(1) Mucosal barrier of the intestine: organisation and role. (2) Mucosal innate immune arsenal: antimicrobial peptides (AMPs).

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Master 2 I²VB: Infectiologie Immunité Vaccinologie Biomédicaments
Master 2 IDOH: Infectious Diseases and One Health

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Mucosal barrier of the intestine: organisation and role

Mucosal innate immune arsenal: antimicrobial peptides (AMPs)

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Mucosal barrier of the intestine: organisation and role

- Morphology
- Histology
- Epithelial cells
- Barrier function
- Survey: sampling, control, information and reaction
- Oral tolerance
- Balance: homeostasis

Mucosal barrier of the intestine : morphology

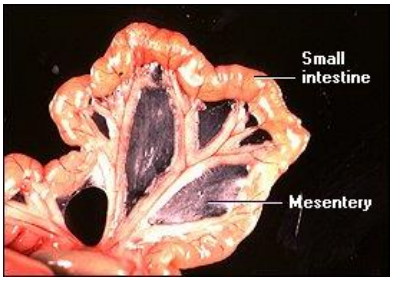
The intestinal tract

- Enormous surface of exchanges: the small intestine exhibits numerous folds (convolutions), and luminal face (internal) of is covered of villi.
- More than 300m^2 of surface in adults (more than a tennis court!).
- In contact with an abundant and complex microbiota : about 10^{14} bacteria (including 10^{12} bact/g of feces in the colon); nearly 1000 bacterial species per individual (metagenomics of the human microbiota)[1].
- Two parts: small intestine (proximal or anterior) and large intestine (distal or posterior).

1 Lepage P. *et al*, 2013, Gut, 62:146–158.

Mucosal barrier of the intestine : morphology

The intestinal tract



- The small intestine: longest part of the tract. Three segments from the stomach to the large intestine

Duodenum (receiving pancreatic, hepatic and bile secretions)

Jejunum (40 to 90% in length according to species)

Ileum (at the junction to the large intestine)

- Approximately 3.5 fold the length of the body (about 6m in human).
- Location of the final steps of the digestion, allowing absorption of nutriments in the blood circulation.
- The large intestine: colon and rectum, storage of undigested elements (feces).

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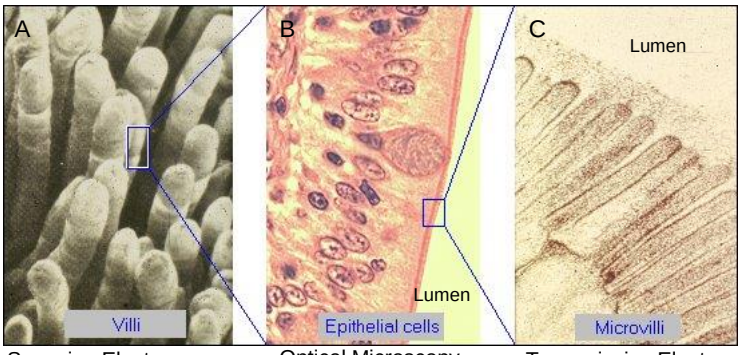
Mucosal barrier of the intestine : histology

At the luminal surface of the intestine



Scanning Electron microscopy | Optical Microscopy (H/E staining) | Transmission Electron Microscopy

Villi: Tissue protrusions towards the lumen (A), covered by epithelial cells (B), themselves protruding microvilli towards the lumen (C).

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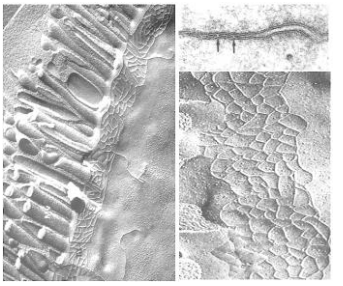
Mucosal barrier of the intestine : histology

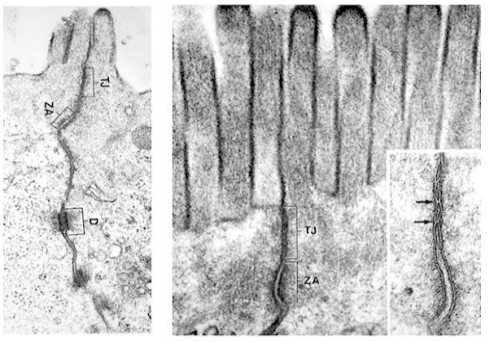
Structure of intestinal epithelial cells

Cohesion and imperviousness: performed by special intercellular junctions

- Tight junctions (TJ)
- Zonula adherens ZA)
- Desmosomes (D)



Cryo -Electron Microscopy



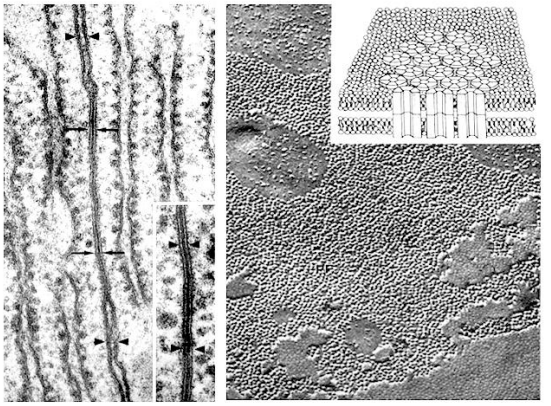
Transmission Electron Microscopy

7

Mucosal barrier of the intestine : histology

Structure of intestinal epithelial cells

Connections between cells performed by specialised intercellular junctions: gap junctions (tubular protein polymers)



Transmission of molecular messages between the cells. Synchronized cells...

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Mucosal barriers : histology

Comparison of epithelial cells between mucosae

Three main morphological types of epithelial cells, according to the mucosae

- Squamous: flat, in alternate stacks. Constitute the sheath of the skin (epiderma) and of internal cavities (mouth, lungs).
- Cuboidal: round-shaped, subject to deformations and ensuring an elastic layer. Bordering the bladder and organs able to retain fluids.
- Columnar: narrow and high cells in single layer. Secretory and absorbing. Bordering the gastro-intestinal tract and forming the ciliated layer of the respiratory tract.

Three types of assembling:

Simples, stratified, pseudo-stratified.

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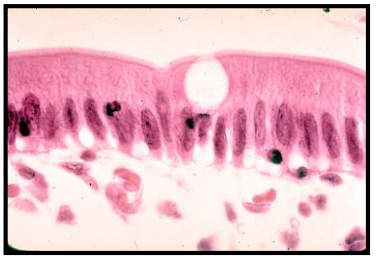
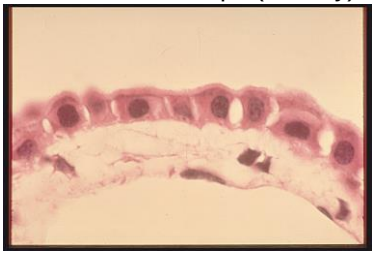
Mucosal barriers : histology

Comparison of epithelial cells between mucosae

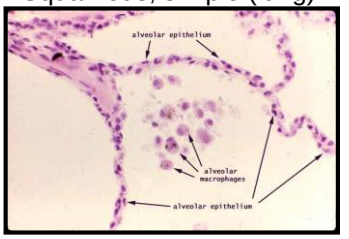
- Columnar, simple (gut)



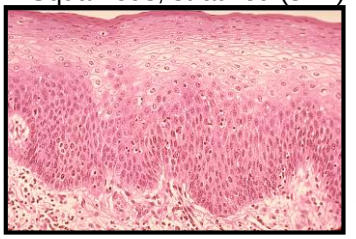
- Cuboidal, simple(kidney)



- Squamous, simple (lung)



- Squamous, stratified (skin)



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Mucosal barriers : epithelial cells

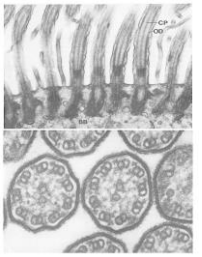
Epithelial cells

- Intestine, 6 types of epithelial cell:

- Enterocytes → absorption
- Enteroendocrine cells → hormone secretion
- Paneth cells → antimicrobial peptide secretion
- tuft cells → taste-chemosensory response
- goblet cells → mucus production
- microfold (M) cells → antigen sampling

- Lungs: ciliated, Goblet cells

Structure of cilia observed by transmission electron microscopy.



