INTERNATIONAL MEDICAL UNIVERSITY
MALAYSIA

Molecular Basis of Gastrointestinal Diseases

Lecture
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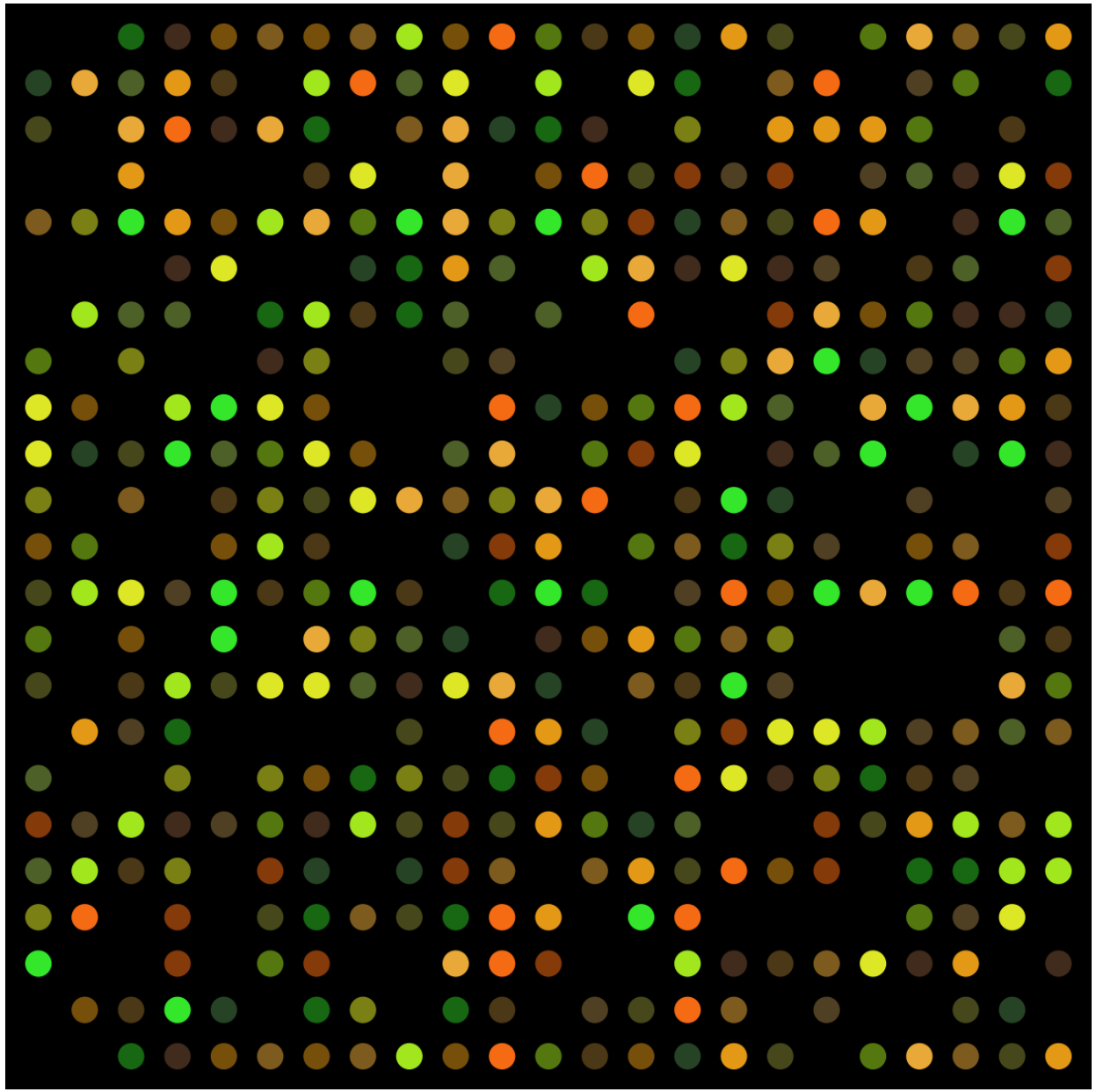


Lesson Outcomes

- Describe the molecular basis of gastrointestinal diseases including
  - Crohn's disease
  - Ulcerative colitis
  - Peptic ulcer (*H. pylori* infection)

