

Cellules de l'épithélium intestinal: structure, fonctions, modèles cellulaires

(UEM907, Master1 « Sciences des médicaments et des
produites de Santé »)

Marie-Françoise Bernet-Camard

- Rappel Anatomie
- Epithélium intestinal: cellules, structures, différenciation, fonctions...
- Modèles: Caco-2, HT-29...
 - Utilisations, intérêts et limites
- Application et analyse de l'article

Archives of Toxicology
<https://doi.org/10.1007/s00204-020-02694-6>

NANOTOXICOLOGY



Small silica nanoparticles transiently modulate the intestinal permeability by actin cytoskeleton disruption in both Caco-2 and Caco-2/HT29-MTX models

Raphaël Cornu¹ · Claire Chrétien¹ · Yann Pellequer¹ · Hélène Martin¹ · Arnaud Béduneau¹


Systeme Gastrointestinal (rappels)

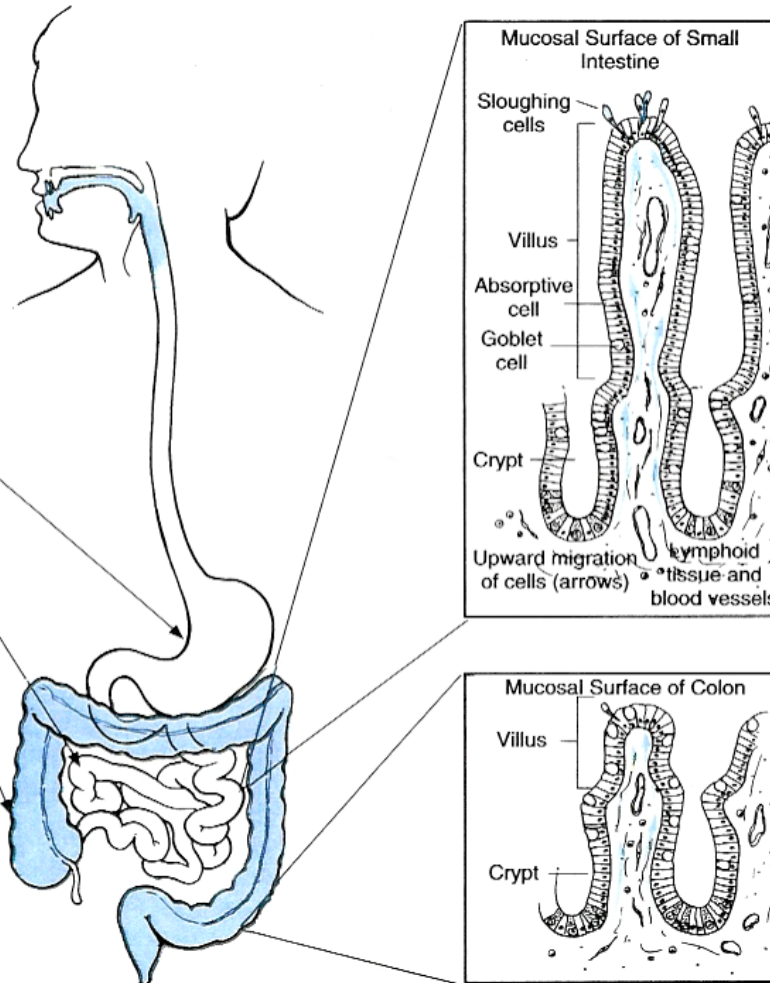
Mouth
 Sloughing cells
 Flow of saliva
 Lysozyme
 sIgA
 Resident microflora
 Lactoferrin

Stomach
 Low pH
 Proteolytic enzymes

Small intestine
 Fast flow
 Mucus, sloughing cells

Colon
 Slow flow
 Mucus, sloughing cells
 Abundant resident microflora

 = High concentration of bacteria (resident microflora)

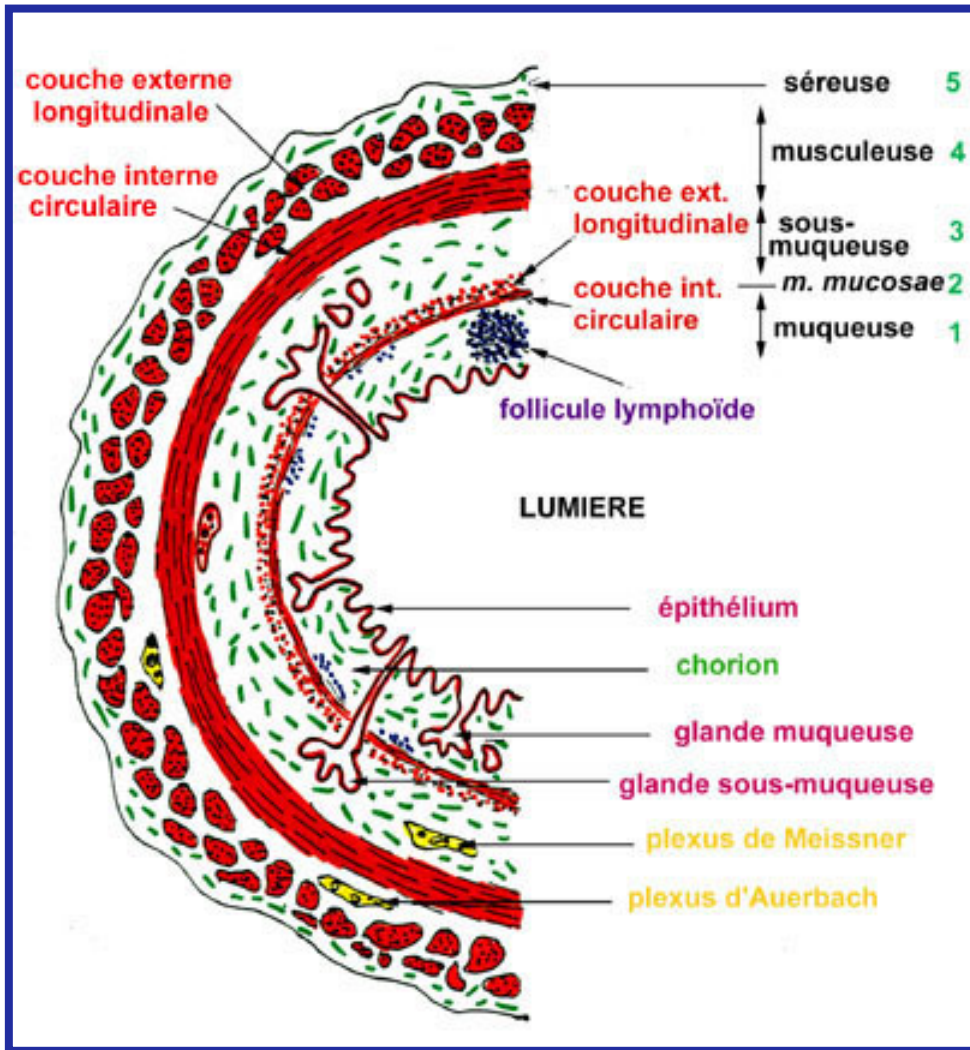


← Intestin grêle

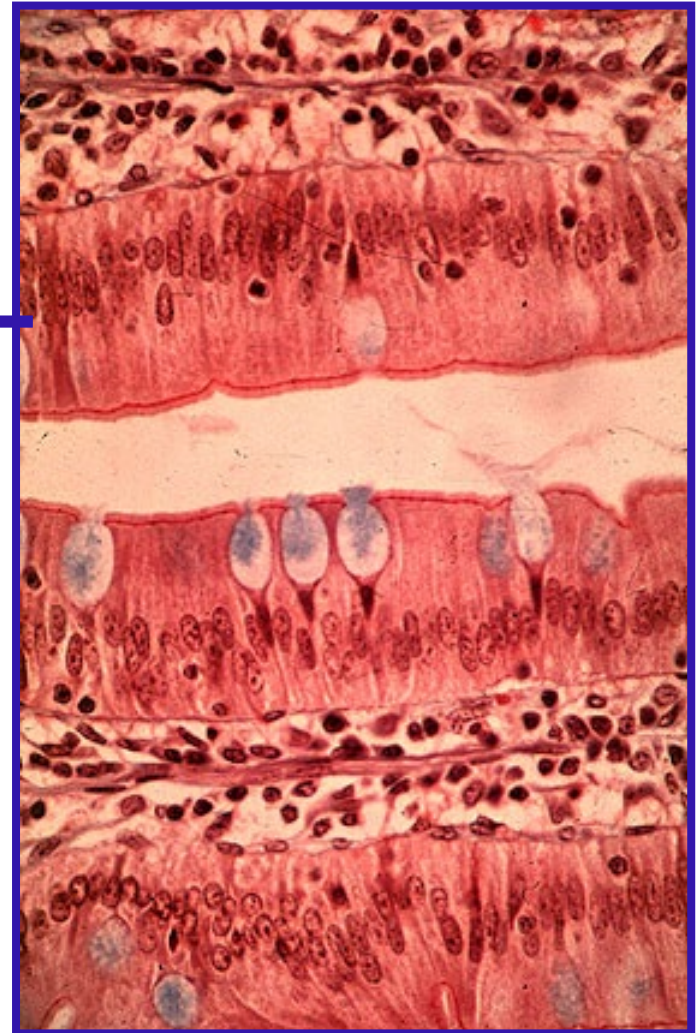
← Colon

➤ Epithélium intestinal: Barrière / échanges (aliments, médicaments, immunité..)

Muqueuse intestinale: Barrière / Échanges



Paroi du tube digestif



Muqueuse intestinale

Muqueuse intestinale: Barrière / Échanges

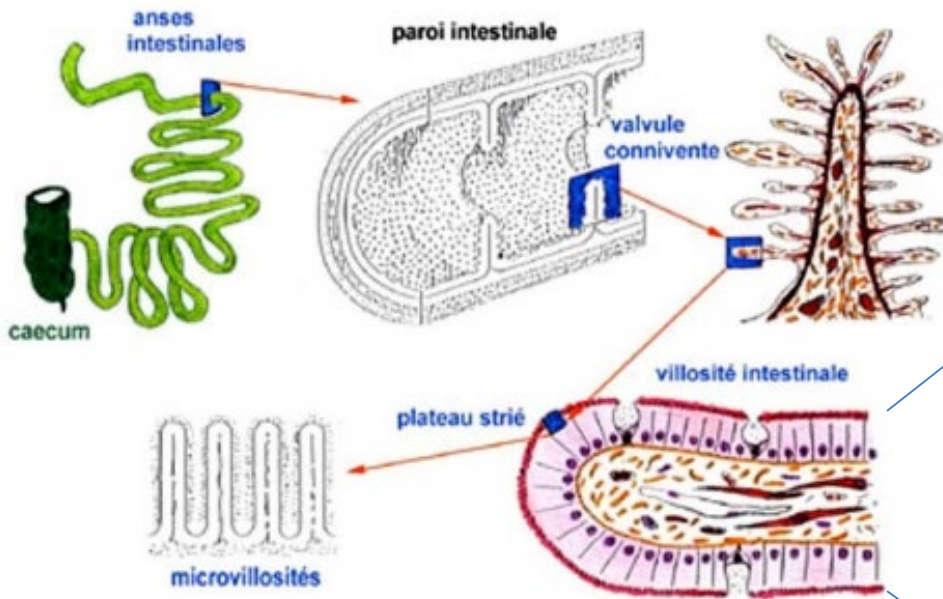
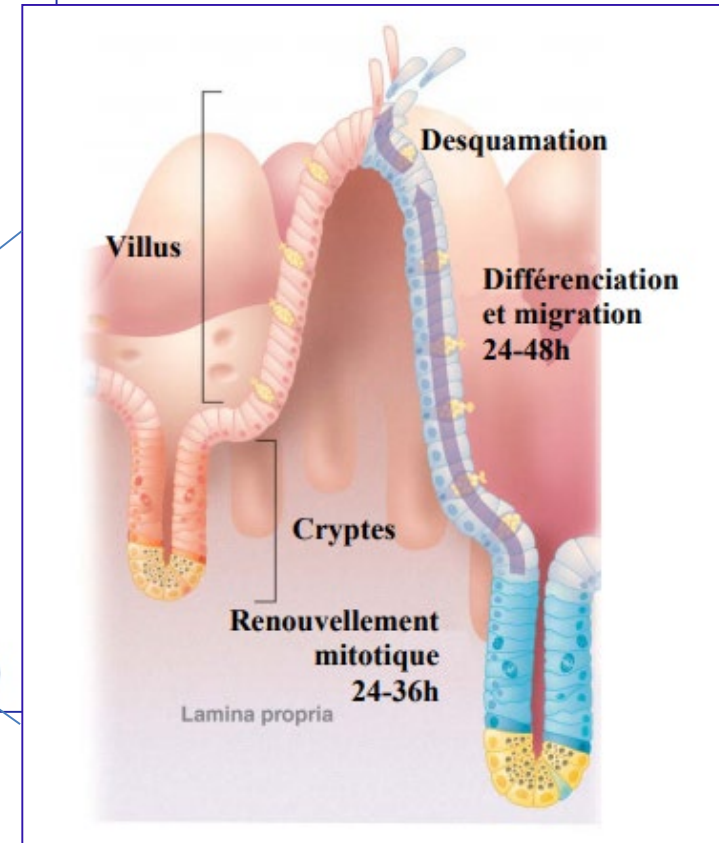


Figure 1 : Organisation de l'intestin

Schématisme des différents niveaux de repliements de l'épithélium intestinal (d'après N.Vacheret)

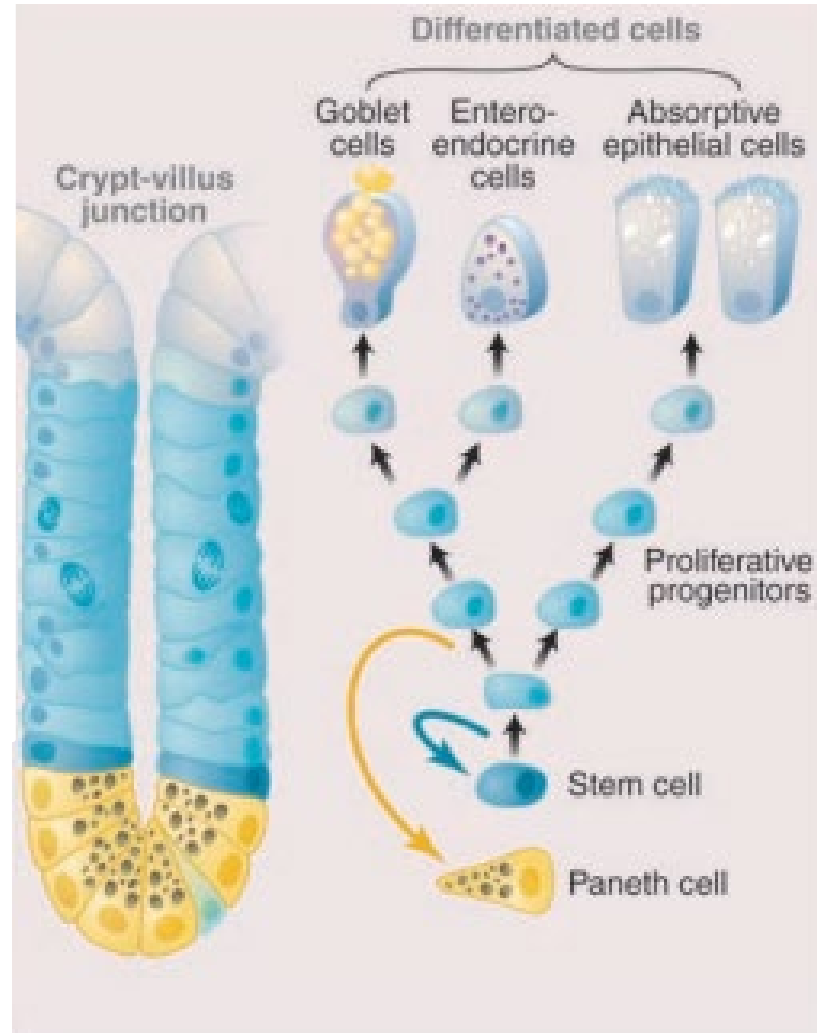
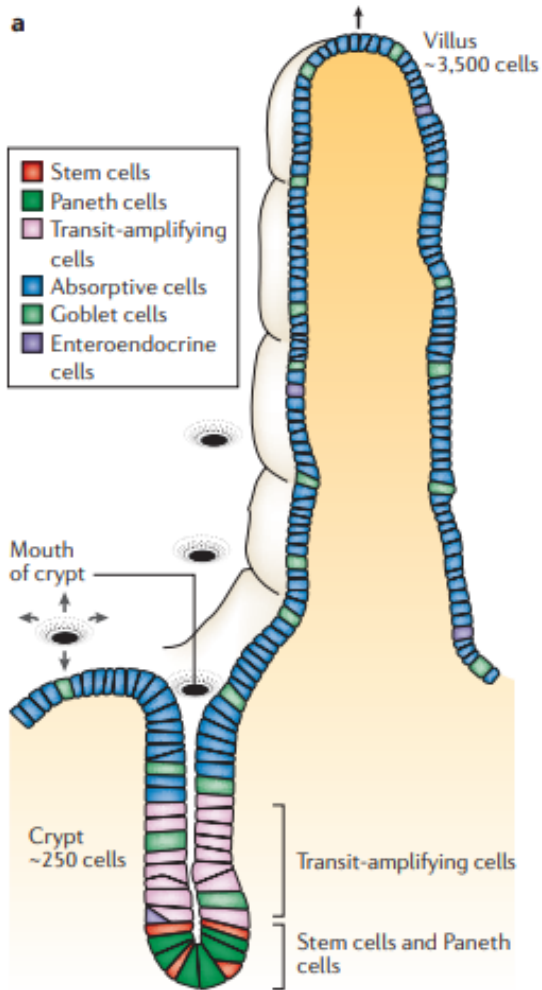


➤ Amplification surface d'échanges

Axe crypto-villositaire intestin grêle
(Radtke et Clevers, 2005)

Muqueuse intestinale:

➤ structure dynamique



Axe crypto-villositaire (Crosnier et coll., 2006)

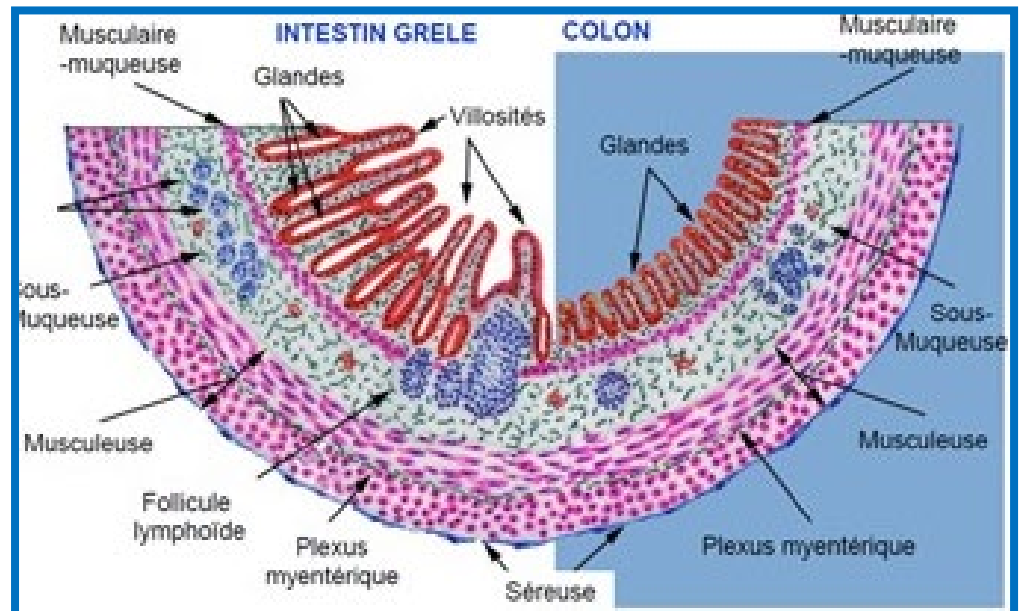
(Radtko & Clevers, 2005)

Intestin grêle/colon

VARIATIONS STRUCTURALES SEGMENTAIRES

	Valvules conniventes	Glandes de Brünner	Villosités	Formations lymphoïdes	Cellules de Paneth
Duodénum	±	+++ (1er duodénum)	longues rectilignes incisées	±	+
Jéjunum	++	0	longues, en doigt de gant	++	++
Iléon	+	0	pyramidales	+++ = plaques de Peyer (*)	+++

* : Description des plaques de Peyer → cours organes lymphoïdes (PCEM2)

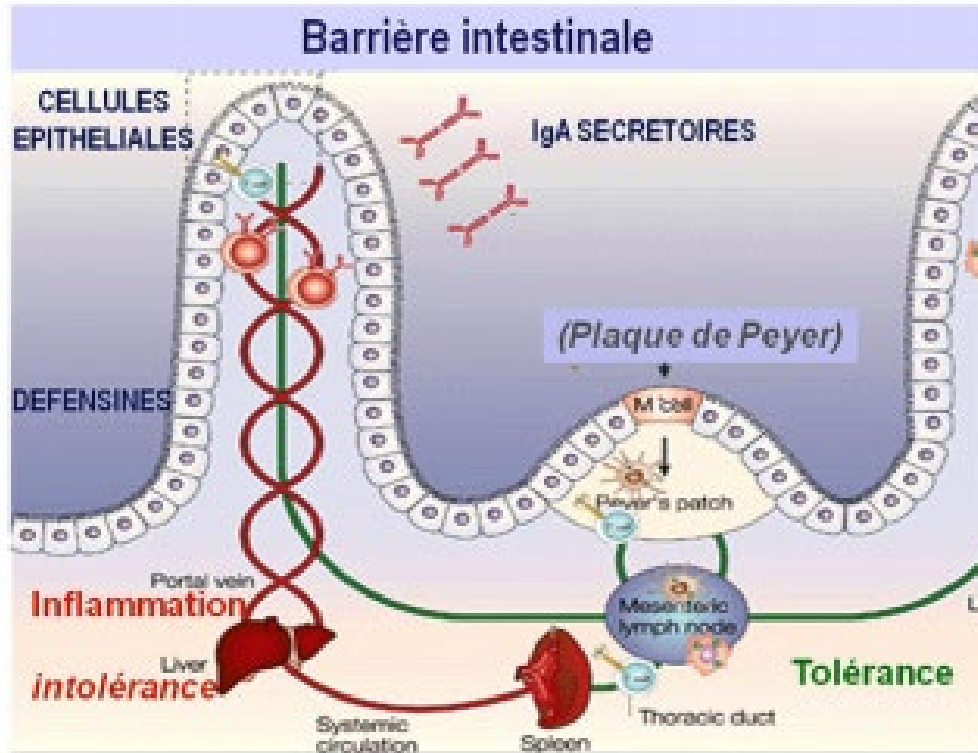


Muqueuse intestinale: Barrière / Échanges

Microbiote

Contrôle de
Perméabilité

Immunité
MALT



Mucus

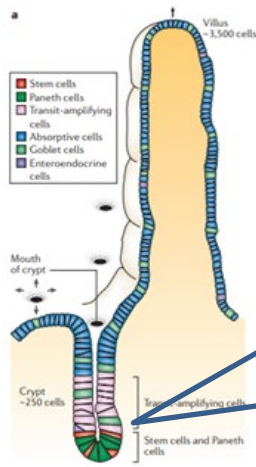
Péristaltisme
intestinal

Peptides
antimicrobiens
IgA

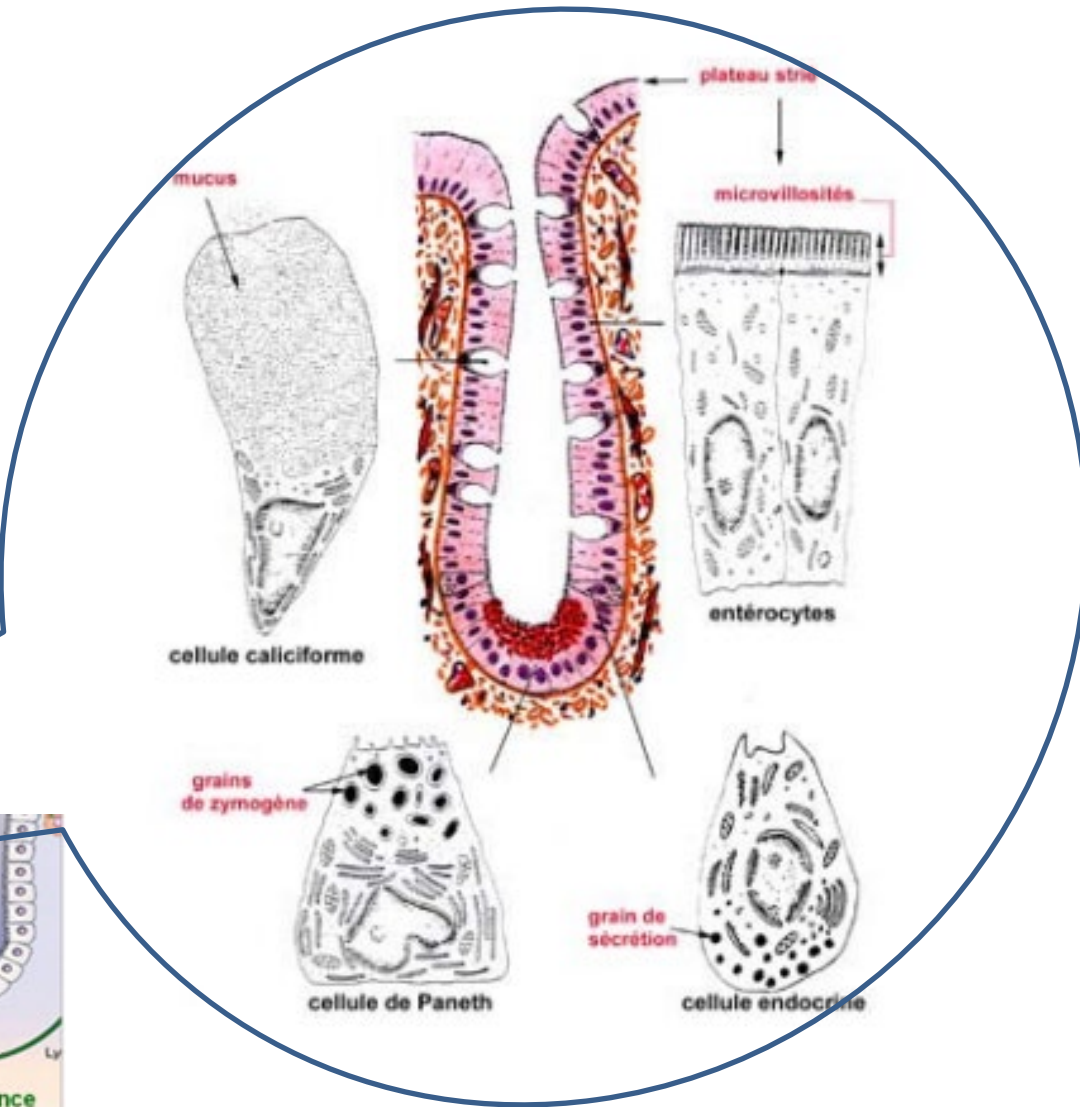
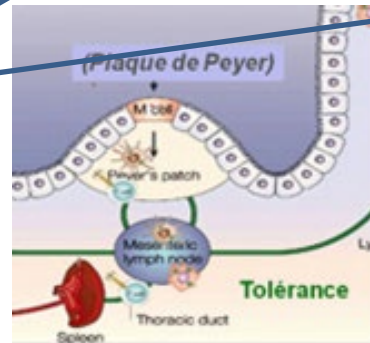
Selon Heyman, 2010

