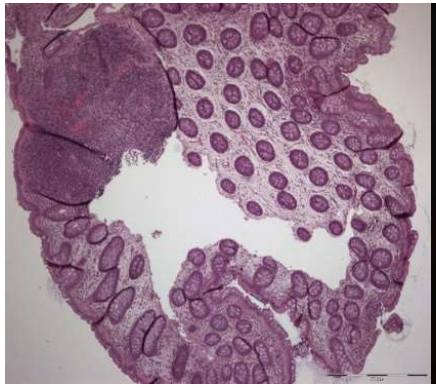
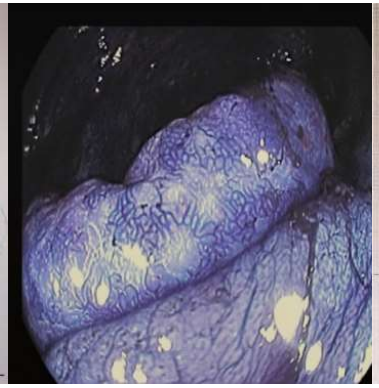
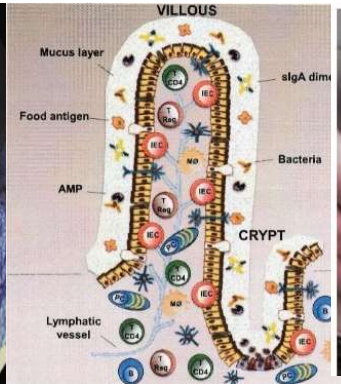
ICoMI VIRTUAL
2nd International Congress of
Micro-immunotherapy
June 2-4, 2022

Mast cell activation in health and disease: Relationship between gut, microbiota and immune- as well as neurovegetative responses

Martin Raithel

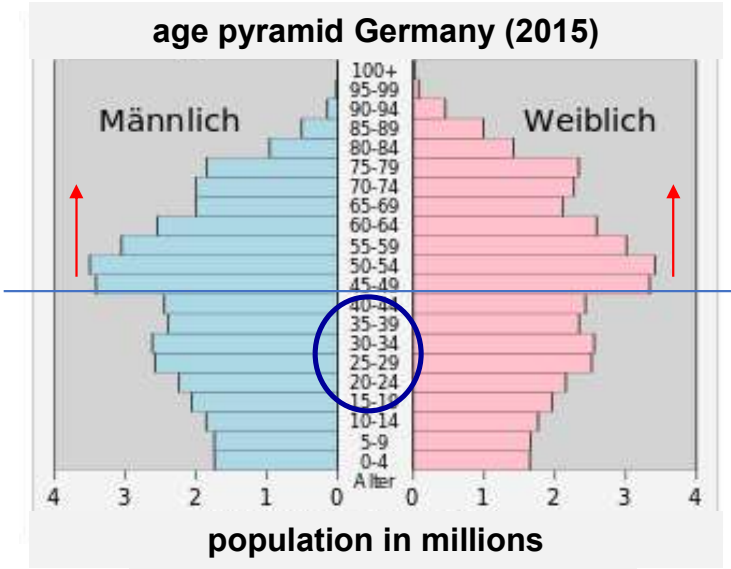
Malteser Waldkrankenhaus St. Marien gGmbH, Erlangen
Gastroenterology, Interventional Endoscopy, Hemato-Oncology, Diabetic- and Metabolic Diseases

www. waldkrankenhaus. de
www.VAEM.eu

Future social challenges
Control of microbiome and mast cells ?

Reducing diversity of human microbiom ↓

↑ Increasing incidence of mast cell diseases



Overaging society

. . .
chronic obstipation
malignancy, malnutrition

structural- & functional deficites

neurodegenerative diseases
. . .

Chronic inflammation
Leaky gut syndrome
Metabolic diseases
. . .

Incidence rates in younger people ↑

. . .
autoimmunity
adipositas, allergies

Barrett esophagus, diabetes

Crohn‘s disease,
ulcerative colitis
. . .

Role of mast cells in gastroenterology and endoscopy

- **Evolution of mast cells in the gastrointestinal tract (GIT)**
  Physiological distribution and involvement of mast cells in homeostasis
  regulation of various physiological functions
  first line defense
  - gastrointestinal infections (FimH-enterobacteria, parasites)

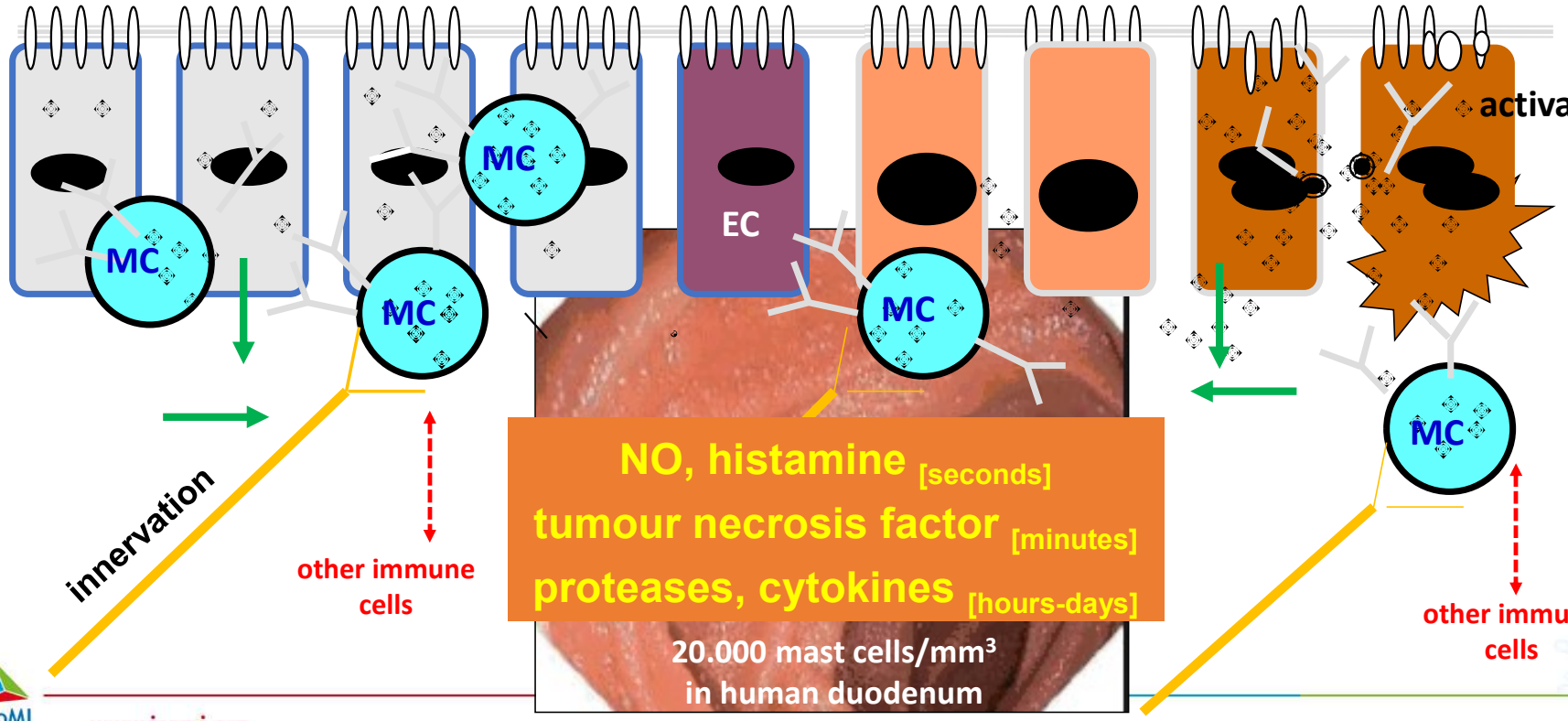
- **Frequent diseases with mast cell hyperplasia, as pathophysiological player or modifier**
  primary involvement
  - gastrointestinally mediated food allergy (e.g. IgE, type I allergy)
  - local or systemic mastocytosis

  primary and/or secondary involvement
  - functional gastrointestinal disease (FGID) and Irritable Bowel syndrome (IBS)
  - mast cell activation syndrome (MCAS)
  - . . .

  secondary involvement
  - celiac disease, as well as in non-celiac gluten/wheat sensitivity
  - Crohn‘s Disease and ulcerative colitis (IBD)
  - eosinophilic gastrointestinal disease (EGIDs)
  - non-IgE mediated gastrointestinally mediated food allergy (e.g. type II, III, IV)
  - . . .

