Prof. Dr. med. Maria J.G.T. Vehreschild
Leiterin Schwerpunkt Infektiologie
Zentrum für Innere Medizin
Universitätsklinikum der Goethe Universität Frankfurt am Main

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Einfluss des Mikrobioms auf Urämietoxine

Potentielle Interessenskonflikte

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Forschungsförderung: 3M, Astellas Pharma, DaVolterra, Evonik, Gilead Sciences, Glycom, Heel, Jennewein, MaaT Pharma, Merck/MSD, Organobalance, Seres Therapeutics

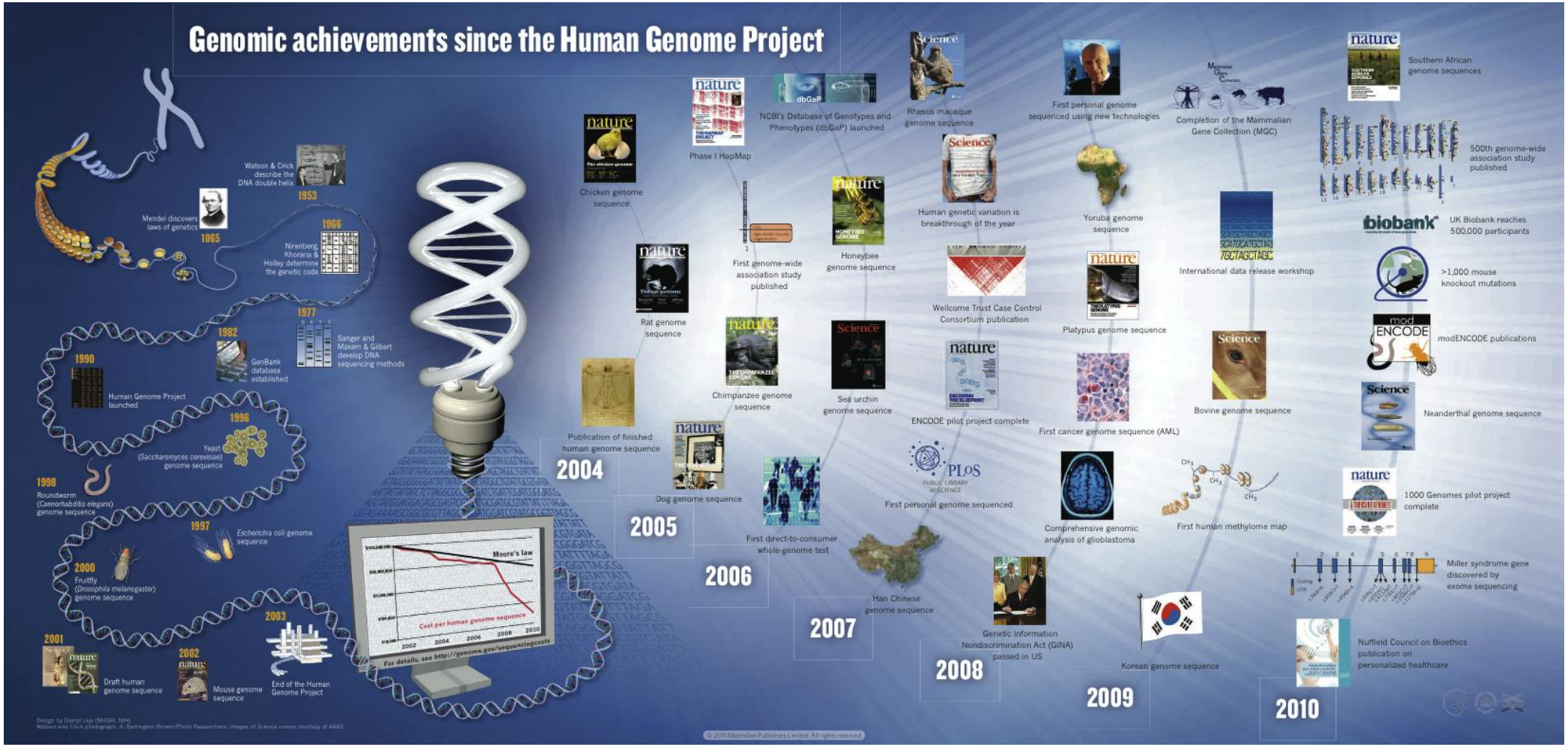
Vortragshonorare: Astellas Pharma, Basilea, Gilead Sciences, Merck/MSD, Organobalance, Pfizer

Beratung: Alb Fils Kliniken GmbH, Arderypharm, Astellas Pharma, DaVolterra, Ferring, MaaT Pharma, Merck/MSD

Definitionen

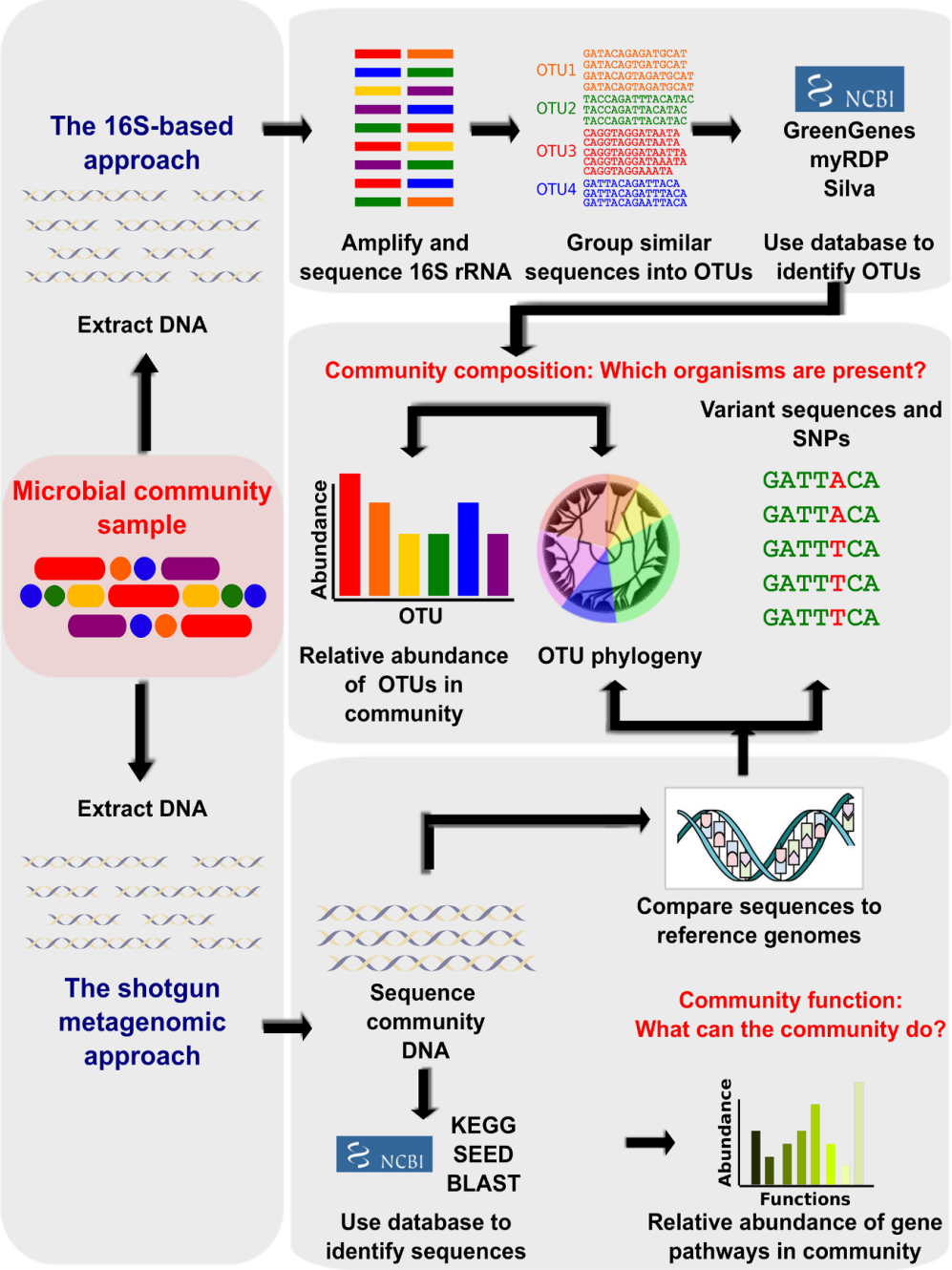
- Die Mikrobiota (Singular!): Gesamtmenge aller den Menschen kolonisierenden Mikroorganismen (Bacteria, Archaea, Eukaryota, Viren)
- Das Mikrobiom: Gesamtheit aller mikrobiellen Gene im menschlichen Organismus
- Der Pathobiont: Ein Mikroorganismus, der unter normalen Umständen symbiotisch mit dem Wirt koexistiert, aber im Rahmen einer Dysbiose zum Pathogen werden kann
- Das Metabolom: Gesamtheit aller Metaboliten im menschlichen Organismus

Sequenzierung als Innovationsmotor



ED Green *et al. Nature* **470**, 204-213 (2011)

Mikrobiomanalyse



Morgan XC et al. PLoS Comput Biol. 2012;8(12):e1002808.