Longitudinal section: basal bodies at the basis of each cilium are formed by centrioles.

Transversal section: 9 doublets of microtubules and 2 central singlets constitute the architecture each cilium.

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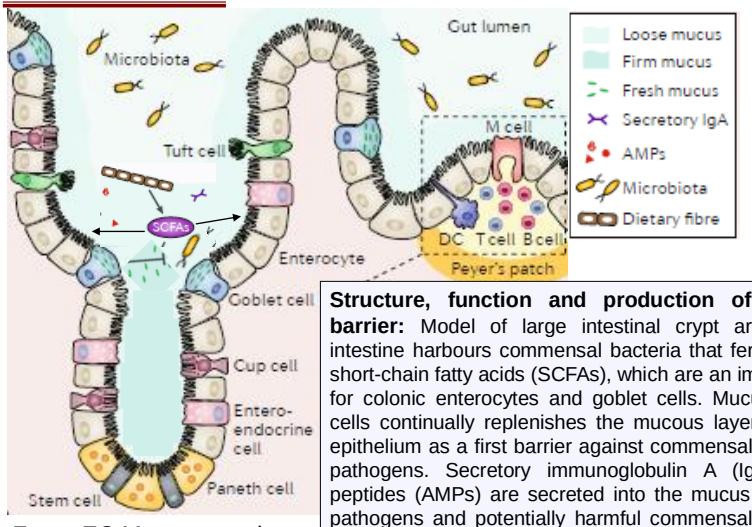
Mucosal barrier of the intestine : epithelial cells

Epithelial cells



From: EC Martens *et al*, Nature Reviews Microbiology (16) 2018.

Structure, function and production of the gut mucosal barrier: Model of large intestinal crypt architecture. The large intestine harbours commensal bacteria that ferment dietary fibre into short-chain fatty acids (SCFAs), which are an important energy source for colonic enterocytes and goblet cells. Mucus secreted by goblet cells continually replenishes the mucous layer that overlies the gut epithelium as a first barrier against commensal bacteria and invading pathogens. Secretory immunoglobulin A (IgA) and antimicrobial peptides (AMPs) are secreted into the mucus as a defence against pathogens and potentially harmful commensal bacteria. The colonic epithelium consists of enteroendocrine cells, tuft cells, cup cells, goblet cells and sentinel goblet cells, which are located at the crypt entrance to reduce bacterial encroachment into the crypt.

Mucosal barrier of the intestine : epithelial cells

Intestinal epithelial cells

- Polarized

Apical pole: facing intestinal lumen.

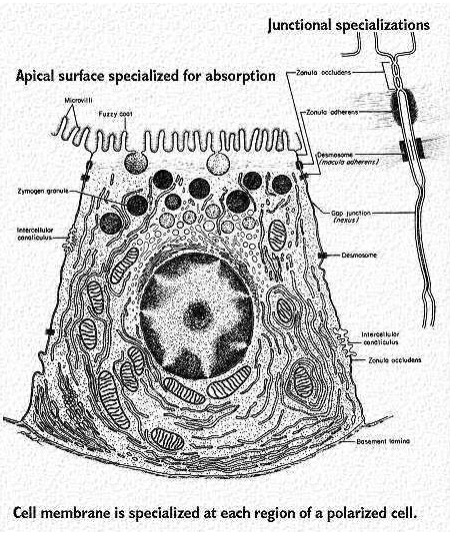
Basolateral pole: facing lamina propria.

Complex network of cytoskeleton microfilaments :

Actin rings at each pole.
Intermediate filaments in all the cytoplasm with some anchoring points at the plasma membrane

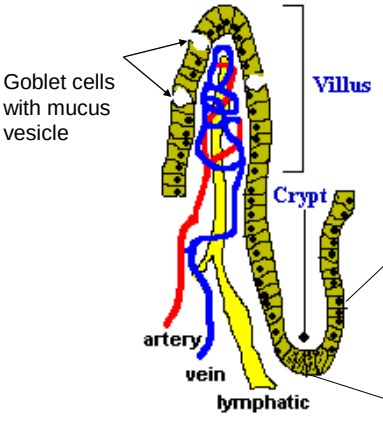
- Limeted lifespan:

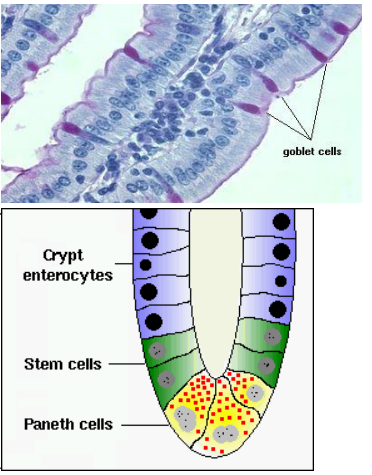
  few days



Mucosal barrier of the intestine : epithelial cells

Intestinal epithelial cells





The renewing of epithelial cells is performed by proliferation, differentiation and migration of stem cells (SC, Lgr5+) from the basis of the crypts towards the top of the villi. At the top of the villi, epithelial cells die by apoptosis and exfoliate. These mechanisms are under the control of EGF, FGF, HGF et ITF.

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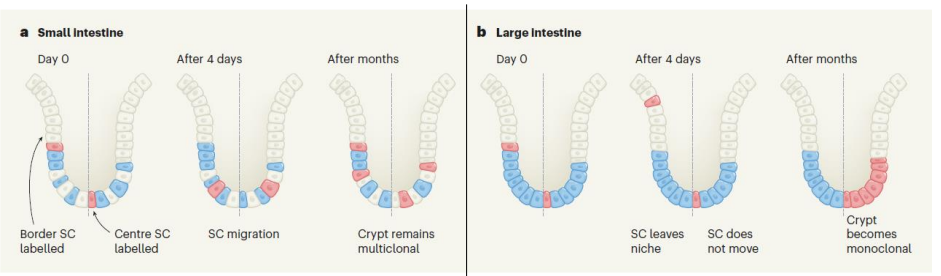
Mucosal barrier of the intestine : epithelial cells

Renewal of intestinal epithelial cells: recent advances

From Azkanaz, M. *et al. Nature* 607, 548–554 (2022)

- Labelling of Lgr5+ stem cell, either at the border or at the basis of the crypt.
- Repeated imaging of the same crypt in a live mouse over many weeks, combined with mathematical modelling:



Active border SC relocation in the small intest : retrograde migration (Wnt-dependent)

Absence of retrograde migration in the crypts of the large intestine

Adult stem cells (Lgr5+) of the intestine: relocation keeps up the numbers.

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Mucosal barrier of the intestine : ontogeny

Morphogenesis and cell differentiation

- The digestive tube is formed from endoderma : week 4 of gestation.
- Monolayer of epithelial cells with tight junctions : week 10.
- Differentiation of Goblet cells for the production of mucus : week 12.
- First synthesis of α-defensins by Paneth cells : week 13; production of lysozyme : week 20.
- Expression of FcRn from week 18 (transfer of passive immunity, IgG in the amniotic fluid around 12 weeks and in the milk).
- Follicles with B cells, T cells area and Peyer's patches : week 19 (after birth in mice).

  Peyer's patches are lymphoid areas covered by a modified epithelium (« M » cells without microvilli but with microfolds).

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Barrier function of the intestine

Components ensuring a barrier function

The goal is to prevent the crossing of pathogens from the lumen to the lamina propria and blood circulation.

Physico-chemical barrier to infections:

Lungs	- muco-ciliary elevator
	- surfactant (air-liquid interface components)
Intestine	- peristaltism
	- acidic pH
	- bile salts
	- thiocyanate
	- gastro-intestinal flow

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Barrier function of the intestine

Components ensuring a barrier function

Extrinsic barrier: to limit the quantity of pathogens reaching the surface of intestinal epithelium.