Gastrointestinal diseases

TYPES

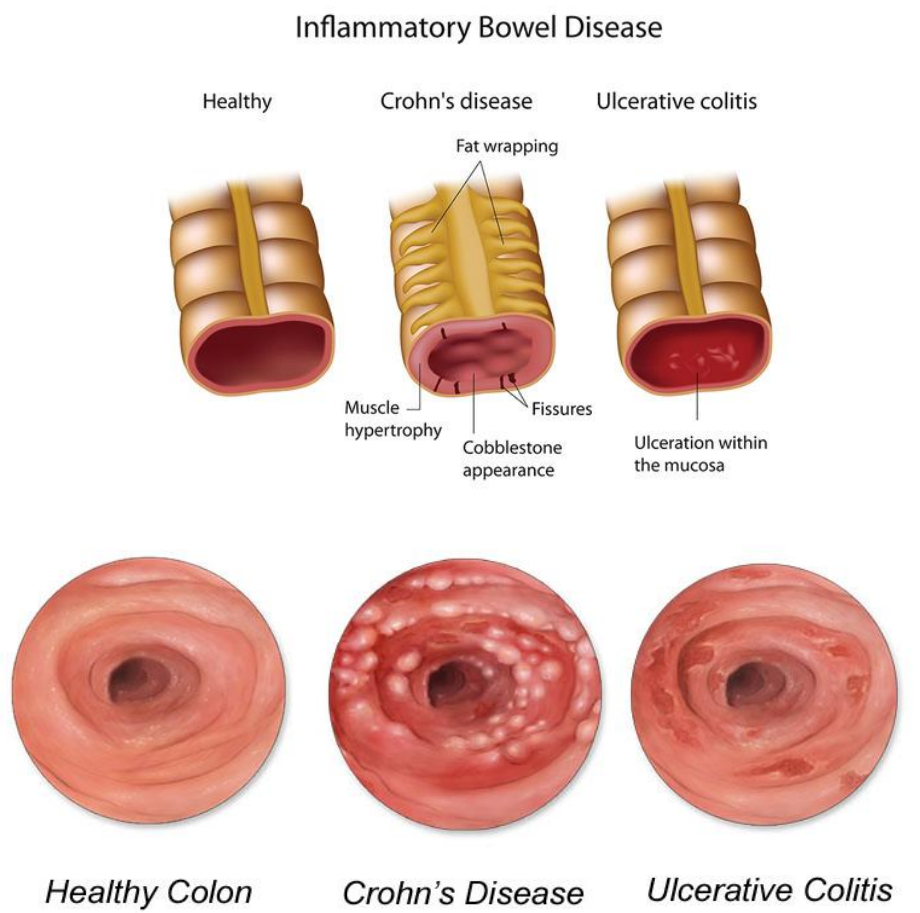
- Oral diseases
  - gingivitis, periodontitis, dental caries
- Oesophageal diseases
  - gastroesophageal reflux, Zenker's diverticulum
- Gastric diseases
  - Peptic ulcer, gastroenteritis, gastritis
- Intestinal diseases
  - Inflammatory bowel disease, coeliac disease, colitis, diverticulitis, constipation, haemorrhoids

