FEMS ONLINE CONFERENCE ON MICROBIOLOGY 2020

Immunopathogenesis and resistance in *Helicobacter pylori* infection

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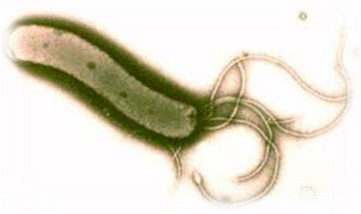
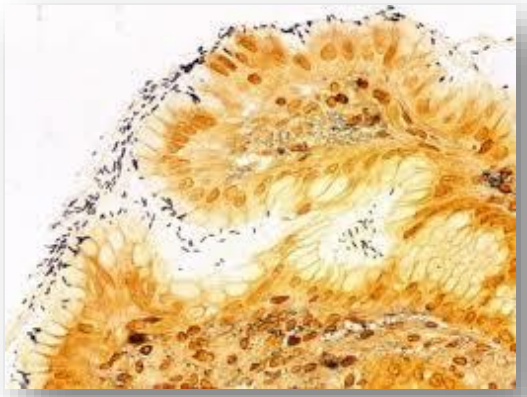
HELICOBACTER DISCOVERY

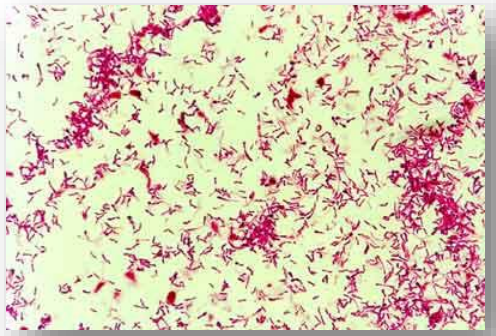
- In 1893 , **Bizzozero**, an Italian pathologist, discovered spiral bacteria in the canine stomach
- In 1896, **Salomon** reported similar findings in the stomachs of cats and mice
- In 1906, In human gaster, in patients with gastric carcinoma, **Krienitz** noticed spiral bacteria
- Modern era in *H. pylori* investigation started with **Marshall and Warren's** work on *H. pylori* in humans, as well as their tenacity to change the dogma of ulcer disease etiology, which finally led to the revision of medical text books.

H. pylori - Introduction

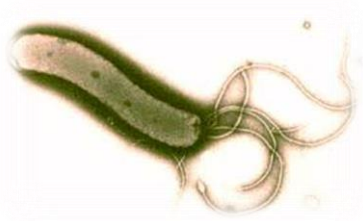


- *H. pylori* - Gram-negative non spore forming, S-shaped or curved bacillus 0.5-0.9 µm by 2-4 µm
- motile - with up to six sheathed polar flagella, microaerophilic

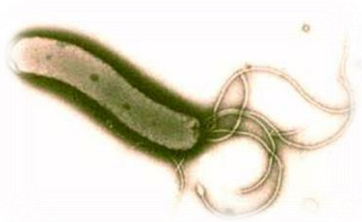




https://www.pharmamicroresources.com/2014/09/h-pylori-bacteria-mutate-to-establish.html



Bacterial Product	Functions	Targeted Host Defense
Urease	Neutralize acidity/regulate inflammation	Gastric acid
Flagellae	Motility/evade TLR5	Gastric peristalsis/immune response
Adhesins	Adherence to gastric epithelium	Gastric peristalsis
Lewis antigens	Adherence to gastric epithelium/molecular mimicry	Host immune response
Arginase	Decrease nitric oxide	Inflammation
AhpC, NapA	Antioxidants	Oxidative stress
LPS	Evade TLR4	Immune response
Dup	Ulcerogenesis	Epithelial cells
DNA	TLR9 signaling	Immune response
VacA	Suppresses T-cell activation	Immune response
CagL	Antigenic variation	Immune recognition



COLONIZATION OF THE GASTRIC MUCOSA

Colonization

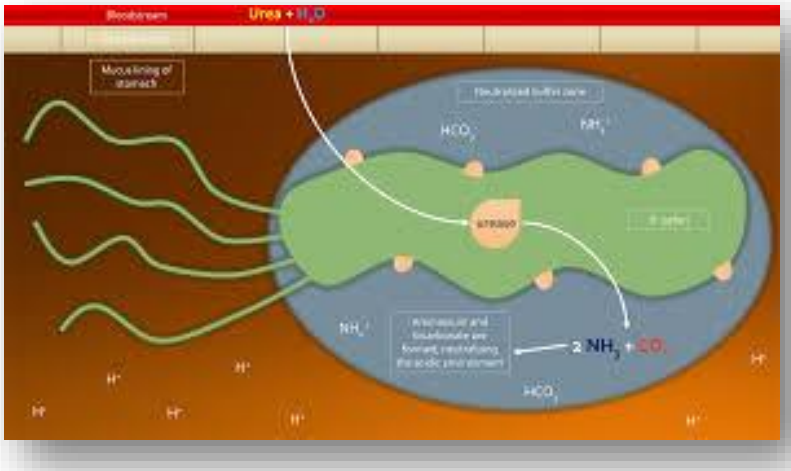
- The initial colonization in the gastric mucus depends on:

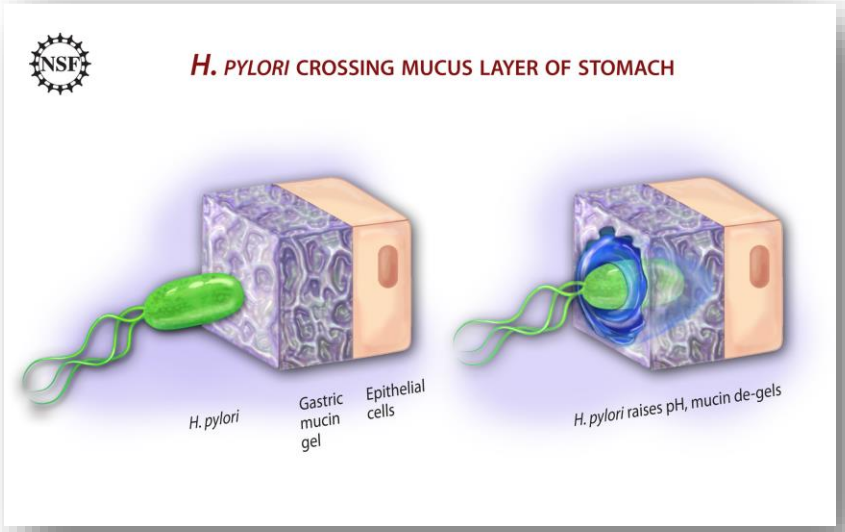
✓ Urease

- Which hydrolyzes urea to ammonia and carbon dioxide, neutralizing the pH, allows the bacterial survival and proliferation

✓ Polar flagella

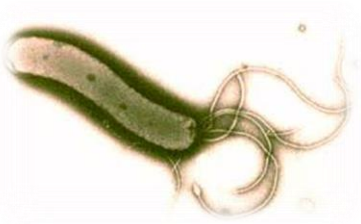
✓ Changes in the bacterial cell morphology lead to effectively penetrating the gastric mucosal barrier and to access to the underlying epithelial cells.

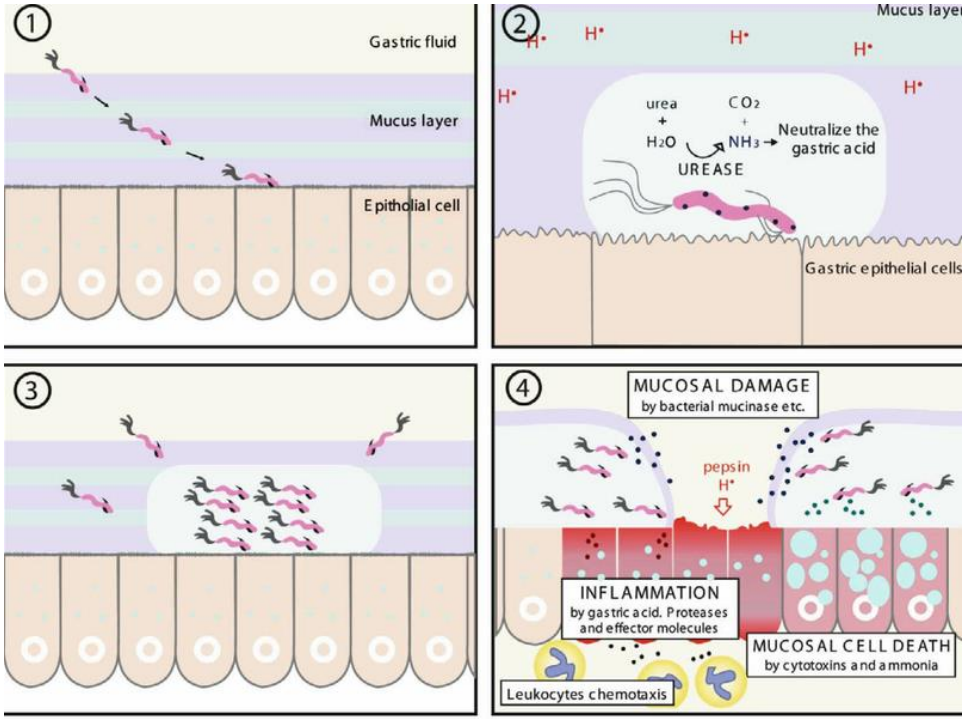




Wikimedia.org

Colonization

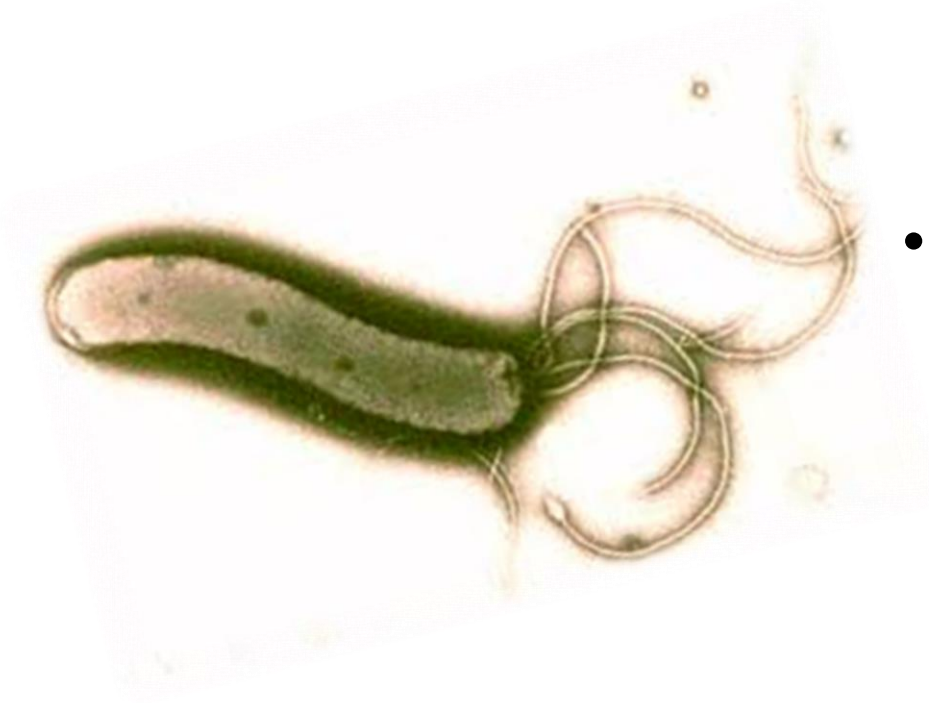




- At low pH, mucin converts into a gel trapping bacteria.
- In the presence of urease, pH will increase - viscosity of the mucin will decrease
- Bacteria get able to swim in mucous layer
- Acute ingestion of *H. pylori* leads to transient hypochlorhydria.
- In addition, in some patients occurs hyperchlorhidria due to gastric inflammation and increased level of **gastrin** and fall in mucosal **somatostatin** level.

