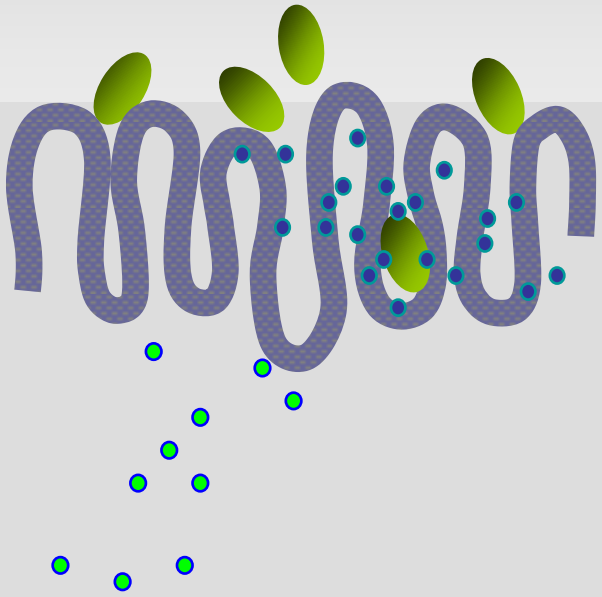
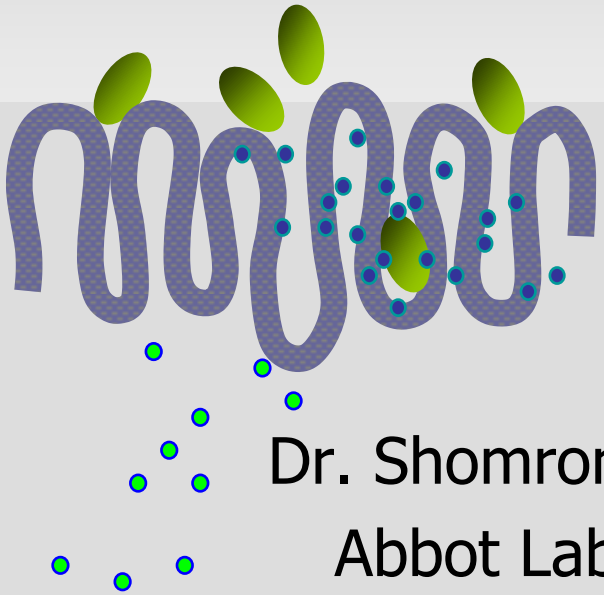
Gut Immunity



Shomron Ben-Horin
Laboratory of Gastro-Immunology
Gastroenterology Department
Sheba Medical Center, Tel-Aviv University, Israel

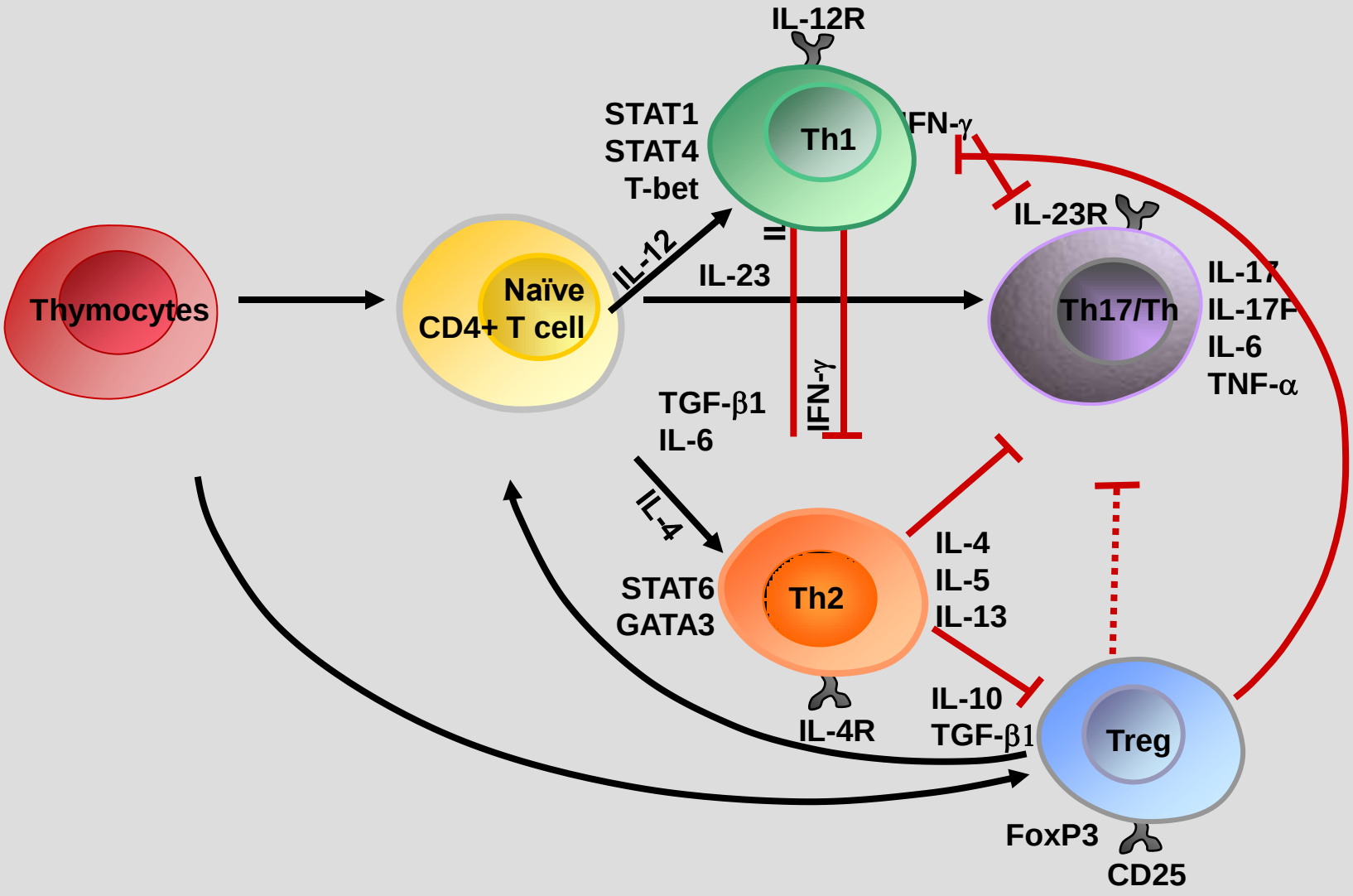
Disclosures



Dr. Shomron Ben-Horin received consultancy fees from:

Abbot Laboratories, Schering-Plough, Optimer Inc.

Immunology made easy



Human host response after exposure to a typical immunology slide

Let’s start with a brief review of :

Gut immune system organization

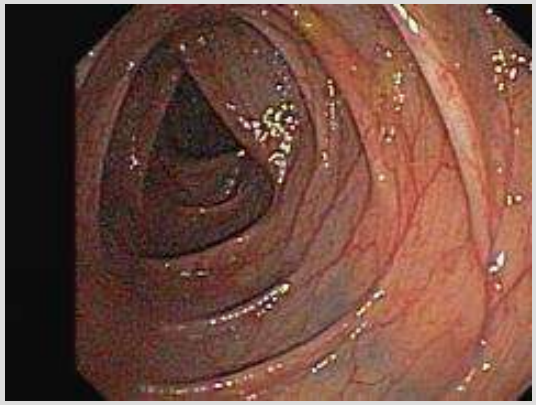
Gut immune system functions

Traditional view -1

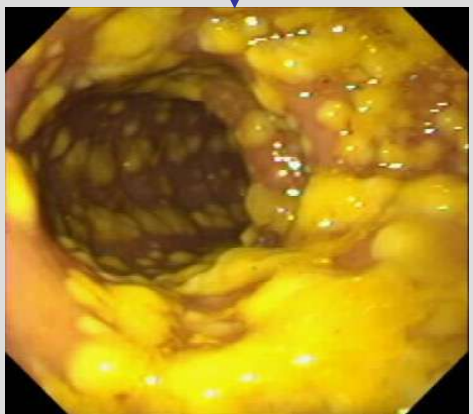
The epithelial functions consist of physical barrier and absorption apparatus

Traditional view -2

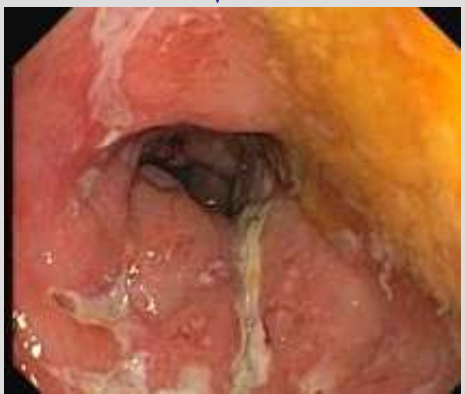
IBD is caused by exaggerated gut immune response (but CDAD is not….)



Immune in-adequacy

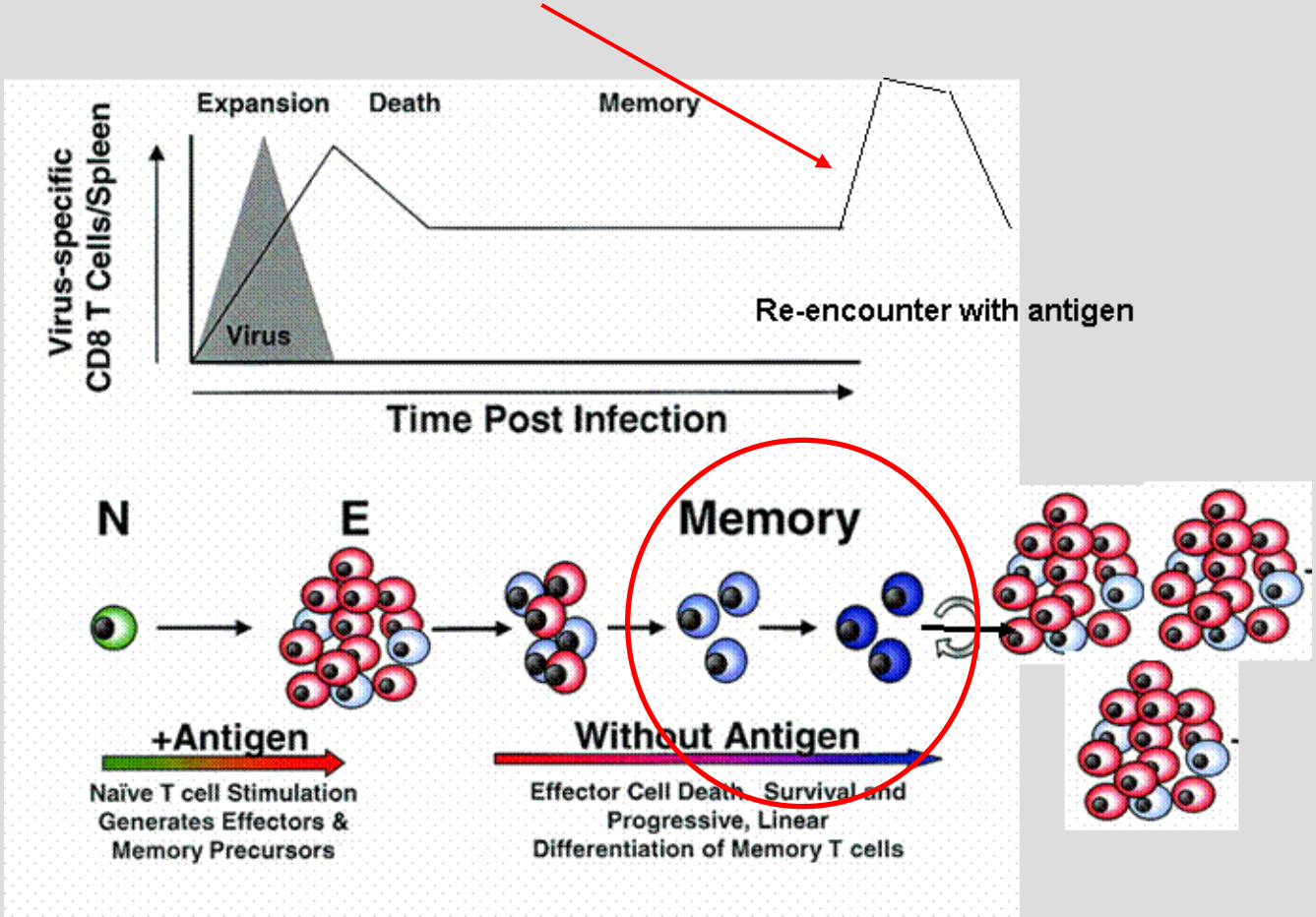


Immune hyper-reactivity



Traditional view -3

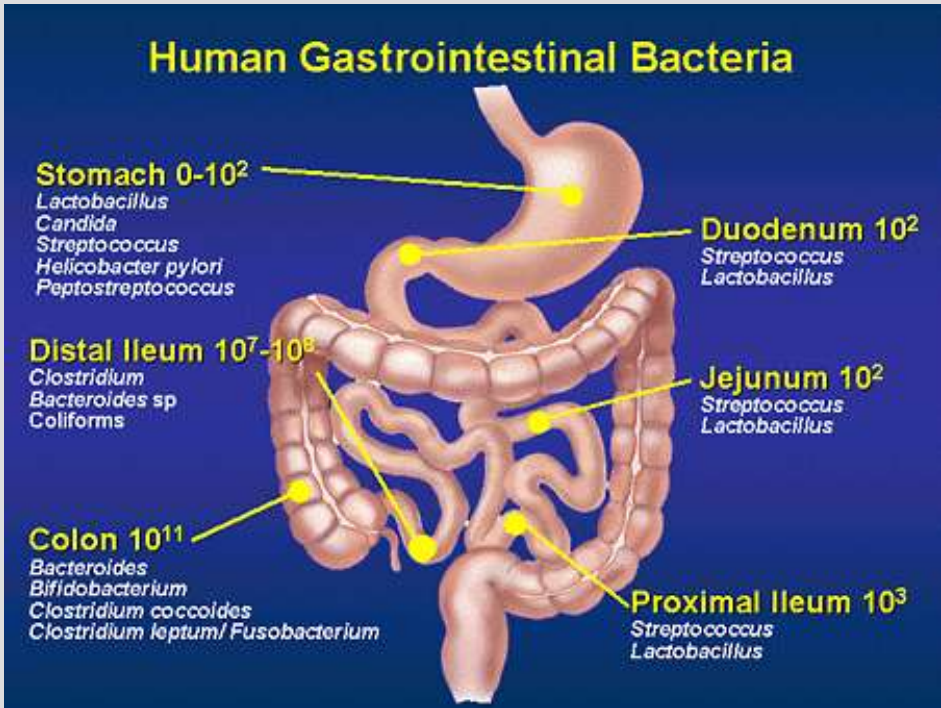
Immune memory is antigen specific



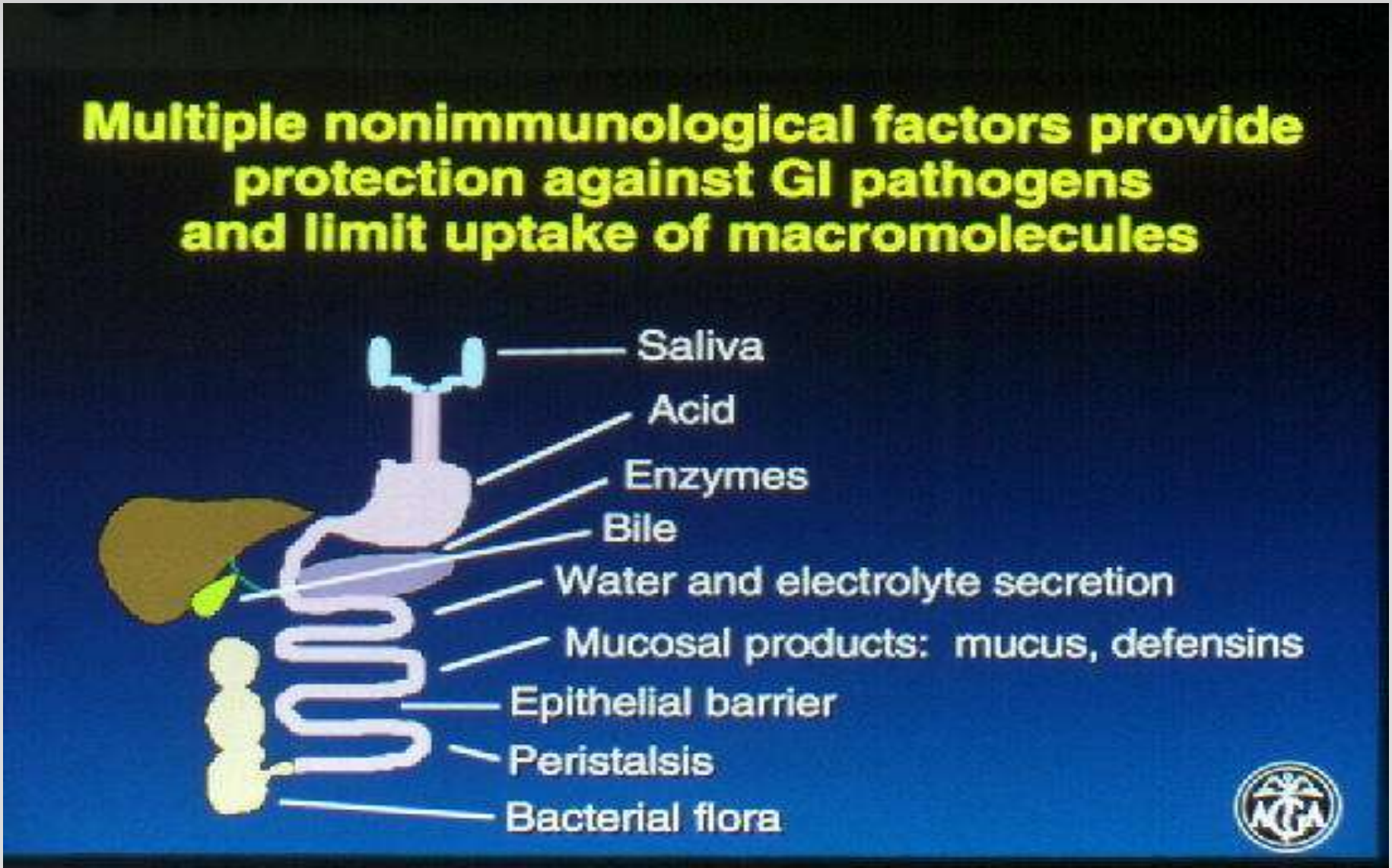
Intestinal Mucosa Defense- Organization

Two types of mechanisms to control pathogens, normal flora (10^{14}) & dietary molecules

Non-Immune
Immune



Intestinal Mucosa Defense: Non-immune



Intestinal Mucosa Defense: Immune

Response type - Immune reaction:

