Children first and always!

KROKIDS

STIFTUNG

zur Unterstützung

chronisch kranker Kinder

Infectious pathomechanisms: improving therapies of chronic diseases in children (and adults)

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Kinder- und Jugendarzt, Neonatologe, Kinder-Gastroenterologe

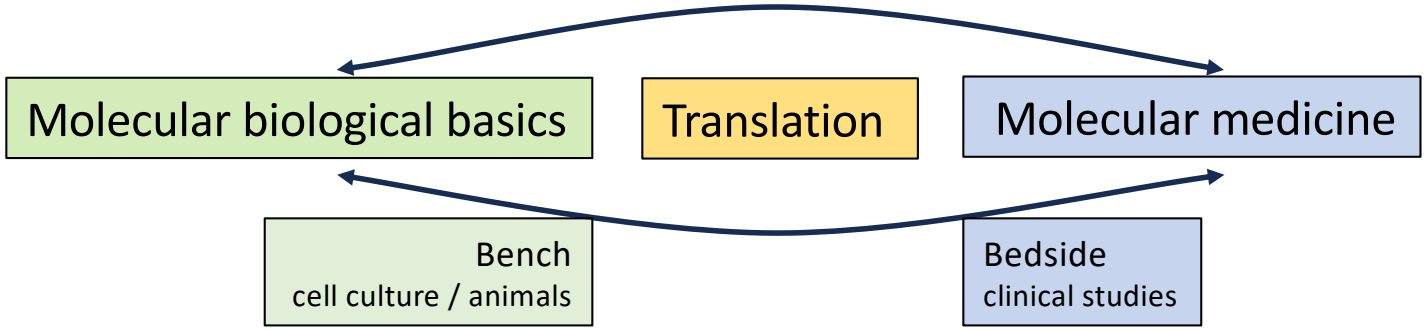
Pediatrics originated in the 19th century from poor and undernourished children with infections.

Infant mortality 1872: 25% ⇢ 2022: 0,3% [Nigeria 7,2%]

► **Chronic Diseases: no cure but constant care** (≈ 4 M children in Germany)

1.1 - 1.2: Infectious agents as tools to dissolve intracellular pathomechanisms

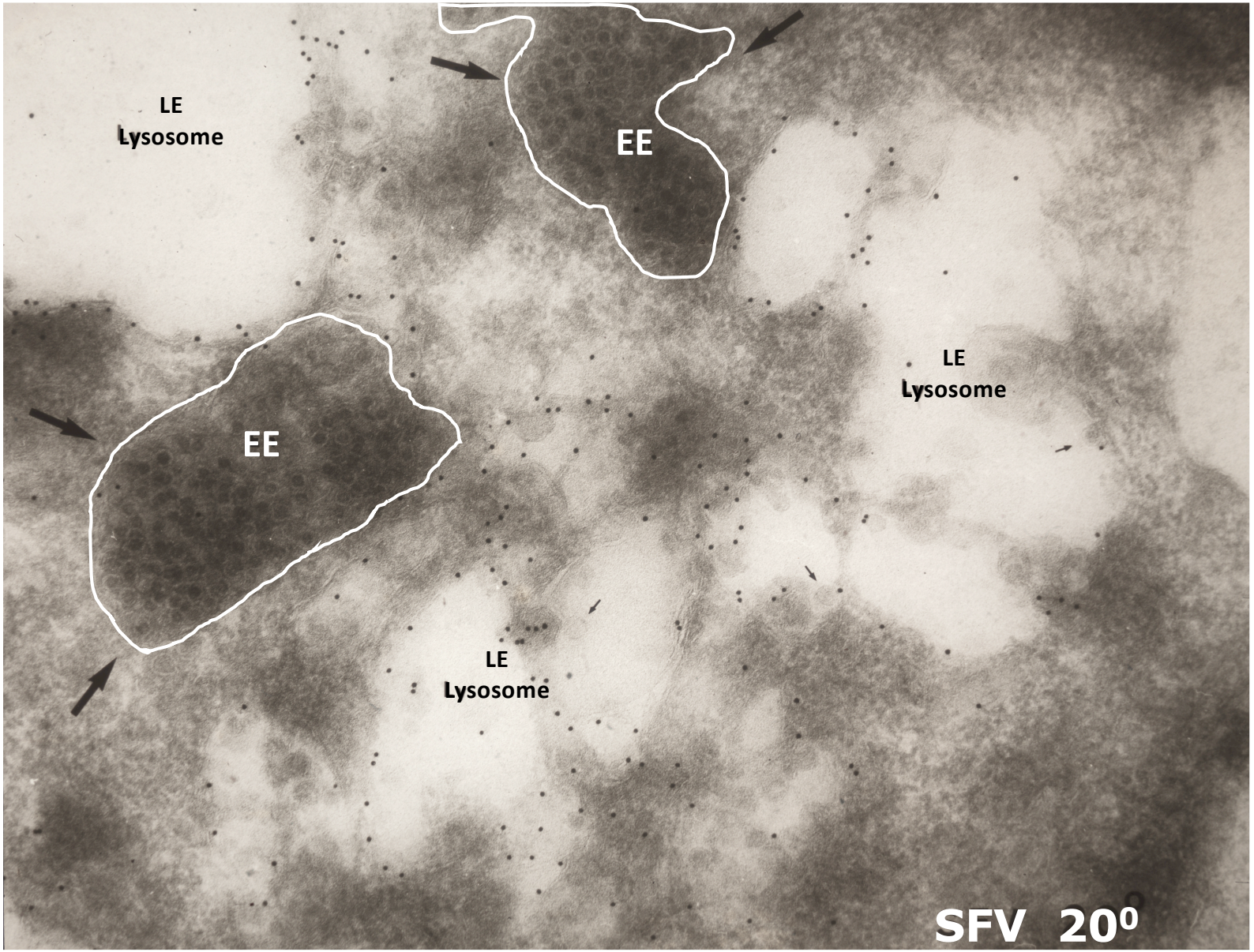
2.1 - 2.5: Intracellular pathomechanisms ⇢ Innovative therapeutic options



Lysosomal Membrane Proteins: SFV as a tool to define early endosomes

(20^{0}C temperature bloc)

J Cell Biol 105: 1227, 1987



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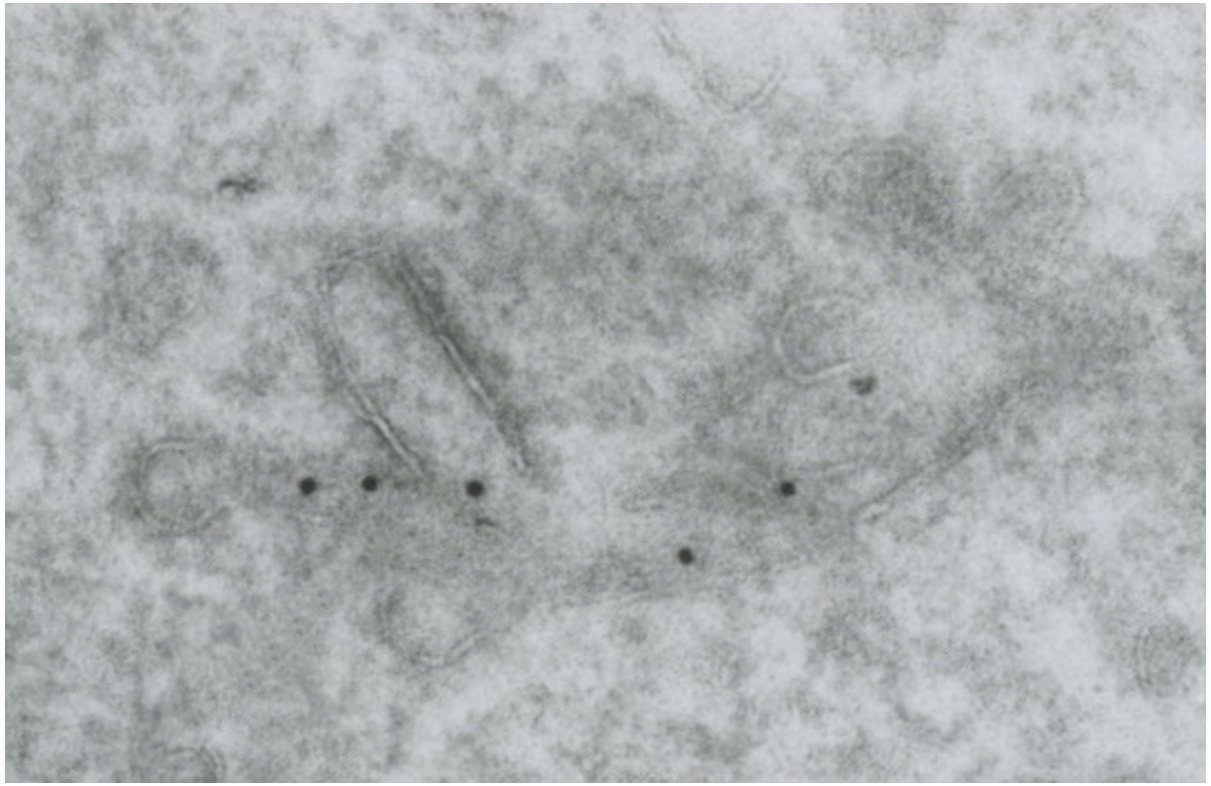
A conformationspecific monoclonal antibody recognizes trimerized hemagglutinin within the rough endoplasmic reticulum of a cell infected with influenza virus.