Multi-Omics Ansatz

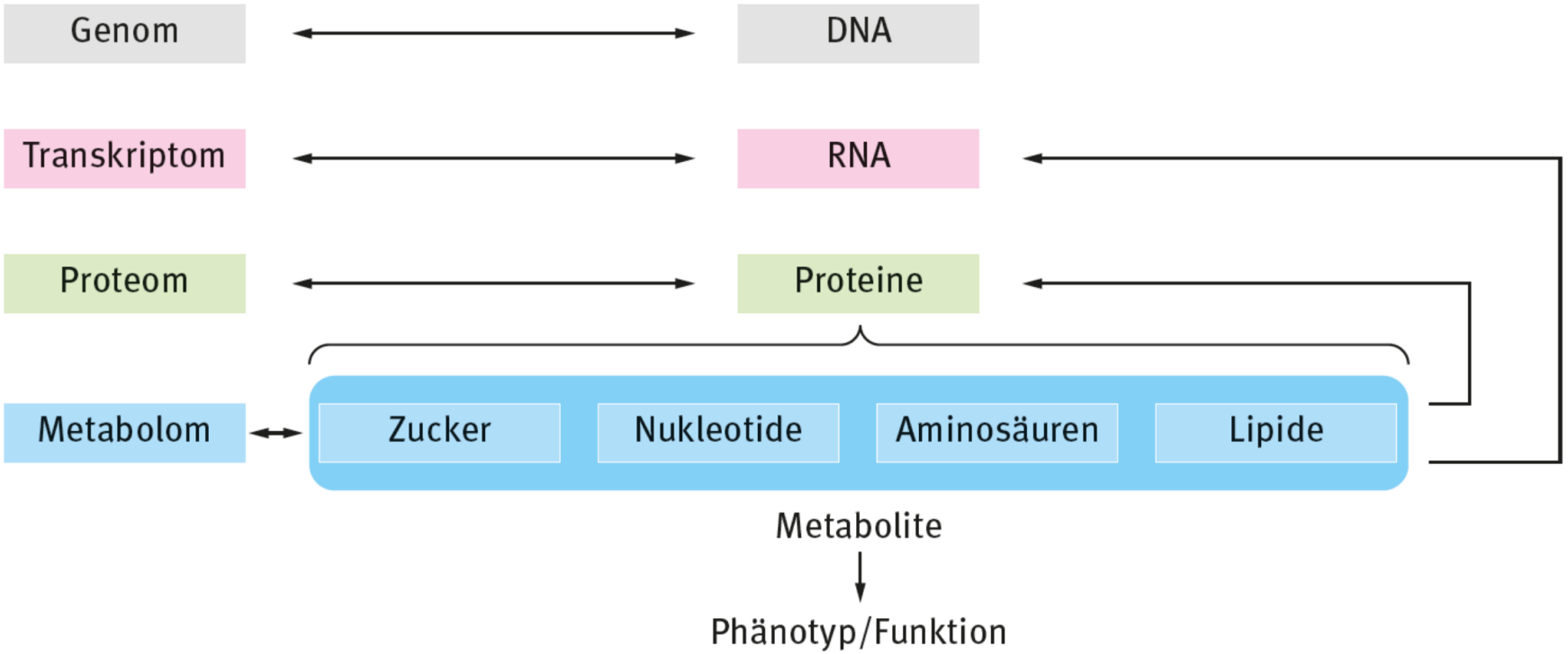
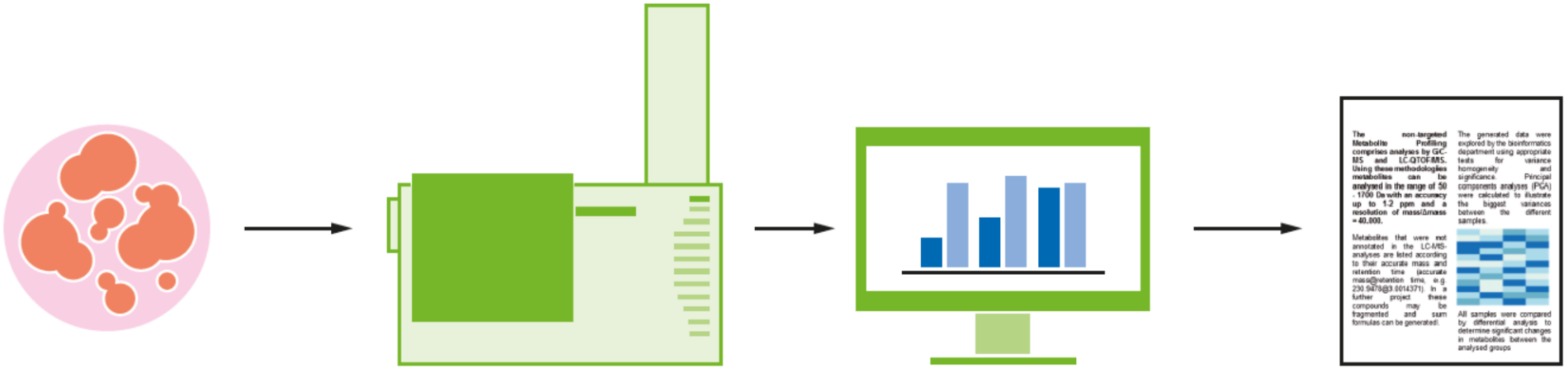


Abb. 2.6: Einordnung des Metaboloms in Relation zu anderen typischen Gebieten der „Omics"-Analyse, Korrelation der unterschiedlichen Felder zur Genom- (Genomics), Transkriptom- (Transcriptomics), Proteom- (Proteomics) und Metabolom- (Metabolomics) Forschung sowie die direkte Nähe des Metaboloms zum Phänotyp/Funktion und Einfluss des Metaboloms auf Proteine und RNA.

Bild aus Stallmach, Vehreschild (Hrsg.) Mikrobiom 2016, de Gruyter

Grundlagen Metabolomanalyse



Probenvorbereitung
Metabolit-Extraktion

LC/MS-Vermessung

Datenprozessierung
und Statistik

Datenanalyse
und Interpretation

Abb. 2.9: Typischer Ablauf eines metabolomischen Experiments von der Probenvorbereitung und Metabolit-Extraktion, gefolgt von der Vermessung, Datenprozessierung und Statistik sowie Datenanalyse und Interpretation.