- Innate response
- Adaptive response

Topographic - Immune compartmentalization:

Topographic Organization of GALT

Mesenteric lymph nodes

Peyer's Patches

Cryptopatches

Solitary lymphoid follicles

Solitary immune cells:

- Intraepithelial lymphocytes (IELs)
- Lamina propria lymphocytes (LPLs)

Atkins D, Nature Rev Immunol 2009

Peyer's patch

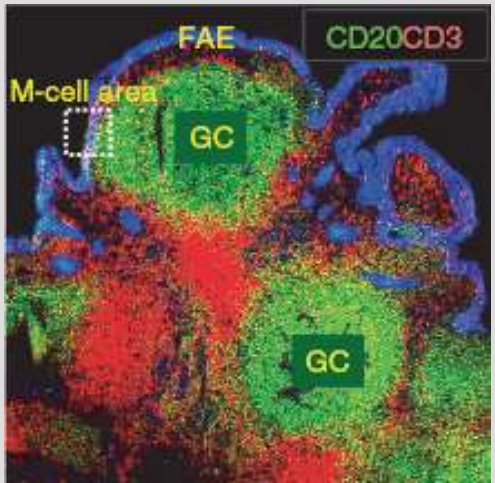
Dome-like protruding into lumen

Sub-epithelial dome (SED) -

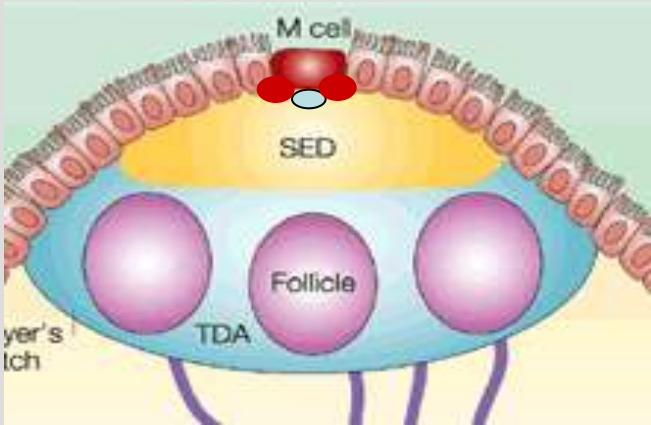
3-4 Follicles (B cell germinal center), inter-follicular region (T-cells), high endothelial venules (HEV) & lymphatics

Villous epithelium & Microfold cells (M cells)

M cells: No villi, thin glycocalyx, basally folded around immune cells. Role: Uptake of Antigens &Transcytosis



Neutra MR, Nature Immunol 2001



Peyer's patch

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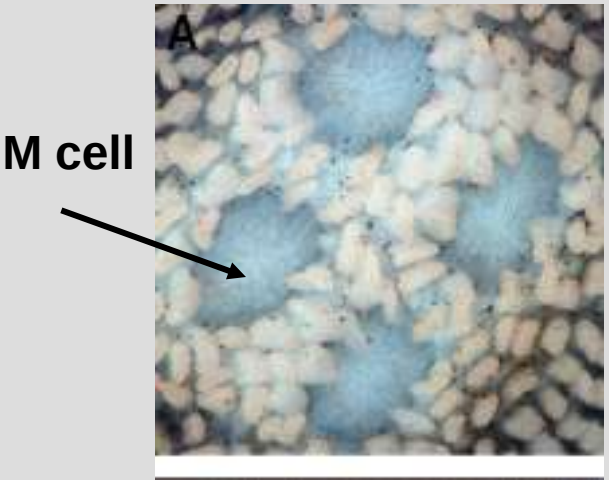
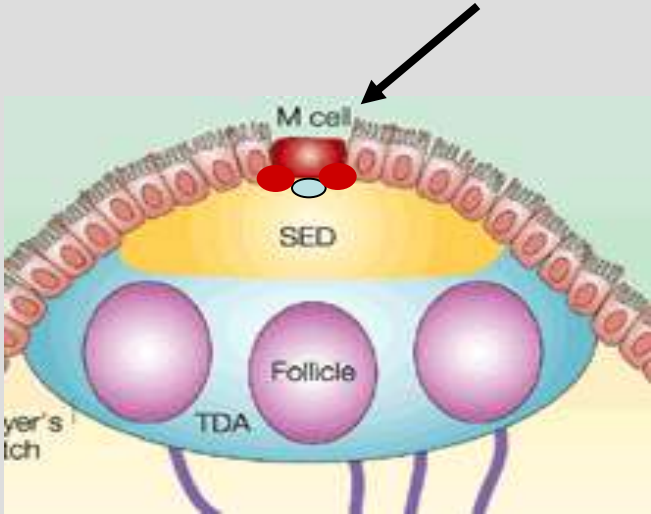


Jang MH, PNAS 2004



Other inductive sites

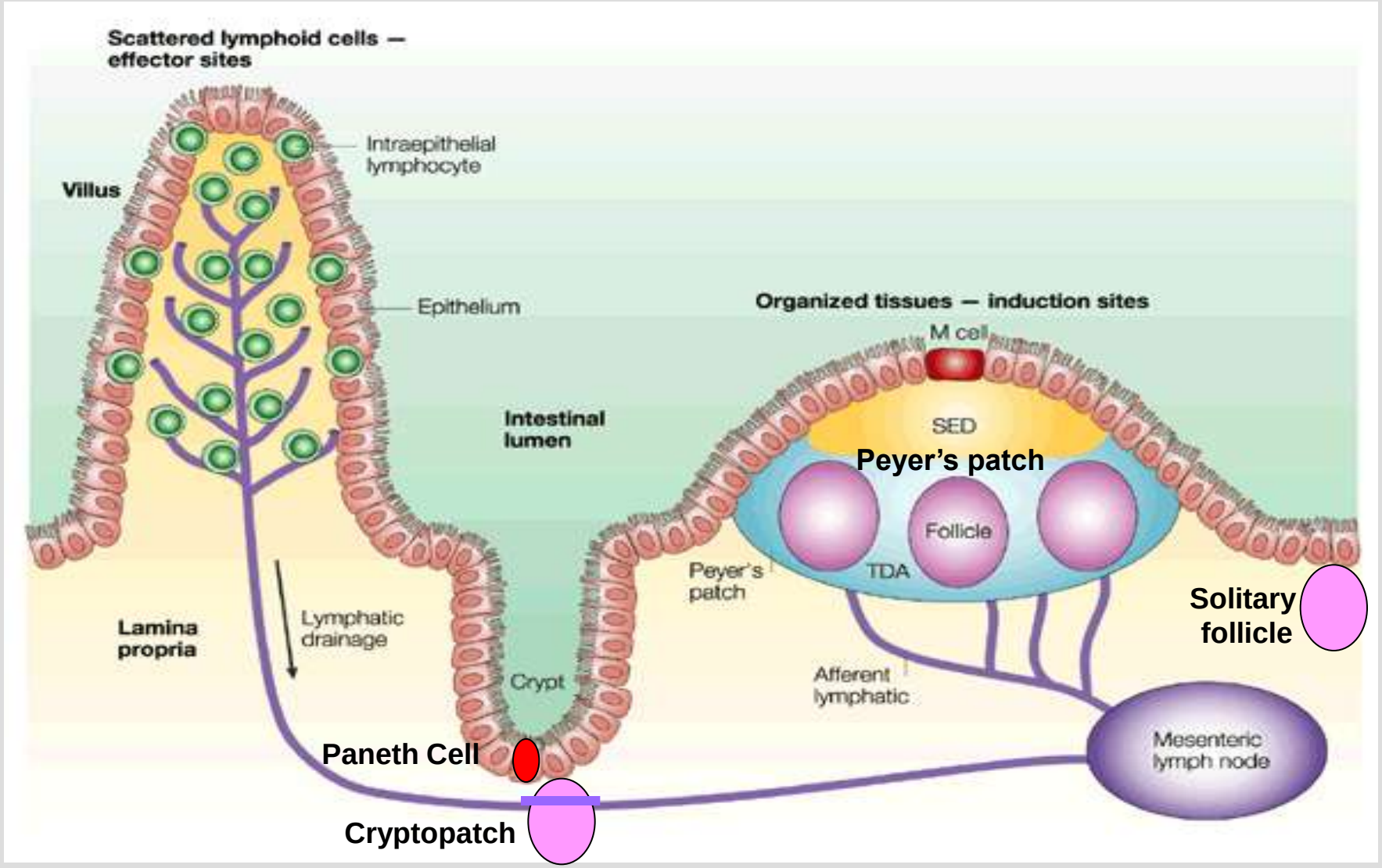
Cryptopatches

- Clusters of hematopoeitic progenitors and dendritic cells
- Role: Extra-thymic development of T-cells

Solitary follicles

- Clusters of lymphocytes - B cells and dendritic cells
- Antigen sampling and presentation >> IgA production

Topographic Organization of GALT - Effectors



Isolated Lymphocytes

Intra-epithelial lymphocytes (IELs) – CD8+ and $\gamma\delta$ T-cells

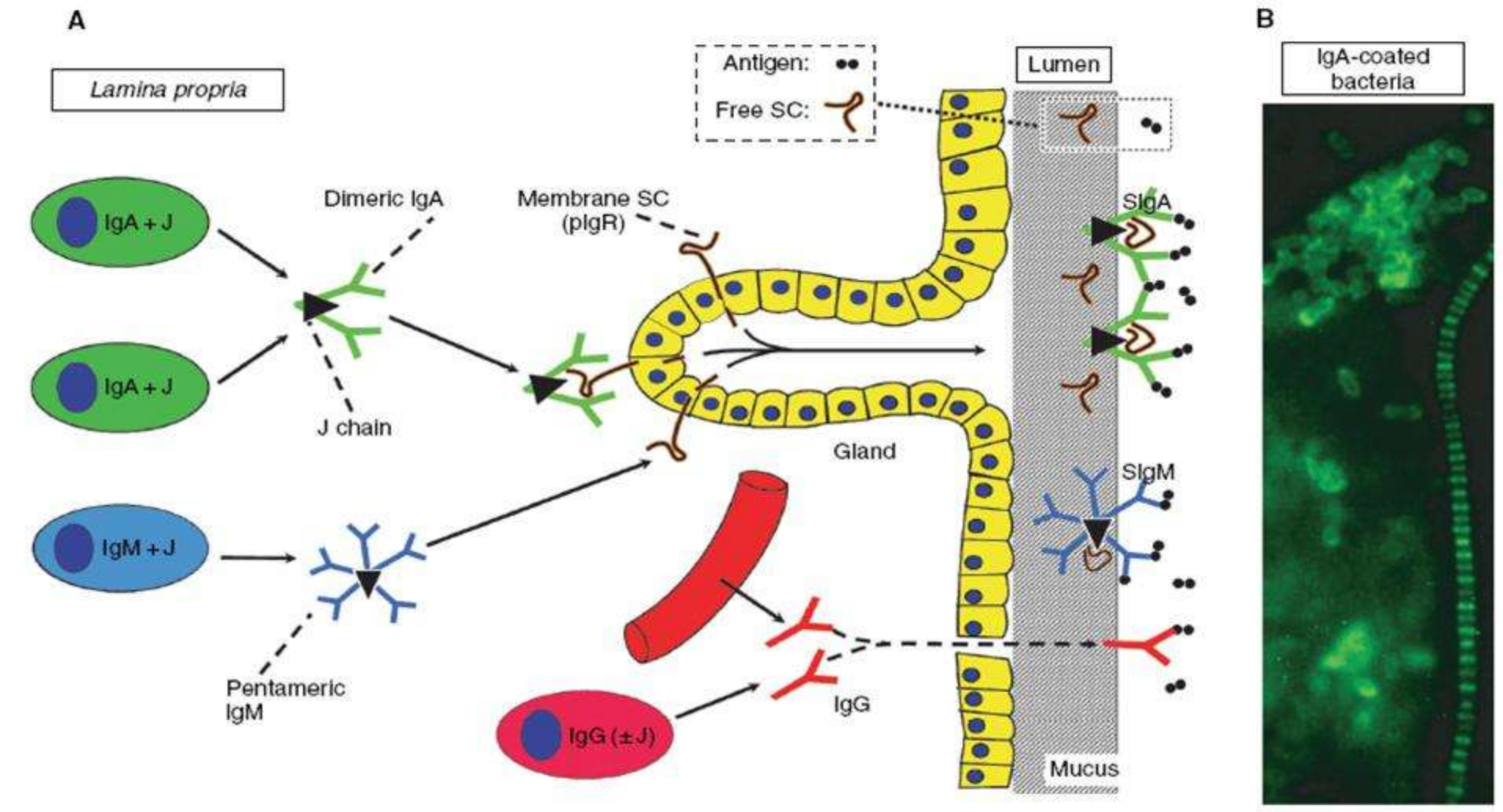
Lamina propria lymphocytes (LPLs) – CD4+ T cells & B-cells

Primed antigen-specific effector cells:

- Excrete pro-inflammatory cytokines
- Exert T-cell cytocytoxicity
- Secrete Immunoglobulines (IgA)

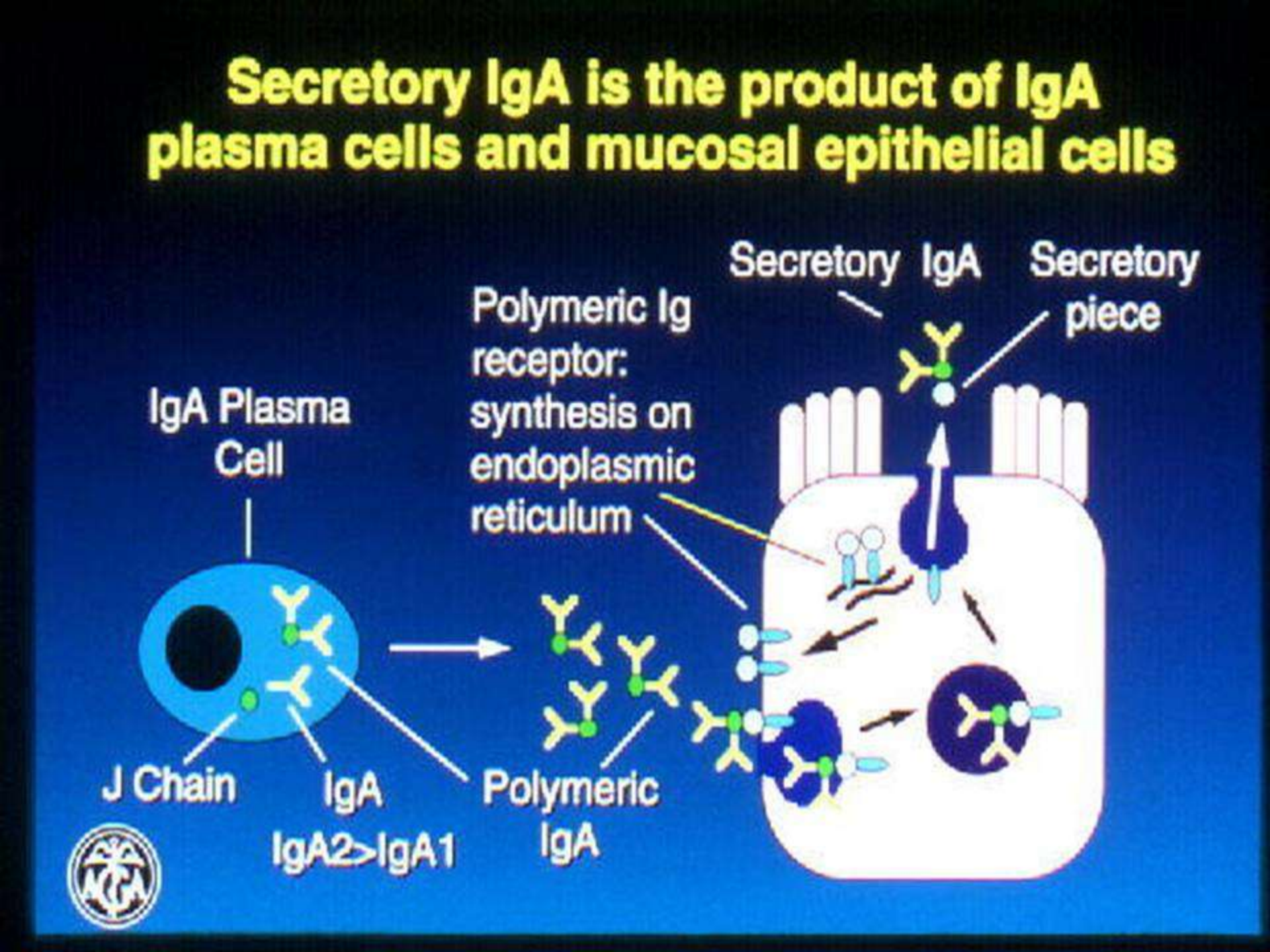
Atkins D, Nature Rev Immunol 2009

Secretory IgA (& pentameric IgM) produced by LP B-cells and actively secreted to gut lumen by epithelium



Suzuki K, Trends Immunol 2008

Secretory IgA is the product of IgA plasma cells and mucosal epithelial cells



Topographic Organization of GALT

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Peyer's Patches
Cryptopatches
Solitary lymphoid follicles

→ Inductive sites

Solitary immune cells:

Intraepithelial lymphocytes (IELs)
Lamina propria lymphocytes (LPLs)