- Premier système de défense de l'Organisme (Ag alimentaires, Micro-organismes....)
- Homéostasie intestinale

Structure de l'épithélium intestinal



Cellules M



Entérocytes

.80% des ζ intestinales
.absorption



.transport transépithélial
.enzymes (mb, glycocalyx)

Cellules à mucus

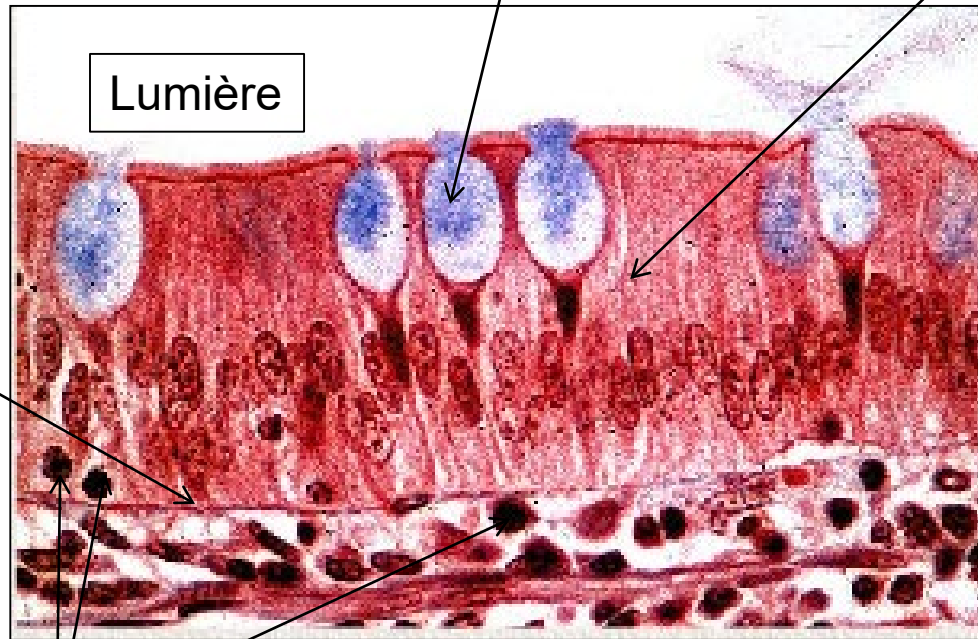
Entérocytes

Lumière

Membrane basale

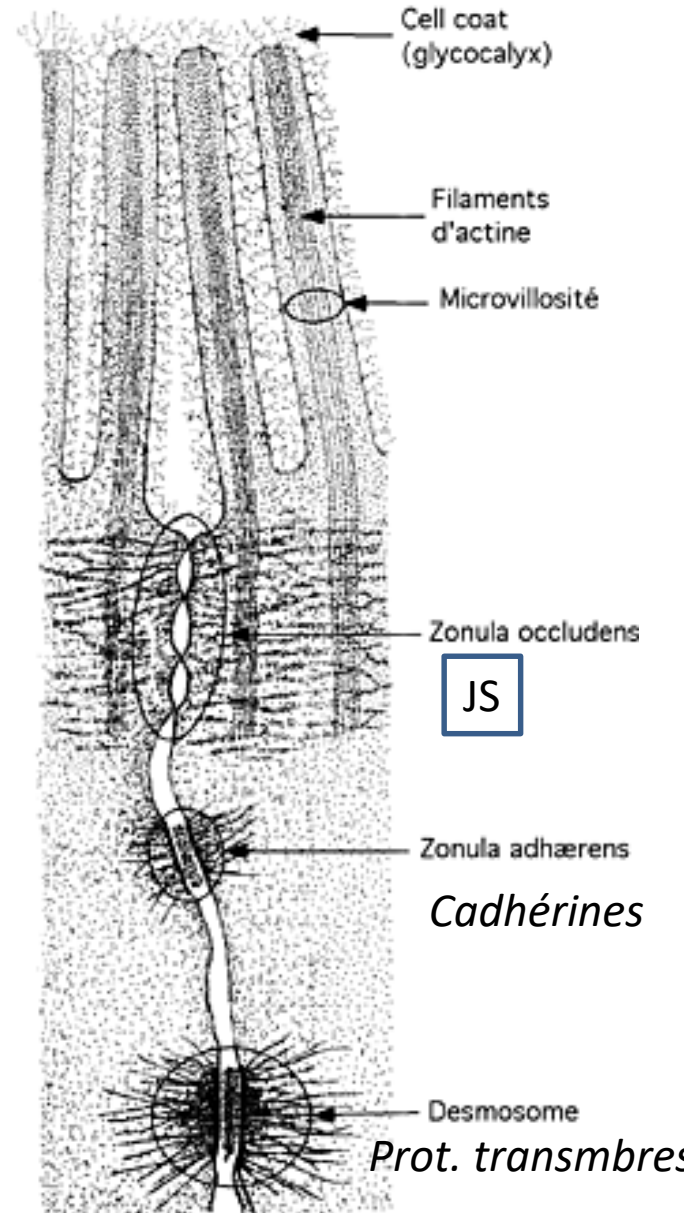
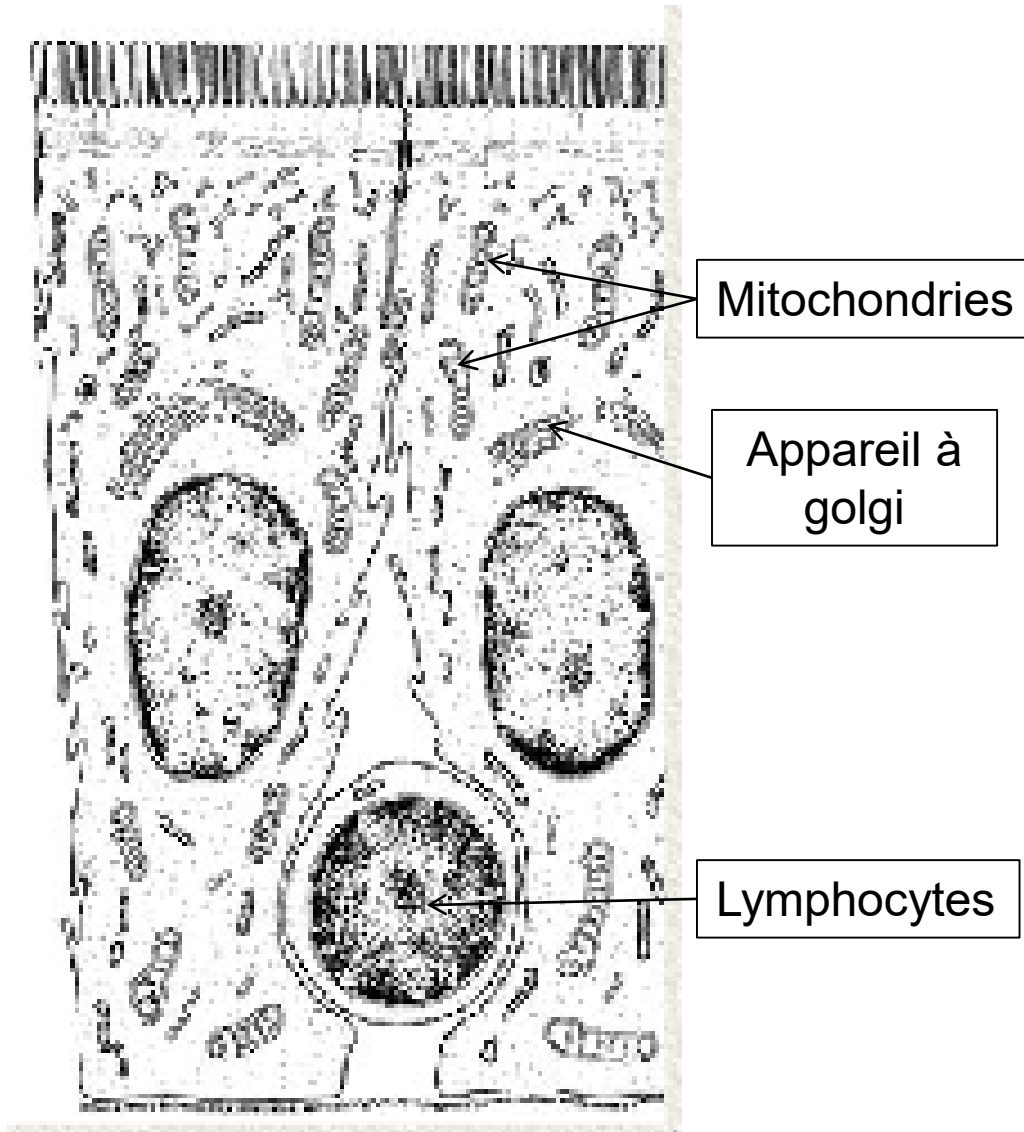
Chorion

Lymphocytes



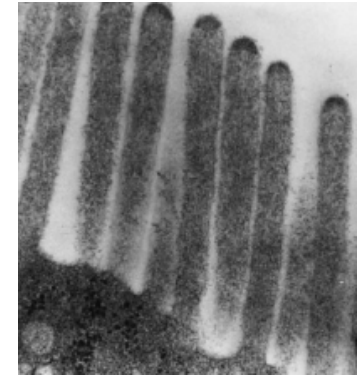
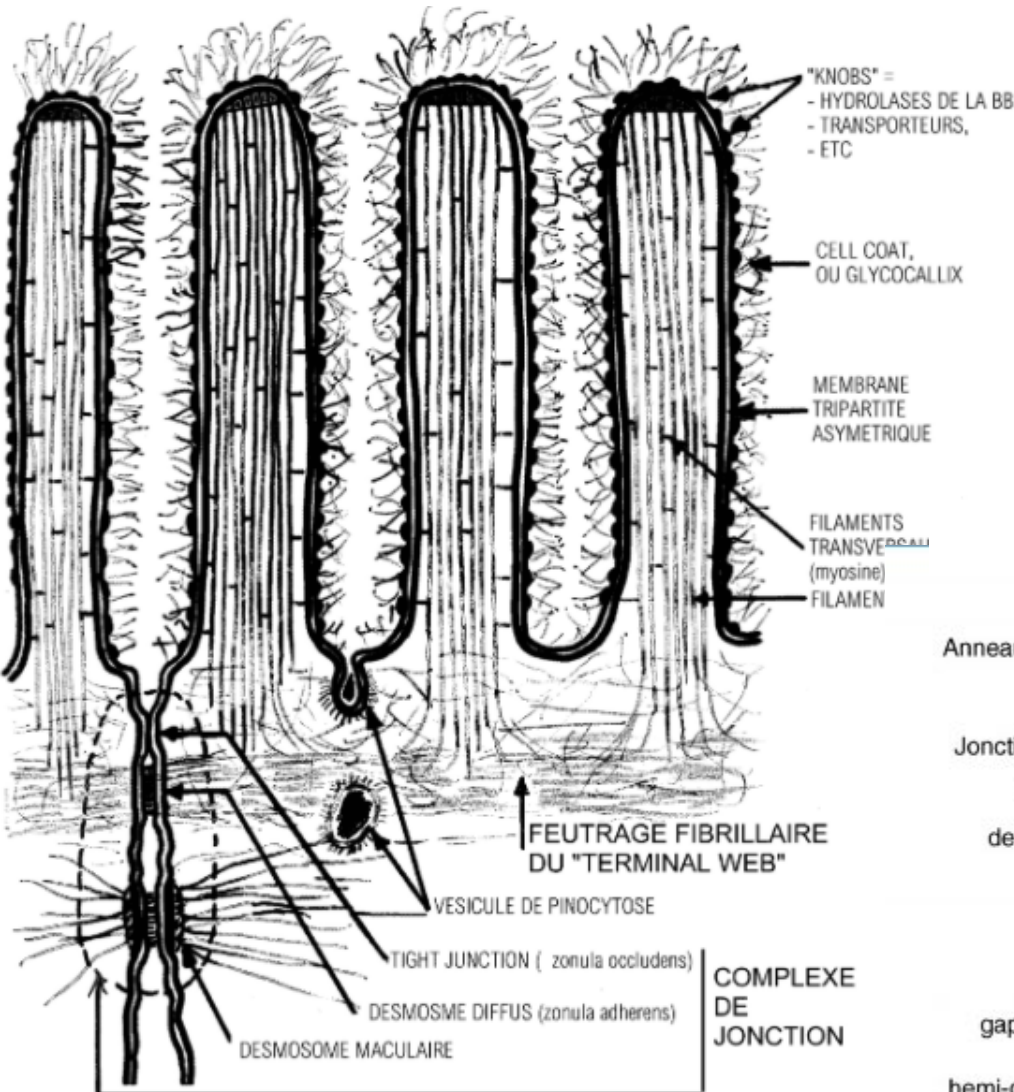
<http://www.chups.jussieu.fr/>

Entérocytes : structure ↔ perméabilité

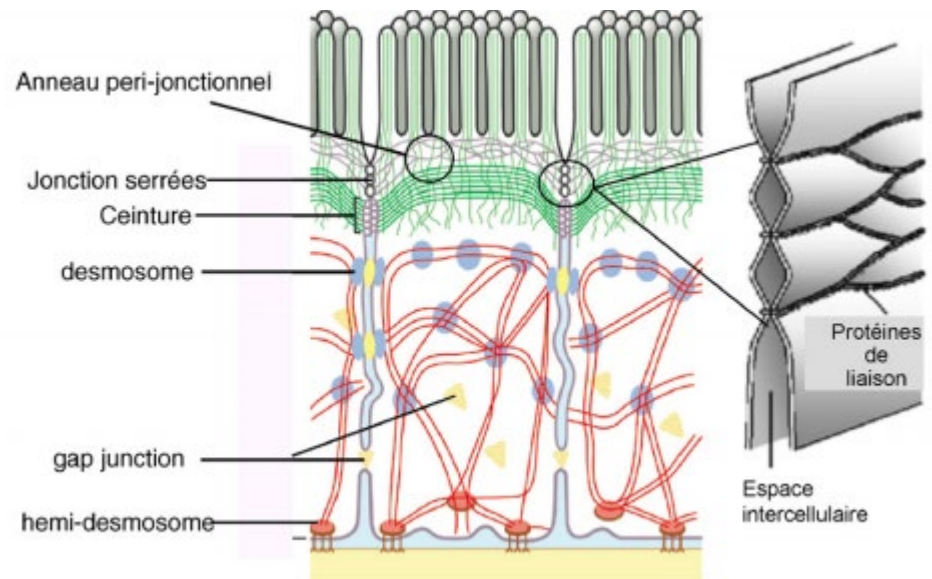


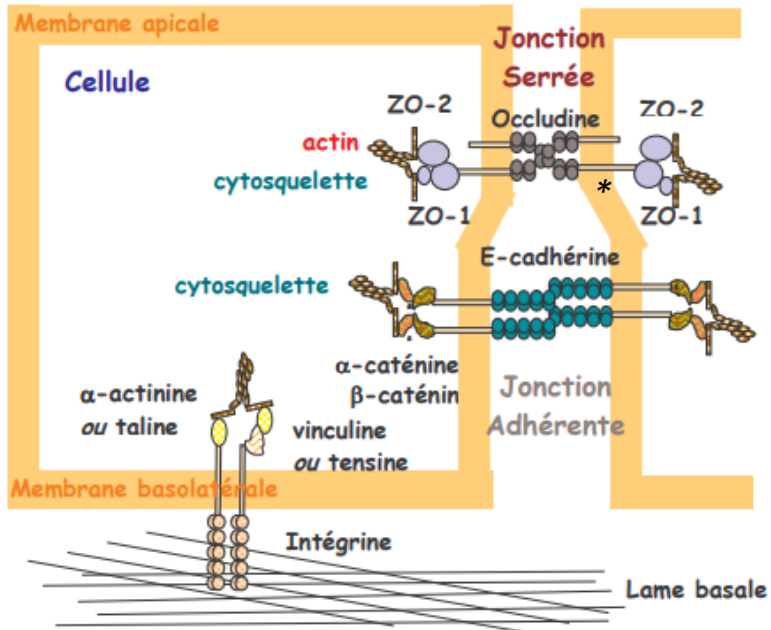
Entérocytes : structure →

Perméabilité Cellules Polarisées



Caco-2
(Jackman et al., 1994)



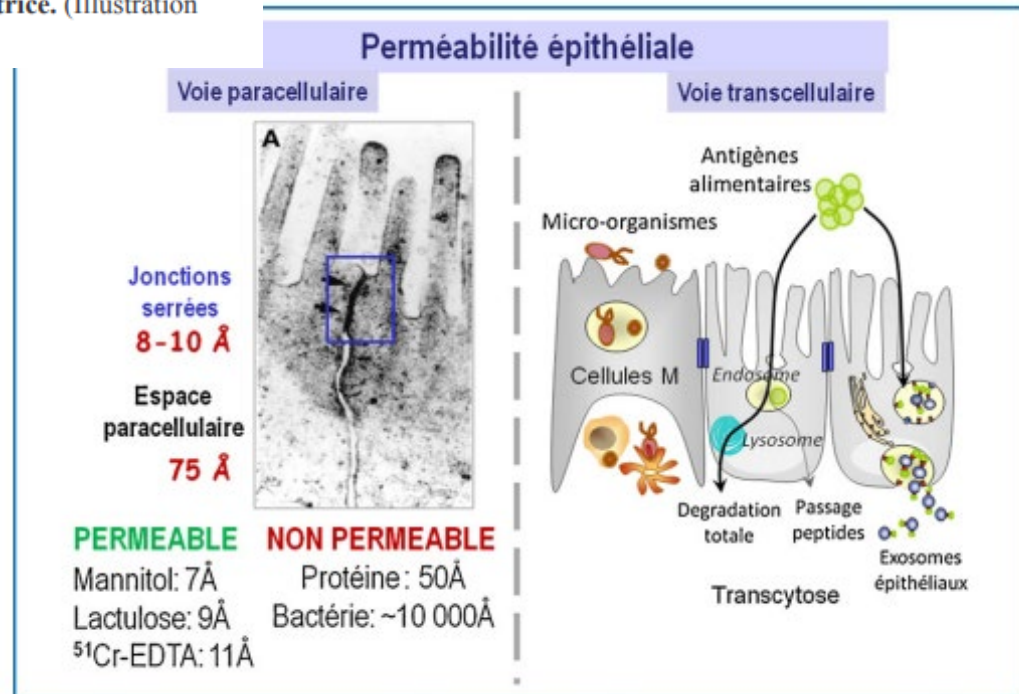


Entérocytes (JS): perméabilité

*actinomyosine

↑↓
absorption

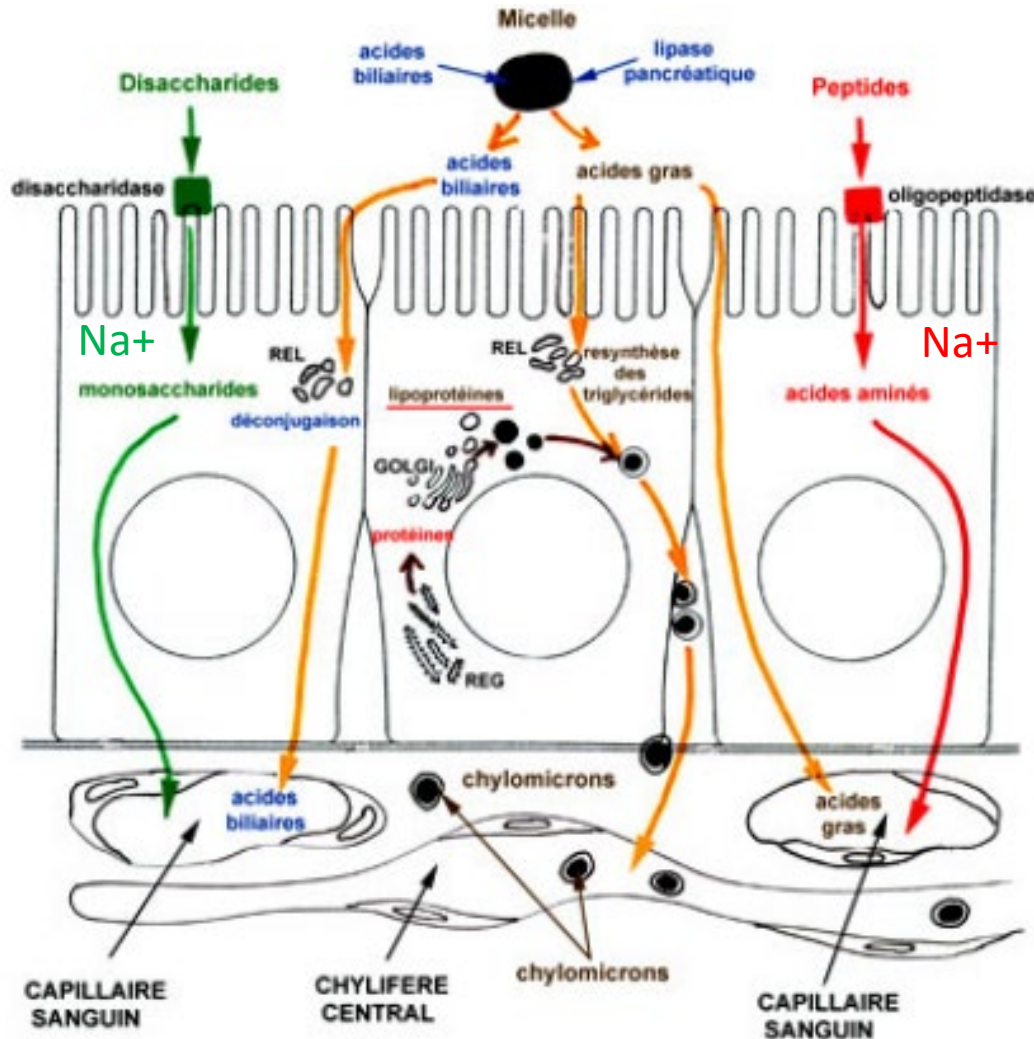
Figure 6 : Les jonctions intercellulaires et les interactions cellule-matrice. (Illustration Lebrun F.)