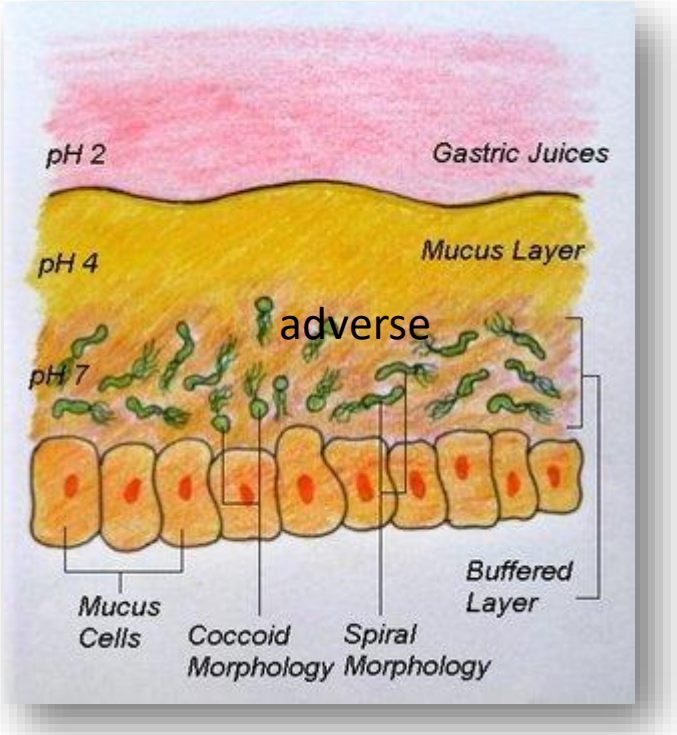
Khan S. *Helicobacter* urease: Niche construction at the single molecule level. J. Biosci 2009; 34(4)

Chemotaxis



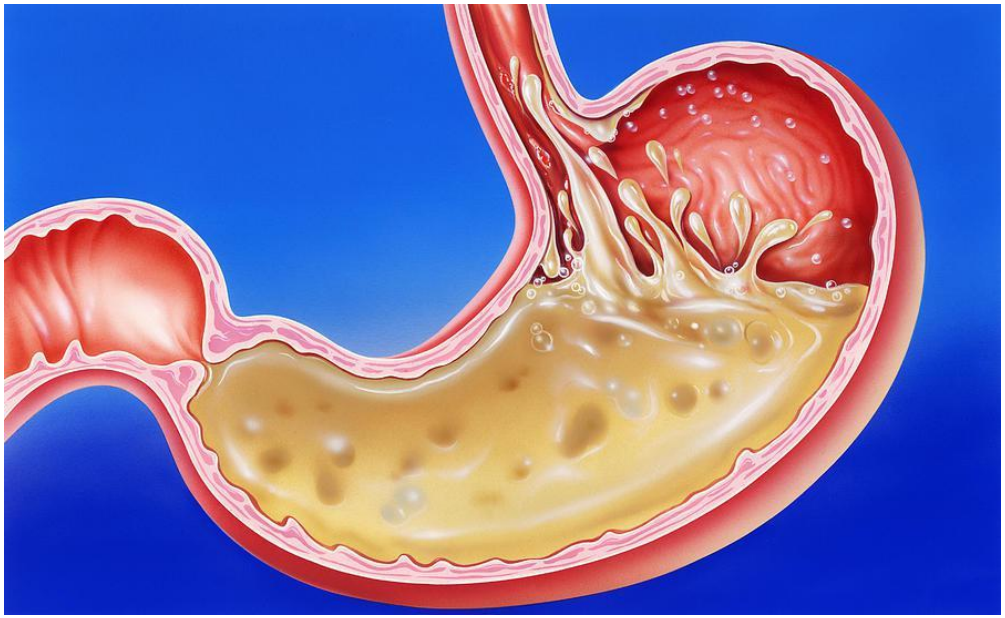
- *H.pylori* expresses several chemotactic receptors (bacterial „noses“) that drag bacteria to the close proximity to gastric epithelial cells
- In the case of deficiency in chemotactic factors, occurs **reduced inflammation** and **defective** T cell responses

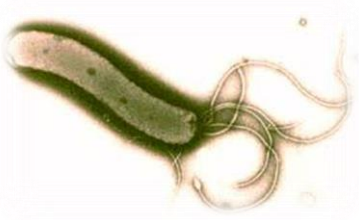
Colonization – final state



H. pylori survive at the wide range of pHs in gaster.

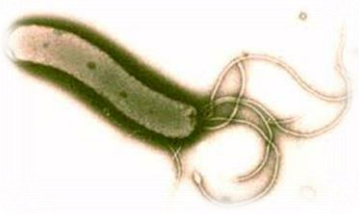
- Helicobacter pylori cells under the stomach mucus layer. © Michelle Wiepjes





SURVIVING

Bacterial survival



- Several factors takes part in this process:

✓DNA repair proteins

✓Catalase

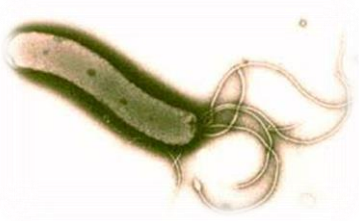
✓Superoxide dismutase

✓Arginase

✓Disabling the autophagy in infected cells

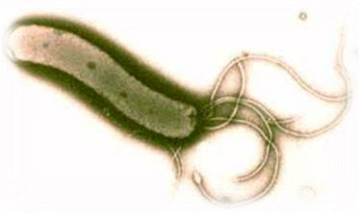
✓Disabling of host cell DNA repair proteins

✓Interaction with host cell micro mRNA

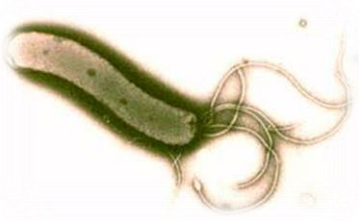


ADHESION

Adhesion

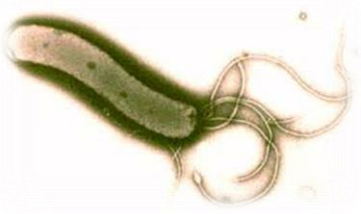


- The BabA and SabA adhesins, OipA, Hop, HomZ, DupA proteins
- BabA binds to the type B blood group antigen in gastric cell
- It facilitates colonization, induces mucosal inflammation and can influence the severity of the disease
- SabA binds to the sialyl-Lewis x/a antigens
- *H. pylori* strains which carry babA, vacAs1 and cagA together are associated with duodenal ulcer and present a higher risk of gastric cancer



VACA AND CAGA PROTEINS

VacA genes

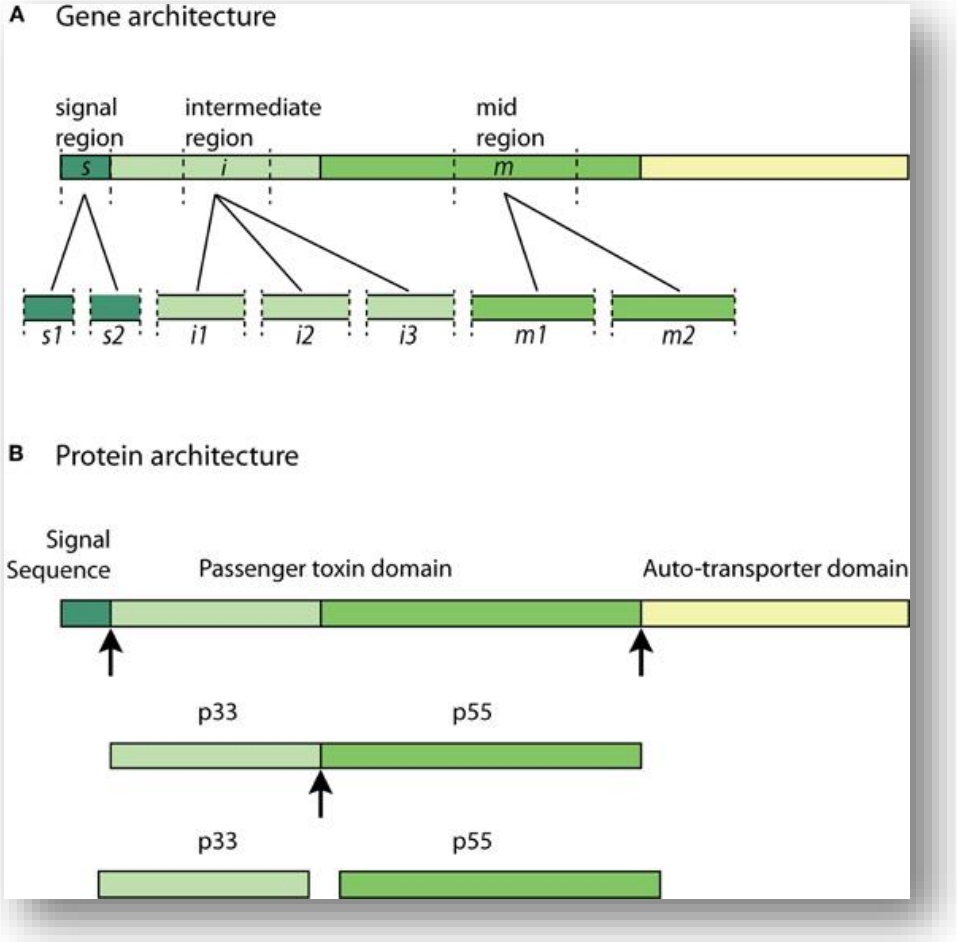


- All *H. pylori* strains carry the *vacA* gene
- That gene shows a diversity in DNA sequences with the variation in the amount of vacuolating activity
- Diversity in sequences are associated with different cell toxicity and severity of disease
- VacA is a secreted pore-forming toxin that enters the host epithelial cells by endocytosis and affects a variety of biological processes.

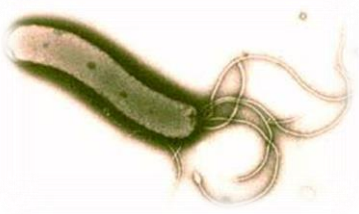
VacA genes

Pattraman S L et al. Vacuolating cytotoxin A (VacA), a key toxin for *Helicobacter pylori* pathogenesis. Front. Cell. Infect. Microbiol. 2012;

There are several regions in *vacA* gene :
Signal (s)
Intermediate (i)
Mid (m)
The s1 type can be further subtyped as s1a, s1b, and s1c.
The m1 type can be subtyped as m1a and m1b.

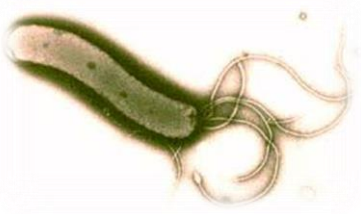


VacA genes



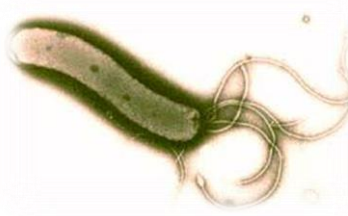
- The v*acA* s1m1 genotype is often associated with PUD and gastric cancer in Western populations
- And with the presence of the *cag*PAI,
- The *vacA* s2m2 genotype is usually linked with the absence of the *cag*PAI

VacA genes



- Recently, it was proposed that an intermediate i region, located between the s and m regions , is associated with gastric cancer.
- The novel d region is associated with gastroduodenal diseases

VacA



- VacA acts through several mechanisms:

✓ Direct cell-damaging effects - cytoskeletal changes and suppression of epithelial cell proliferation

✓ Inducing apoptosis of epithelial cells

✓ Blocking phagosome maturation in macrophages

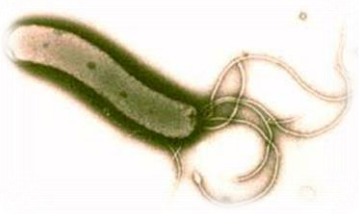
- And there are some effects on immunity:

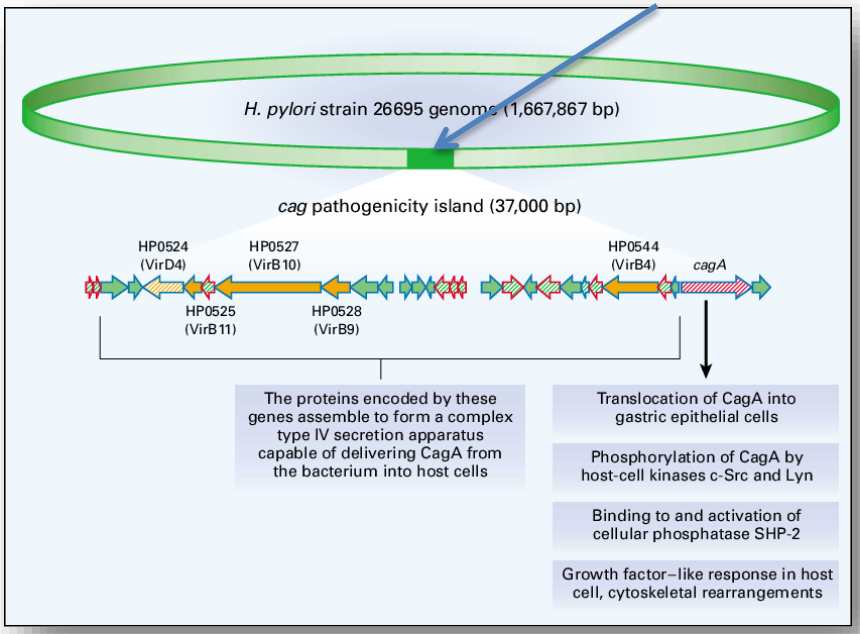
✓ Inhibition of antigen presentation,

✓ blocking T cell proliferation,

✓ downregulates Th1 functions – immune supression

*cag*PAI

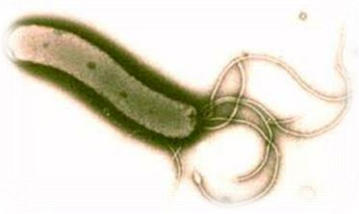




- There is *cag*PAI in some *H. pylori* strains chromosome.