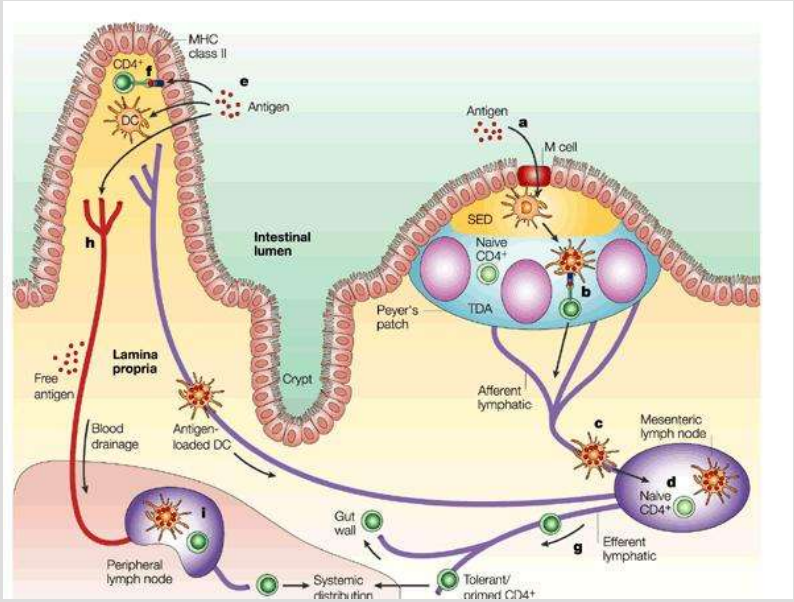
→ Effector sites

Atkins D, Nature Rev Immunol 2009

Intestinal effector T & B cells – Education/migration

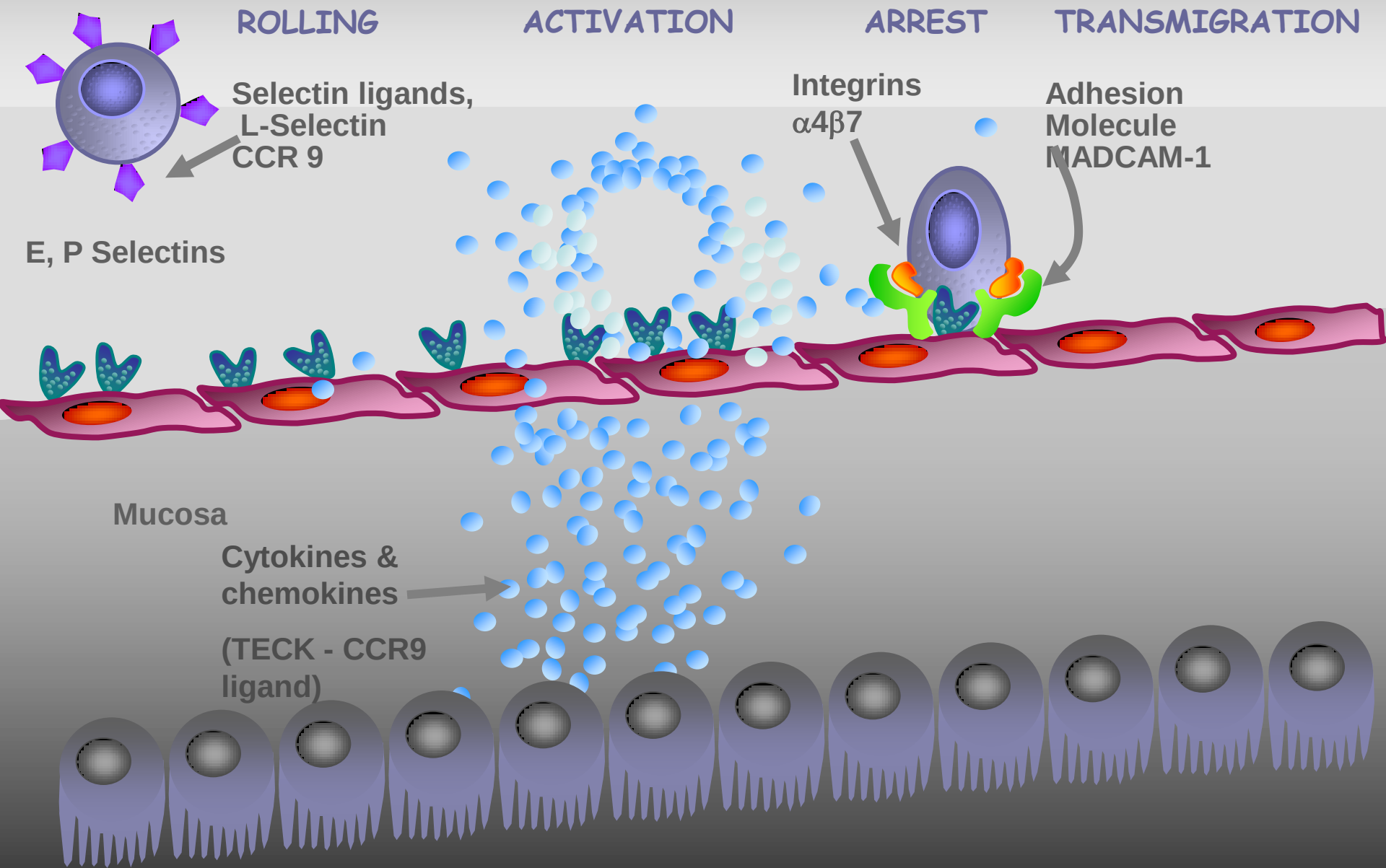
1. Naïve T & B cells presented with antigen by macrophages/DCs in Peyer's patches or mesenteric lymph nodes >> effector cells

2. Exit through efferent lymph vessels into circulation/spleen

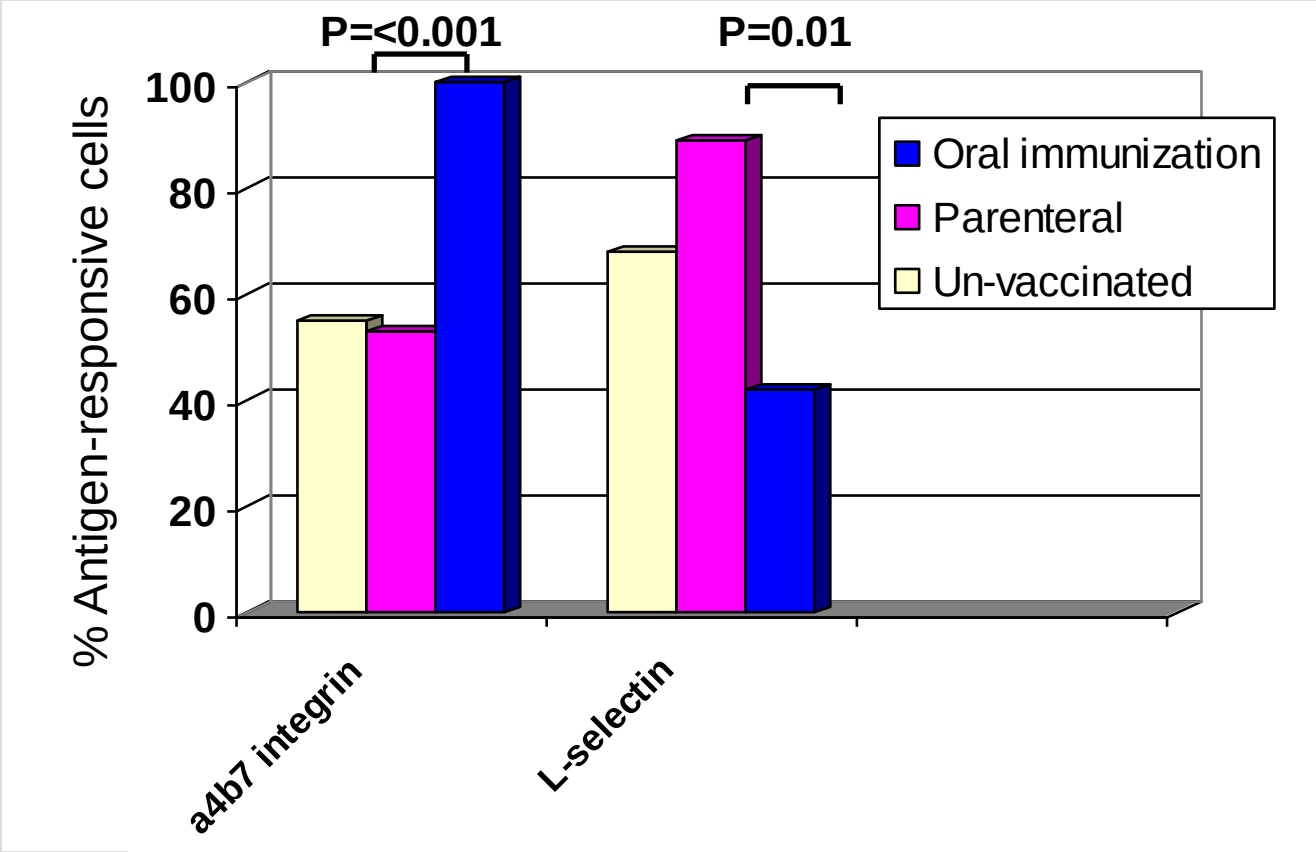


So how do they return to intestinal wall ?

Migration of Cells into Tissues

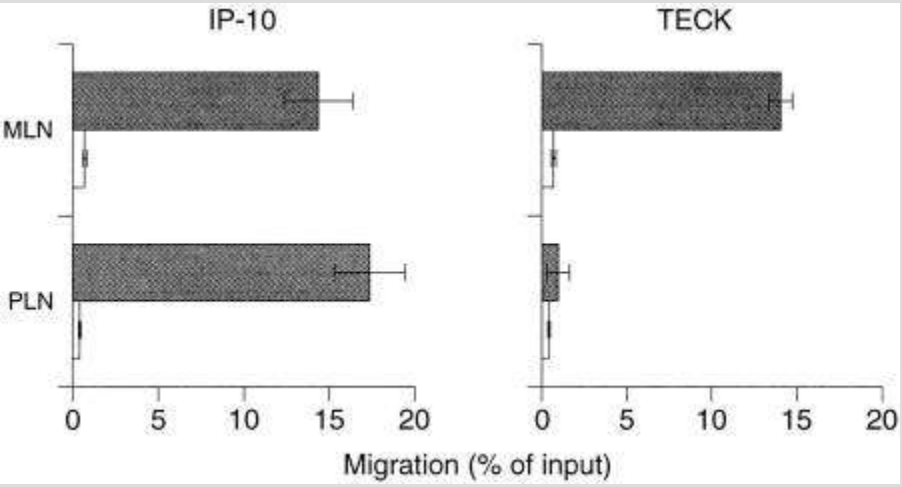


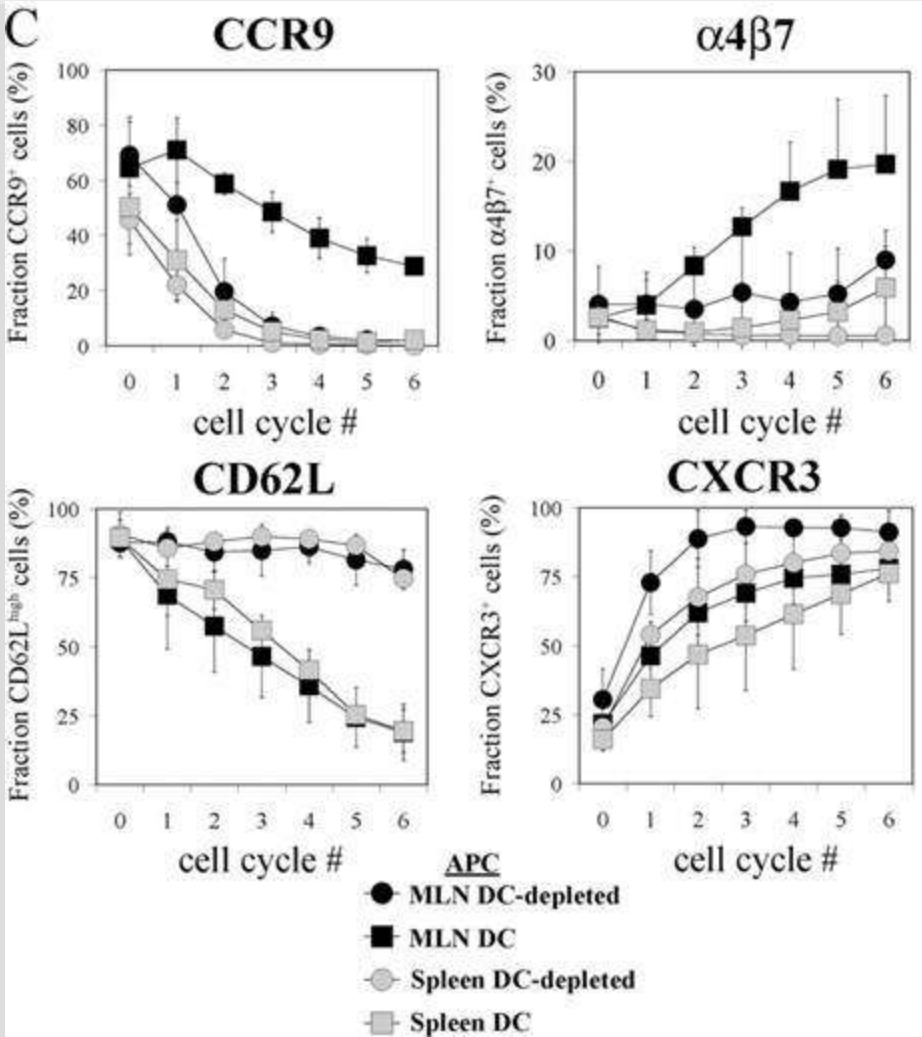
Oral immunization with S.typhy Ty21a induce antigen-responsive cells expressing a4b7 integrin, whereas parenteral vaccination does not



Kantele A, J Immunol 1999

Intestinal dendritic cells in Peyer patches & mesenteric LN prime memory T-cells to express gut-homing molecules: $\alpha4\beta7$ integrin and CCR9 chemokine receptor





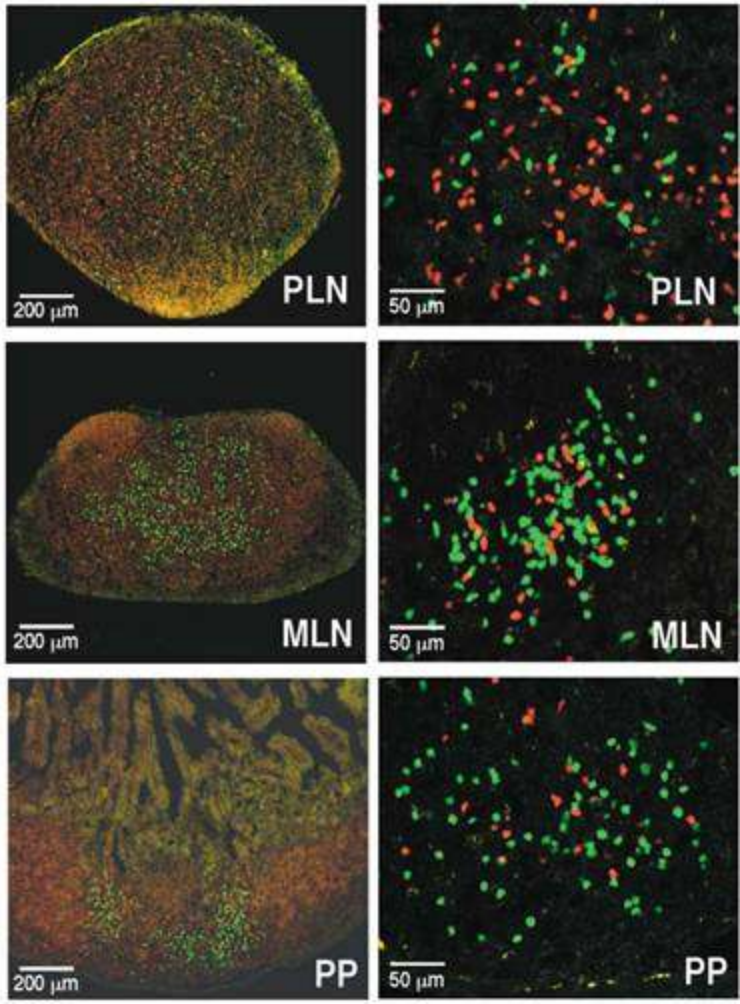
Mora JR, Nature 2003
Campbell DJ, J Exp Med 2002
Johansson-Lindbom B, J Exp Med 2003

CD4+ cells primed by dendritic cell in the mesenteric lymph nodes preferentially migrate back to the GALT

CD4+ cells primed by MLN DC (labeled with green or by PLN DC (red – TRITC labeled)
After injection into mice

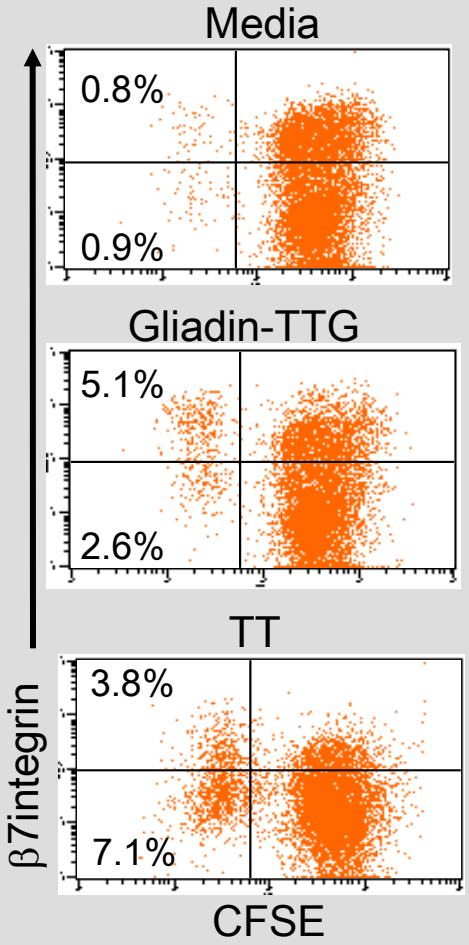
>>

T cells primed by MLN DC(Green labeled) are Much more able to migrate back to Peyer Patch



Iwata M, Immunity 2004

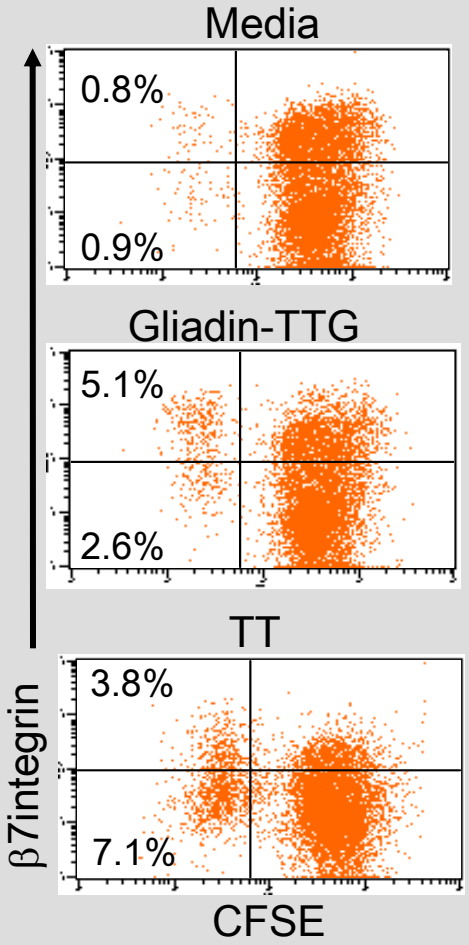
Gliadin-TTG specific CD4+ memory T-cells, but not Tetanus specific cells, preferentially express the gut-homing β7 integrin



