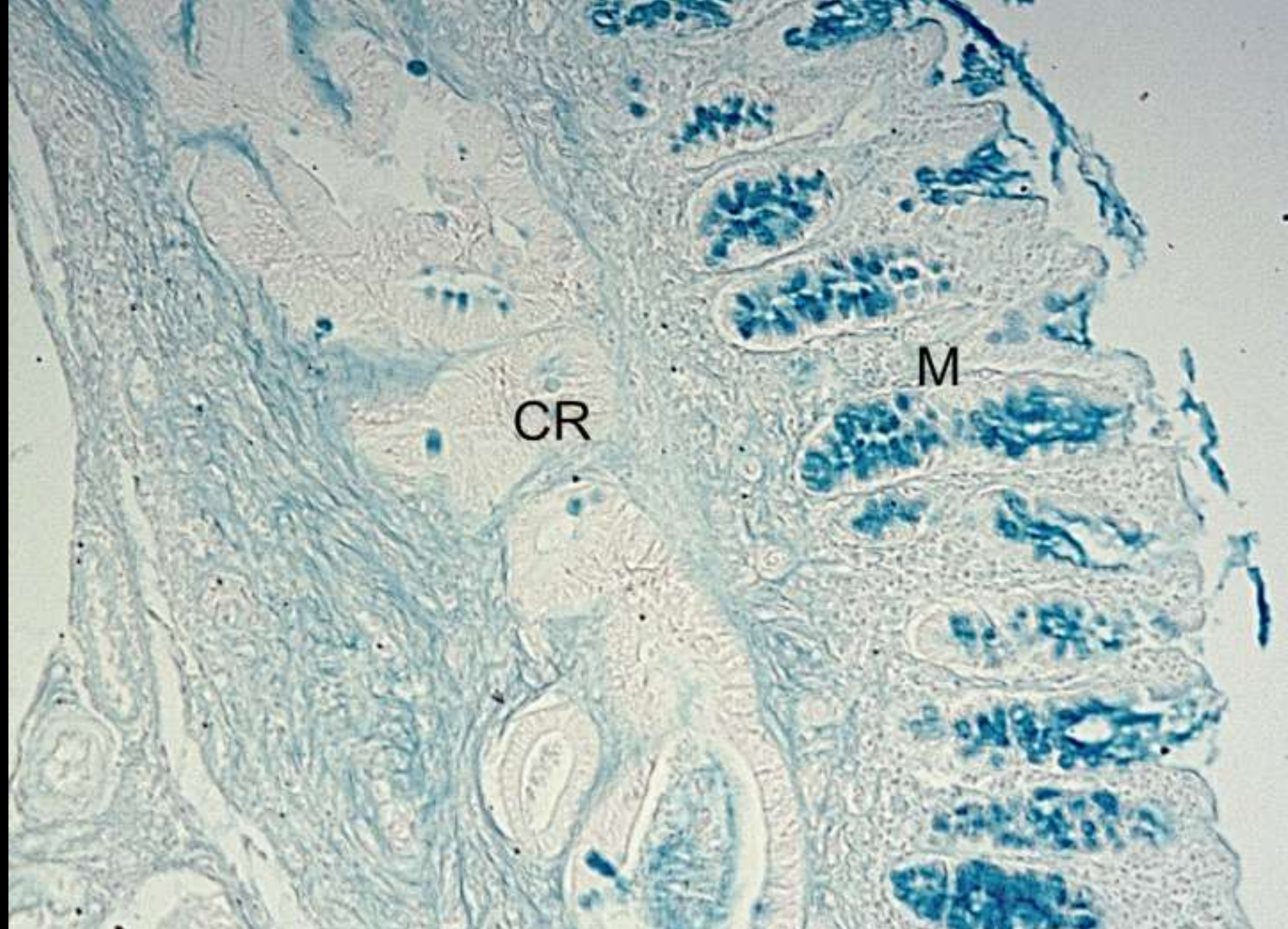

CV
GF


Mucosa with increased
collagen fibers in rat with
chronic colitis
induced by DSS
CHRONIC COLITIS (RAT, DSS)
1 month after induction
Picrosirius staining for collagen (red)


CR
F
AOM INDUCED COLON CANCER (RAT)

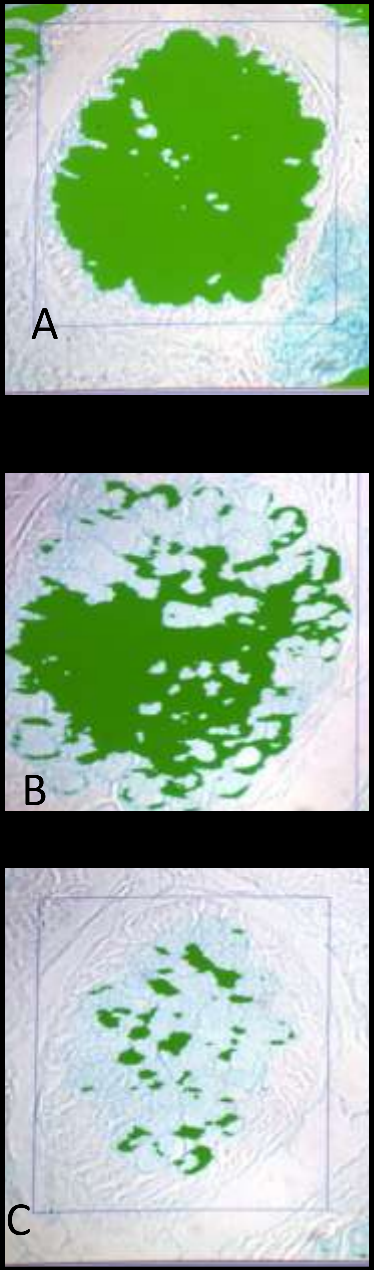


MUCIN

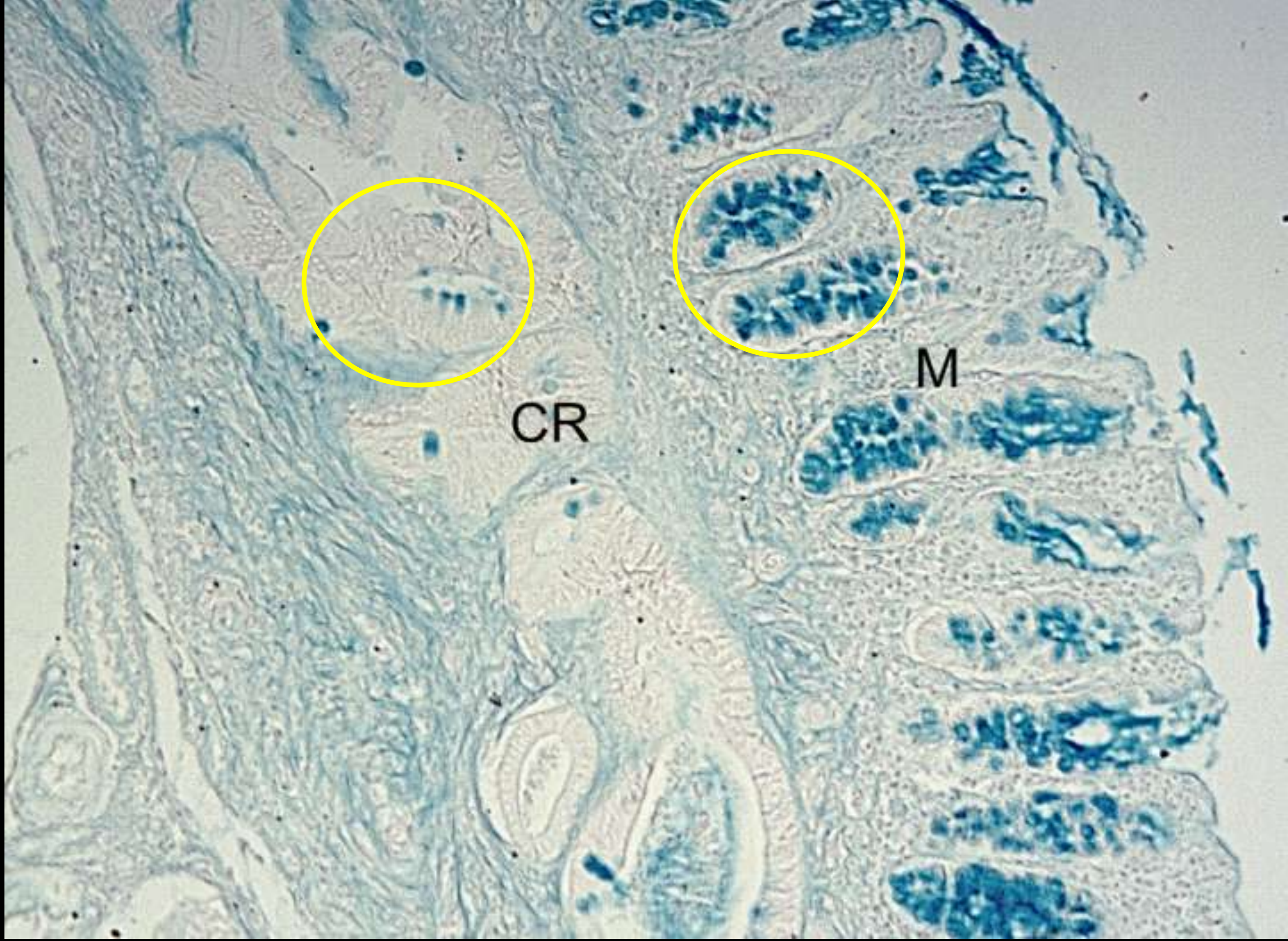


A – healthy mucosa
B – adenoma
C – cancer

Changes in Alcyan blue staining of mucin in the rat colonic mucosa crypts during progression of carcinogenesis
(AOM induction of cancer)