- Proteolysis and acidic stomach
- Peristaltism
- Mucus layer
- Antibodies
- Commensal flora

Intrinsic barrier: physical characteristics of the epithelium, antimicrobial factors, production of defensins.

Immune equipment:

Intra-epithelial lymphocytes, IEL
Peyer's patches: dome with M cells surrounding lymphoid follicles
Disseminated lymphoid follicles
Immune cells of the lamina propria

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Barrier function of the intestine

Components ensuring a barrier function

Commensal intestinal flora

- Help for digestion (*Ruminococcus*, *Fibrobacter* in ruminants).
- Degradation of glycoproteins of the mucus (mutual profit: nutriments source for the flora and clearing of the mucus).
- Competition towards pathogens: competition for nutriments, production of bacteriocins, interference with signals of the quorum sensing.
- Angiogenesis and development of innate immunity[1].

1 L. Hooper *et al.*, 2001, Science, 291: 881-884.

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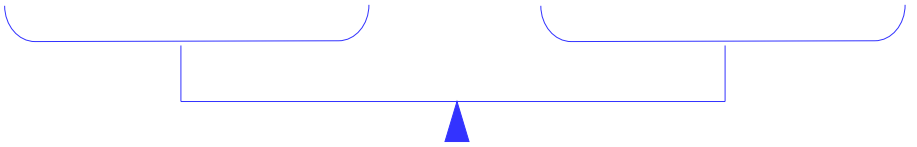
Barrier function of the intestine

Components ensuring a barrier function

Prevent an excessive response to food antigens and to the harmless commensal flora (tolerance)

Establish a protective immune response against intestinal pathogens (reaction)



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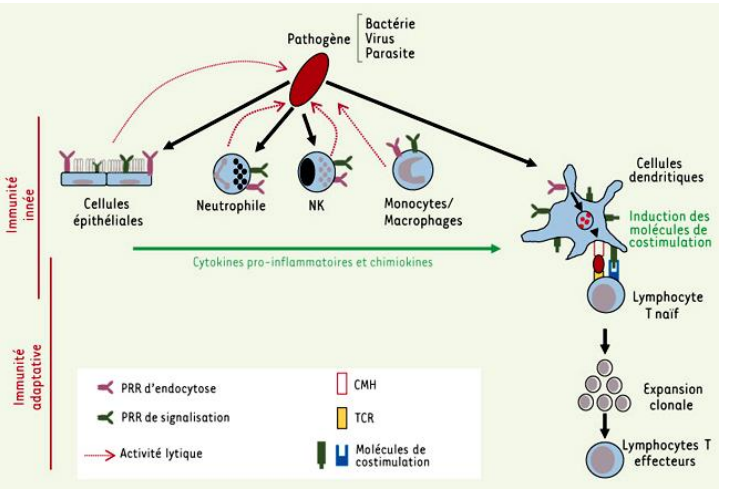
Intestinal survey

Sampling, control, information and reaction

Sampling allows immediate reaction if pathogen recognition: innate immunity
Call for specific help if outflanking enemy: adaptive immunity



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Intestinal survey

Sampling and control

Discrimination between beneficial commensal flora and harmful pathogens:
recognition of molecular patterns at the surface of microbes (MAMPs) by receptors (PRR)

MAMPs = Microbe-associated molecular patterns
- LPS
- lipoteichoic acid
- peptidoglycans
- lipoproteins...

PRR = pattern-recognition receptors

Toll-like receptors — On enterocytes, phagocytes and dendritic cells (DC)
- TLR1-TLR2 -> Lipoproteins
- TLR4 -> LPS
- TLR5 -> Flagellin
- TLR9 -> non-methylated CpG motifs of bacterial DNA

Nod-like receptors (NLRs) — Intracytoplasmic
- NOD1, NOD2 (NF-kB pathway)
- NLRP3, NLRC4 (Caspase 1 pathway)

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Intestinal survey

Sampling and control

Innate Immunity

Molecular pattern of microbe	Source	Pattern recognition receptor of innate immunity	Principal innate immune response
dsRNA	Replicating viruses	Toll-like receptor? **TLR3, *TLR7*, TLR8**	Type I interferon production by infected cells
LPS	Gram-negative bacterial cell wall	Toll-like receptor/CD14 **TLR4/CD14**	Macrophage activation
Unmethylated CpG nucleotides	Bacterial DNA	Toll-like receptor **TLR9**	Macrophage activation
N-formylmethionyl peptides	Bacterial proteins	*N*-formylmethionyl peptide receptors	Neutrophil and macrophage activation
Mannose-rich glycans	Microbial glycoproteins or glycolipids	1. Macrophage mannose receptor 2. Plasma mannose-binding lectin	1. Phagocytosis 2. Opsonization, complement activation
Phosphorylcholine and related molecules	Microbial membranes	Plasma C-reactive protein	Opsonization, complement activation

Abbreviations: dsRNA, double-stranded RNA; LPS, lipopolysaccharide.

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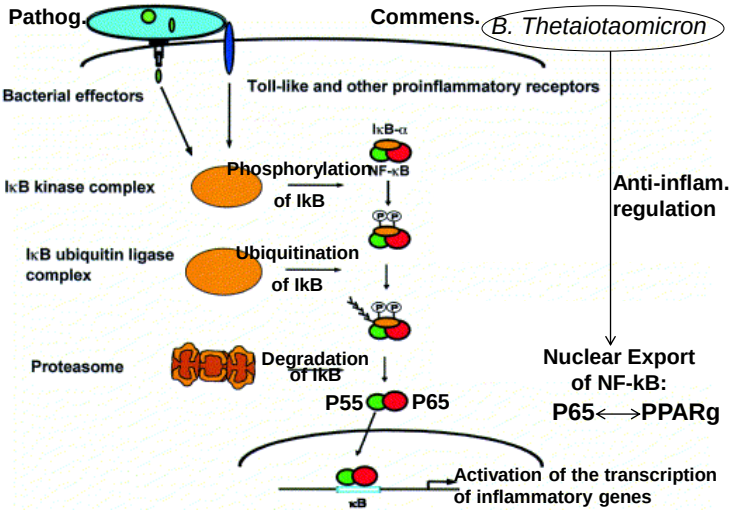
Intestinal survey

Information and reaction

Signals transduced by PRR trigger inflammatory response (inflam. cytokines) and the production of chemokines to recruit phagocytes.



Effect of TLR-ligand interaction: activation of the **NF-κB pathway**

Expression of genes of the inflammatory response

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Intestinal survey

Information and reaction

Stimulation of the epithelium by PAMPs: activation of the NF-κB pathway.

Expression of IL-8 (CXCL8), IL-1b, GMCSF, CXCL1 et MCP-1 (CCL2).

Recruitment of polynuclear neutrophils, macrophages and activation of the inflammatory response : IL-1, IL-6, TNF-a, …

Innate immunity cells are called to fight: phagocytes first!

Intestinal survey

Over-reaction

Intestinal immune pathologies : inbalanced situations

- IBD (inflammatory bowel disease): Crohn 's disease and ulcerative colitis.

  Reaction to commensal bacteria.

  Abnormally enhanced access to PRR of enterocytes.

  « Hyper-response » to MAMPs (NOD2= susceptibility gene to Crohn's disease) [1,2].

  Development of IBD in IL-2 deficient mice.

