Overview

1. "Basic" mechanisms of self-tolerance

- central tolerance
- peripheral tolerance

2. Mucosal immunology

- role of innate immunity in tolerance to foreign
- oral tolerance

3. Analyzing tolerance in clinical disease settings

- autoimmune disease vs. DTH reactions
- pathogenetic role of genetic and microbial factors
- where does tolerance fail and how can it be assessed?

2. Mucosal immunology

MALT: mucosa-associated lymphoid tissues

BALT: bronchi-associated lymphoid tissues

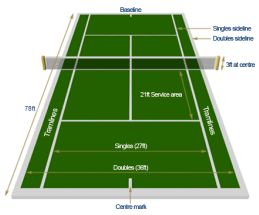
NALT: nasopharynx-associated lymphoid tissues

GALT: gut-associated-lymphoid tissues

MALT of the urogenital tract

- barriers between internal and external environments

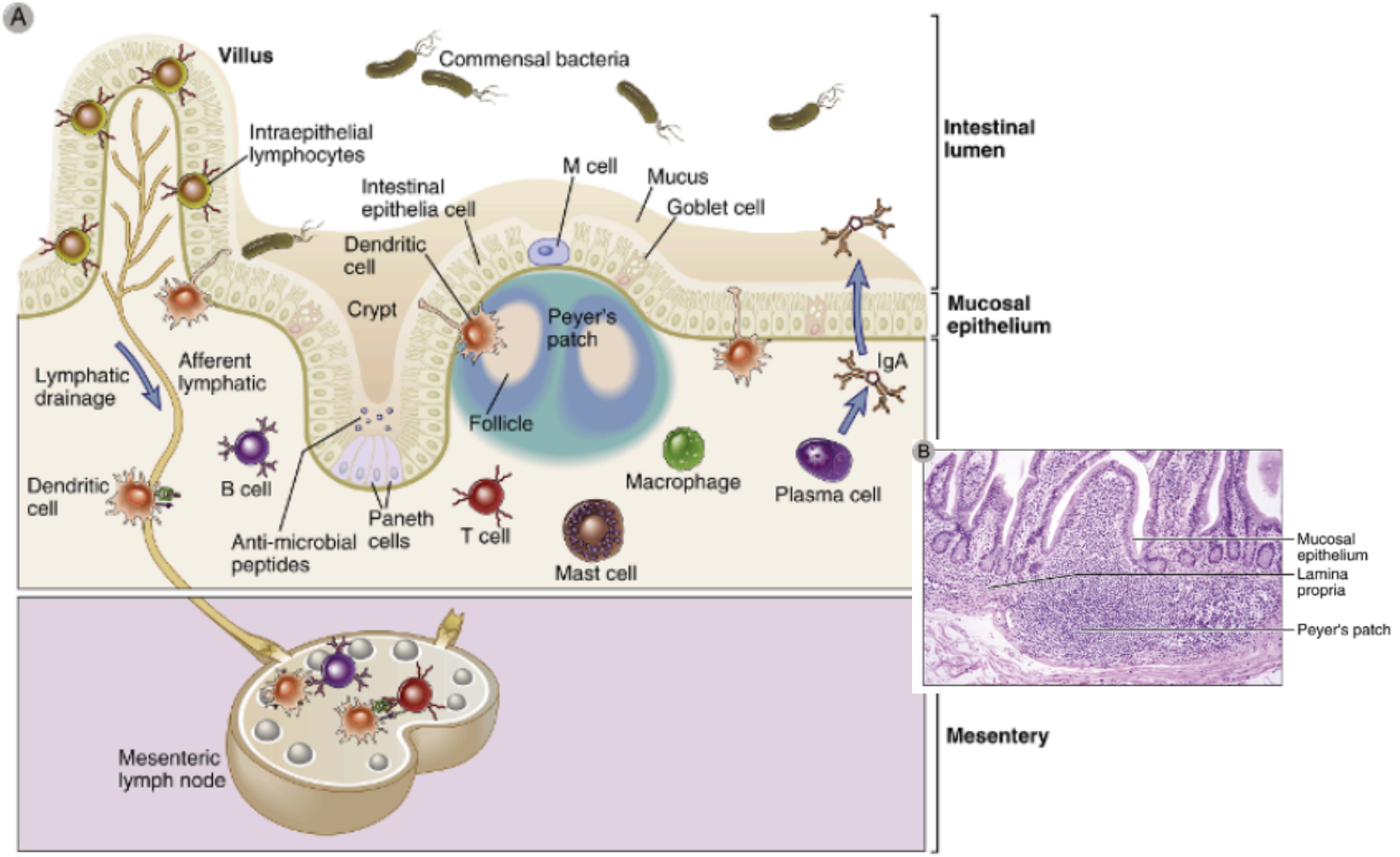
> 200 m^2



- confronted with a vast array of harmless and potentially harmful antigens

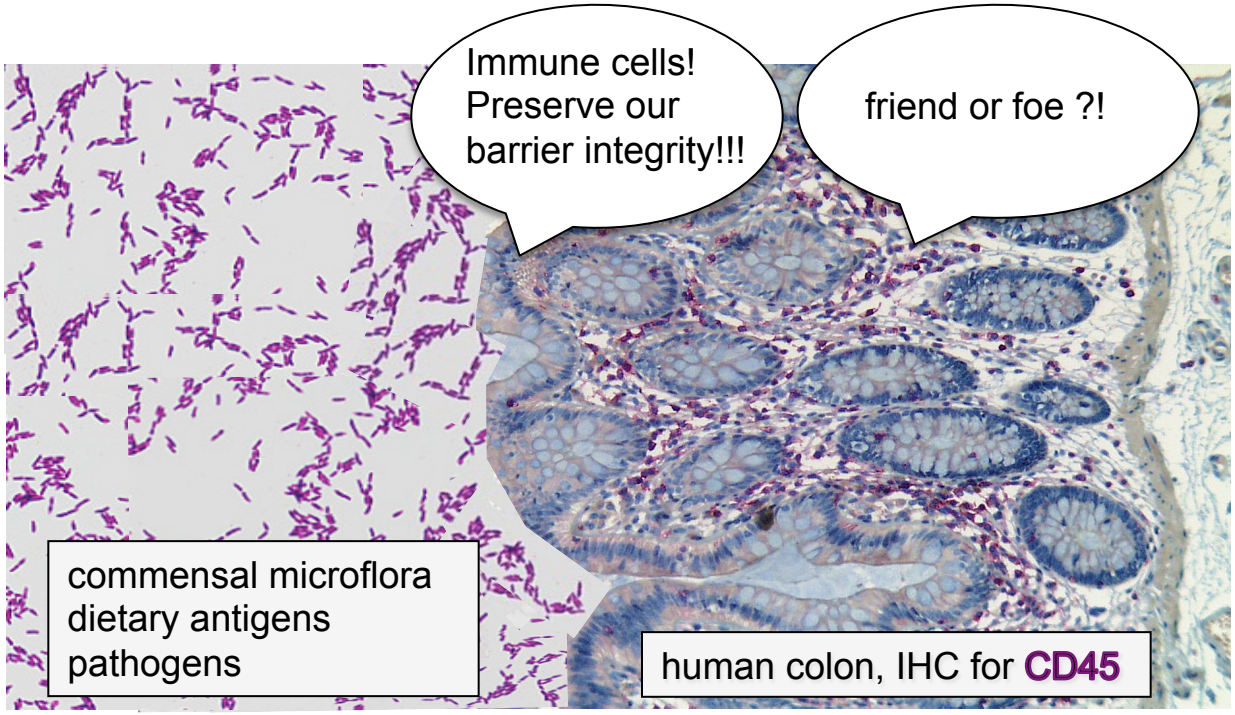
- contain lymphoid inductive and effector sites with specialized immune cells

Mucosal immunology: Organization of the GALT



Mucosal immunology: The difficult task of the GALT

…when two supersystems are confronted….



(abundant) commensal microbes and food antigens need to be tolerated while (rare) pathogens have to be recognised and fighted off

