PreCorso 2012
Firenze, 23 luglio 2012

Infezione da *Helicobacter pylori* e cancro gastrico

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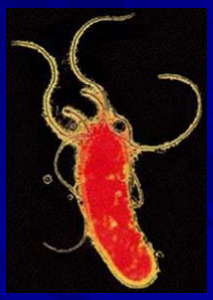
Helicobacter pylori



- Quando è stato scoperto?
- Chi l'ha visto per primo?
- Da quanto tempo "conosce" l'uomo?
- Perché avvistato e non scoperto?
- Coincidenze delle grandi scoperte?
- Come vive nello stomaco?
- Quante persone sono infettate? iIlustri pazienti?
- Tutti gli infettati sono malati?
- E l'ospite (uomo) infettato che fa?
- Le Relazioni Pericolose .....

The Nobel Prize in Physiology or Medicine 2005

"for their discovery of the bacterium *Helicobacter pylori* and its role in gastritis and peptic ulcer disease"

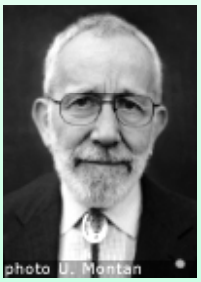
b. 1951

Barry J. Marshall
Australia

Helicobacter pylori Research Laboratory, QEII Medical Centre; University of Western Australia
Australia

J. Robin Warren
Australia

Perth, Australia

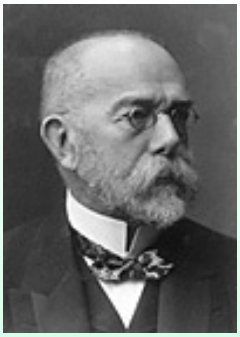


b. 1937

E 100 anni prima Premio Nobel a chi?

The Nobel Prize in Physiology or Medicine 1905

"for his investigations and discoveries in relation to tuberculosis"



Robert Koch

Germany
Institute for Infectious Diseases
Berlin, Germany

b. 1843
d. 1910

Helicobacter pylori
quando è stato scoperto?

Cultured for the first time on Easter Thursday 1982

–Patient 37, 70y.o. male

–DU, GU, artificial valve, anticoagulants

MRSA epidemic at Royal Perth

–Overworked microbiology technologists

– No time to check the culture on Saturday

–Not examined until Tuesday Not examined until Tuesday

–Gram negative rods seen in pure culture

We had been using the right methods for

–Cultures were being discarded after 48 hours

From the 2005 Nobel Lecture by Barry Marshall

… e 100 anni prima?

Die Ätiologie der Tuberkulose.[1)]

(Nach einem in der Physiologischen Gesellschaft zu Berlin am 24. März 1882 gehaltenen Vortrage.)

Von

Dr. R. Koch,

Regierungsrat im Kaiserl. Gesundheitsamt.

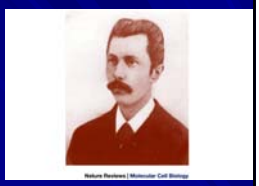
Die von Villemin gemachte Entdeckung, daß die Tuberkulose auf Tiere übertragbar ist, hat bekanntlich vielfache Bestätigung, aber auch anscheinend wohlbegründeten Widerspruch gefunden, so daß es bis vor wenigen Jahren unentschieden bleiben mußte, ob die Tuberkulose eine Infektionskrankheit sei oder nicht. Seitdem haben aber die zuerst von Cohnheim und Salomonsen, später von Baumgarten ausgeführten Impfungen in die vordere Augenkammer, ferner die Inhalationsversuche von Tappeiner und anderen die Übertragbarkeit der Tuberkulose gegen jeden Zweifel sichergestellt und es muß ihr in Zukunft ein Platz unter den Infektionskrankheiten angewiesen werden.

Wenn die Zahl der Opfer welche eine Krankheit fordert, als Maßstab für ihre Bedeutung zu gelten hat, dann müssen alle Krankheiten, namentlich aber die gefürchtetsten Infektionskrankheiten, Pest, Cholera usw. weit hinter der Tuberkulose zurückstehen.

The first report on the etiology of tuberculosis was presented on March 24, 1882 in Berlin.

Helicobacter
chi l'ha visto per primo? quando?