Mucosal mast cells (MMC) in the human gastrointestinal tract (GIT)
Primary immune cells located in mucosal surfaces and intestine

Evolutively very old and widespread cell type



Different stages of life

1) Resting cell
2) Partly activated
3) Fully activated
4) Inhibited or controlled, or stabilized cell
5) cell death (apoptosis, necrosis)

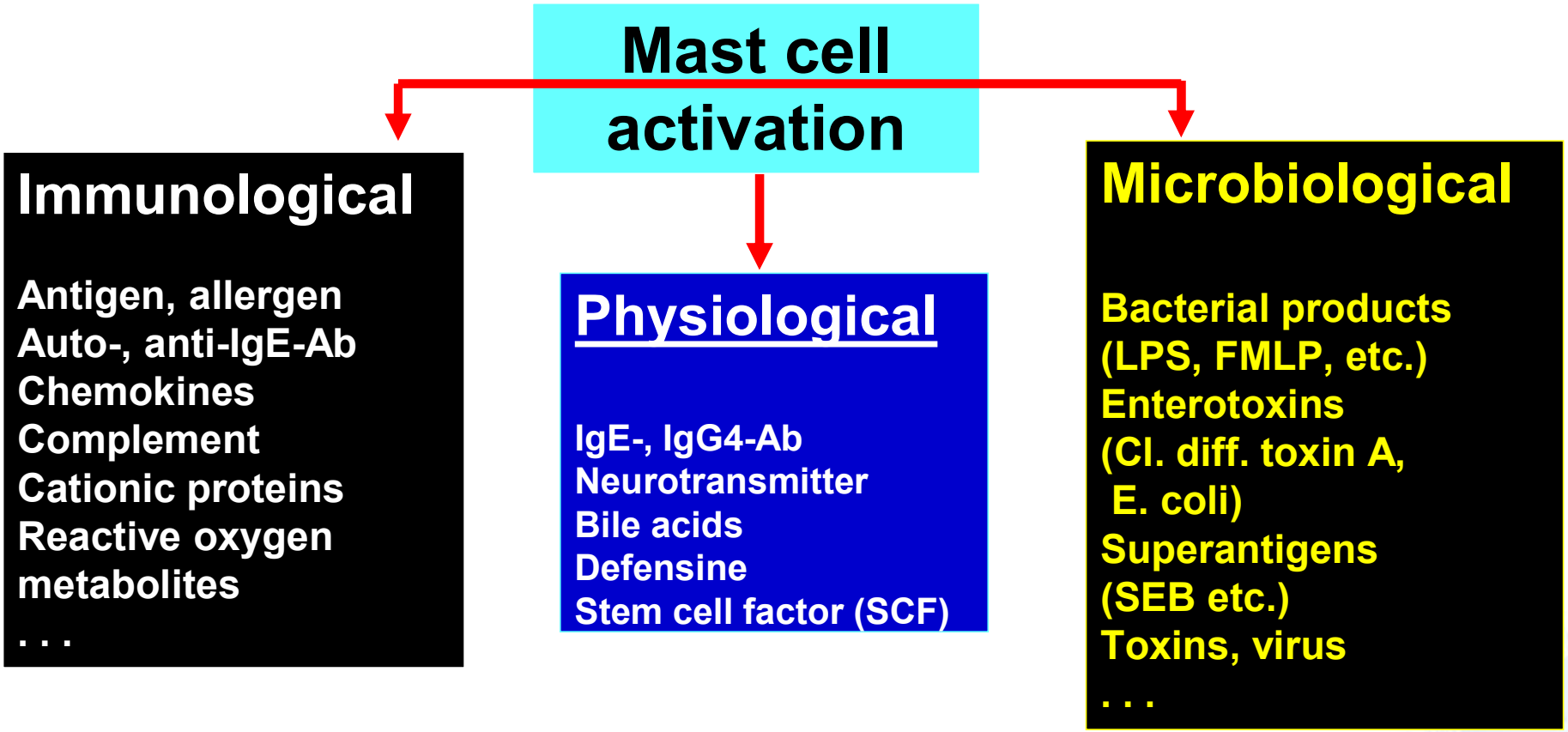
Physiological functions of mast cells in non-malignant gastrointestinal tissues

- **Gastric acid secretion - histamine**
  Gastric mucosal protection (vasodilation, mucus) – prostaglandins
- **Among others, important regulator of gut barrier tightness**
- **Regulation of motility – stomach, jejunum, ileum, sigmoid colon**
- **Induction of chloride secretion in intestinal epithelial cells - histamine**
- **First line of defense to bacterial invasion by phagocytosis of bacteria with subsequent mediator secretion and rapid induction of immune cell activation (e.g. neutrophils)**
- **Immunoregulatory functions on innate and adaptive immune response**
- **Effects on neurotransmission (histamine, serotinin CNS/ENS)**
- **Tissue repair, angiogenesis and wound healing**

- Mast cells account for 2-3% of mononuclear cells patrolling the lamina propria
- Mast cells are at all body sites that come into frequent contact with the external milieu
- MZ contain or may produce more than 200 biochemically and immunologically active secretory products
- „heparinocytes“
- **Mast cells function as „early warning system“**

Quist RG et al. Activation of mast cells by bile acids. Gastroenterology 1991; 101: 446-456
Malaviya R et al. Mast cell phagocytosis of FimH-expressing enterobacteria. J Immunol 1994; 152: 1907-1914
Binder HJ et al. Selected summaries: Bactericidal activity of mast cells. Gastroenterology 1994; 107: 893-894
Bertaccini G, Corruzzi G. An update on histamine H3 receptors and gastrointestinal functions. Dig Dis Sciences 1995; 40(9): 2052-2063

Potential pathogenic stimuli for mast cell degranulation



→ Pathologically increased activation in mast cell activation syndrome (MCAS)
Sub-threshold triggers of physical stimuli (heat, pressure, osmolality) as well as neurovegetative influences (anxiety, emotions, stress) etc. may trigger mast cell degranulation

Molderings GJ, Homann J, Raithel M, Frieling Th. For the Interdisciplinary Multicenter Research Group on Systemic Mast cell activation disease (MCAD), Germany. Correspondence: Toward a global classification of mast cell activation diseases. *J Allergy Clin Immunol* 2011; 127(5): 1311 - 1312

Inhibition and down-regulation of mast cell function

Mucosal and connective tissue mast cell populations

Immunological

ITIM-bearing receptors
- FcγRIIb, gp49B1,
- signal regulatory protein-α (SIRP-α),
- TGFβ receptor

Non-ITIM-bearing receptors
- CD 200
- G-protein receptors

Physiological

Normal extra- and intracellular levels of calcium, magnesium and iron
. . .