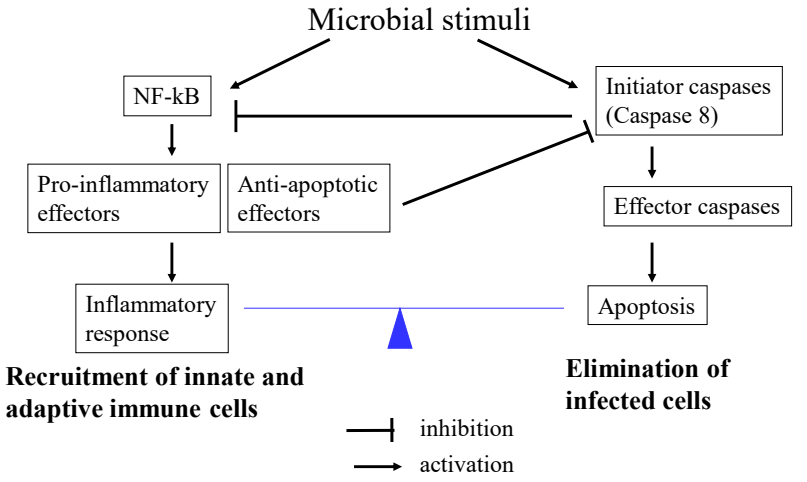
  Induction of human β-defensins 2 and 3 is blocked during Crohn's disease.

1 Y. Ogura *et al.*, 2001, Nature, 411: 603.
2 J. P. Hugot *et al.*, 2001, Nature, 411: 599.

Intestinal survey

Information and reaction

Signals transduced by PRR may also trigger a pro-apoptotic response leading to programed cell death.



Intestinal survey

Information and reaction

Inocuity of the commensal flora ?

- Weak exposure of molecular patterns (MAMPs):
  commensal MAMPS are covered by IgAs [1].
- low number of accessible PRR at the apical surface of enterocytes.
- Negative regulation of NF-κB pathway by commensal bacteria [2].
  - ➡ Signals delivered to limit inflammatory responses of the epithelium: « inflammatory suppression ».

1 AJ Macpherson & T Uhr, Science 2004, 303:1662-1665.

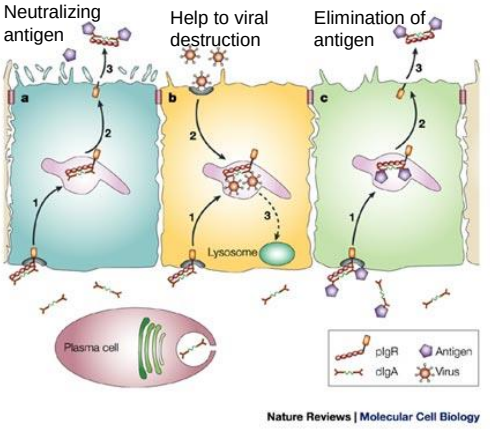
2 A Neish et al., Science 2000, 289: 1560-1563.

Intestinal survey

Information and reaction

« Camouflage » of the commensal flora : role of secretory IgA

Produced mainly by B220+ IgM+ lymphocytes, plasmacytes.



1 and 2: binding of dimeric IgA (dIgA) to polyIg receptor (pIgR) at the basolateral side of the epithelium and transcytosis (via endosomal network).
3: Cleavage of the large extracellular domain of pIgR, releasing secretory IgA linked to the secretory component into the intestinal lumen.

Prevent host invasion by intestinal flora

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Intestinal survey

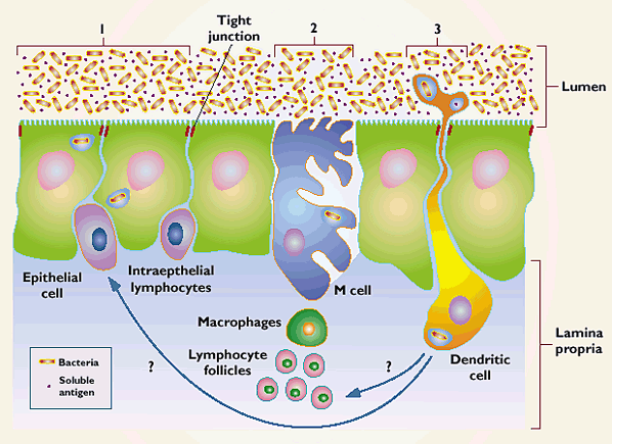
Information and reaction

The epithelial crossing of microorganisms is possible but under control by sampling:

1. Direct crossing, through epithelial cells.

2. Crossing through M cells surrounding lymphoid follicles.

3. Capture by dendritic cells expanding dendrites between epithelial cells into the lumen.



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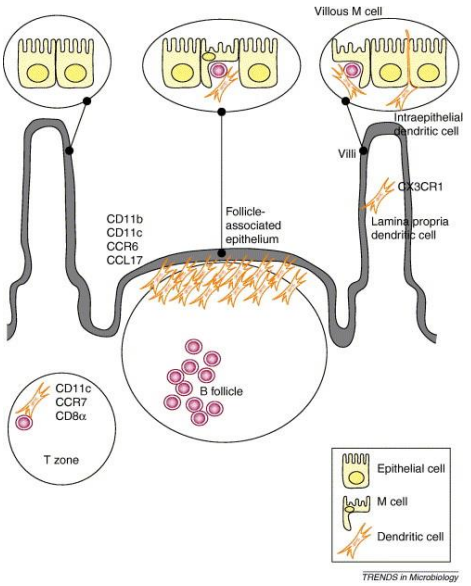
Intestinal survey

Information and reaction



M cells are able of « super capture »
- Disorganized brush border
- Reduced glycocalyx
- No secretion of mucus
- Expression of CCL20

Recruitment of immature dendritic cells beneath the dome.

Mucosal dendritic cells considered as « super sentinel cells ».

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Intestinal survey

Information and reaction towards specific immune response

T cell response: development of specific immunity towards pathogen-derived antigens.

Intra-epithelial lymphocytes (IEL): mostly CD8+, TCR$\alpha\beta$ or TCR $\gamma\delta$ positive with restricted repertoire ⟷ non conventional MHCI of intestinal epithelial cells (IEC).

IELs express $\alpha E\beta 7$ integrin interacting with E-cadherin of IECs (intercellular adhesion).

T-lymphocytes of the lamina propria are effector / memory cells coming from the migration of primed T-cells:
Priming by dendritic cells (DC) in lymphoid follicles and draining lymph nodes
→ recirculation and mucosal homing of T-cells.

The trafficking of primed lymphocytes : via the lymph → thoracic duct
then blood circulation and mucosal homing from high endothelial venules.

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Intestinal survey

Information and reaction towards specific immune response

Circulation of lymphocytes

T and B lymphocytes stimulated in the MALT (mucosa associated lymphoid tissue) acquire receptors for mucosal homing: $\alpha 4\beta 7$ integrin.

$\alpha 4\beta 7$ integrin of primed lymphocytes	⟷	MadCAM-1 adressin of high endothelial venules
CCR9/CCR10 of primed lymphocytes	⟷	CCL25 et MEC (mucosa-associated epithelial chemokine)

Settlement of specific immunity

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Oral tolerance

Tolerance to food antigens and to commensal flora

The first encounter of an antigen (Ag) in the intestine triggers an inhibitory process to prevent specific cellular responses to this Ag (TCD4+ et TCD8+) following systemic immunization with the same Ag.

↓

Immunosuppression

Prevention of a delayed hypersusceptibility to food antigens and to the commensal flora.

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Oral tolerance

Mechanism

- Small intestine mucosae represents the site of induction of oral tolerance.
- Epithelial cells express non conventional MHC I et II molecules and do not possess co-stimulation molecules necessary for Ag presentation → induction of anergy ?
- Intestinal T lymphocytes of the lamina propria are mostly effector/memory cells and not naive.
- Epithelial cells that have encountered an intestinal Ag may become suppressive towards specific CD8+ T cells by secretion of IL10 and of TGFβ .

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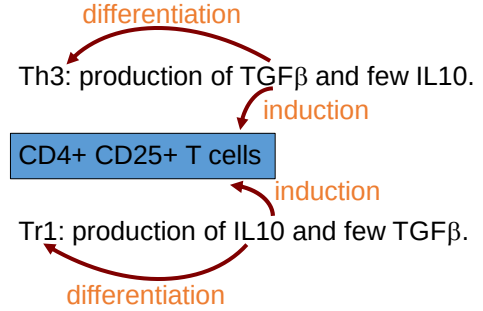
Oral tolerance

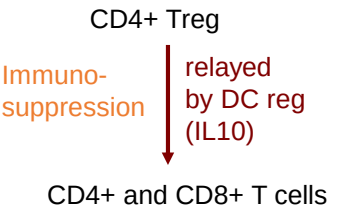
Mechanism

According to the dose of antigen:

- Low dose: immuno-suppression mediated by regulatory T cells (Treg).
- High dose: immuno-suppression mediated by anergy/deletion of Ag-specific T cells.