Bild aus Stallmach, Vehreschild (Hrsg.) Mikrobiom 2016, de Gruyter

Immunphänotypisierung und funktionelle Analysen

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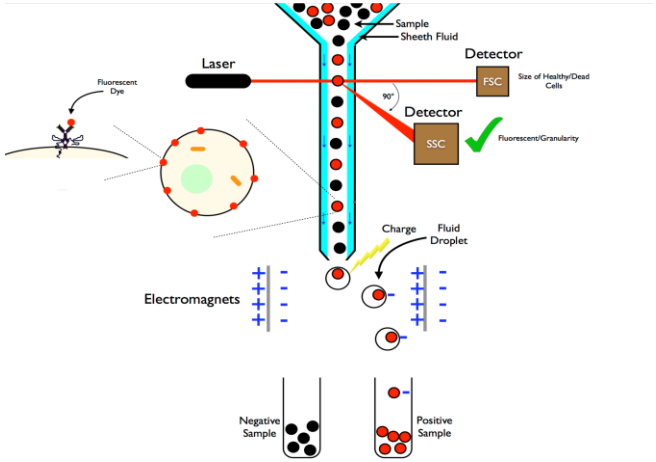
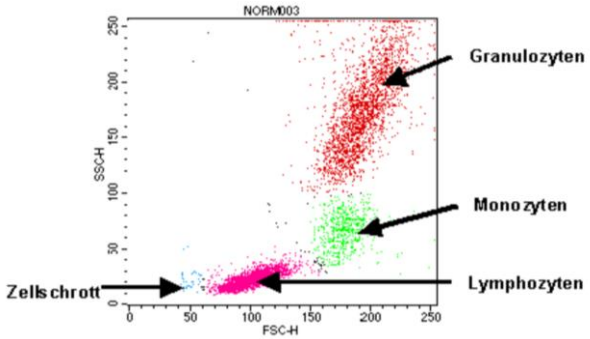
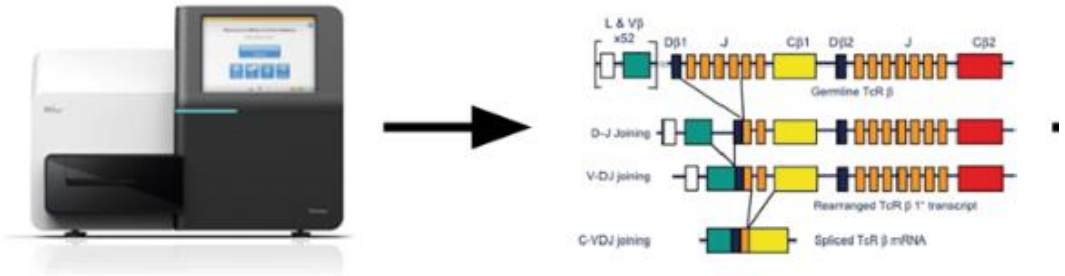
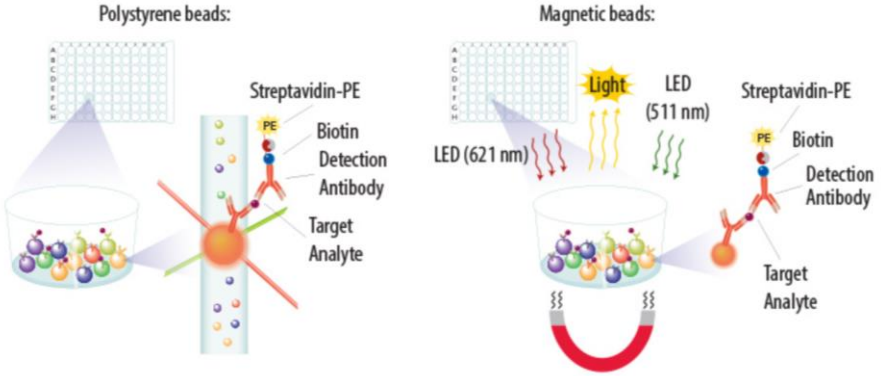




Klassische Durchflusszytometrie



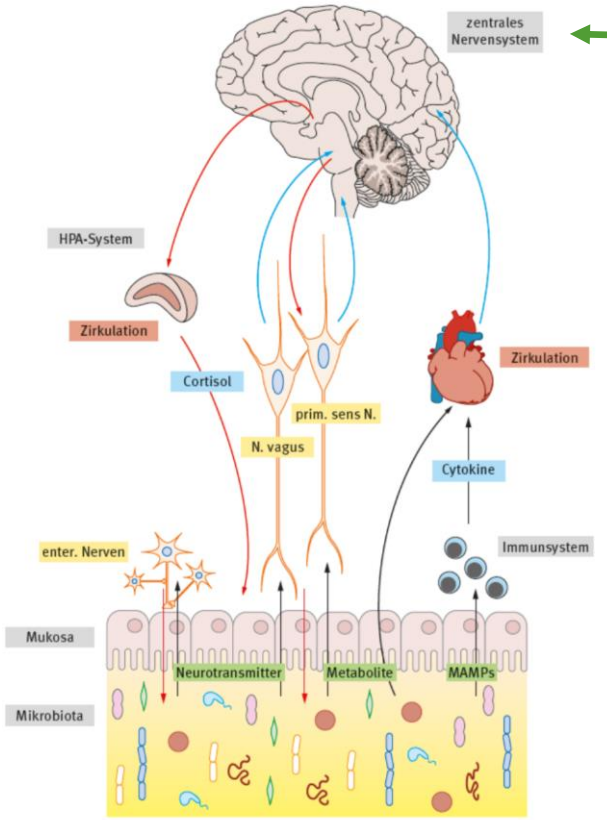
Zellsortierung mit nachfolgender Sequenzierung des Immunglobulin- und T-Zellrepertoires



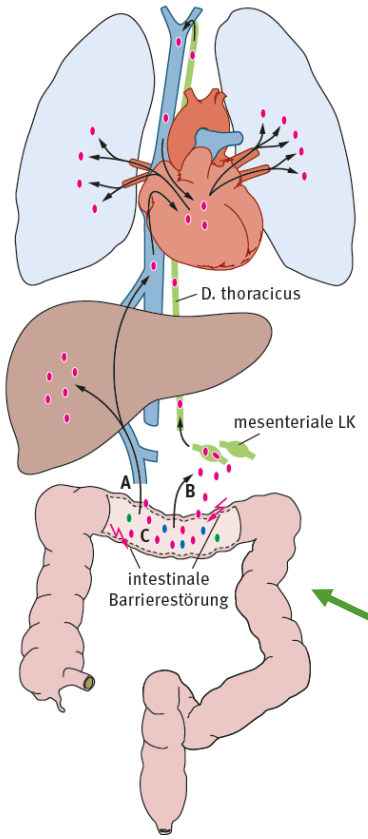
Zytokinanalysen

Bilder aus www.bio-techne.com; www.ruhr-universitate-bocum.de; Sabban 2011, PhD thesis, The University of Sheffield; Reindl-Schweighofer, Nephro Script 2016