Microbiota & nutrients

SCFAs – act & inhibit
→ butyrate inhibits degranulation & TNF production

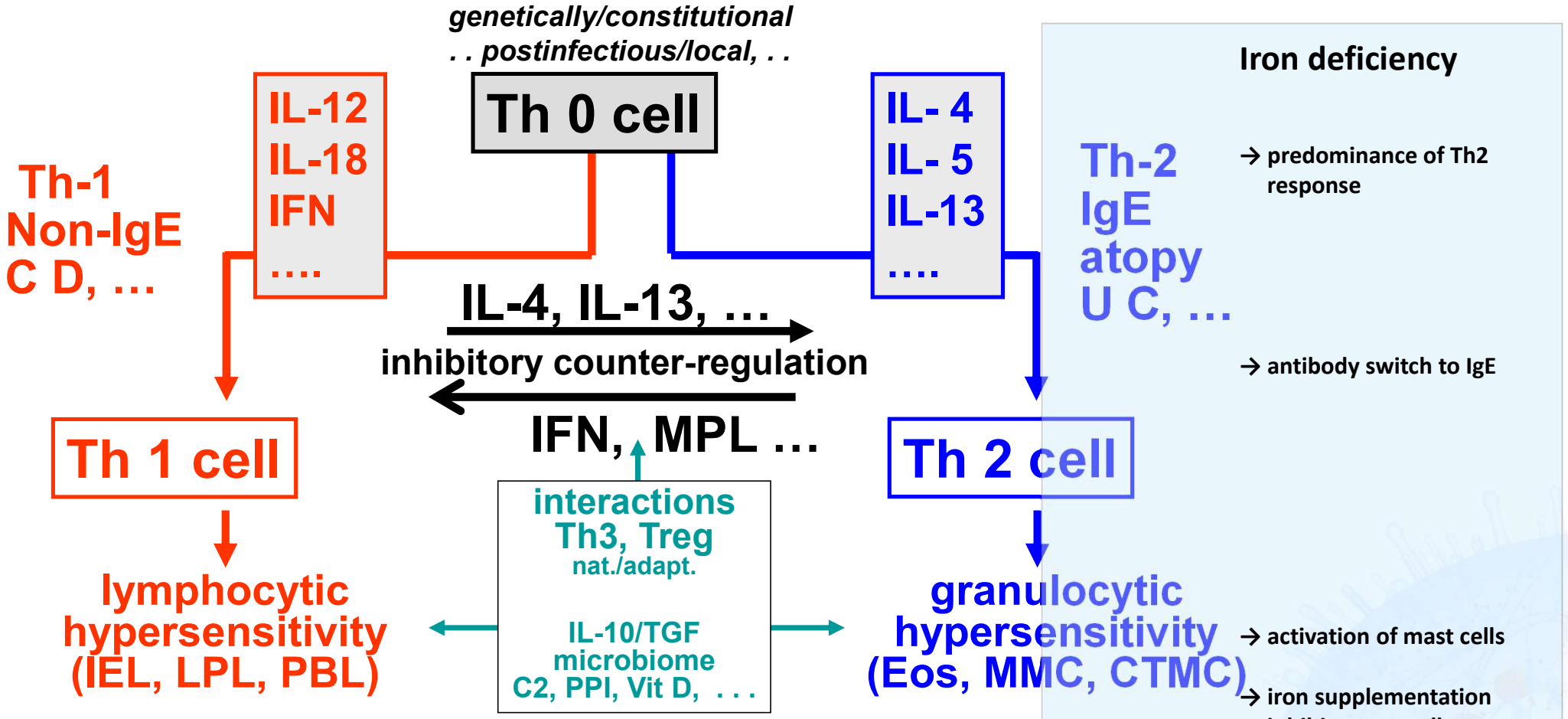
SCFA inhibits histone deacetylation
→ FOXP3+ CD4+ T cells, inhibiting histamine release

→S- & I-type lectins (Siglecs, Siglec-8) inhibit IgE-dependent MC activation
. . .

→ Physiological down-regulation and suppression of mast cells takes place in the microenvironment of a healthy gut by nutrients, metabolites and constituents of the microbiome as well as physiologcal neural transmission

Afrin L et al. MCAD and microbiotic interactions. Clin Ther 2015
Rea K et al. Microbiome: A key regulator of stress and neuroinflammation. Neurobiology Stress 2016; 4: 23-33

Immune regulation in allergy & inflammation targeting lymphocytic and granulocytic cell populations in the GIT



Roth-Walter F et al. Cow's milk protein beta-lactoglobulin confers resilience against allergy by targeting complexe iron into immune cells. J Allergy Clin Immunol 2020
Roth-Walter F. Gezielte Mikroernährung gleicht einen funktionellen Eisenmangel beim Allergiker aus. Allergologie 2021; 44(5): 349-355

IgE-dependet food allergy or gastrointestinally mediated food allergies grade I-IV°

as a simple model of mast cell activation via $Fc_{epsilon}$-RI/II

Local gastric expression of IL-4 mRNA in Hp-positive patients with food allergy

Early allergic phase (< 1 - 6h):
histamine, PAF, serotonin, TNF, prostaglandines, leucotrienes etc

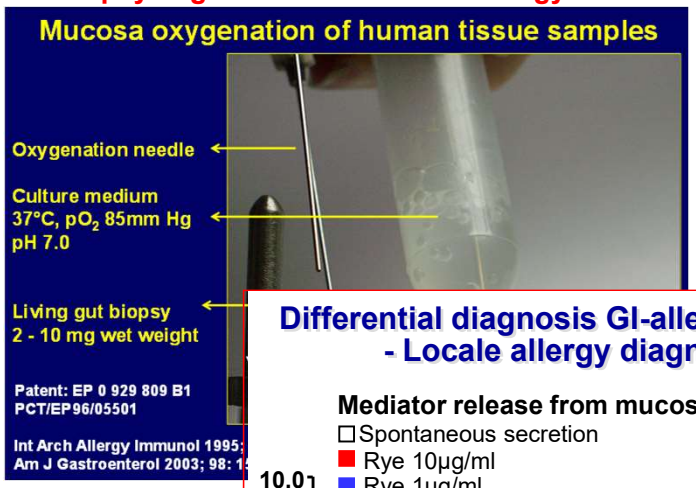
Late allergic phase (> 6 - 12h - 72h):
GM-CSF, chemotaxins ECF, NCF, tryptase, E C P, cytokines

Konturek PC, Rienecker H, Hahn EG, Raithel M. Helicobacter pylori as protective factor against food allergy. Med Science Mon 2008; 14(9): CR453 – CR458

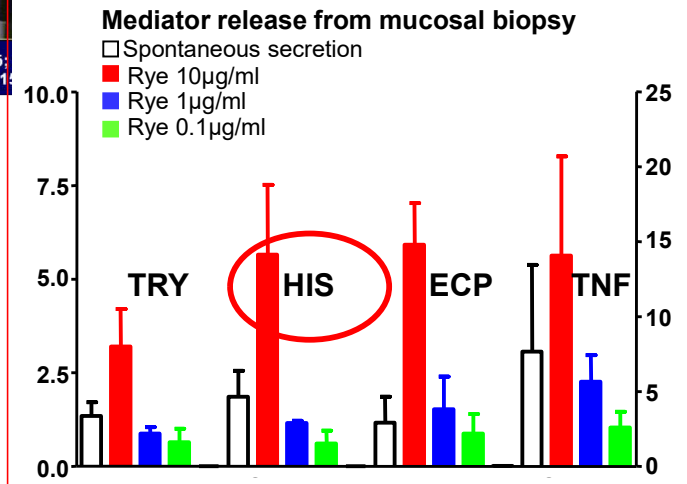
Role of endoscopy in immunology

Functional proof of immediate local mediator release with clinical consequences

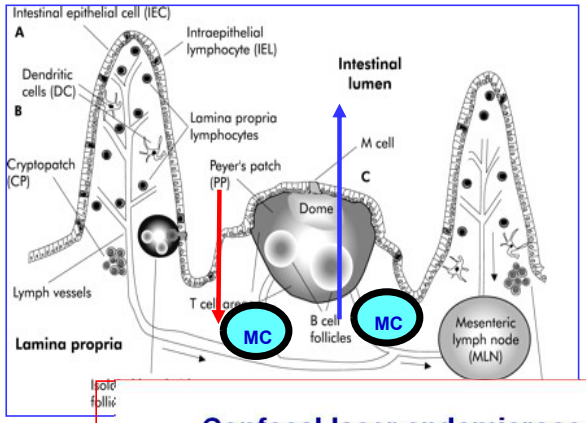
Baenkler HW, Lux G. Antigen-induced histamine release from duodenal biopsy in gastrointestinal food allergy. Ann Allergy 1989



Differential diagnosis GI-allergy and/or IBD
- Locale allergy diagnostics -



Locale (seronegative), IgE-mediated GI-allergy
complete remission > 5 years after avoidance