Cell 53: 197, 1988

CPS Deficiency: Lancet 346, 8989:1530, 1995 ⇢ x 30

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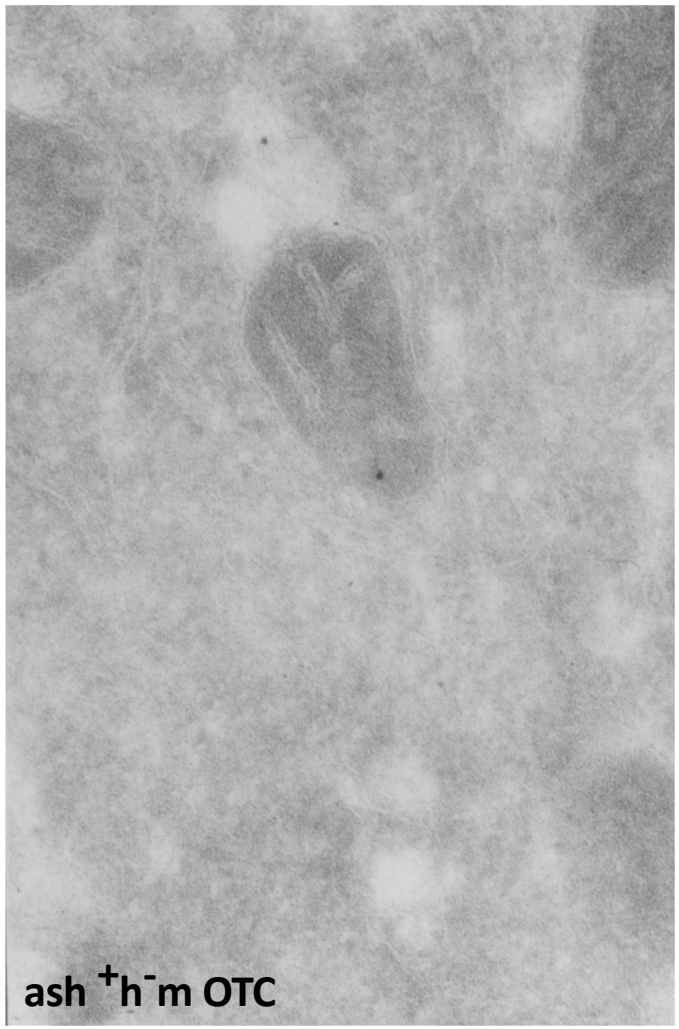
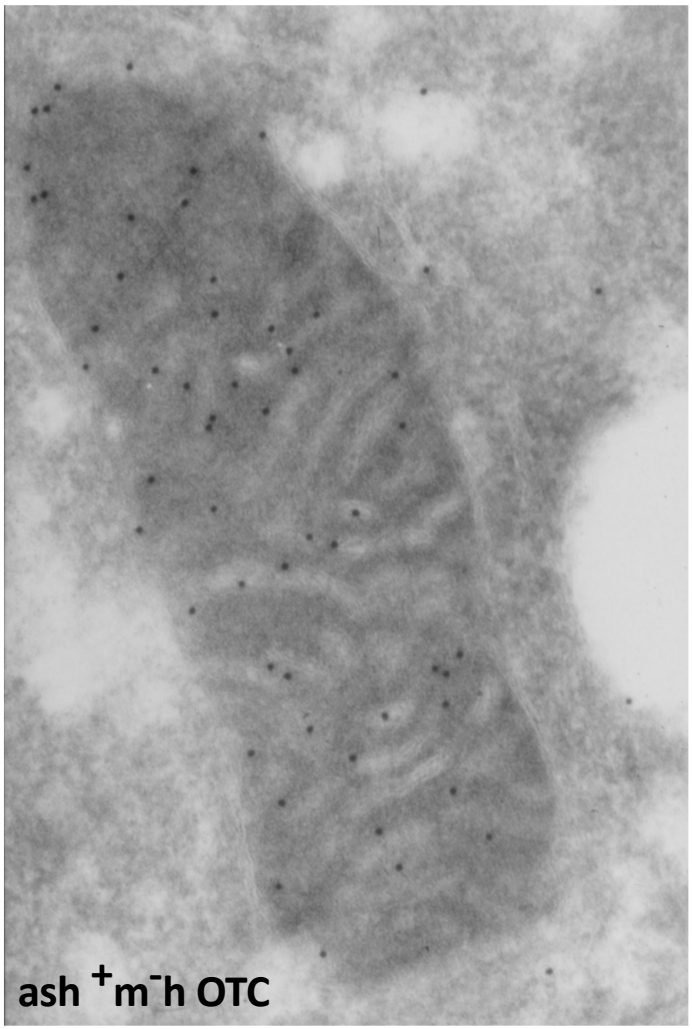
Gene therapy in OTC deficiency with an adenoviral vector: Importance of species-specific leader sequence for import and efficacy





OTC = **O**rnithine **T**rans**c**arbamylase

Human Gene Therapy 12: 1035, 2001

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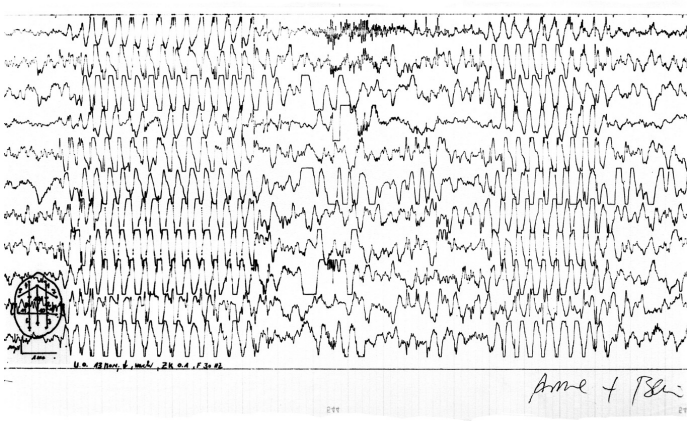
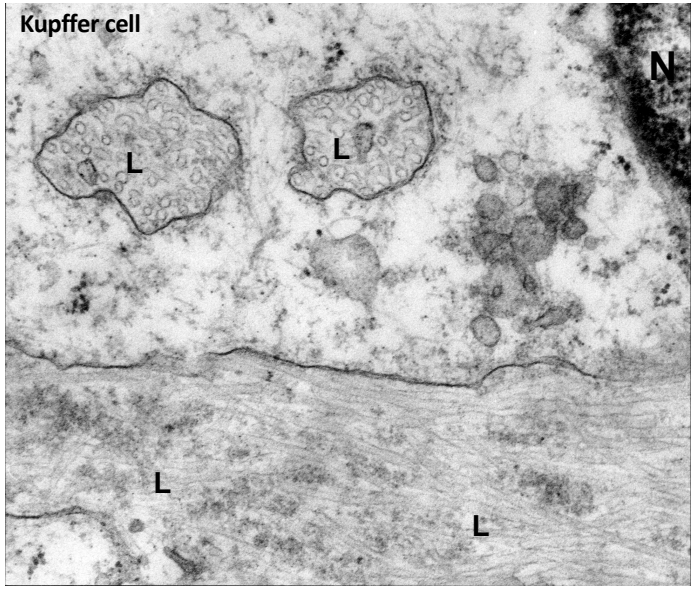
Infant with Type 2 Gaucher Disease

→ G202R Mutation of Glucocerebrosidase





Consanguinity
Failure to thrive
Seizures resistent to therapy
Hepatosplenomegaly
Psychomotoric retardation
Death after 14 months of life

Glucocerebrosidase activity: 22 (14) % in leukocytes
25 (13) % in fibroblasts

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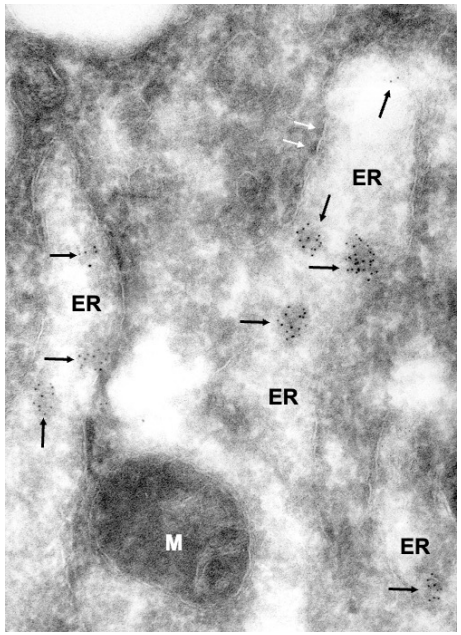
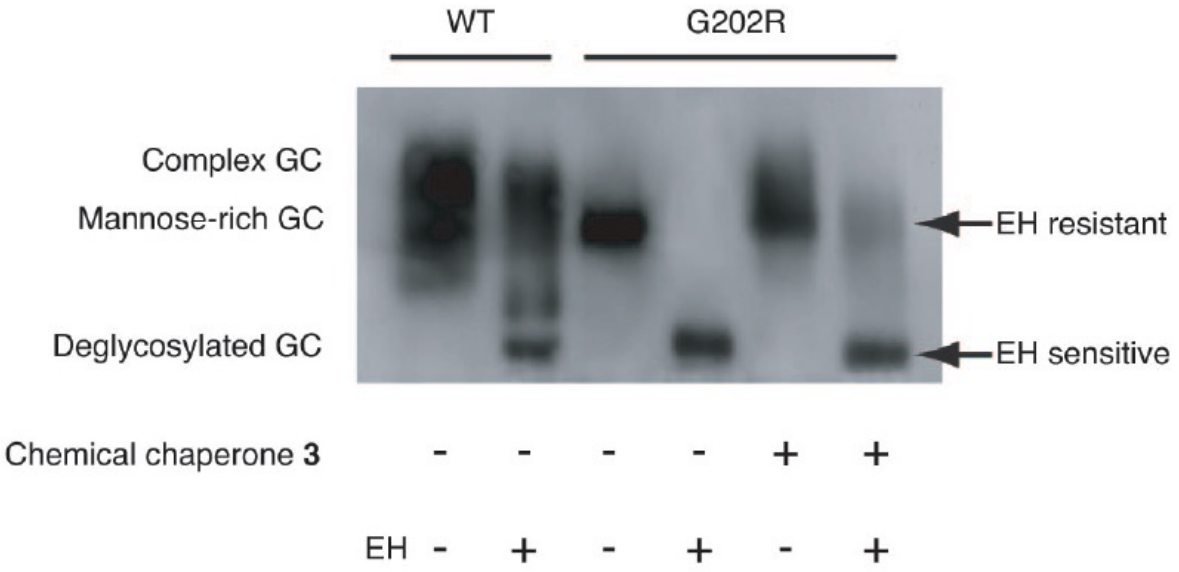
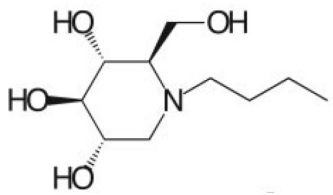
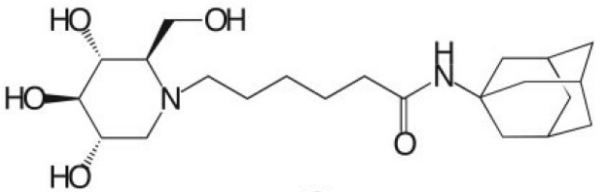
CHAPERONE = cell- and ER-permeable small molecules crossing the blood-brain barrier
→ improve stability (folding), concentration and transport to lysosome