- Suerbaum et Michetti. *Helicobacter pylori* Infection. The New England Journal of Medicine. 2002; 347(15):1175-86.

CagA



- CagA is highly immunogenic protein associated with:

✓ cell injury,

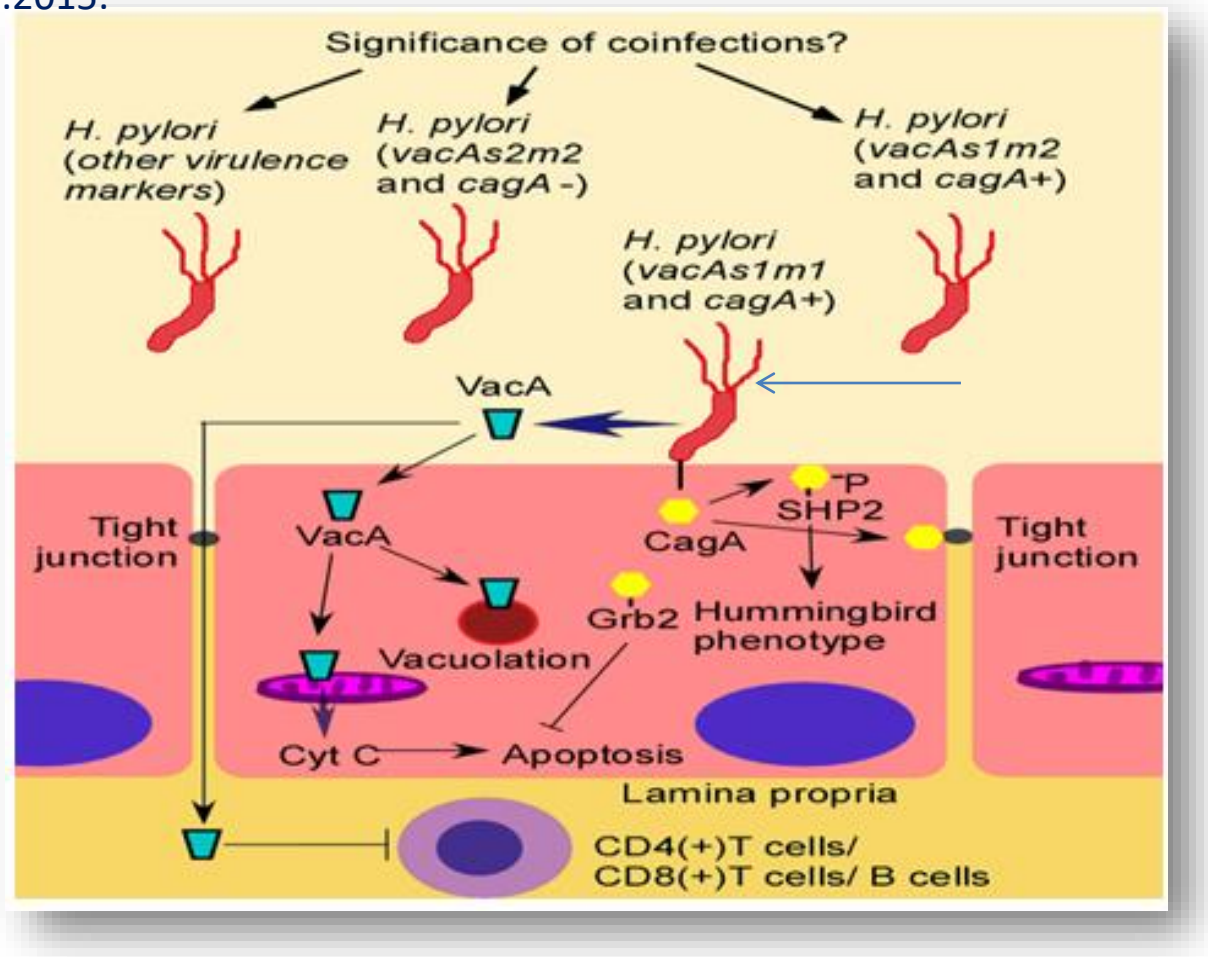
✓ duodenal ulcers,

✓ and gastric adenocarcinoma.

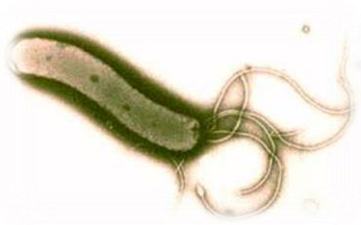
- When phosphorylated, modulates migration, spreading, and adhesion of bacteria to the epithelial cells
- CagL expressed on the pilus of the T4SS is required for CagA translocation.
- It interacts with integrin on epithelial cells that translocate bacterial CagA.

Hummingbird phenotype

Chattopadhyay S et al. The VacA and The CagA of Helicobacter Pylori: Two Multitasking Proteins of a Multitasking Bacterium . Journal of Gastrointestinal Disorders and Liver function.2015.



CagA



- CagA contributes to:

✓ alteration of signaling transduction

✓ affecting host cell physiology

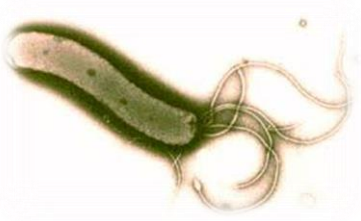
✓ disruption of intercellular junctions

✓ loss of cell polarity

✓ promotion of inflammation

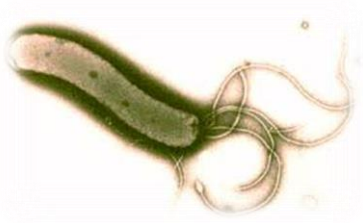
✓ dysregulation of cellular apoptosis and proliferation

CagA



- CagA is protein phosphorylated on specific tyrosine residues within repeating pentaaminoacid Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs.
- So far, four different motifs have been described according to the amino acid sequence: A, B, C, D.

CagA



- Variation in the number of cagA EPIYA motif variants present in the same biopsy correlated with peptic ulcer
- Occurrence of two or more EPIYA-C motifs is associated with atrophy in the gastric mucosa
- CagA has emerged as a major virulence factor for gastroduodenal disease severity, including an INCREASED CANCER RISK

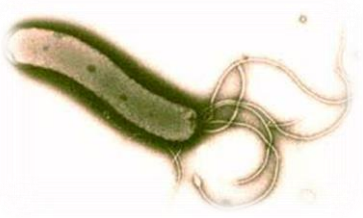
ABBREVIATIONs OF THE aa NAMES

Helicobacter pylori Life Cycle
Samuel Mckenzie News Medical Life Science

- Glutamic acid (E)
- PROLINE* (P)
- ISOLEUCINE (I)
- TYROSINE* (Y)
- ALANINE (A)

- (*) Essential only in certain cases

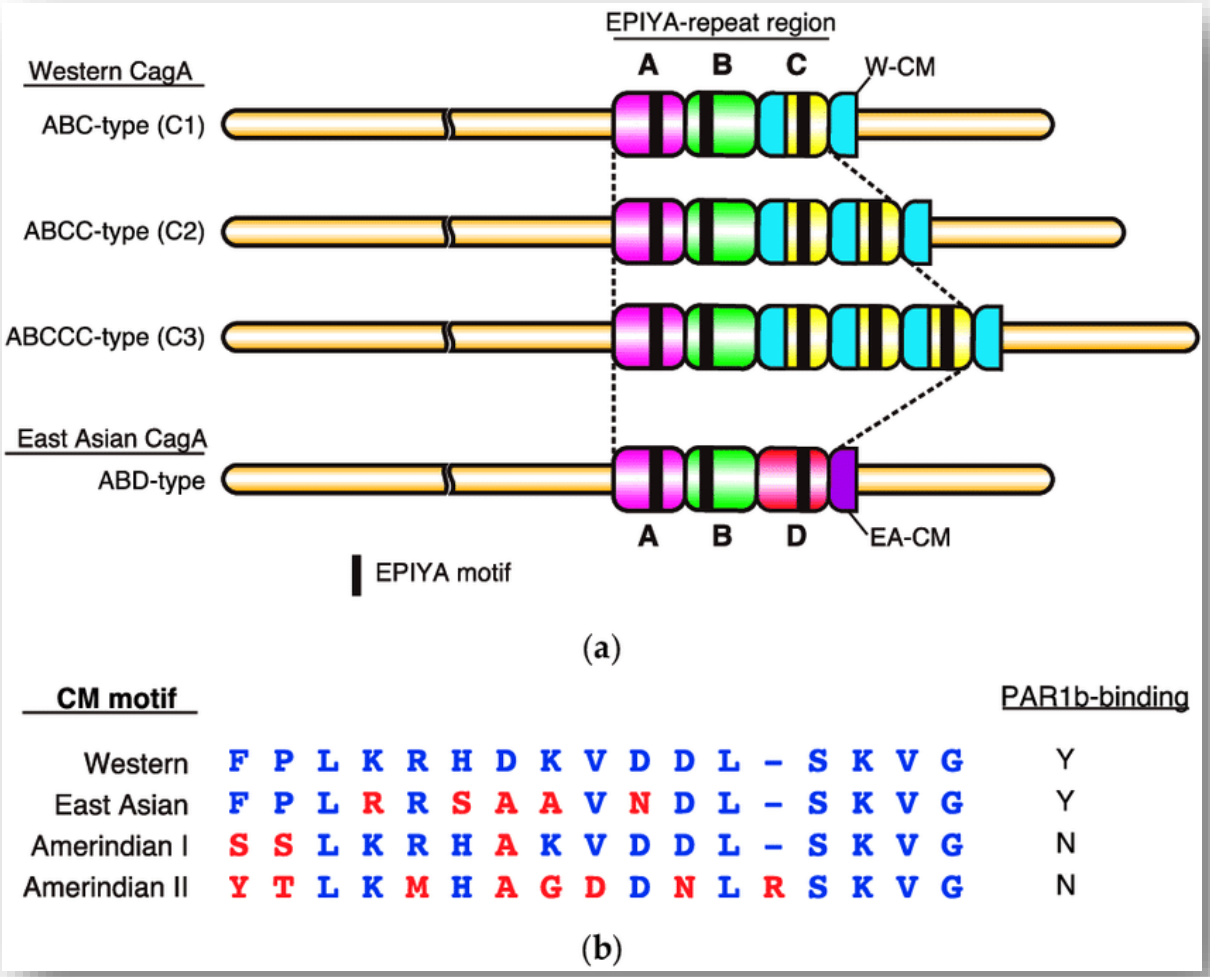
CagA



- In WESTERN-TYPE isolates: EPIYA-A and –B motifs , are followed by varying numbers of EPIYA-C motifs
- In EAST ASIAN-TYPE: EPIYA-A and -B are, commonly followed by an EPIYA-D motif
- Variation in number of repeating EPIYA-C motifs at the C-terminus of the protein may alter the biological activity of CagA in phosphorylation-dependent as well as phosphorylation-independent ways.
- Number of **cagA EPIYA-C** motifs and the tyrosine phosphorylation status of CagA are important risk factors for gastric cancer among Western strains