IBD: Crohn’s Disease and Ulcerative colitis

Crohn’s Disease and Ulcerative Colitis

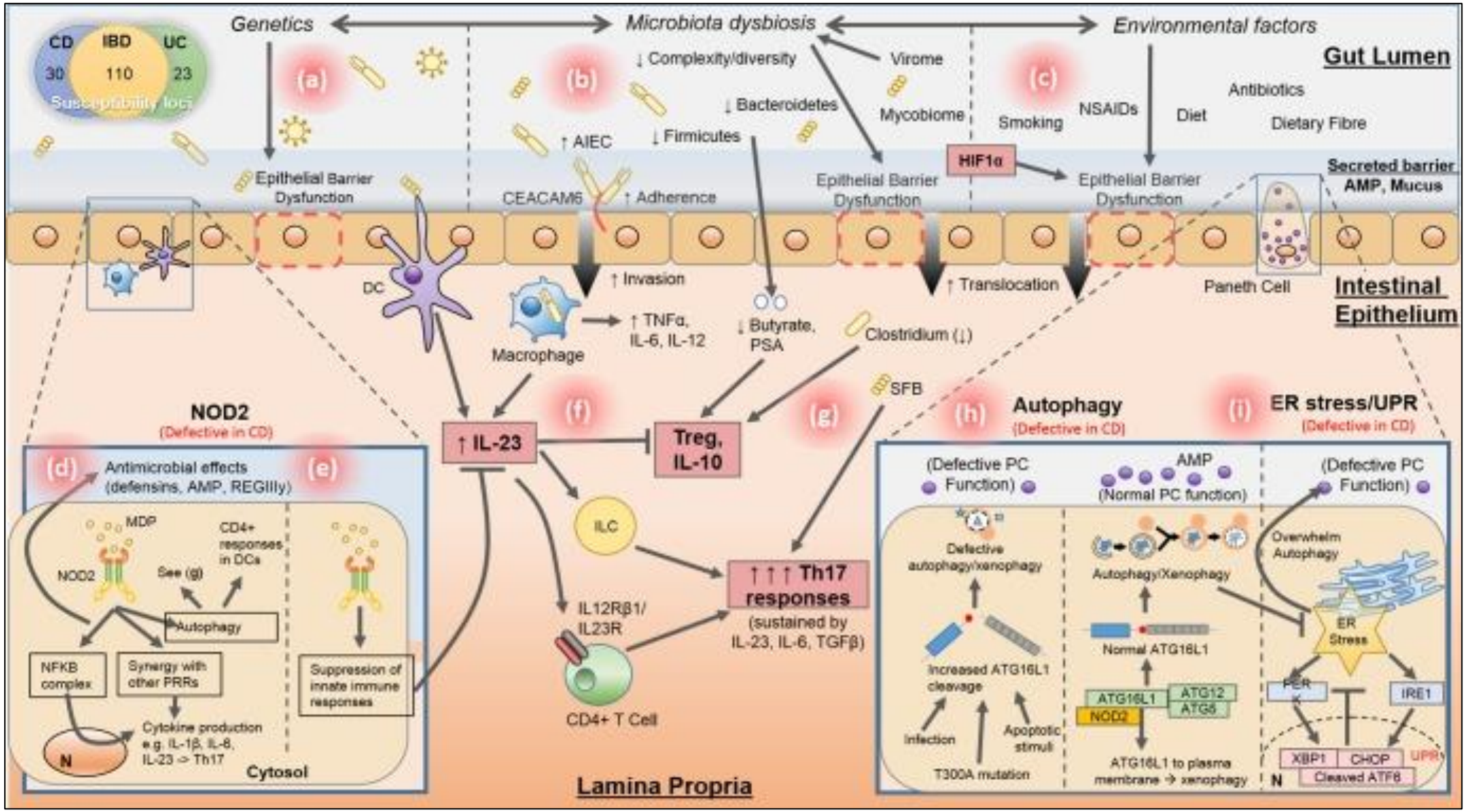
Background

- Inflammatory bowel diseases
- Our gut is home to an enormous number of bacteria and these bacteria can become harmful if they penetrate the wall of intestine
- The thin, continuous layer of epithelium lines the intestinal surface creating a barrier that prevents bacteria from crossing that border
- The mechanisms that control the integrity of the epithelium and contribute to maintaining a healthy gut have remained unknown



https://www.youtube.com/watch?v=gnZEge78_78

Molecular mechanisms in the pathogenesis of Crohn's disease (CD)



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4447044/pdf/biolrep-07-44.pdf

Molecular Mechanisms - IBD

- NOD2 (nucleotide-binding oligomerization domain 2)
  - A cytosolic pattern recognition receptor (PRR) that controls immunity against intracellular bacteria in intestinal epithelial cells
  - Defects in NOD2 function leads to weakening of innate immunity and anti-bacterial defence
  - Genetic polymorphism of NOD2 gene – present in patients with IBD
- Autophagy
  - a lysosomal degradation pathway that is essential for cellular survival, differentiation, development, and homeostasis
  - Bacteria is sequestered into double membrane-coated autophagosomes that subsequently fuse with endosomes and lysosomes (xenophagy)
  - autophagy genes (ATG16L1, IRGM, and LRRK2)
  - Defects contribute to IBD

Molecular Mechanisms - IBD

- IL-23/Th 17 and IL-10
  - IL-23: secreted by macrophages and DCs
  - IL-23 enhances Th17 response, reduces differentiation of Treg cells, and anti-inflammatory IL-10 production
  - IL-23 sustains Th17 responses (together with IL-6 and transforming growth factor-beta, TGF-β) that release IL-17
  - Massive infiltration of Th17 cells in the inflamed intestinal mucosa of IBD
  - Unrestrained Th17 activity and excessive IL-17 leads to IBD
  - IL-10-deficient mice develop spontaneous colitis in contact with gut commensal microbiota
  - Genetic variants of the IL-10 gene are associated with IBD
  - Other cytokines: IL-1, IL-6, IL-33, TNF-α

Molecular Mechanisms - IBD

- Microbiota dysbiosis
  - Gut microbiota: total 10^{12} in number
  - Change in microbial composition and diversity has impact on IBD occurrence
  - Decrease in Firmicutes including Bacteroidales, *Faecalibacterium*, and Clostridiales (e.g. *F. prausnitzii*) and increase in Enterobateriaceae (e.g. adherent invasive *Escherichia coli* [AIEC])

Define the terms 'microbiota dysbiosis' and 'microbiota symbiosis'.

Current treatment

- ✓Anti-inflammatory agents
  - ✓5-Aminosalicylate (5-ASA) – effective in UC but not in CD
  - ✓Corticosteroids – used in UC
- ✓Immunosuppressive agents
  - ✓Azathioprine – in both CD and UC
  - ✓Methotrexate - in CD
  - ✓Cyclosporine- in UC
- ✓Biological agents (Anti-TNF therapy)
  - ✓Infliximab, adalimumab, golimumab – inhibitors of TNF-α
  - ✓Etanercept – decoy receptor to TNF-α