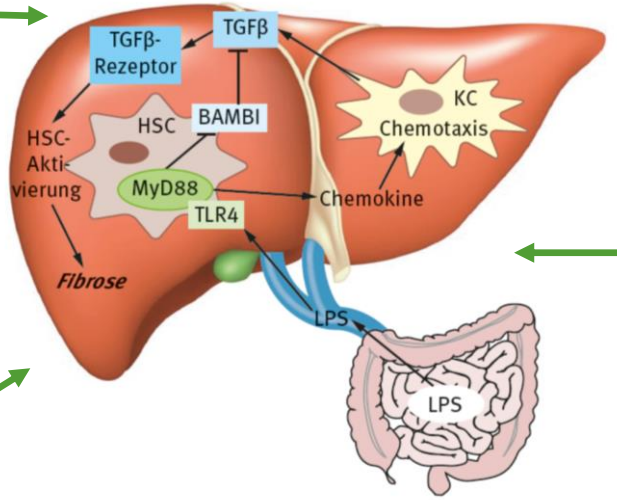
Regulation



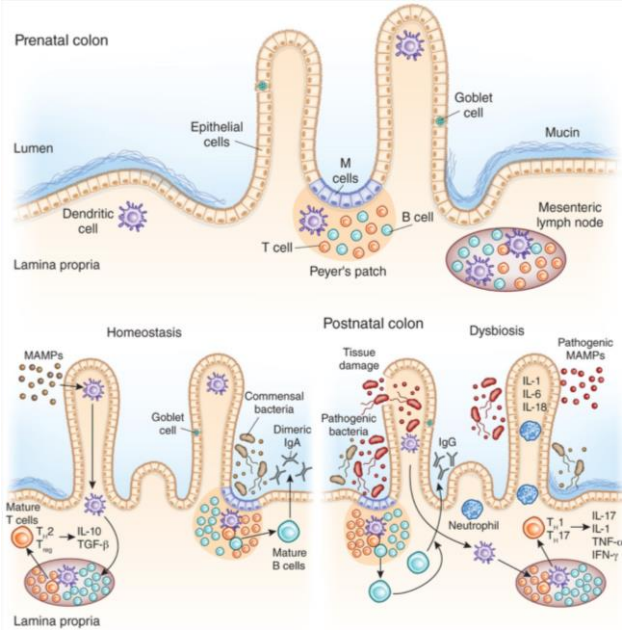
Darm-Hirn Achse



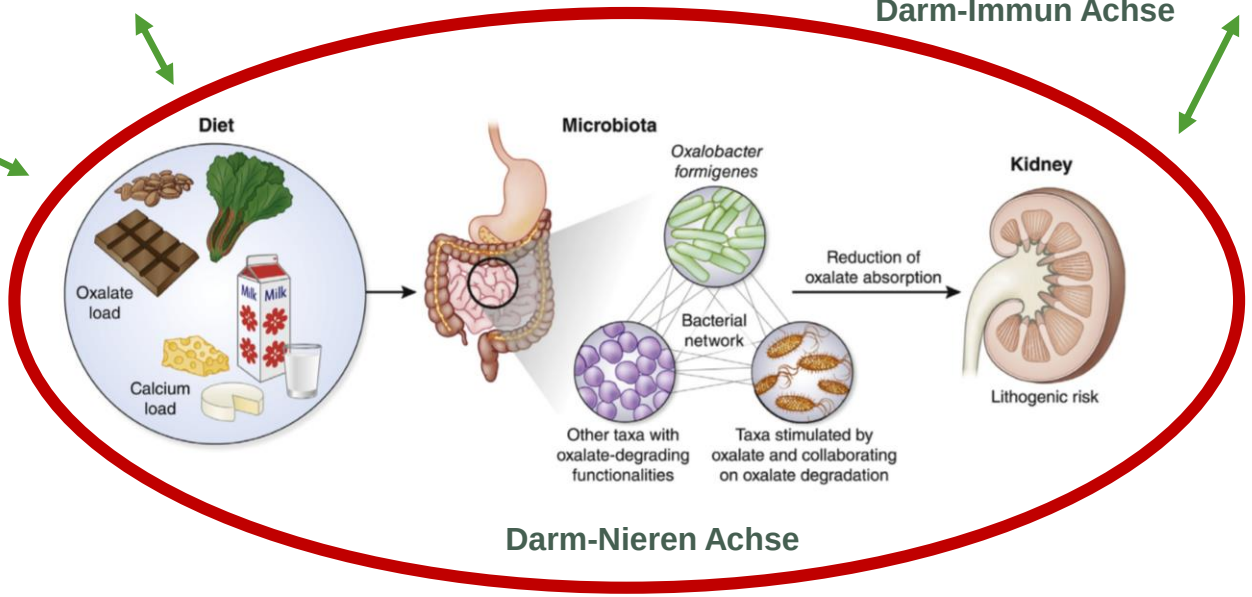
Darm-Lungen Achse



Darm-Leber Achse



Darm-Immun Achse



Darm-Nieren Achse

Bilder aus
Stallmach, Vehreschild (Hrsg.) Mikrobiom 2016, de Gruyter
Tamburini. Nat Med. 2016 Jul 7;22(7):713-22.
Ticinesi et al. Kidney Int. 2019 Jul;96(1):25-27.

Erste Arbeiten zu Urämietoxinen und Mikrobiom

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THE ROLE OF THE MICROBIAL FLORA IN UREMIA

I. SURVIVAL TIMES OF GERMFREE, LIMITED-FLORA, AND CONVENTIONALIZED RATS AFTER BILATERAL NEPHRECTOMY AND FASTING

BY ALBERT EINHEBER, PH.D., AND DARRYL CARTER,* M.D.

(*From the Department of Germfree Research, Division of Surgery, Walter Reed Army Institute of Research, Washington, D. C.*)

Einheber et al. J Exp Med. 1966;123(2):239-250.
Keimfreie anephrische Mäuse leben länger als Mäuse mit einer definierten oder einer Standardmikrobiota

CLINICAL RESEARCH www.jasn.org

Colonic Contribution to Uremic Solutes

Pavel A. Aronov,* Frank J.-G. Luo,† Natalie S. Plummer,† Zhe Quan,‡ Susan Holmes,§ Thomas H. Hostetter,‡ and Timothy W. Meyer†

Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites

William R. Wikoff[a], Andrew T. Anfora[b], Jun Liu[b], Peter G. Schultz[b,1], Scott A. Lesley[b], Eric C. Peters[b], and Gary Siuzdak[a,1]

Aronov et al. J Am Soc Nephrol. 2011;22(9):1769-1776. und Wikoff et al. Proc Natl Acad Sci USA. 2009;106(10):3698-3703.
Die Mikrobiota des Kolons ist essentiell für die Synthese verschiedener Urämietoxine.

Mikrobiom bei Chronischer Nierenerkrankung

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Table 1. Alterations of the Gut Microbiome in CKD

Study Group	Methods	Gut Microbiota Features Associated With CKD	Reference
		Human Studies	
CKD patients (n = 22), with (n = 12) and without (n = 10) GI symptoms	Antroduodenojejunal manometry, culture	Abnormal motility and bacterial overgrowth in small intestine of CKD patients	151
Hemodialysis patients (n = 8)	Bacterial cell count	Increased counts of both aerobic (~10^6 bacteria/mL) and anaerobic (~10^7 bacteria/mL) organisms in the duodenum and jejunum (which are normally not colonized heavily by bacteria in healthy individuals)	152
ESRD patients (n = 24) vs healthy persons (n = 12)	Phylogenetic microarray	Highly significant differences between the ESRD and healthy control groups in the abundance of >200 bacterial operational taxonomic units belonging to 23 bacterial families	23
ESRD patients (n = 24) vs healthy persons (n = 12)	Phylogenetic microarray and hypergeometric distribution tests	Expansion of bacterial families possessing urease, uricase, and *p*-cresol– and indole-forming enzymes; depletion of bacteria possessing short-chain fatty acid forming enzymes	49
ESRD patients (n = 10) vs healthy persons (n = 8)	16S rDNA amplification and DNA pyrosequencing	Disturbed composition of microbiota characterized by overgrowth of aerobic bacteria such as *Enterobacteria* and *Enterococci* species (~100 times higher in ESRD patients); of the anaerobic bacteria, ESRD patients had significantly lower numbers of *Bifidobacteria* and higher numbers of *Clostridium perfringens*	24
ESRD patients (n = 52)	16S rDNA amplification and pyrosequencing	Increased bacterial translocation in plasma of ESRD patients; bacterial DNA concentration positively correlated with plasma levels of CRP and IL-6	153
		Experimental Animal Studies	
5/6 nephrectomy (n = 6) or sham-operated rats (n = 5)	Phylogenetic microarray	Significant difference in abundance of 175 bacterial operational taxonomic units between uremic and control animals, most notably as decreases in the Lactobacillaceae and Prevotellaceae families	23
Uremic (n = 20) and control (n = 20) Wistar rats	Culture	Impaired intestinal mucosa barrier with significantly more bacterial translocation in uremic than in control animals	154