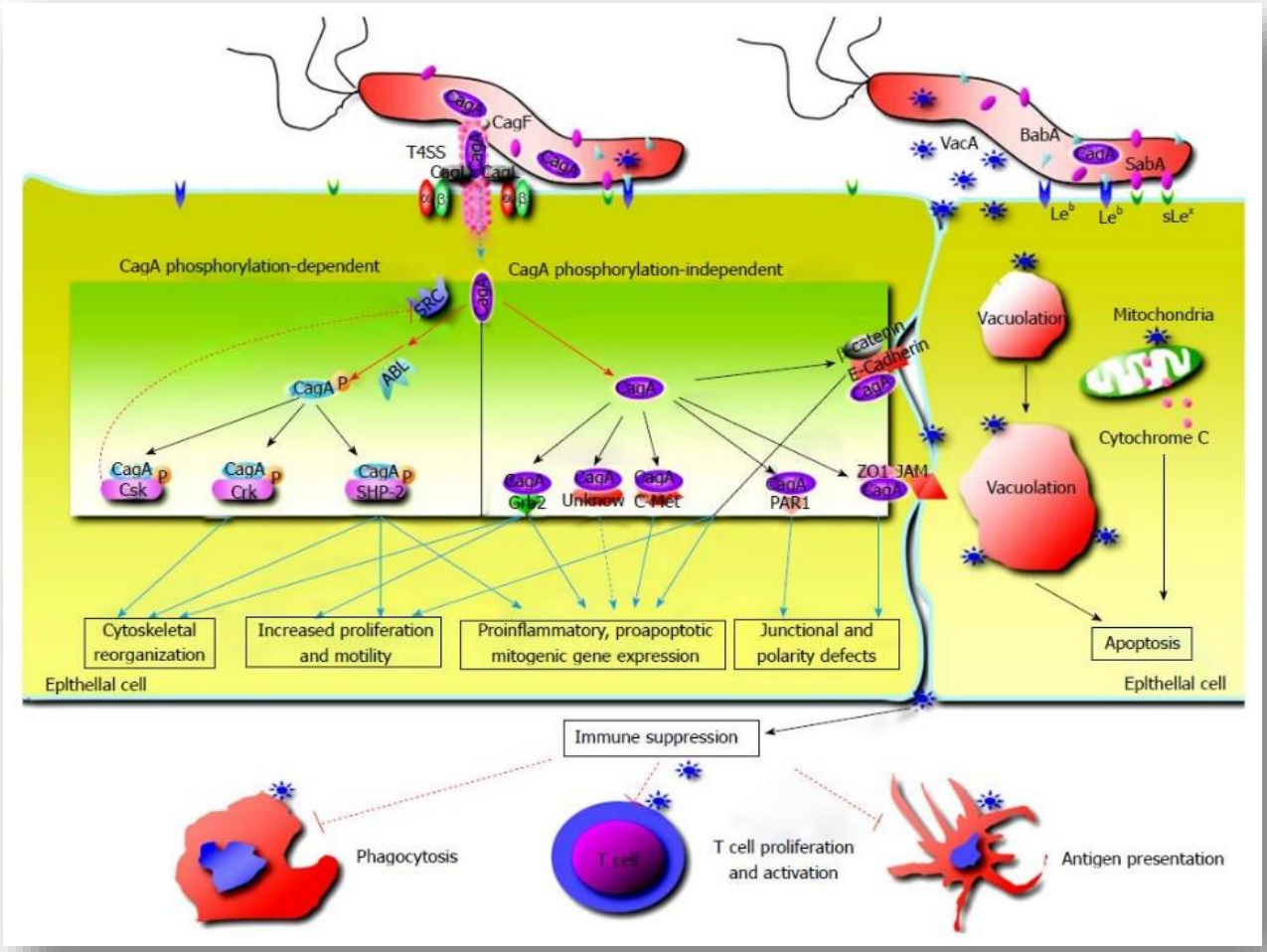
EPIYA motifs

Zhang R-G et al. Role of Helicobacter pylori infection in pathogenesis of gastric carcinoma. World J Gastrointest Pathophysiol. 2016; 7(1): 97-107.



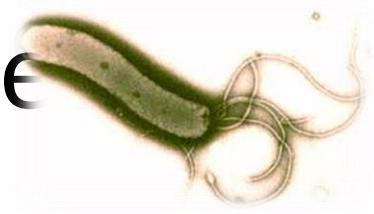
Summary of VacA and CagA activity

Zhang R-G et al. Role of Helicobacter pylori infection in pathogenesis of gastric carcinoma. World J Gastrointest Pathophysiol. 2016; 7(1): 97-107.



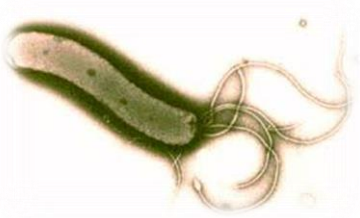
INNATE IMMUNE RESPONSE

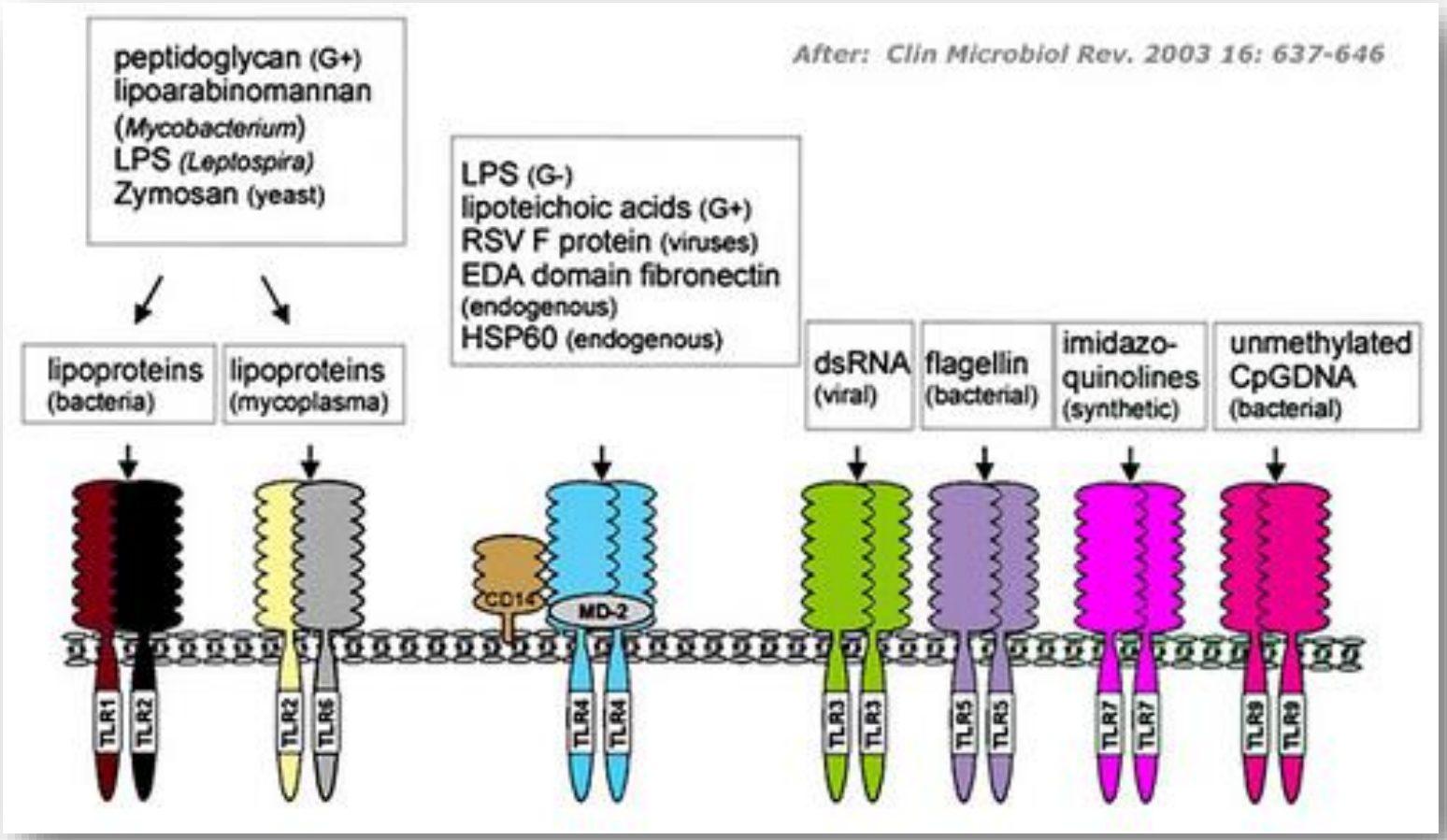
Innate Immune response



- The first defensive barriers against *H. pylori* is the mucus
- The immune responses against *H. pylori* are initiated by the recognition of the pathogen associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) on epithelial and innate immune cells
- This process is followed by initiation of the adaptive immune responses

PRR -TLRs




peptidoglycan (G+)
lipoarabinomannan
(Mycobacterium)
LPS (Leptospira)
Zymosan (yeast)
After: Clin Microbiol Rev. 2003 16: 637-646
LPS (G-)
lipoteichoic acids (G+)
RSV F protein (viruses)
EDA domain fibronectin
(endogenous)
HSP60 (endogenous)
lipoproteins
(bacteria)
lipoproteins
(mycoplasma)
dsRNA
(viral)
flagellin
(bacterial)
imidazo-
quinolines
(synthetic)
unmethylated
CpGDNA
(bacterial)
CD14
MD-2
TLR1
TLR2
TLR2
TLR6
TLR4
TLR4
TLR3
TLR3
TLR5
TLR5
TLR7
TLR7
TLR9
TLR9

Innate Immune response

Portal-Celhay C et Perez-Perez GI. Immune responses to Helicobacter pylori colonization: mechanisms and clinical outcomes Clinical Science (2006) 110, 305–14

- Colonization of the stomach by *H. pylori* elicits an **inflammatory** response and recruits:

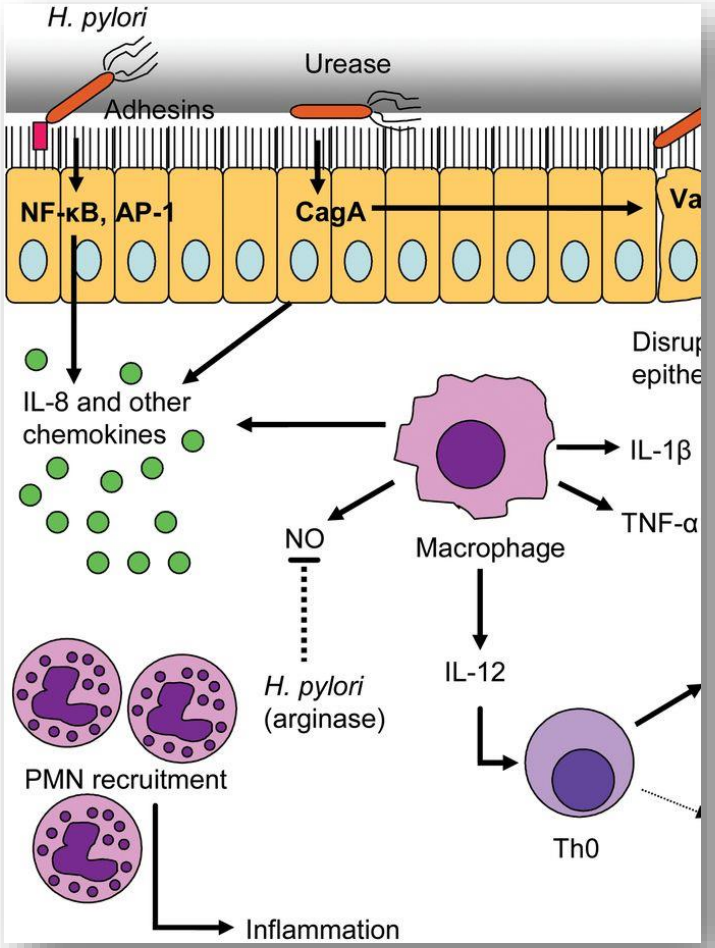
✓ neutrophils,

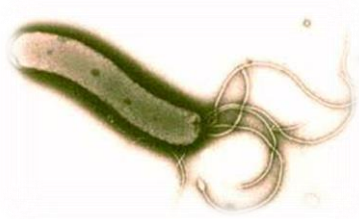
✓ lymphocytes,

✓ macrophages

✓ and dendritic cells (DCs)

- to the gastric mucosa

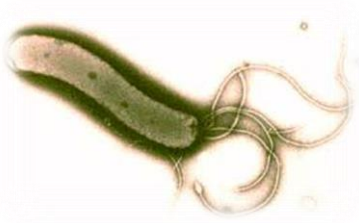




EVASION OF NONSPECIFIC IMMUNE RESPONSE

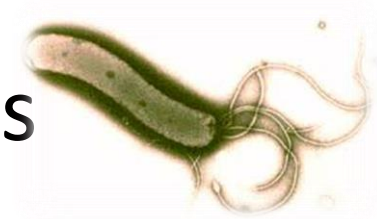
Evasion of immune response

- LPS escapes the TLR4 recognition
- The less activity of LPS in *H. pylori* is related to its compound content.
- The recognition of non-LPS ligands by TLR2 leads the innate immune responses to induction of anti-inflammatory responses with IL-10 production
- Flagellin can modify the N terminal recognition domain of TLR5 and



- Escape the innate immune responses

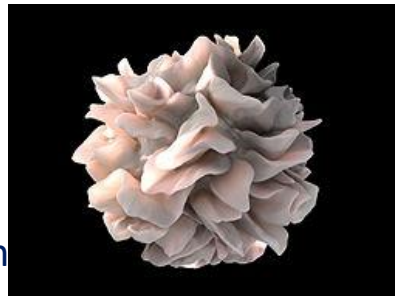
Evasion from RLRs, CLRs, NLRs



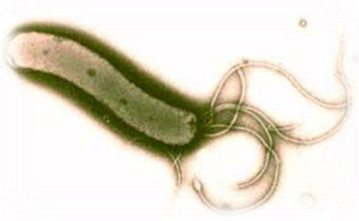
- The RIG-I like receptors (RLRs) is a subfamily of PRRs
- RLRs act as cytoplasmic sensors of PAMPs.
- Evasion from C-type lectin receptors (CLRs) recognition enables that *H.pylori*
- inhibits the signaling complex leading to suppression of pro-inflammatory cytokine production.
- Consequently, recognition of *H. pylori* with NLRs, leads to the activation of inflammasome complex and the downstream signaling pathways, essential for controlling *H.pylori* infection.