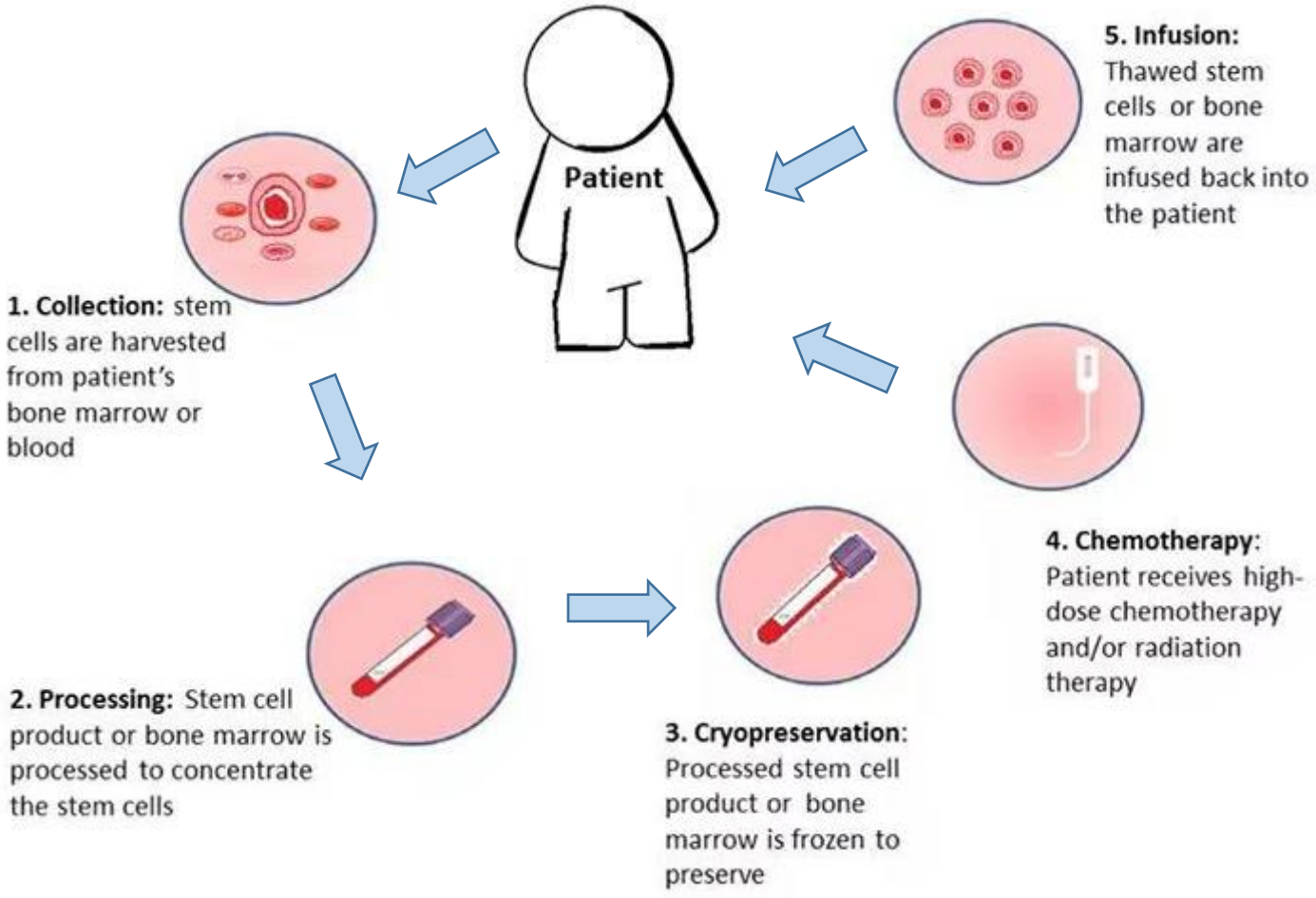
Emerging therapeutic options

- ✓Targeting cytokines in IBD
  - ✓ Tocilizumab – anti-IL-6
  - ✓ Ustekinumab – anti-IL-23
- ✓Targeting Th17
  - ✓ Vidofludimus - supresses IL-17 expression
- ✓Inhibition of lymphoid cell homing
  - ✓ Natalizumab, vedolizumab – targeting $\alpha 4 \beta 7$ integrins
  - ✓ Etrolizumab, PN-943 (oral), carotegrast methyl (oral) – newer anti-integrins agents under development/trial
- ✓JAK inhibitors
  - ✓ Tofacitinib – inhibition JAKs (preferably JAK1 and JAK3) of the tyrosine kinase family involved in cytokine signalling

Emerging therapeutic options

- ✓Restoring microbiota symbiosis
  - ✓Treating AIEC with antibiotics
  - ✓Restoring the ‘healthy’ microbiota – probiotics, fecal microbiota transplantation (FMT)
- ✓Gene transfer therapy for regulatory cytokines such as IL-10 - being explored and yields positive preliminary results
- Autologous hematopoietic stem cell transplantation – resetting the mucosal immune response