Heyman, 2010

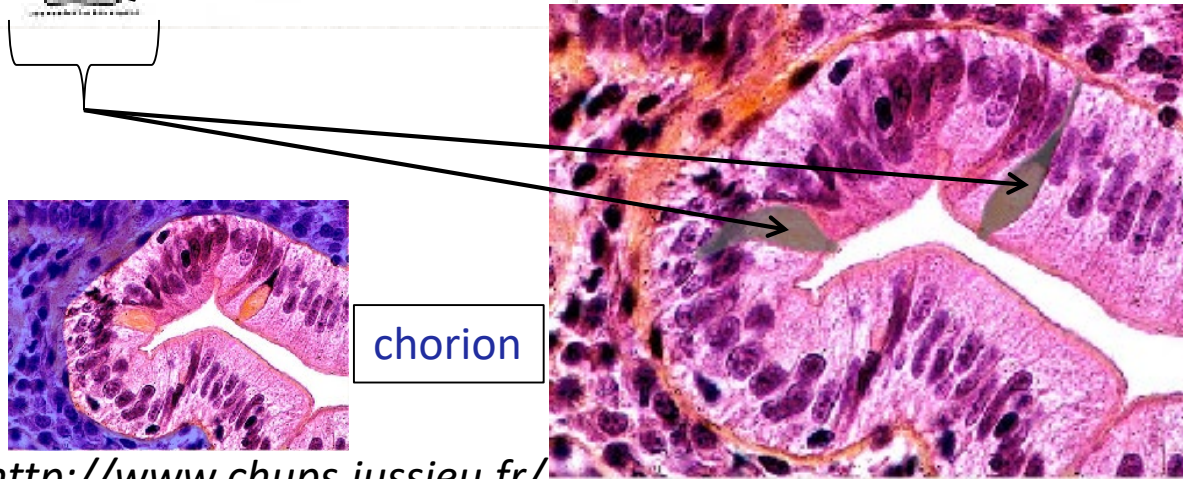
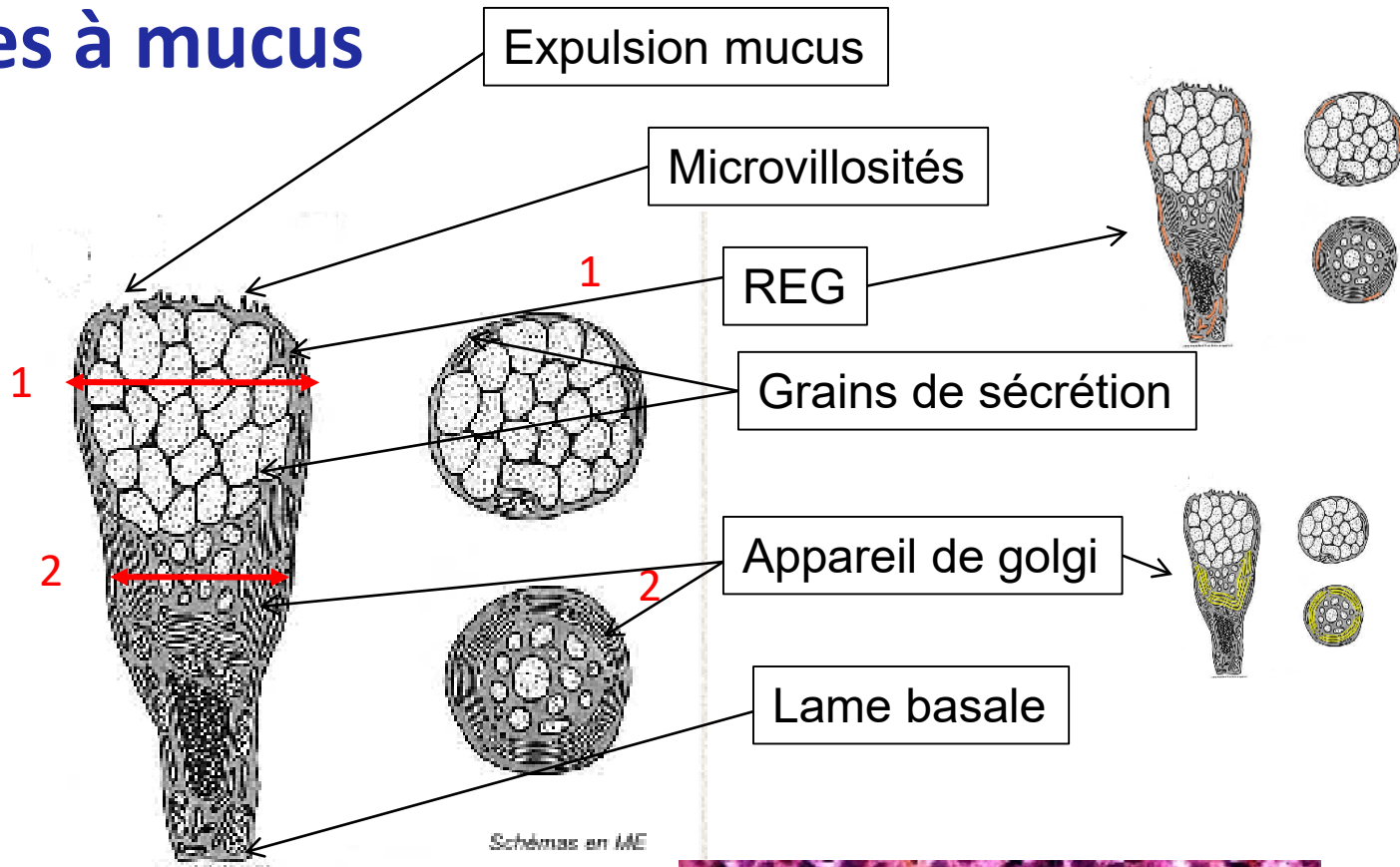
+ ...réseau microtubules

Entérocytes: métabolisme et absorption (ultimes étapes)



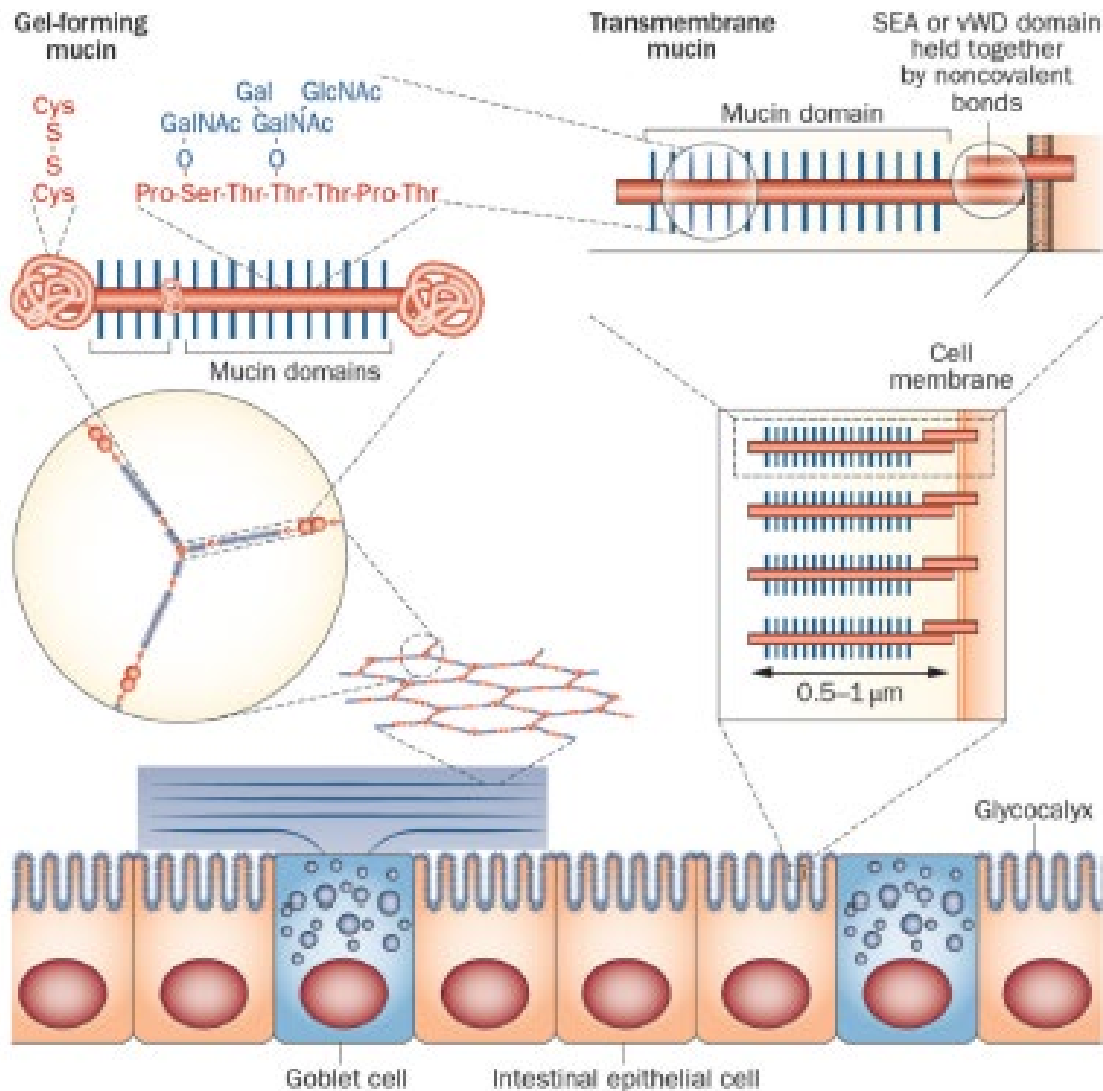
Selon N. Vacheret, UCB Lyon

Cellules à mucus



++ Colon

Mucus



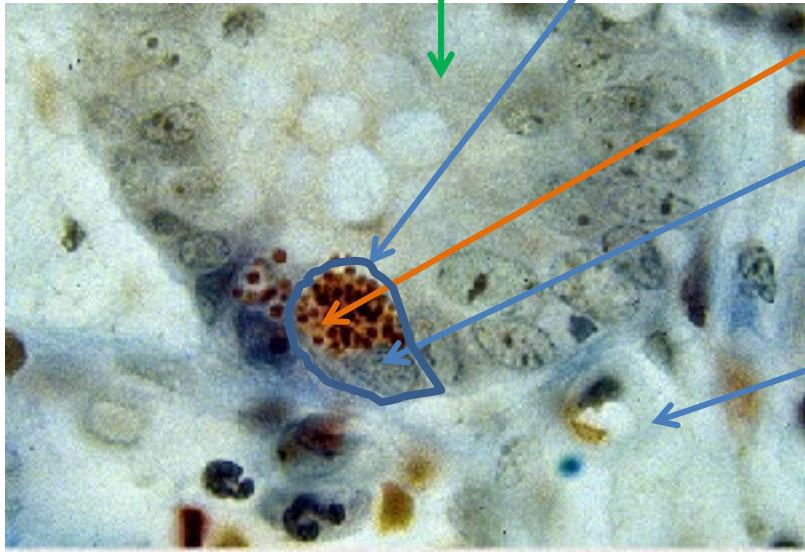
Mucin ⁺ (and type)	Cell type expression	Function
MUC1 (transmembrane)	Epithelial cells	Signalling, protection
MUC2 (gel-forming)	Goblet cells Paneth cells	Protection, lubrication, entrapment
MUC3 ^{II} (transmembrane)	Enterocytes	Apical surface protection
MUC4 (transmembrane)	Epithelial cells Goblet cells	Signalling, protection
MUC5AC (gel-forming)	Mucous cells	Protection, lubrication, entrapment
MUC6B (gel-forming)	Mucous cells Goblet cells	Protection, lubrication, entrapment

.....

Cellules de Paneth



Glande de Lieberkühn



Granulations

Noyau

Chorion

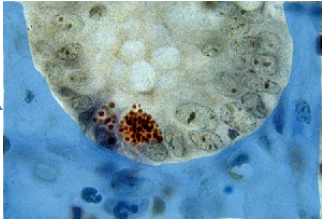
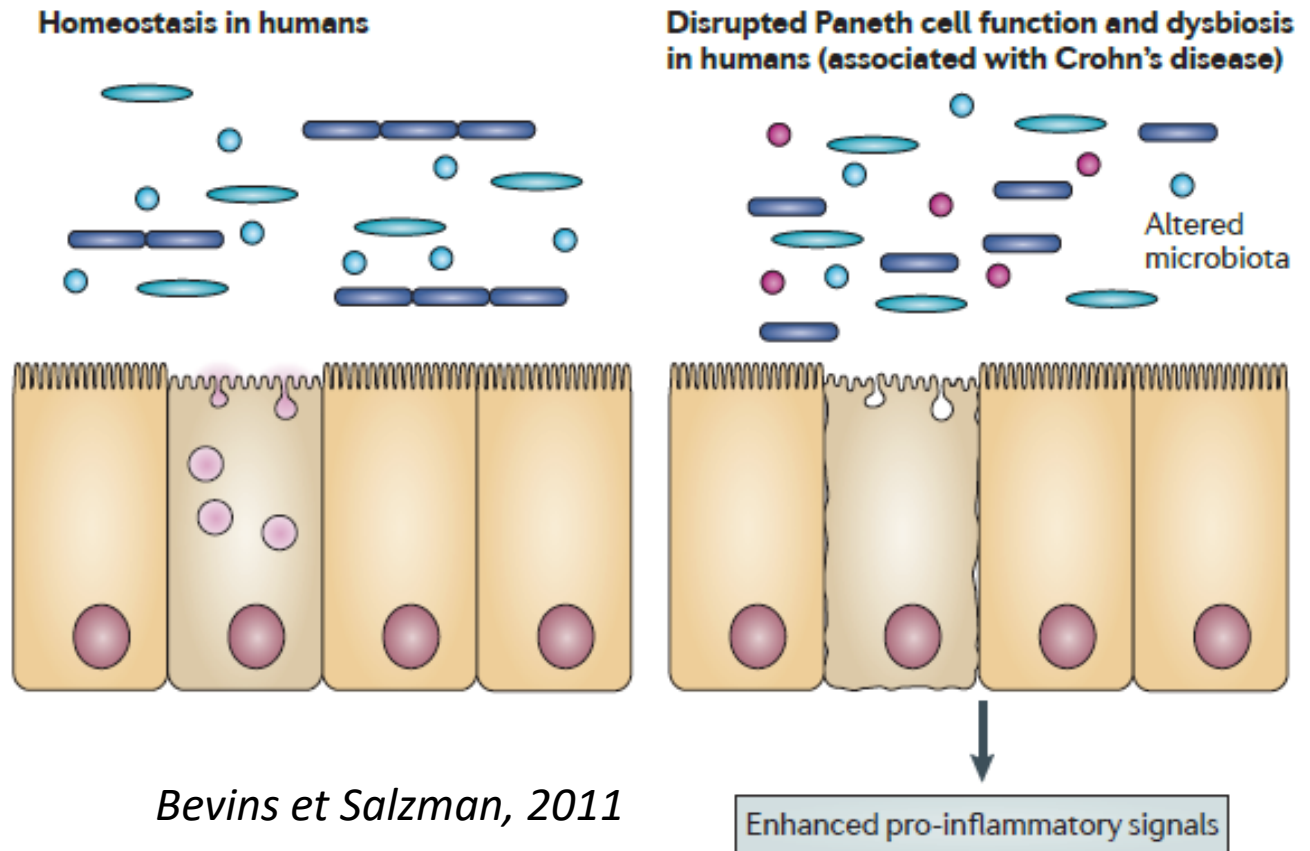


Table 1 | Paneth cell antimicrobials

Name	Biochemical classification	Antibacterial activities
α-defensins	Antimicrobial peptides	Against Gram-positive and Gram-negative bacteria
CR5 peptides (in mice)	Antimicrobial peptides	Against Gram-positive and Gram-negative bacteria
Lysozyme C	β -1,4-glycosidase	Against Gram-positive and (to a lesser extent) Gram-negative bacteria
sPLA2	Phospholipid-sn-2 esterase	Against Gram-positive bacteria only

Cellules de Paneth : fonctions

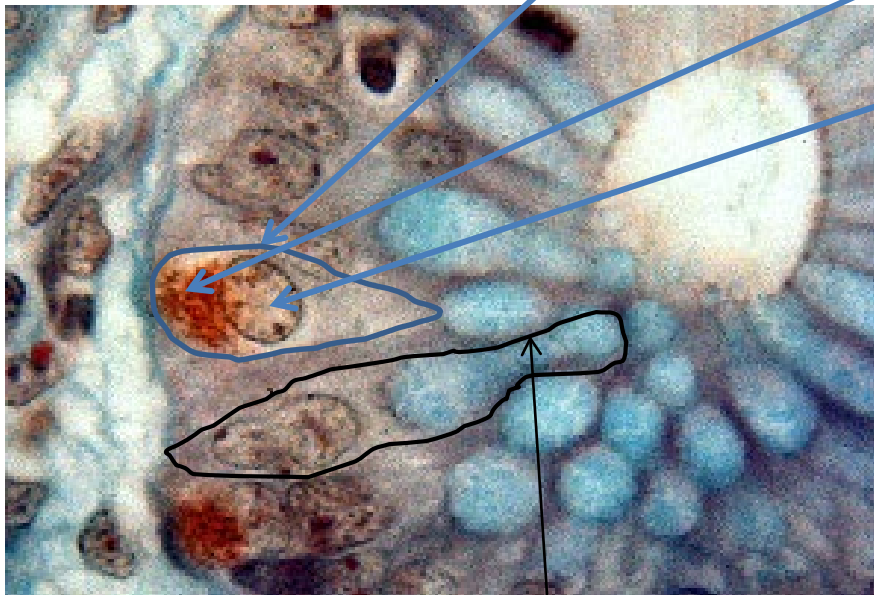
- ✓ Homéostasie intestinale
- ✓ Inflammation



Cellules entéro-endocrines

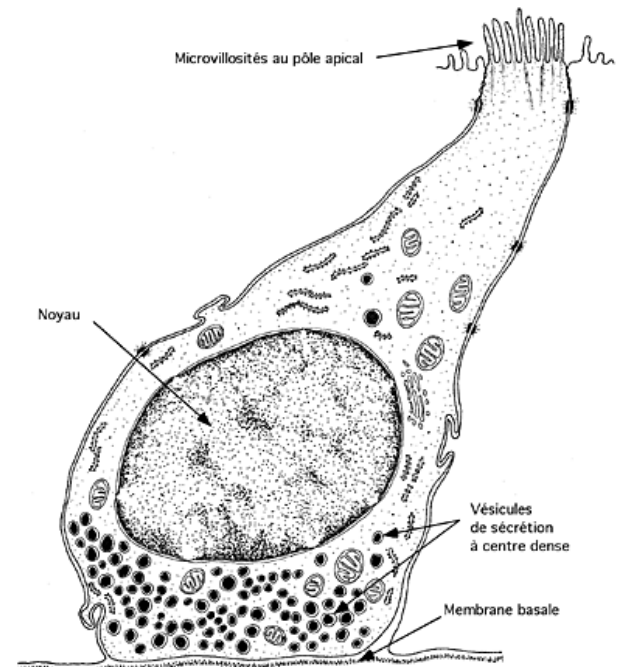
Granulations

Noyau



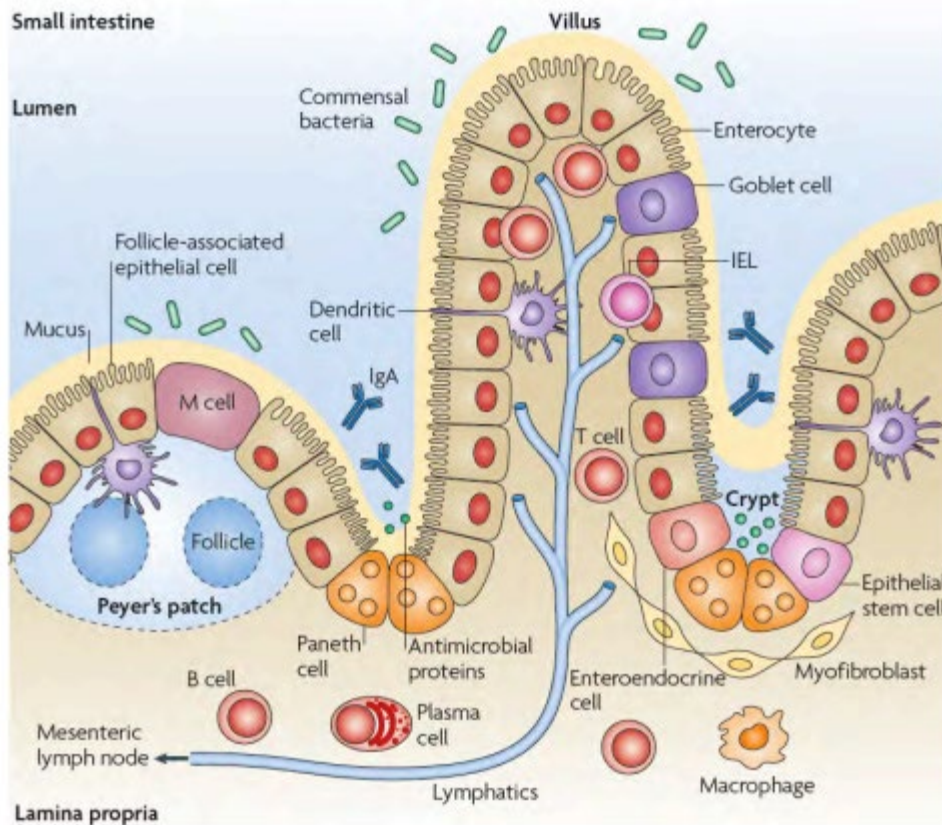
Cellule à mucus

- ++ cryptes
- Hormones: cholécystokinine, Gastric inhibiting peptide, sécrétine....
- Glutamate?