collateral tissue damage is a “no go” in order to prevent loss of sequestration and a potentially deleterious uncontrolled influx of antigens

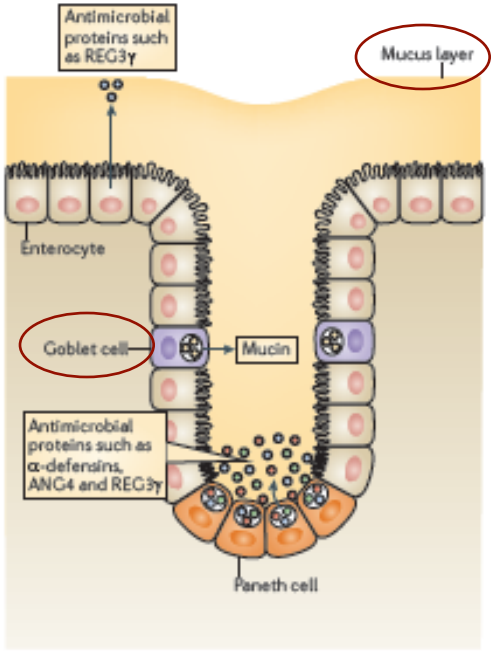
Mucosal immunology: Multi-layered adaptations of the GALT

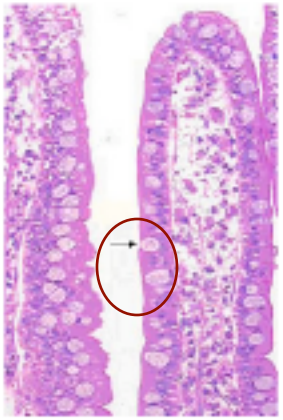
- ➢ intestinal epithelial cells
- • intestinal intraepithelial lymphocytes
- ➢ intestinal macrophages
- ➢ intestinal dendritic cells
- • intestinal innate lymphoid cells
- • intestinal B cells
- • intestinal CD4 T cells

tolerance mechanisms at the level of innate immunity also exist !

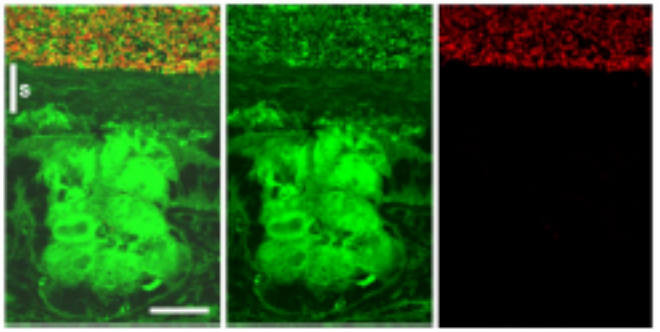
Multi-layered adaptations of the GALT: Intestinal epithelial cells

The first line of defense: the mucus layer





colon: two different mucus layers
the inner layer is devoid of bacteria !



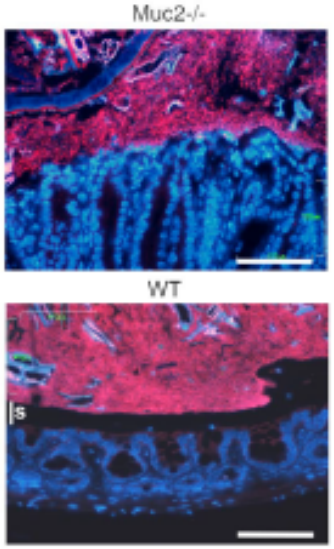
green: anti-MUC2
red: FISH using a bacterial probe

Johansson et al. PNAS 2008

functions of mucus layer:

- outer layer: providing optimal symbiotic habitat
- inner layer: segregation of bacteria,
  local concentration of antimicrobial peptides and IgA

- small intestine: delivery of tolerogenic functions to dendritic cells Cerutti et al. Science 2013