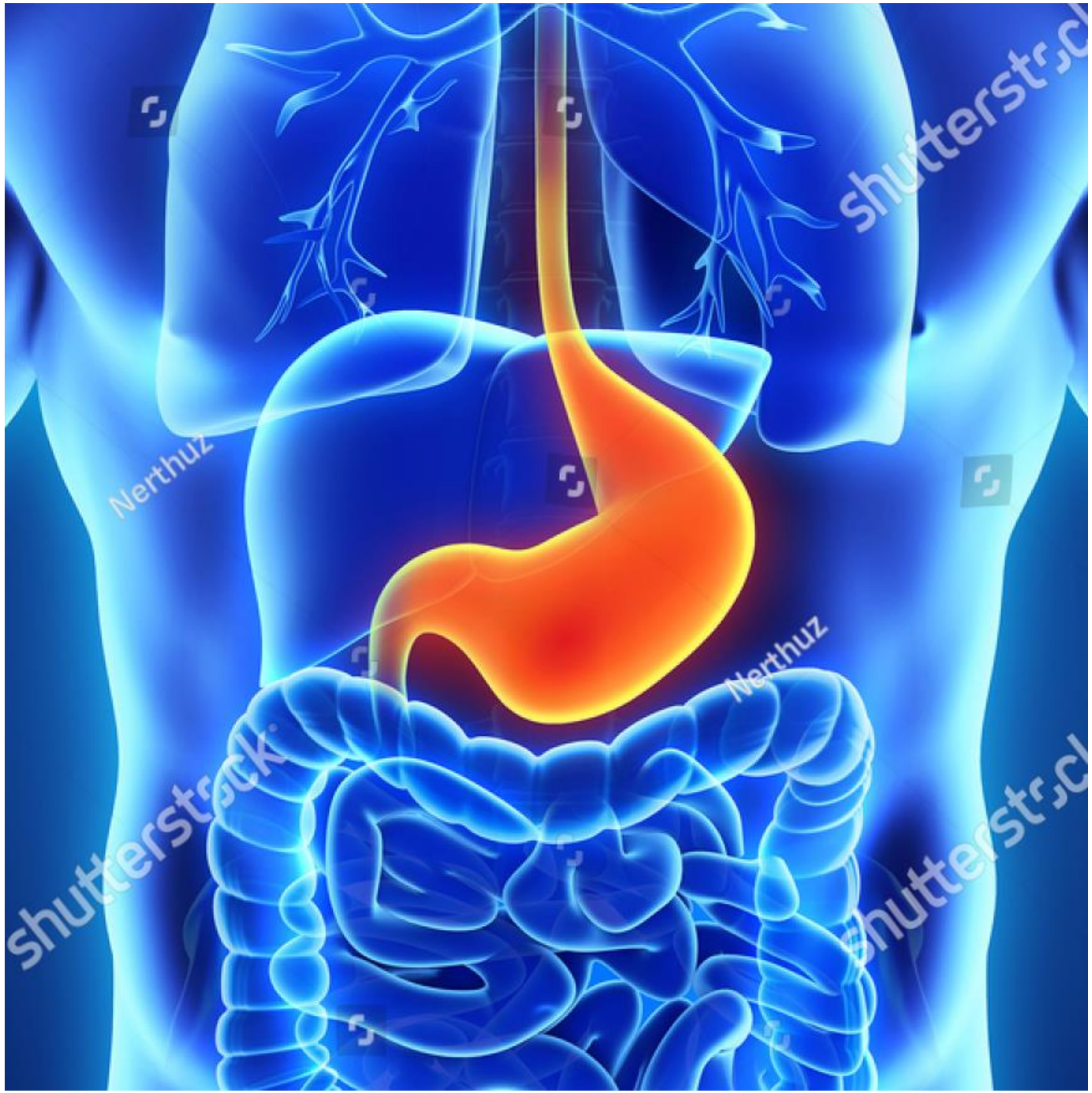
Emerging therapeutic options



The autologous stem cell transplant process

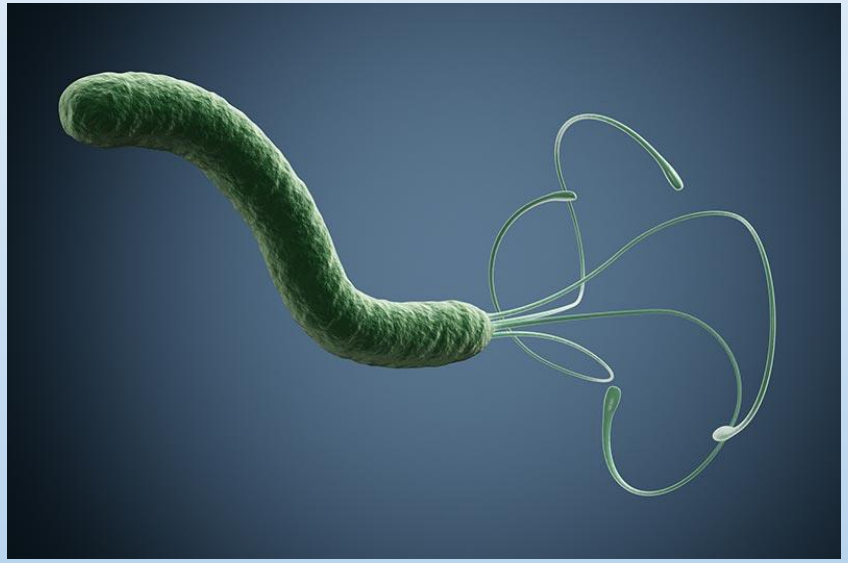
http://www.patient-help.com/28706/stem-cell-transplant-multiple-myeloma

Helicobacter pylori and peptic ulcers



Helicobacter pylori

- A gram-negative, micro-aerophilic and spiral bacterium (with 4–6 tunicate flagella) infecting nearly half of the world population
- Important role in pathogenesis of gastritis, peptic ulcer and gastric cancer
- *H. pylori* infection - associated with 70% of gastric ulcers and up to 80% of duodenal ulcers
- The only main reservoir of *Helicobacter pylori* is human being
- Prevalence of *H. pylori* infection - as high as 80% in adults



https://www.bu.edu/research/articles/physicists-uncover-swimming-secrets-of-h-pylori-bacteria/