Tolerogenic phenotype of DCs

Wikimedia.com



- There is hypothesis that the suppression is caused by efficient DC re-programming toward a tolerance-promoting phenotype
- These DC cells have the capacity to induce Tregs cell production
- In addition, CagA+ *H. pylori* promotes IL-10 expression, inhibiting DC maturation and inducing DCs to have a tolerogenic phenotype which can suppress the proliferation of CD4+ and CD8+ effector T cells
- The conseqence is the suppression of *H pylori*-specific Th17 and Th1 response and chronic colonization of the stomach

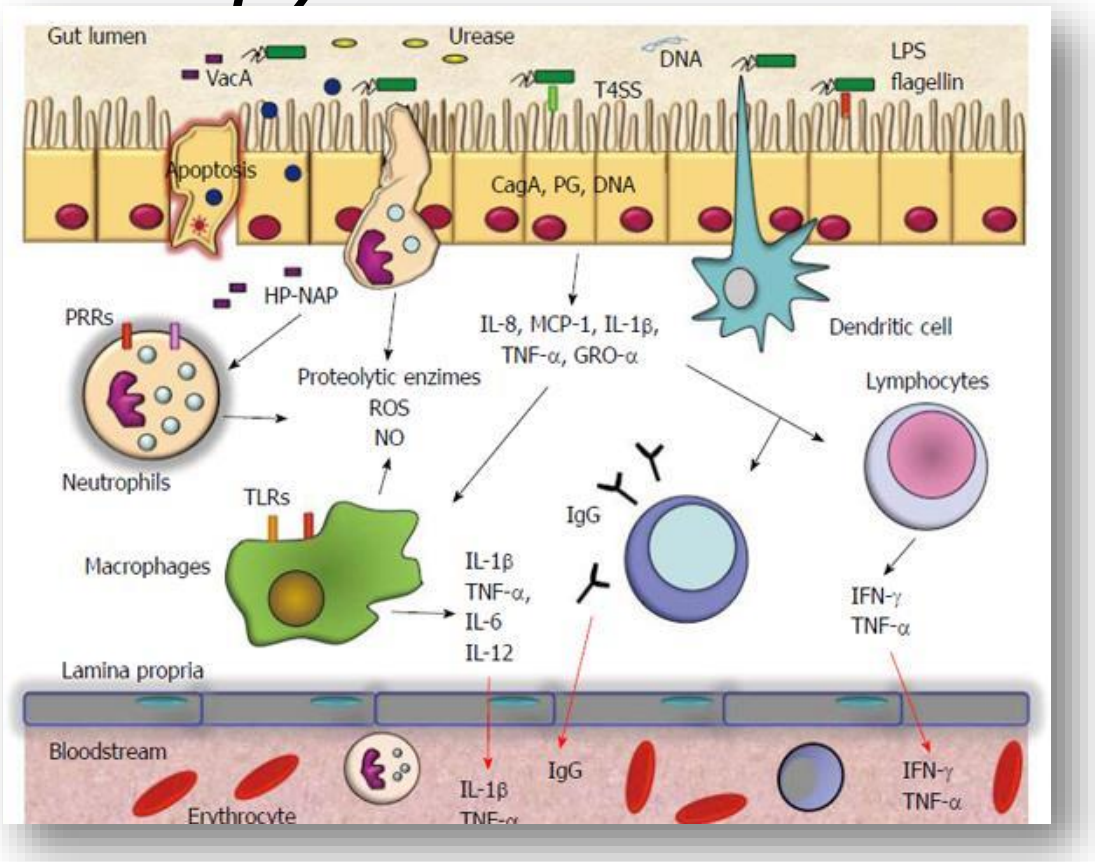


ADAPTIVE IMMUNE RESPONSE

Adaptive immune response

- The activation of human gastric DCs by *H. pylori* directs naive CD4+ T cells to Th1 differentiation through IL-12 production
- This process enables secretion of cytokines such as IL-1, IL-6, TNF-α and IFN-γ
- Due to activation of the transcription factors T-bet (T box expressed in T cells) and Stat 4 (signal transducer and activation of transcription factor 4).

Adaptive response in *Helicobacter pylori* infection

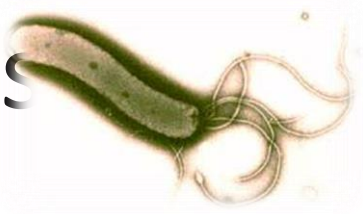


L Álvarez-Arellano, C Maldonado-Bernal. *Helicobacter pylori* and neurological diseases: Married by the laws of inflammation. World J Gastrointest Pathophysiol. 2014; 5(4): 400-404

Adaptive immune response

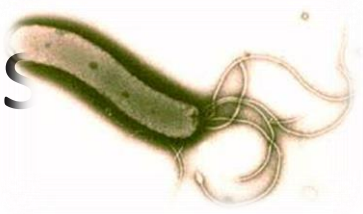
- IL-18, is a Th1-related cytokine promotes IFN-γ production by T cells and natural killer (NK) cells in the presence of IL-12 and is correlated with gastric inflammation.
- The initial Th1 response is aimed to eradicate the *H. pylori* infection.

Adaptive immune respons



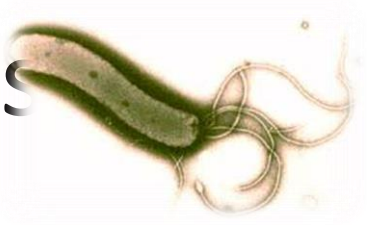
- The role of Th17 cells in mucosal host defense is associated with the production of IL-17, IL-8 and GM-CSF (granulocyte macrophage colony-stimulating factor)
- *H. pylori*-stimulated DCs are potent inducers of Th17 cells by regulating IL-1β/IL-23 production,
- CagA may play an important role in mediating this process

Adaptive immune respons



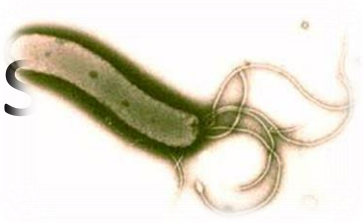
- IL-8 recruits neutrophils that are critical for the clearance of the bacteria
- IL-21 is a cytokine that modulates the differentiation of CD4+ and CD8+ T cells
- IL-21 promotes Th17 differentiation and IL-10 production, and it inhibits the generation of potentially pathogenic Th1 and Th17 effector cells.

Adaptive immune respons



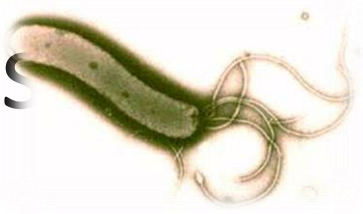
- A Th2 response has been observed in patients with intestinal metaplasia and intestinal-type gastric cancer, suggesting that Th2 cells could be involved in different outcomes of *H. pylori* infection
- Predominant Th2 immune response is characterized by IL-13 secreting cells.

Adaptive immune respons



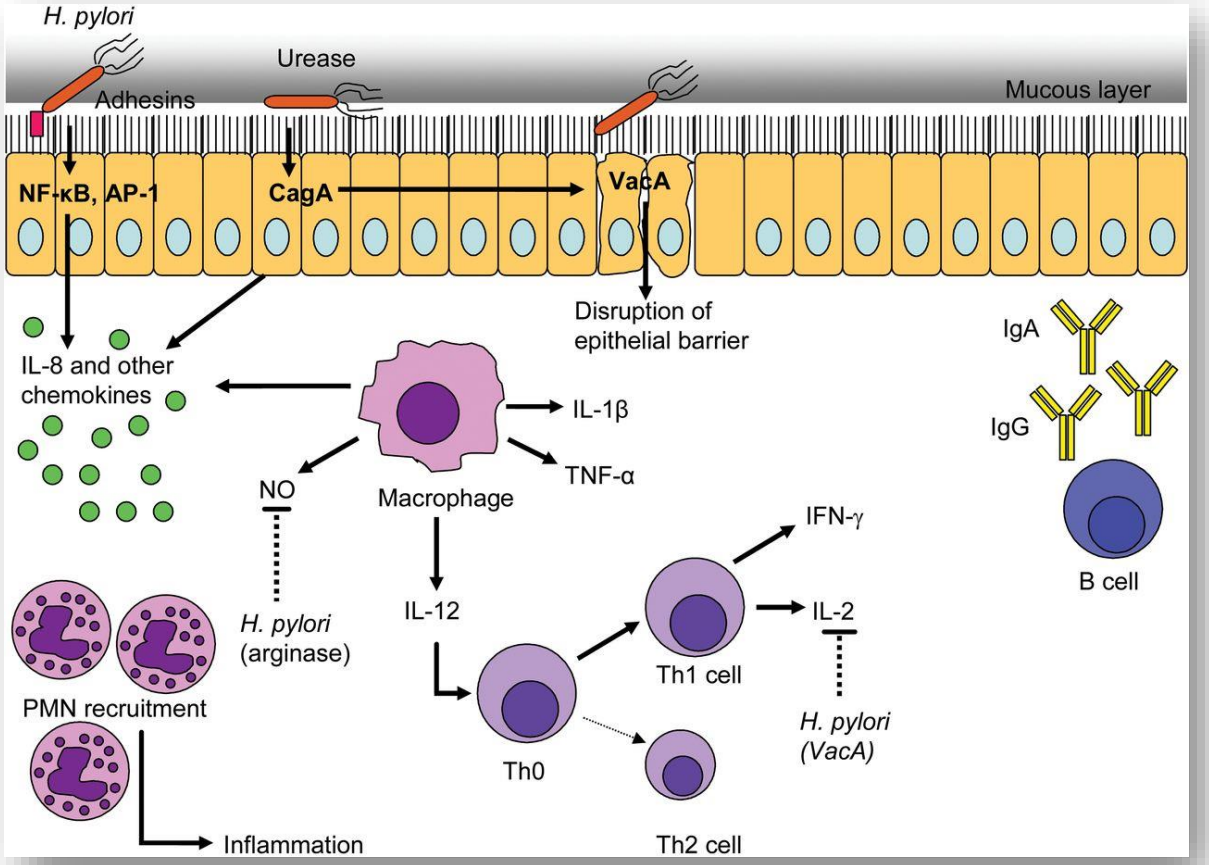
- The immune response was originally considered as a Th1 response but Th17 and Tregs have been introduced in *H.pylori* infection
- In addition, Th17 cells appear to be essential in the clearance of *H. pylori*