Johansson et al. PNAS 2008

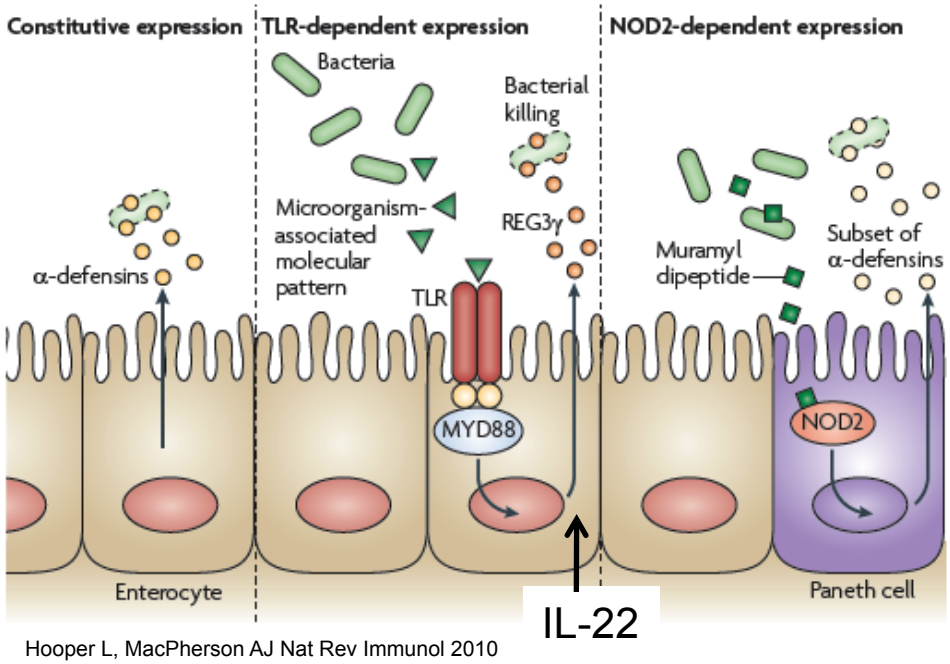
Muc2$^{-/-}$ mice:

penetrance of bacteria

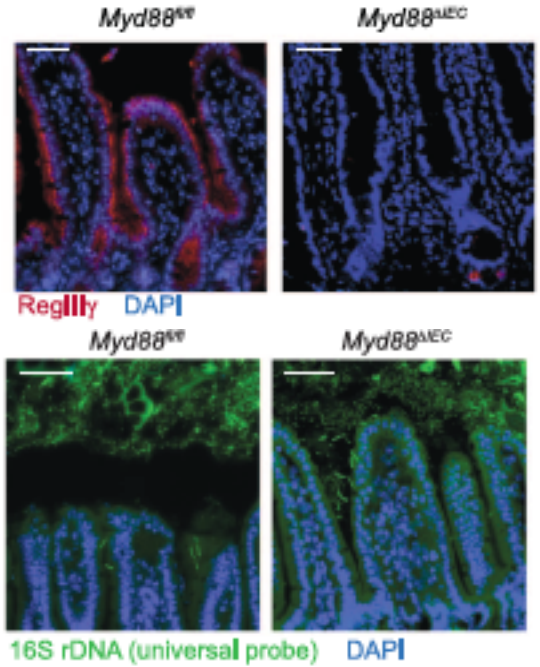
spontaneous colitis !

Multi-layered adaptations of the GALT: Intestinal epithelial cells

Production of anti-microbial proteins further limits bacterial-epithelial contact



Hooper L, MacPherson AJ Nat Rev Immunol 2010



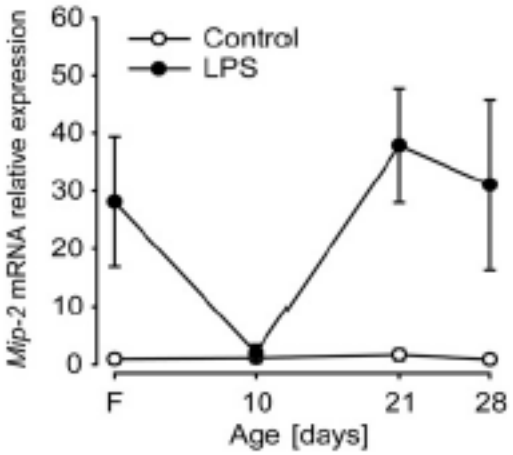
Vaishnava et al. Science 2011

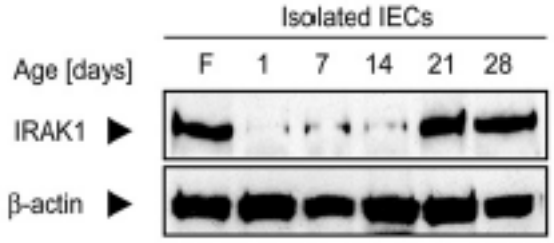
- defective anti-microbial peptide expression in experimental models (IL-22$^{-/-}$ or MyD88$^{-/-}$ mice): increased susceptibility to intestinal inflammation

- ATG16L1 is a disease susceptibility locus in Crohn's disease - disruption of ATG16L1 in mice results in a disturbed Paneth cell granule exocytosis

Multi-layered adaptations of the GALT: Intestinal epithelial cells

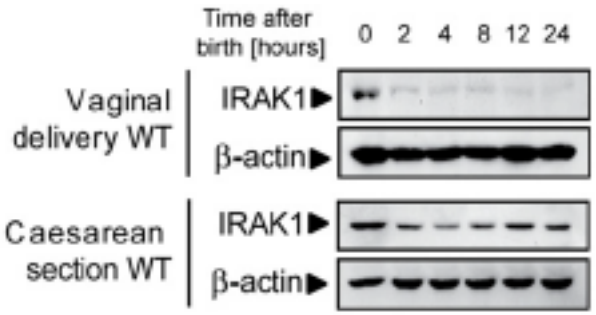
Highly regulated expression of TLRs during postnatal development:

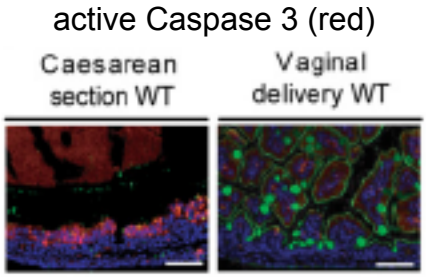




Chassin C et al. Cell 2010

immediately after birth,
IEC respond to LPS exposure
but then transiently acquire
endotoxin tolerance due to active
downregulation of IRAK1





lack of tolerisation of the
neonatal epithelium in
Caesarean section-born
mice results in massive
epithelial apoptosis after
oral exposure to E. Coli

first contact of neonatal IEC with colonizing bacteria is crucial to simultaneously allow for maturation of the intestinal immune system and to protect from inappropriate pro-inflammatory immune activation until vital structures have been established

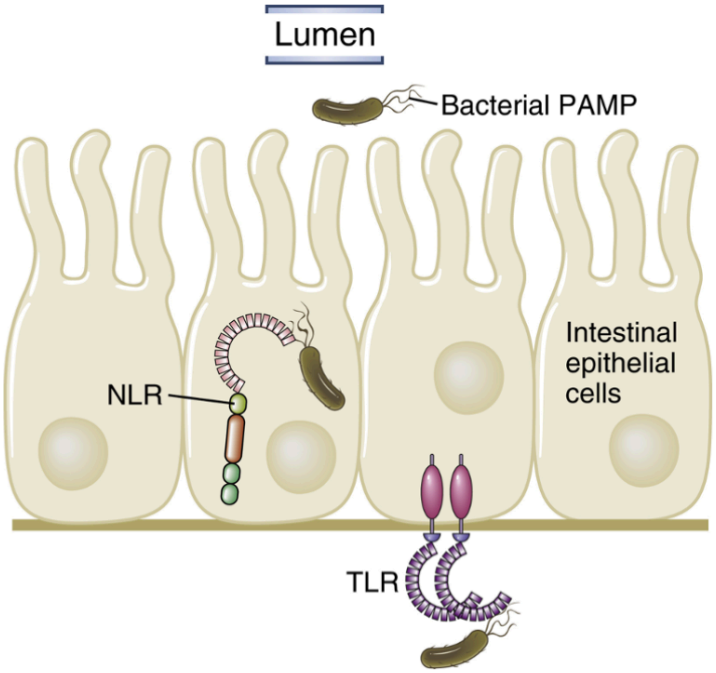
relevant in pathogenesis of necrotising enterocolitis in pre-term infants?

Multi-layered adaptations of the GALT: Intestinal epithelial cells

Highly regulated and compartmentalized expression of pattern recognition receptors also in mature enterocytes:

NLR family receptors for bacterial flagellin are located in the cytosol

TLR5 is only expressed on the basolateral, not the apical side



…allows for discrimination of commensal flora vs. invading pathogens!

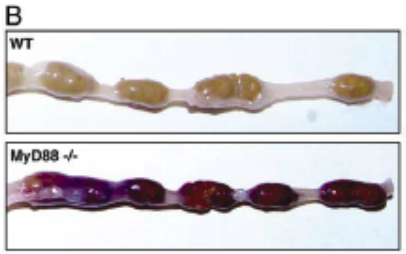
Multi-layered adaptations of the GALT: Intestinal epithelial cells

…however, “tonic” TLR signaling is vital for intestinal homeostasis!

Cell, Vol. 118, 229–241, July 23, 2004, Copyright ©2004 by Cell Press

Recognition of Commensal Microflora by Toll-Like Receptors Is Required for Intestinal Homeostasis

Seth Rakoff-Nahoum,[1] Justin Paglino,[2] Fatima Eslami-Varzaneh,[3] Stephen Edberg,[2] and Ruslan Medzhitov[1,*]



MyD88$^{-/-}$ mice suffer from an exaggerated DSS-induced colitis due to a lack of microbial TLR-induced tissue protective factors (and anti-microbial peptides) in the intestinal epithelium

NATURE|Vol 446|29 March 2007

Epithelial NEMO links innate immunity to chronic intestinal inflammation

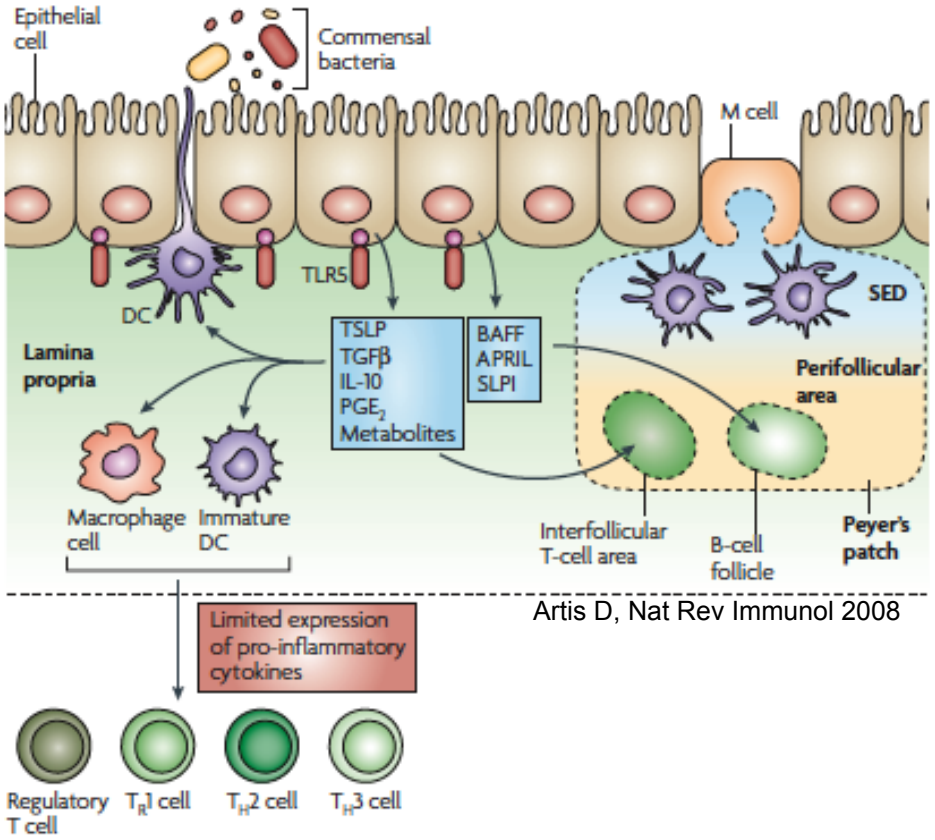
(Nemo = IκB kinase γ)