Cellules M

Effecteurs immunitaires de la muqueuse intestinale



Epithelium:

- Enterocytes
- Goblet cells
- Paneth cells
- Intra-Epithelial Lymphocytes

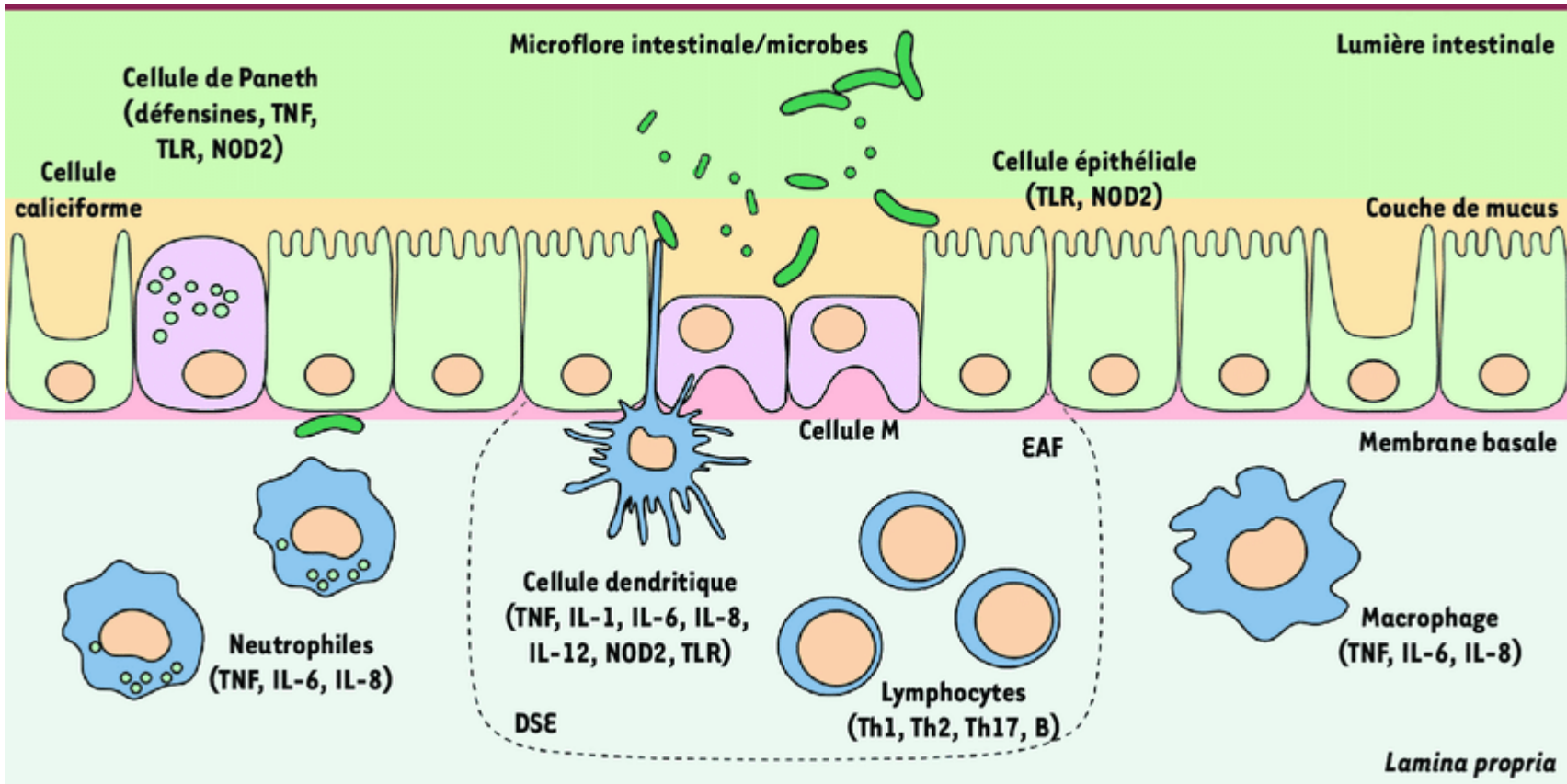
Lamina propria:

- Lymphocytes T CD4+ / T CD8+
- Plasmocytes IgA+
- DC
- Macrophages
- Innate Lymphoid cells

Plaques de Peyer:

- DC
- Lymphocytes T CD4+ / T CD8+
- Lymphocytes B

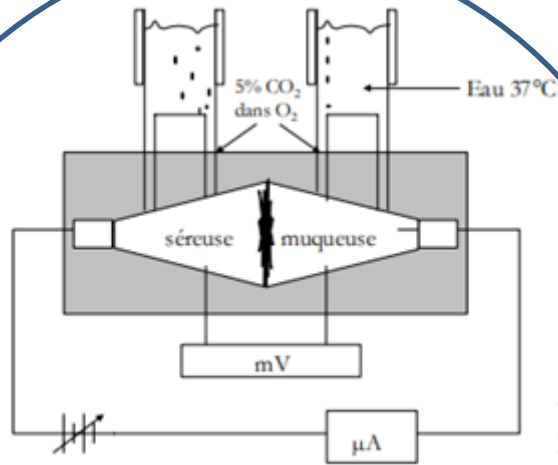
Synthèse sur les mécanismes de défense (exemple des MICI)



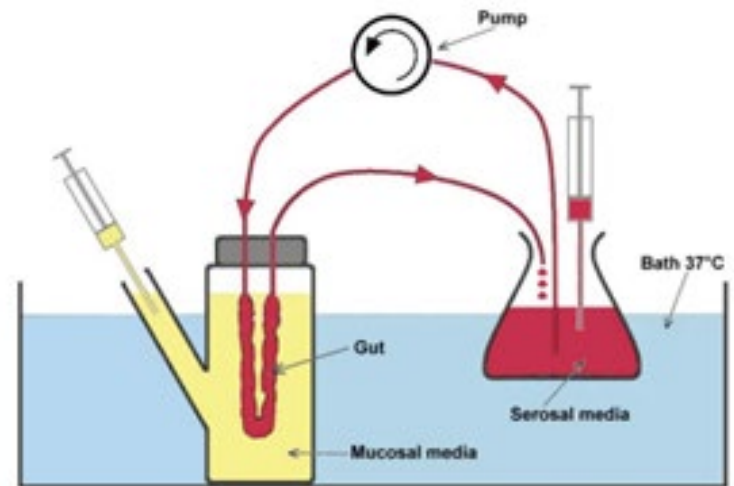
Quels modèles d'études ?

- Objectifs de ces études
- *in vivo* et *in situ* techniques avec différents modèles animaux → extrapolation ?
- *ex-vivo* modèles de tissus excisés humains ou animaux
- Modèles de culture cellulaire *in vitro*

MODELES *IN VITRO*:*EX VIVO*

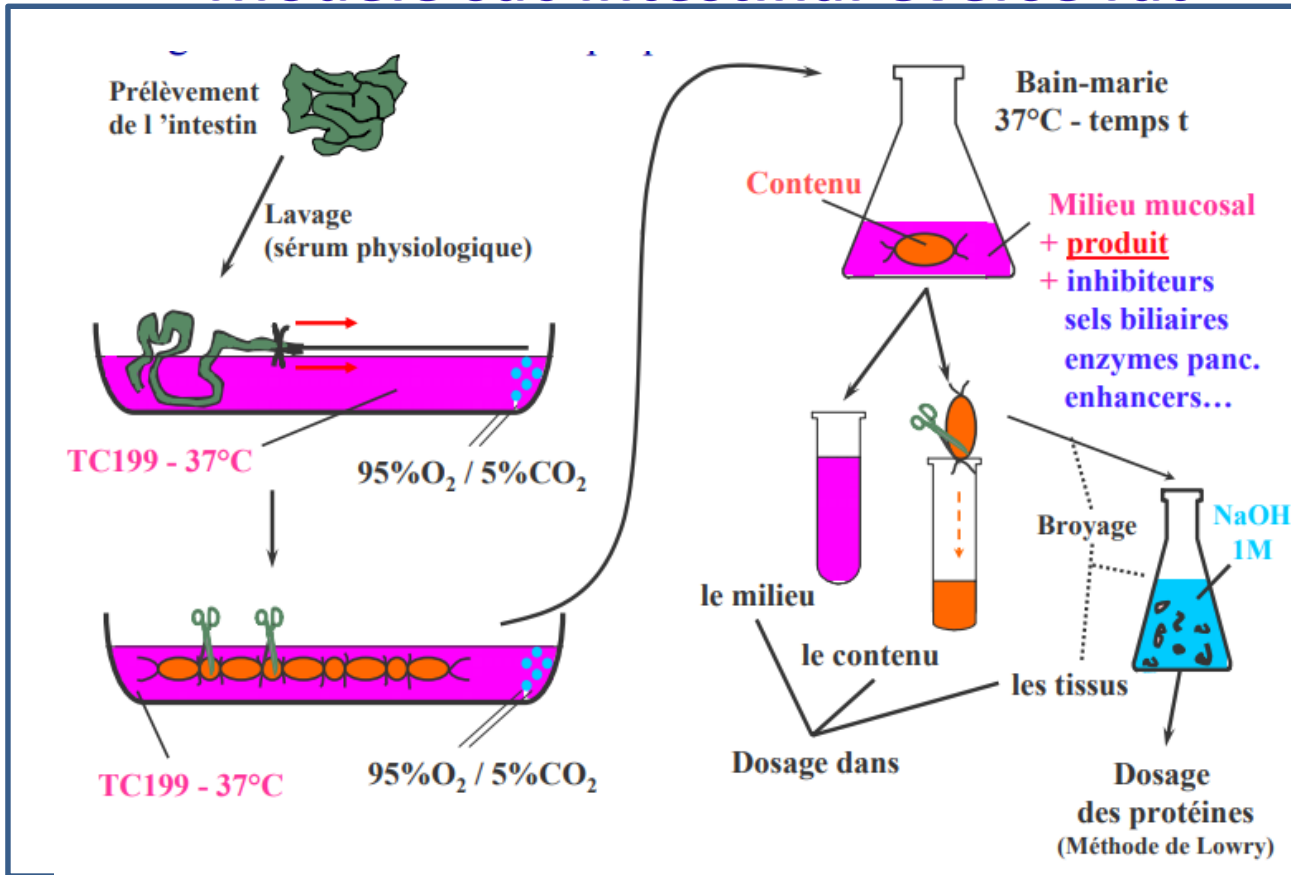


Chambre de Ussing

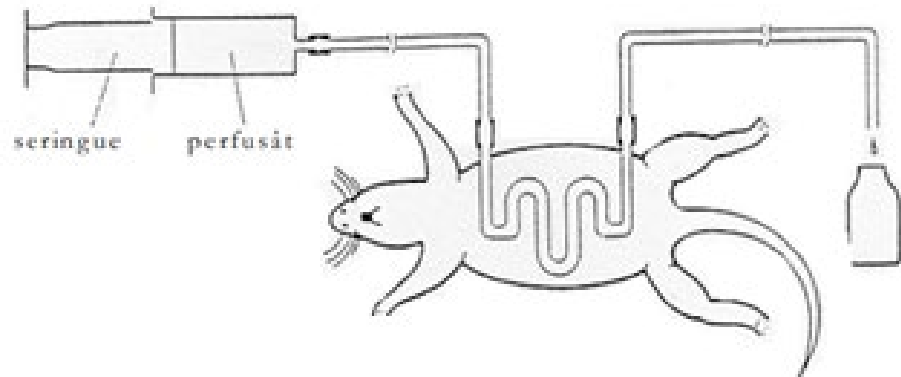


Segment intestinal éversé perfusé

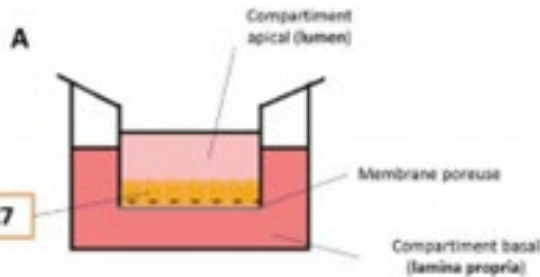
Modèle sac intestinal éversé rat



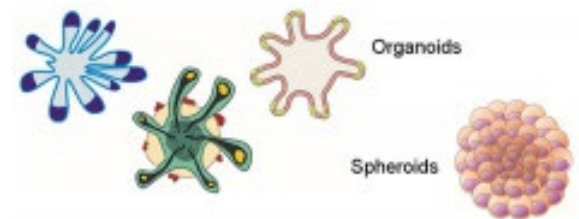
Système de perfusion *in situ* chez le rat



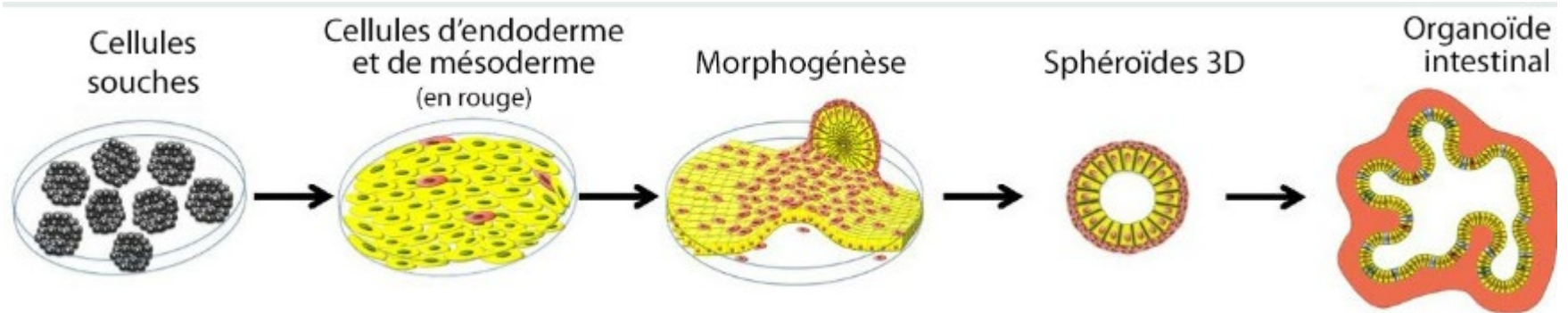
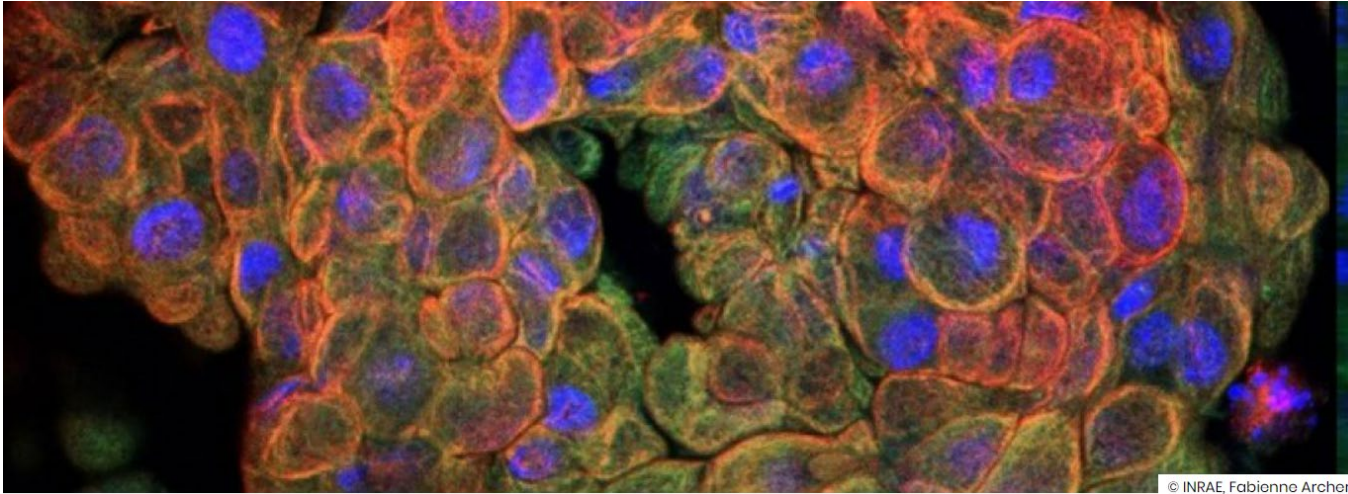
Modèles de cultures cellulaires



- ✓ Outil essentiel avant essais in vivo et essais cliniques
- ✓ Rendu possible grâce aux progrès de la culture cellulaire
- ✓ Etudes en toxicologie, pharmacologie, biologie cellulaire...
- **Cultures primaires** (cellules indifférenciées et différenciées)
- **Lignées de culture cellulaire** (Fogh)
- Évolution: **co-culture** (+s lignées), 2-D
- **Organoïdes**, modèles in vitro 3-D
- ✓ Nombreux avantages



Organoïdes: avenir?



Selon Welles and Spence, Development, 2014, 141:752)

Modèles cellulaires intestinaux

Cell line	Origin	Derived from	Purchased from ATCC at passage number
Caco-2	Caucasian male, 72 years	Colorectal adenocarcinoma	18
C2BBel	Caucasian male, 72 years	Colorectal adenocarcinoma	47
HT29	Caucasian female, 44 years	Colorectal adenocarcinoma	128
T84	Male, 72 years	Colorectal carcinoma, metastatic site (lung)	53
FHC	13 weeks gestation	Normal colon	16

➤ Avantages (si conditions rigoureuses de culture):

Essais à long terme

Reproductibilité

➤ Limites



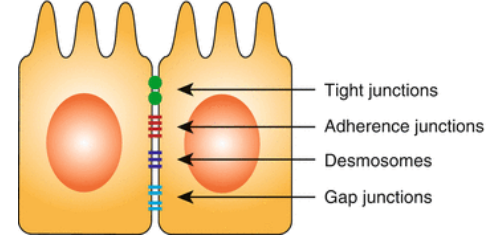
PROTOCOLES:

- > Impact sur cellules
- > Absence de contaminations

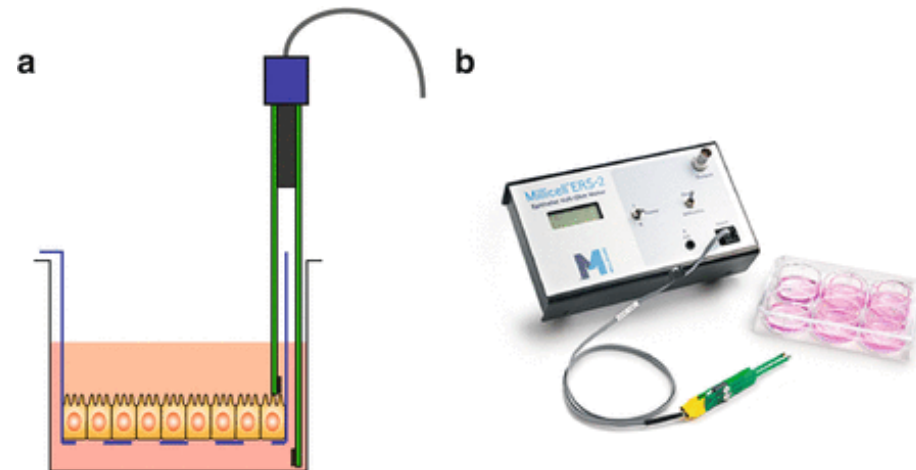
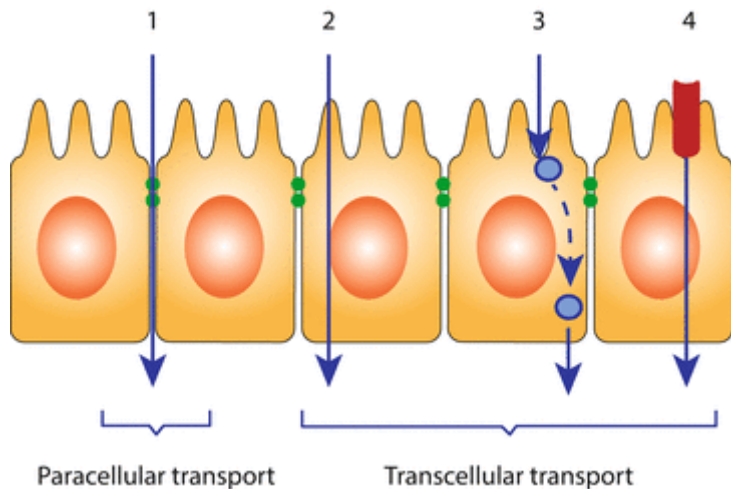
- Solutions salines et milieux de culture définis
- pH et CO₂
- Sérum (veau foetal...)
- Conditions variables selon lignées / sélection de clones
- Evolution en fonction du nombre de passages (gestion « stock »..)
- Laboratoire dédié (PSM, matériel dédié, procédures à suivre, tests...)



Caco-2 (caractéristiques 1)



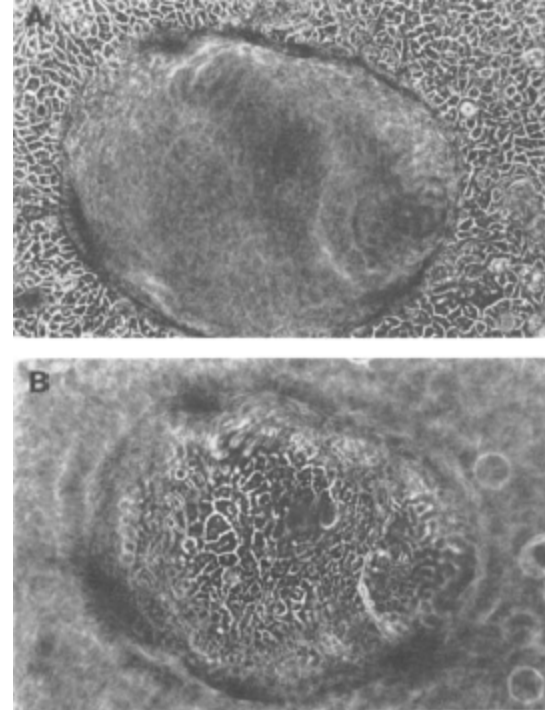
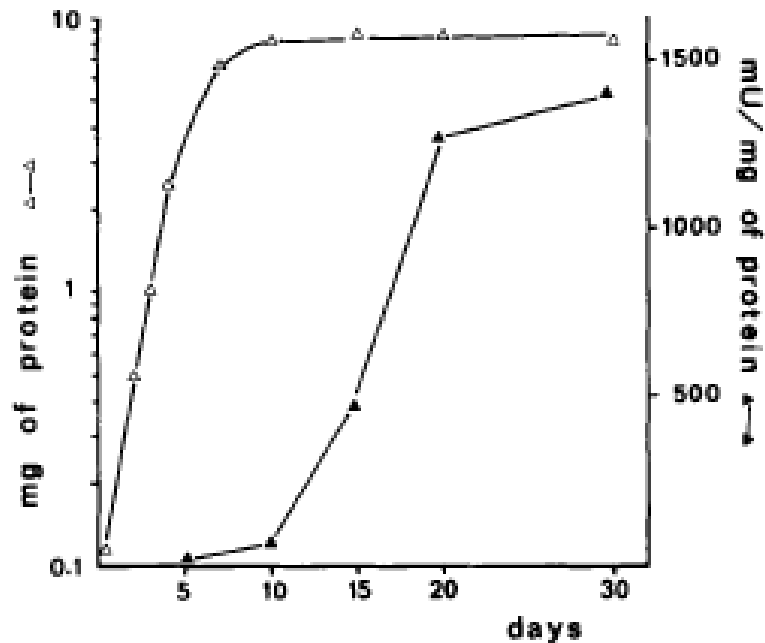
- ✓ **Monocouche polarisée** (pôle apical et basal), **Bordure en brosse**
- ✓ **Différenciation au cours de la culture: J5 → J15**
- ✓ **Résistance électrique transépithéliale importante**
- ✓ **Enzyme digestives**: peptidases membranaires et dissaccharidases de l'intestin grêle (lactase, aminopeptidase N, saccharase-isomaltase et dipeptidylpeptidase IV)



$$R_{\text{sample}} - R_{\text{blank}} = R_{\text{cell layer}}$$

$\Omega \text{ cm}^2$

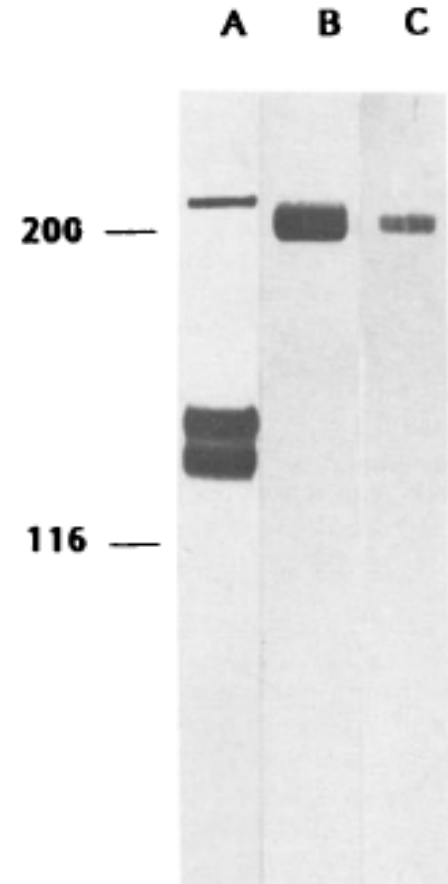
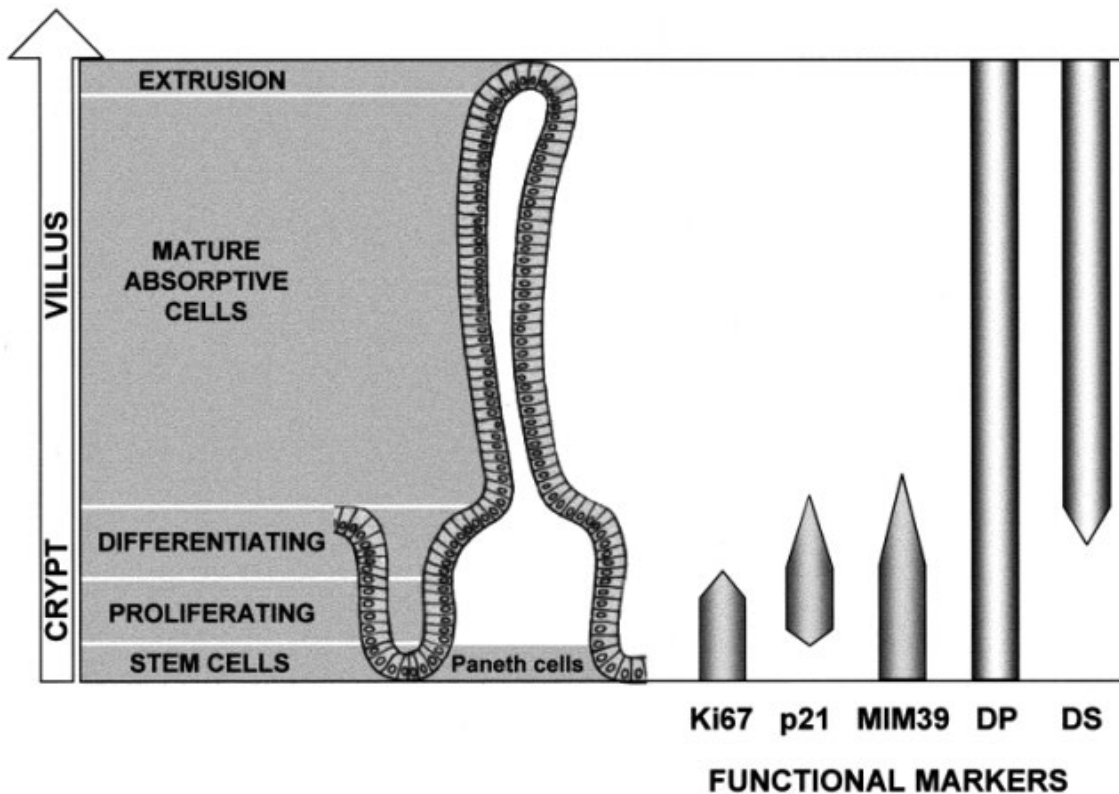
Différenciation spontanée (après confluence)