Pathophysiology of *H. pylori* infection

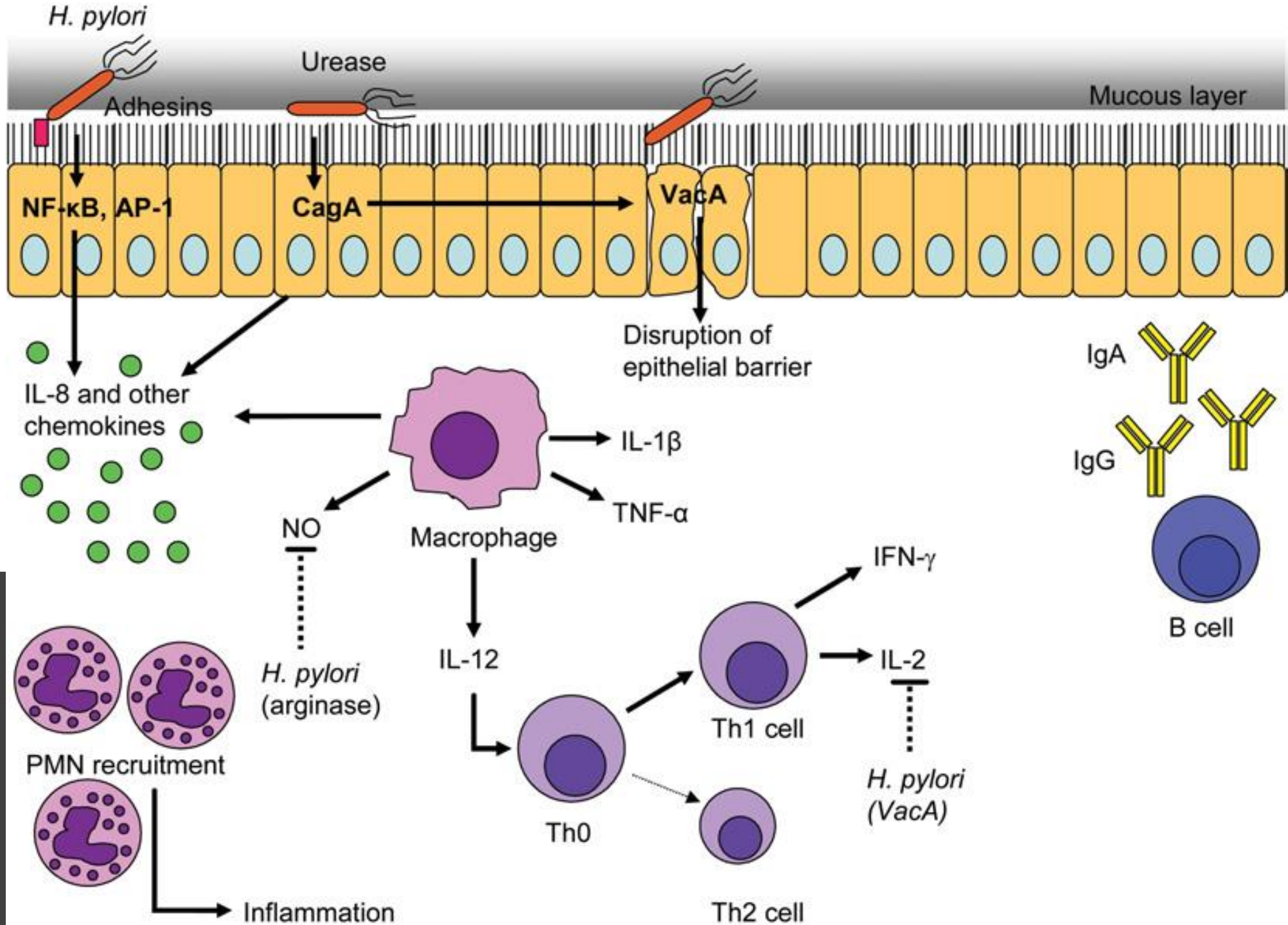
- Involves both innate and adaptive immune responses
- Innate immune responses
  - Colonization is helped by urease and flagellae
  - Colonization activates NOD1 (Nucleotide-binding oligomerization domain-containing protein 1) and triggers expression of pro-inflammatory genes
  - Infiltration of polymorphonuclear and mononuclear cells
  - Binding via TLR2, TLR4, TLR5 and TLR9

What is the difference between 'innate' and adaptive' immunity?

Pathophysiology of *H. pylori* infection

- Adaptive immune responses
  - Activation of Th1 and Th2 cells
  - Systemic and local antibody production
  - Inflammatory cytokines: IFN-γ, IL-1, IL-2, IL-6, IL-8, IL-12 and TNF-α
  - release of reactive oxygen species mediates injury to mucosa
  - Injury – DNA damage, apoptosis of gastric epithelial cells

H pylori pathogenesis and the inflammatory response



https://www.researchgate.net/publication/7310597_Immune_responses_to_Helicobacter_pylori_colonization_Mechanisms_and_clinical_outcomes/figures?lo=1