Nojirimycin: antibiotic (from Streptomyces), iminosugar, glycosidase inhibitor









***N*-butyl-deoxynojirimycin**:
- ⇨ Miglustat (ZAVESCA®)
- ⇢ Gaucher Disease
- ⇢ Niemann-Pick Disease Typ C

Chaperone 3:
N-hexanoic acid adamantly amide deoxynojirimycin

J Pathol 188 (4): 407, 1999
Am Chem Society 1(4): 235, 2006

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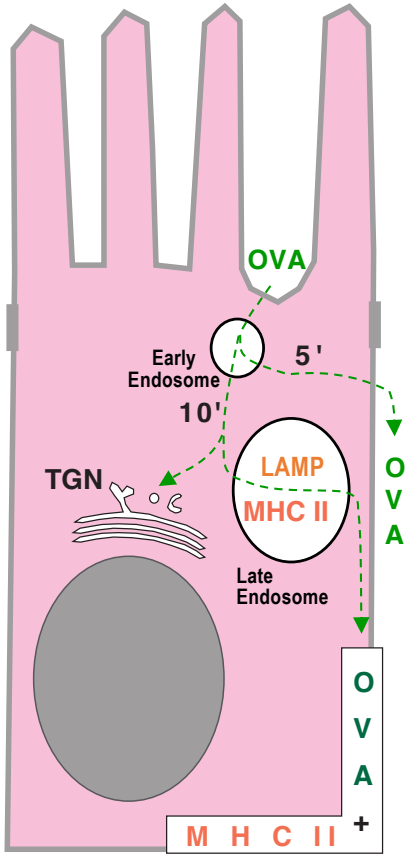
Tolerance Induction

Transport of OVA into MHC class II⁺late endosomes

(in-vivo mouse model: "naive" enterocytes)

BALB/c → Tolerance Induction

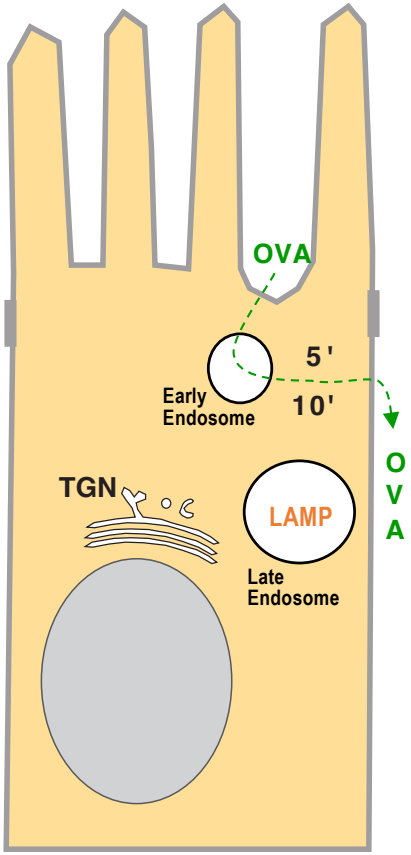


Antigen processing in late endosomes

↓

Antigen presentation on basolateral membrane

SCID → Tolerance Failure



→ Generation of oral tolerance in the infant‘s small bowel

→ Bacterial colonization?

Gastroenterology 118: 128, 2000

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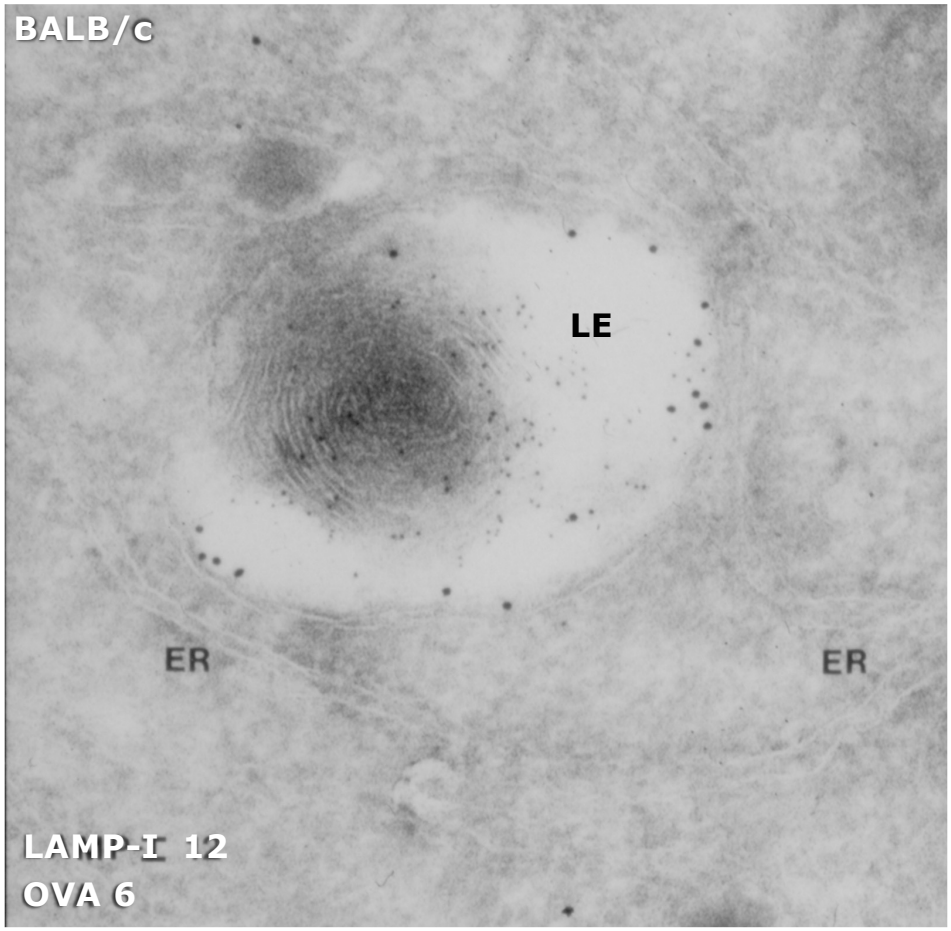
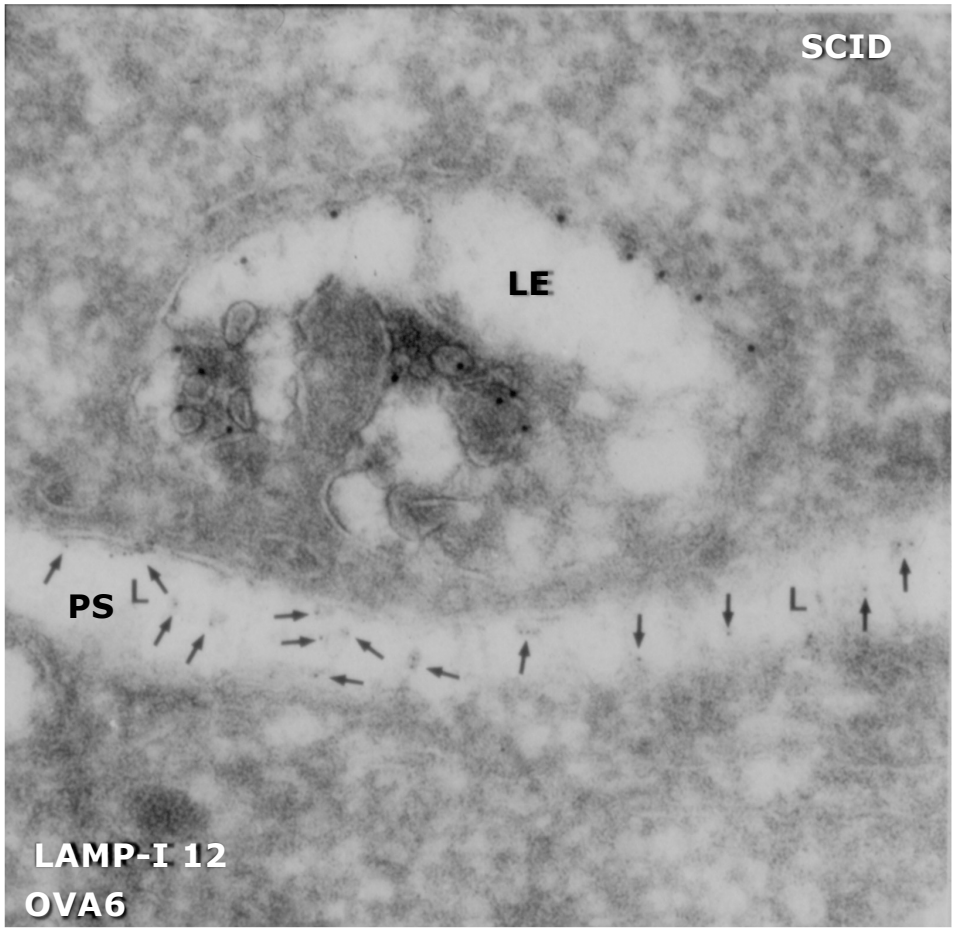
Allergy: tolerance induction by targeting of allergens into LE of enterocytes