(Pinto, 1983)

Variation sucrase activité BB en fonction culture

Axe crypto-villose de l'intestin grêle



Pageot and al., *Microsc.Res.Tech.*, 2000.49:394-406)
Adapté Beaulieu 1988

Sucrase-isomaltase dans
fractions enrichies BB
A: jéjunum adulte
B: Caco-2 J15 (P70)
C: Colon foetus

Études TEER / Transport (inserts)

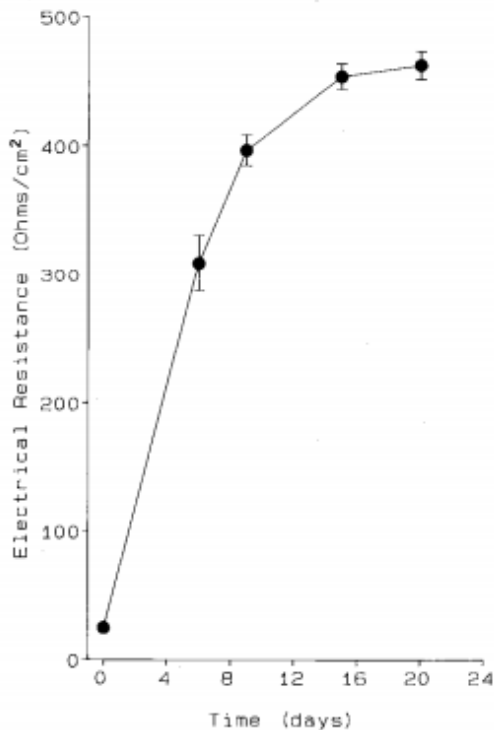


Fig. 5. The transepithelial electrical resistance of Caco-2 cells on nitrocellulose filters with time. Results \pm S.E.M. for 12 samples are shown.

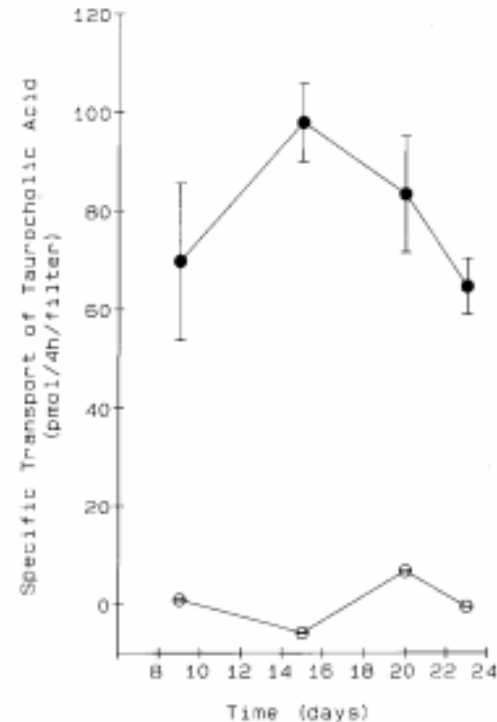
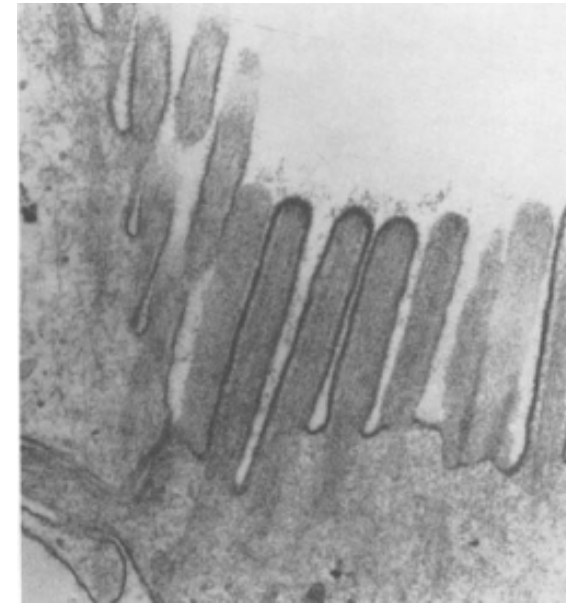
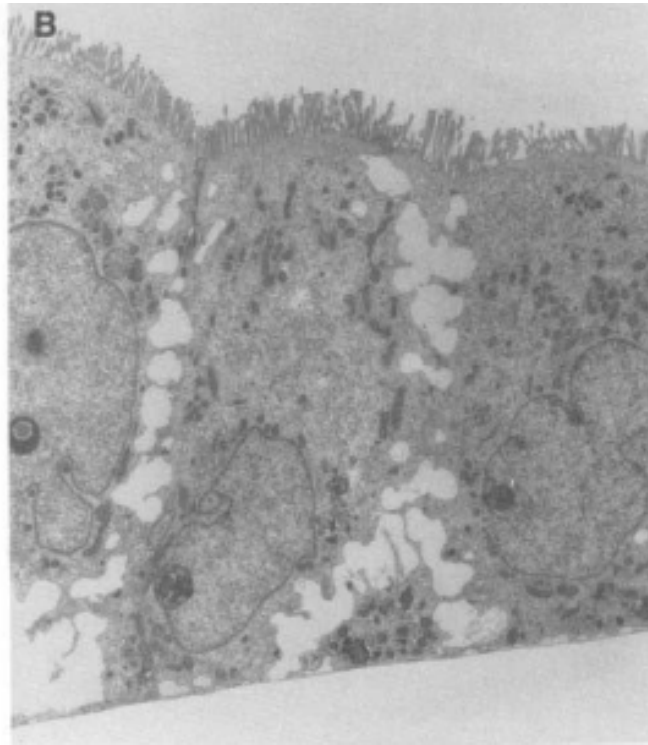
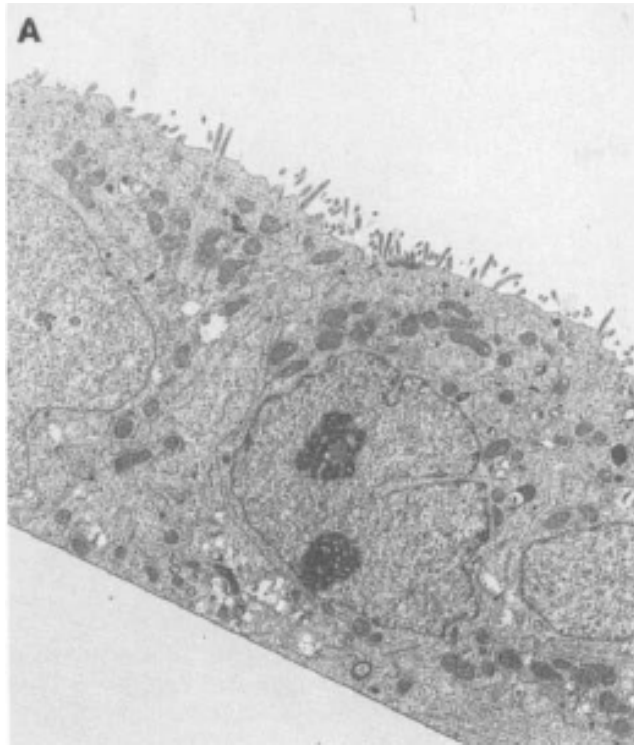


Fig. 6. The specific transport of [¹⁴C]taurocholic acid across Caco-2 cells cultured on nitrocellulose filters. Specific transport of taurocholic acid (apical to basolateral, (●); basolateral to apical, (○)). Result \pm S.E.M. for 3 samples are shown.

Wilson et coll, J. Control. Release, 1990

- Nombre repiquages
- Corrélation absorption médicaments in vivo, notamment jéjunum (surtout ++ transport passif intracellulaire)

Expression de la bordure en brosse en fonction de la différenciation

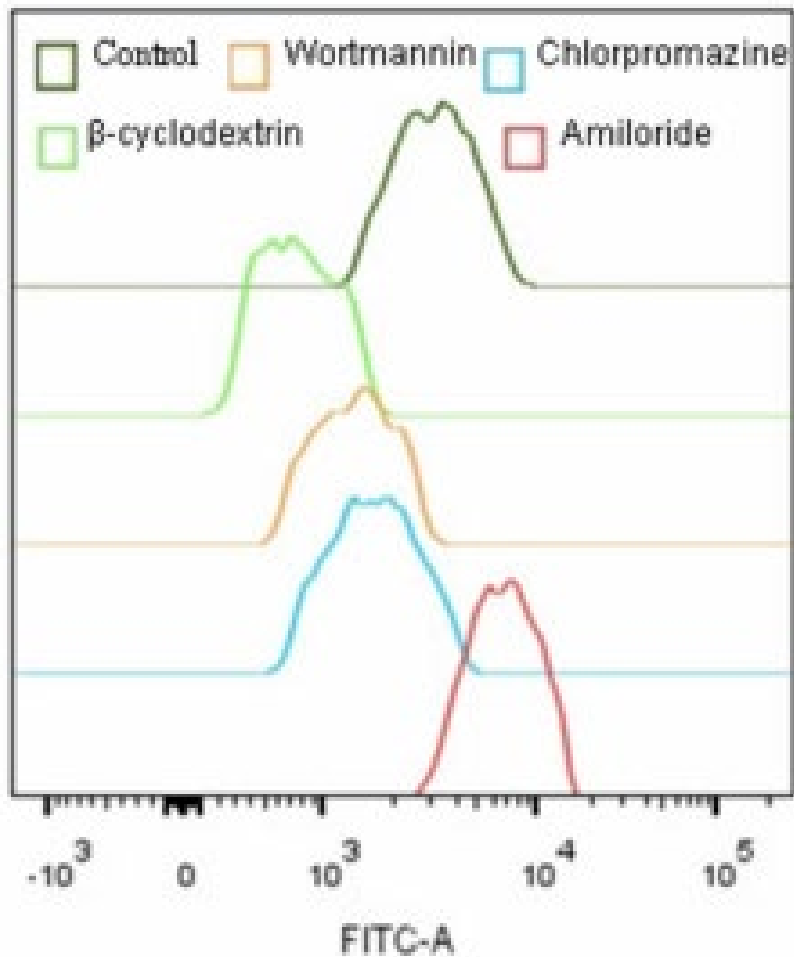


A : J5
B : J10
C : J15

Caco-2 (caractéristiques 2)

- ✓ **Transport actif** : acides aminés, sucres, vitamines, hormones
- ✓ **Transport ionique membranaire**: Na⁺/K⁺ ATPase, H⁺/K⁺ ATPase, Echangeur Na⁺/H⁺, co-transporteur Na⁺/K⁺/Cl⁻, canaux Cl⁻ apicaux
- ✓ **Transporteurs non-ionique membranaire**: glycoprotéine P (P-gp)...
- ✓ **Récepteurs**: vitamine B12, D3, EGFR (epidermal growth factor receptor), transporteurs sucres GLUT1, GLUT2, GLUT3, GLUT5, SGLT1
- ✓ **Production cytokines**: IL-6, IL-8, TNF- α , TGF- β 1, TSLP (thymic stromal lymphopoietin), IL-15
- ✓ **PAS DE MUCUS**

Essais cellulaires pour l'étude du transport des médicaments (2.1)

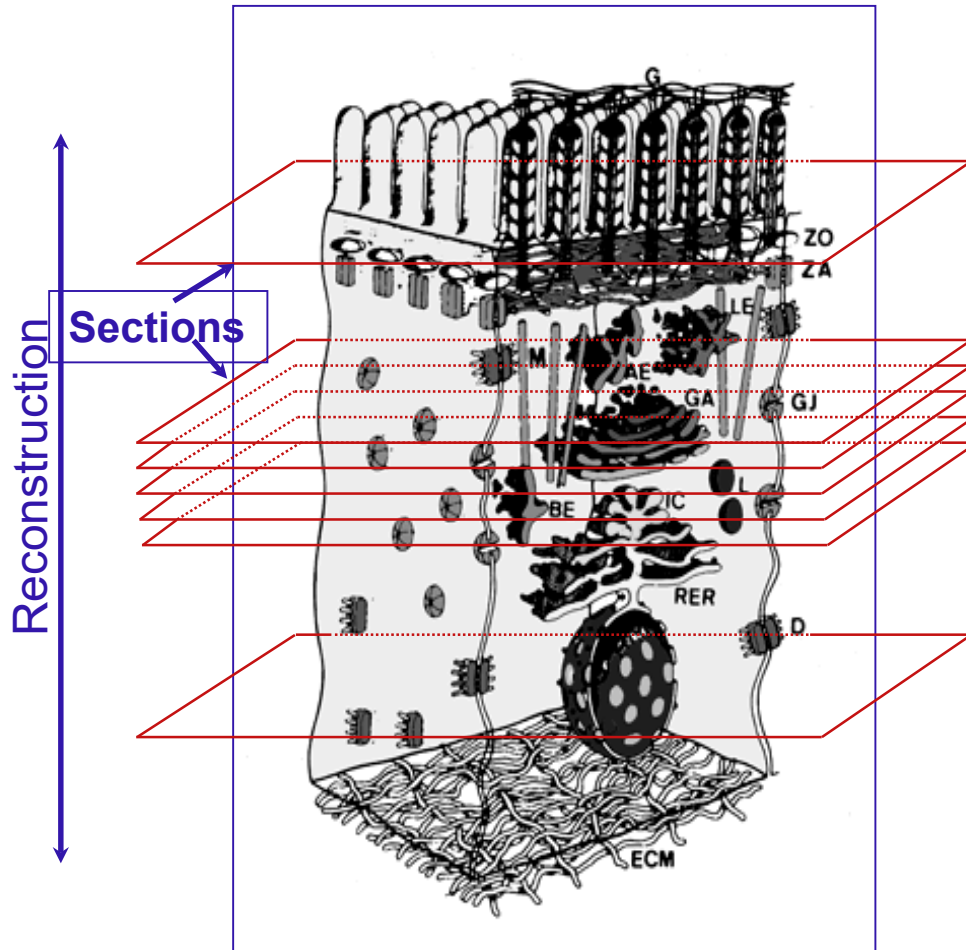


Screening de plusieurs mol. pharmaco

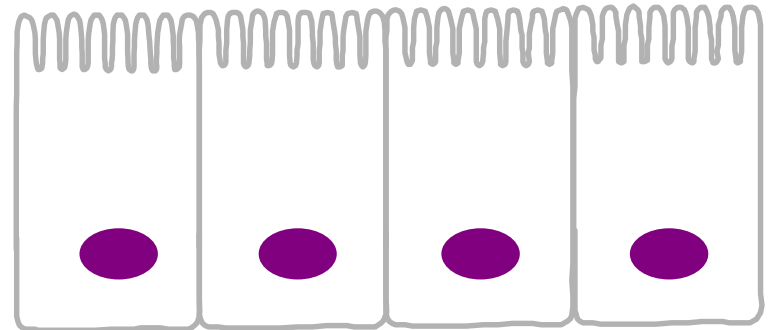
Experimental Evaluation of the Transport Mechanisms of Pig IFN- α in Caco-2 Cells, Liu et al., Front. Pharmacol., 2017

→ endocytose via les raft-lipiques

CONFOCAL LASER SCANNING MICROSCOPY (2.2)

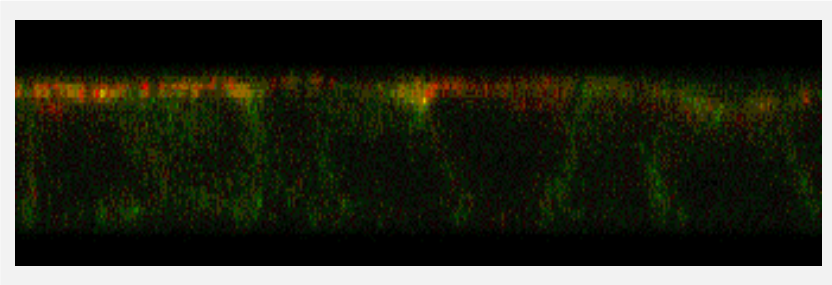


Luminal space



Serosal space

IMMUNOLABELLING

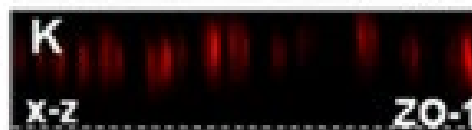
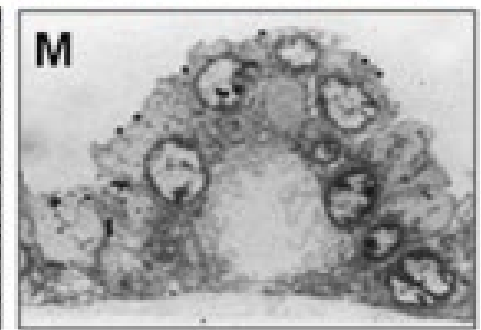
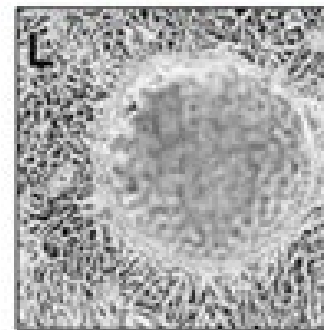
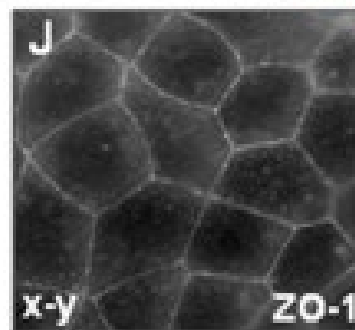
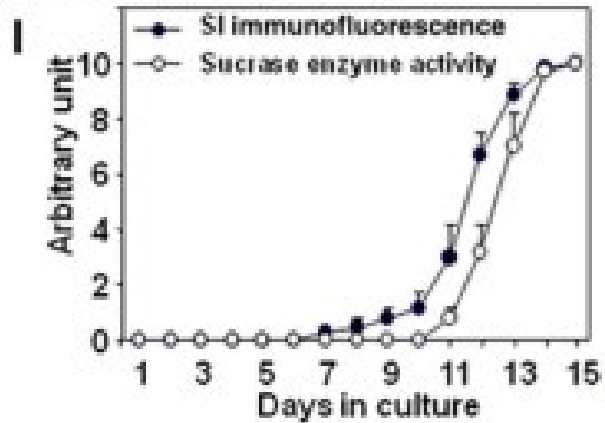
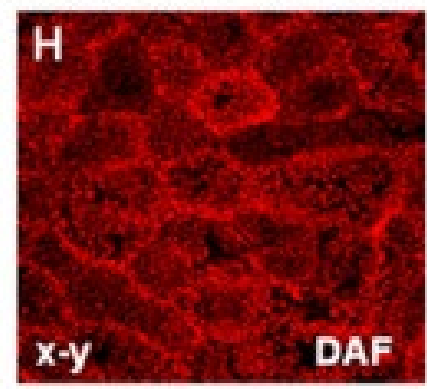
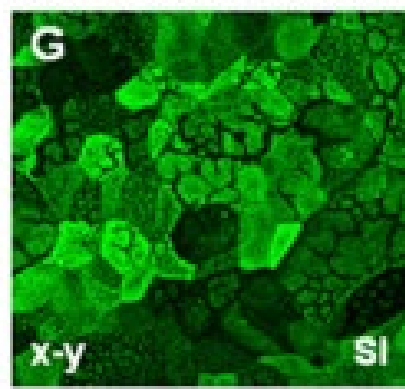
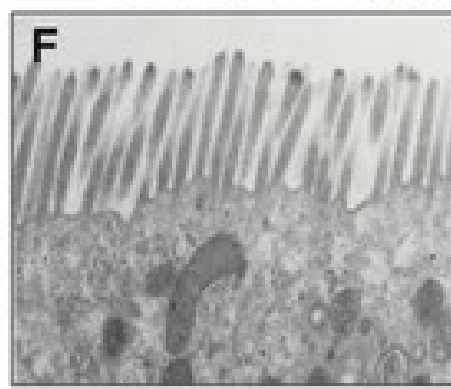
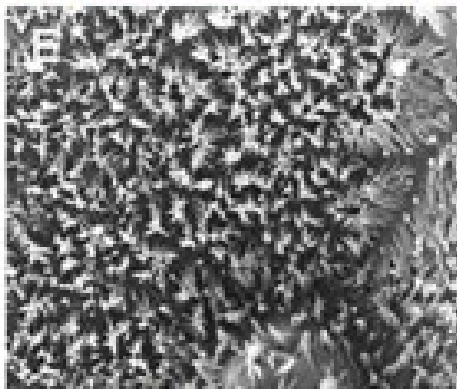
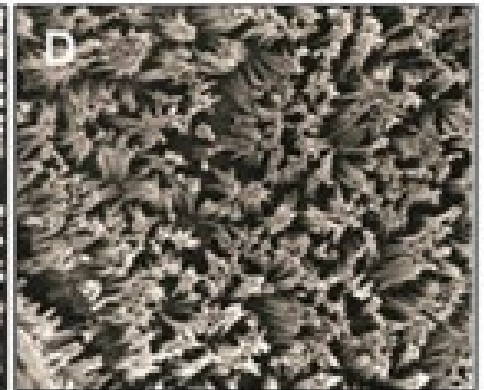
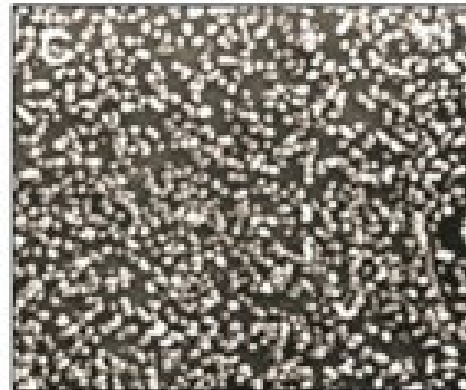
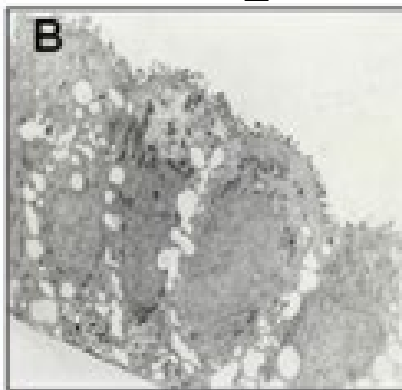
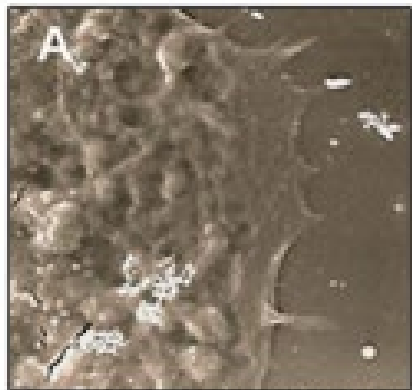