- Giulio Bizzozzero
- 1893, *Arch f mikr Anat* 42: 82-152. "Ueber die schschlauchformigen drusen des magendarmkanals und die bezienhungen ihres epithels zu dem oberflachenepitel der schleimhaut."
- 1892, *Atti della Reale Accademia delle Scienze di Torino* 28: 233-251. "Sulle ghiandole tubulari del tubo gastroenterico e sui rapporti del loro epitelio coll'epitelio di rivestimento della mucosa."



Helicobacter
altri avvistamenti

- La presenza di batteri spiraliformi nello stomaco fu descritta a più riprese nel secolo scorso, tra gli altri da Freedberg nel 1940 a Steer e Colin-Jones nel 1975.
- Ito descrisse e fotografò batteri spiraliformi in biopsie derivate dal suo stomaco, nel suo famoso Textbook of Physiology del 1966.
- Tra gli anni 20 e 50 del '900 altri scienziati, quali Luck e Fitzgerald, comprovarono la presenza di attività enzimatica di tipo ureasica nello stomaco di gatti e cani.
- Nel 1959 Lieber dimostrò che l'attività ureasica gastrica poteva essere soppressa con una terapia antibiotica a base di tetracicline. Nel 1968 Delluva osservò che animali mantenuti in condizioni asettiche non presentavano attività ureasica nello stomaco.
- Ma…la presenza di batteri nello stomaco e l'attività ureasica non furono poste in relazione all'insorgenza di patologie gastroduodenali

Modificato da Warren e Marshall 2005 Nobel Lecture

H. pylori
perché avvistato e non scoperto?

- Acid environment kills organisms
- The normal stomach is sterile
- Bacteria seen are contaminant passing through, dead or secondary to gastric lesions such as peptic ulcer. Just a secondary infection, due to gastritis
- "If it is true, why were they not recognised before…"

(From the 2005 Nobel Lecture by Robin Warren)

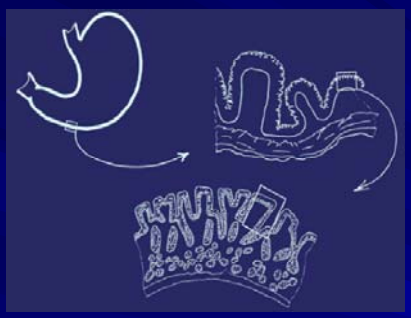
… perché?

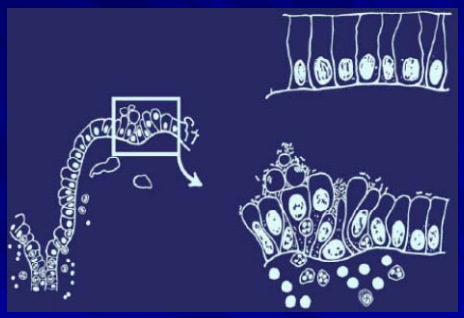
- "L'uomo, per sua natura, ha più paura della verità che della morte…"

Soren Kierkegaard

- "…Il più grande ostacolo alla conoscenza non è l'ignoranza bensì l'illusione della conoscenza" *Daniel Boorstein*

Warren First Report





(From the 2005 Nobel Lecture by Robin Warren)

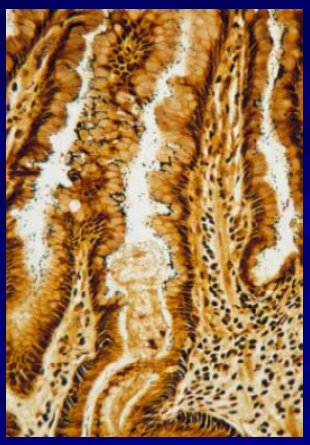
Conclusion:

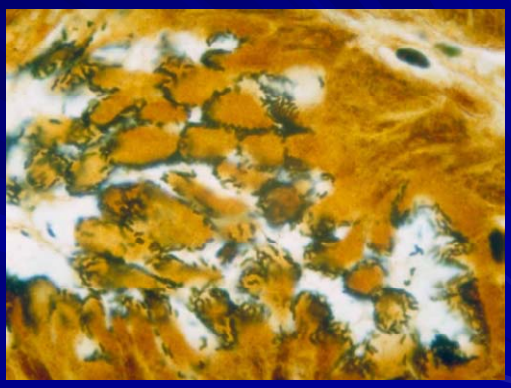
There is chronic gastritis with a small erosion. The quality of the surface mucus appears slightly more dense than normal in many areas, and it contains numerous bacteria in close contact with the surface epithelium. These bacteria have the morphology of Campylobacter. They appear to be actively growing and not a contaminant. I am not sure of the significance of these unusual findings, but further investigation of the patient's eating habits, gastro-intestinal function and microbiology may be worthwhile.