Gastroenterology 118: 128, 2000

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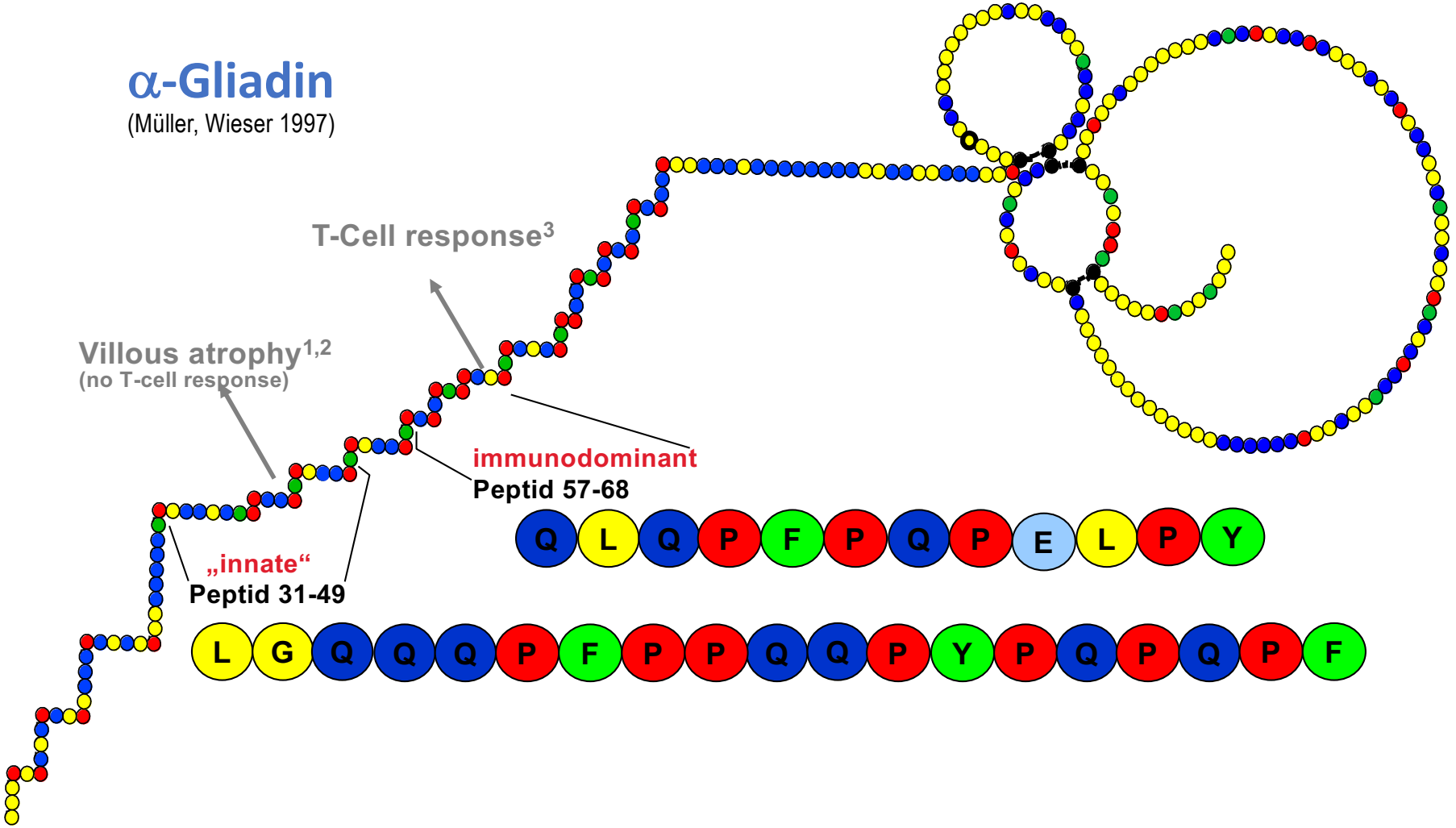

[1] Sturgess et al., Lancet 343: 758, 1994
[2] Arentz-Hansen et al., Gastroenterology 123: 803, 2002
[3] Anderson et al., Nature Medicine 6: 337, 2000

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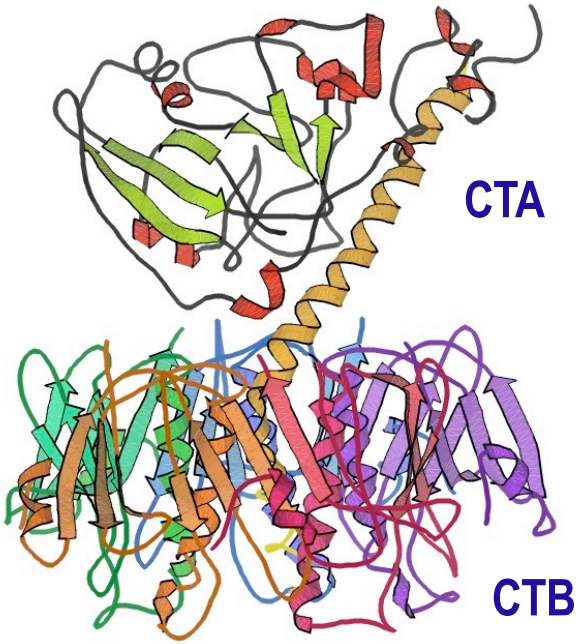
Cholera Toxin, Subunit B

- Homopentamer
  Binding to GM1 ganglioside (⟶lysosome)
  non-toxic

- Mucosal adjuvant for oral tolerance and autoimmune diseases



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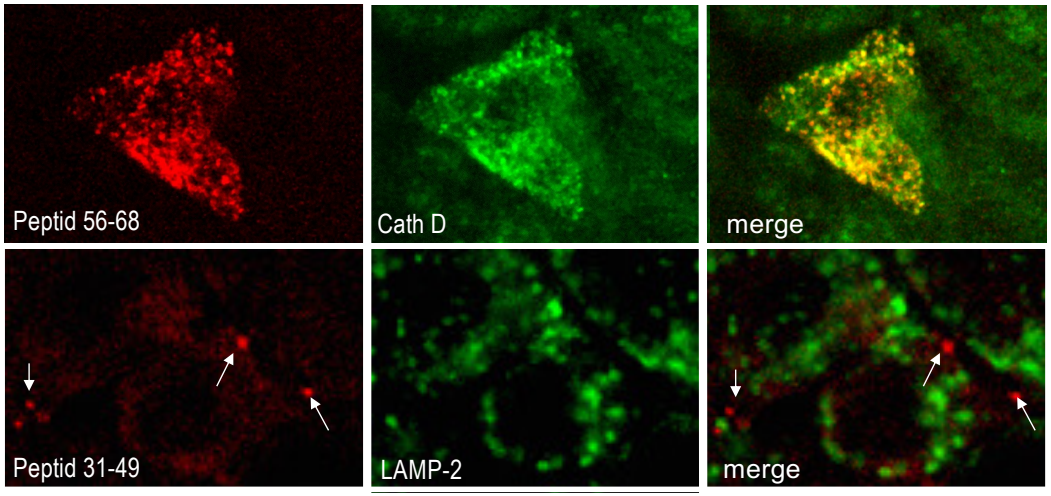
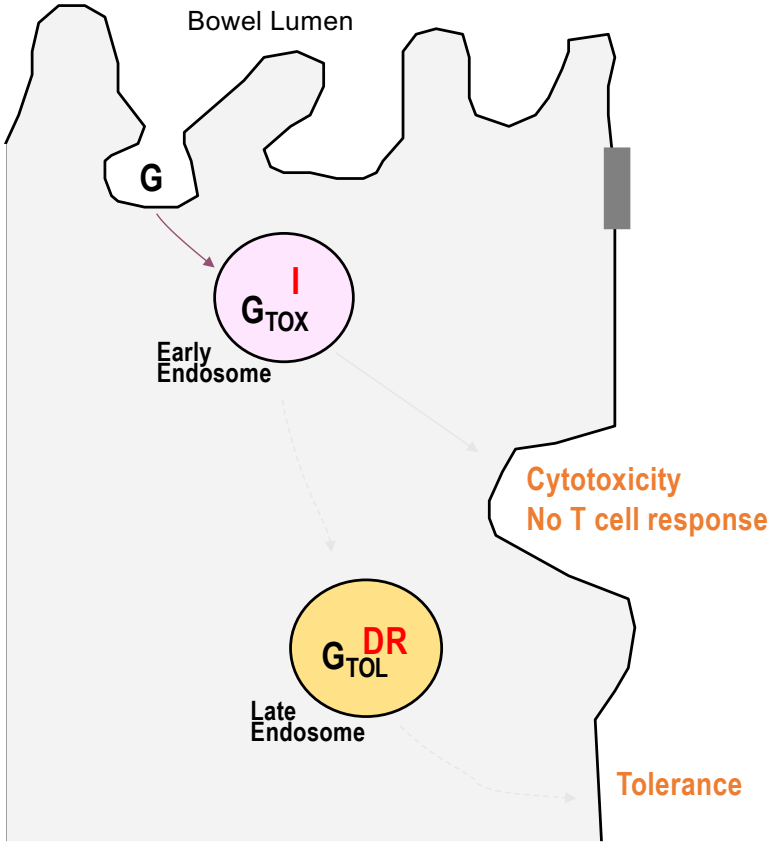
Antigen Presentation