H. pylori virulence factors

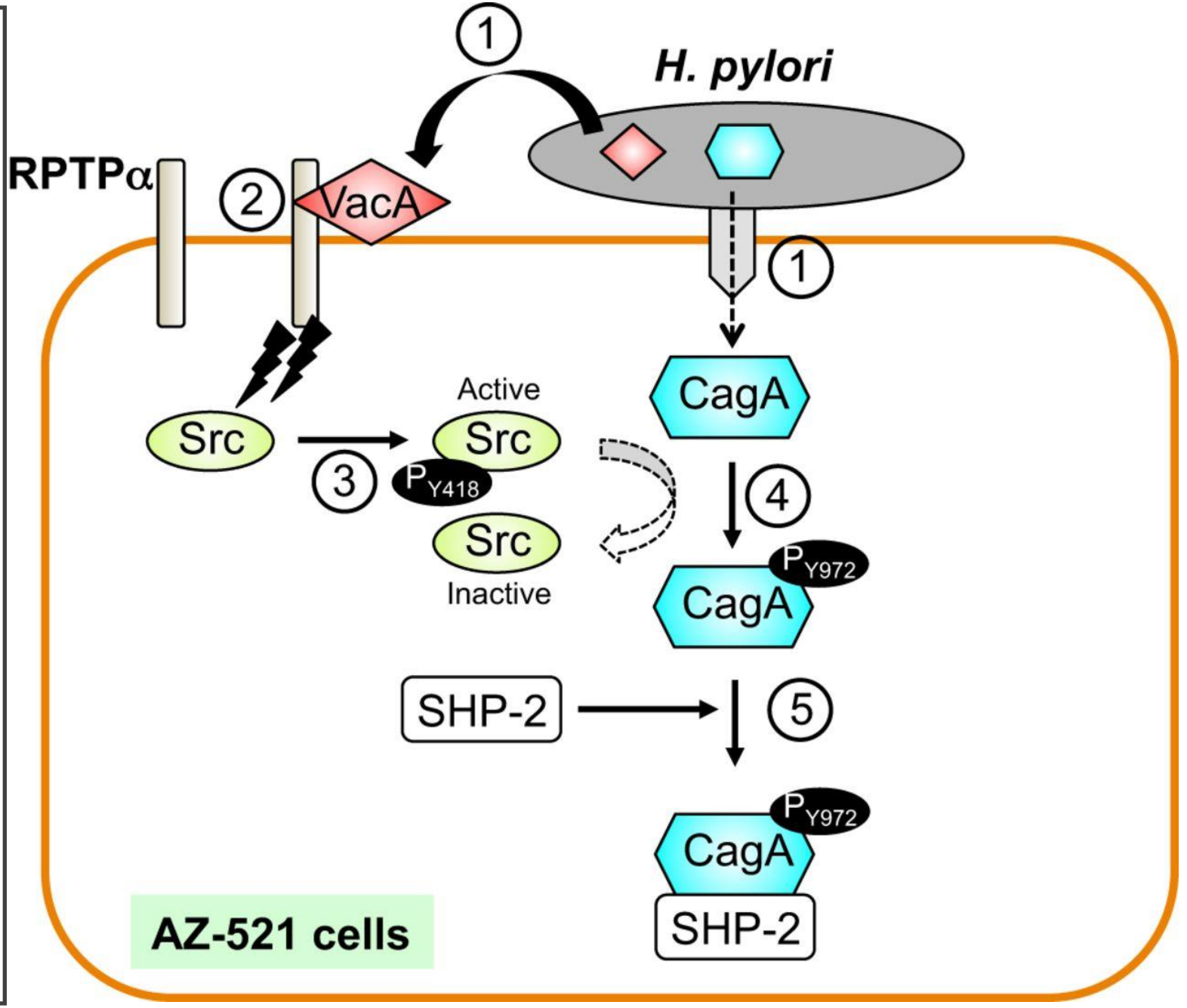
- Major virulence factor - cag-PAI (pathogenicity island): a 40 kb genomic fragment containing ORFs (open reading frames) that represent 31 genes
- 2 gene products serve as important virulence factors: CagA and VacA
- CagA
  - type IV bacterial secretion system (T4SS) - deliver the immunodominant CagA protein - growth-factor-like cellular response and cytokine production by the host cell
  - *H. pylori* cagA+ strains - increased risk for severe gastritis, atrophic gastritis, peptic ulcer and gastric cancer

H. pylori virulence factors

- VacA
  - Encodes vacuolating cytotoxin
  - A secreted protein toxin responsible for the gastric epithelial erosion
  - Genetic polymorphism - different forms of vacA exhibit varied phenotypes and have particular associations with gastroduodenal diseases.
  - vacA s1/m1 strains - most closely associated with gastric carcinoma

Molecular interaction of VacA and CagA in *H. pylori* infection

http://dmm.biologists.org/content/9/12/1473



Treatment of *H. pylori* infection

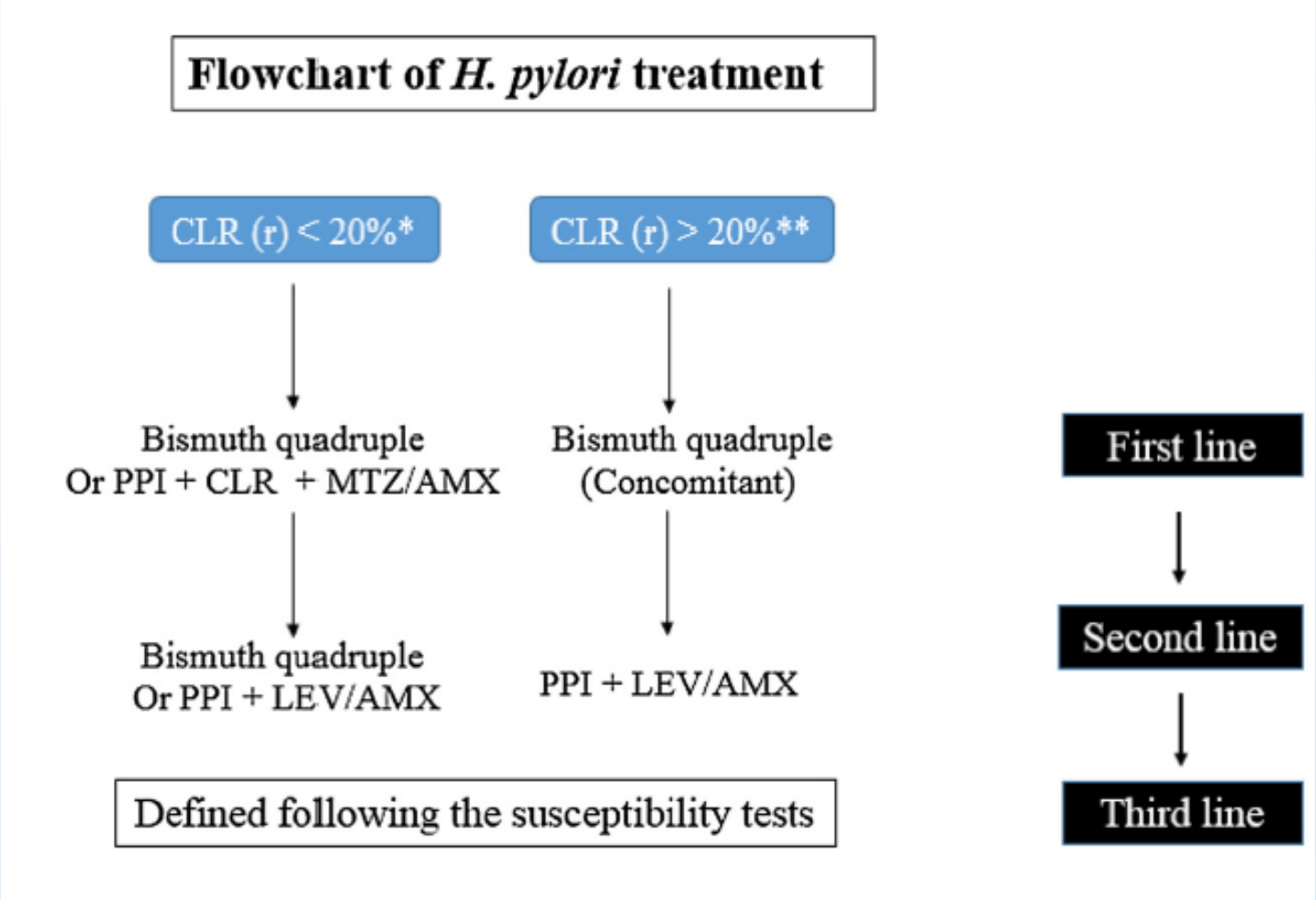
- Successful elimination of *H. pylori* - reduce the risk of development of duodenal and gastric ulcers as well as gastric cancer
- Current treatment - standard multidrug regimens
- Challenges
  - poor treatment adherence
  - Emergence of drug resistance
  - Drug-drug and drug-food interactions