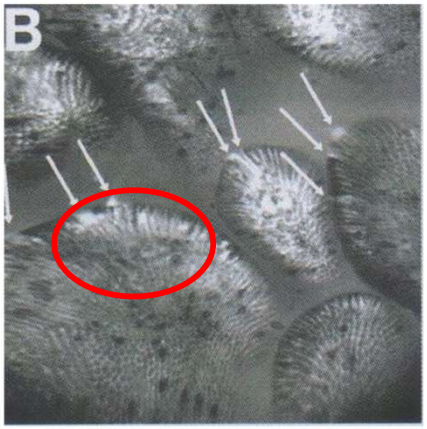


allergens
antigens
…

antibodies IgE, IgA, …
mediators histamine, tryptase, TNF, …

Confocal laser endomicroscopy (CLE) in IBS
Effects of CLE-identified foodstuffs on IBS

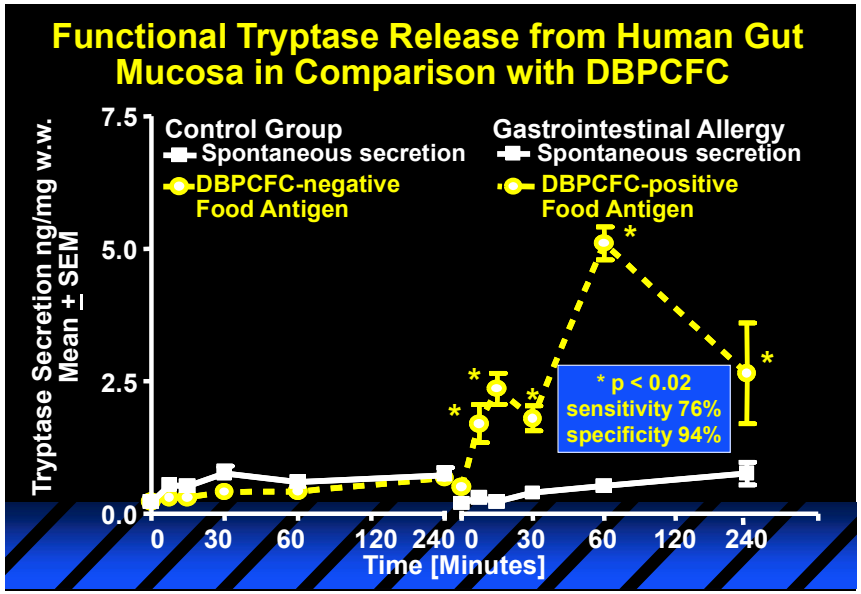
- 22/36 pts. (61.1%) = CLE+ group showed reactions in CLE
  - Wheat 13/36 (36.1%)
  - Milk 9/36 (25.0%)
  - Yeast 6/36 (16.6%)
  - Soy 4/36 (11.1%)
- Response rate to individual diet of CLE-identified antigens
  - symptom score reduction >50% in 19/22 pts. (86.3%) < 4 weeks
- After 3 months symptom reduction in
  - 5 pts. 50-70% ↓
  - 11 pts. 80-90% ↓
  - 6 pts. 100% ↓



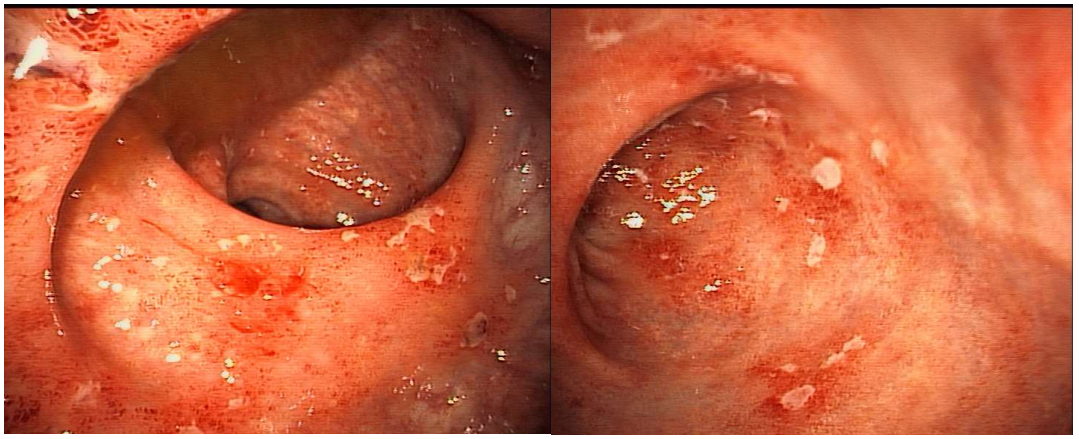
Raithel M et al. Colorectal mucosal histamine release by mucosa oxygenation in comparison with established clinical tests in GI allergy (GMA). W J Gastroenterol 2006; 12(29): 4699–4705

Fritscher-Ravens A et al. Many Patients With IBS Have Atypical Food Allergies Not Associated With Immunoglobulin E. Gastroenterology 2019 Jul; 157: 109–18.e5

Allergen-mediated mast cell activation in gut
Pathophysiological consequence of significant degranlation

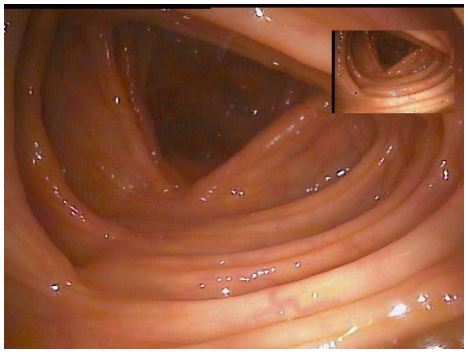


Raithel M et al.
Analysis and Characteristics of Mast Cell Tryptase and Eosinophilic Cationic Protein from Human Gut Mucosa in Gastrointestinal Allergy.
In Wüthrich B, Ortolani C (eds): Highlights in Food Allergy. Monographs in Allergy, Basel, Karger, 1996; 32: 143 - 156

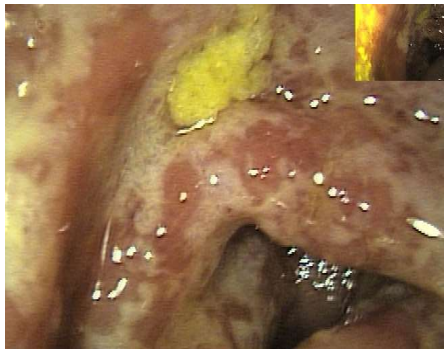


24 hours after positive double-blind oral food challenge wit nut mix
Hypotonia, fever, bloody diarrhoea and pain

Raithel M, Hahn EG, Baenkler HW. Gastrointestinal allergies. *Dtsch Ärzteblatt Int* 2002; 99: A 780 – 786