Adaptive immune respons

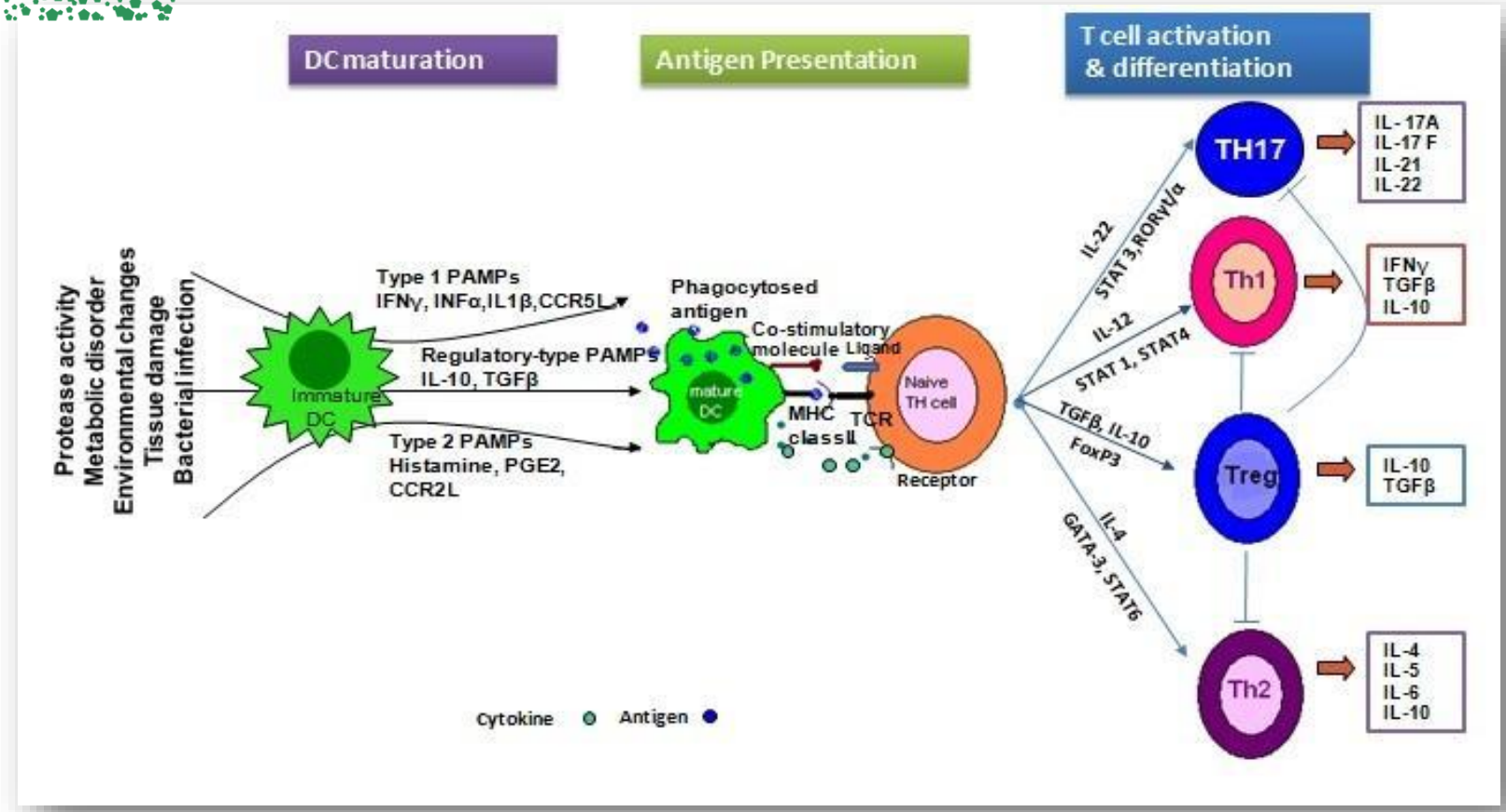


- The expression of the intracellular pro-inflammatory cytokine IL-32 is induced in gastric epithelial cells in a Cag PAI-dependent manner
- IL-32 activates NF-κβ and stimulates the production of cytokines and chemokines
- IL-32 high levels are also related to human gastritis and gastric cancer.

The inflammatory response in *Helicobacter pylori* infection



Activation of T cells



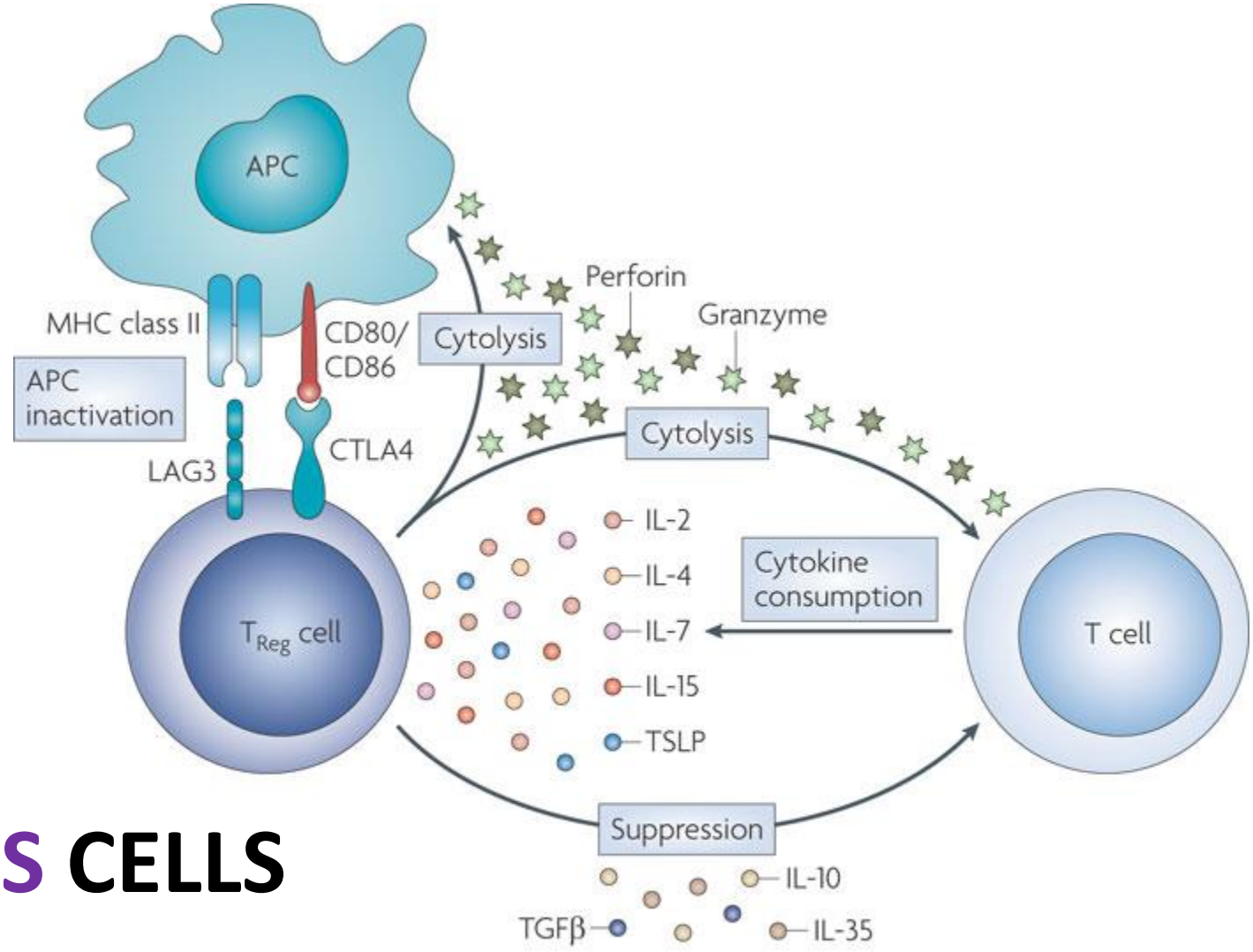
L Álvarez-Arellano, C Maldonado-Bernal. *Helicobacter pylori* and neurological diseases: Married by the laws of inflammation. World J Gastrointest Pathophysiol. 2014; 5(4): 400-4

Helicobacter pylori infection and MMPs induction

- *H. pylori* infection and MMPs induction involve both cellular and humoral immune response
- Phosphorylated CagA activates a series of signaling molecules including inflammatory cytokines, ROS, MMPs leading to aberrant cellular function.
- TH17 stimulates MMPs through IL-17 and IL-21.
- MMPs play important role in cancer genesis

- Unveiling the

TREGS CELLS



Rochman Y, Spolski R, Leonard WJ.New insights into the regulation of T cells by gamma(c) family cytokines. Nat Rev Immunol 2009; 9:480-90

Nature Reviews | Immunology

TREGS

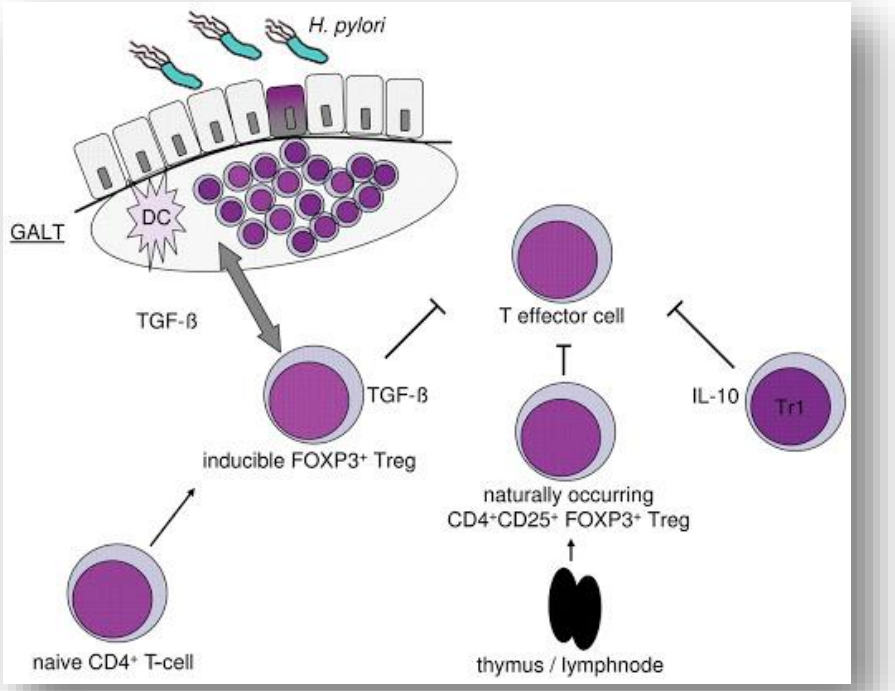
forkhead box Fox p3 protein

Wikimedia Transcription factors and intracellular receptors

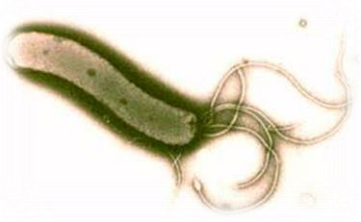
- Despite inflammation,
- the clearance of *H. pylori* is often incomplete due to the activation of CD4+CD25hi forkhead box protein 3 (Foxp3+) regulatory T cells (Tregs).
- **Treg cells are potent suppressors of T cell effector function and regulate the balance between immunity and infection.**

Immunological determinants of H. pylori-associated inflammation

- H. pylori-induced gastritis is characterized by a predominant Th1/Th17 response
- It is regulated by Treg cells

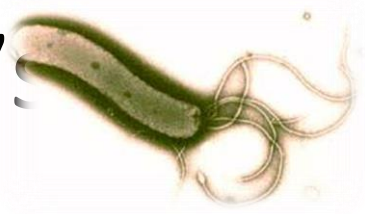


KANDULSKI A et al. Role of Regulatory T-cells in H. pylori-induced Gastritis and Gastric Cancer ANTICANCER RESEARCH 30: 1093-1104 (2010



EVASION OF SPECIFIC IMMUNE RESPONSE

Evasion of Th1 and Th17



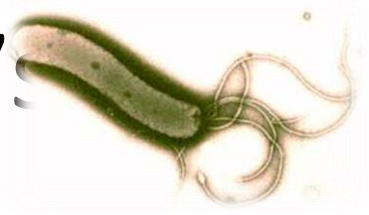
- *H.pylori* virulence factors contributing in interfering with T cell responses are:

✓VacA,

✓γ-glutamyltranspeptidase (GGT),

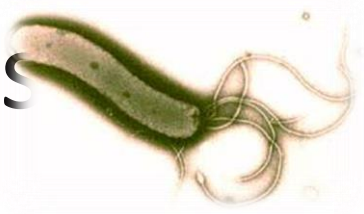
✓arginase

Evasion of Th1 and Th17



- Receptor for VacA is integrin β2 (CD18) on T cells
- VacA inhibits the proliferation of T cells interfering with the signaling pathway of TCR, IL-2 and upstream molecules
- VacA also prevents nuclear translocation of the nuclear factor of activated T cells (NFAT) transcription factor, and consequently inhibits transcription of specific T cell genes