- Celiac Toxic Reaction → Dendritic Cells (HLA-DQ 2/8)
- Oral Tolerance → Enterocytes (HLA-DR / Late Endosomes)

HT-29, intestinal epithelial cells





Gut 59: 300, 2010

KROKIDS STIFTUNG zur Unterstützung chronisch kranker Kinder

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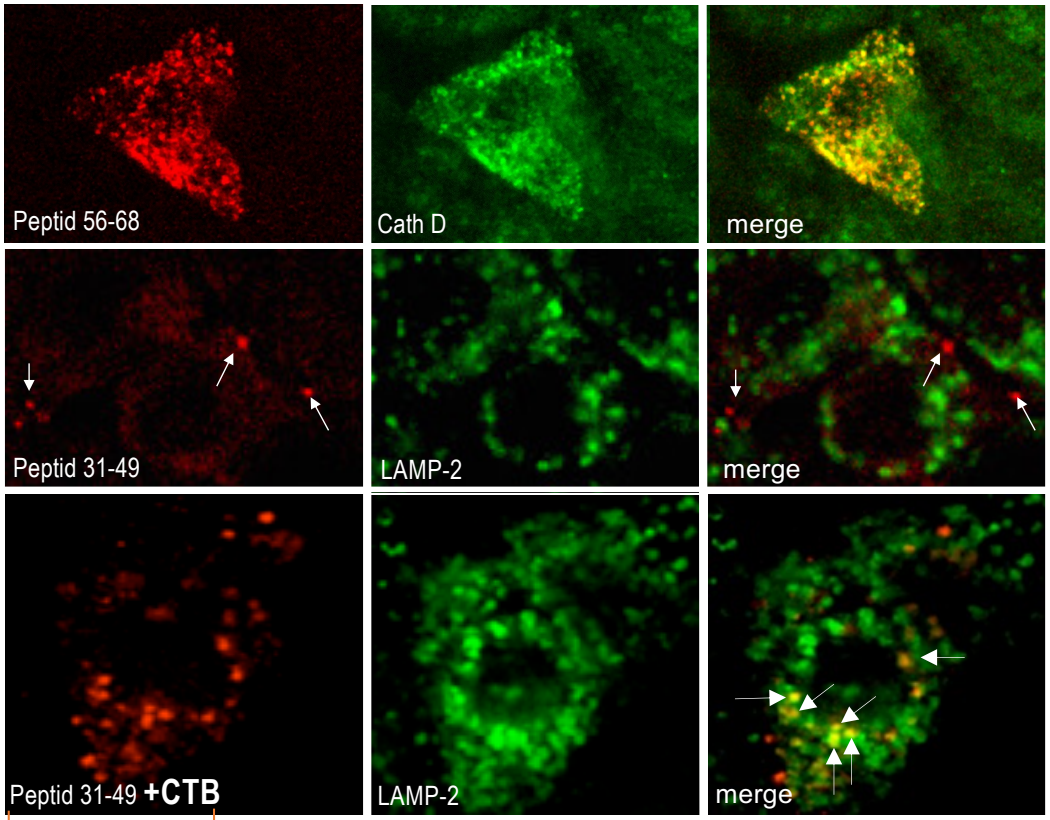

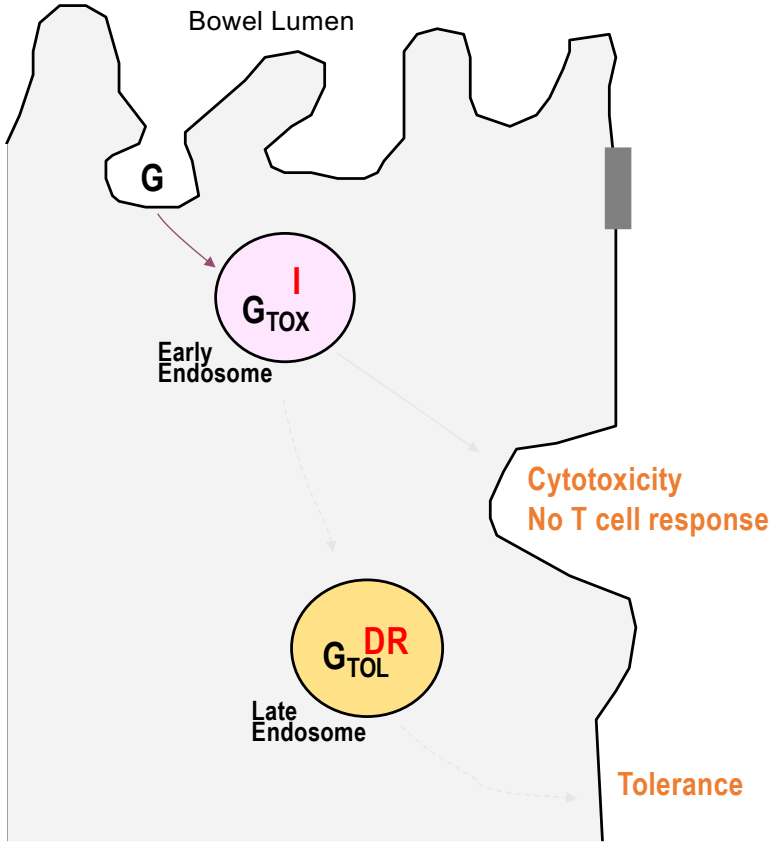

Gut 59: 300, 2010

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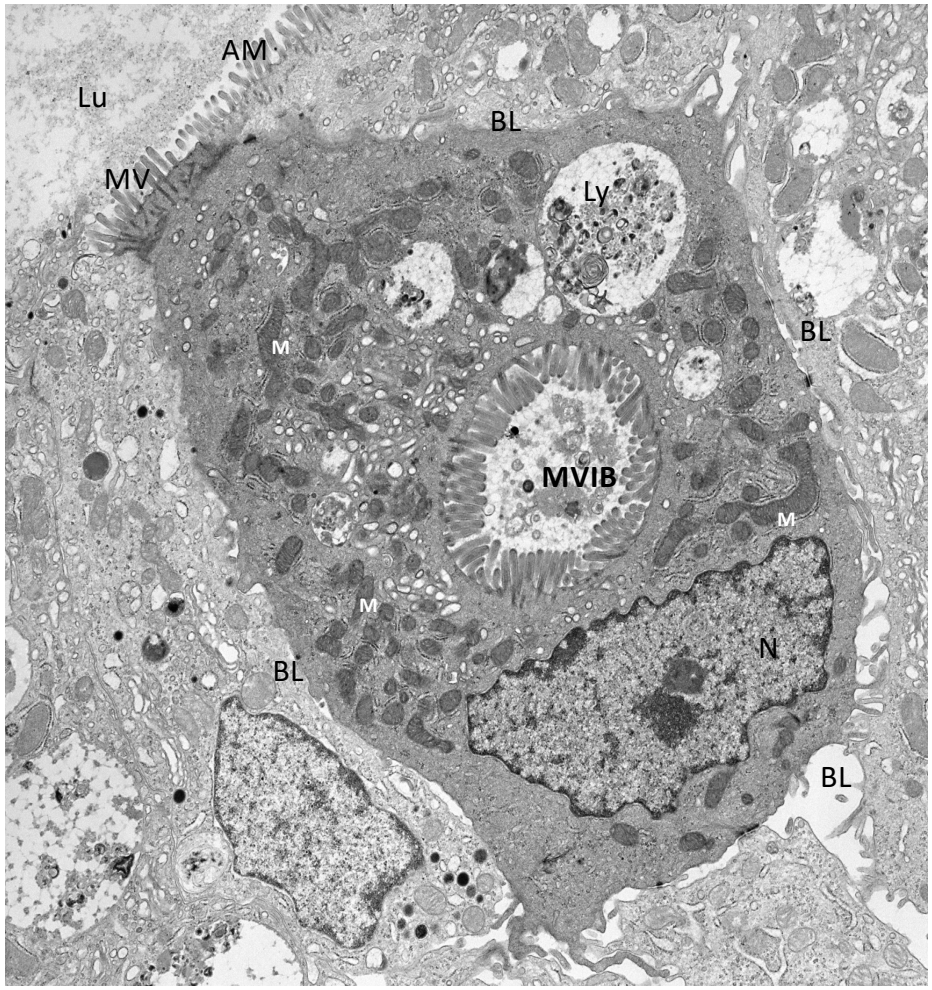
Microvillus Inclusion Disease

- Infants lifelong dependent on parenteral nutrition
- Defect of the biosynthetic pathway

  Davidson et al. Gastroenterology 75, 783, 1978
  Cutz et al. NEJM 320: 646, 1989
- Internalization of cationized ferritin and OVA into Microvillus Inclusion Bodies
  ⇨ increased autophagocytosis

  Gut 51: 514, 2002
- Mutations of myosin Vb and syntaxin 3

  Müller et al. Nat Genet 40: 1163, 2008
  Wiegerinck et al. Gastroenterology 147: 65, 2014