Regulatory T cells: CD4+ Treg





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Balance: homeostasis

Components ensuring a barrier function

Maintain intestinal homeostasis : state of equilibrium

Prevent an excessive response to food antigens and to the harmless commensal flora (tolerance)

Establish a protective immune response against intestinal pathogens (reaction)

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Cytokines orchestrate the immune status

Balance Homeostasis

Inbalance Inflammation

Immune cells nomenclature :

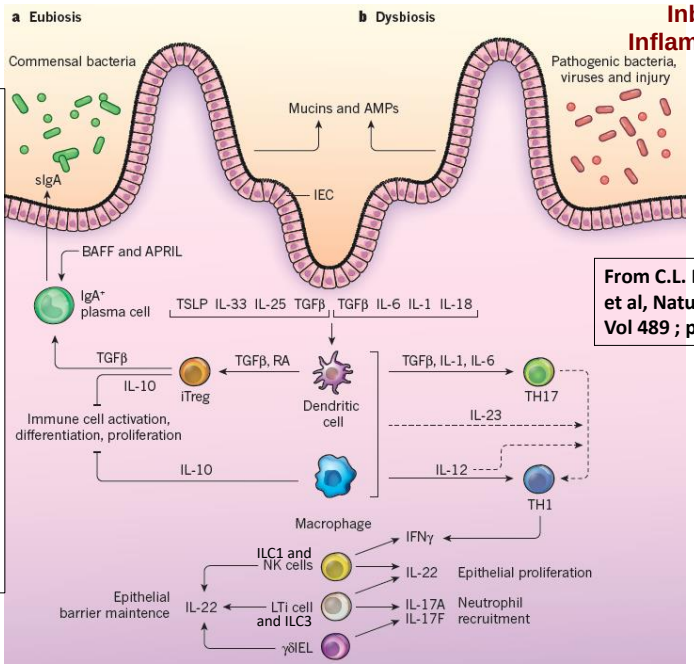
iTreg=induced regulatory T cell

TH=T helper

ILC= innate lymphoid cell

Lti=lymphoid tissue inducer

IEL=intra-epithelial lympphocyte



From C.L. Maynard et al, Nature, 2012 Vol 489 ; p231

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Master 2 I^{2}VB: Infectiologie Immunité Vaccinologie Biomédicaments
Master 2 IDOH: Infectious Diseases and One Health

Mucosal innate immune arsenal: antimicrobial peptides (AMPs)

- Characteristics
- Producing cells
- Structures
- Biological activity

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Characteristics of AMPs

Ancestral components of innate immunity

- *In natura*: more than 2800 antimicrobial peptides registered in 2017 (>2000 from animals). Antimicrobial peptide database: http://aps.unmc.edu/AP/main.php
- Essential components of mucosal innate immunity: intrinsic barrier function.
- Two major families of AMPs in animals :

Cathelicidins: large diversity of primary and secondary structure, but a highly conserved pro-region (N-term domain cathelin, C-term domain LL37 in humans).

Defensins: α, β, or θ. About 50 α and 90 β in mammals.

- The two α and β subfamilies of defensins are encoded by a cluster of at least 8 genes on the chromosome 8p23 in humans.
- Antimicrobial peptides are ≤ 5 kDa (30 to 50 AA) with a positive net charge and an amphipathic character.
- Most of AMPs display direct bactericidal activity with large spectrum (Gram+ and Gram-), at micromolar concentration *in vitro*.

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Characteristics of AMPs

Ancestral components of innate immunity

Other antimicrobial factors:

In mice: CRS (cryptdin related sequence) peptides organized as homo- or hetero-dimers.

BPI protein of 55kDa, component of polynuclear neutrophils, able to bind to lipid A of the LPS and to disorganize the outer membrane of Gram- bacteria.

Intestinal enzymes with antimicrobial activity: PLA2 and lysozyme, in the granules of Paneth cells and of neutrophils.

Angiogenin 4 (REG3 gamma).

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Characteristics of AMPs

Ancestral components of innate immunity

AMPs are highly conserved throughout evolution.

- Found in plants and animals (invertebrates and vertebrates).
- From moluscs to birds and to humans: β-defensins are the most ancestral, α-defensins appeared in mammals and θ-defensins restricted to primates.
- Analogy of signal sequences of avian β-defensins and of cytotoxic peptides of snake venom [1].
- Structural analogy of platypus venom peptides, of the bovine neutrophil β-defensin 1 and of a neurotoxic peptide of sea-anemone.
- First defensins described more than 25 years ago from neutrophils extracts [2].

1- Zhao *et al*., 2001, Infect. Immun. 69: 2684-2691.
2- Ganz T… Lehrer RI, 1985, J Clin Invest. 76:1427-35.

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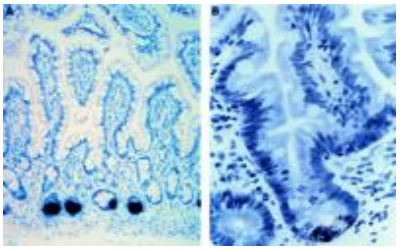
AMPs producing cells

Intestinal epithelial cells

Defensins identified from Paneth cells: α-defensins in the secretory granules.



Localization of HD5 mRNA (left, silver staining) and peptide (right, brown peroxydase product) [1].

→ **Strategic position to protect multipotent stem cells of the epithelium**

Differentiated enterocytes of the large intestine can produce β-defensins and cathelicidins.

1 G. Diamond & C.L. Bevins, Clin. Immunol. Immunopathol. 1998, 88: 221-225.
E. Porter et al. Infect. Immun. 1997, 65: 2389-2395.

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AMPs producing cells

Intestinal epithelial cells

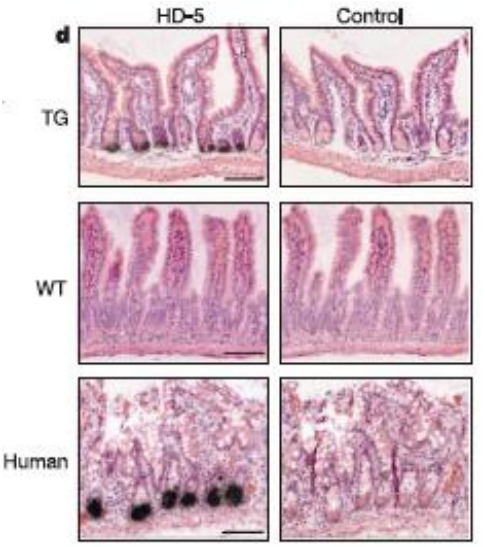
Paneth cells granules contain α-defensins that can be released upon stimulation.

Transgenic mice for human α-defensin 5 (HD5) exhibit low intestinal colonization by *Salmonella* when inoculated orally, by comparison to control mice [1].

Protection against enteric salmonellosis in transgenic mice expressing a human intestinal defensin

Nita H. Salzman*†, Dipankar Ghosh†‡, Kenneth M. Huttner§, Yvonne Paterson‖ & Charles L. Bevins‡

1 Salzman N *et al*, 2003, Nature, 422: 522-526



HIS, antisense RNA probe

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AMPs producing cells

Intestinal epithelial cells

Epithelial cells express β-defensins constitutively or inducibly (hBD=human beta-defensin):

- hBD1, hBD3 and hBD4 expressed constitutively by small and large intestine epithelial cells.

- hBD2 expression induced by inflammatory cytokines, by IL-17 [1], by PAMP receptors pathways: TLR2 and TLR4 [2], NOD2/CARD15 [3].