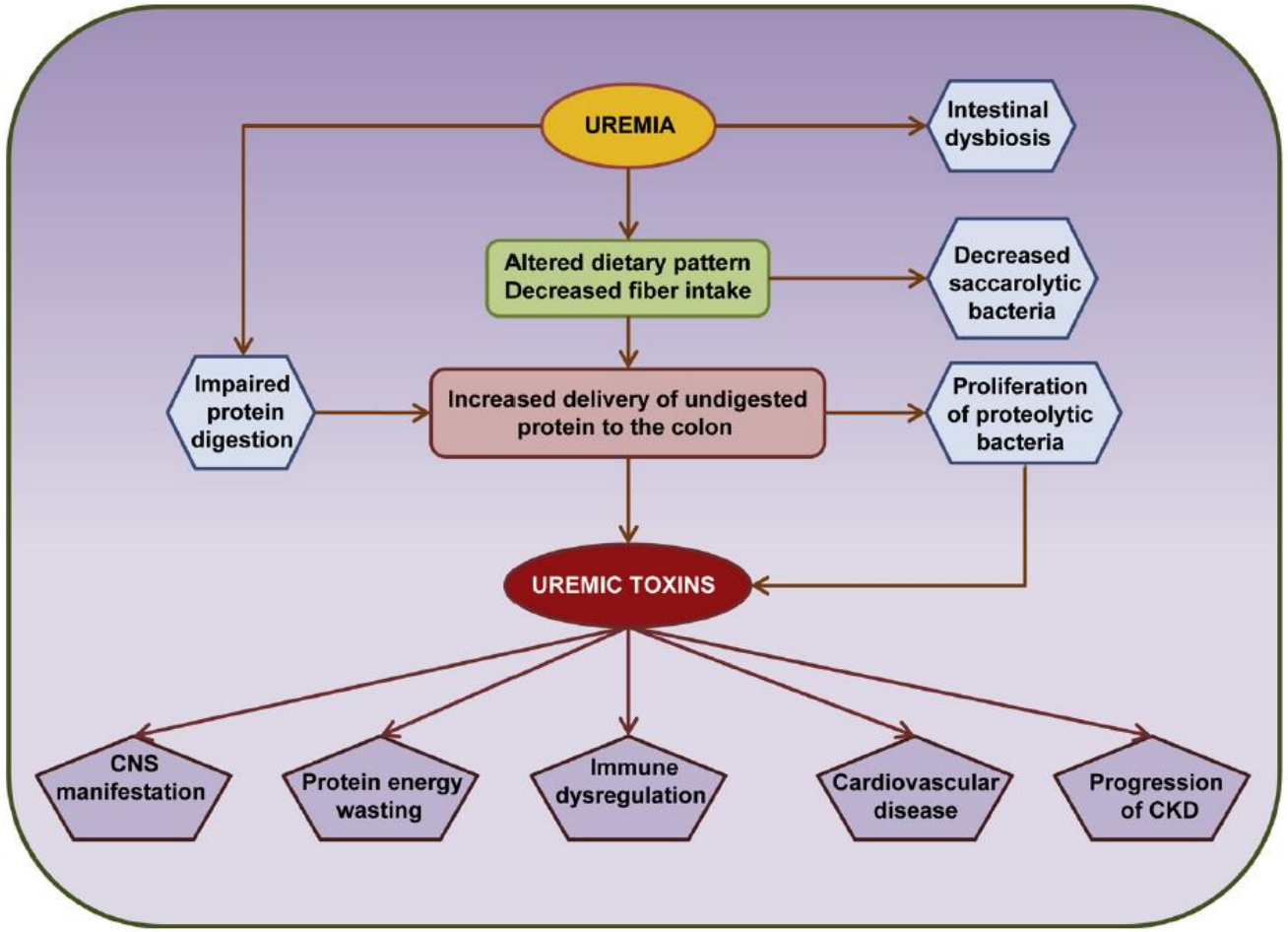
Abbreviations: CKD, chronic kidney disease; CRP, C-reactive protein; ESRD, end-stage renal disease; GI, gastrointestinal; IL, interleukin; rDNA, ribosomal DNA.

Erste Fallserien und Studien am Tier kartographieren das Mikrobiom bei chronischer Niereninsuffizienz:

- Abnormale Darmmotilität und Überwucherung des Dünndarms
- Erhöhter Anteil aerober Bakterien im Kolon
- Erhöhte Rate an bakteriellen Translokationen durch die Darmwand und reaktiver Entzündungsaktivität

Ramezani et al. Am J Kidney Dis. 2016 Mar;67(3):483-98.

Pathomechanismen Dysbiose → Urämietoxine



Entstehung von Urämietoxinen:

- ↓ Intestinale Resorption Proteine
- ↑ Proteinanteil im distalen Darm
- ↑ Anteil proteolytischer Bakterien
- ↑ Proteinfermentation
- ↑ Urämietoxine

Ramezani et al. Am J Kidney Dis. 2016 Mar;67(3):483-98.

Mikrobiota und Metabolite/Toxine

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Mikrobiota-assoziierte Urämietoxine

Name	Source	Class
Indoxyl sulphate	Tryptophan	Protein-bound
Indole-3 acetic acid	Tryptophan	Protein-bound
p-cresyl sulphate	Phenylalanine, tyrosine	Protein-bound
TMAO	Choline, l-carnitine, PPC	Water-soluble
Phenylacetylglutamine	Phenylalanine	Water-soluble

Abbreviation: PPC, phosphatidylcholine.

Zuordnung relevanter Bakterien

↑ **Tryptophanase possessing families**
Clostridiaceae
Enterobacteriaceae
Verrucomicrobiaceae

Increased indoxyl sulphate and *p*-cresyl sulphate

Fatty acid (butyrate) forming ezymes ↓
Lactobacillaceae
Prevotellaceae

Decreased production of short-chain fatty acids

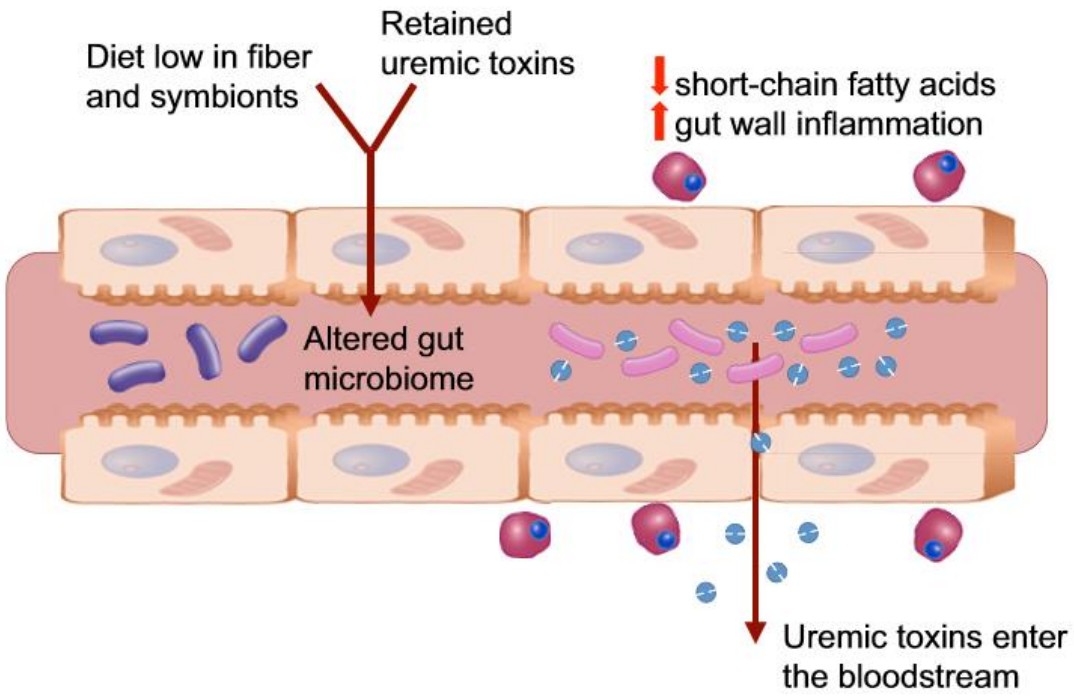
↑ **Urease possessing families**
Alteromonadaceae
Cellulomonadaceae
Clostridiaceae
Dermabacteraceae
Enterobacteriaceae
Halomonadaceae
Methylococcaceae
Micrococcaceae
Moraxellaceae
Polyangiaceae
Pseudomonadaceae
Xanthomonadaceae