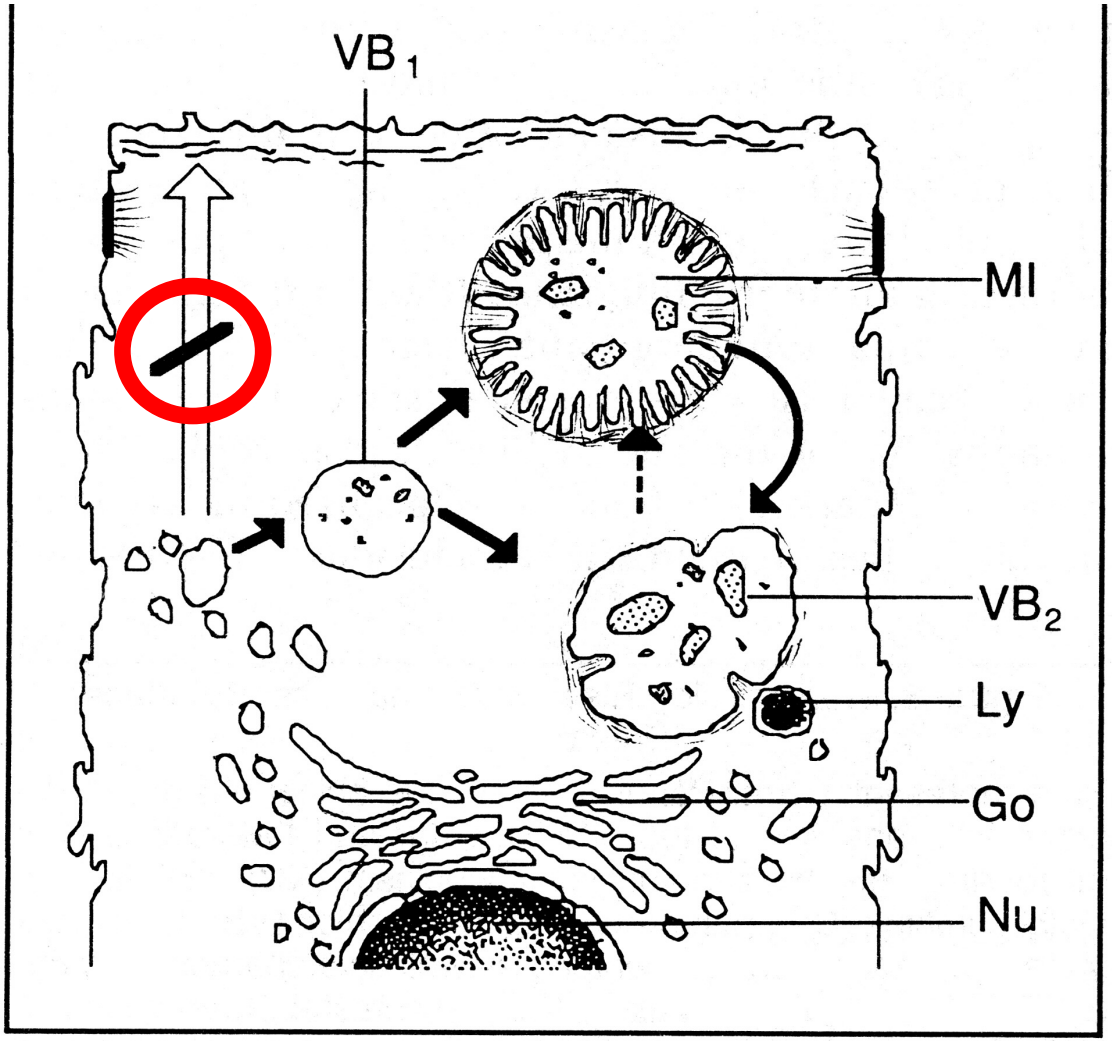


Lu, Lumen; MV, Microvilli; AM, Apical Membrane; BL, Basolateral Membrane; M, Mitochondrium; Ly, Lysosome; N, Nucleus; **MVIB, Microvillus Inclusion Body.**

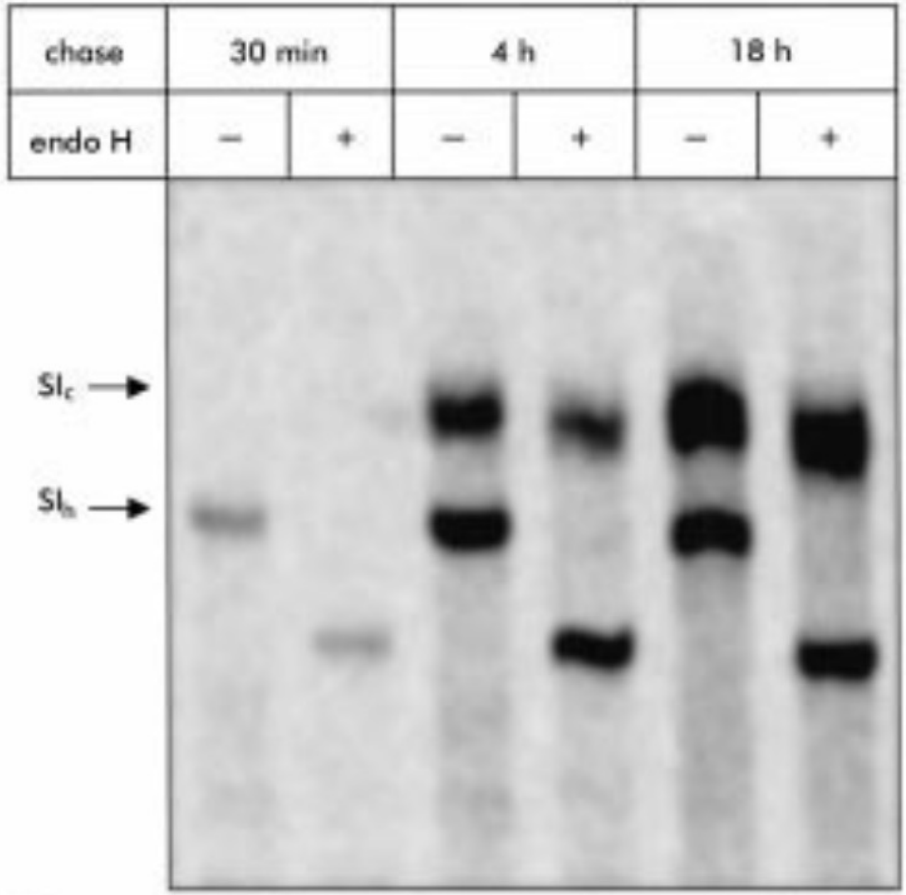
Cutz E, Rhoads M, Drumm B, Sherman PM, Durie PR, Forstner GG:

Microvillus inclusion disease: An inherited defect of brush-border assembly and differentiation.

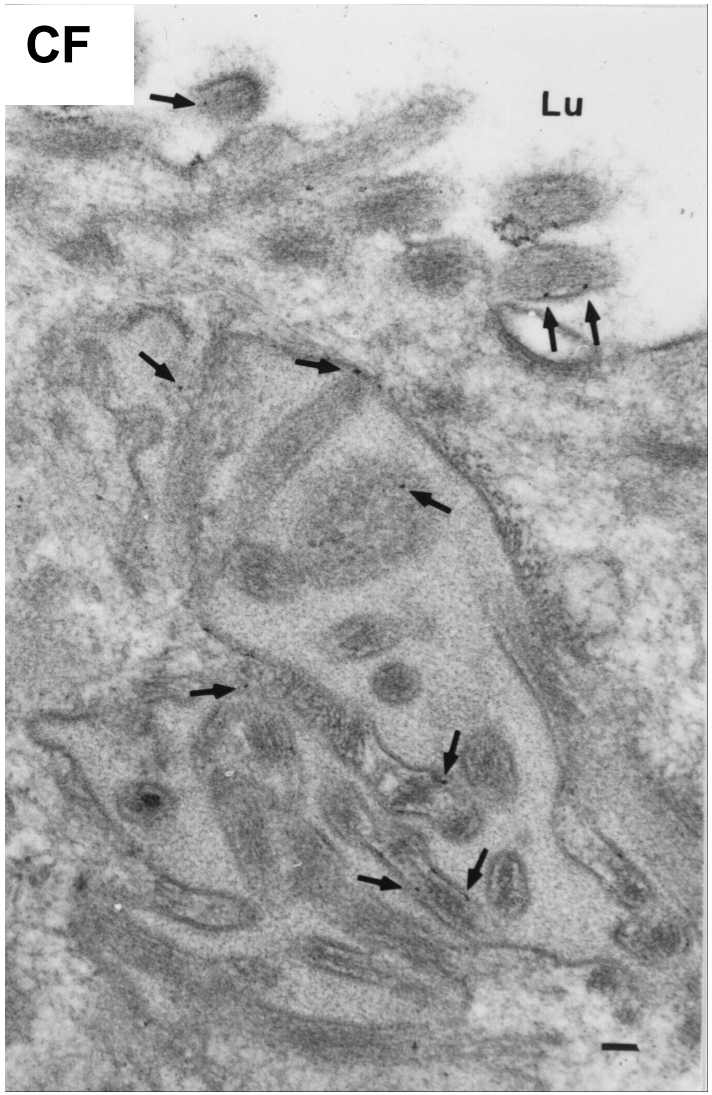
N Engl J Med 1989; 320: 646–51



"Acid phosphatase, a lysosomal marker, has not been localized in large vesicular bodies or microvillus inclusions." *Davidson, Cutz, Hamilton, Gall, Gastroenterology 75: 783, 1978*



Normal conversion rates of the endo H sensitive mannose rich precursor forms of these proteins (SI_h) to the endo H resistant complex glycosylated and mature species (SI_C)



Internalization of cationized ferritn (CF) attached to the microvilli into MVIB within 5 min