- hBD2 expression stimulated by BCG [4], by *Campylobacter jejuni* [5]…

1- Kao et al. 2004, J. Immunol. 173 : 3482-3491.
2- Vora et al. 2004, J. Immunol. 173 : 5398-5405.
3- Voss et al. 2006, J. Biol. Chem. 281 : 2005-2011.
4- Mendez-Samperio et al. 2006, Cell Immunol. 239, 61-66.
5- Zilbauer et al. 2005, Infect. Immun. 73 : 7281-7289.

AMPs producing cells

Reguation of expression

- Cathelicidins are less regulated by TLR and/or cytokines.
- Cathelicidins are positively regulated by histones acetylation.
- Cathelicidins are positively regulated by vitamin D (1,25 D3).
- Di-hydroxy vitamin D3 is able to positively regulate TLRs expression.

→ Feed back loop to amplify production of various AMPs

AMPs producing cells

Polynuclear granulocytes, neutrophils

In human PMNs :

- 4 α -defensins stored in the azurophilic granules that will merge with phagosome.

- 1 cathelicidin (LL-37) stored in secondary specific granules and delivered to extracellular milieu following degranulation.

The type and the number of AMPs in PMNs of mammals vary according to species.

Secretion of AMPs by PMNs and epithelial cells may be simultaneous (20 AMPs identified in the human skin).

Most of the AMPs are produced as pro-peptides: maturation by cleavage of the pro-region.

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Structure of AMPs

Secoondary structures

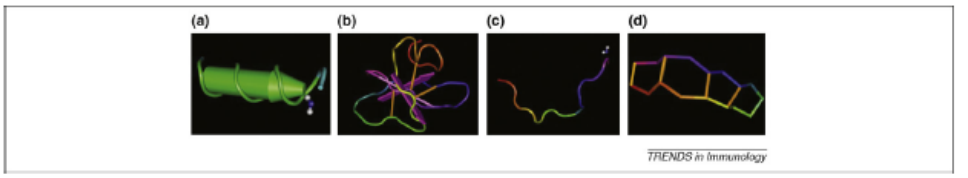

Figure 1. Structure of selected antimicrobial peptides (AMPs). AMPs are present in a wide variety of structural conformations, such as peptides with α-helix structures, peptides with β-sheet structures stabilized by disulfide bridges or peptides with extended or loop structures. **(a)** α-helix. NMR-structure of the LL-37 core peptide of cathelicidin bound to detergent micelles (PDB ID: 2FBS). **(b)** β-sheet. Solution structure of the defensin hBD2 by two-dimensional proton nuclear magnetic resonance spectroscopy (PDB ID: 1FQQ). **(c)** extended structure. NMR structure of the bovine antimicrobial peptide indolicidin bound to dodecylphosphocholine (DPC) micelles (PDB ID: 1G89). **(d)** loop structure. 3D structure of a cyclic defensin from the leukocytes of rhesus macaques (PDB ID: 1HVZ). PDB ID: ID of peptide structure in Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank (http://www.rcsb.org/pdb/home/home.do). The style of LL37 in (a) is shown as secondary coloring shortcuts, whereas the styles of peptides in (b-d) are shown in rainbow coloring shortcuts.

Highly compact peptides:
α-helix conformation (mainly cathelicidins and some α -defensins) or β-sheet conformation (β-defensins), stabilized by disulfide bridges, or cyclic conformation (θ-defensins), composed of two hemi-defensins (in primates).

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Structure of AMPs

Secoondary structures

Rabbit α-defensin

RK-1

HNP-3

Dimeric human α-defensin

Monomeric human β-defensin

hBD-1

RTD-1

Rhesus Macaque θ-defensin

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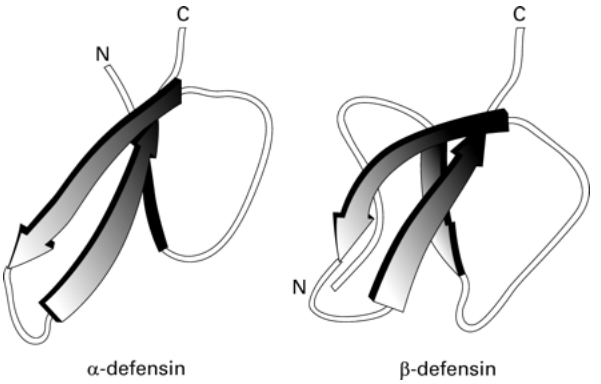
Structure of AMPs

Secoondary structures

Beta sheet, characteristic of α- and β-defensins.



α-defensin

β-defensin

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Structure of AMPs

Secoondary structures

Comparison of the sub-families of vertebrates defensins

	Structure	Size (kDa)	AA number	Cys pairing	Origin
α-defensins	β sheet	3,5-4	29-35	1-6, 2-4, 3-5	hum., rabbit, rat, mice
β-defensins	β sheet	4-6	38-42	1-5, 2-4, 3-6	hum., bov., chicken sheep, pig...
θ-defensines	cyclic	2	18	1-4, 2-5, 3-6	primates

The connectivity of the six cysteine residues in the sequence to constitute three disulfide bridges stabilizing the 3D structure : define the sub-family of defensins.

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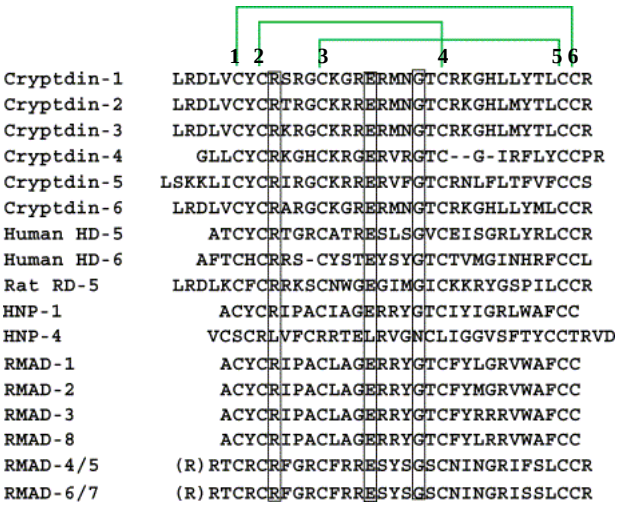
Structure of AMPs

α-defensins primary structure

Structural homologies linked to sequence homologies

Cysteine number 1 is the first at the N-terminal extremity.

In mice there are twenty isoforms of α-defensins called cryptdins.



Structure of AMPs

α-defensins mature peptides

Production of the active form by cleavage of the pro-region to release active peptide

In mice, cryptdins are matured in the granules of Paneth cells, with the protease MMP7 (matrix metalloprotease matrilysin).

proCrp1:

```
20                          43 44          53 54    58 59
DPIQNTDEETKTEEQPGEDDQAVS↓VSFGDPEGTS↓LQEES↓LRDLVCYCRSRGCKGRERMNGTCRKGHLLYLCCR
```

proHD5:

```
20                                    55 56      62 63
ESLQERADEATTQKQSGEDNQDLAISFAGNGLSALR↓TSGSQAR↓ATCYCRTGRCATRESLSGVCEISGRLYRLCCR
```

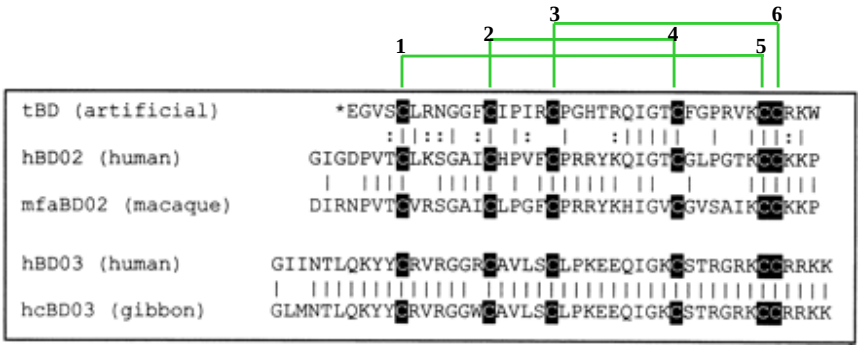
In humans, the pro-form of HD5 is secreted, then matured by trypsin in the intestinal lumen. The active form can also be secreted [1].