MUCIN



A – healthy mucosa
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Changes in Alcyan blue staining of mucin in the rat colonic mucosa crypts during progression of carcinogenesis
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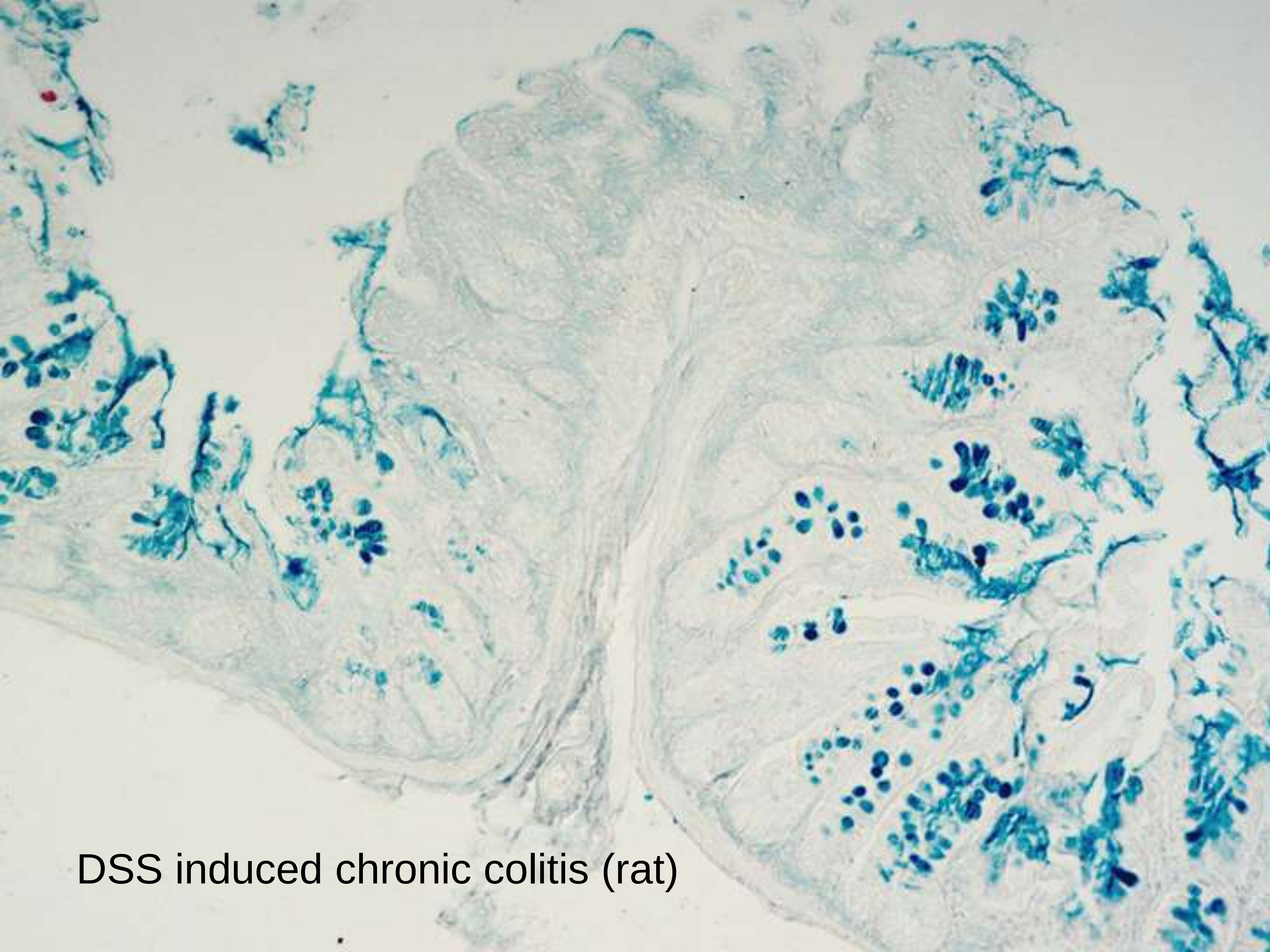
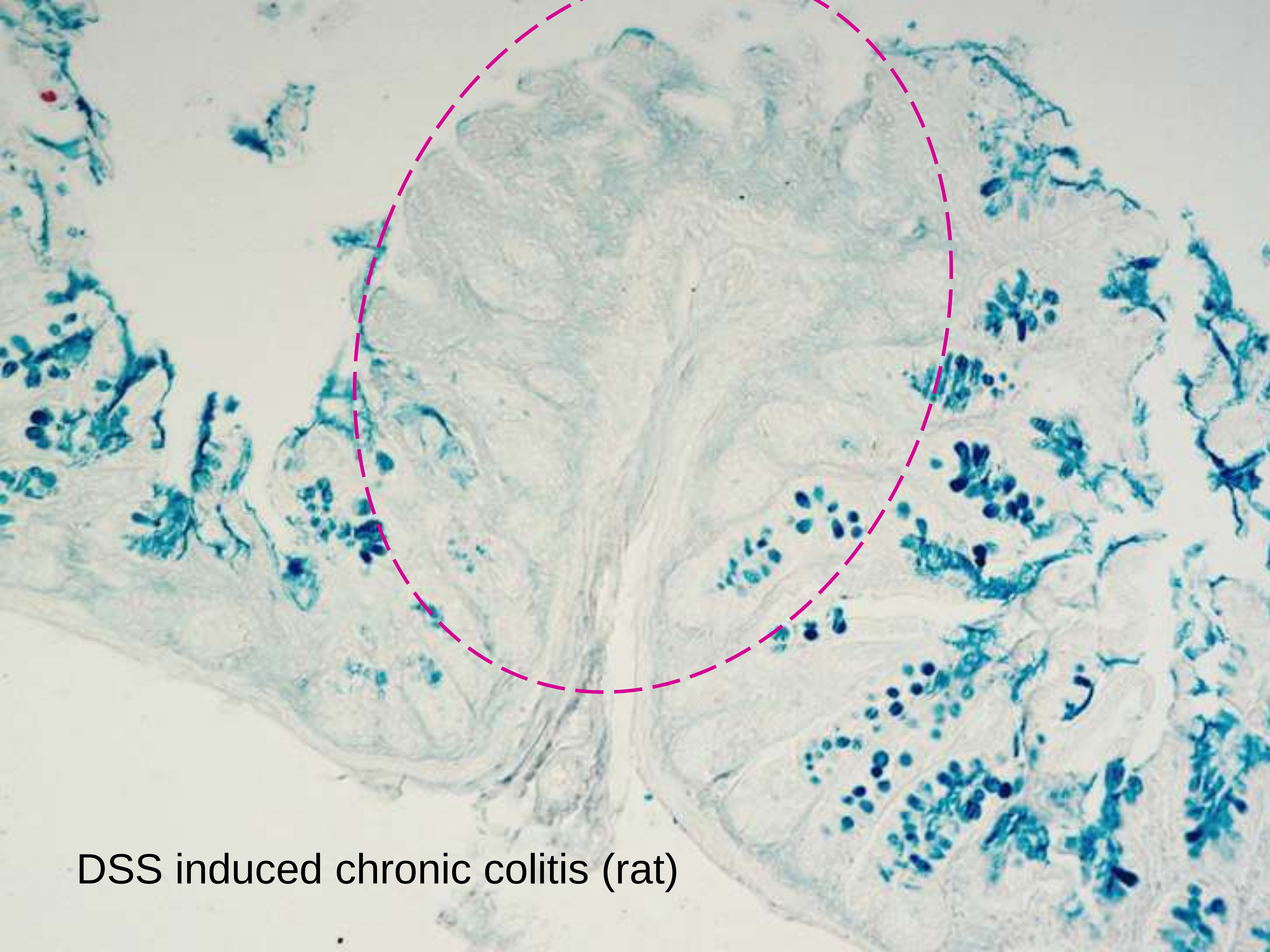
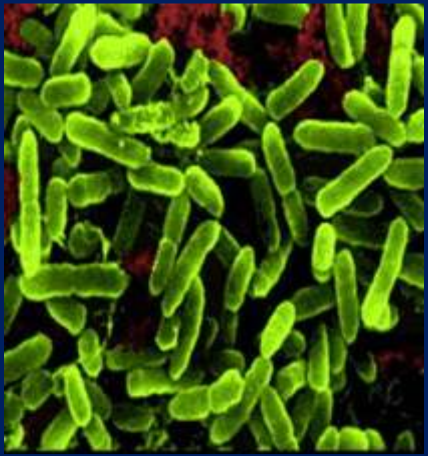
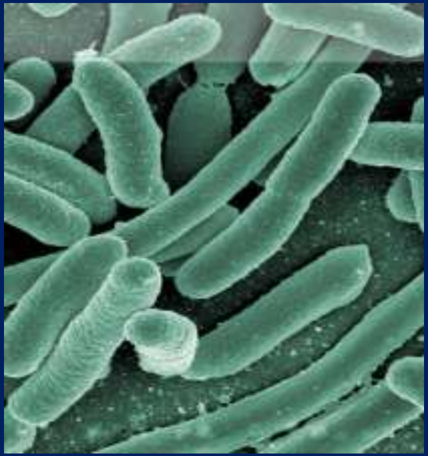
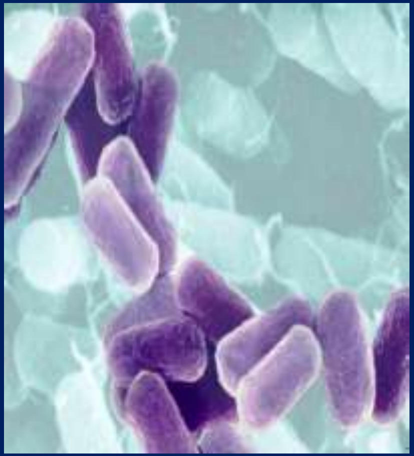