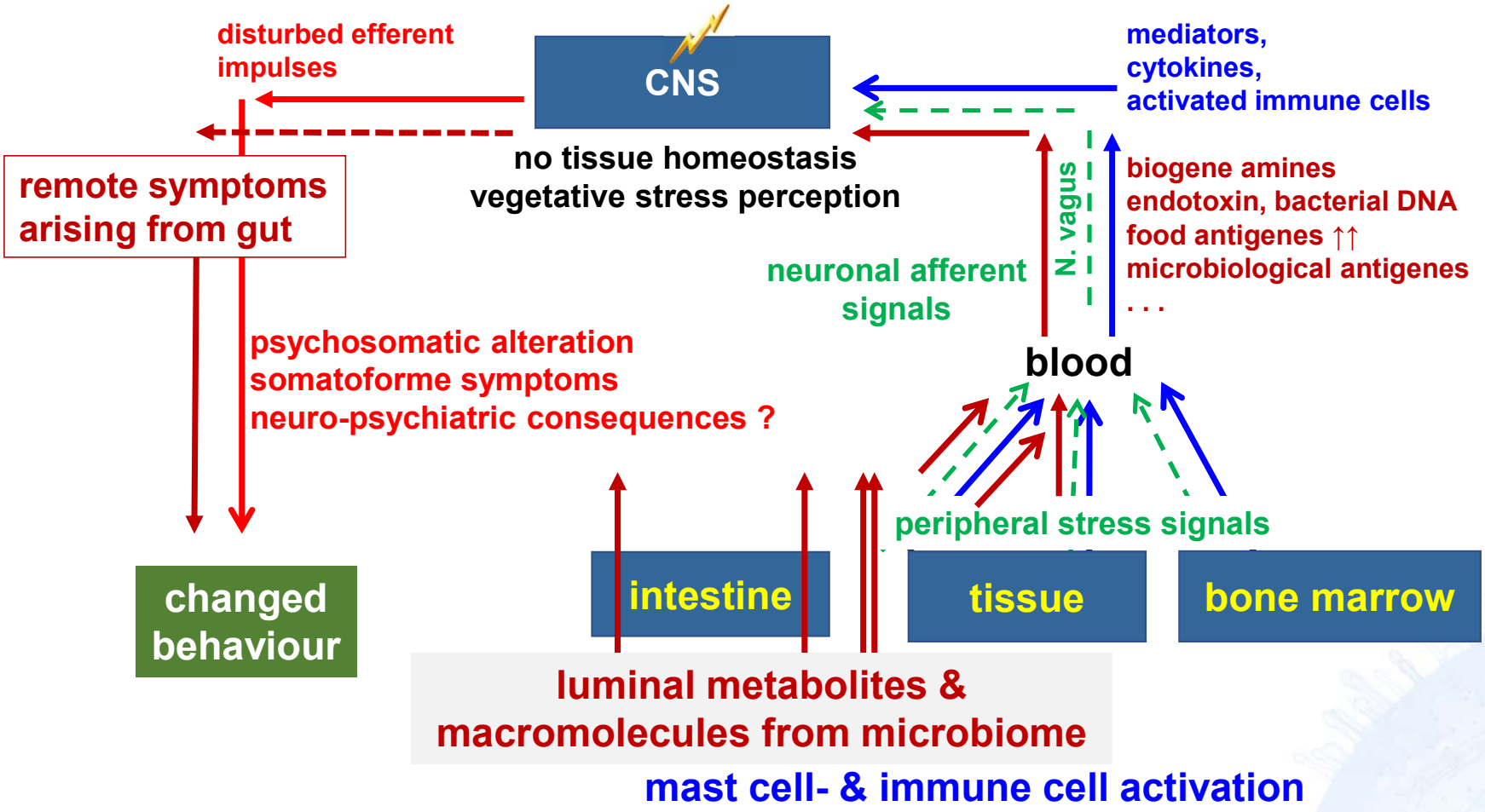
Right hemicolon before food chalenge with rye



Right hemicolon 14 hours after open rye challene

Left hemicolon 14 hours after food challenge

Problem of CNS involvement in non-erosive types of leaky gut as demonstrated in recurrent local allergic- or mast cell reactions



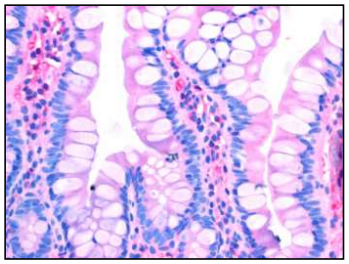
Afrin LB, Pöhlau D, Raithel M et al. B*rain Behav Immunol 2015 Jul 8. Pii: S0889-1591*
Kelly JR et al. Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. Front. Cell. Neurosci 2015; 9: Article 392

Modern approach for endoscopic immune diagnostics at the GI-tract

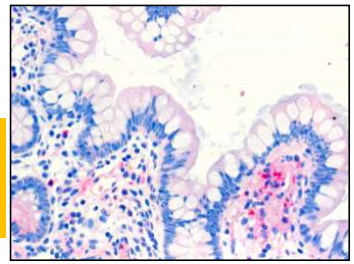
Non-allergic hypersensitivity
Food intolerance

Histology & immunostaining for DAO

normal red DAO-cóntaining epithelial cells and tissue



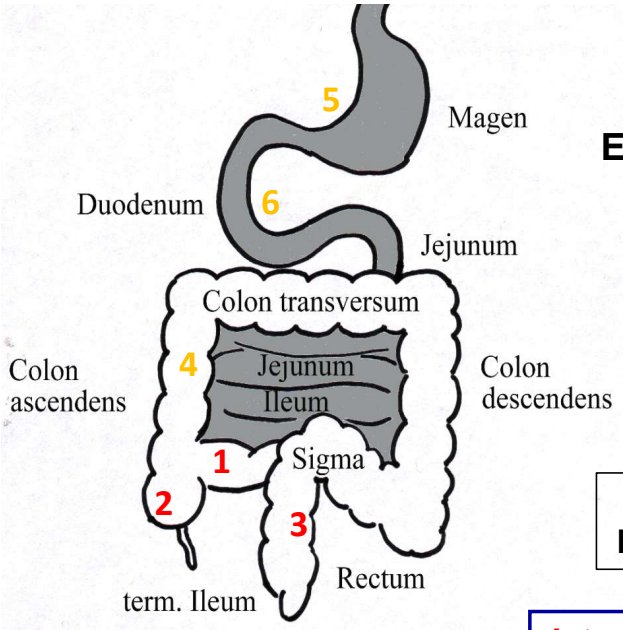
pale epithelial cells without red staining (loss/reduction DAO)



Indication for intolerance to biogenic amines

GI endoscopy & biopsies

Locations for endoscopically guided segmental gut lavage



Allergic hypersensitivity
Gastrointestinal food allergy

Endoscopically guided lavage within the gut

Work-up of lavage fluid
local food-specific IgE-antibodies & mediators

- **Intestinal IgE** indication of segmental type I allergy
- **ECP/Tryptase** indication of eos. gastroenteritis mastocytosis
- **TNF** indication of other Inflammation or non-IgE allergy

Schwab, Raithel, Hahn et al. Am J Gastroenterol 2001; 96: 508 – 514 Raithel et al. Deutsches Ärzteblatt 2002, 99: A780 – 787
Raithel M, Finzel S, Heussinger N, Rieker RJ, Baenkler HW. Gastrointestinally mediated food allergy causing Spondyloarthritis-like disease. Arch Clin Gastroenterol 6(2): 049-053
DOI: https://dx.doi.org/10.17352/2455-2283.000078

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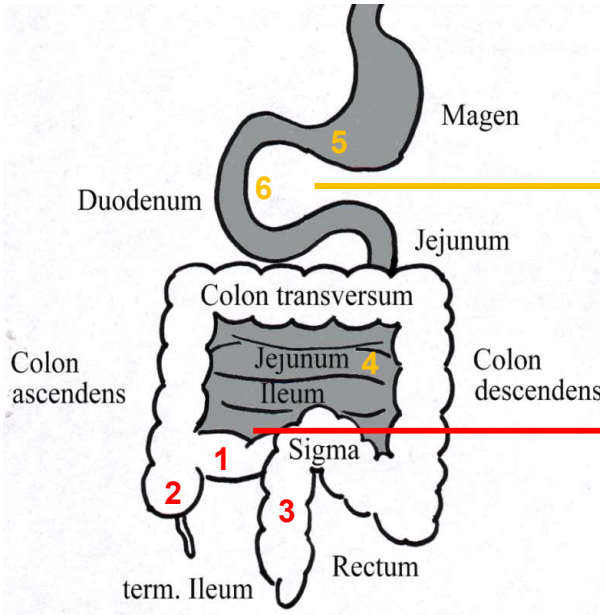
Endoscopically guided segmental gut lavage for luminal immune diagnostics at the gastrointestinal compartment

Lokalisationen zur endoskopisch gesteuerten segmentalen Lavage



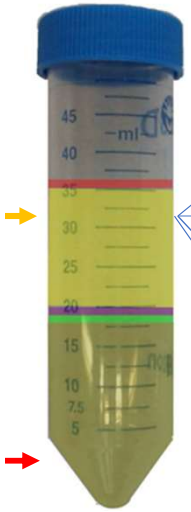
Anatomische Spülorte:

1. Unterer GI-Trakt:
(i.a. Erwachsene)
- **terminales Ileum (1)**
- **Coekum/Appendix (2)**
- **Rekto-sigmoidaler (3) Übergang**