Arianna Nenci[1,2]*, Christoph Becker[3]*, Andy Wullaert[1], Ralph Gareus[1], Geert van Loo[2], Silvio Danese[4], Marion Huth[2], Alexei Nikolaev[3], Clemens Neufert[3], Blair Madison[5], Deborah Gumucio[5], Markus F. Neurath[3]* & Manolis Pasparakis[1,2]

Mice with an epithelial-specific inhibition of NFkB develop spontaneous intestinal inflammation due to impaired antimicrobial peptide expression, increased epithelial cell apoptosis and increased translocation of bacteria

Multi-layered adaptations of the GALT: Intestinal epithelial cells

by the production of a variety of cytokines and other factors, the intestinal epithelium can actively modulate immune responses and “educate” local immune cells towards tolerance



Artis D, Nat Rev Immunol 2008

e.g. production of:

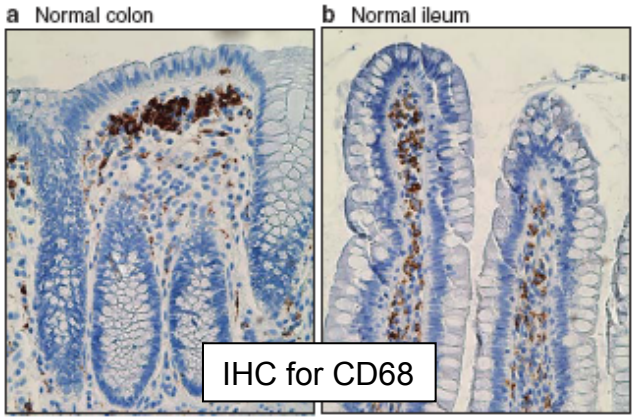
TGFβ
IL-10
TSLP
retinoic acid
April
BAFF

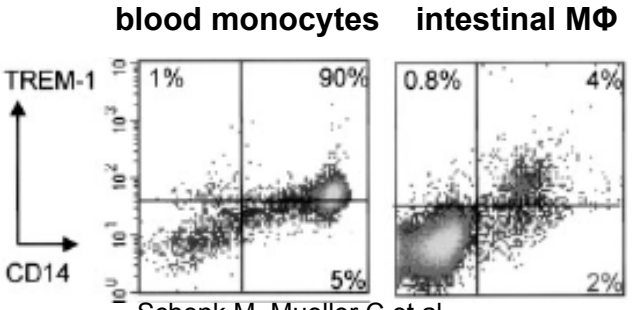
modulation / conditioning of DC

bias of Th differentiation into Th2 / T_{reg}

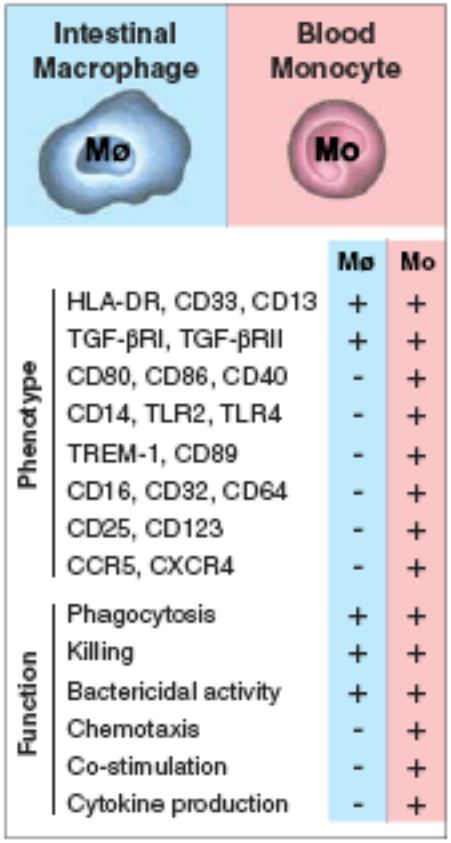
promotion of B cell IgA switching

Multi-layered adaptations of the GALT: Intestinal macrophages

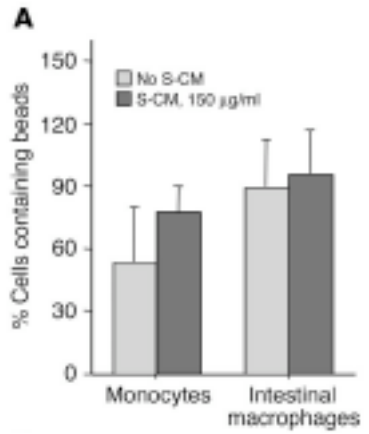




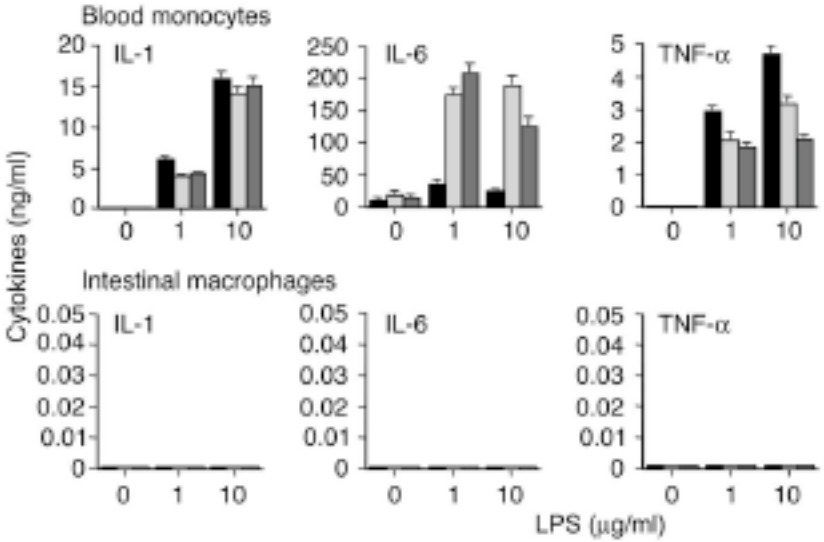
Schenk M, Mueller C et al.,
J Immunol 2005