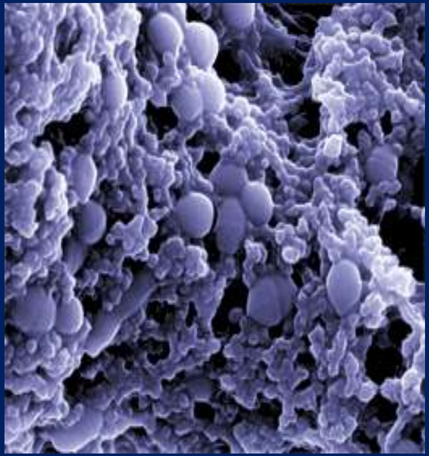
DSS induced chronic colitis (rat)


DSS induced chronic colitis (rat)









http://www.optimallivingfoods.com/my-bacteria.html

Billions of bacteria live in our gut, that they colonize shortly after birth. The relationship with the organism is mostly of mutual benefit, and they shape our immune system throughout life

However, the intestine is faced with the dilemma of being **constantly exposed to a large variety of environmental antigens, microbes and pathogens.** As such the intestinal mucosa must be protected against pathogenic invasion and/or exaggerated inflammatory responses.

Simultaneous presence of numerous commensal bacteria and potentially fatal pathogens. The process by which the intestinal immune system distinguishes between those commensal micro-organisms which present no danger (and in contrast may be essential for our health) and true pathogens remains unclear

The mucosal immune system

Tlaskalová-Hogenová H et al: ***Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases****. Immunol Lett. 2004 May 15;93(2-3):97-108.*

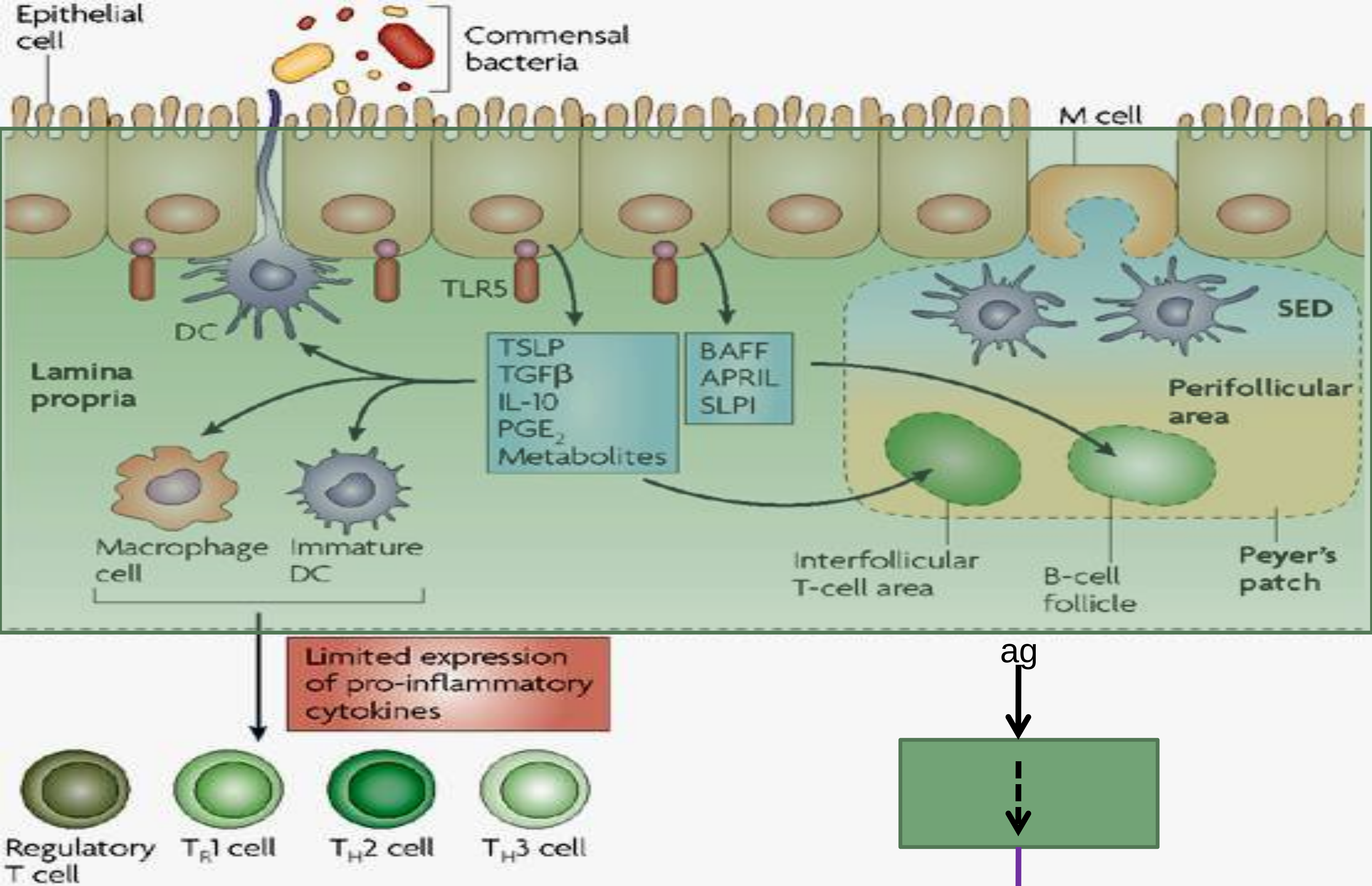
The mucosal immune system has developed specialized regulatory, anti-inflammatory mechanisms for **eliminating or tolerating non-dangerous, food and airborne antigens and commensal micro-organisms (oral, mucosal tolerance)**.

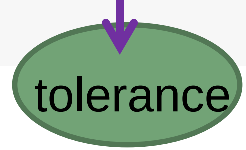
The mucosal immune system must provide local defense mechanisms against environmental threats (e.g. invading pathogens). This important requirement is fulfilled by several mechanisms of mucosal immunity: strongly developed **innate defense mechanisms** ensuring appropriate function of the **mucosal barrier**.