2. Oberer GI-Trakt:
(Kinder & spezielle Fragestellungen)
- **Jejunum (4)**
- **Antrum (5)**
- **Duodenum (6)**

3. Mundhöhle
(orale Lavage)

Analyse der Spülflüssigkeit nach Konzentrierung (Cap-FEIA)



Aktivität Eosinophile & Mastzellen

Intestinales Gesamt-IgE („allergischer Darm“)

Intestinales spezifisches IgE (Darm- bzw. Segment-bezogene IgE-Sensibilisierung auf Lebensmittel

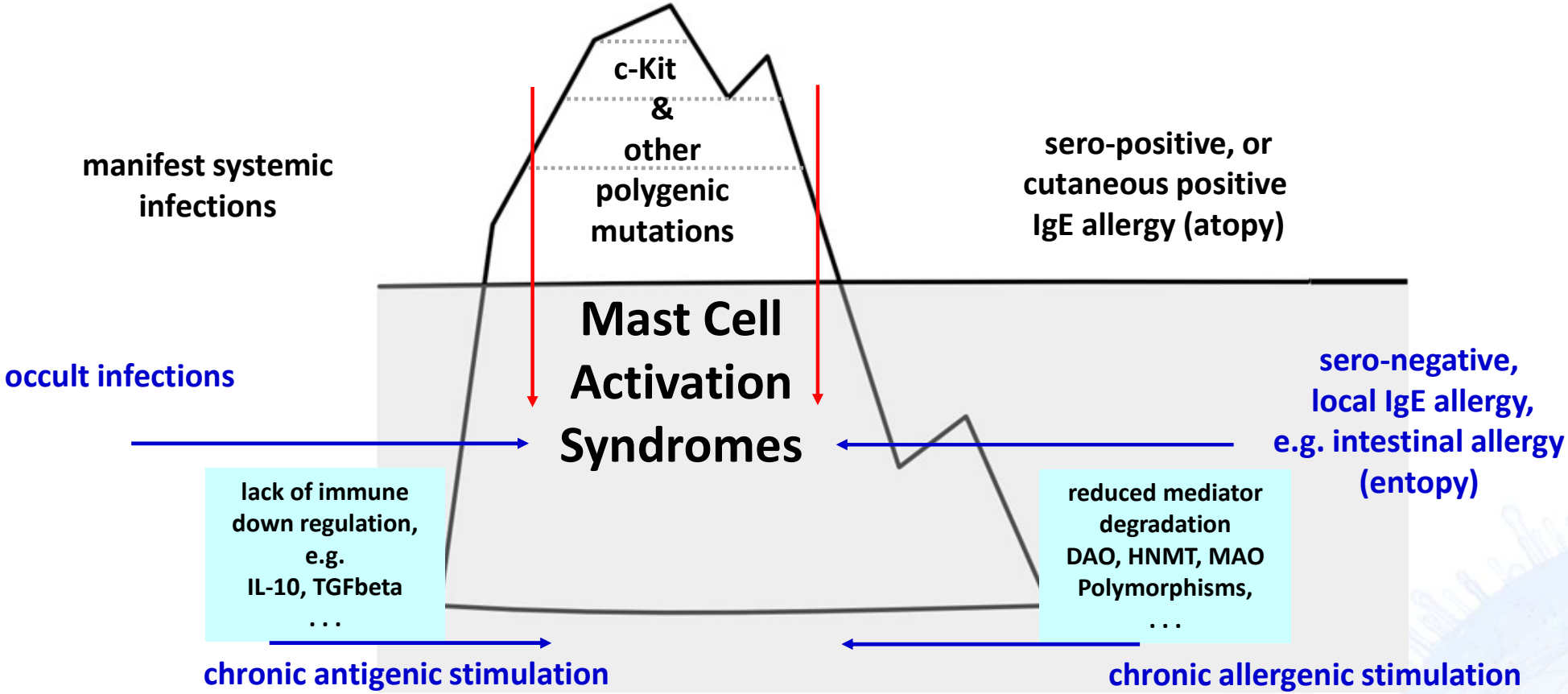
TNFalpha

Gesamtprotein (Bezugsparameter)

Biopsieentnahme immer erst nach Lavage
IgE-AK aus Blut ➔ falsch positive Werte

Schwab, Raithel, Hahn et al. Am J Gastroenterol 2001; 96: 508 – 514 Raithel et al. Deutsches Ärzteblatt 2002, 99: A780 – 787
Raithel M, Finzel S, Heussinger N, Rieker RJ, Baenkler HW. Gastrointestinally mediated food allergy causing Spondyloarthritis-like disease. Arch Clin Gastroenterol 6(2): 049-053 ;DOI: ttps://dx.doi.org/10.17352/2455-2283.000078

Emerging understanding of genetic and phenotypic drivers in mast cell activation disease (MCAD)



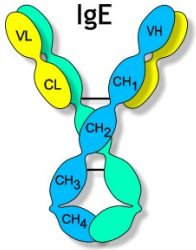
disorders featuring inappropriate mast cell activation

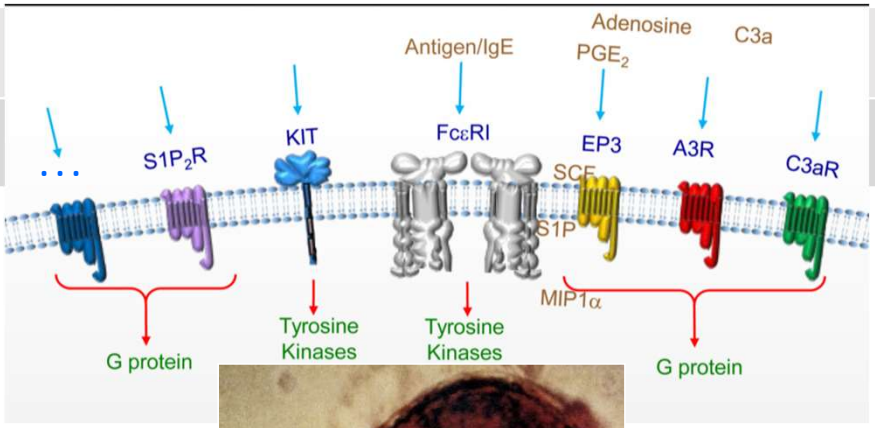
Irritable bowel syndrome (IBS),
Functional gastrointestinale diseases (FGID) and
Mast cell activation syndrome (MCAS)

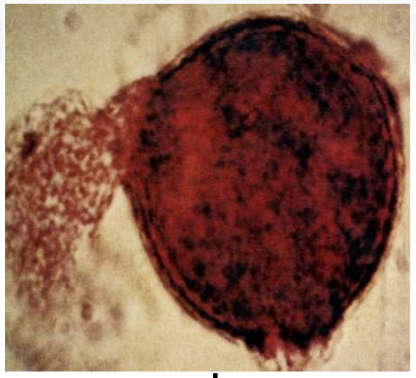
as a complex model
due to various, partly yet unkown, etiologies (triggers)

Various stimuli und stages of mast cell secretion
Anaphylactic degranulation *versus* selective secretion

Allergy		Inflammation
Antibody (IgE)		Various triggers







Immediate pulsatile histamine & mediator secretion and, usually consecutive degradation

↓

Sub-total anaphylactic degranulation of all mediators with a rapid kinetics

←

↓

Secretion of only 1-2 mediators responsible for functional complaints (?)