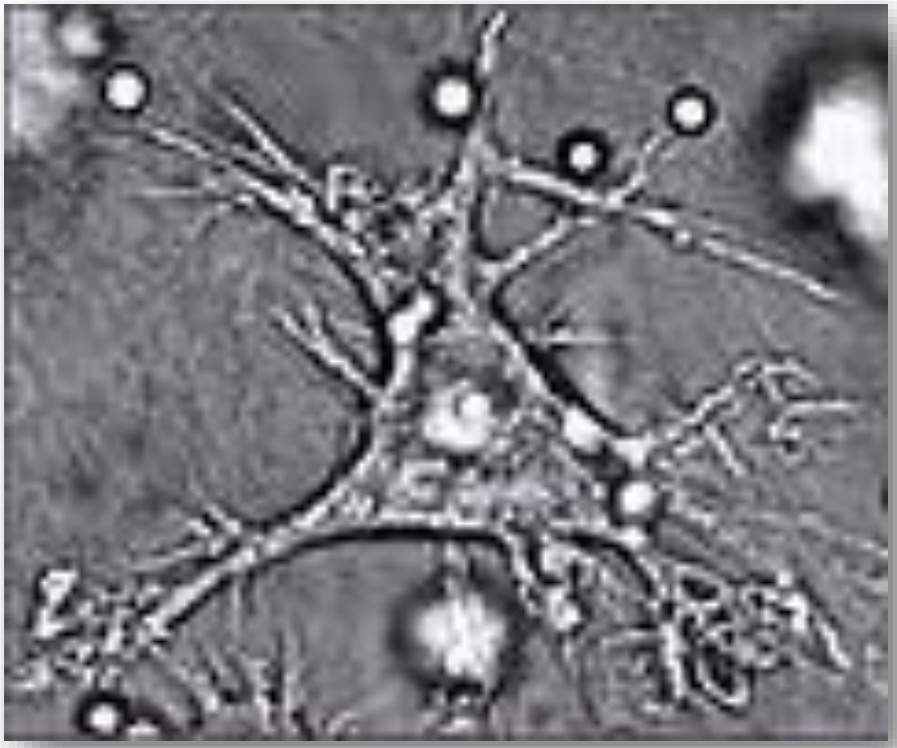
Deviation in T cell respons



- GGT and VacA from *H. pylori* indirectly affect the activity of T lymphocytes and promote the differentiation of effector CD4+ T cells to Tregs
- Tregs can suppress cytokine proliferation and production of other T cells
- Tregs cells have been implicated in limiting inflammatory responses to *H. pylori*
- They are producing anti-inflammatory cytokines such as IL-10 and TGF-β that,
- Result in pathogen persistence to limit tissue injury.
- This process is positively correlated with chronic gastritis and gastric adenocarcinoma

*H.Pylori t*olerogenic programming in DCs

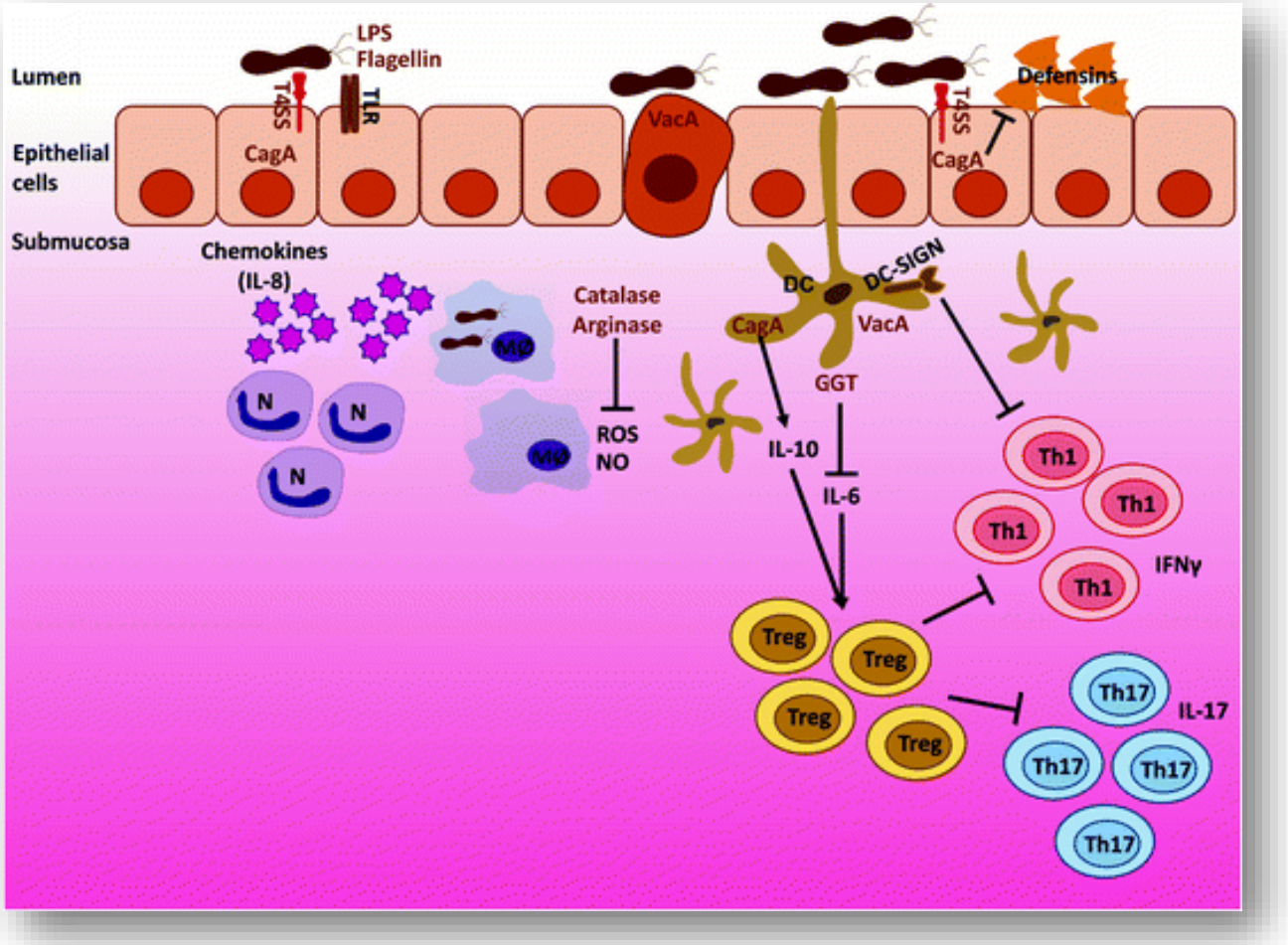
- *H. pylori*-induced tolerogenic DCs are not able to induce effector functions in naive T cells, and these cells became very efficient in inducing Tregs
- Dominant Treg-induced Response is *H. pylori* persistance.
- So, *H.pylori* is controling
- chronic infection conditions

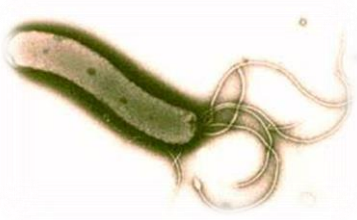


EM Dendritic cell
wikimedia.com

Immune Evasion Strategies and Persistence of Helicobacter pylori

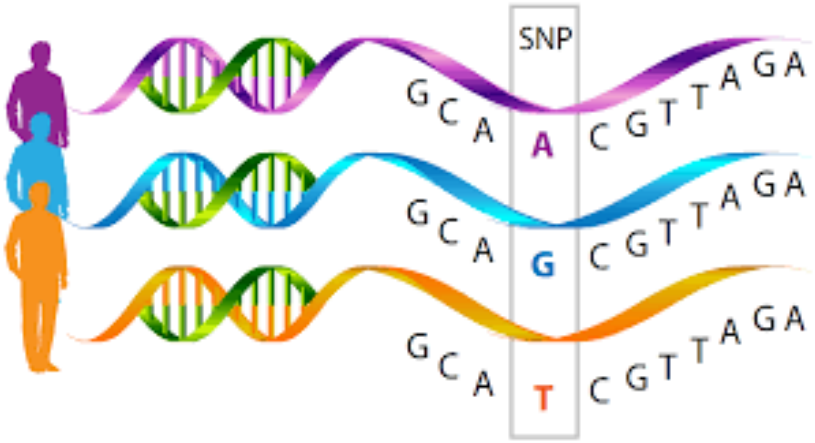
Molecular Pathogenesis and Signal Transduction by Helicobacter pylori pp 53-71 Markus Gerhard 2017 Raquel Mejías-Luque