J. R. Warren 1979

H. pylori

black bacilli line the pits, easily seen (silver stain)





(From the 2005 Nobel Lecture by Robin Warren)

Lancet Letters 1983

A new species

Bacteria linked to gastritis

- "since the new bacteria are associated with gastritis as described by Warren, then they may play a role in other poorly understood gastric diseases i.e. peptic ulcer and gastric cancer."

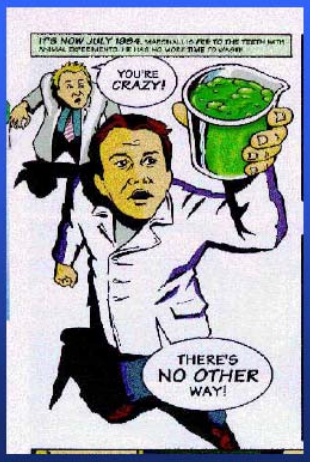
From the 2005 Nobel Lecture by Barry Marshall

Koch's Postulates …

1. **The same organism must be present in every case of the disease.**
2. **The organism must be isolated from the diseased host and grown in pure culture.**

3. **The isolate must cause the disease, when inoculated into healthy, susceptible animal.**

Koch's Postulates and *H. pylori*.....

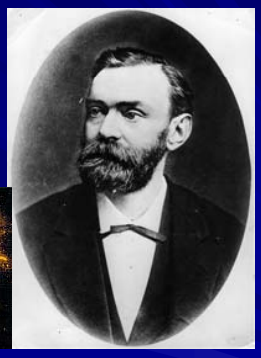


An attempt to Fulfill Koch's Postulates for *Campylobacter pyloridis*

Med J. Aust 1984

From the 2005 Nobel Lecture by Barry Marshall

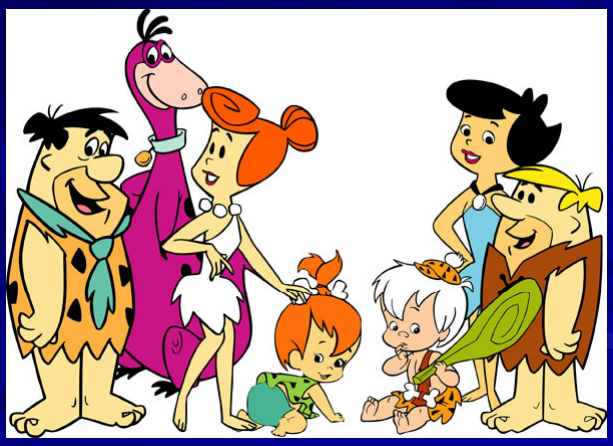
H. pylori e pazienti nei secoli



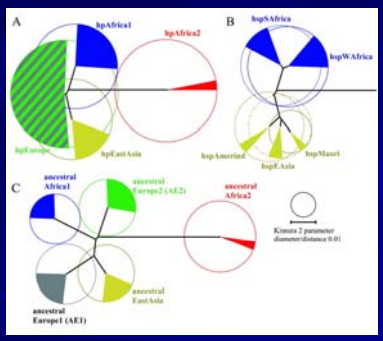
- Napoleone, a 52 anni, morì di "ulcera antrale maligna" (cancro gastrico). Così suo padre, suo nonno, almeno un fratello, una sorella.
- Alfred Nobel soffriva di ulcera peptica!
- James Joyce morto di ulcera duodenale perforata.