1 D Ghosh *et al*., 2002, Nature Immunol. 3: 583-590.

Structure of AMPs

β-defensins primary structure

Structural homologies linked to sequence homologies



Biological activity of AMPs

Antimicrobial activity

Large spectrum of antimicrobial activity: bacteria, fungi, protozoan parasites and virus

- *Escherichia coli*
- *Staphylococcus aureus*
- *Salmonella typhimurium*
- *Pseudomonas aeruginosa*
- *Listeria monocytogenes* …

Large anti-bacterial spectrum: Gram+ and Gram-

Demonstration *in vivo*: KO mice for the genes of AMPs

- Camp -/- mice: susceptible to *Streptococcus*, herpes simplex virus, *Escherichia coli* and vaccinia virus.
- mBD1 -/- mice: susceptible to *Haemophilus influenzae*, *Staphylococcus*.

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Biological activity of AMPs

Antimicrobial activity measurement

Determination of the Minimum Inhibitory Concentration (MIC) by radial diffusion assay

Bacteria added to LB 0.03% - type1 agarose underlay gel

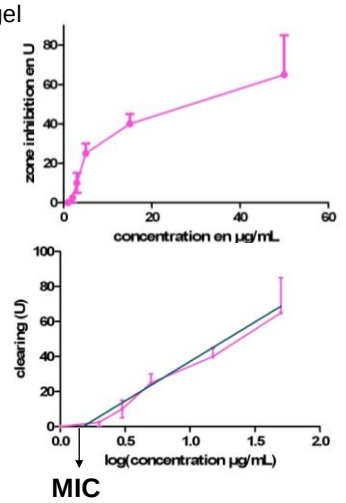
Peptide loaded into each well

Peptide diffusion, 3h at 37° C

Rich medium overlay added

+16h at 37° C

E. coli ATCC 25922



Biological activity of AMPs

Mechanisms of antimicrobial activity

Disturbance and rupture of membranes of pathogens.

- Cationic molecules-> affinity for the negative charges at the surface of bacterial membranes (more than on the surface of eukaryotic cells).
- Amphipathic molecules (analogous to detergents) -> disorganization of membranes and rupture of electrochemical gradients.

➡ Micro-organisms with high osmotic pressure « blow out ».

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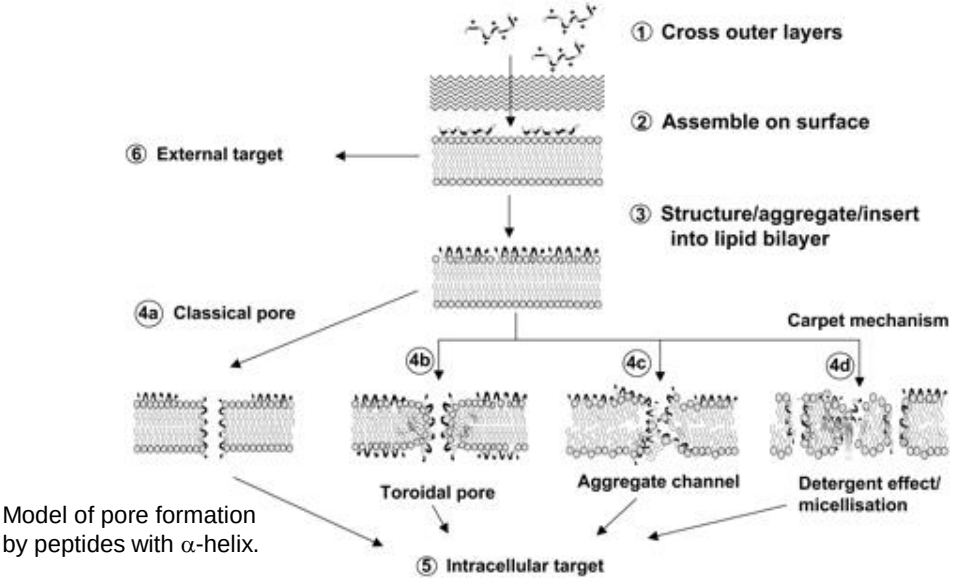
Biological activity of AMPs

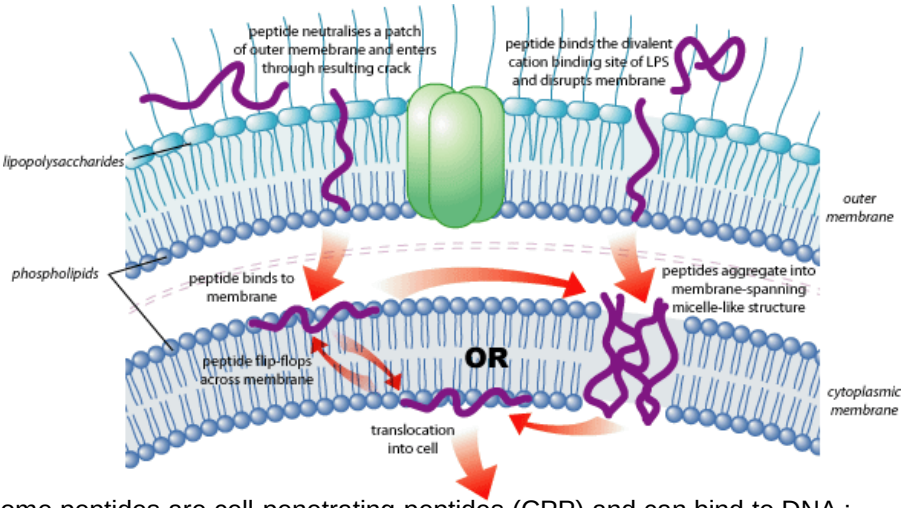
Mechanisms of antimicrobial activity



Model of pore formation by peptides with α-helix.

Biological activity of AMPs

Mechanisms of antimicrobial activity



Some peptides are cell-penetrating peptides (CPP) and can bind to DNA : inhibition of replication, transcription, and bacterial destruction.

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Biological activity of AMPs

Mechanisms of antimicrobial activity

No deleterious effect on host cells: hypothesis

- Less negative charges at the surface of eukaryotic cells : phospholipids with negative polar head (PS) are found in the inner leaflet of the lipid bilayer.
- Cholesterol content of eukaryotic (not prokaryotic) membranes ensures a rigid lipid bilayer less prone to disorganization.
- Protective role of serum proteins, of the extracellular matrix.
- Cytotoxic concentrations for eukaryotic cells are largely above microbicidal concentrations (MIC LL37 and β-defensins in the order of µM, cytotoxic concentration > 30µM) .

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Biological activity of AMPs

Resistance of microbes to antimicrobial activity

Reduce the net negative charge of the bacterial membrane

→ **limit the access of cationic AMPs**

- Modification of the phosphatidyl glycerol with L-lysine (*mprF* gene of *S.aureus*).
- Alanylation of teichoic and lipoteichoic acid (*dlt* operon).
- Acylation of the lipid A of LPS (*phoP-phoQ* genes, *pagP* gene of *Salmonella*).