Ben-Horin S et al, J Leuk biol 2006

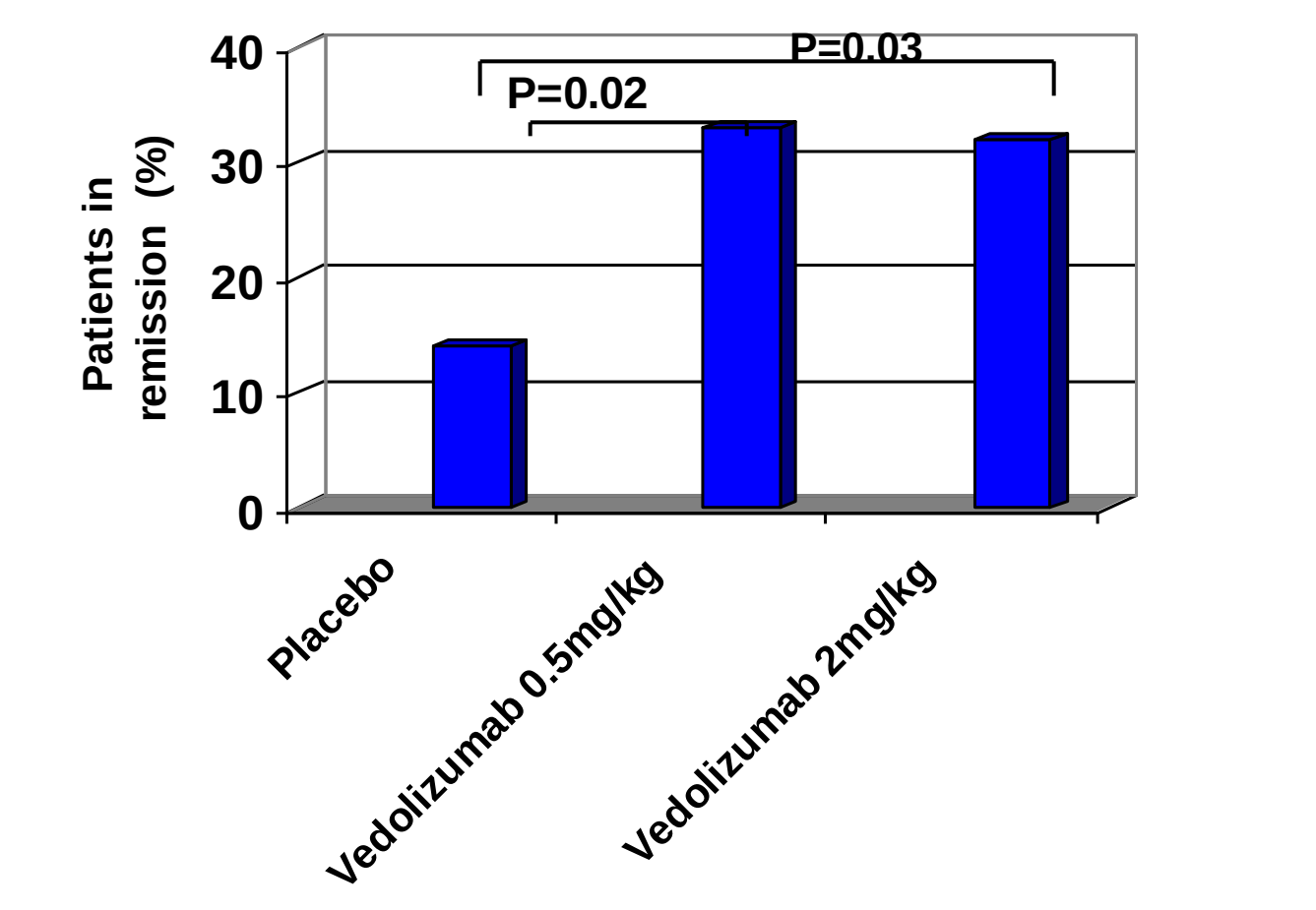
NEW view 1: Immune memory is antigen-specific….AND tissue-specific

Gliadin-TTG specific CD4+ memory T-cells, but not Tetanus specific cells, preferentially express the gut-homing β7 integrin



Ben-Horin S et al, J Leuk biol 2006

Anti α4β7 integrin mAb (Vedolizumab) induces remission in moderately active ulcerative colitis



Feagan B, N Engl J Med 2005

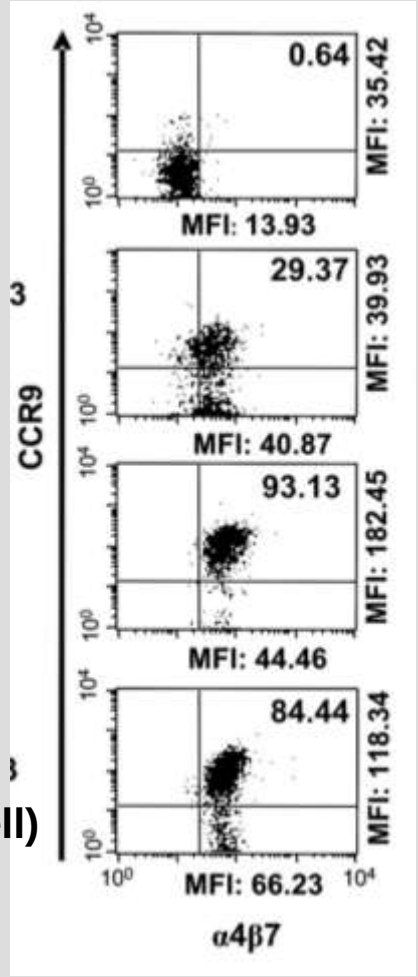
Intestinal epithelial cells (m-IC) induce expression of gut homing phenotype by T-cells in the absence of intestinal dendritic cells

CD8+ cells only

CD8+ cells & bone marrow DC

CD8+ cells & bone marrow DC & gut epithelial cells

CD8+ cells & bone marrow DC & gut epithelial cells (transwell)



Edele F, J Immunol 2008

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Cryptopatches
Solitary lymphoid follicles

→ Inductive sites

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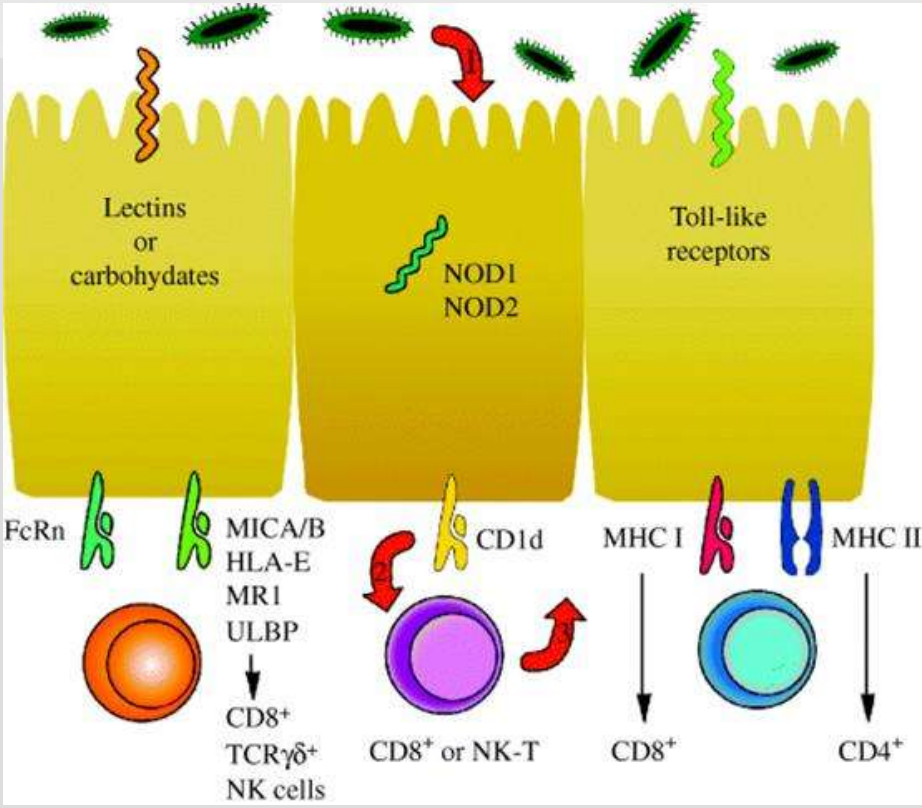
→ Effector sites

Epithelium ??

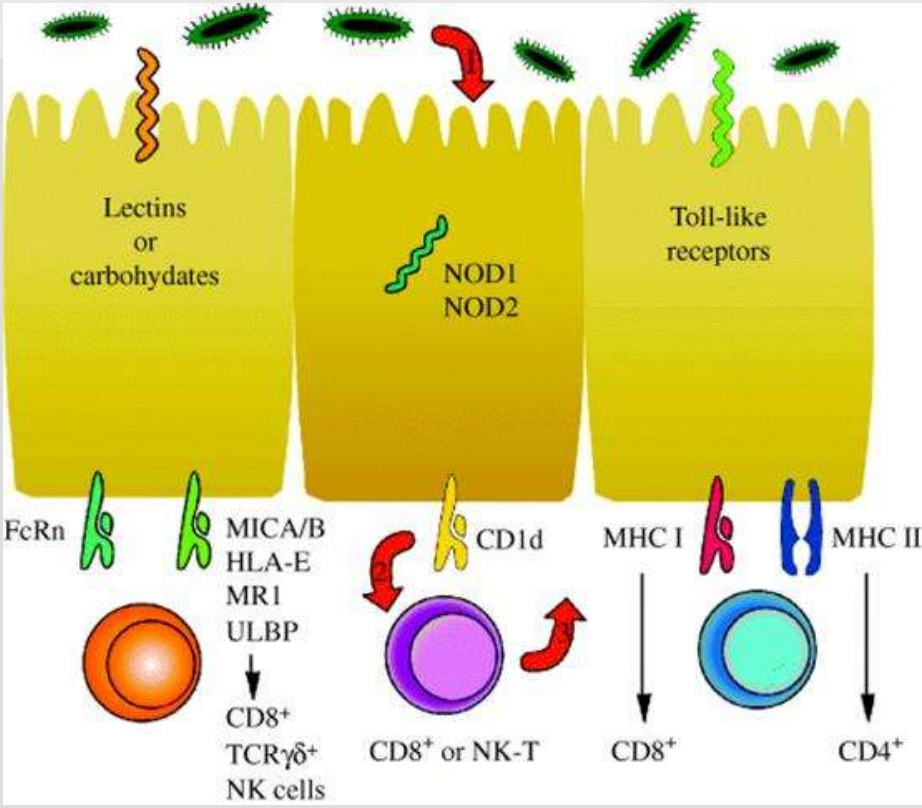
Intestinal Epithelium expresses bacterial sensing molecules



Giraradin SE, Science 2003

Intestinal Epithelium expresses bacterial sensing molecules

NOD1 – Recognition of Gram- bacterial wall tri-peptide
NOD2- Recognition of Gram-/+ bacterial wall MDP
>> Activation of MAPK-NFkB pathways & Secretion of chemokines/peptides



Giraradin SE, Science 2003

Toll-Like Receptors: Recognition of Pathogen associated molecular patterns PAMP)

Peptidoglycan (G+)
Lipoprotein
Lipoarabinomannan (Mycobacterial)
LPS (Leotosopia)
LPS (Porphyromonas)
GPI (Trypanosoma cruzi)
Zymosan Yeast)

LPS (G-)
Lipoteichoic acids (G+)
RSV F Protein

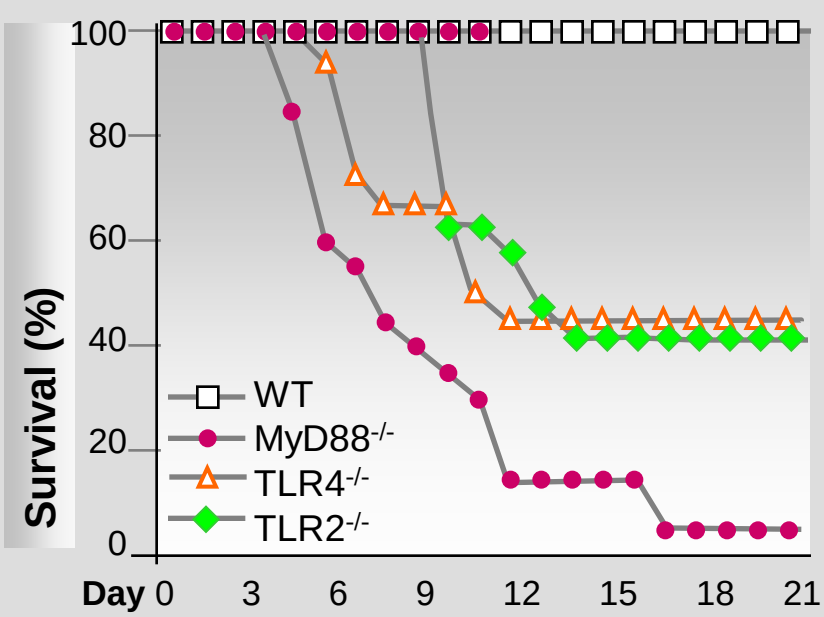
dsRNA

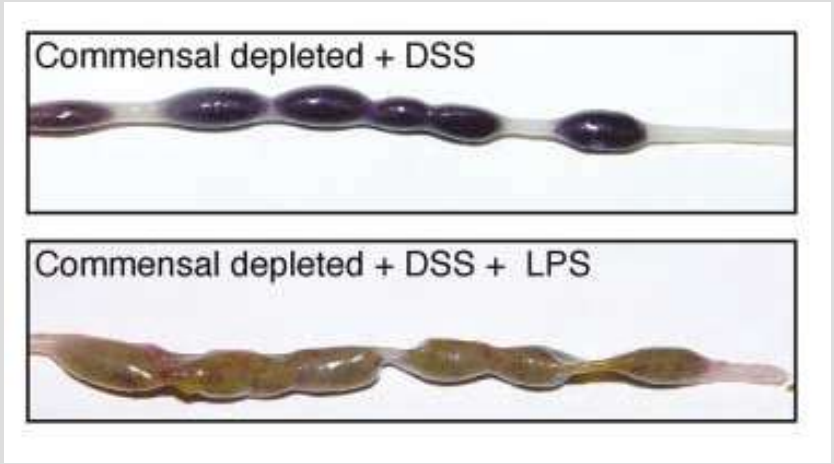
Flagellin

Unmethylated CpG DNA

Artis D, Nature Rev Immunol 2008

Commensal triggering of TLR maintains epithelial homeostasis and protects against DSS colitis



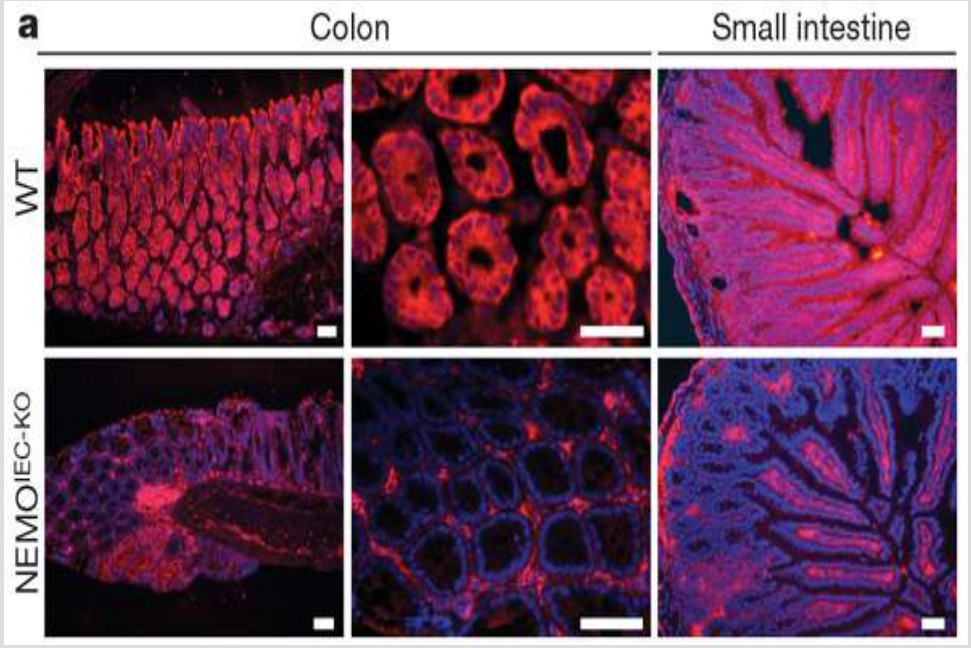


Deficient TLR signaling increases mortality after DSS colitis

Depletion of intestinal flora increases mortality after DSS colitis, and LPS restores protection

Rakoff-Nahoum S, Cell 2004

Selective NFkB ablation in intestinal epithelium of mice by knocking out NEMO (IKK-γ) leads to innate and later adaptive immune cell inflitrate and intestinal inflammation



Nenci A, Nature 2007

Murine models validity for human studies

Arch Gynecol - **Links**

The effect of intraperitoneal colchicine on the formation of peritoneal adhesions in the rat

Shapiro I, Granat M, Sharf M.

A double blind study was carried out to determine the effect of colchicine on the formation of intraperitoneal adhesions **in the rat**. Adhesions were produced by a standard method in 92 rats.

The animals were than divided at random into two groups. One group (47 rats) was treated with intraperitoneal colchicine, whilst the other, the control group (45 rats) was treated with intraperitoneal normal saline in the same volume, frequency and duration.