→

Slow, chronic persistent histamine -, and/or specialised mediator secretion, with a chronic impuls for mediator degradation

↓

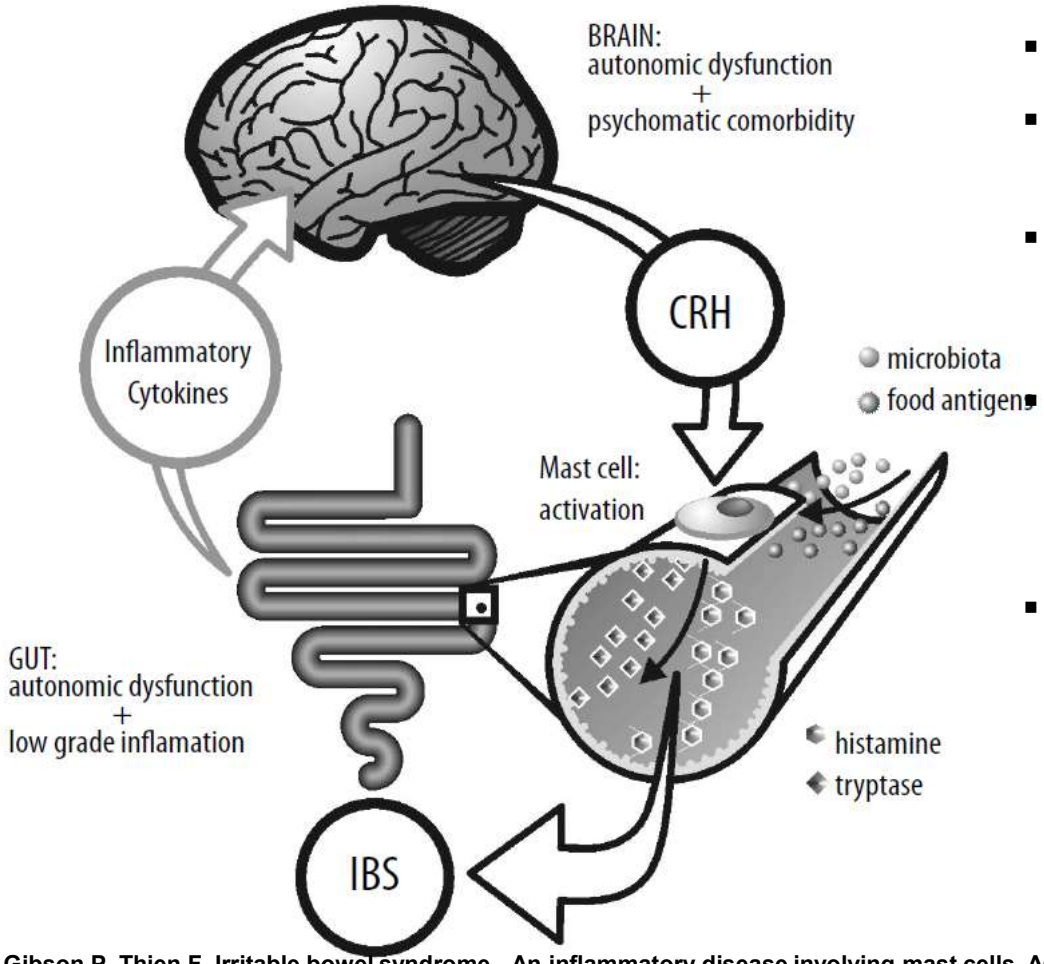
Selective piece-meal degranulation of a few or more particular mediators with a protracted kinetics

Modified und adapted from Gilfillan AM and Beaven MA. Regulation of mast cell responses in health and disease. Crit Rev Immunol. 2011 ; 31(6): 475–529

Neurovegetative regulation, immune homeostasis and role of microbiome in mast cell driven disease

The Crucial Pathogenetic Role of Mast Cells (MC) in Irritable Bowel Syndrome – Brain-Gut Axis and its` interaction

The Brain – Gut axis

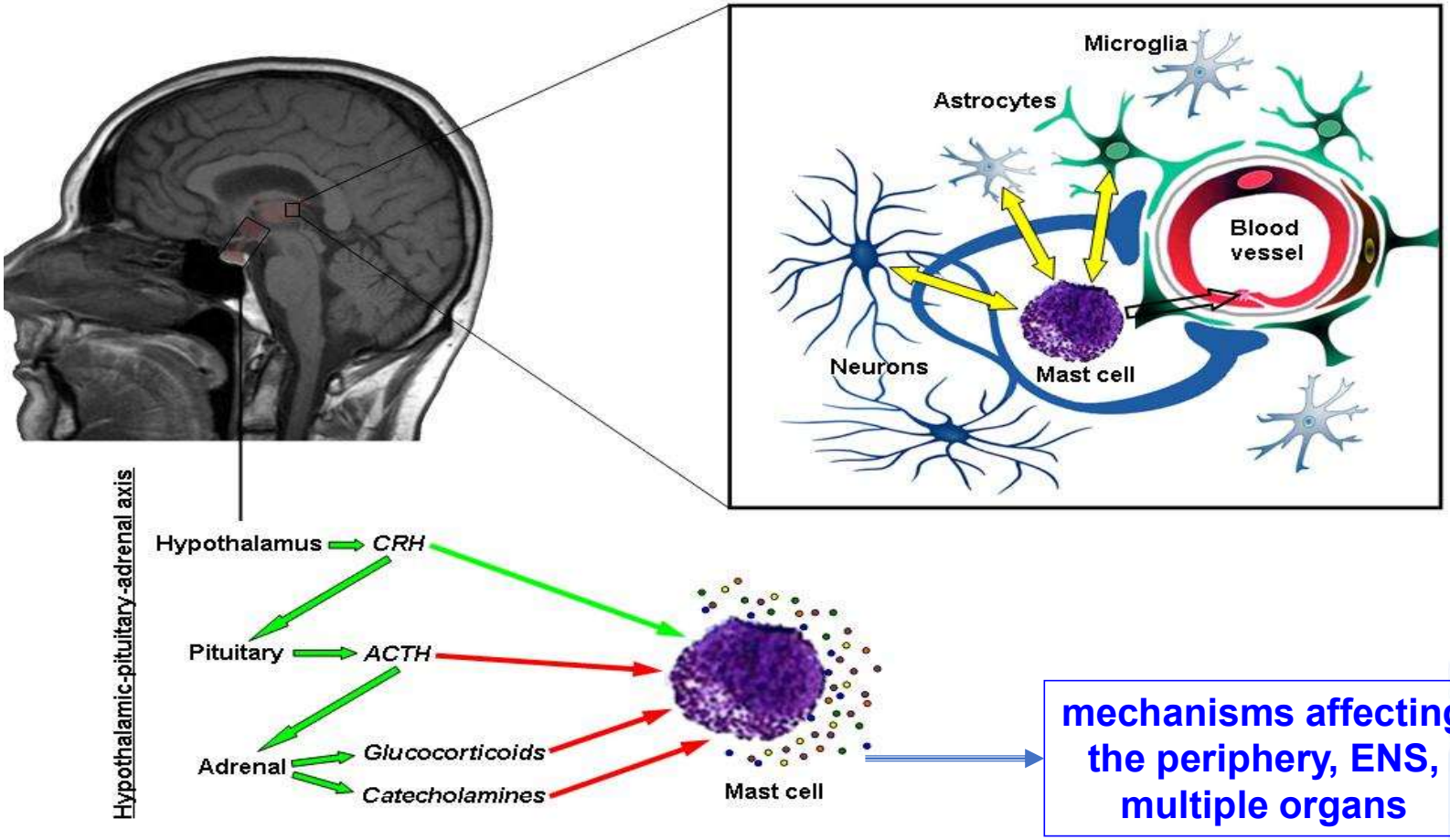


- Proximity of MCs to enteric nerves
- bidirectional communication between CNS/ENS and the gut.
- MCs respond to neuropeptides & neurotransmitters
- MCs synthesize and secrete molecules that modulate neural responses (e.g., CRH, SP, serotonin, nerve growth factor)
- Human studies have revealed abnormal colonic motility mimicking IBS after injection of CRH.