Defense mechanisms at the mucosal barrier

- existence of unique types of lymphocytes (CD8+ B7+, γδT cells, etc.) and their products
- transport of polymeric immunoglobulins through epithelial cells into secretions (**sIgA**)
- migration and homing of cells originating from the mucosal organized tissues in mucosae and exocrine glands

The important role of commensal bacteria in development of optimally functioning mucosal immune system was demonstrated in **germ-free animals** (using gnotobiological techniques)



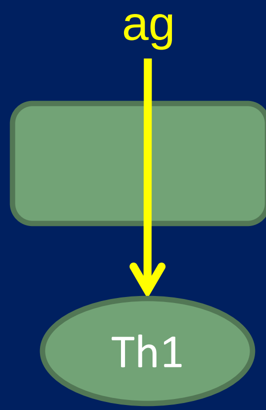


Nature Reviews Immunology **4**, 478-485 (June 2004) | doi:10.1038/nri1373

OPINION: Interactions between commensal intestinal bacteria and the immune system

Andrew J. Macpherson[1] and Nicola L. Harris[1]

Nicklin S, Miller K: ***Local and systemic immune responses to intestinally presented antigen.*** *Into Arch Allergy Appl Immunol. 1983; 72(1):87-90*



Antigens presented directly to GALT
either by an injection into the Peyer's patches
or via a mechanically damaged lamina propria,
stimulated a rapid and vigorous immune response.

Systemic effect: antigen-specific humoral antibody response in the serum

Local effect: antigen-specific immunoglobulin A response in the bile.

CONCLUSIONS: GALT is capable of reacting to foreign materials penetrating the gut wall

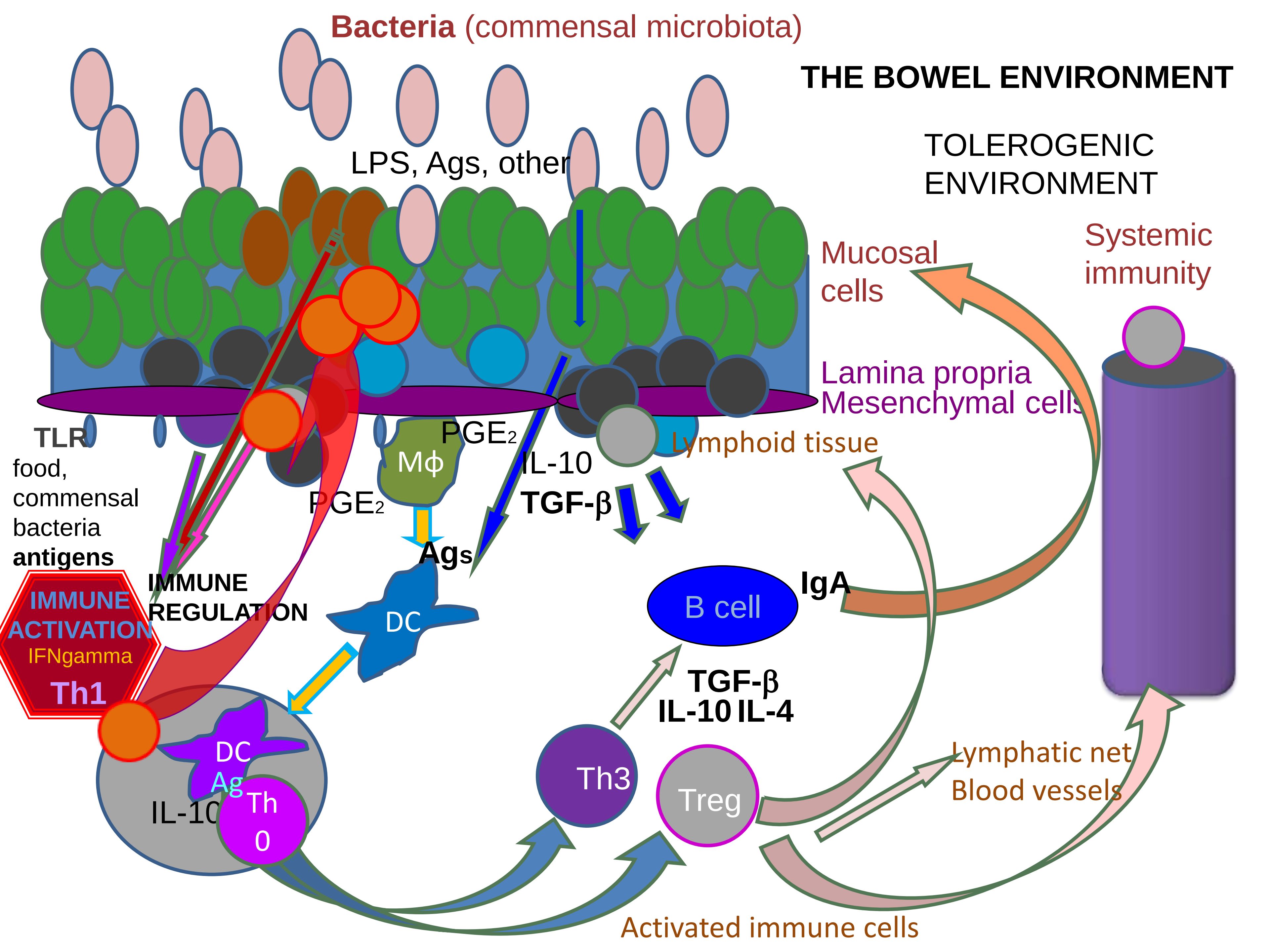
García-Lafuente A, et al.: **Modulation of colonic barrier function by the composition of the commensal flora in the rat** . Gut, 2001; 48(4):503-7

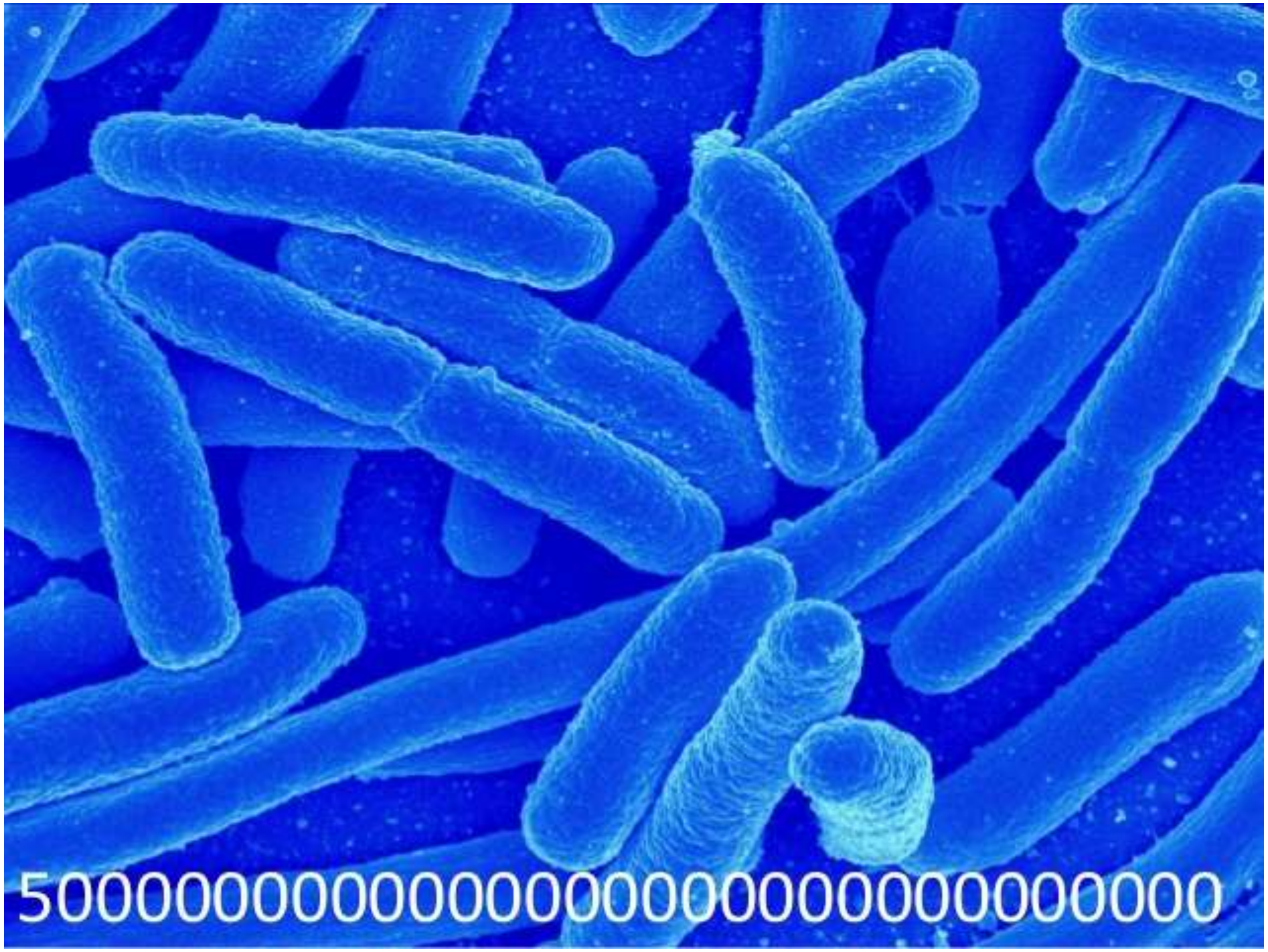
- Escherichia coli, Klebsiella pneumoniae, and Streptococcus viridans significantly increased lumen-to-blood clearance of mannitol
- Lactobacillus brevis had the opposite effect and reduced permeability to mannitol
- Bacteroides fragilis did not induced significant changes. Permeability to dextran was not altered by any of the strains tested.
- **CONCLUSIONS: Certain commensal bacteria can modify colonic wall permeability to luminal substances.**

Baba N, et al.: **Commensal bacteria trigger a full dendritic cell maturation program that promotes the expansion of non-Tr1 suppressor T cells.** J Leukoc Biol. 2008 Aug;84(2):468-76.