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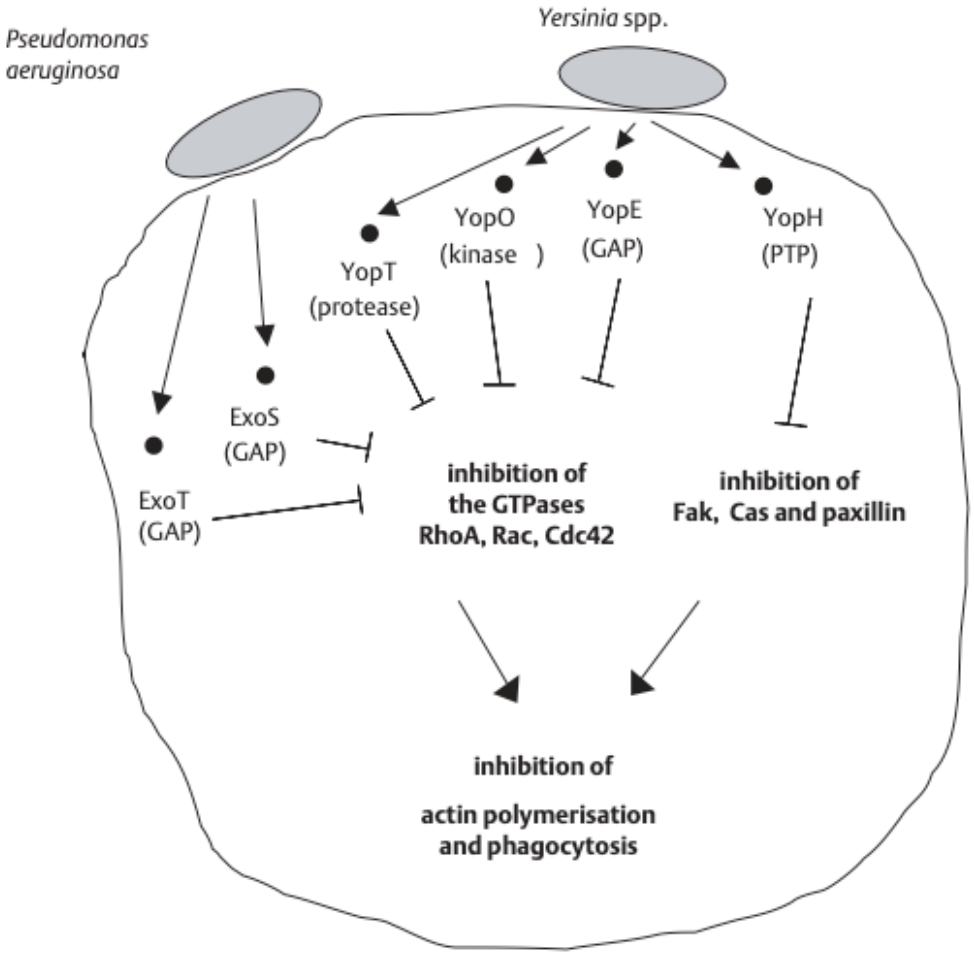
Inhibition of autophagocytosis: pharmacological therapy?



Bacterial pathogens of Yersinia or Pseudomonas aeruginosa are able to inject proteins into the cytosol of infected cells, thereby inhibiting actin poly-merization and susequently phagocytosis.

Infectious/toxic proteins

- ⇨ latrunculine, cytochalasine (actin)
- ⇨ vinca alkaloids (microtubule)
- ⇨ filipin, methyl-ß-cyclodextrin, genistein (lipid rafts)
- ⇨ chlorpromazine (clathrin)
- ⇨ amiloride (Na+/H+ exchange, macropinocytosis)

Z. Gastroenterol 44: 667, 2006

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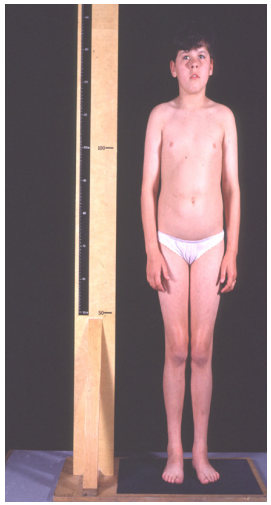
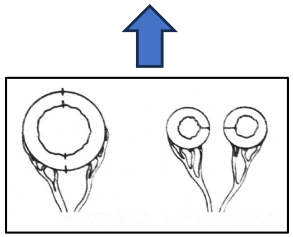
Short Bowel Syndrome (SBS)

1982

38cm Jejunum - No ICV, All colon



2004



Bianchi Op

A. Bianchi / Manchester

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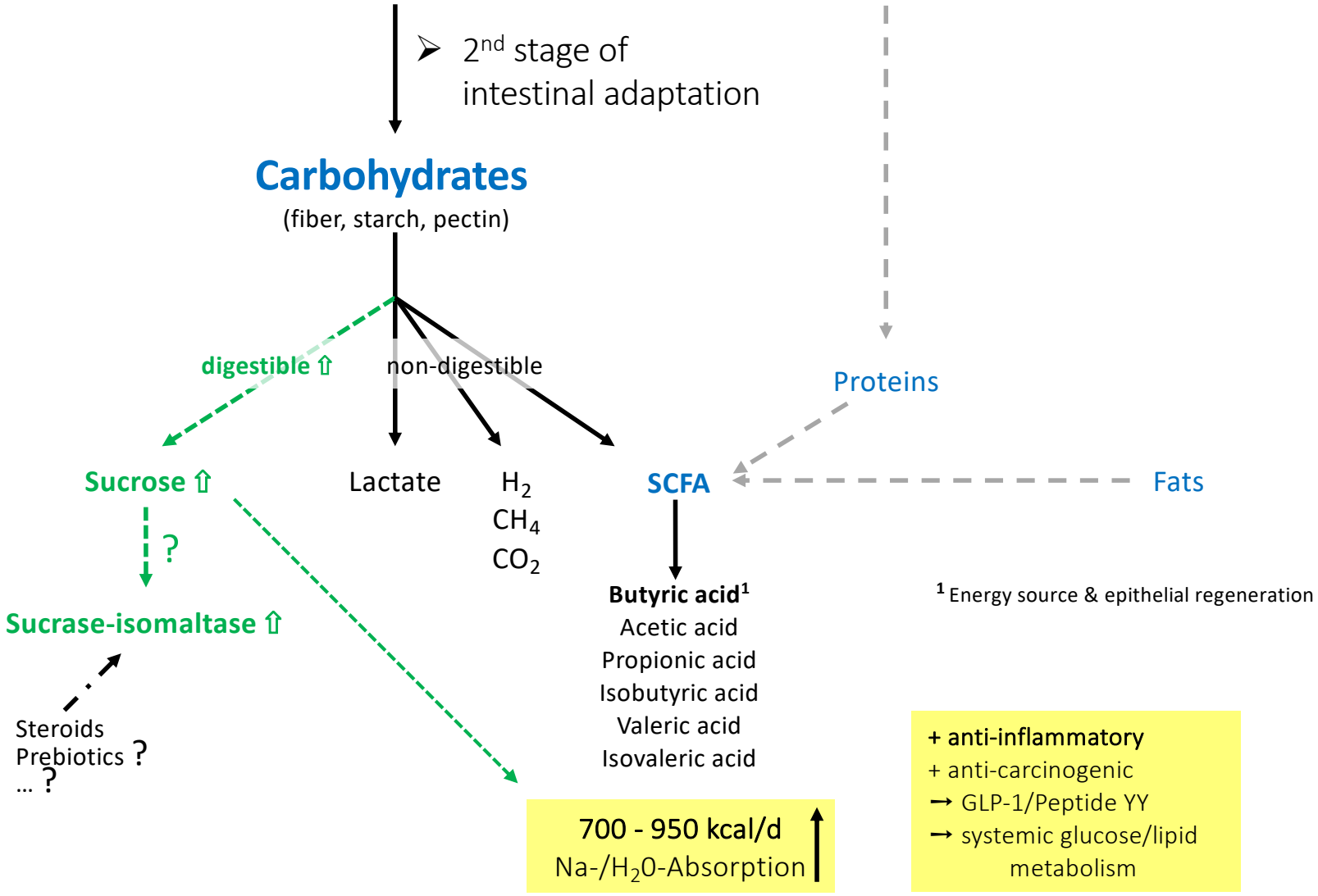
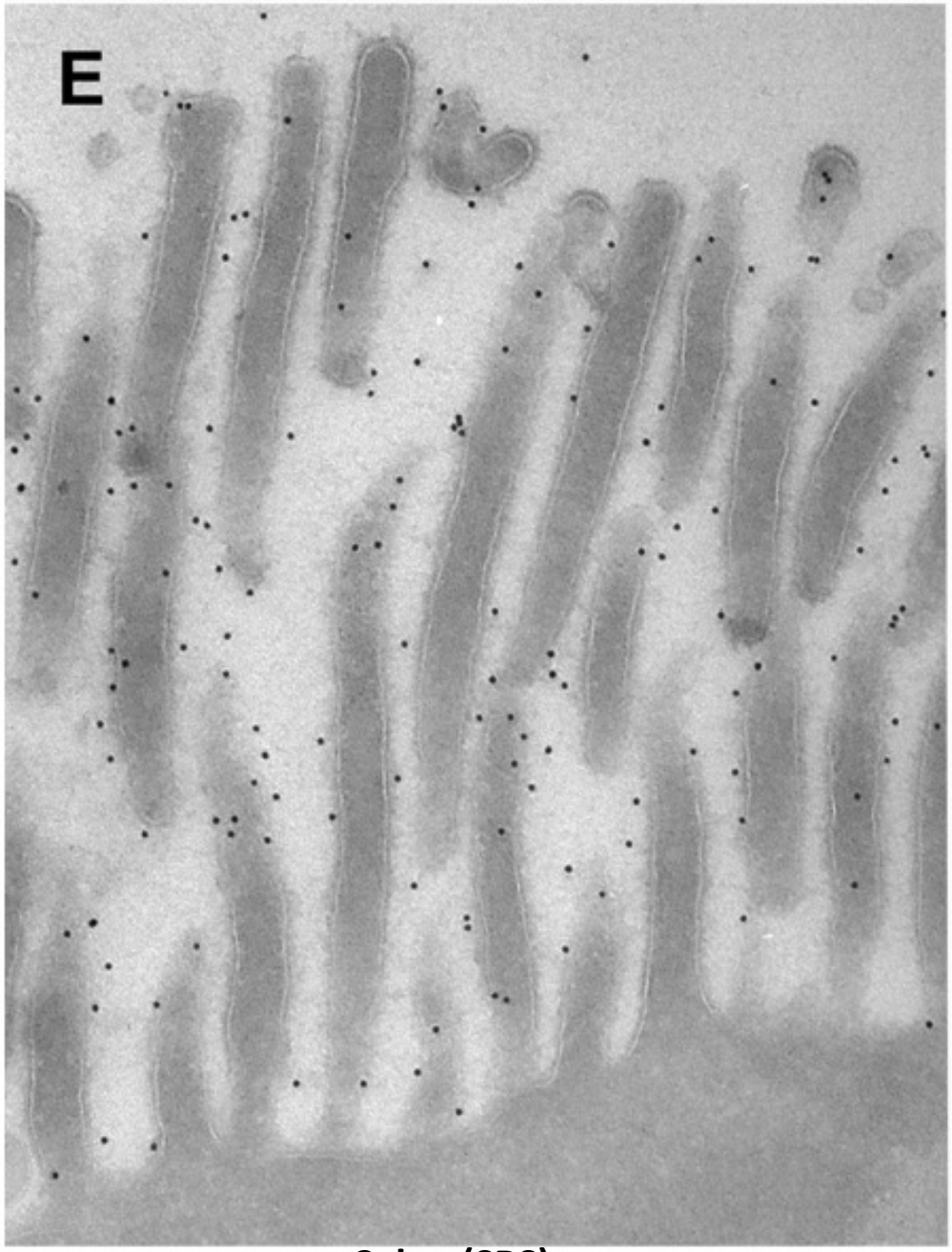
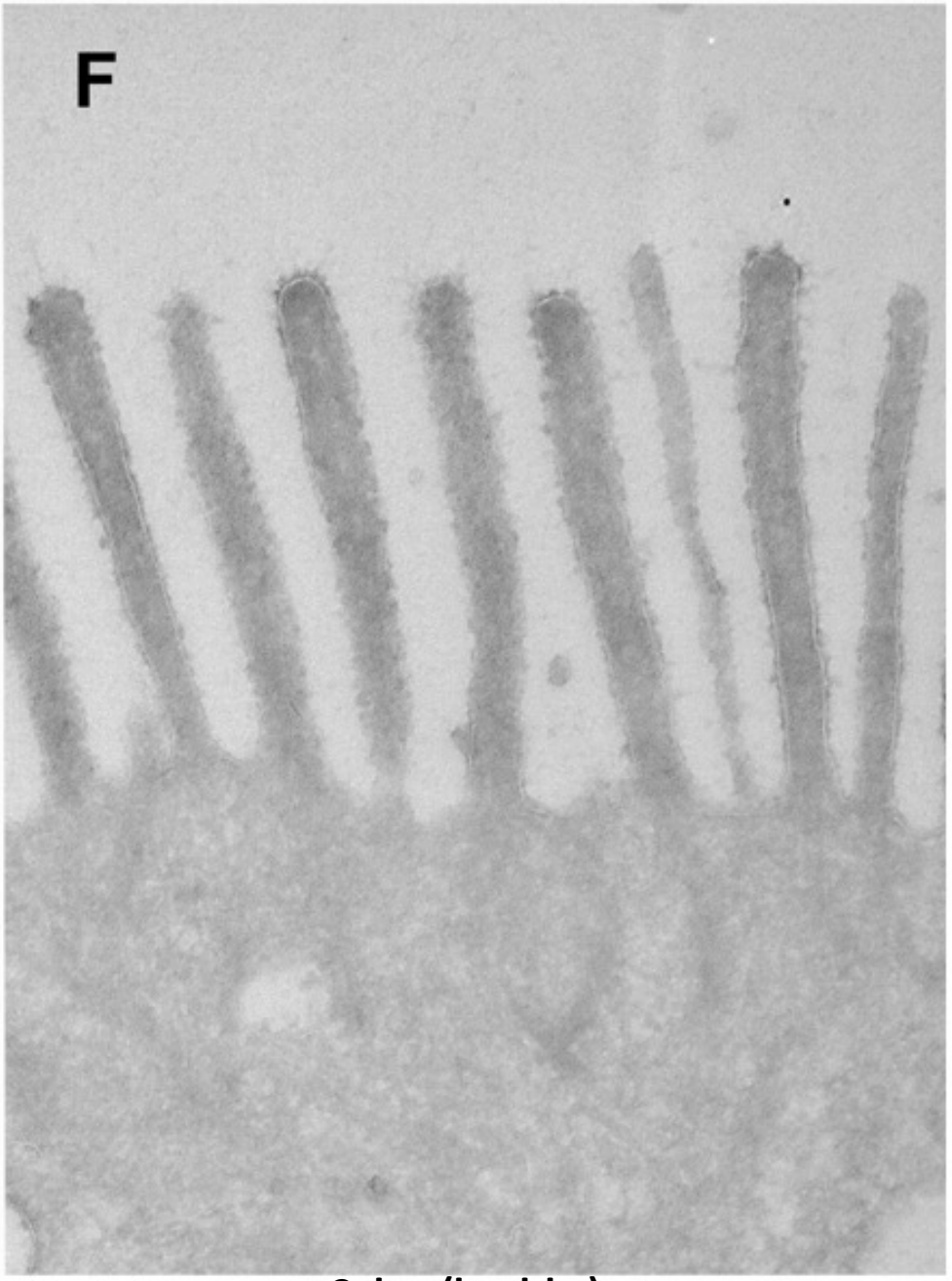
Colon: Bacterial (anaerobic) Fermentation