Two kinds of adhesions were found. 1. "Surface adhesion" - consisting of two serosal surfaces attached together; their area was significantly smaller (p less than 0.00003), and their number was lower (p less than 0.03) in the colchicine group than in the control group. 2. "Filamentous adhesions" - thin, elastic cords connecting omentum or pelvic fat bodies to other viscera. We concluded that post-operative treatment with colchicine reduces the formation of intraperitoneal adhesions in the rat

Murine models validity for human studies

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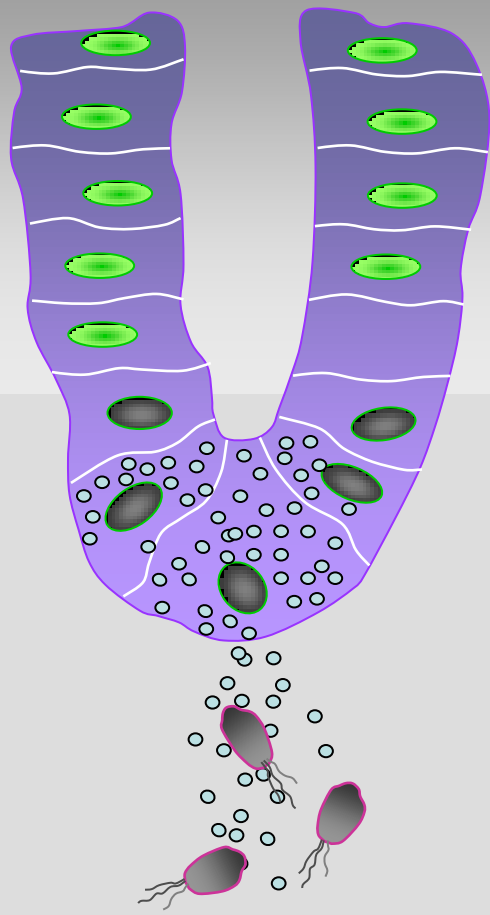
Paneth cell

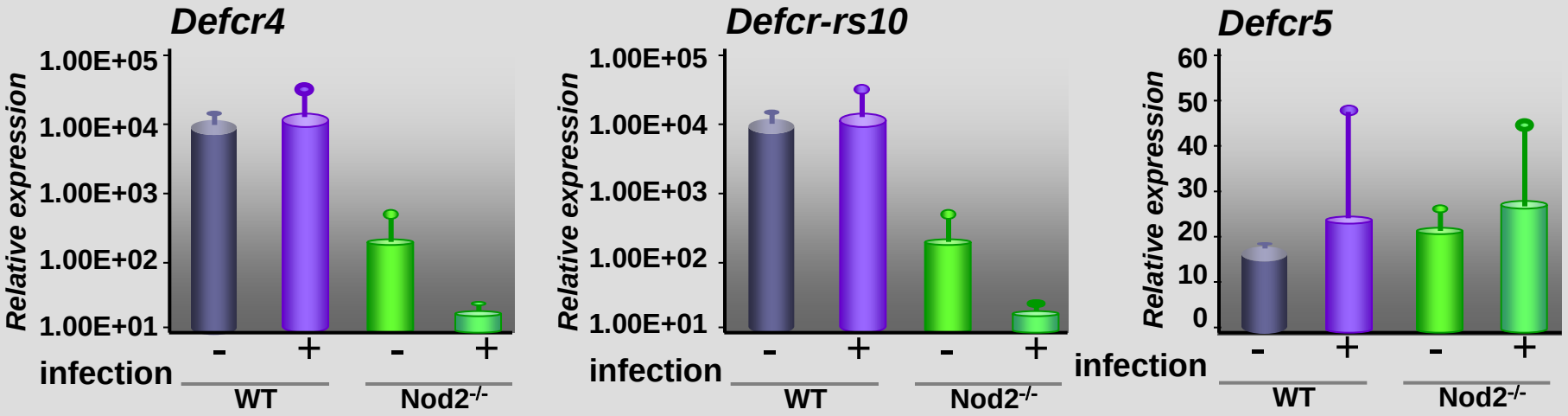


Specialized epithelial cells at base of crypts

Respond to certain patterns of bacteria

Secrete anti-bacterial peptides (Defensins)

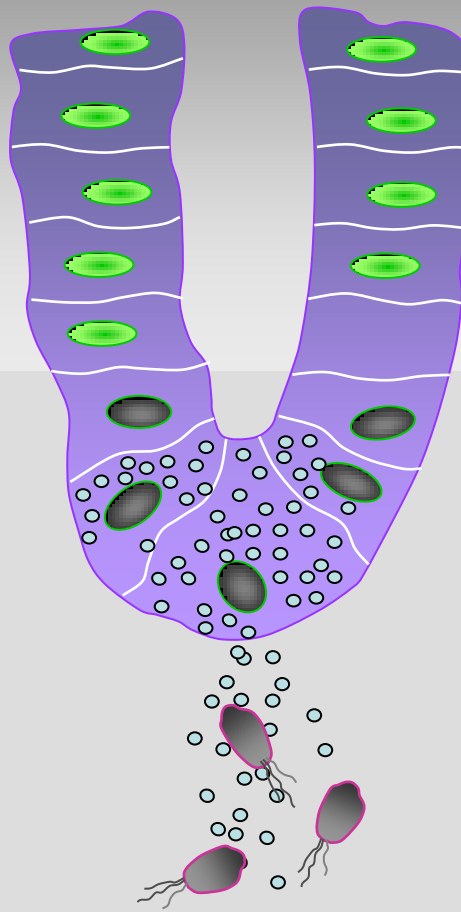
NOD2 Knockout mice have defective expression of Defensins after Listeria infection



- Does the Nod2 defect induce loss of function and reduces innate immunity→ exposure to bacterial infections mainly in the TI region?

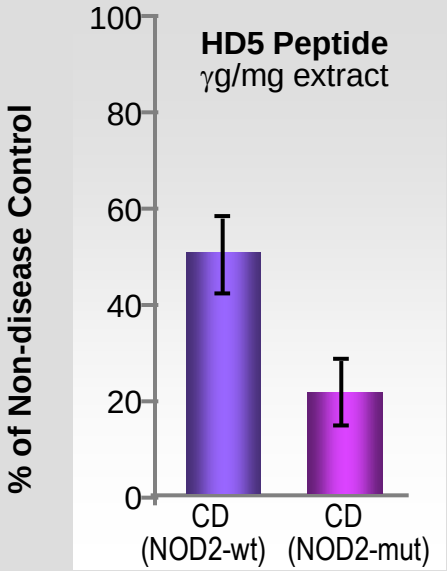
Kobayashi KS, Science 2005

Paneth cell



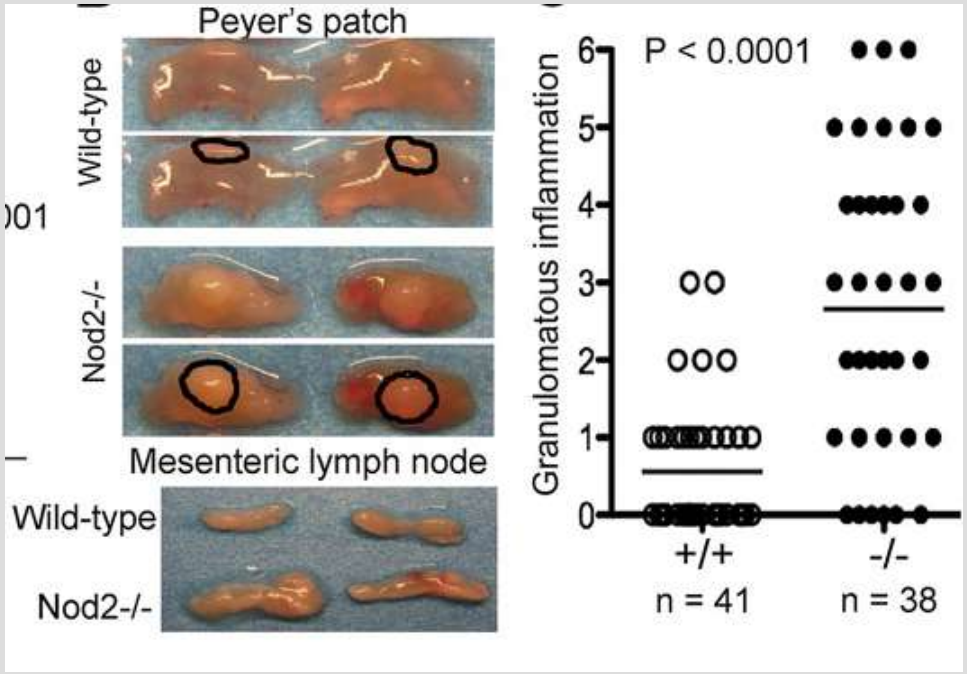
NOD2 mutations found in 15% Crohn's patients and associated with diminished mucosal α-defensin expression

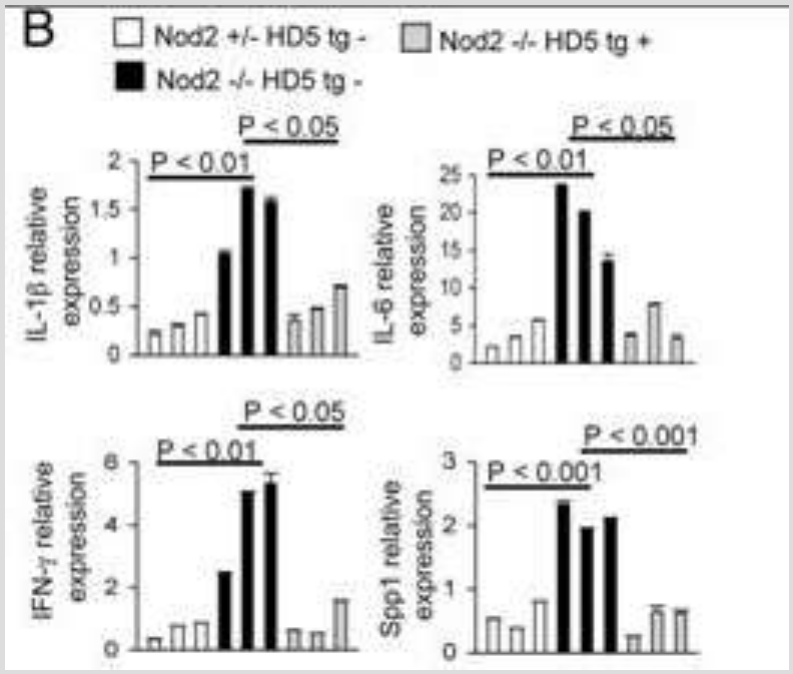
>> reduced control of luminal bacteria ??



Wehkamp J, PNAS 2005

NOD2- mice develop H. hepaticus granulomatous ileitis in Peyer patches, reversed in transgenic human defensin mice

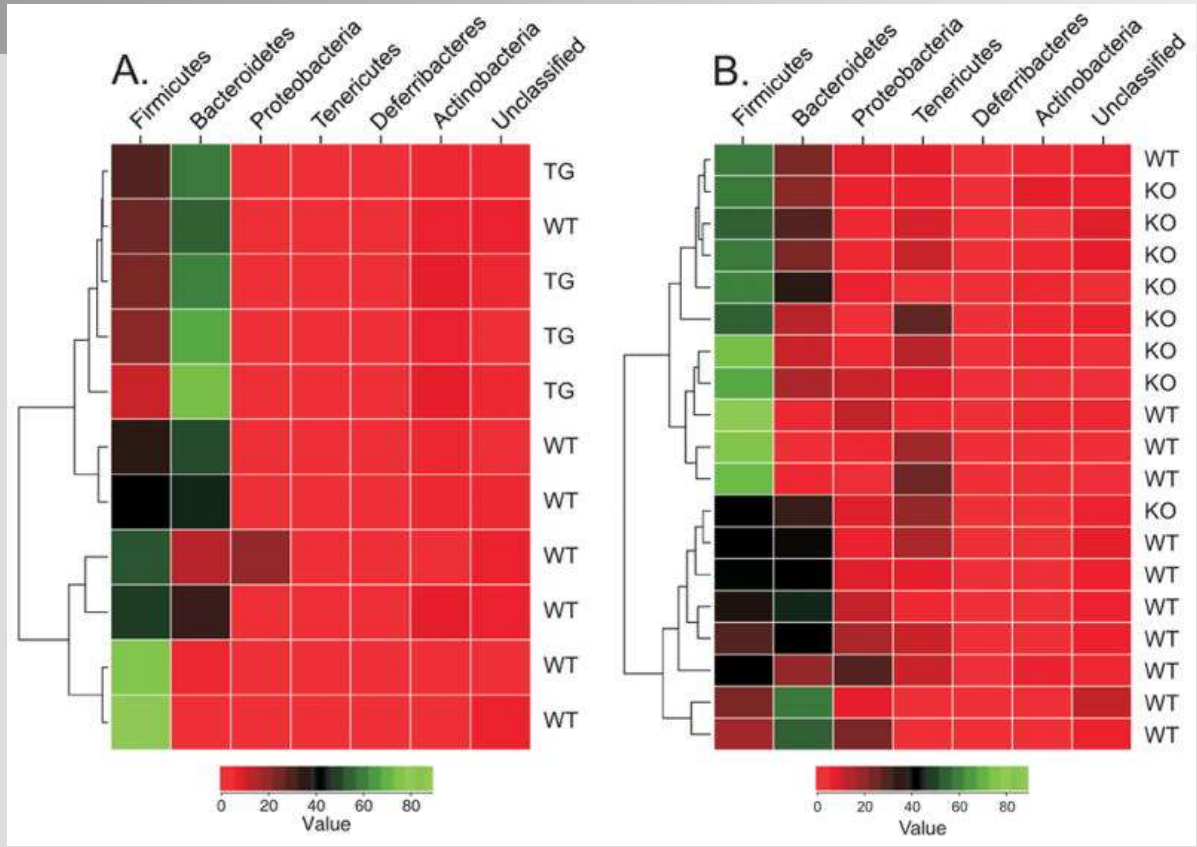




WT bone marrow transfer does not restore function >> Epithelial dependency

Biswas A, PNAS 2010

The α-defensins impact the microbial composition of the gut

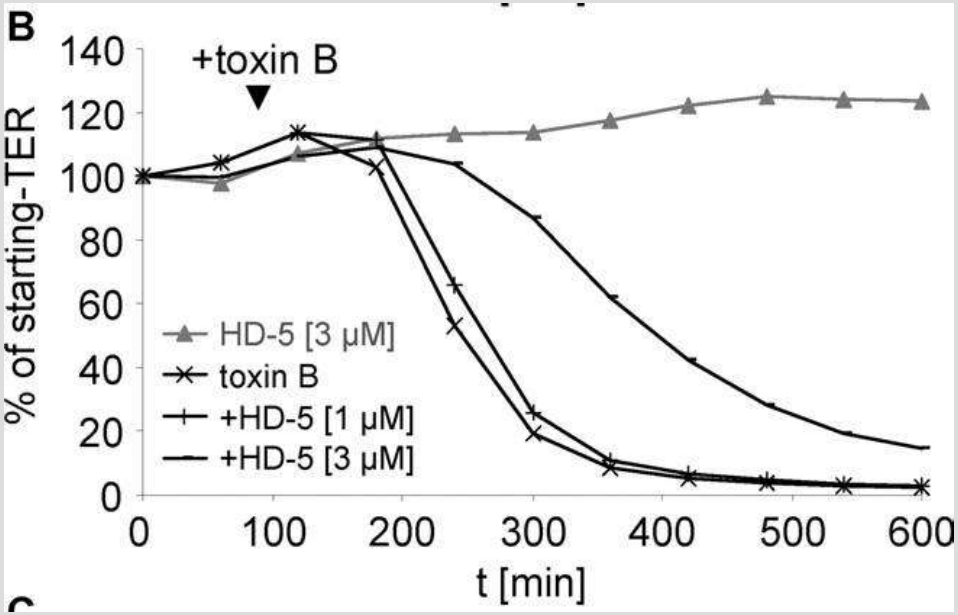


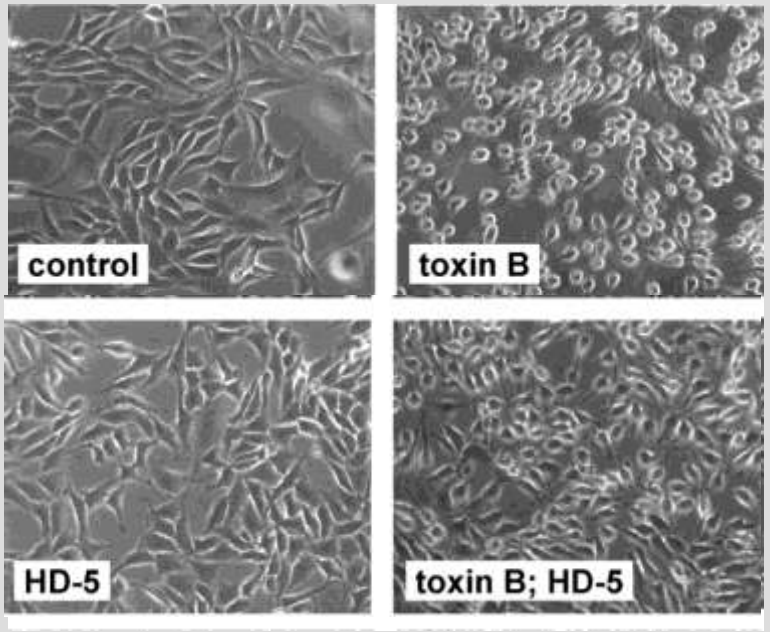
Defensin Knock-outs

Transgenic defensin mice

Salzmann NH, Nature Immunol 2010

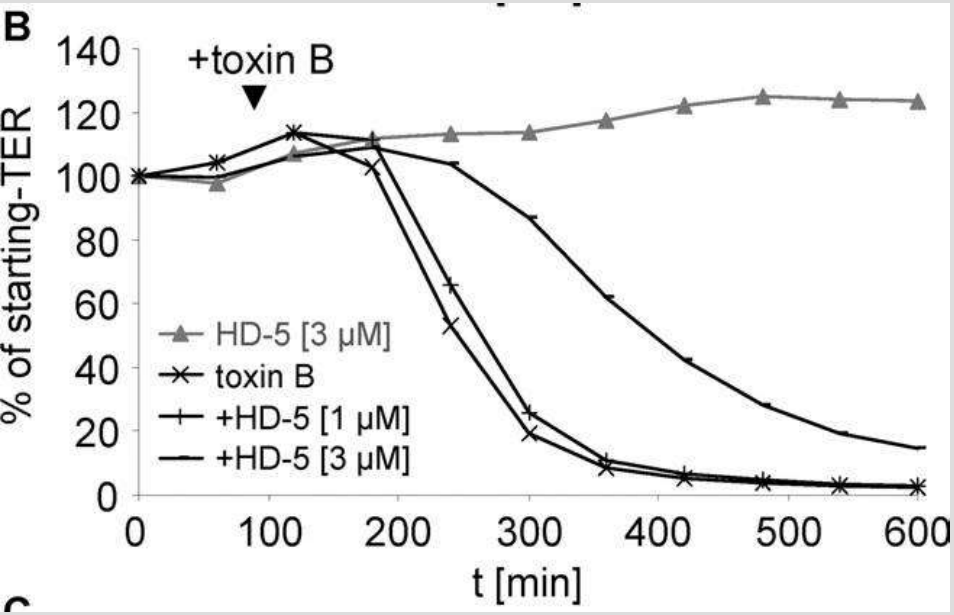
Pre-incubation of intestinal epithelium with α-defensins ameliorates C.difficile Toxin B injury

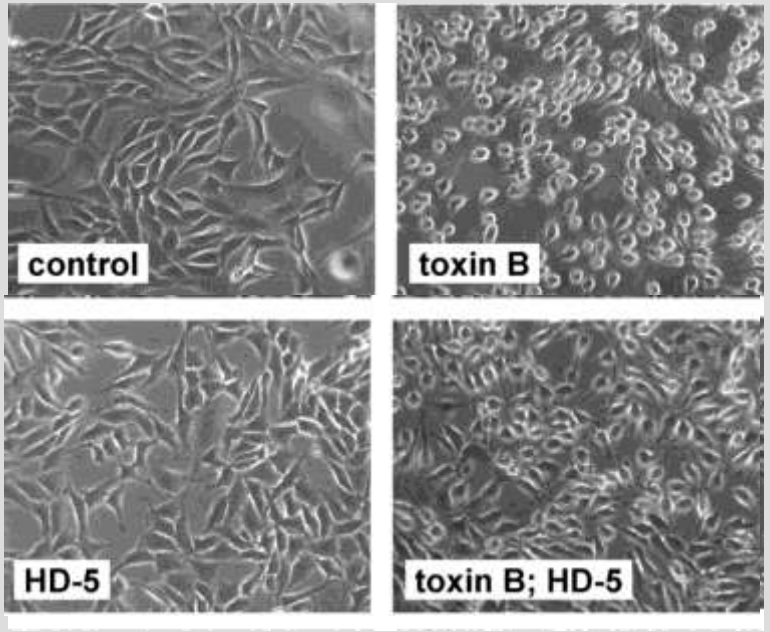




Giesmann T, Gastroenterology 2008

Pre-incubation of intestinal epithelium with α-defensins ameliorates C.difficile Toxin B injury