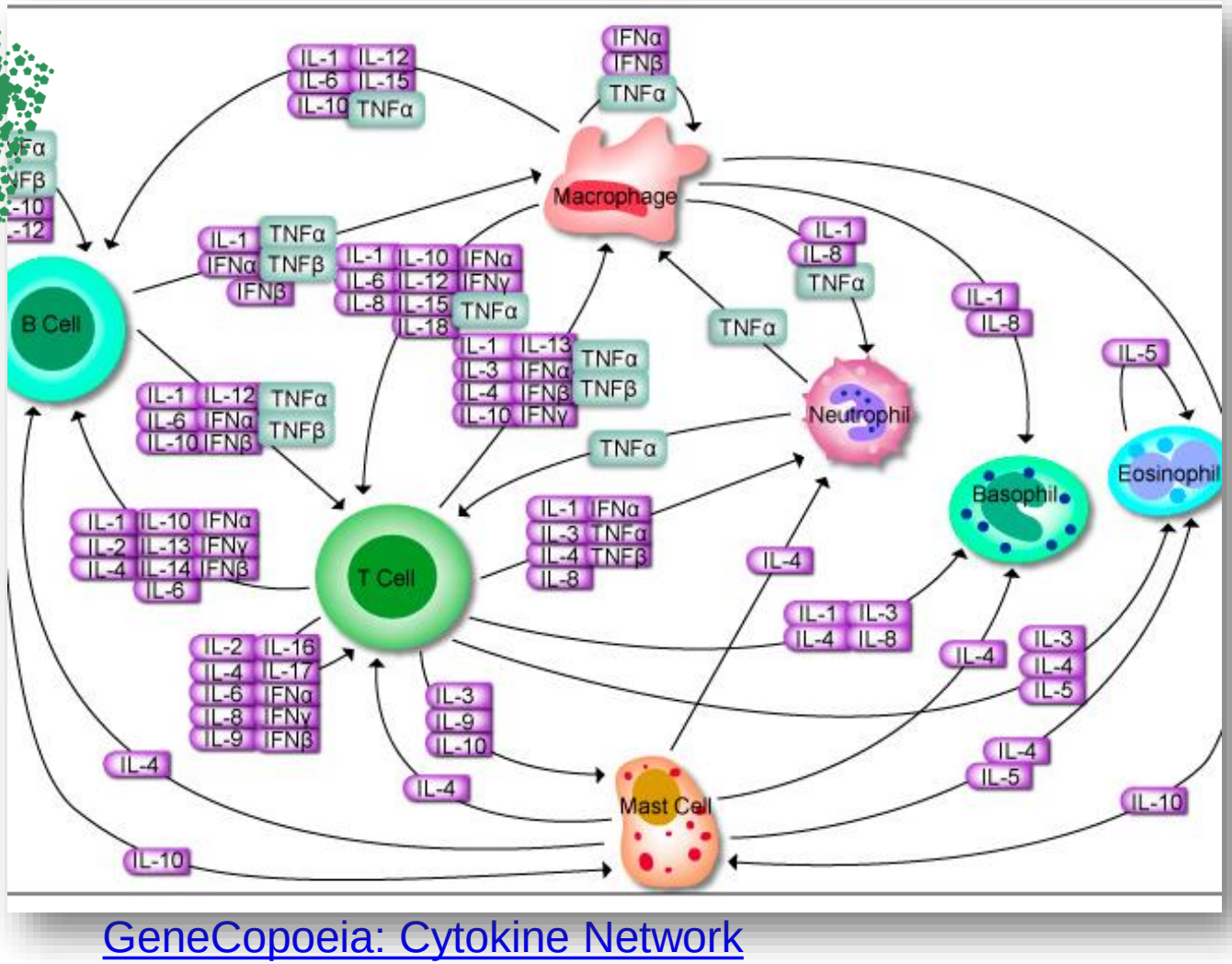


HOST FACTORS

Polymorphism of Genes for cytokines



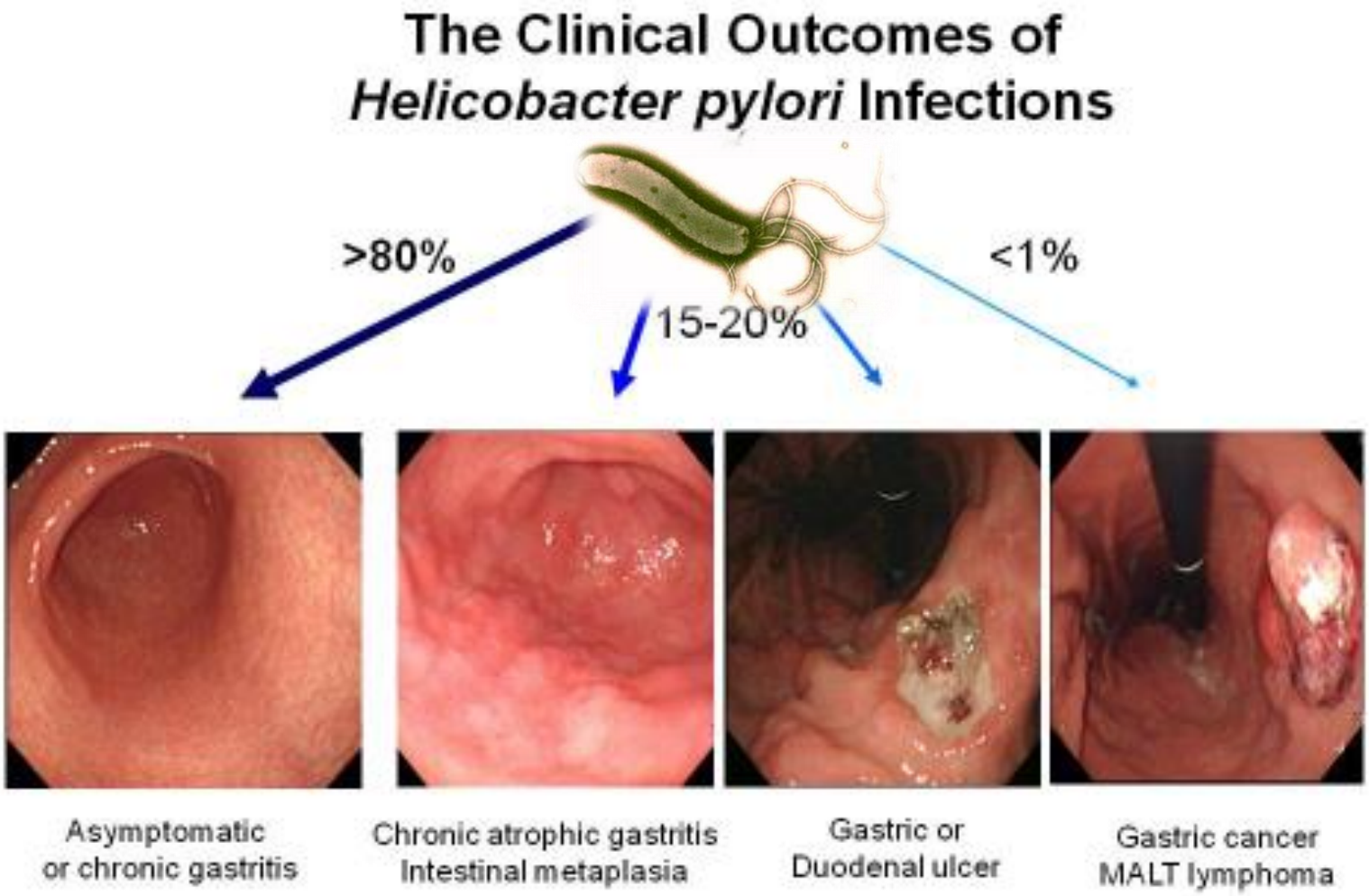
- The most frequently studied gene polymorphisms are in the IL-1, IL-10 and TNFα genes.
- Interleukin-1 gene cluster polymorphisms are associated with an increased risk of both hypochlorhydria and gastric cancer
- IL-10 8 SNPs are associated with spontaneous production, suggesting that IL-10 polymorphisms can modulate the levels of cytokine production and can thus modify the risk of infection.

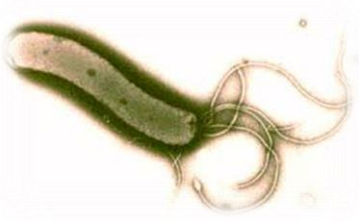


GeneCopoeia: Cytokine Network

Cytokines are molecules with pleiotropic, redundant, synergistic, addition, antagonistic activity….eternal interplay

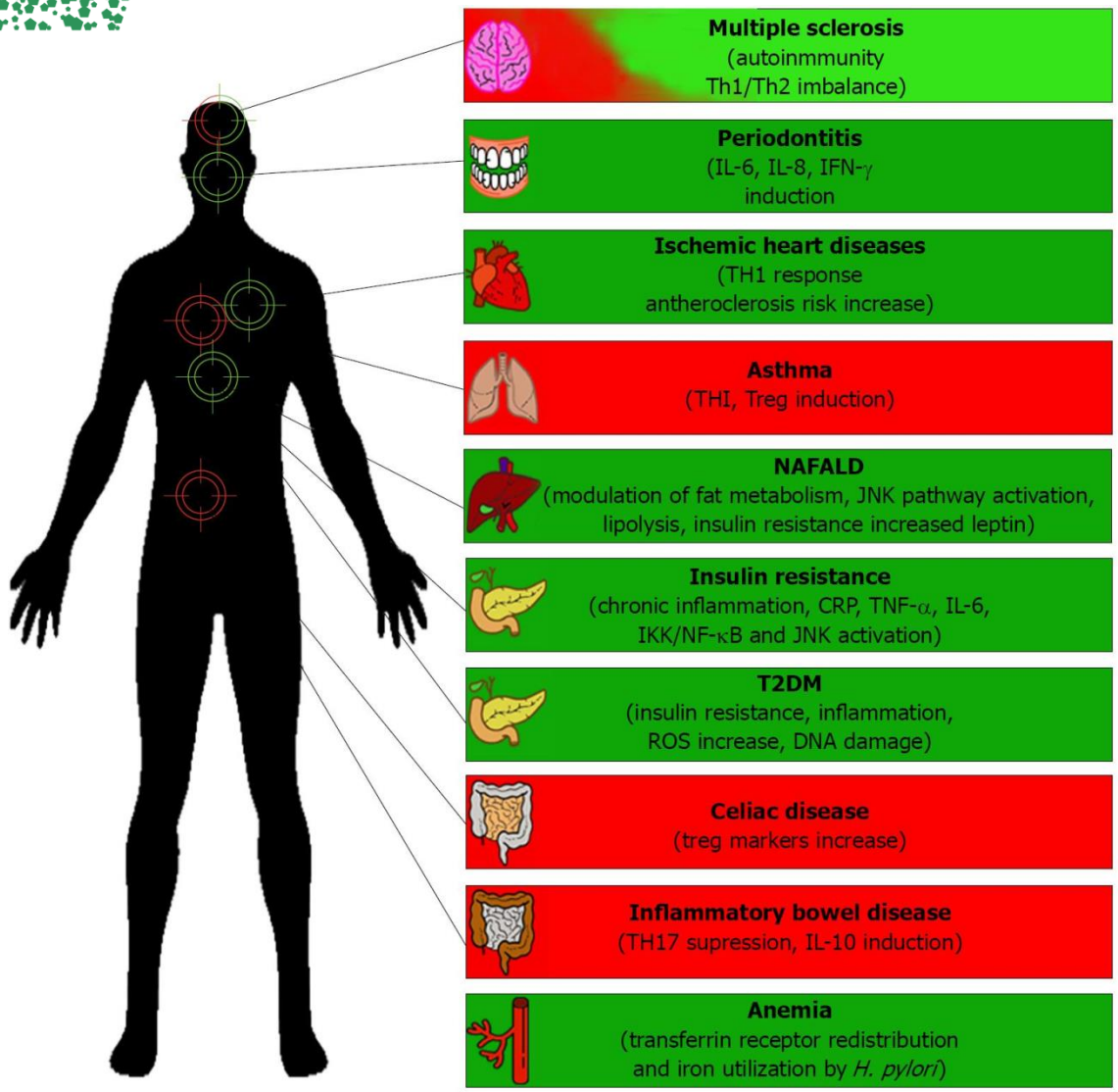
https://www.facebook.com/pg/Helicobacter-Pylori-H-Pylori-1425787414353825/community/?ref=page_internal

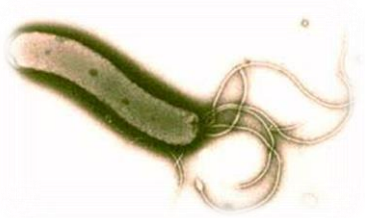


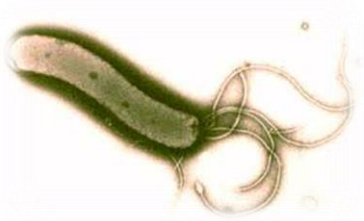


H. PYLORI AND EXTRA GASTRIC MANIFESTATIONS

Bravo D et al. *Helicobacter pylori* in human health and disease: Mechanisms for local gastric and systemic effects. World J Gastroenterol 2018; 24(28): 3071-89

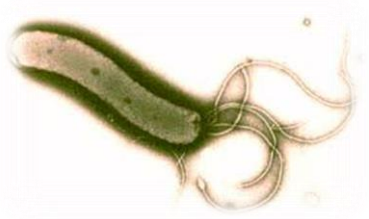






VACCINES

Vaccines



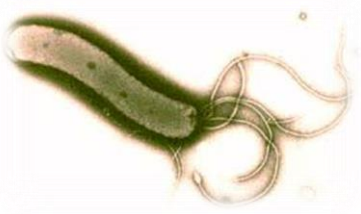
Approaches was applied in *H. pylori* vaccine in past and may be examined in future.

Past experimental approaches to *H. pylori* vaccine			
Vaccine (Antigen + Adjuvant)	Route	Result	Ref
Urease + LT	Oral	Decrease of bacterial load in vaccinated groups	Michetti et al. (1999)
Whole cell + dmLT	Oral	No bacterial clearance	Kotloff et al. (2001)
Urease or HP0231 + Salmonella Ty21a	Oral	Bacterial clearance in both vaccinated and control	Aebischer et al. (2008)
Urease + LT	Oral	Efficacy 72%	Zeng et al. (2015)
CagA, VacA, NAP + Alum	IM	Clearance equivalent between vaccinated and	Malfertheiner et al. (2018)
Newer experimental approaches to *H. pylori* vaccine			
Multi-epitope DNA-prime/peptide (EpiVax)	IN	Some therapeutic protection in mice	Moss et al. (2011)
GGT (Imevax/IMX101 X)	IP	Protection against allergic asthma	Oertli et al. (2013)
Lactococcus lactis expressing CTB and urease (Probiotic vaccine)	Oral		Li et al. (2014)
UreI-UreB + CTB (Urease epitope vaccine)	Oral	Limited protection in BALB/c mice	Yang et al. (2015)
Lp220 + CTB	IP	Limited protection in BALB/c mice	Li et al. (2016)
A non-pathogenic Vibrio cholerae strain engineered to express HpaA, UreB and FlaA (Helicovaxor®)	Oral	Introduced as oral inactivated vaccine	Tobias et al. (2017)

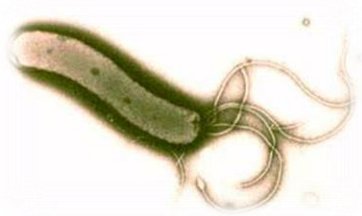
CTB, cholera toxin subunit B; dmLT, double mutant LT; GGT, gamma-glutamyl-transpeptidase; IM, intramuscular; IN, intranasal; IP, intraperitoneal; LT, *Escherichia coli* heat-labile enterotoxin; UreI-UreB, *H. pylori* urease I and urease B.

Karkhah A et al. Helicobacter pylori evasion strategies of the host innate and adaptive immune responses to survive and develop gastrointestinal diseases. Microbiological Research 218 (2019); 49-57

Vaccines

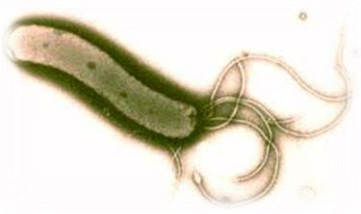


- However, it seems that solution is not next door.
- In one study, repeated experimental reinfection of two subjects indicates that *Helicobacter pylori* infection does not promote an immune response protective against future reinfection.
- In addition, they indicate difficulties for vaccine development, especially therapeutic vaccines.



H. PYLORI RESISTANCE TO ANTIMICROBIALS

Resistance



- In Asia-Pacific region, the prevalence of clarithromycin, levofloxacin, and metronidazole resistance was 21, 27, and 45%, respectively.
- According to the results of one study group, in Europe, *H. pylori* resistance rates for adults were 17.5% for clarithromycin, 14.1% for levofloxacin and 34.9% for metronidazole.
- For clarithromycin and levofloxacin, percentage of resistance was significantly higher in Western/Central and Southern Europe (>20%) than in Northern European countries (<10%).
- For ciprofloxacin, levofloxacin, clarithromycin and metronidazole, in Niš, Serbia, the rate of resitance in patients after unsuccsessful therapy, was at least, twice higher (unpublished data,T. Babić).

Thank you very much for the attention