Dendritic cells (DCs) orchestrate the immune response establishing immunity versus tolerance.

Commensal bacteria instructed DCs to convert naive CD4+ T cells into hyporesponsive T cells that secreted low or no IFN-gamma, IL-10, and IL-17 and instead, displayed suppressor function.


Bacteria (commensal microbiota)
THE BOWEL ENVIRONMENT
TOLEROGENIC ENVIRONMENT
LPS, Ags, other
Mucosal cells
Systemic immunity
Lamina propria
Mesenchymal cells
Lymphoid tissue
TLR
food, commensal bacteria antigens
PGE2
Mϕ
PGE2
IL-10
TGF-β
Ags
IMMUNE ACTIVATION
IFNgamma
Th1
IMMUNE REGULATION
DC
B cell
IgA
TGF-β
IL-10 IL-4
DC
Ag
IL-10
Th 0
Th3
Treg
Lymphatic net
Blood vessels
Activated immune cells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Definitions

- **Microbiota:** microbial community.
- **Microbiome:** can refer to microbiota but can also refer to collective genomes and gene products of microbes living within and on humans.
- **Metagenome:** collection of genomes within complex microbial communities and human DNA, some also include RNA and proteins and other metabolites.
- **Biodiversity** is a measure of the complexity of a community. Includes number of taxa (richness) and their range of abundance (evenness).

Johnson, Pediatrics, 2012
Weinstock, Nature, 2012

The human gut metagenome has at least 100 times as many genes as "us."

There are 10 to 100 trillion microbes in our GI tract, most in the distal gut.

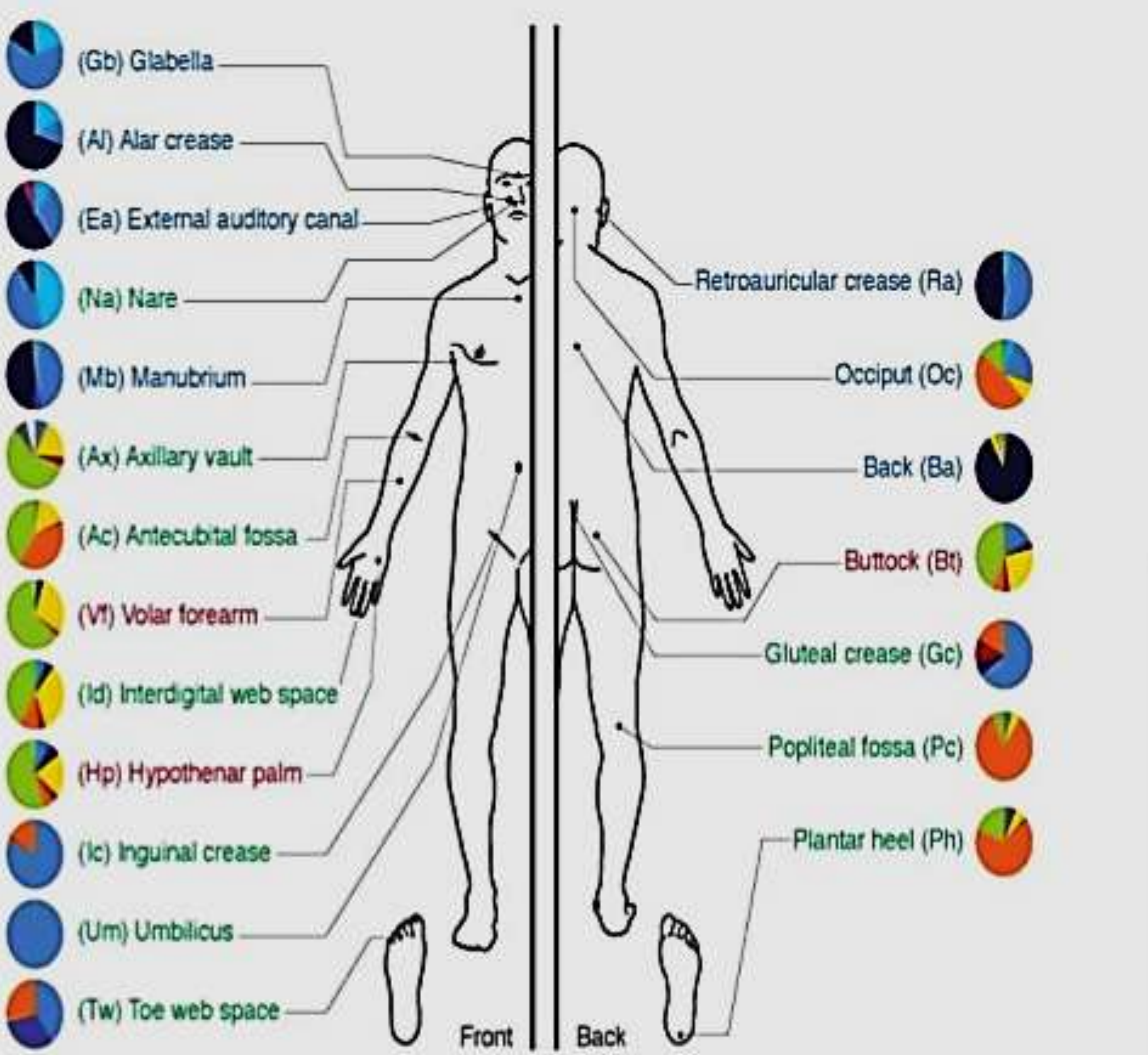
Two phyla (Bacteroidetes and Firmicutes) make up >90% in adults.

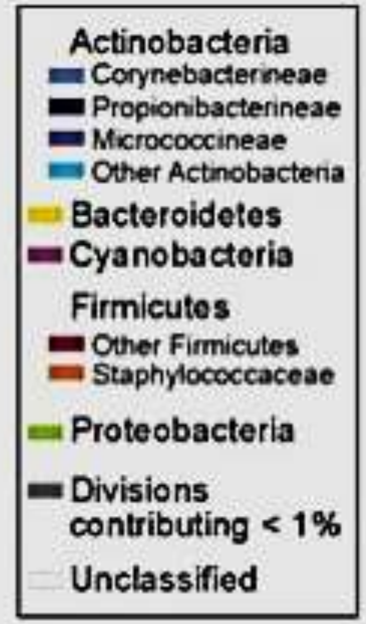
In 2007, the NIH Human Microbiome Project was formed.

Gill, Science, 2006

HOW MANY DIFFERENT ORGANISMS ARE NORMALLY IN OUR BODY?

- Mouth ; > 600 Species
- Skin : > 600 Species
- Intestine : (Cecum/ colon) : 8,000 genera
- Vagina : > 200 Species





We Are Really More Bug than Man.......

INTESTINAL MICROBIOME

- >1,000 species but most in adults are from 2 phyla: Firmicutes and Bacteroidetes
- Outnumber human somatic cells by factor of 10^2
- Total Weight: 1-2 kg
- 60% of total fecal content
- Concentration: ~1012/gram in colon
- •Total #: ~1014

THE HUMAN GUT FLORA

TABLE. Major Bacteria and Archaea Phyla and Genera Found in the Human Gut Microbiota[a]

Phyla	Representative genera
Bacteria	
Firmicutes	*Ruminococcus*
	Clostridium
	Peptostreptococcus
	Lactobacillus
	Enterococcus
Bacteroidetes	*Bacteroides*
Proteobacteria	*Desulfovibrio*
	Escherichia
	Helicobacter
Verrucomicrobia[b]	
Actinobacteria	*Bifidobacterium*
Cyanobacteria[b]	
Synergistes[b]	
Archaea	
Euryarchaeota	*Methanobrevibacter*

[a] Prokaryotic phyla were identified by using an alignment of the 18,348-sequence dataset from reference 18.