J3

TEM_J15

J10

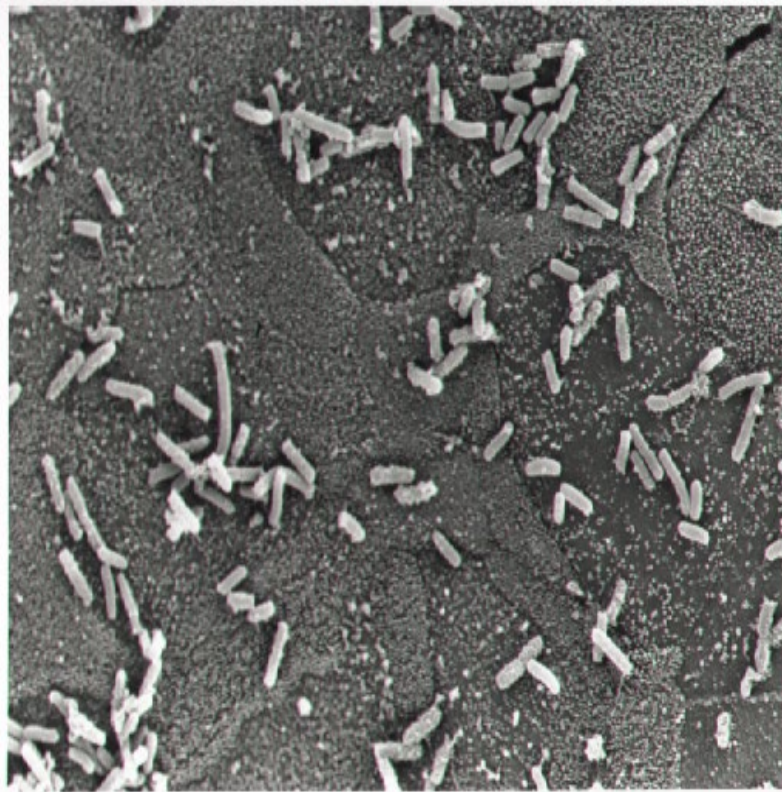
J15



Caco-2 / TC7

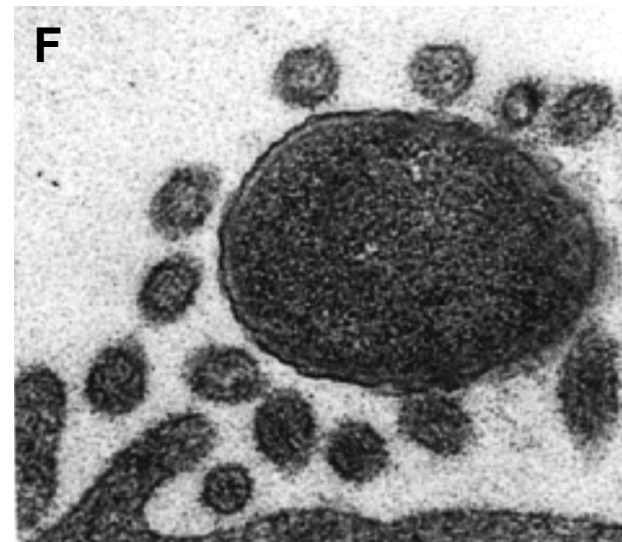
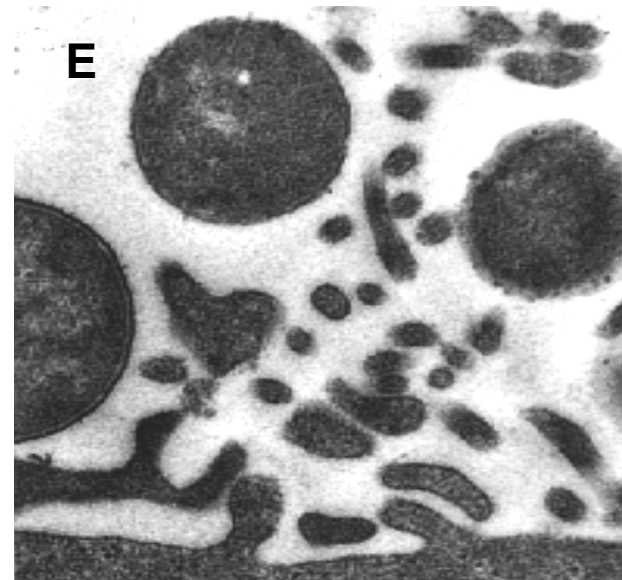
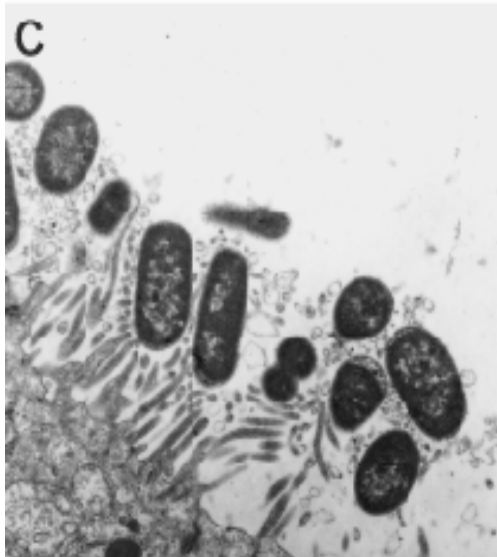
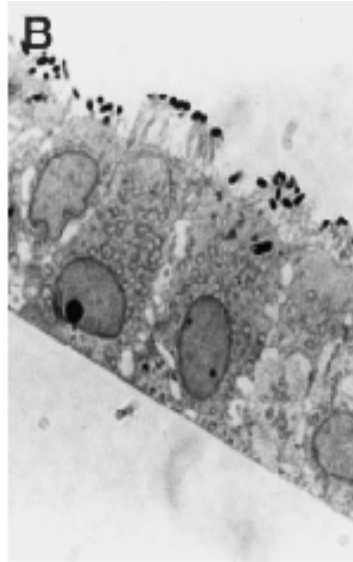
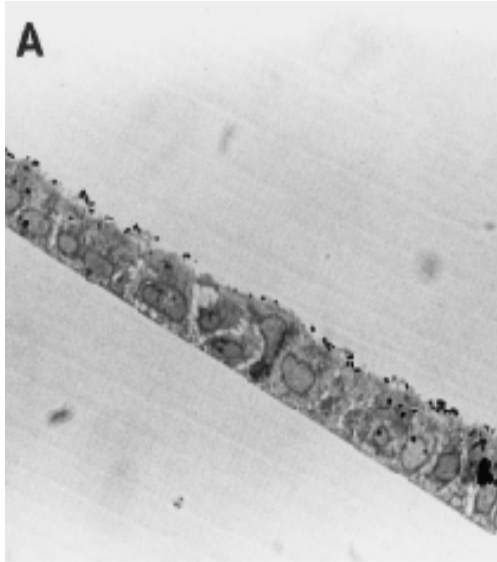
(selon Lievin et coll, 2013)

***Escherichia coli* d'adhésion diffuse de la famille Afa/Dr (Afa/Dr DAEC)**



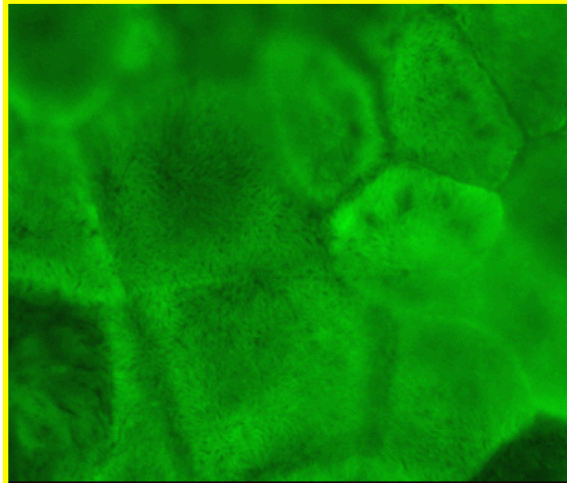
Adhésion diffuse du DAEC C1845 sur les cellules Caco-2, *Bernet et coll.*

BRUSH BORDER LESIONS IN Afa/Dr DAEC C1845-INFECTED HUMAN INTESTINAL CELLS

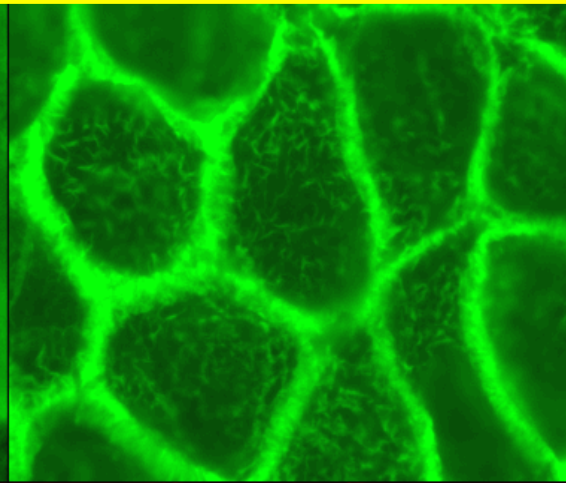


ALTERATIONS IN MICROVILLOUS F-ACTIN AND VILLIN BY Afa/Dr DAEC STRAIN C1845 IN POLARIZED HUMAN INTESTINAL CELLS

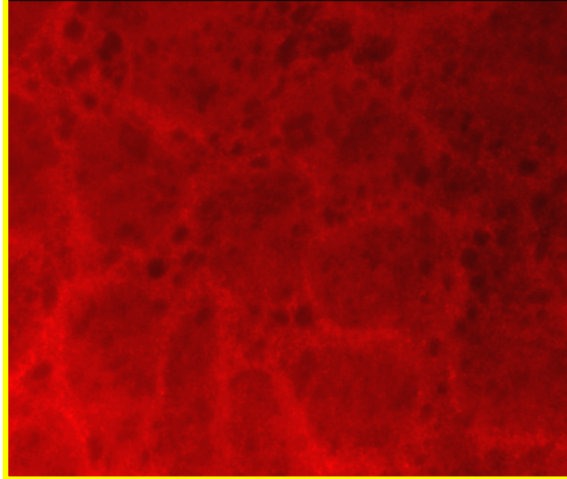
F-ACTIN
CONTROL



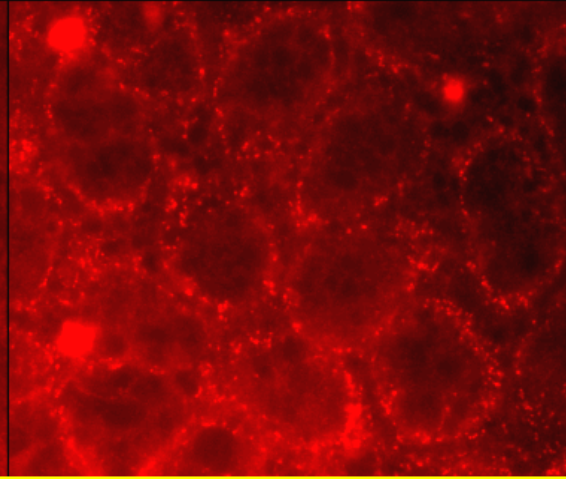
F-ACTIN
INFECTED



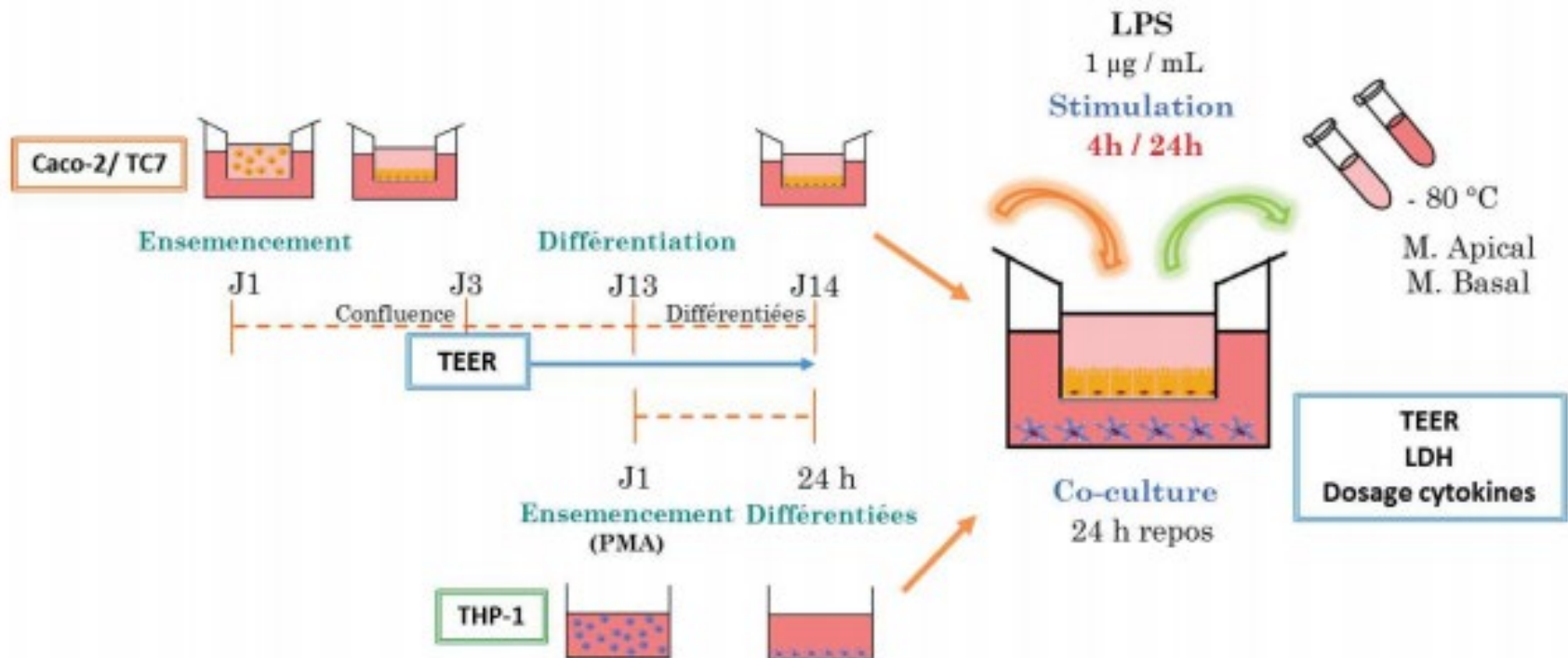
VILLIN
CONTROL



VILLIN
INFECTED



Etude induction inflammation sur Co-culture Caco-2/TC7



Avantages et limites Caco-2

Avantages	Inconvénients
Lignée cellulaire établie donc facilement utilisable	Grande variabilité interlaboratoire et au-delà d'un grand nombre de passages en culture
Compréhension des mécanismes de passage passif vs actif ou para- vs transcellulaire	Épithélium formé souvent imperméable (TEER Caco-2 égal à 50 vs 100 <i>in vivo</i>)
Mise en évidence du rôle et de la caractérisation des transporteurs	Transporteurs souvent sous-exprimés dans le modèle Caco-2
Étude d'un grand nombre de principes actifs, y compris ceux solubles dans des solvants organiques (DMSO)	Corrélation limitée avec les mécanismes de dissolution aqueux <i>in vivo</i>

- Différenciation spontanée, études ++ biosynthèse, expression polarisation....
- Attention extrapolation (un seul type cellulaire, pas de mucus, cellules cancéreuses (Glucose...), pas tous les récepteurs, enzymes... (à vérifier))

HT29: lignées...

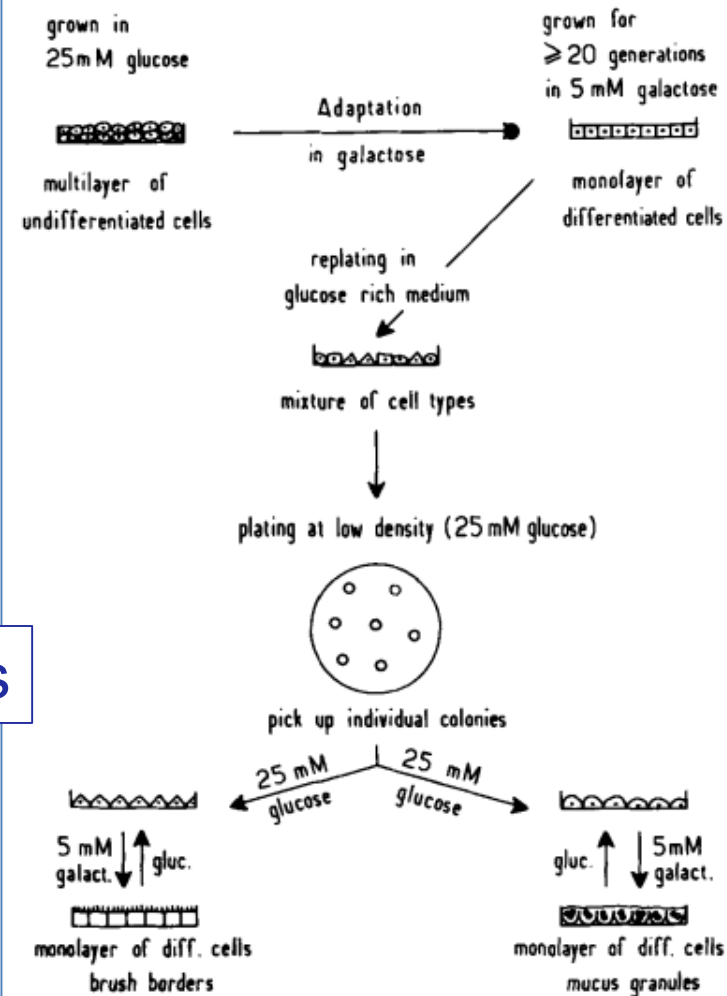
- Fogh 1964 → femme caucasienne de 44 ans tumeur adénocarcinome colique humaine
- **phénotype indifférencié, multicouches** de cellules non polarisées (f taux hydrolases, F taux consommation Glc)
- Modèle unique d'études des mécanismes de différenciation cellulaire (galactose, butyrate de Na, MTX, FU...)

Clone HT-29.cl16E	Homogeneous subpopulations of goblet cells forming in culture monolayers of polarized cells producing mucins and secreting mucus ^b
HT29-18N2 clone	
HT29-MTX	
HT29-SB	
HT29-FU	Mixed population of enterocyte-like cells (90%) and randomly distributed, mucin-secreting cells (10%) ^b

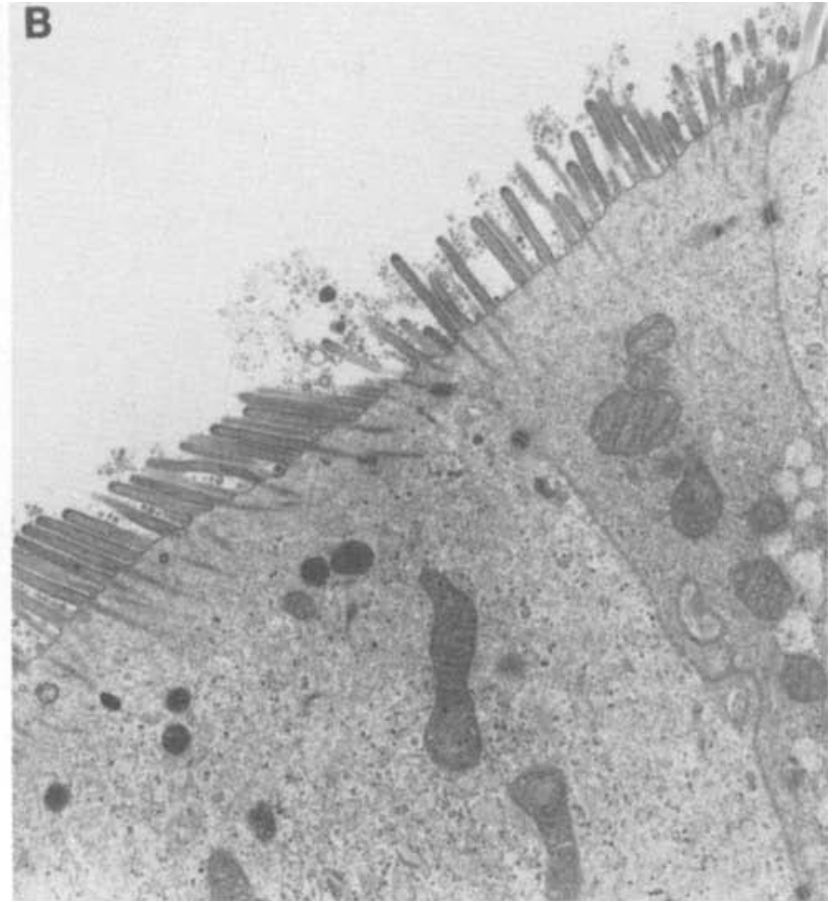
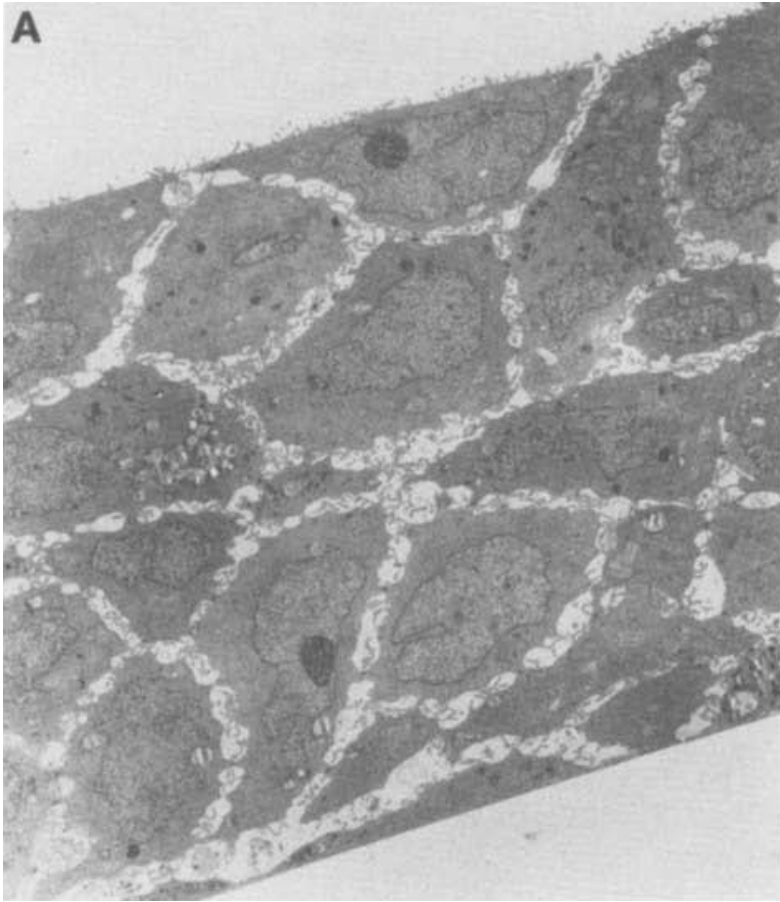
HT29: lignées...