Weber B, Mueller C et al.
Sem Immunopathol 2009



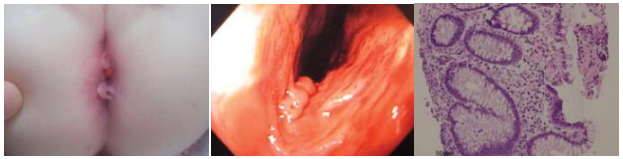
Smythies et al., JCI 2005



…are continuosly replenished from blood monocytes and educated by the local tissue environment (e.g. IL-10, TGFβ) to acquire the typical properties of resident intestinal Mϕ: IL-10 production, highly efficient phagocytosis and bacterial killing in the absence of pro-inflammatory cytokine secretion!

Multi-layered adaptations of the GALT: Intestinal macrophages

mutations in the human IL-10R gene: very-early-onset colitis



Moran CJ, Inflamm Bowel Dis 2013

IL-10-deficient (IL-10$^{-/-}$) mice: spontaneous intestinal inflammation

Interleukin-10 Receptor Signaling in Innate Immune Cells Regulates Mucosal Immune Tolerance and Anti-Inflammatory Macrophage Function

Dror S. Shouval,[1,2,25] Amlan Biswas,[1,2,23] Jeremy A. Goettel,[1,2,23] Katelyn McCann,[1,23] Evan Conaway,[3] Naresh S. Redhu,[1,2,23] Ivan D. Mascanfroni,[4] Ziad Al Adham,[5] Sydney Lavoie,[1] Mouna Ibourk,[1] Deanna D. Nguyen,[6,7] Janneke N. Samsom,[8,23] Johanna C. Escher,[9,23] Raz Somech,[10,12,23] Batia Weiss,[11,12,23] Rita Beier,[13,23] Laurie S. Conklin,[14,23] Christen L. Ebens,[15,23] Fernanda G.M.S. Santos,[16,23] Alexandre R. Ferreira,[16,23] Mary Sherlock,[17,23] Atul K. Bhan,[18,19] Werner Müller,[20] J. Rodrigo Mora,[6,7] Francisco J. Quintana,[4] Christoph Klein,[21,23] Aleixo M. Muise,[5,23] Bruce H. Horwitz,[2,3,23] and Scott B. Snapper[1,7,22,23,*]

Macrophage-Restricted Interleukin-10 Receptor Deficiency, but Not IL-10 Deficiency, Causes Severe Spontaneous Colitis

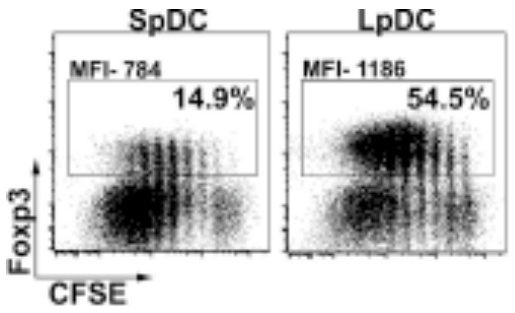
Ehud Zigmond,[1,2] Biana Bernshtein,[1] Gilgi Friedlander,[3] Catherine R. Walker,[4] Simon Yona,[1] Ki-Wook Kim,[1] Ori Brenner,[5] Rita Krauthgamer,[1] Chen Varol,[2] Werner Müller,[4] and Steffen Jung[1,*]

Immunity 2014

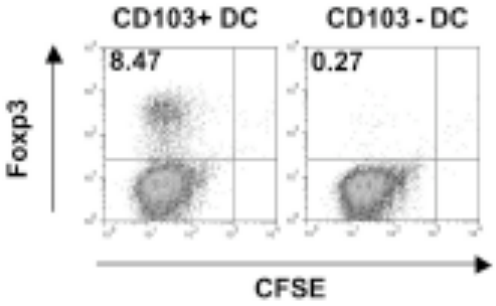
lack of IL-10 receptor signaling in intestinal macrophages prevents their anti-inflammatory conditioning & leads to intestinal inflammation

Multi-layered adaptations of the GALT: Intestinal dendritic cells

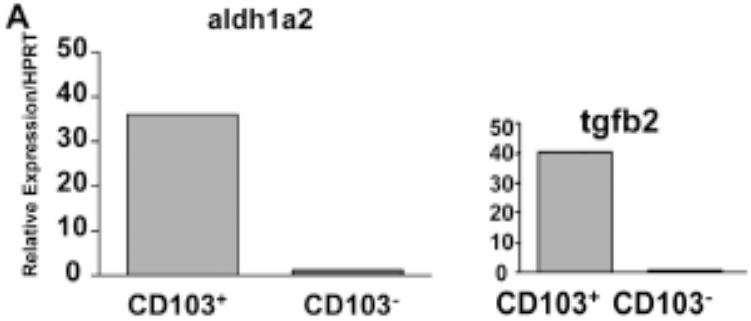
- DC in Lamina Propria (LP), Mesenteric Lymph nodes (MLN), Peyer's patches (PP)
- various subsets, two main subsets: $CD103^+$ vs. $CD103^-$ DC



LP-DC efficient inducers of $Foxp3^+$ T_{regs}



capacity lies within the $CD103^+$ subset...