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NEW view 2: epithelium is a barrier…but also an immune organ

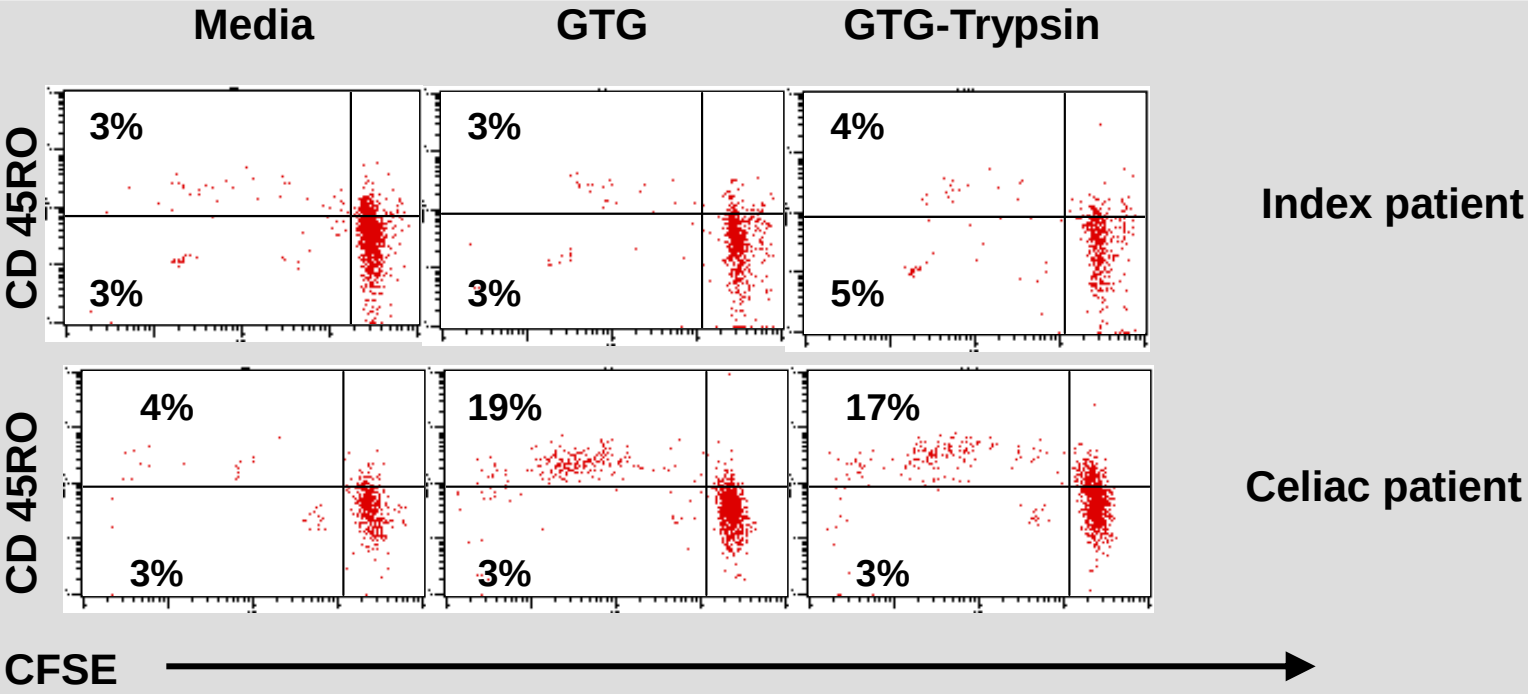
Intestinal enterocytes induce expression of FoxP3 in activated CD4+ cells and expansion of Treg population

Dotan I, Am J Gastrointest Physiol, 2008

Intestinal enterocytes up-regulate MICA expression and IL-15 secretion leading to Intra-epthelial CD8+ T-cell activation and epithelial damage after exposure to gliadin in celiac

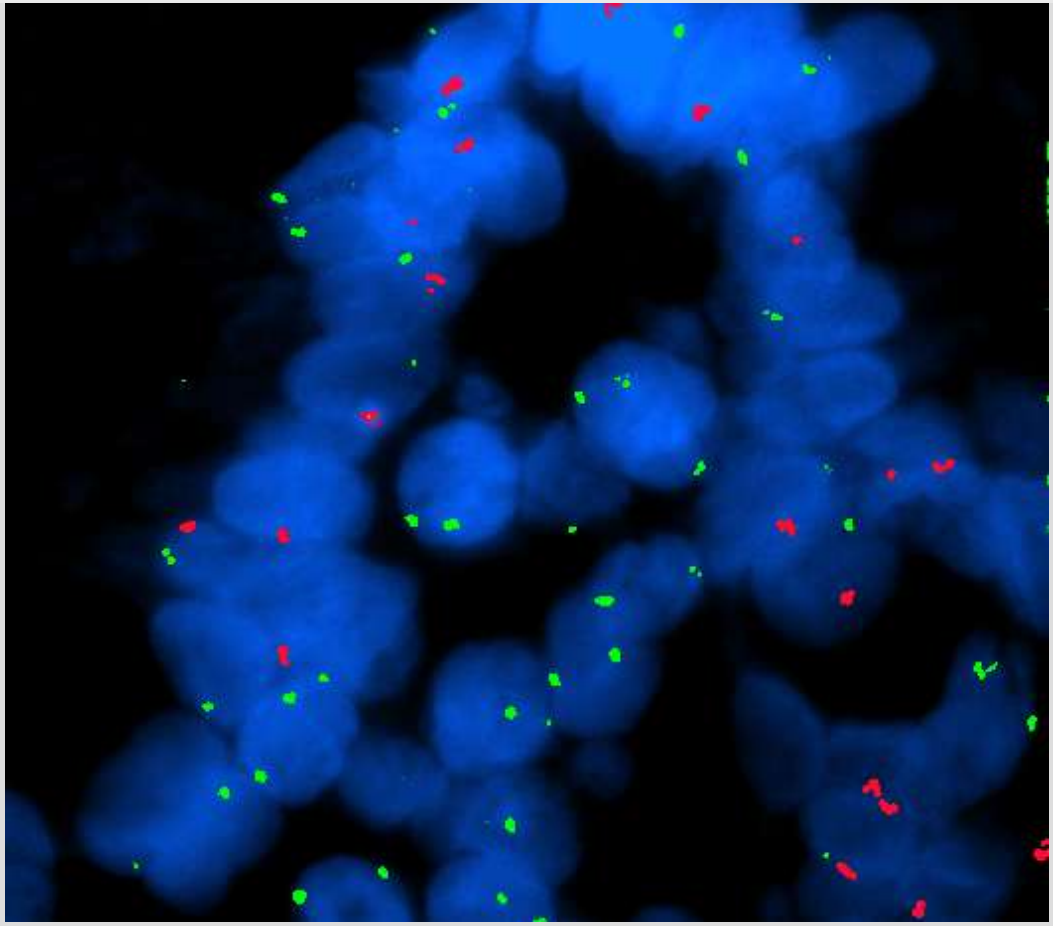
Kagnoff MF, J Clin Invest 2007

In a bone-marrow transplanted celiac : No immune memory to celiac antigens



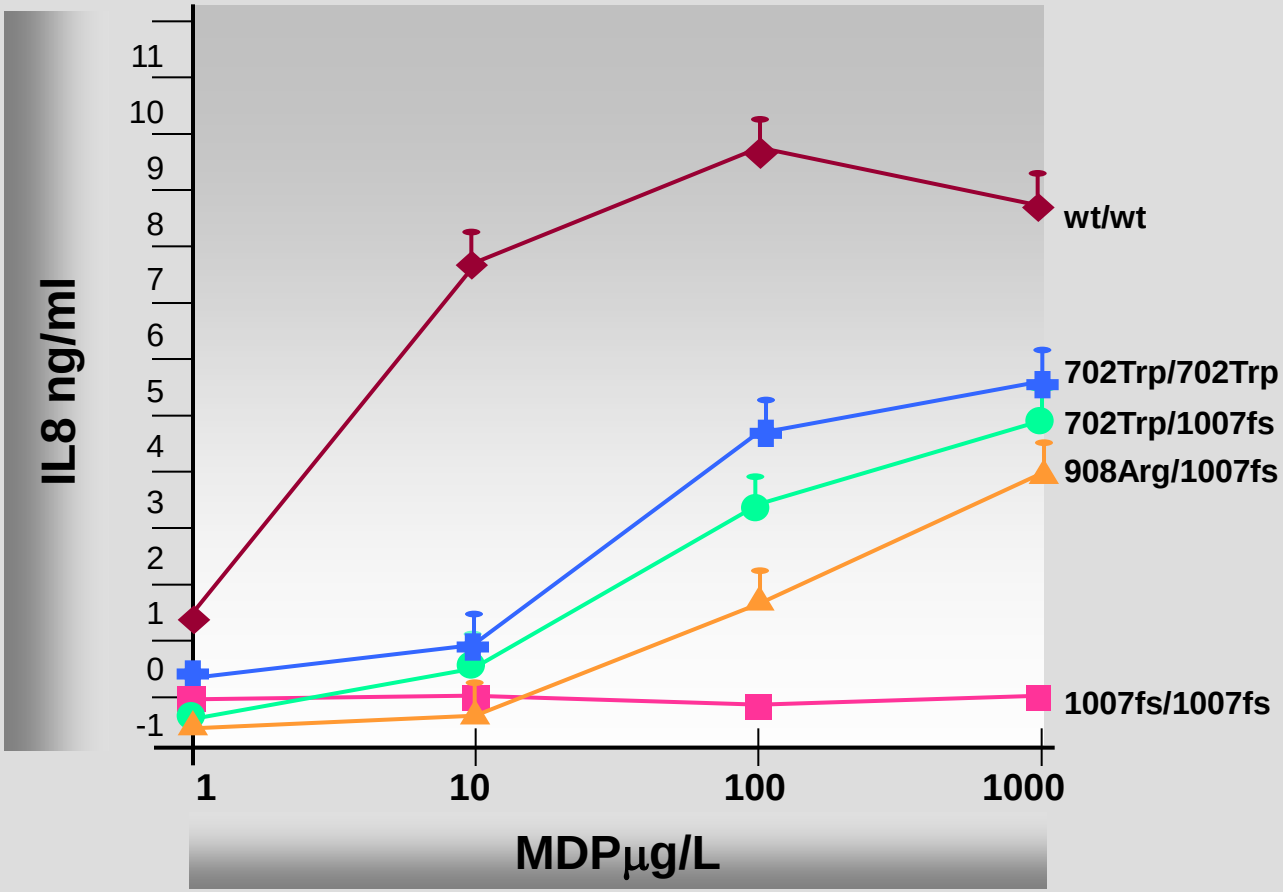
Ben-Horin S et al, Submitted

In a bone-marrow transplanted celiac : Intestinal epithelial-lymphocyte chimerism



Ben-Horin S et al, Submitted

Mutated NOD2 Monocytes Mount Defective Immune Responses: Innate system dysfunction in Crohn's disease?



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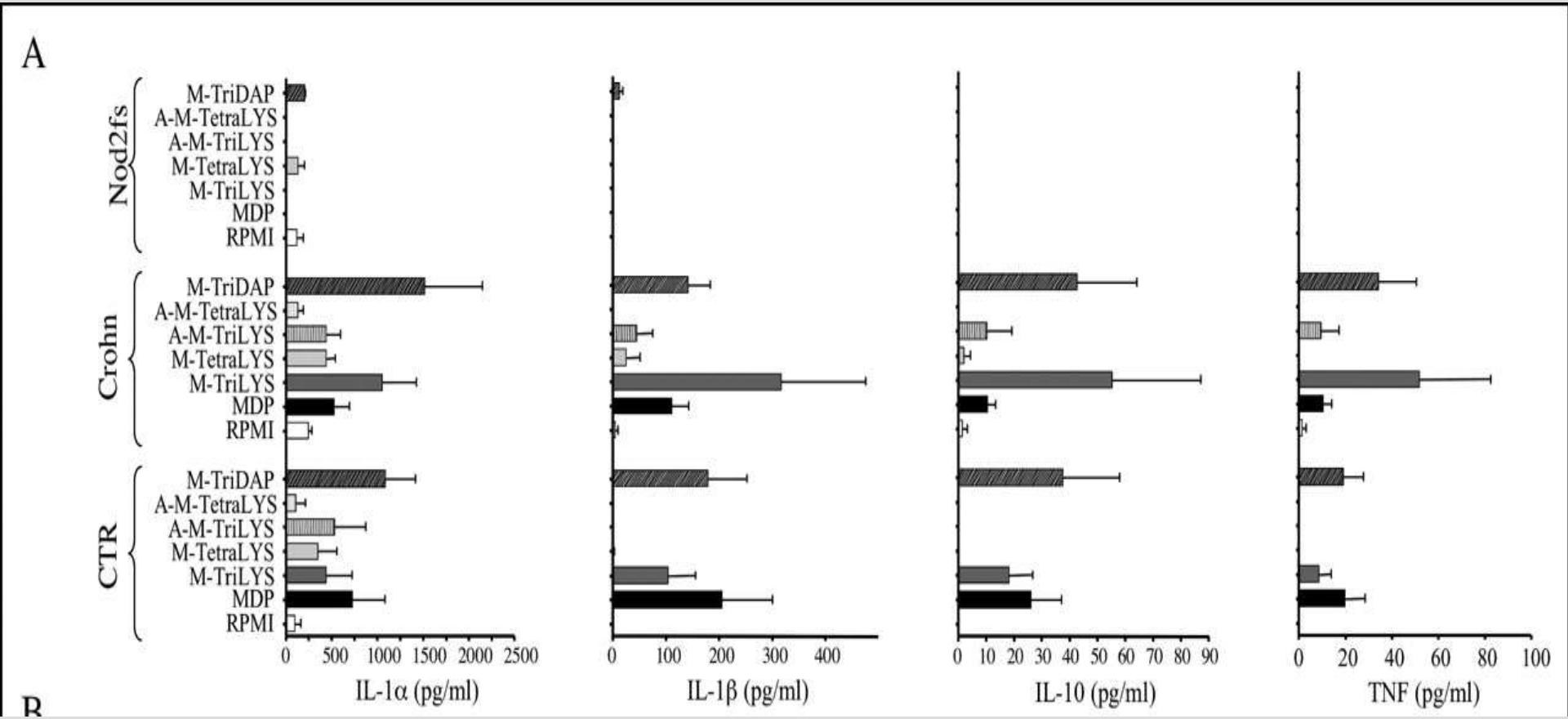
PBMCs' response to MDP and peptidoglycans is reduced in Crohn's patients homozygous for the NOD2 mutation (Nod2fs) compared to controls (CTR) & Crohn's patients without defects in Nod2 (Crohn)



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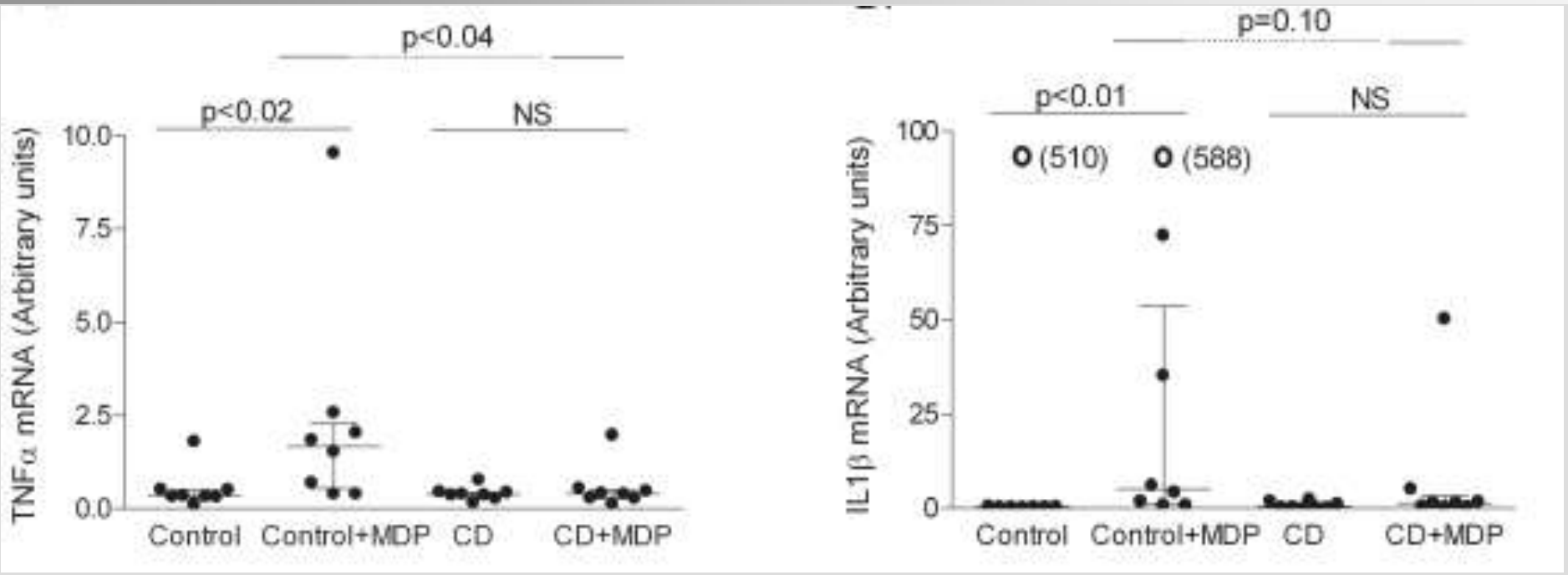
Diminished response to MDP is also present in monocytes from Crohn's patients without NOD2/CARD15 mutations