Increased metabolism of urea to ammonium hydroxide

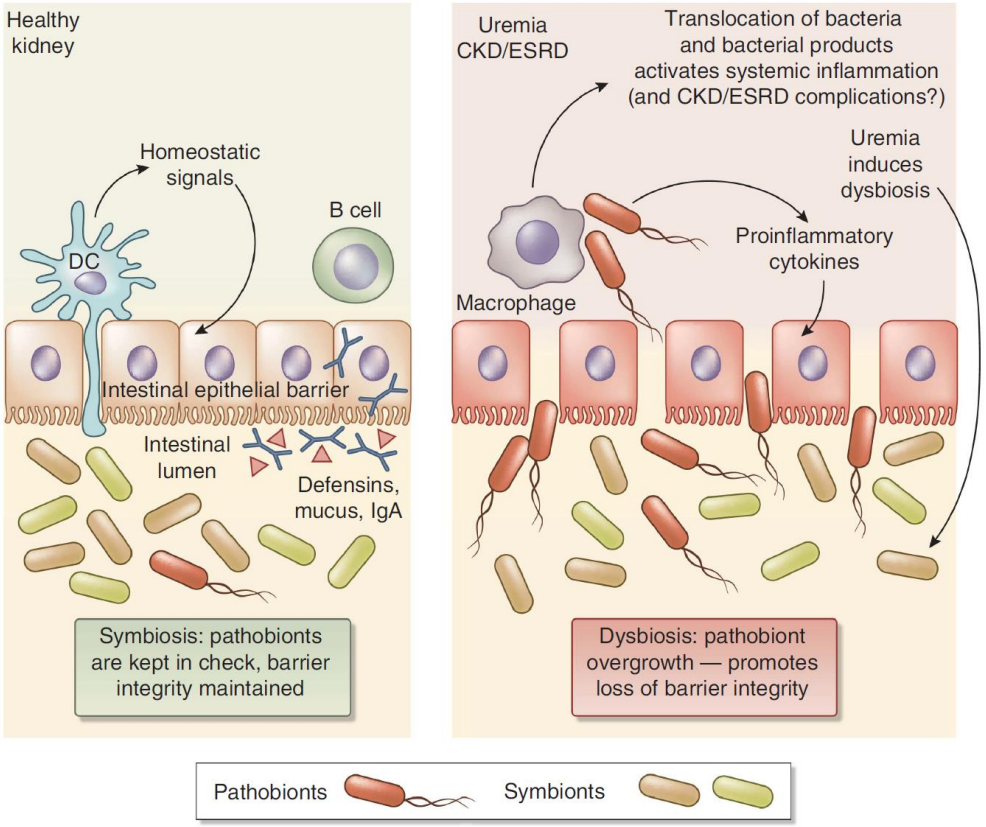
$$CO(NH_2)_2 + H_2O \rightarrow CO_2 + 2NH_3$$
$$NH_3 + H_2O \rightarrow NH_4OH$$

Lau et al. Clin Sci (Lond). 2018 Mar 9;132(5):509-522.

Mikrobiota und Inflammation



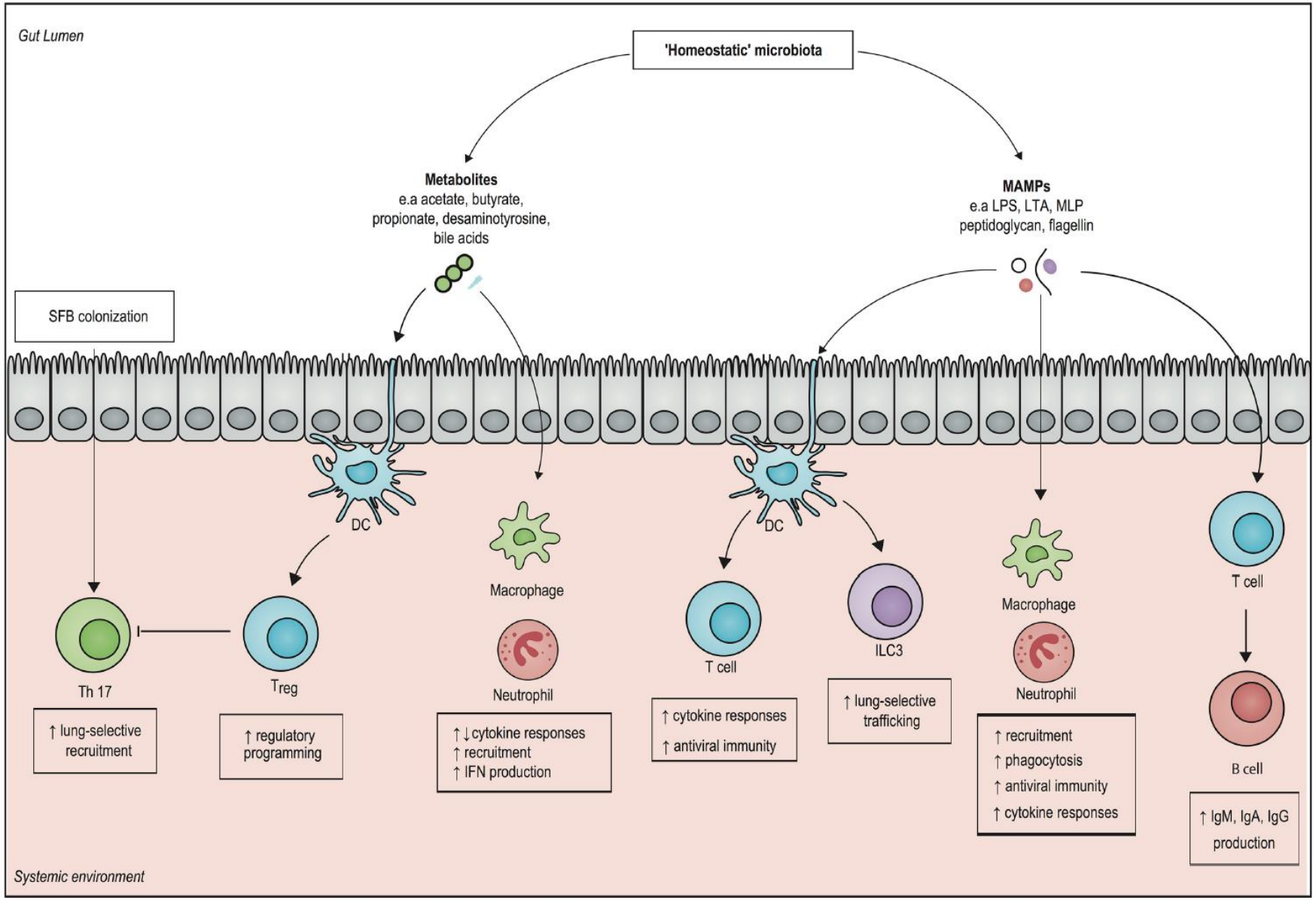
Gestörte Darmepithelbarriere
durch Butyratmangel