Sucrase



Colon (SBS)



Colon (healthy)

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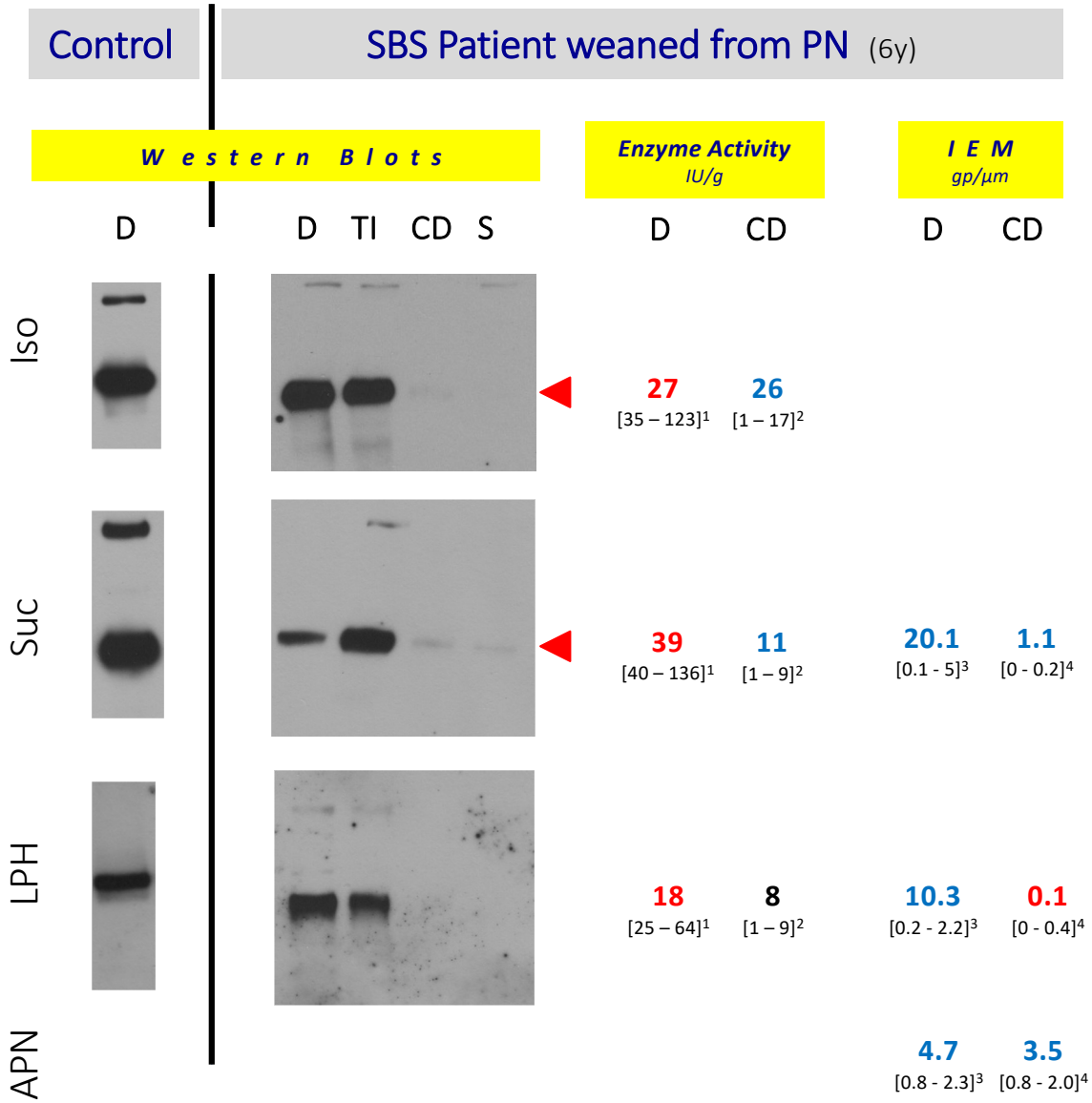

- gastroschisis, ileal atresia
- small bowel: 54 ➞ 105 cm (+ Bianchi at 1y)
- 60 cm distal colon

Iso = *Isomaltase*
Suc = *Sucrase*
Lac = *Lactase*
APN = *Aminopeptidase*
D = *Duodenum*
TI = *Terminal Ileum*
CD = *Colon Descendens*
S = *Sigma*
DS = *Disaccharidases*
IEM = *Immunoelectron microscopy*

[1] *Normal Range Duodenum (healthy controls)*
[2] *Mean ± SD Colon (SBS patients)*
[3] *Mean ± SD Duodenum (healthy controls)*
[4] *Mean ± SD Colon (healthy controls)*

TAKE HOME MESSAGE:

Translational research can be very productive and innovative!
⇨ therapies to cure and even to prevent chronic diseases

Thank you for your attention !