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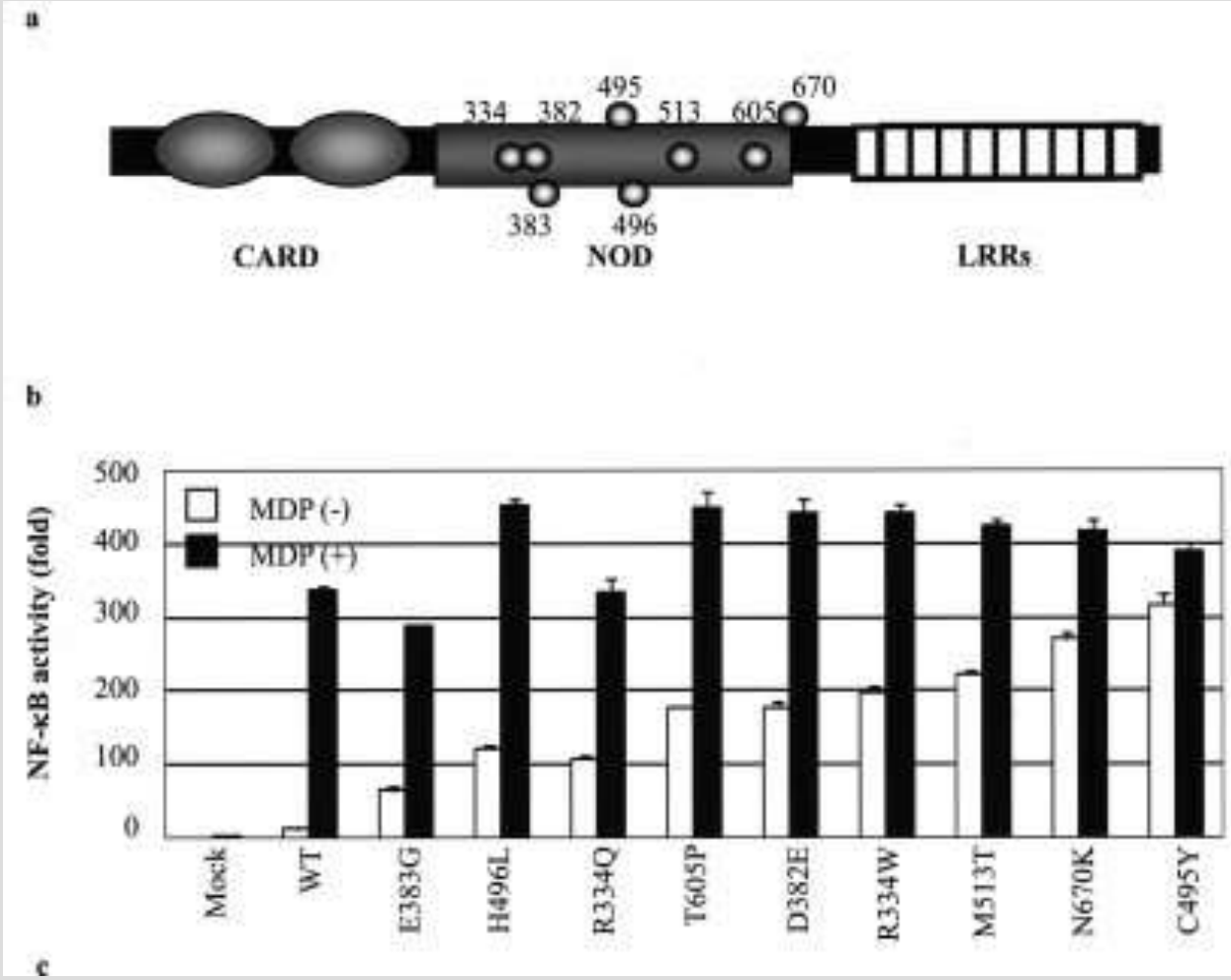
NOD2 activating mutations cause constitutive NFkB activation and early-onset sarcoidosis (Blau syn)



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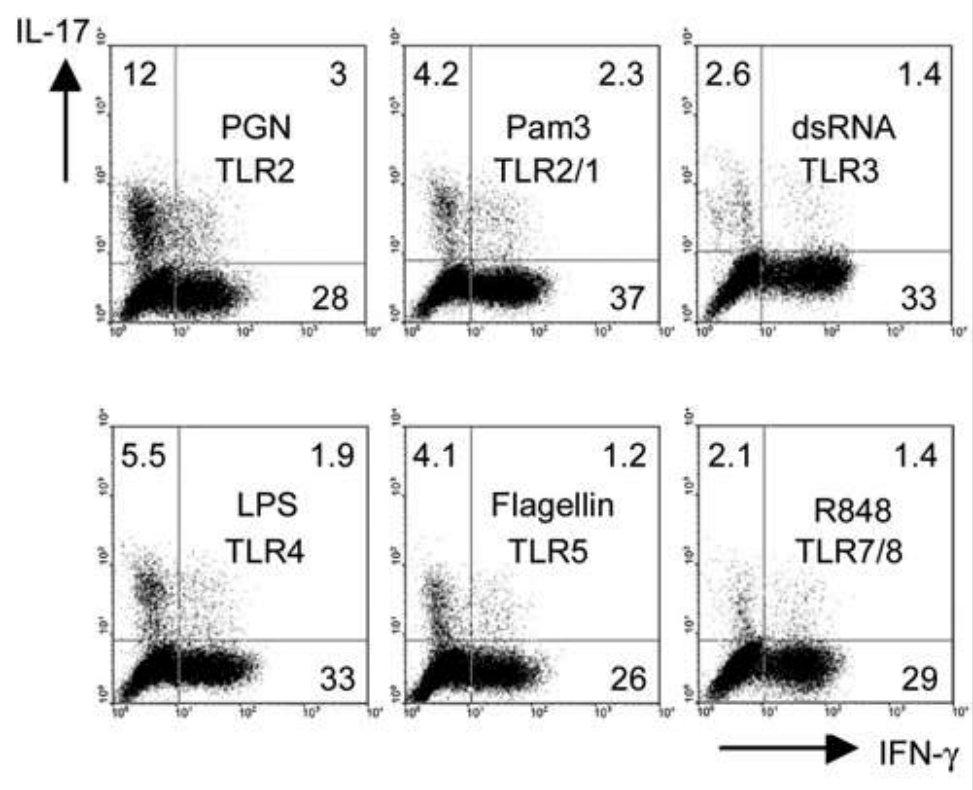
TLR2 & NOD2 signaling cause dendritic cells to induce IL-17 expression by memory T-cells



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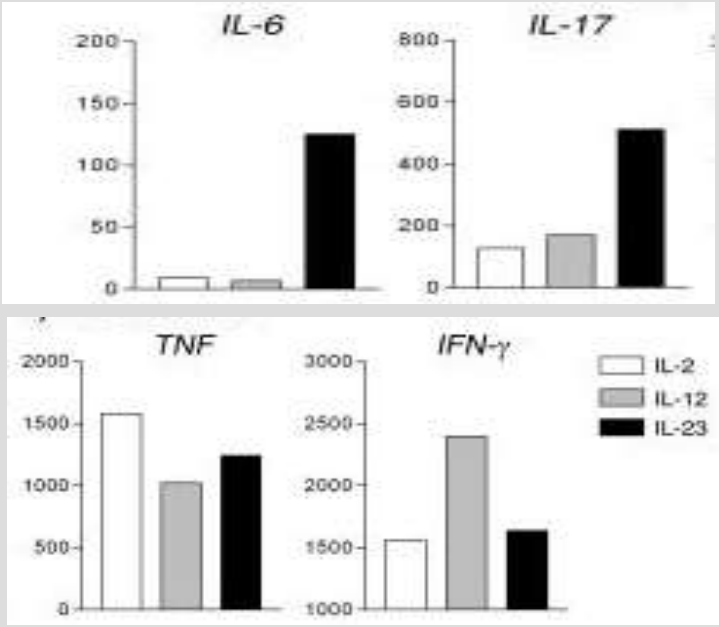
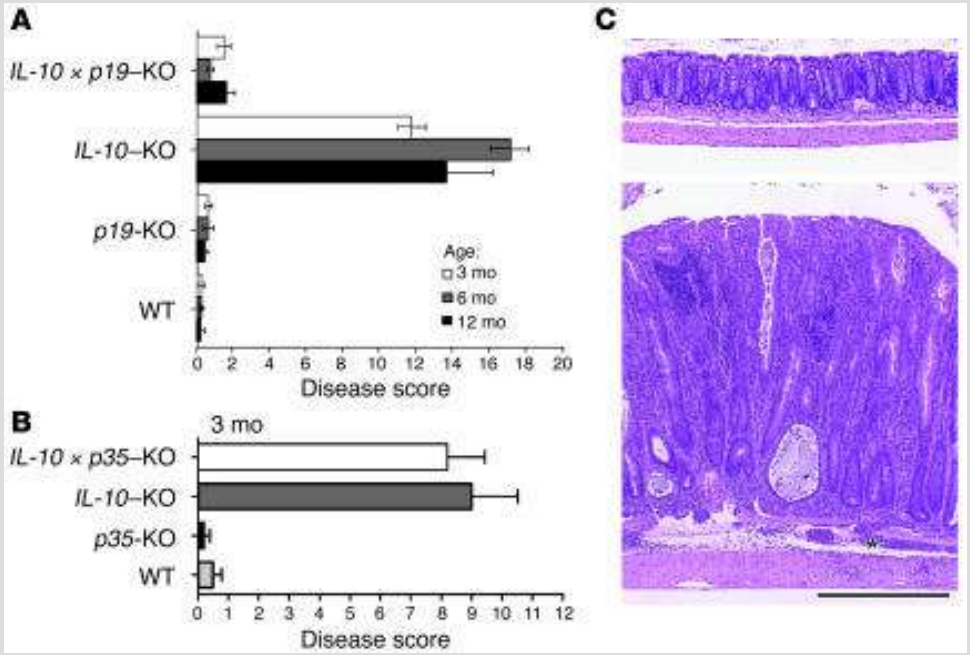
Th17 memory T-cells are induced by IL-23 and mediate intestinal inflammation in IL-10 KO mice model





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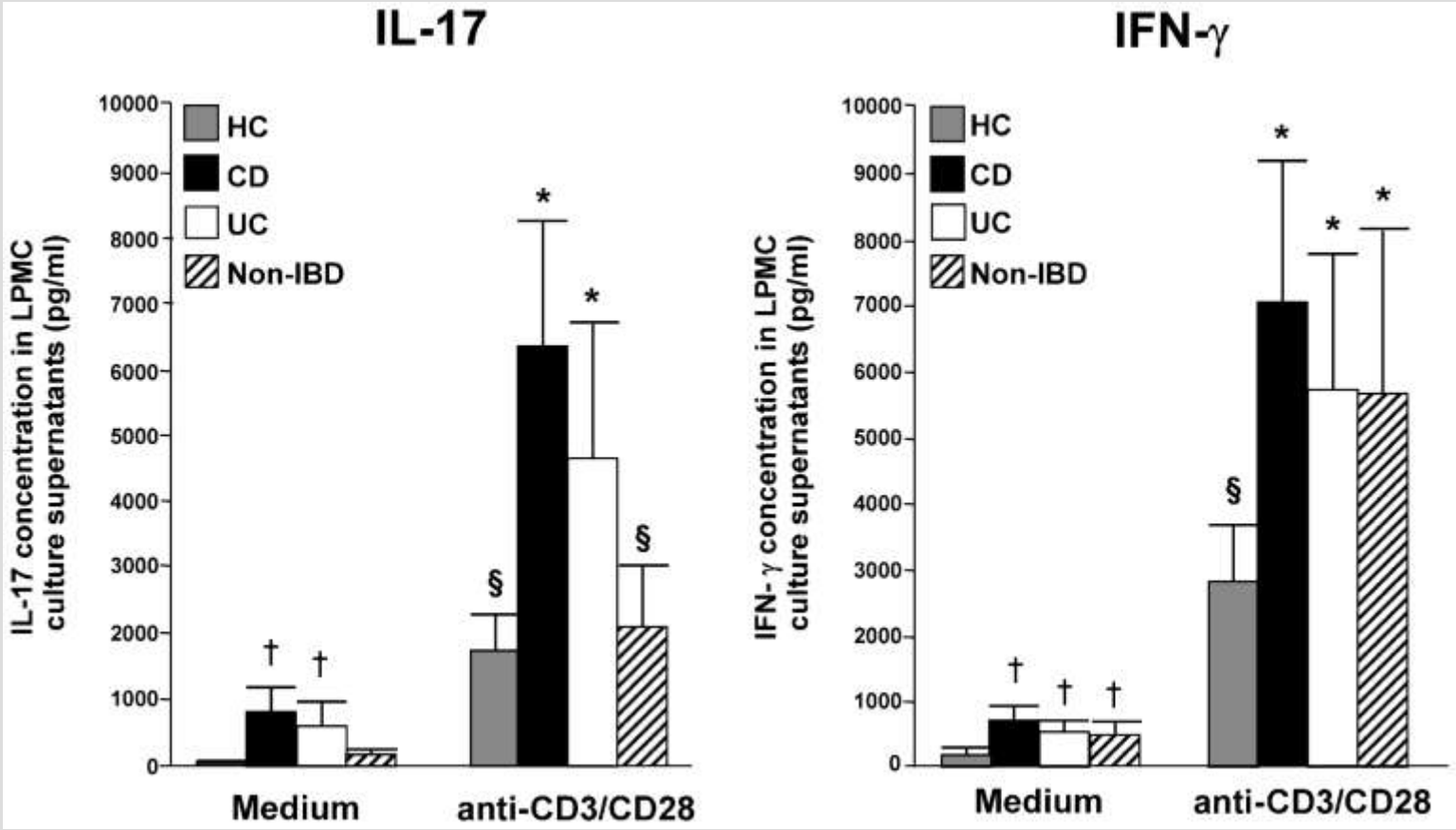
Secretion of IL17 by lamina propria mononuclear cells (LPMCs) is increased in patients with Crohn's disease (CD) & ulcerative colitis (UC) compared to non-IBD patients patients and healthy controls (HC)



Rovedatti L , Gut 2009

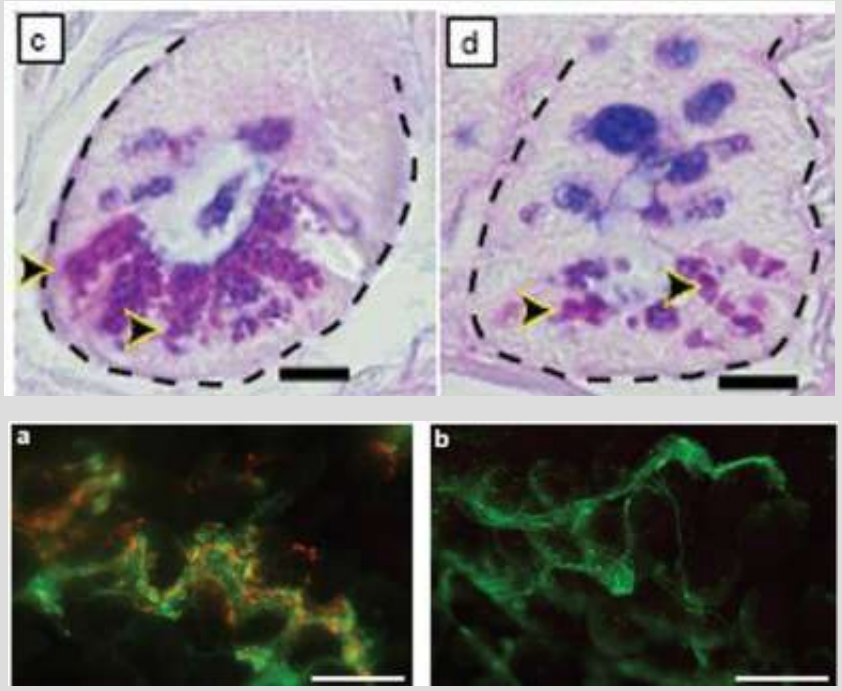
Defective Autophagy in ATG16L1 mutated mice & Crohn's patients results in disordered paneth cells and reduced lysozyme secretion