[b] Not related to any known genera.

DiBiase, et al. Mayo Clin Proc 2008;83:460-469

Benefits of the normal flora

1. **Synthesize and excrete vitamins**
   Vitamin K and Vitamin B12

2. **Prevent colonization by pathogens**
   competing for attachment sites or for essential nutrients

3. **May antagonize other bacteria**
   the production of substances which inhibit or kill non-indigenous species(nonspecific fatty acids, peroxides, bacteriocins).

4. **Stimulate the development of certain tissues**
   i.e., intestines, certain lymphatic tissues, capillary density

5. **Stimulate the production of cross-reactive antibodies**.
   Low levels of antibodies produced against components of the normal flora are known to cross react with certain related pathogens, and thereby prevent infection or invasion.

Carbohydrate fermentation and absorption

Digest starch, plant fiber, pectin into SCFAs (short chain fatty acids) viz. acetic acid, propionic acid, butyric acid. Digest proteins like collagen, elastin.

Repression of pathogenic microbial growth

Competition for nutrition, attachment. Produce bacteriocins , Lactic acid.

Metabolic function

HCA (heterocyclic amines)

Preventing inflammatory bowel disease

SCFAs prevent IBD

Preventing allergy

Allegies = *C. difficile* and *S. aureus* > *Bacteroides* and *Bifidobacteria*

Ecology.....

Babies delivered Normally are dominated by ***Lactobacillus, Prevotella,* and *Atopobium*** ....

Babies delivered by Cesarian section have microbiota that of the maternal skin community like ***Staphylococcus.***

Prevotella is related with carbohydrates and simple sugars.

Bacteroides enterotypes is associated with animal proteins, amino acids and saturated fats.

What can damage Gut Flora

- Antibiotics
- Steroids, The Pill
- Other Drugs
- Stress
- **Poor Diet**
- Infections
- Disease
- Bottle Feeding
- Old Age
- Pollution
- Radiation
- Alcohol
- Toxic Chemicals
- Dental Work

Table 1 **Intestinal microbiota-associated diseases, syndromes, or other aberrations, with summaries of multiple studies that support an association between the microbiota and the indicated aberration.**

Aberration	Most relevant observations and potential correlation	References
Crohn's disease	Diversity decrease – reduced *F. prausnitzii*	Kaser et al. 2010[51]; Sokol et al. 2009[52]; Willing et al. 2010[53]
Ulcerative colitis	Diversity decrease – reduced *A. muciniphila*	Png et al. 2010[54]; Kaser et al. 2010[51]; Lepage et al. 2011[55]
Irritable bowel syndrome	Global signatures – increased *Dorea* and *Ruminococcus*	Salonen et al. 2010[36]; Saulnier et al. 2011[56]; Rajilić-Stojanović et al. 2011[13]
Clostridium difficile infection	Strong diversity decrease – presence of *C. difficile*	Grehan et al. 2010[57]; Khoruts et al. 2010[58]
Colorectal cancer	Variation in *Bacteroides* spp. – increased fusobacteria	Sobhani et al. 2011[59]; Wang et al. 2012[60]; Marchesi et al. 2011[61]
Allergy/atopy	Altered diversity – specific signatures	Stsepetova et al. 2007[62]; Bisgaard et al. 2011[63]; Storrø et al. 2011[64]
Celiac disease	Altered composition, notably in small intestine	Nistal et al. 2012[65]; Di Cagno et al. 2011[66]; Kalliomäki et al. 2012[67]
Type 1 diabetes	Signature differences	Vaarela 2011[68]; Giongo et al. 2011[69]; Brown et al. 2011[70]
Type 2 diabetes	Signature differences	Larssen et al. 2010[71]; Wu et al. 2010[72]; Kootte et al. 2012[73]
Obesity	Specific bacterial ratios (*Bacteroidetes/Firmicutes*)	Ley et al. 2006[74]; Turnbaugh et al. 2009[10]; Musso et al. 2011[75]

De Vos, Nutrition Review, 2012

Table 1. Effect of Various Diets on the Intestinal Microbiota

Dietary intervention	Change in microbiota
High-fat diet	Bacteroidetes ↓
	Firmicutes ↑
	Proteobacteria ↑
High-fat/High-sugar diet	Bacteroidetes ↓
	Firmicutes ↑ (Erysipelotrichi, Bacilli)
Protein-rich/Saturated fats	"*Bacteroides*" enterotype
Carbohydrate-enriched diet	"*Prevotella*" enterotype
Self-reported vegetarians	"*Prevotella*" enterotype
High fiber content	Bacteroidetes ↑, Actinobacteria ↑
	Firmicutes ↓, Proteobacteria ↓
Western diet	"*Bacteroides*" enterotype
Vegetarian lifestyle	*Bacteroides* spp. ↓, *Enterobacteriaceae* spp. ↓
	Bifidobacterium spp. ↓, *Escherichia coli* ↓