…is associated with production of TGFβ and the vitamin A-derivative retinoic acid

intestinal $CD103^+$ DC have a propensity to induce $Foxp3^+$ T_{regs} and are critically required for induction of oral tolerance

Sun et al., JEM 2007
Coombes et al., JEM 2007

Mucosal immunology: Oral tolerance

The phenomen:

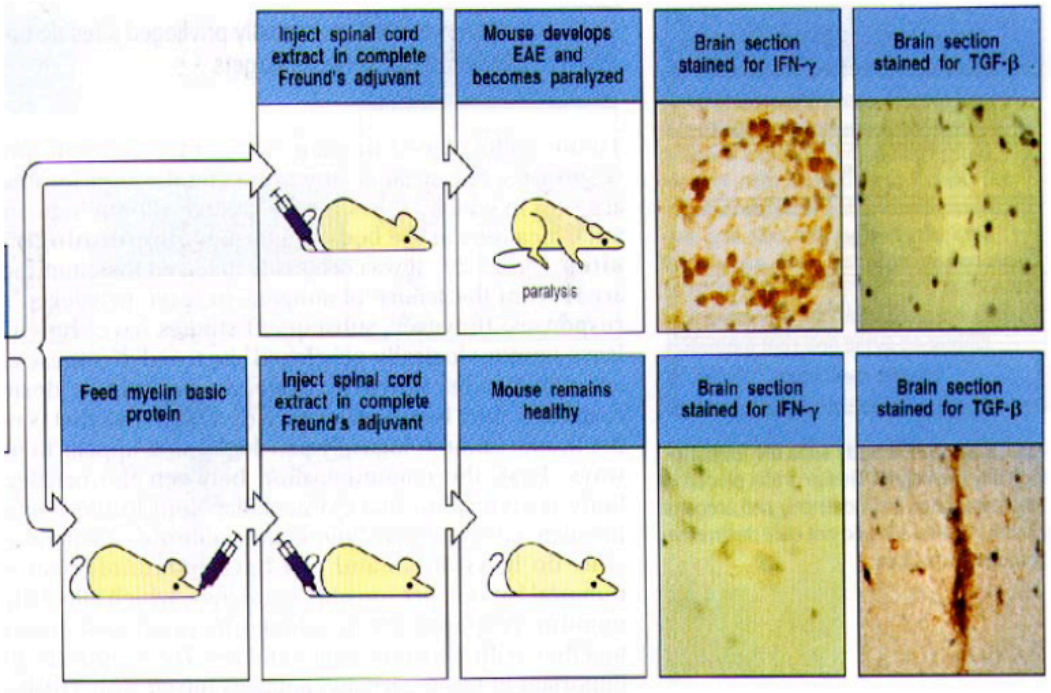
Oral administration of antigen can lead to suppressed immune responses to peripheral challenge with the same antigen

SUPPRESSION OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS BY ORAL ADMINISTRATION OF MYELIN BASIC PROTEIN AND ITS FRAGMENTS[1]

PAUL J. HIGGINS AND HOWARD L. WEINER[2]

THE JOURNAL OF IMMUNOLOGY

Vol. 140, 440–445, No. 2, January 15, 1988
Printed in U.S.A.



Janeway / Travers

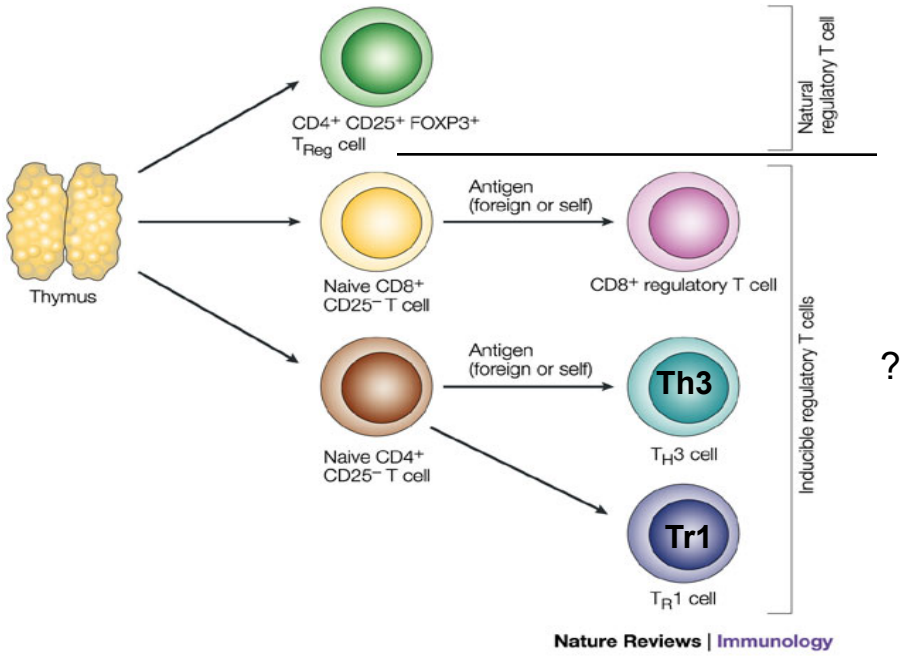
tolerance can be transferred by T cells

TGFβ appears to be involved

Mucosal immunology: Oral tolerance

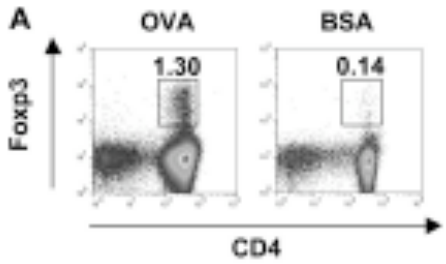
The (likely) mechanism:

- (clonal deletion)
- induction of regulatory T cells



?

	induction	cytokine
nT_{reg} (Foxp3+)	IL-2, TGFβ	TGFβ, IL-10, cytokine-independent suppression ?
Th3 (Foxp3+)	TGFβ	TGFβ, IL-10, IL-4
Tr1 (Foxp3-)	IL-10	IL-10, TGFβ, IFNγ
iT_{reg} (Foxp3+)	TGFβ, IL-2, retinoic acid	TGFβ, IL-10,… cytokine-independent suppression ?



feeding of ovalbumin (but not BSA) to Ovalbumin-TCR tg mice (lacking endogenous Tregs) *de novo* induces Foxp3+ CD4+ T_{regs} cells in the MLN Coombes et al., J Exp Med 2007