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Biological activity of AMPs

Resistance of microbes to antimicrobial activity

Secretion of proteases for the degradation of AMPs

- External membrane protease of *S. Typhimurium* (*pgtE*)
- V8 protease and aureolysin of *S. aureus* (targeting LL37)

Efflux pumps to remove AMPs

- *mtrCDE* gene of *Neisseria gonorrhoeae*
- *qacA* gene of *Staphylococcus aureus*

Globally limited bacterial means

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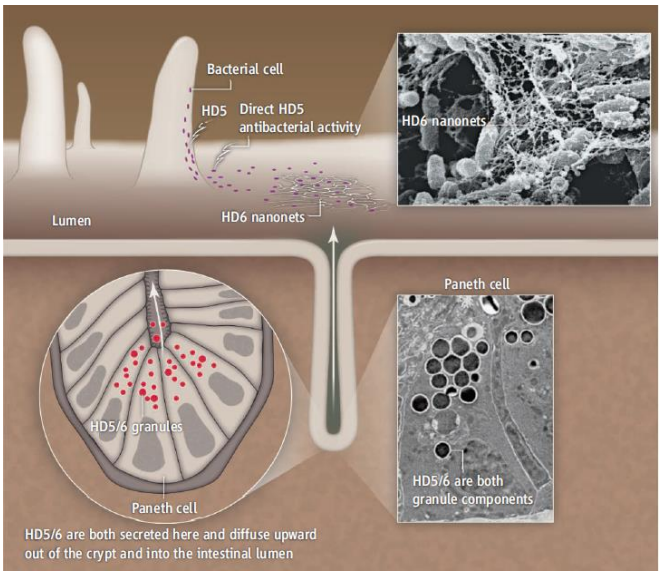
Biological activity of AMPs

A unique human α-defensin 6: not antimicrobial



HD6 forming fibers and associating with HD5: Nanonets trapping bacteria

Hiutung Chu et al., Human α-Defensin 6 Promotes Mucosal Innate Immunity Through Self-Assembled Peptide Nanonets. Science 2012 Vol 337: 477.

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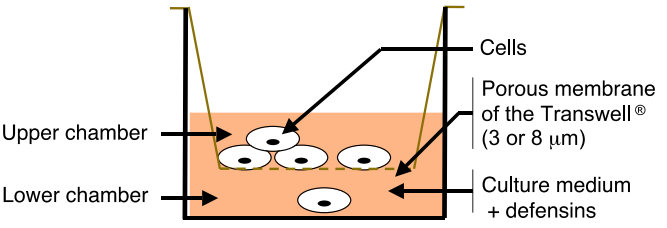
Biological activity of AMPs

Much more than antimicrobial: chemotactic activity

Measurement of the chemotactic activity: cell migration assay using the « Transwell® » system.



Counting of the cells in the lower chamber.

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Biological activity of AMPs

Immuno-modulatory activity: chemotactism

hBD2 and CCL20 display similar 3D structure and share the same affinity for the CCR6 receptor → Recruitment of dendritic cells (DC) and T cells.

HNP1 and HNP2 (α-defensins) → Recruitment of monocytes
hBD3 and hBD4 (ß-defensins)

LL37 (cathelicidin) → Recruitment of neutrophils (feed-back loop)

With chemotactic activity for DCs and T cells, defensins may link innate and adaptive immunity.

Remark: Conversely, some chemokines display direct bactericidal activity analogous to AMPs.

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Biological activity of AMPs

Immuno-modulatory activity

Regulation of inflammation by stimulation of cytokines production

CXCL8 (IL-8)
CCL2 (MCP1) — Chemokines: influx of phagocytes
IFNα… — Antiviral effectors

hBD2, hBD3, hBD4 and cathelicidin induce the production of
- IL-6 (inflammatory)
- IL-10 (anti-inflammatory)
- MCP1 (CCL2)
- MIP3α (CCL20)
- RANTES (CCL5)

  Chemokines: influx of phagocytes, of Ag-presenting cells, of lymphocytes…
- IL-18 (inducer of IFNγ, major activator of macrophages)

Possible adverse effect: Inhibition of the production of cytokines

Competition with ligands, disturbance of membrane microdomains with receptors : cathelicidin.

Refs: Niyonsaba *et al.* 2005, J. Immunol, 175: 1776-84; Niyonsaba *et al*. 2007, J. Invest Dermatol 127: 594-604; Di Nardo *et al*. 2007, J. Immunol. 178:1829)

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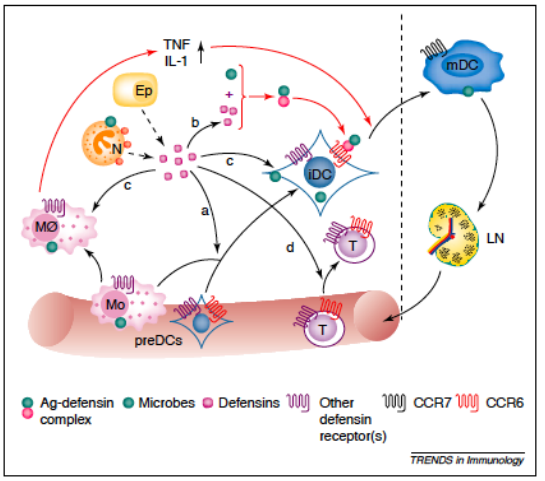
Biological activity of AMPs

Immuno-modulatory activity

Enhanced adaptive immunity

Induction of expression of co-stimulation molecules (CD80, CD86, CD40) on Ag-presenting cells (DC): hBD2 via TLR4; hBD3 via TLR1 and TLR2
(Biragyn A et al Science, 2002; Funderburg et al, PNAS USA, 2007)

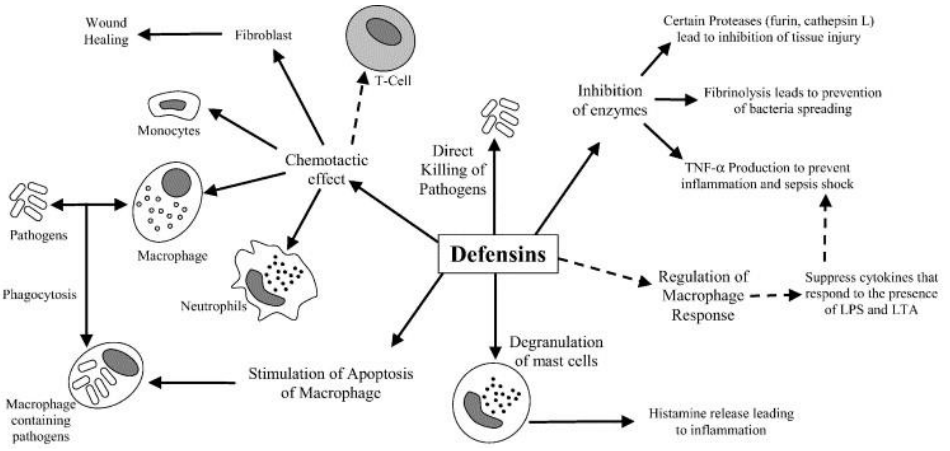
Defensins from neutrophils (N) and epithelial cells (Ep) ---->:
a. recruit pre-DC
b. bind to Ag, priming capture by immature DC => mature DC
c. stimulate DC maturation
d. favor influx of specific effector lymphocytes (T) primed in the lymph nodes (LN)



Biological activity of AMPs

Immuno-modulatory activity

Beyond antimicrobial and immuno-modulatory



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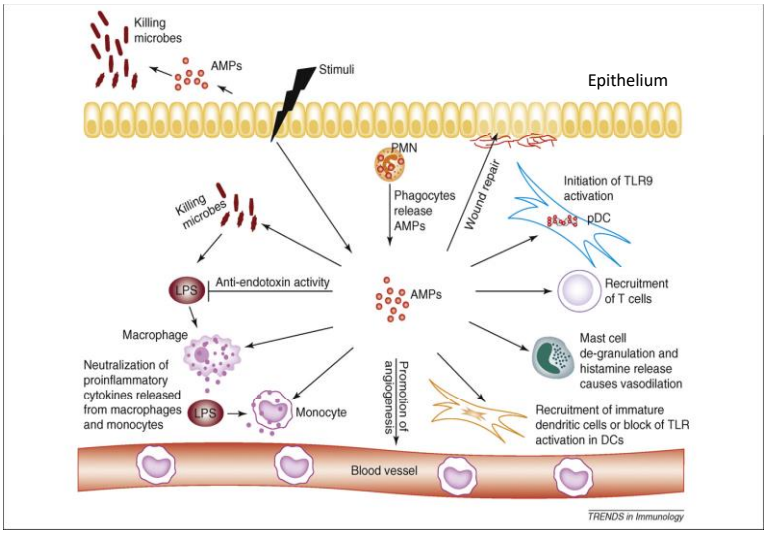
Biological activity of AMPs

Immuno-modulatory activity

Beyond antimicrobial and immuno-modulatory



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