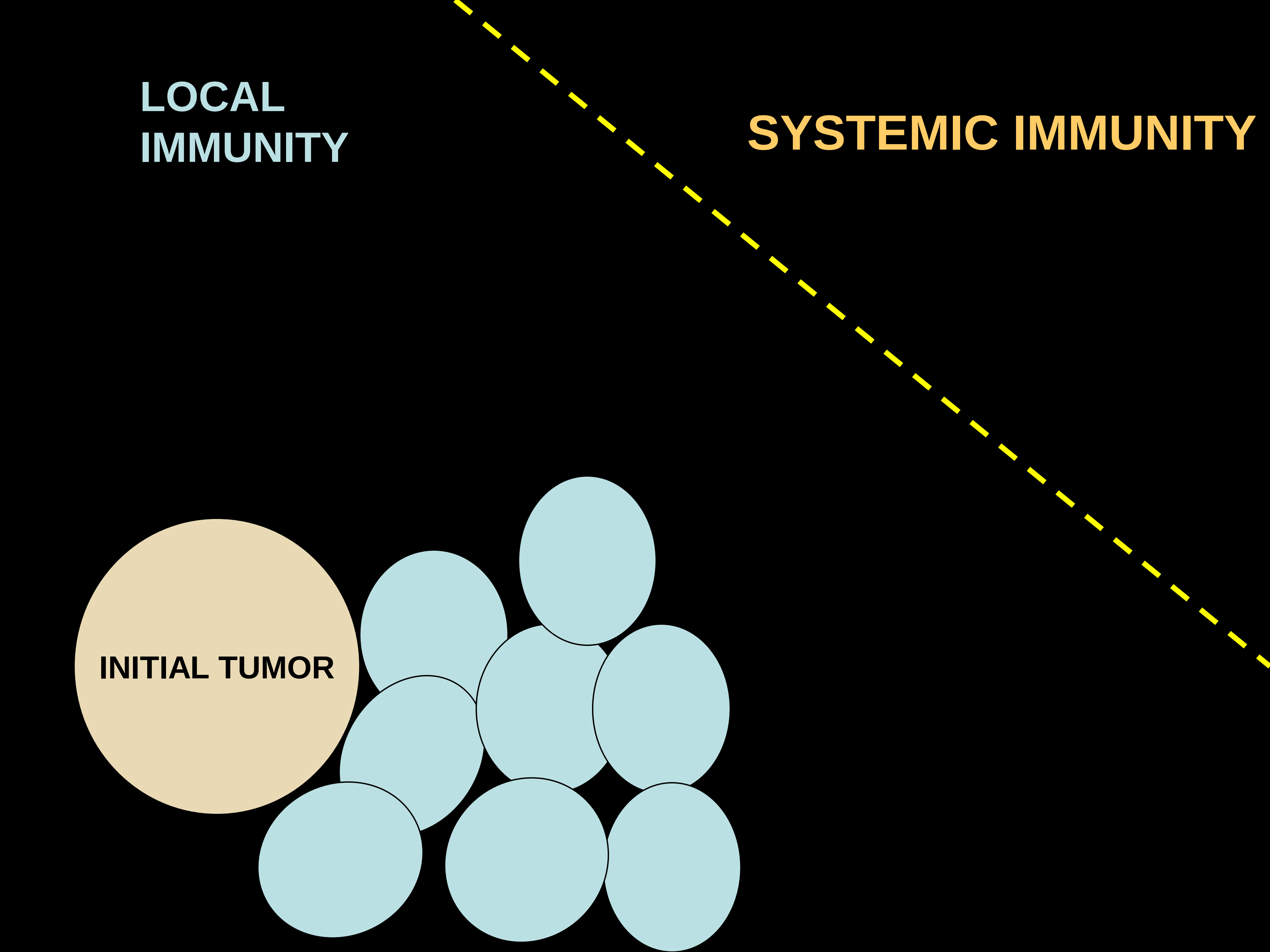
Moschen, Gut and Liver, 2012

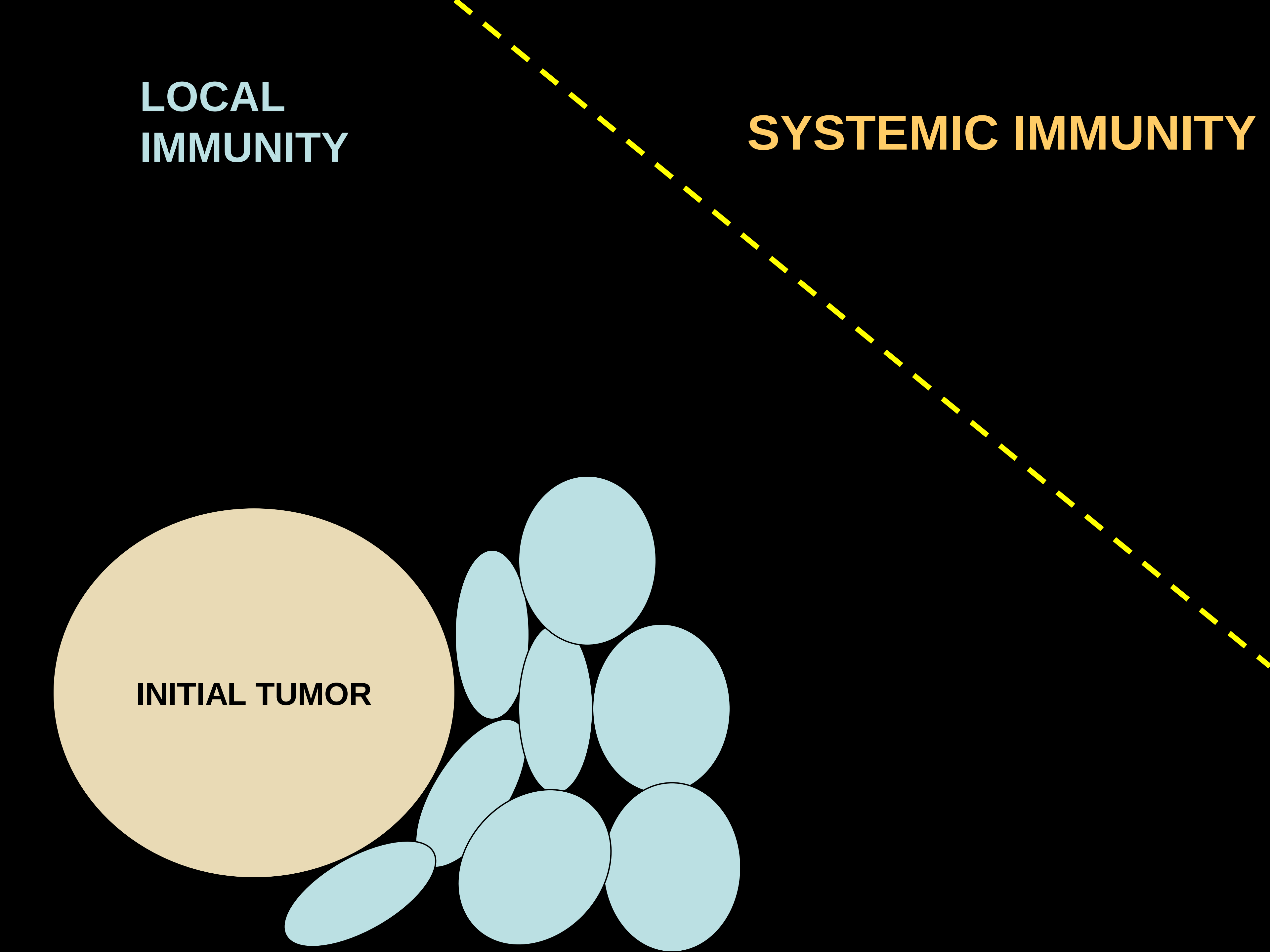
Gut microbiome may alter obesity.

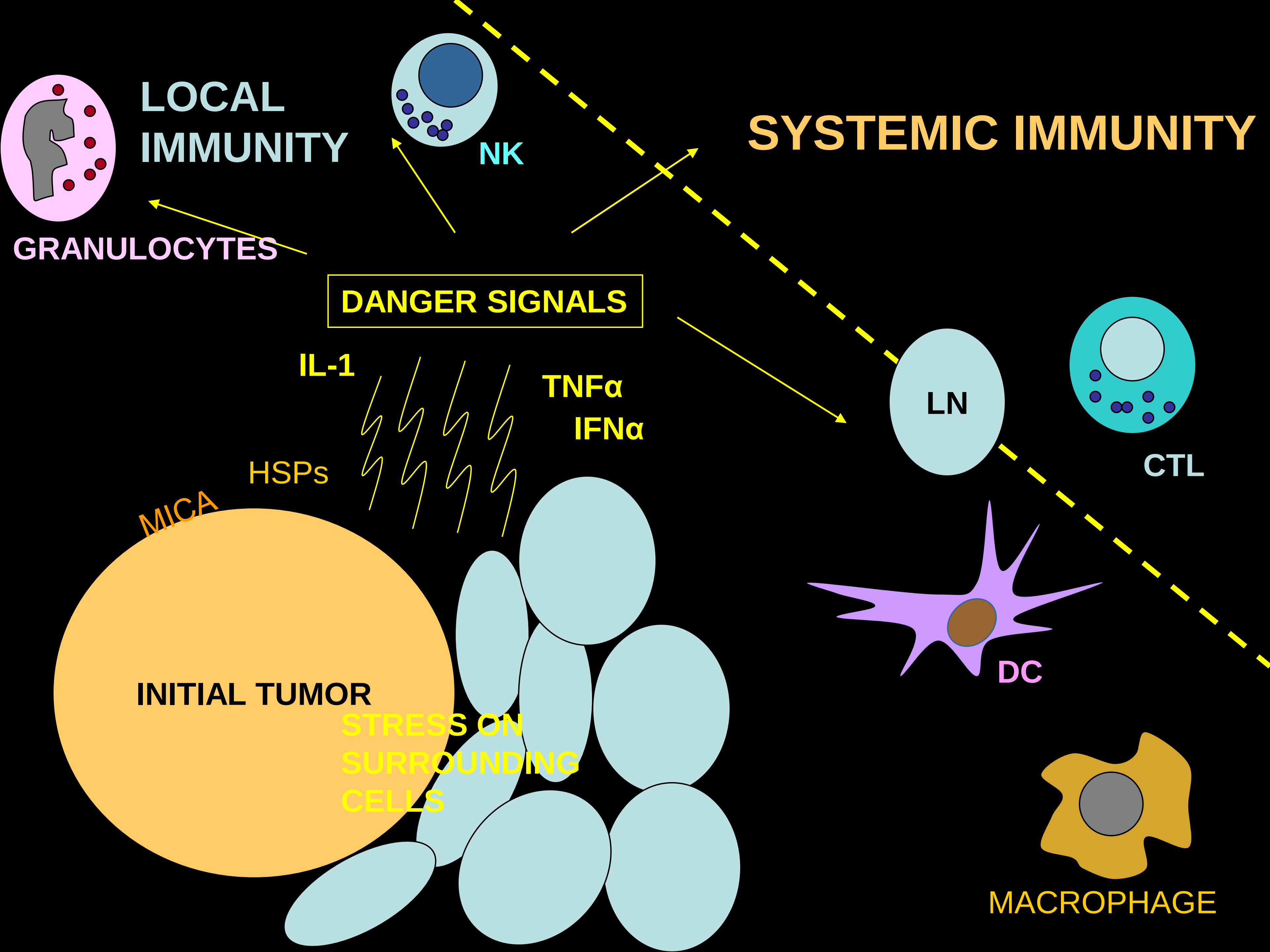
- Mice and human microbiotas have Firmicutes and Bacteroidetes dominating.
- Transplantation of an "obese microbiota" to germ free mice results in increased adiposity compared to transplantation of a lean microbiota.
- Mice fed high calorie Western diet for 8 weeks increased levels of Firmicutes and decreased Bacteroidetes; this has been seen in humans as well.