Wild type

ATG knock-out

Crohn's without ATG16L1 mutation

Crohn's with ATG16L1 mutation



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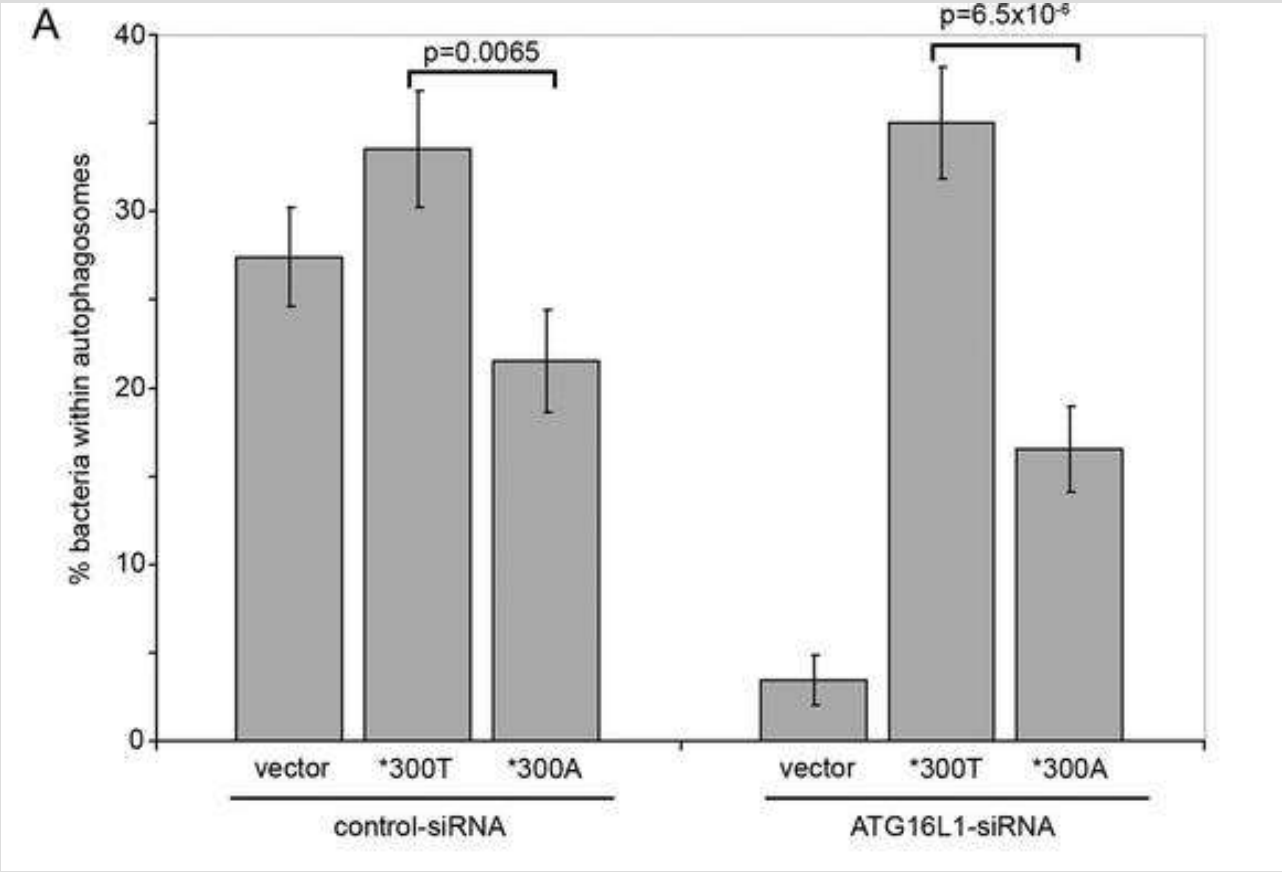
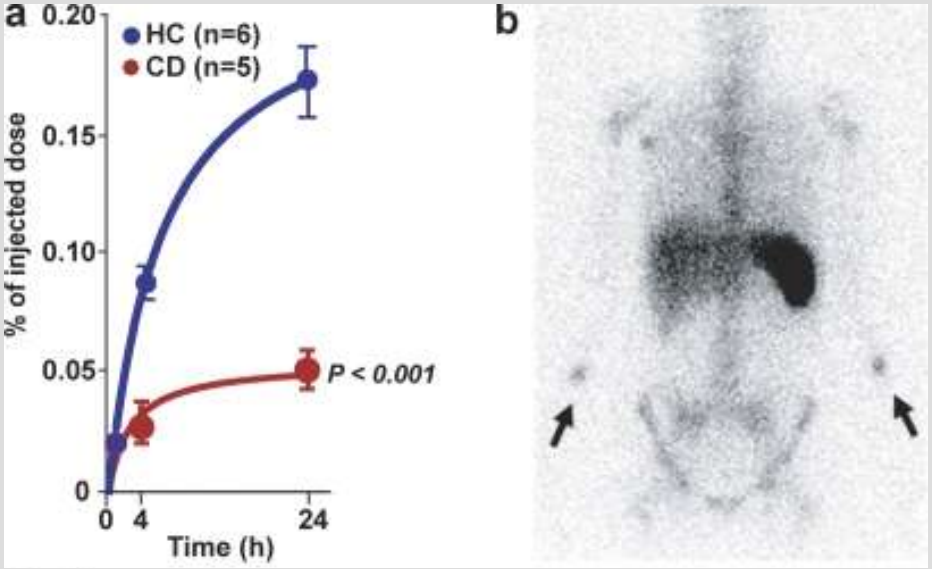
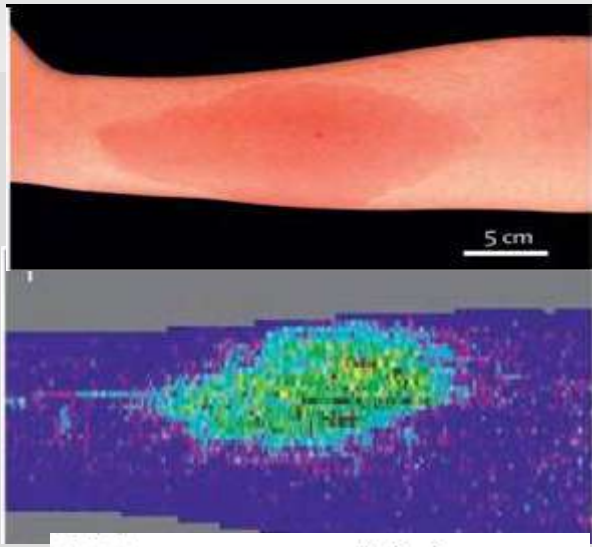
siRNA silencing of ATG16L1 results in reduced Salmonella Typhimurium internalization into autophagosome

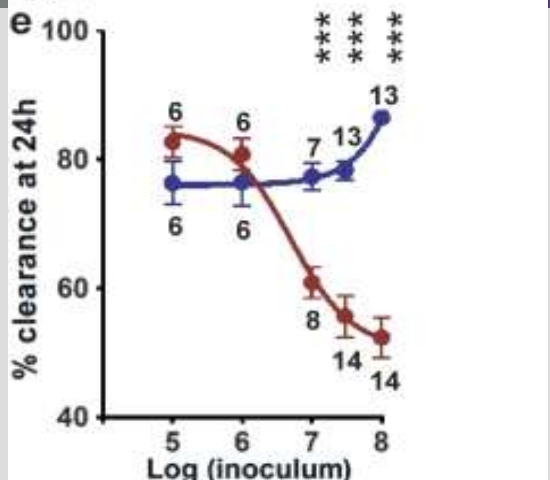


Kuballa P, PLoS One 2009

Crohn's patients in remission have decreased recruitment of PMN & reduced clearance of E.coli injected into forearm







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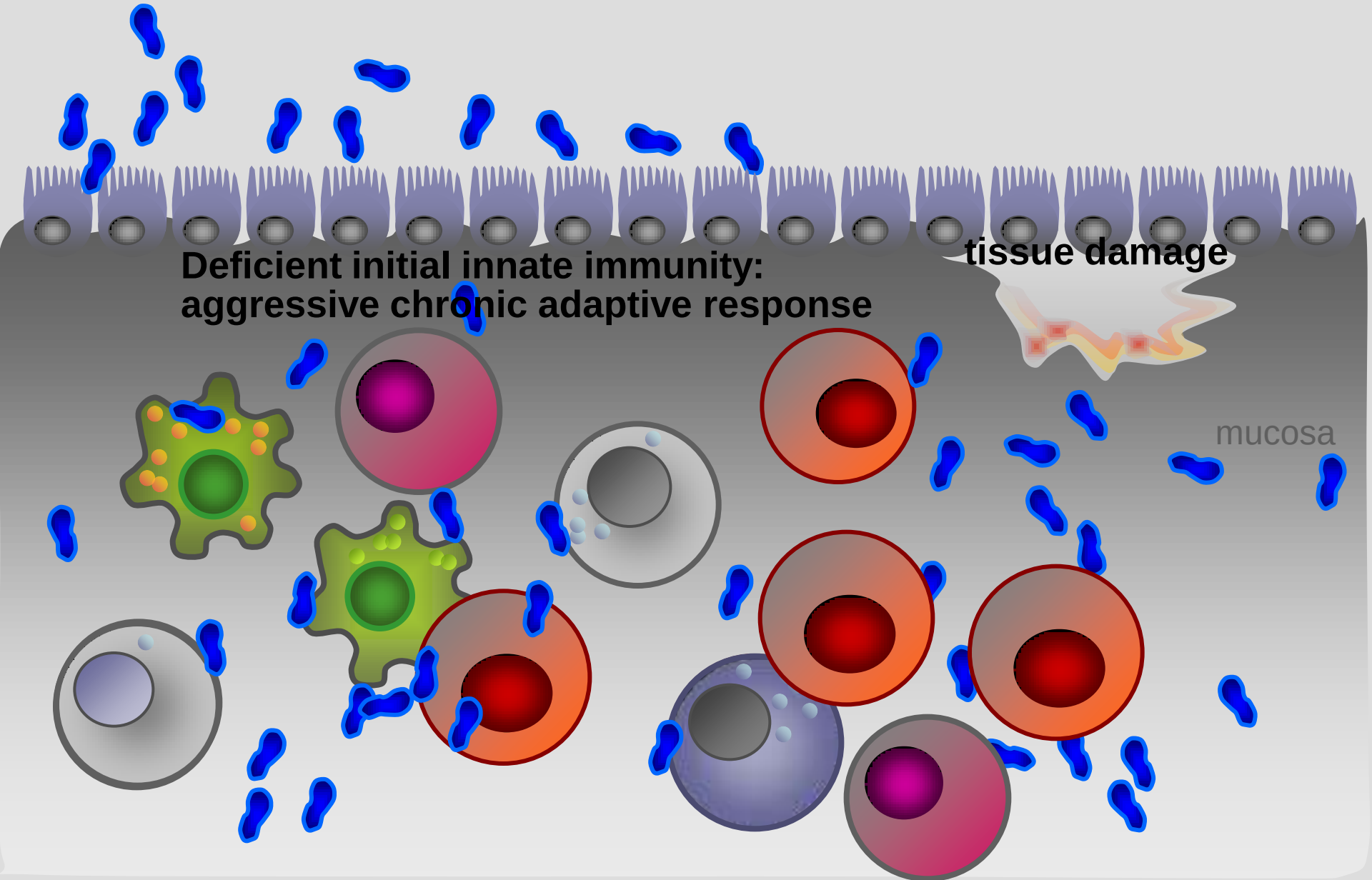
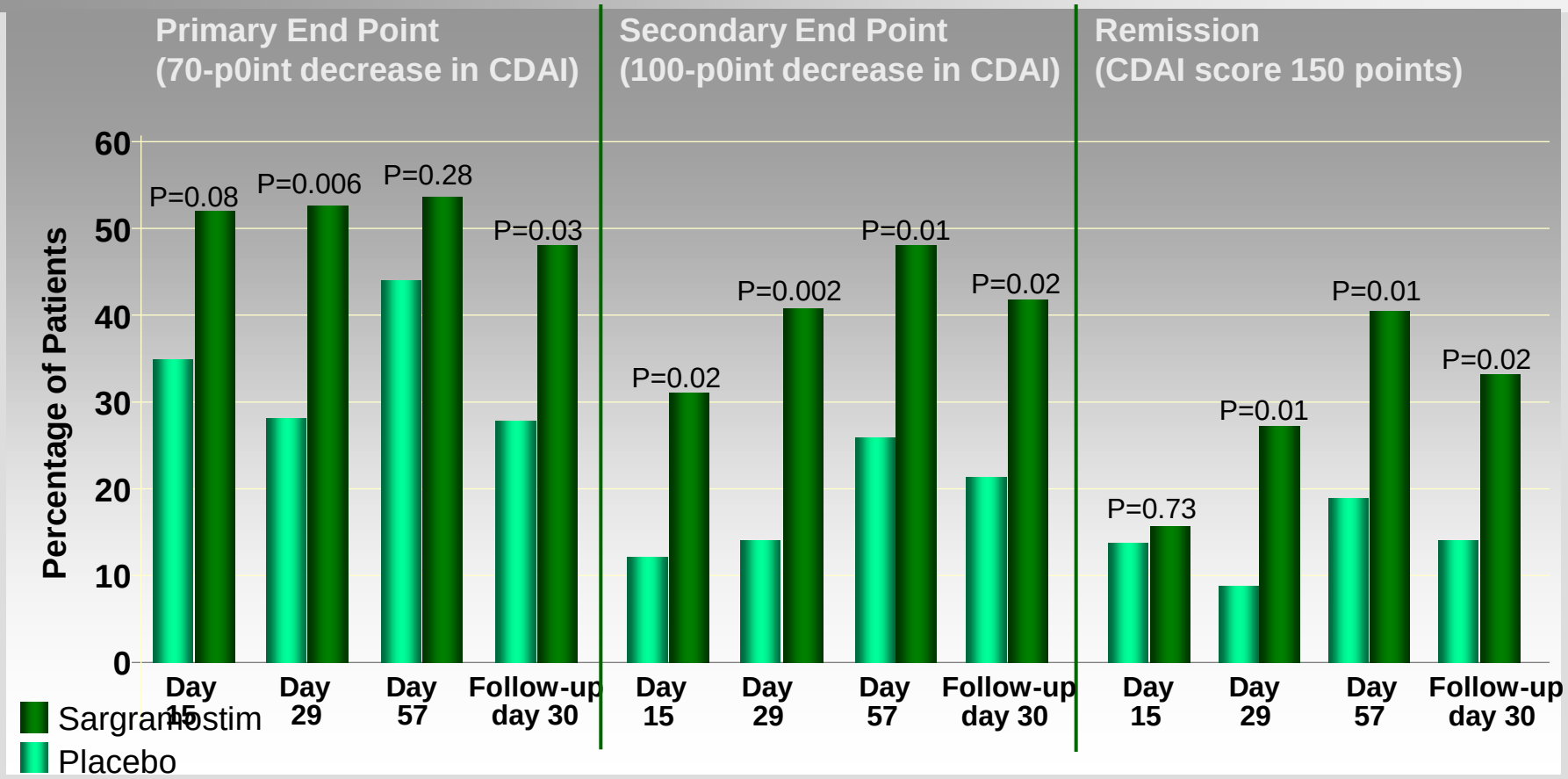
Deficient Innate Immunity in Crohn's disease ?

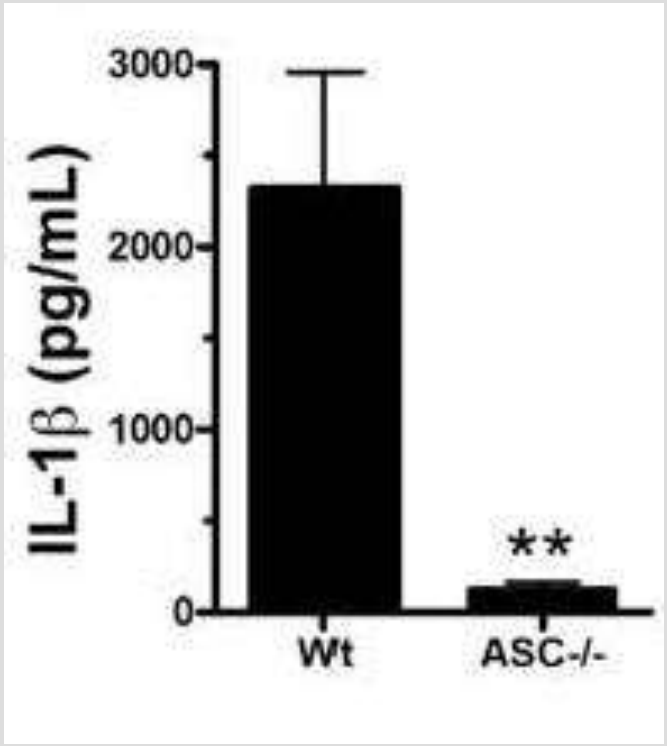


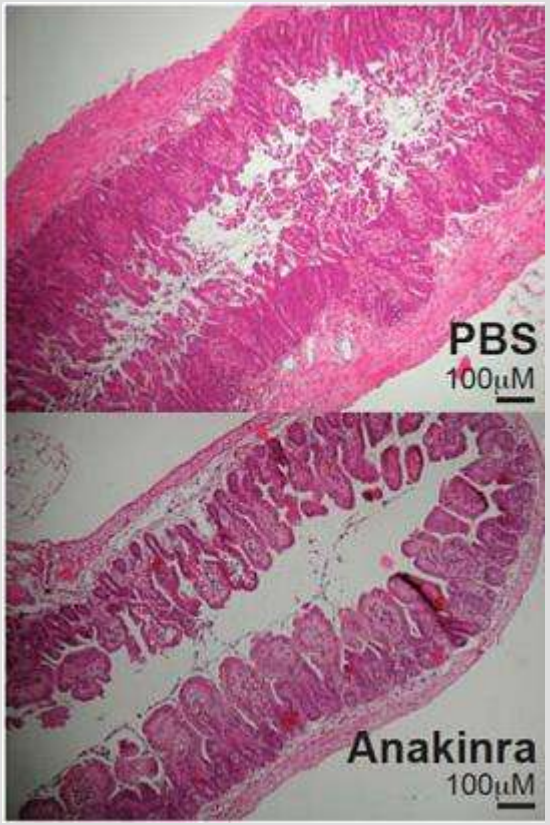
GM-CSF Confers Therapeutic Advantage in Crohn's Disease



Korzenik JR, N Engl J Med 2005

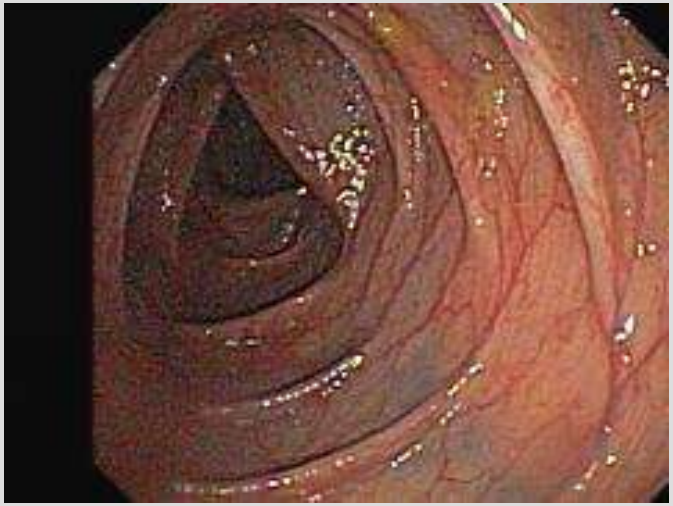
TcdA & TcdB trigger IL-1β secretion by NLRP intra-cellular inflammasome, and disease is reversed by IL-1β blockade



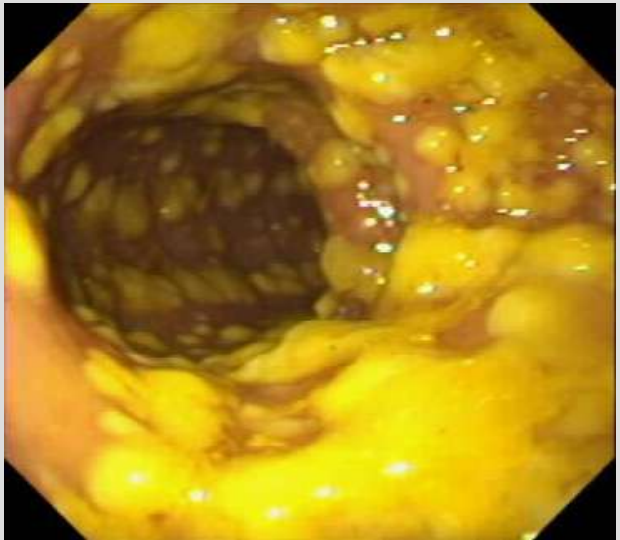


Ng J, Gastroenterology 2010

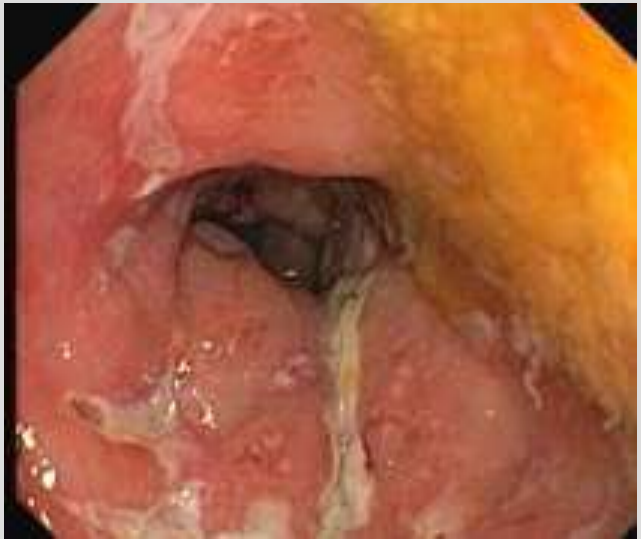
Transforming normal colon to inflammation



Immune in-adequacy



Immune hyper-reactivity



Thank you for your attention