Mucosal immunology: Oral tolerance

Evidence exists that oral tolerance mediated by T_{regs} is operative in humans

Journal of Immunology, 1994, 152:

Oral Tolerance in Humans

T Cell but Not B Cell Tolerance After Antigen Feeding[1]

Steffen Husby,[2*] Jiri Mestecky,[†] Zina Moldoveanu,[†] Stephen Holland,[*] and Charles O. Elson[3*]

Table 1. *Delayed skin test response to KLH*[a]

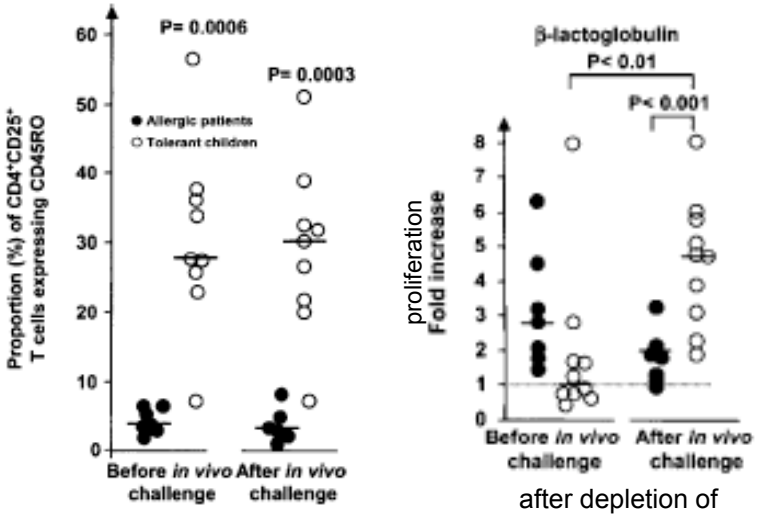
Group	24 h Mean (range)	24 h Number of positive/total	48 h Mean (range)	48 h Number of positive/total
KLH-fed	1.2 (0–10)	1/8	0 (0–0)	0/8
Controls	11.9 (0–23)	7/8	6.6 (0–20)	5/8
p-value	0.007		0.038	

[a] KLH (10 μg) was injected intracutaneously and the reaction measured as induration (mm) at 24 and 48 h after the injection.

J. Exp. Med. Volume 199, Number 12, June 21, 2004

Allergen-responsive $CD4^+CD25^+$ Regulatory T Cells in Children who Have Outgrown Cow's Milk Allergy

Malin R. Karlsson,[1] Jarle Rugtveit,[1,2] and Per Brandtzaeg[1]



after depletion of CD4+ CD25+

GASTROENTEROLOGY 2007;132:1705–1717

Severe Food Allergy as a Variant of IPEX Syndrome Caused by a Deletion in a Noncoding Region of the *FOXP3* Gene

TROY R. TORGERSON,[*] AVRIEL LINANE,[*] NICOLETTE MOES,[‡,§] STEPHANIE ANOVER,[*] VÉRONIQUE MATEO,[‖] FRÉDÉRIC RIEUX-LAUCAT,[‖] OLIVIER HERMINE,[¶] SHASHI VIJAY,[*] ELEONORA GAMBINERI,[#] NADINE CERF-BENSUSSAN,[§] ALAIN FISCHER,[‖,**] HANS D. OCHS,[*] OLIVIER GOULET,[‡,§] and FRANK M. RUEMMELE[‡,§]

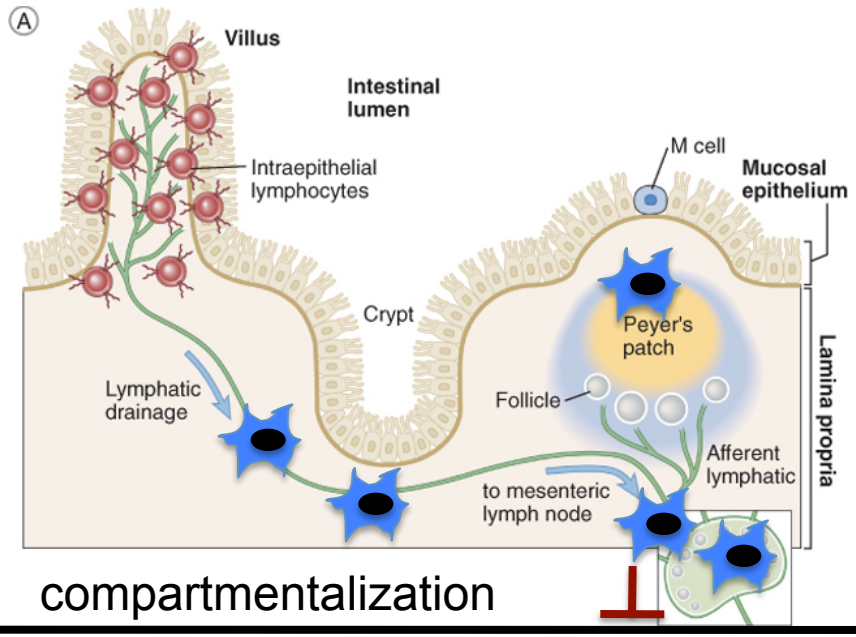
Mucosal immunology: Oral (mucosal) tolerance

Allergen-specific immunotherapy / desensitization

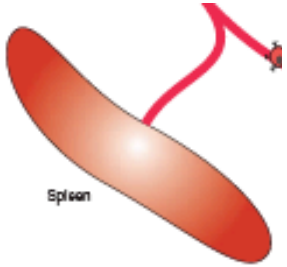
repetitive administration of antigens via the oral, sublingual (nasal) route or the skin

inhibition of basophil and mast cell activation
skewing of Th2 cells to allergen-specific T_{regs}
shift from IgE to IgG4

Mucosal immunology: Oral tolerance does not apply to commensal flora



the mesenteric fire wall:



mucosal DC loaded with commensal bacteria do not traffic beyond the mesenteric lymph node!

MacPherson et al. Nat Rev Immunol 2004

the systemic immune system is ignorant, rather than tolerant of the intestinal commensal flora!