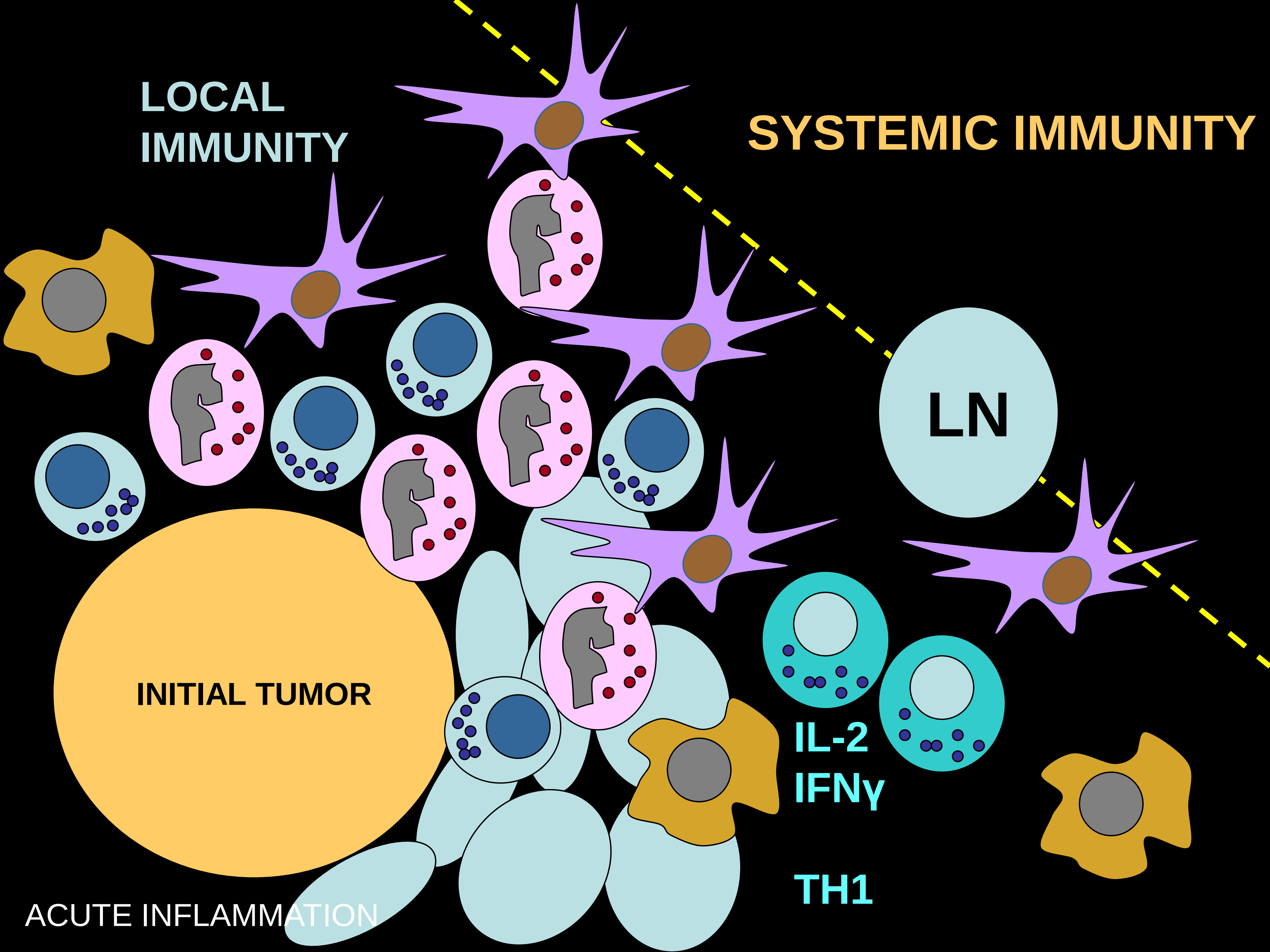
Reinhardt, JPGN, 2009
Ferrer, Env Micro, 2012

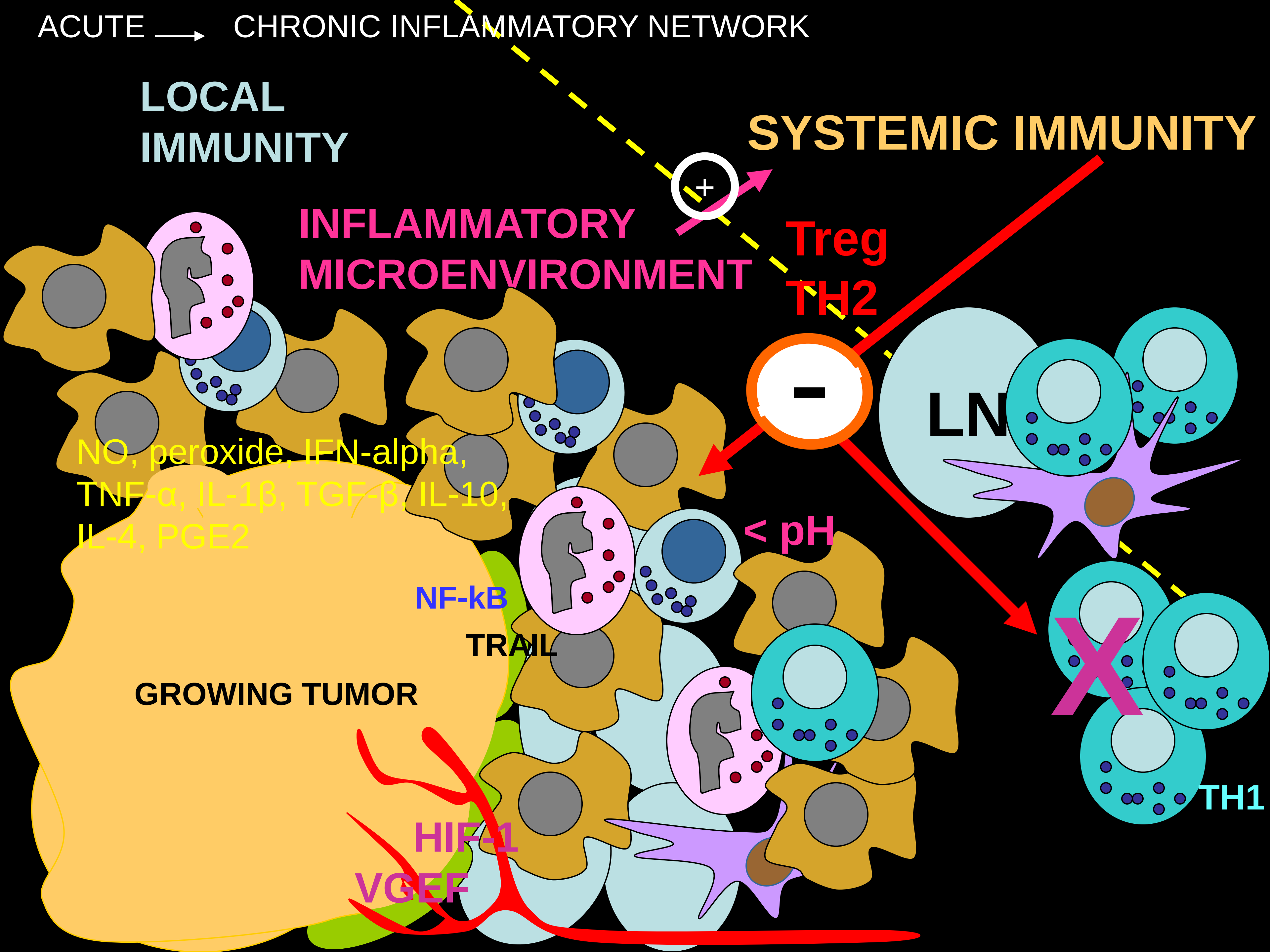
TUMOR IMMUNOLOGY


LOCAL
IMMUNITY
SYSTEMIC IMMUNITY
INITIAL TUMOR


LOCAL
IMMUNITY
SYSTEMIC IMMUNITY
INITIAL TUMOR


LOCAL IMMUNITY
GRANULOCYTES
NK
SYSTEMIC IMMUNITY
DANGER SIGNALS
IL-1
TNFα
IFNα
HSPs
MICA
INITIAL TUMOR
STRESS ON SURROUNDING CELLS
LN
CTL
DC
MACROPHAGE


LOCAL
IMMUNITY
SYSTEMIC IMMUNITY
LN
INITIAL TUMOR
IL-2
IFNγ
TH1
ACUTE INFLAMMATION


ACUTE → CHRONIC INFLAMMATORY NETWORK
LOCAL IMMUNITY
SYSTEMIC IMMUNITY
INFLAMMATORY MICROENVIRONMENT
+
Treg
TH2
-
LN
NO, peroxide, IFN-alpha, TNF-α, IL-1β, TGF-β, IL-10, IL-4, PGE2
< pH
NF-kB
TRAIL
GROWING TUMOR
X
TH1
HIF-1
VGEF

CR COLON (rat)