Translokation von Pathobionten

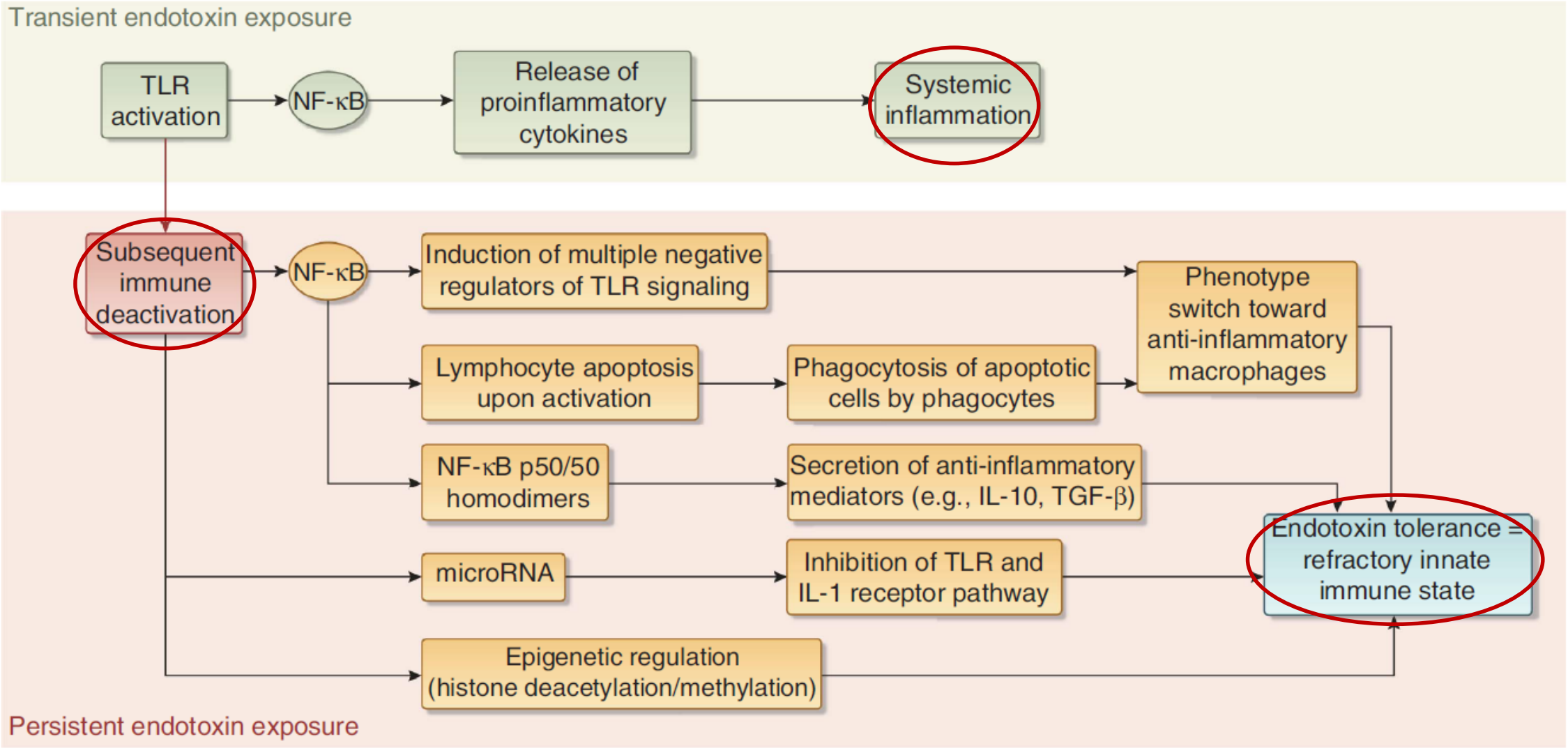
Lau et al. Clin Sci (Lond). 2018 Mar 9;132(5):509-522.
Anders et al. Kidney Int. 2013 Jun;83(6):1010-6.

Interaktion mit der Darm-Immun Achse



Haak et al. Front Immunol. 2018 Sep 10;9:2042.

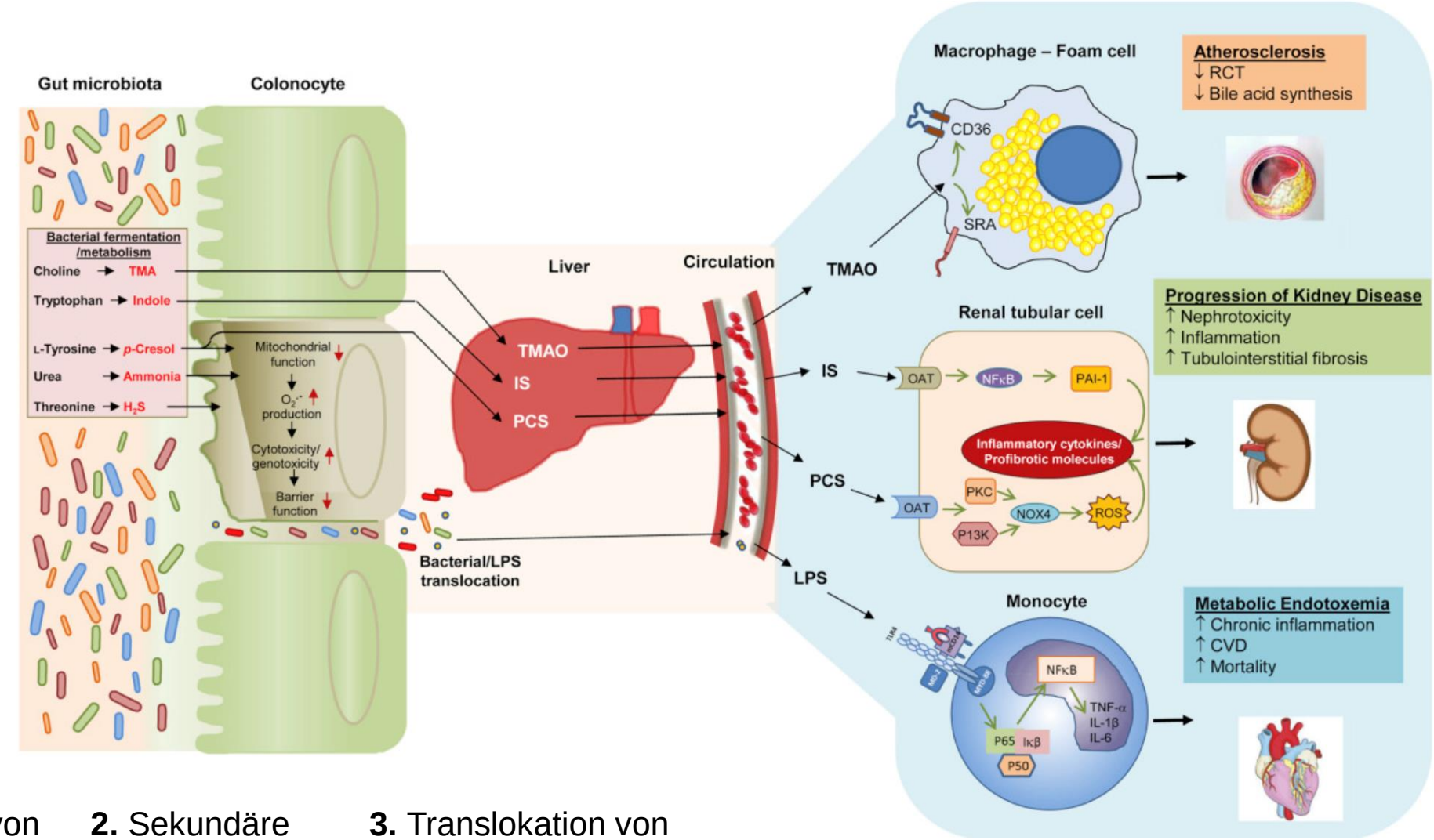
Einfluss von Endotoxinen auf die Immunantwort



Anders et al. Kidney Int. 2013 Jun;83(6):1010-6.

Klinische Relevanz

Ramezani et al. Am J Kidney Dis. 2016 Mar;67(3):483-98.