Philpott H, Gibson P, Thien F. Irritable bowel syndrome - An inflammatory disease involving mast cells. Asia Pac Allergy 2011;1:36-42

Stress – Brain – Gut – Mast cell axis may involve the whole body

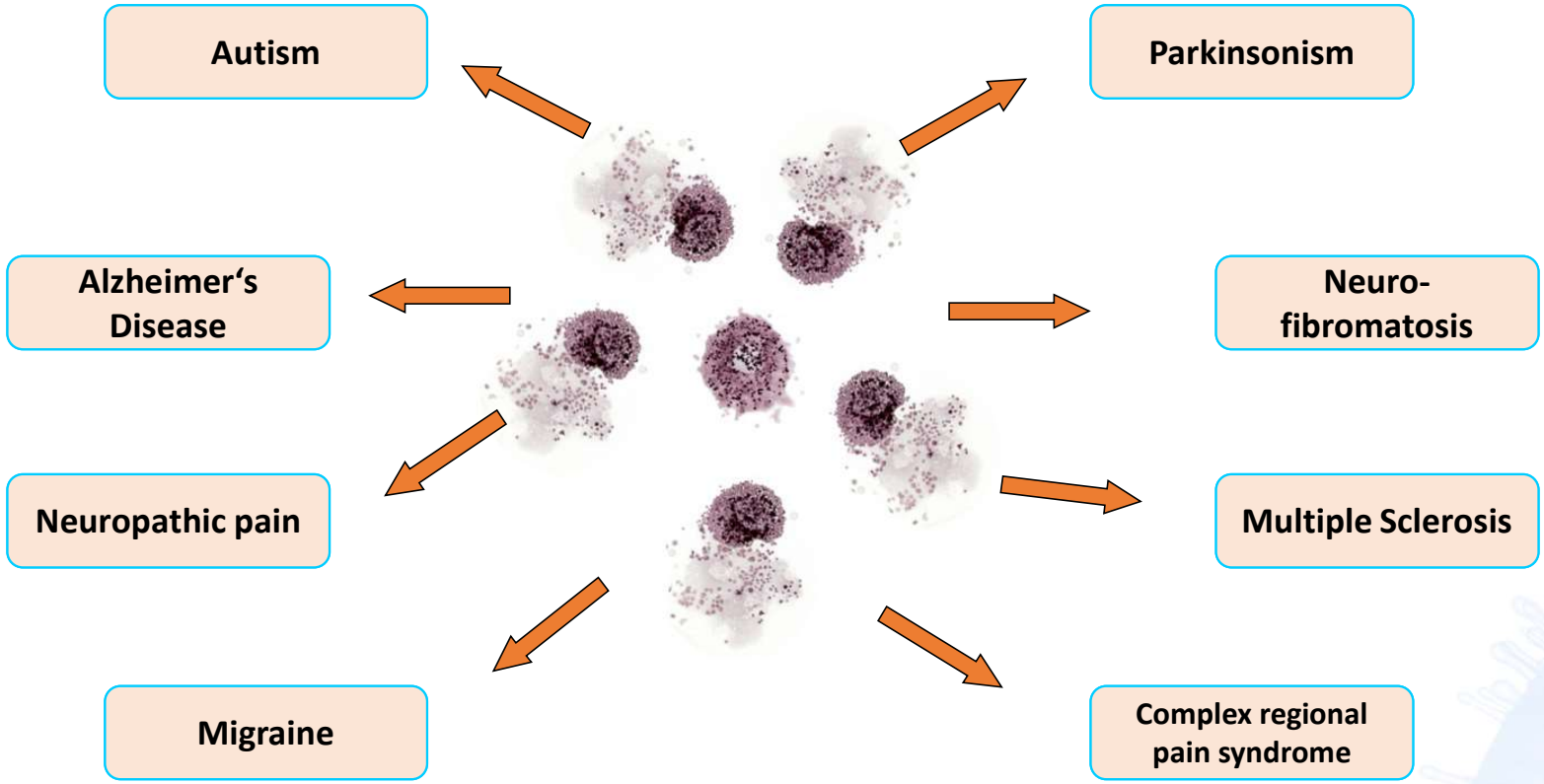
mechanisms within the CNS



mechanisms affecting the periphery, ENS, multiple organs

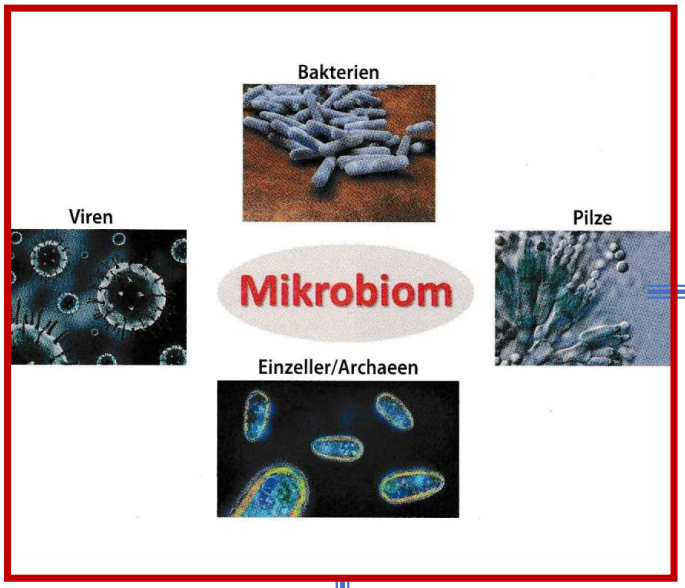
The Crucial Pathogenetic Role of Mast Cells in Neuroinflammatory and Psychiatric Diseases

Neurological and psychiatric diseases
for which pathogenetic involvement of mast cells has been demonstrated



Differential effects of microbiome on mast cell populations microglia and nervous system

selective secretory triggers
z.B. E. coli, histamine secretion



ENS, CNS
epithelial cells, …

No anaphylactic degranulation

ENS, CNS
immune cells, …

selective secretory inhibitors
z.B. E. coli IgE degranulation

microglia

Development, function, maturation

Afrin L et al. MCAD and microbiotic interactions. Clin Ther 2015
Rea K et al. Microbiome: A key regulator of stress and neuro-inflammation. Neurobiology Stress 2016; 4: 23-33

Potential effects of pre- and probiotics as well as fecal microbiome transfer (FMT) on mast cell regulation

apoptosis, activation degranulation

selective mediator expression & - secretion
TNF ↓ und PGJ2 ↑

proinflamm. cytokines ↓
antiinflamm. cytokines ↑

barrier disruption (TRY)

interaktion with nerves, neurotransmitters

bactericidal & tumouricidal effects

Nutrition
fibers – carbohydrates
FOS, GOS, lectines,
short chain fatty acids
polyphenole,
phytotherapeutics . . .

E. coli Nissle 1917

Saccharomyces

Bifidobacteria

Faecalibacterium

Lactobacillus

Lactococcus

Akkermansia

. . .

Lutgendorff F et al. Probiotics prevent intestinal barrier dysfunction in acute pancreatitis in rats via induction of ileal mucosal glutathione biosynthesis. PLoS One 2009;4:e4512
Afrin L et al. MCAD and microbiotic interactions. Clin Ther 2015
Rea K et al. Microbiome: A key regulator of stress and neuroinflammation. Neurobiology Stress 2016; 4: 23-33

Conclusions
Mast cell activation in health and disease

- Mast cells are **primitive immune cells** that appear early in evolution. They are early connected with the gastro-intestinal tract due to regulation of several physiological functions and their ability to react within seconds when ongoing infections are imminent.

- Mast cells represent a **first line of defense along the GI-tract and in other mucosal surfaces** due to their ability to phagocytose bacteria and to activate subsequent immune cells and mechanisms.

- Stimulation of mast cells may cause various types of degranulation (anaphylactic, piece-meal, selective) and local tissue damage, functional disturbances and/or systemic reactions. Down-regulation of mast cells appears thus to be an upcoming important issue to control mast cell activity in several disease conditions.

- Gastrointestinal food allergy, Irritable bowel syndrome, functional diseases and mast cell activation syndrome are often coupled with mast cell hyperplasia and/or signs of activation. While in IgE-mediated GI-allergy the triggering mechanism is focused on the $Fc_{epsilon}$ receptor, the exact triggers are not yet defined in the other conditions.

- However, a sub-population of patients with MCAS have been found to produce local IgE antibodies at the mucosal/gastrointestinal level, resulting in persistent secondary activity of mast cells when allergens are not identified.
- By interaction of mast cells with the microbiome and enteric nerves mast cell products may have profound influence on neurovegetative regulation, mood, pain perception, gut barrier and behaviour.