- Phénotype polarisé (% Caco-2):
 - différenciation de même type (mais plus longue)
 - Activités enzymatiques BB + faibles
 - Pas de dôme
- Selon sélection, composition différente:
 - HT29-CL 16E (butyrate de sodium)
 - HT-29 MTX (méthotrexate)
 - HT-29 FU (fluorouracile)
- HT29-MTX: étude activité mucines, réponses aux infections bactériennes...
- HT29-MTX-Caco-2 cocultures

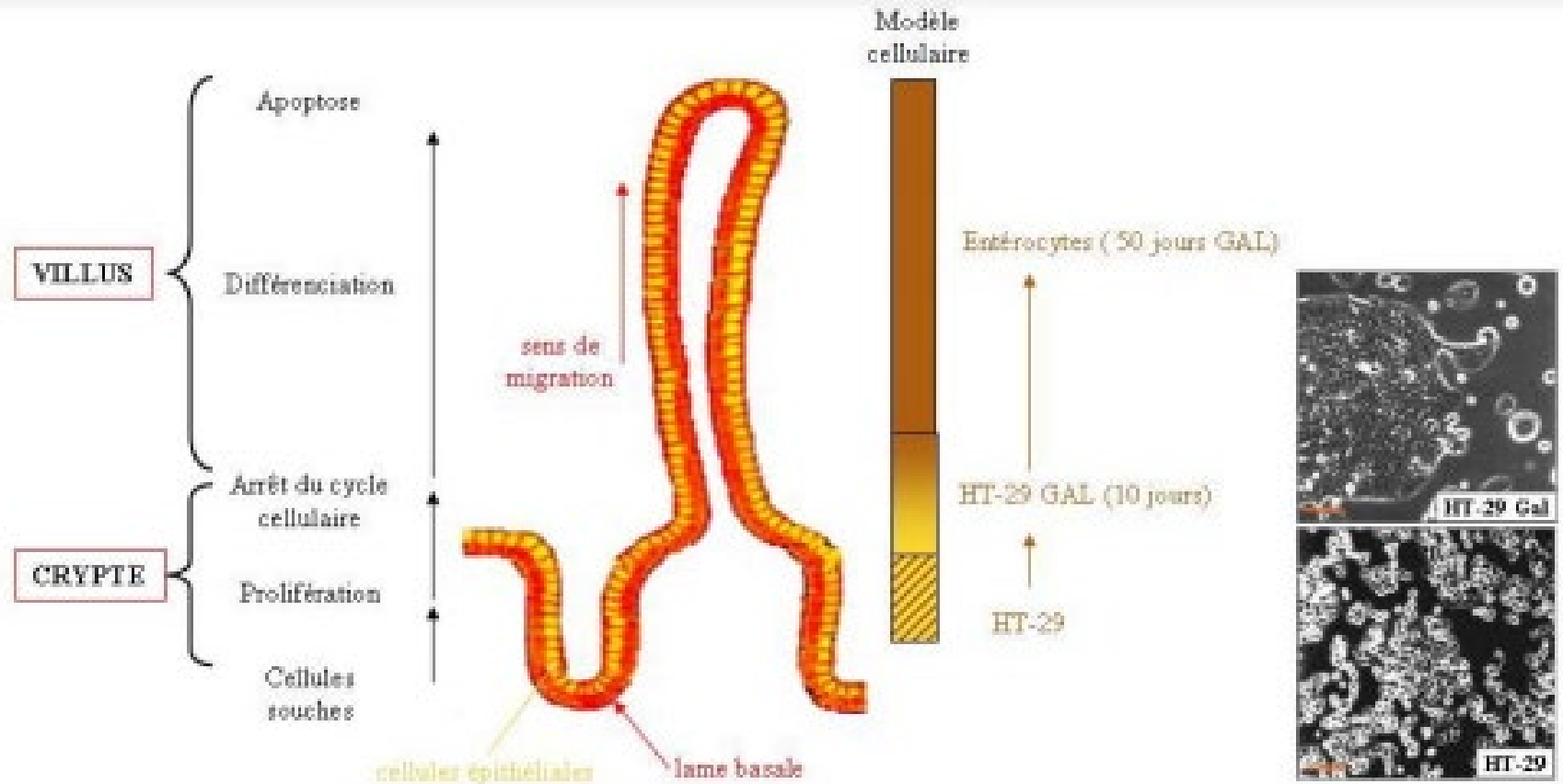
mucus



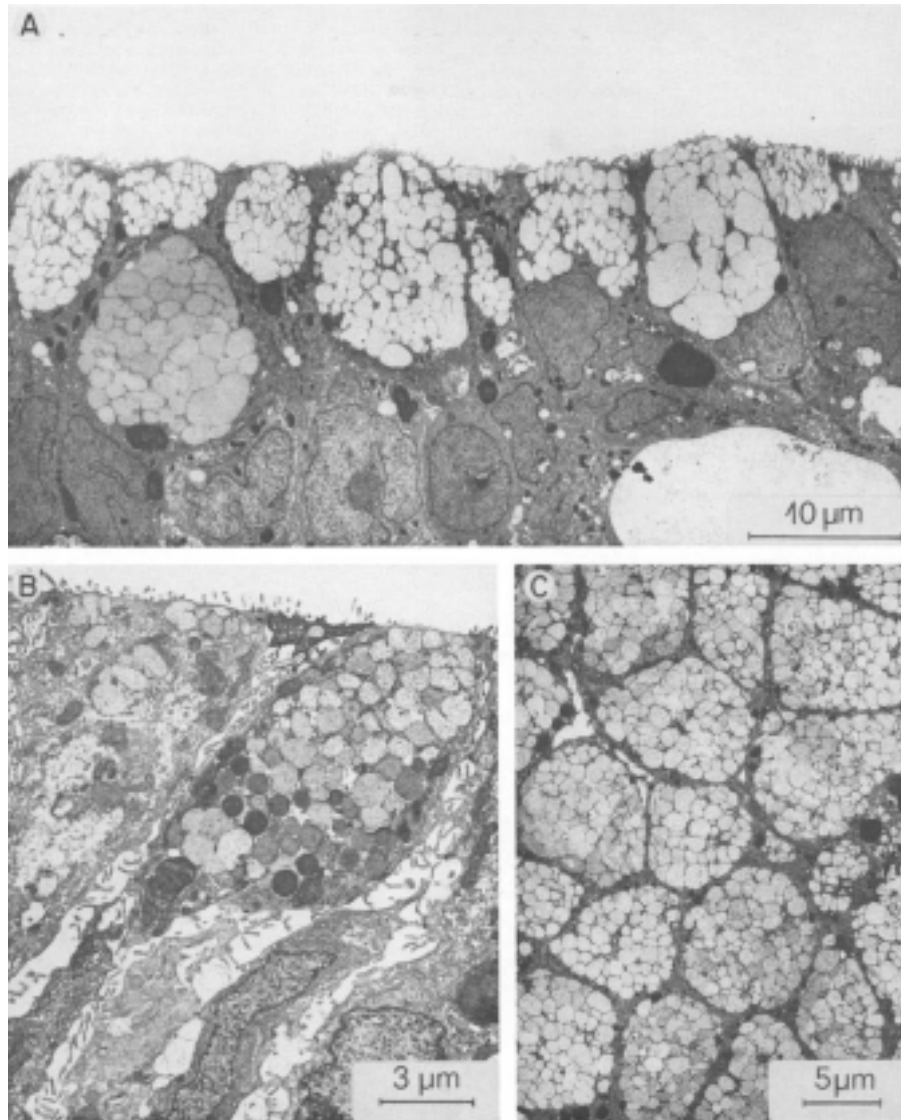
HT29



HT29: modèle de différenciation entérocytaire

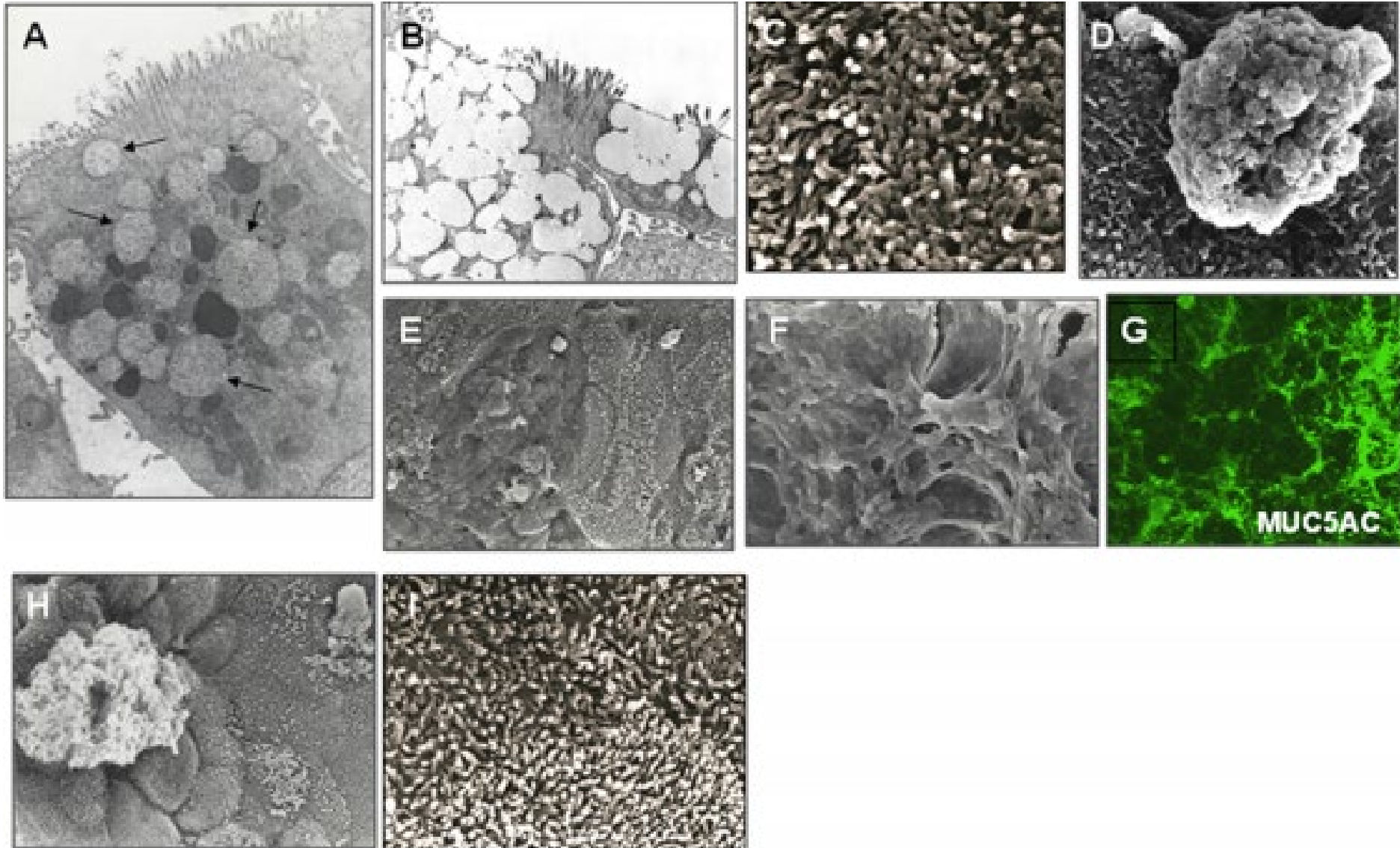


HT29-16E

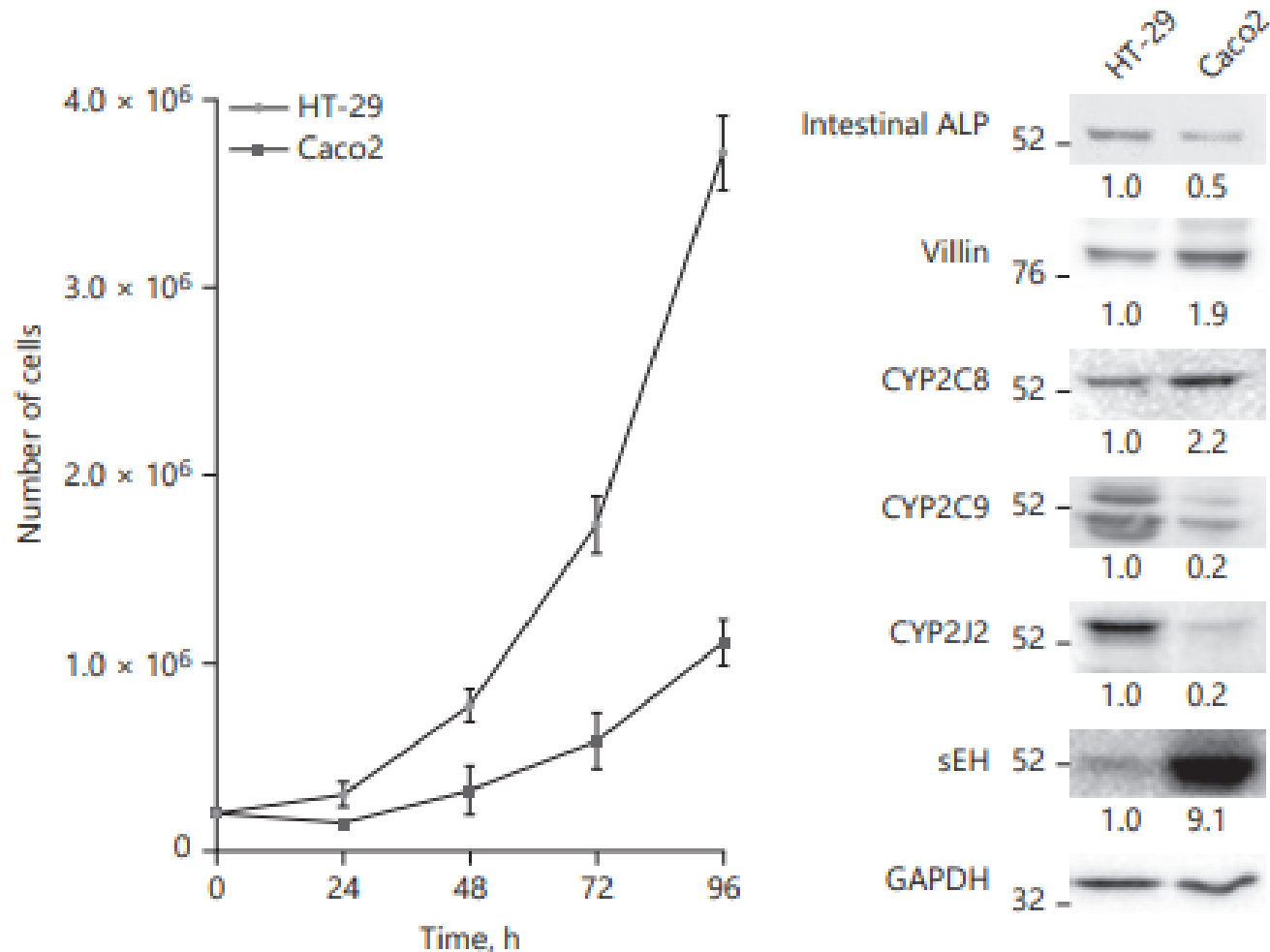


Beaulieu and al., Microsc.Res.Tech., 2000.49:394-406)

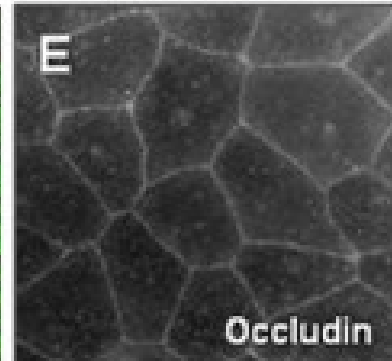
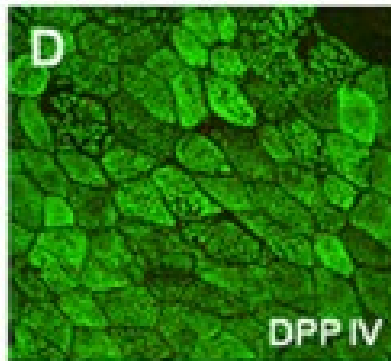
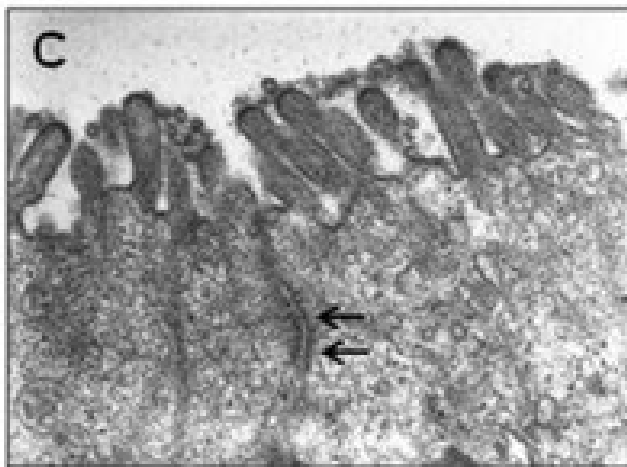
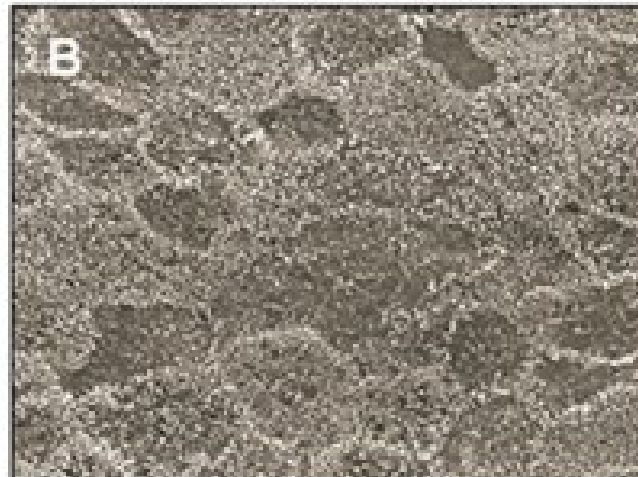
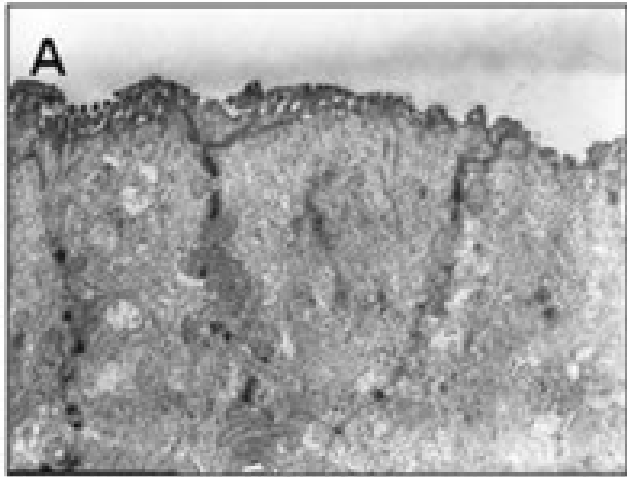
HT29-MTX (A-G) / HT29-FU(H,I)



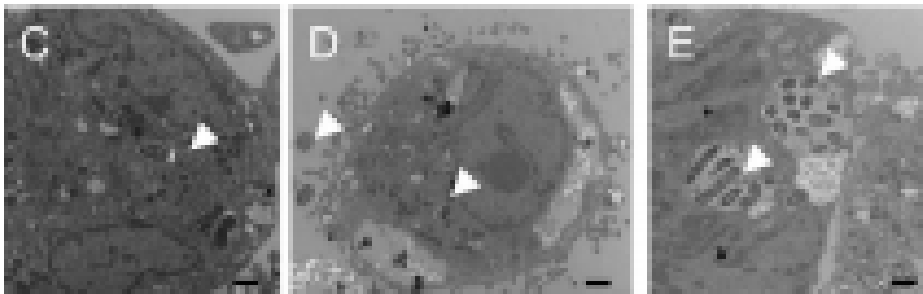
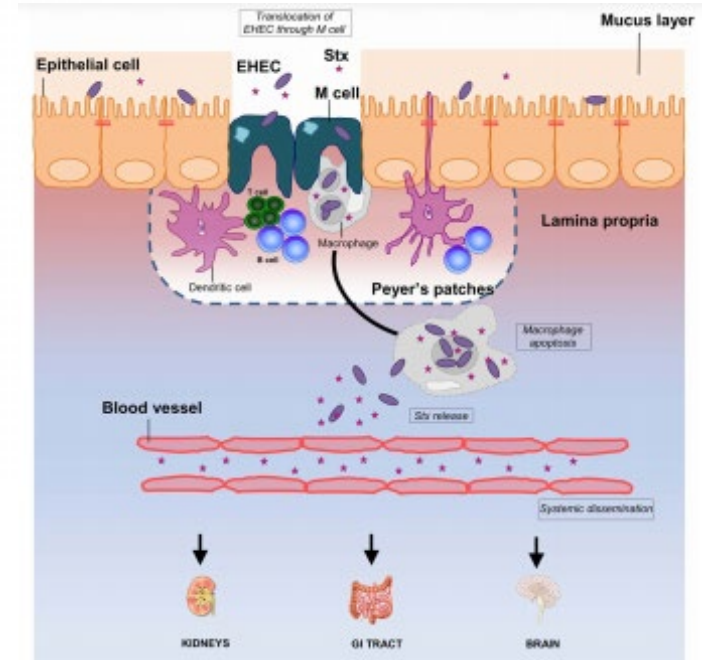
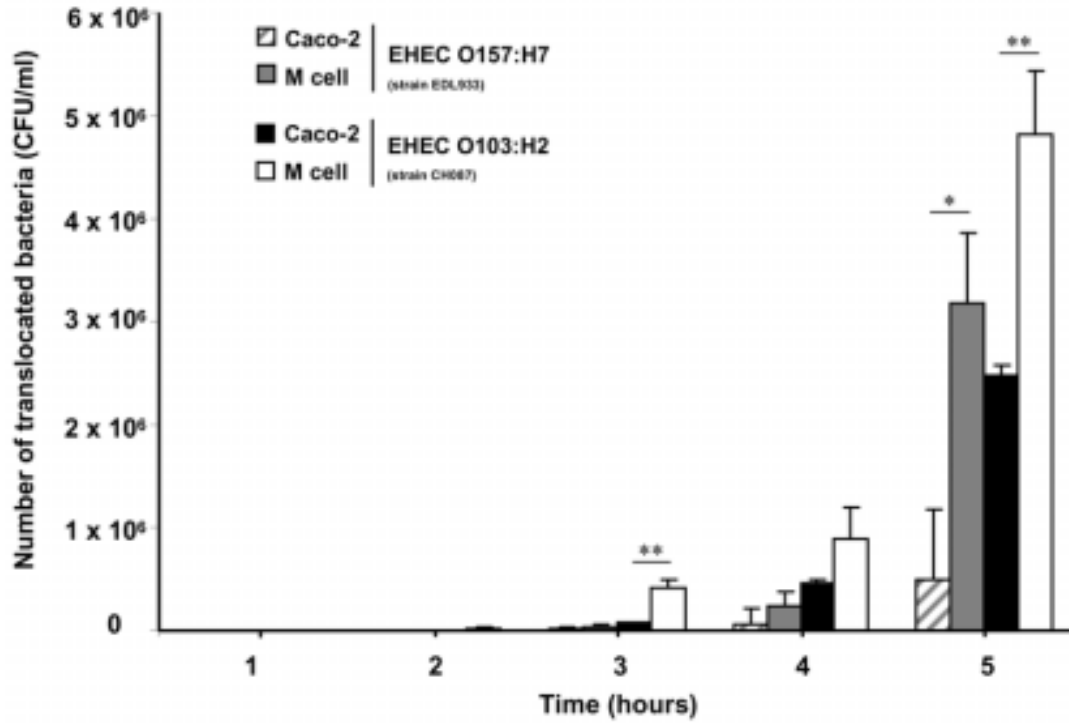
Études sur la différenciation cellulaire intestinale (physio et cancéreuse)