1. Entstehung von Urämietoxinen durch bakterielle Fermentation

2. Sekundäre Verstärkung der Dysbiose durch Urämietoxine

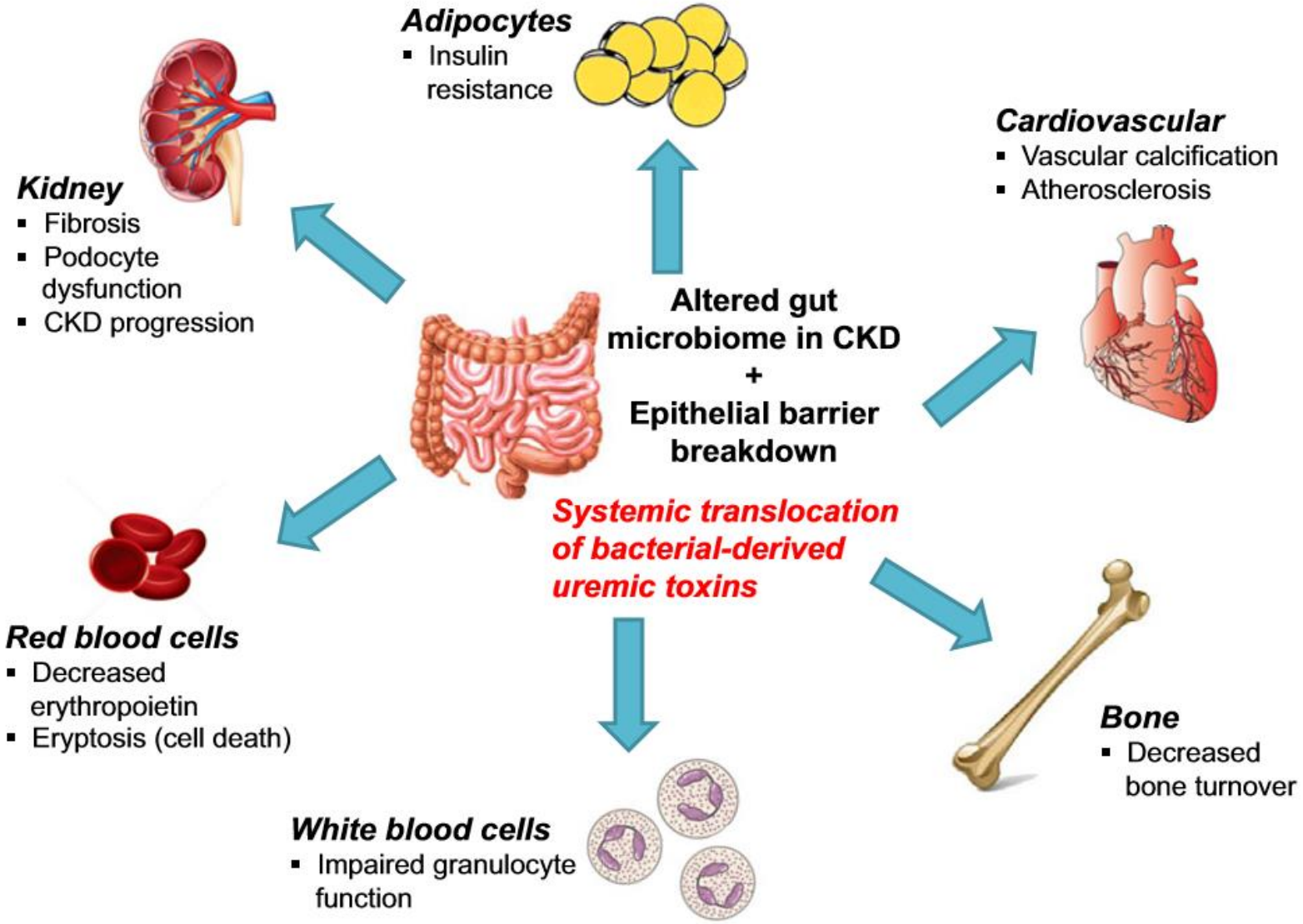
3. Translokation von Bakterien

4. Hepatische Aktivierung von Urämietoxinen

5. Aktivierung pathologischer Pathways in verschiedenen Endorganen

6. Endorganschäden und Infektionen

Spektrum Endorganschäden



Lau et al. Clin Sci (Lond). 2018 Mar 9;132(5):509-522.

Therapeutische Perspektive

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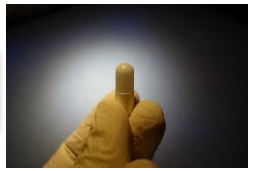
Development of prebiotics

Synthetic prebiotics

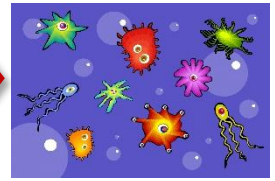
Optimization of administration

-80°C storage
Lyophilization

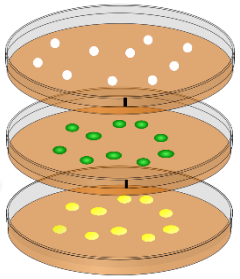


Capsules

Classical FMT



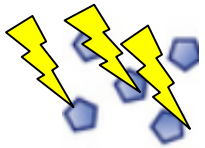
Modified ecosystem



Defined ecosystem

Development of probiotics

Bacterial metabolites



Synthetic metabolites

Development of postbiotics

Therapeutische Perspektive

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Table 3. Treatment Options for Uremic Toxins and Inflammation

Intervention	Patient Type	Comments	Reference
	Probiotic		
Oligofructose-enriched inulin	Healthy participants (n = 50)	↓ Urinary excretion of *p*-cresol	72
Inulin/oligofructose	Obese women (n = 30)	↓ Endotoxemia	64
Oligosaccharides	Elderly participants (n = 74)	↓ TNF-α mRNA and IL-6 mRNA; ↓serum soluble CD14	73
	Prebiotic		
Bifobacterium bifidum, *Bifobacterium catenulatum*, *Bifobacterium longum*, and *Lactobacillus plantarum*	Peritoneal dialysis patients (n = 39; 21 in probiotics group and 18 in placebo group)	↓ TNF-α, IL-5, IL-6, and endotoxin; ↑ IL-10; preserve residual kidney function	138
Oligofructose-enriched inulin	Hemodialysis patients (n = 22)	↓ Serum *p*-cresyl sulfate and generation rate	58
	Synbiotic		
Probiotic along with inulin, oat bran, pectin, and resistant starch	Trauma patients (n = 65)	↓ Rate of systemic inflammatory response, syndrome, infections, severe sepsis, and mortality	74
Galacto-oligosaccharides, *Lactobacillus casei*, and *Bifobacterium breve*	Hemodialysis patients (n = 7)	↓ Serum *p*-cresol	143
Probinul neutro	CKD patients stages 3-4 (n = 30)	↓ Total plasma *p*-cresol	139
	Oral adsorbent		
AST-120	Adult patients with moderate to severe CKD (n = 132)[146]; adult patients with moderate to severe CKD (n = 2035)[148]	↓ Indoxyl sulfate in a dose-dependent manner[146]; unable to demonstrate beneficial effect of AST-120 on progression of CKD[148]	146,148
	α-Galactosidase Inhibitor		
Acarbose (an inhibitor of the α-glucosidase enzymes)	Healthy volunteers (n = 9)	↓ Serum concentrations of *p*-cresol; ↓urinary excretion of *p*-cresol; ↑fecal excretion of nitrogen increased	160
	Genetically Engineered Bacteria		
Microencapsulated genetically engineered live *Escherichia coli* DH5 cells	Uremic rats	↓ Plasma urea concentration; ↓plasma ammonia concentration	145
	Ongoing Studies		
SYNERGY-HMW inulin, fructo-oligosaccarides and galacto-oligosaccarides plus strains from the *Lactobacillus*, *Bifidobacteria*, and *Streptococcus* genera	Patients with moderate to severe CKD stages 4-5 (n = 37)	Ongoing study aiming to assess effectiveness of synbiotics on the synthesis of uremic toxins indoxyl sulfate and *p*-cresyl sulfate	149
Arabinoxylan-oligosaccharides (AXOS)	Patients with CKD stage 3b-4	Ongoing study aiming to assess whether AXOS can decrease intestinal generation and serum concentrations of microbial metabolites in patients with CKD	161

Abbreviations: CKD, chronic kidney disease; HMW, high-molecular-weight; IL, interleukin; mRNA, messenger RNA; TNF-α, tumor necrosis factor α.

- Erste klinische Studien zeigen gemischte Effekte
- Ergebnisse erlauben noch keinen Transfer in die klinische Praxis

Ramezani et al. Am J Kidney Dis. 2016 Mar;67(3):483-98.

Zusammenfassung

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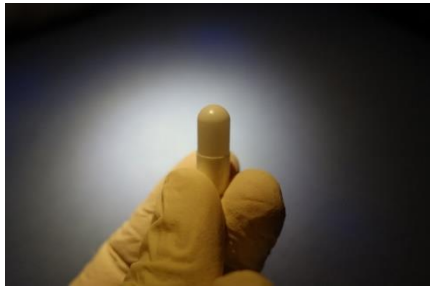
- Durch Sequenzierung verbesserte Darstellung der Mikrobiota
- Multi-OMICS Analysen als wichtige Komplementierung für die Identifizierung funktioneller Zusammenhänge
- Mikrobiota-assoziierte Urämietoxine tragen zur Morbidität von Patienten mit chronischer Niereninsuffizienz maßgeblich bei:
  - Entstehung durch erhöhte Fermentation von Proteinen im Darmlumen →
  - Sekundäre Verstärkung einer Dysbiose durch Urämietoxine selbst →
  - Translokation von Bakterien und hepatische Modifikation der Urämietoxine →
  - Aktivierung dysregulatorischer Pathways in den Endorganen →
  - Multiple Endorganschäden und Infektionen
- Therapeutische Perspektive über Modulation der Mikrobiota



FMT Kapsel

UNIKLINIK KÖLN

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Clinical Infectious Diseases Team

Clinical Microbiome Research Group

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