Current treatment

- First-line therapy
  - Triple therapy – 2 antibiotics + proton pump inhibitor (PPI) for 10-14 days
    - PPI (esomeprazole) + clarithromycin, + amoxicillin/metronoidazole
    - Choices depend on local resistance pattern
  - Quadruple therapy – PPI + bismuth salicylate + metronidazole + tetracycline for 10-14 days
    - In regions with high resistance to clarithromycin
- Second-line therapy
  - Quadruple therapy (see above)
  - Levofloxacin-based triple therapy
    - PPI + levofloxacin + amoxicillin
  - Moxifloxacin-based triple therapy or metronidazole-based triple therapy

Current treatment

- Third-line therapy
  - Performing an antibiotic susceptibility test in order to select the next antimicrobial drug
  - Rescue therapies based on rifabutin, rifaximin, levofloxacin, or sitafloxacin
  - Example: rifabutin (150 mg), amoxicillin (1 g), and ciprofloxacin (500 mg) twice daily for 14 days



https://www.sciencedirect.com/science/article/pii/S2213716517300097?via%3Dihub

New perspective in treatment

- Alternative strategies - needed to complement existing treatments and increase their efficacy
- Promising alternatives:
  - Probiotics
  - Vaccine
  - Natural products

New perspective in treatment

- Probiotics
  - Members of the genera *Bifidobacterium*, *Lactobacillus*, and *Streptococcus*
  - Effects: provide defense against pathogens, maintain stomach homeostasis, reduce *H. pylori* survival, and trigger an immune response
  - Mechanisms
    - Pro-fermentation – producing lactic acid, decreasing the stomach's pH and inhibiting *H. pylori's* urease activity
    - Inhibition of pathogen adhesion, stimulation of mucin production
    - Modulating the production of pro-inflammatory cytokines, reducing local inflammation

New perspective in treatment

- Vaccines
  - Under development – animal studies and a few clinical trials
  - Challenges
    - enormous genetic diversity of the pathogen
    - complexity of the host's immune system
  - Target antigens that have been investigated
    - Urease
    - recombinant factors generated by CagA, VacA, and neutrophil-activating protein
  - Current state – not much success and more studies needed

New perspective in treatment

- Natural products
  - Broccoli, garlic, green tea, licorice, honey, propolis, and curcumin
    - Used as adjuvant therapies
    - Mechanisms – anti-inflammatory, antimicrobial, anti-oxidant
  - Current state – more scientific evidence is required to support their use

What is 'adjuvant' therapy?

Summary

- Molecular basis of gastrointestinal diseases including
  - Crohn’s disease
  - Ulcerative colitis
  - Peptic ulcer (*H. pylori* infection)

References

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Online Discussion

Helicobacter pylori infection is one of the most common infections in human beings worldwide.

Immunisation against *H pylori*, once thought to be impossible, is now widely considered the only practical approach to large-scale elimination of the bacterium from susceptible populations.

Nonetheless, developing a successful vaccine is proving to be more difficult than earlier thought, perhaps because *H pylori* colonises the gastric mucosa without crossing the epithelium, making the bacterium inaccessible to many immune effector mechanisms.

In many studies, immunisation not only prevented new *H pylori* infection but also cured animals of ongoing infection, paving the way for design of both prophylactic and therapeutic vaccines.

Discuss the strategies in vaccine development for *H pylori* infection. Describe some of the current and novel vaccine candidates under development and discuss their efficacy and prospect for future clinical use.

Thanks