T84



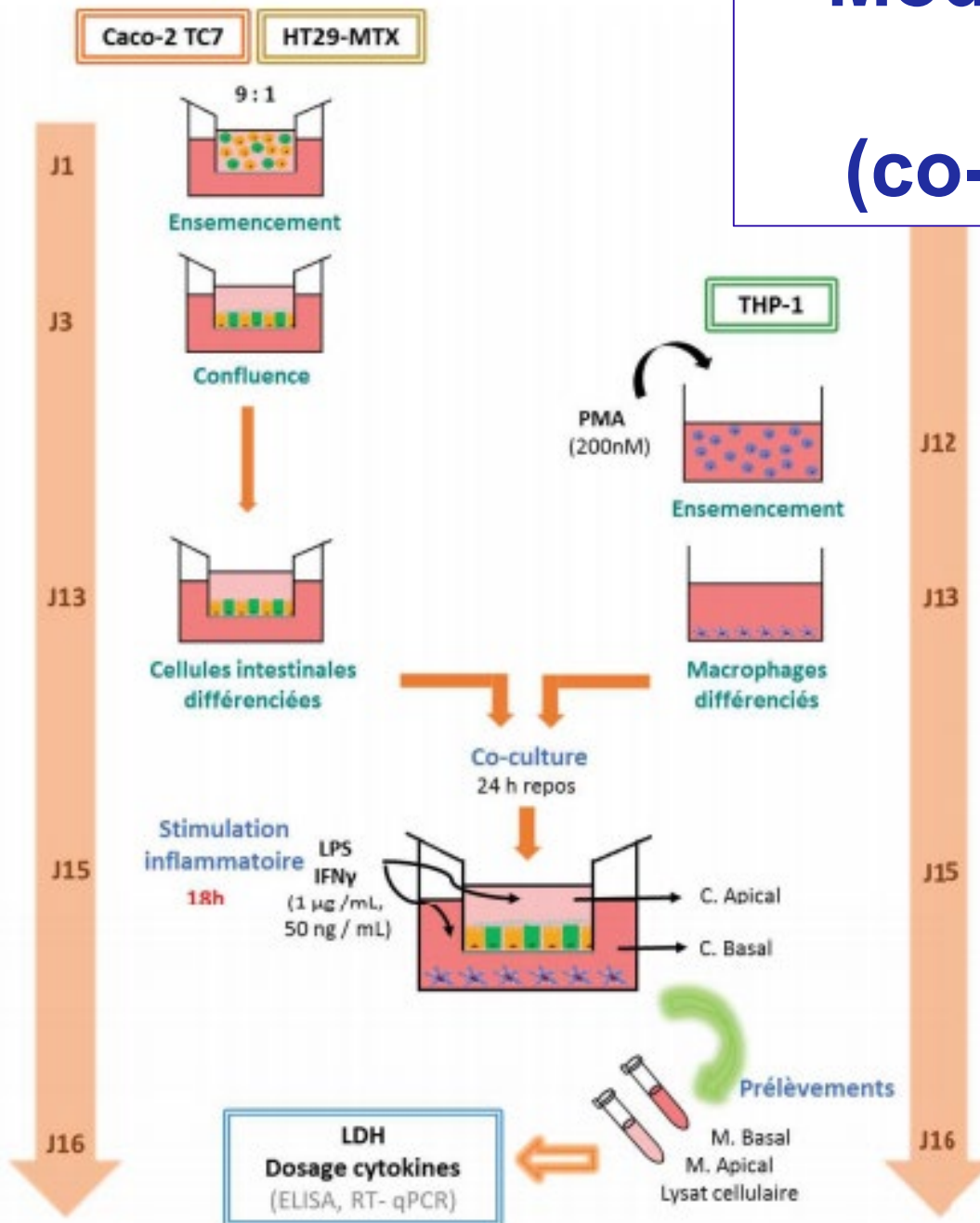
Étude sur modèle cellulaire de cellules M



THP1

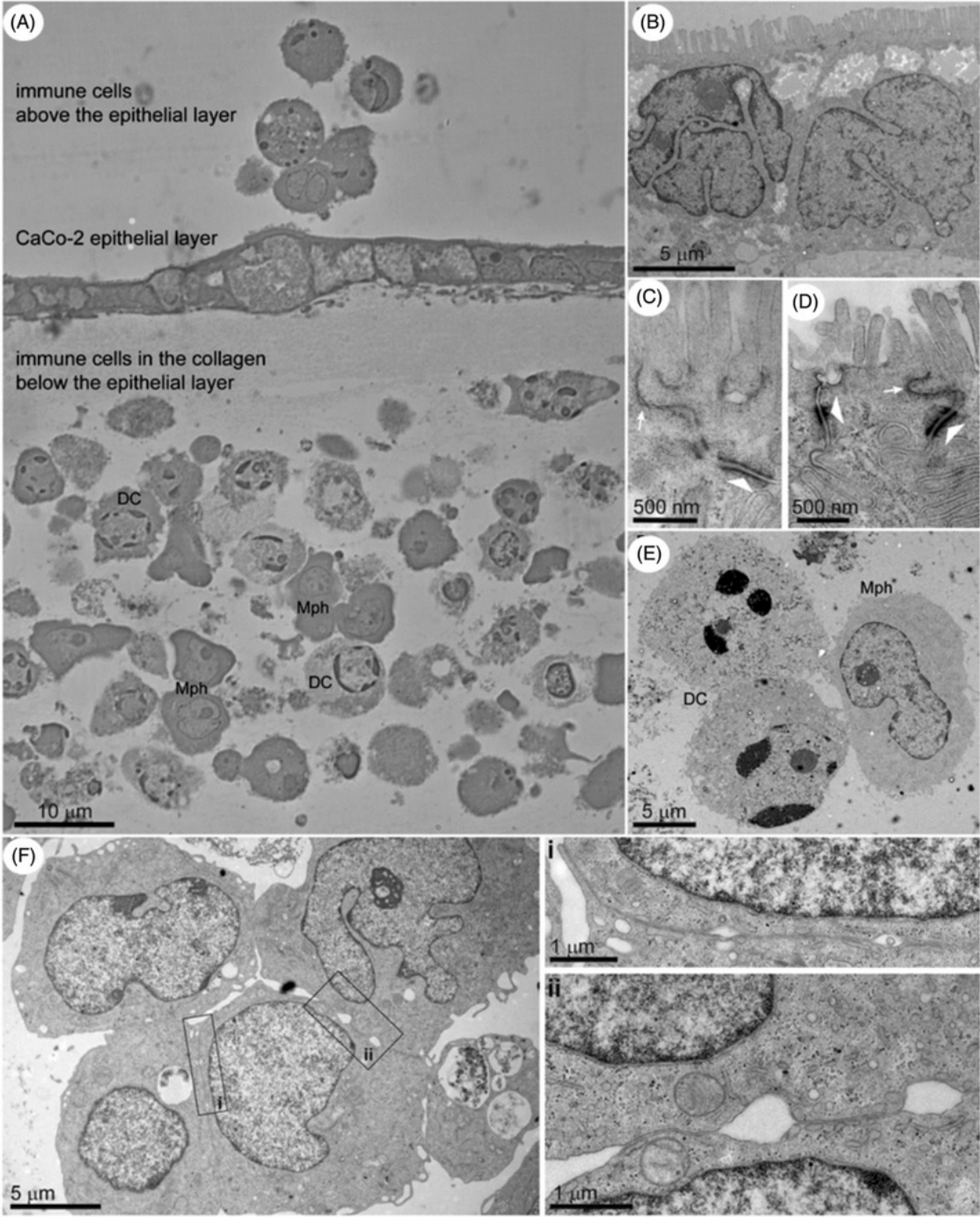
Etienne-Mesmin et coll., PLoS One 2011

Modèle de stimulation inflammatoire (co-culture 3 lignées)



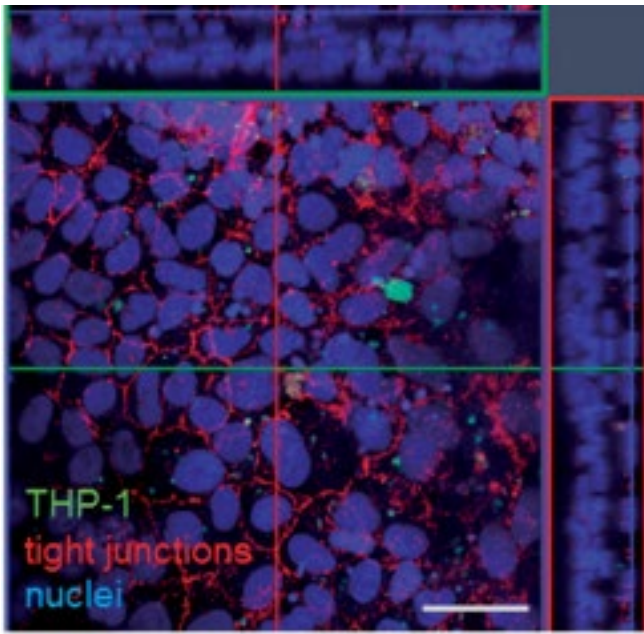
Selon Ponce de Léon Rodriguez

A 3D co-culture of three human cell lines to model the inflamed intestinal mucosa for safety testing of nanomaterials

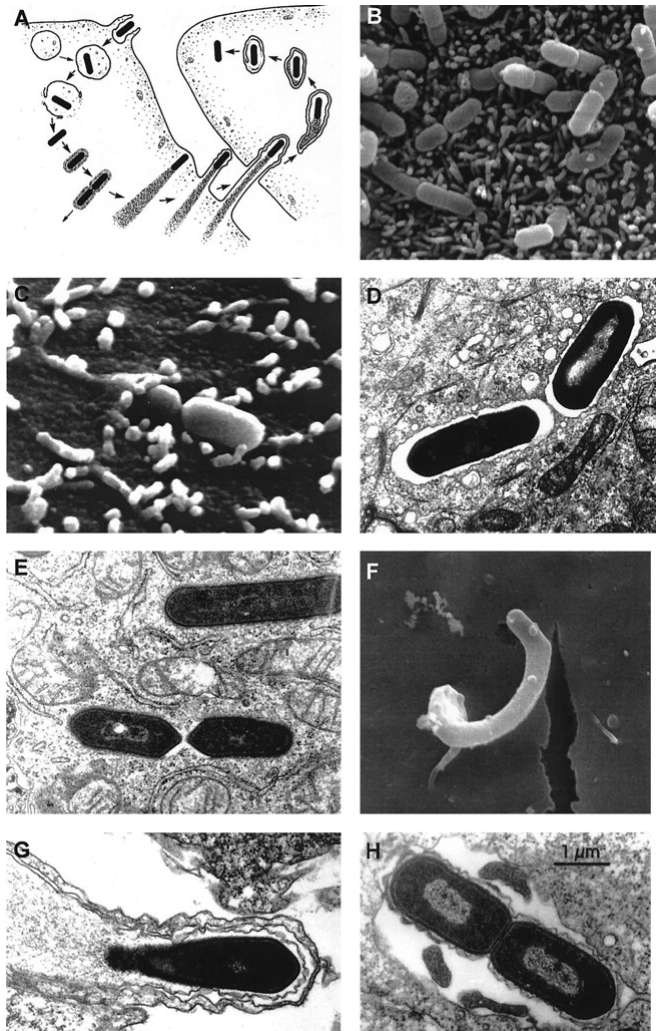


(IL-1β)

Modèle 3D-coculture



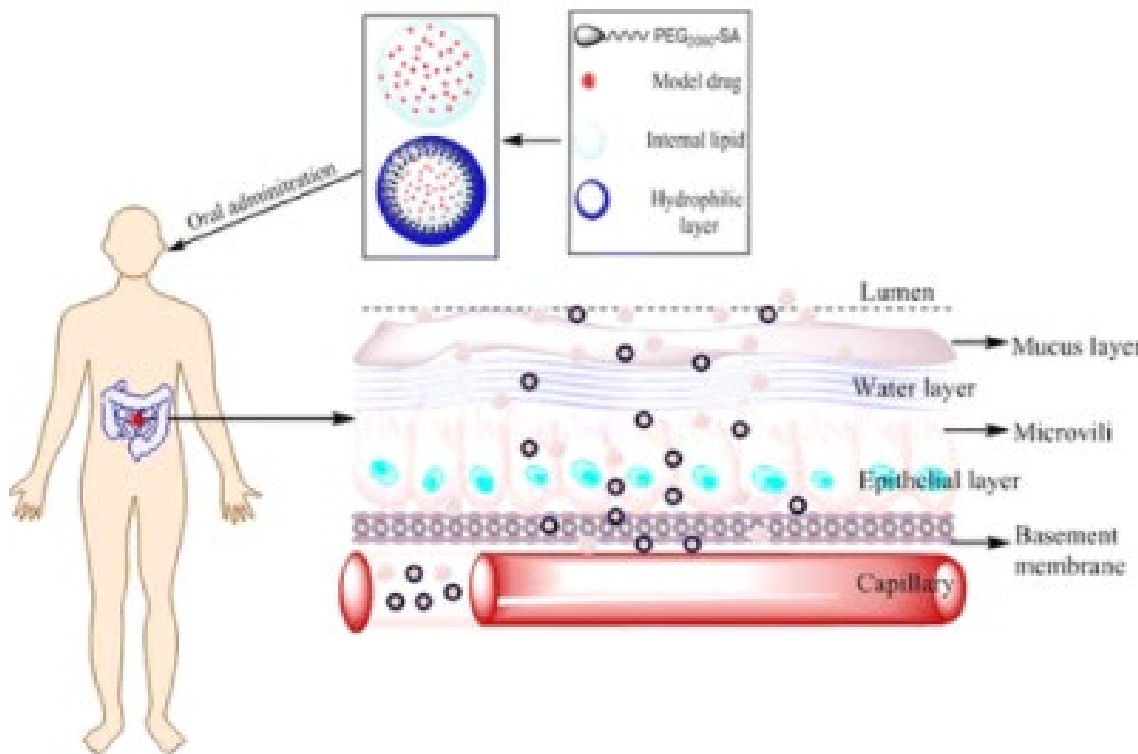
Mécanismes de virulence de *Listeria*



Tilney et coll., 1989

José A. Vázquez-Boland et al. Clin. Microbiol. Rev. 2001;
doi:10.1128/CMR.14.3.584-640.2001

Mise au point nanoparticules: exemple d'utilisation d'un modèle de co-culture



- ✓ Transport et absorption de diverses Nanoparticules PEGylées ou non
- ✓ Processus actif (action d'inhibiteurs)
- ✓ PEGylation augmente biodisponibilité pour voie Orale (comparaison des lignées (Caco-2 et Caco-2/HT29): ++mucus

Improved Transport and Absorption through Gastrointestinal Tract by PEGylated Solid Lipid Nanoparticles (Yuan et coll., Mol. Pharmaceutics, 2013)



Small silica nanoparticles transiently modulate the intestinal permeability by actin cytoskeleton disruption in both Caco-2 and Caco-2/HT29-MTX models

Raphaël Cornu¹ · Claire Chrétien¹ · Yann Pellequer¹ · Hélène Martin¹ · Arnaud Béduneau¹

Bien qu'ayant de multiples applications industrielles et biomédicales et étant parmi les nanomatériaux les plus produits, les silices amorphes soulèvent de nombreuses interrogations quant à leur toxicité chronique chez l'Homme. L'équipe de Cornu *et coll.* en 2020 (Arch. Toxicol., 2020, 94(4) :1191-1202) a choisi d'utiliser des modèles de culture cellulaire pour tenter d'apporter des réponses à cette problématique. Ils ont ainsi testé la toxicité orale de nanoparticules de silice amorphe de tailles différentes (10 à 200 nm) à 1 et 10 mg.ml⁻¹ ainsi qu'un additif alimentaire E551 (mélange de nanoparticules de silice de tailles différentes). Le choix a été fait d'utiliser la lignée Caco-2 ainsi qu'un modèle de co-culture Caco-2/HT29-MTX.

- De quelles cellules les Caco-2 et les HT29_MTX sont-ils les modèles? Argumentez
- Quels est l'intérêt d'utiliser deux modèles dont un modèle de co-culture?
- Dans un premier temps, les auteurs ont testé la viabilité cellulaire (cf ci-dessous). Pourquoi 2 tests différents? Que peut-on en conclure?

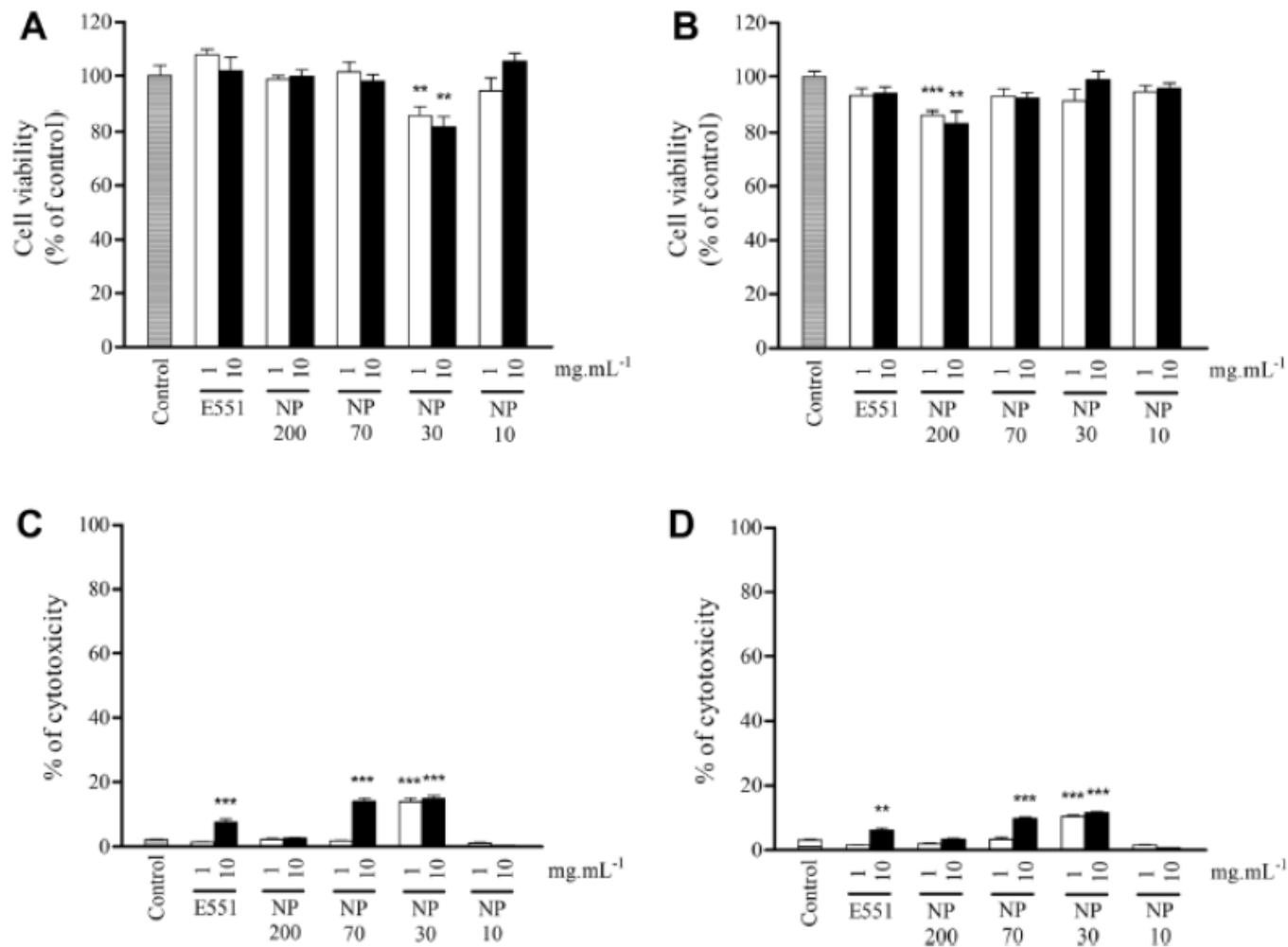


Fig. 2 Effect of the silica NPs (1 and 10 mg mL⁻¹) on cell viability in Caco-2 (**a, c**) and co-culture Caco-2/HT29-MTX (**b, d**) after 2 h exposure. Cell viability was determined by an MTT assay (**a, b**) and cytotoxicity was determined by an LDH assay (**c, d**). Results are expressed as percent of control medium (transport buffer without any

NP) for cell viability assay. For LDH activity, results were expressed as % of cytotoxicity. Data are means \pm SEM from triplicate of three independent cultures. ** $p \leq 0.01$ and *** $p \leq 0.001$ with respect to control

□ Pourquoi choix TEER? Conclusion entre les deux lignées? Apport courbe E?

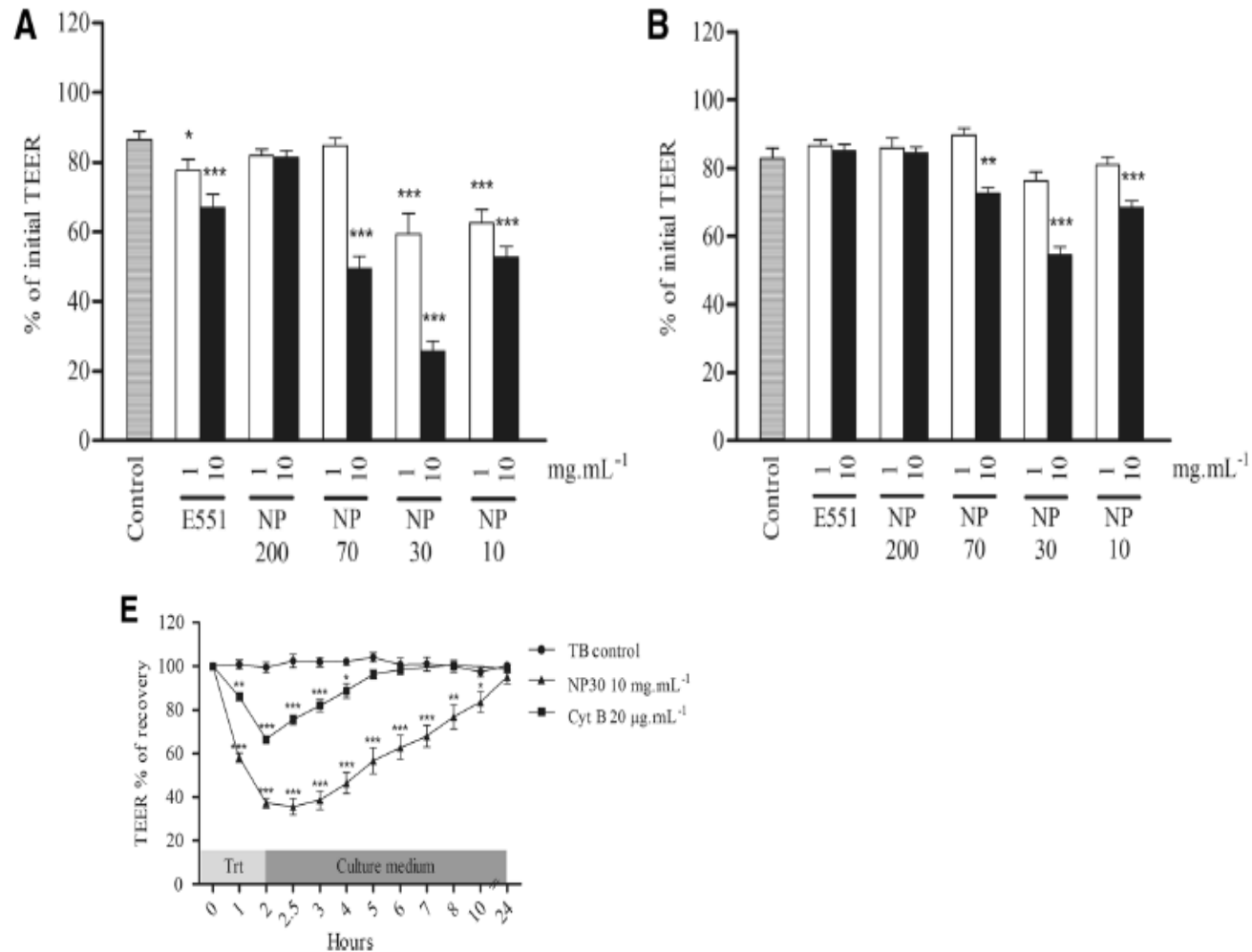


Fig. 3 Effect of the silica NPs (1 and 10 mg mL⁻¹) on cell permeability in Caco-2 (a, c) and co-culture Caco-2/HT29-MTX (B and D) after 2 h exposure. Cell permeability was determined by TEER measurement (a, b) and assessment of Lucifer-Yellow paracellular transport (c, d); data are means ± SEM from triplicate of at least four

independent cultures. TEER monitoring of Caco-2 cells exposed to NP30 (10 mg mL⁻¹) and Cyt B (20 µg mL⁻¹). “Trt” means treatment (e); data are means ± SEM from duplicate of at least three independent cultures. **p* ≤ 0.05, ***p* ≤ 0.01 and ****p* ≤ 0.001 with respect to control