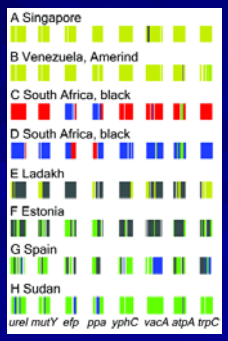
H. pylori e pazienti nei secoli

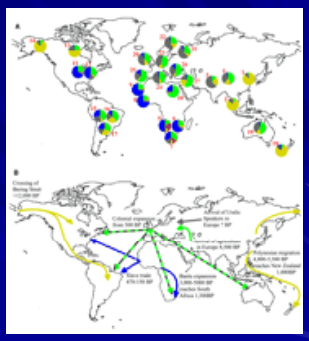
da quanto tempo "conosce" l'uomo?



Traces of human migrations in *H. pylori* populations







Falush et al, *Science 2003*

H. pylori and related clinical outcomes

***Helicobacter pylori* infection**

↓

Superficial gastritis

↓

Chronic gastritis

↻ Chronic active gastritis ↓ MALT B-cell Lymphoma

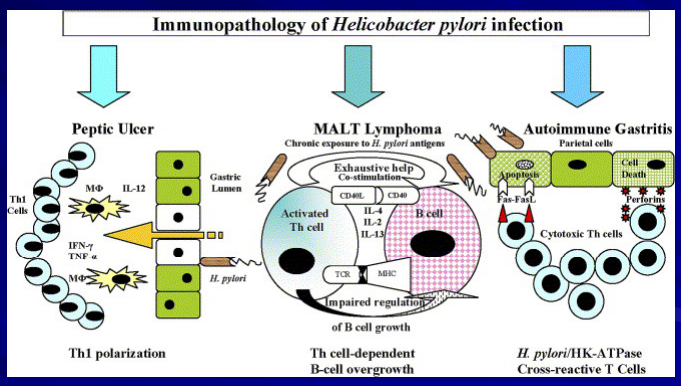
↓ Peptic ulcer

↻ Atrophic gastritis ↓ Gastric adenocarcinoma

"..Qui pourrait ne pas frémir en songeant aux malheureux qui ont causé une seule liaison dangereuse ..."

P. Choderlos De Laclos

Les liaisons dangereuses



D'Elios *et al.*, *J Immunol* 1997; *Gastroenterology* 99, 2001; *PNAS* 2003; *J Exp Med* 2003 *Clin Dev Imm 2012 Trends Mol Med* 2004; *J Clin Invest* 2006; *Helicobacter* 2009 *Gut 2011, Blood 2011, Cancer Immunol Immther* 2009, *Expert Rev Anti Infect Ther* 2010

DIAGNOSI

Per la diagnosi indispensabile eseguire GASTROSCOPIA che ha un duplice scopo:

- ACCERTARE L'ULCERA
- EFFETTUARE BIOPSIE PER ESCLUDERE LA PRESENZA DI CANCRO

Diagnosis

- Endoscopy reveals the ulcer and allows biopsy and citology
- Biopsy for histopathology
- Brushing for cytology mandatory in gastric ulcers to exclude malignancy
- Biopsies can be taken to detect *H. pylori* by urease test, histological analysis or culture
- ^{13}C Urea breath test (non invasive test)

Tests for *Helicobacter pylori* diagnosis

Invasive tests

- Histology
- Culture
- Real time PCR
- Rapid Urease Test

Non invasive tests

- Urea Breath test
- Stool Test
- Serology

D'Elios et al, Expert Rev Anti Infect Ther 2010

TERAPIA ULCERA PEPTICA

- Eradicazione *Helicobacter pylori*
- Inibitori pompa protonica (PPI)
- Farmaci anti-H2
- Agenti citoprotettivi
- Preparazioni contenenti bismuto
- Analoghi delle prostaglandine

TERAPIA ERADICANTE per *Helicobacter pylori*

Triplice terapia per una-due settimane con:

PPI	20 mg per due al dì
CLARITROMICINA	500 mg per due al dì
AMOXICILLINA	1 gr per due al dì

Suggested anti - HP therapeutic regimens

- Lansoprazole 30 mg twice daily + amoxicillin 1 g twice daily (or tetracycline 500 mg four times daily if allergic) + metronidazole 400 mg three times daily, altogether for 1 week
- Lansoprazole 30 mg twice daily + amoxicillin 1 g twice daily (or tetracycline 500 mg four times daily if allergic) + clarythromycin 500 mg twice daily, altogether for 1 week

MAASTRICHTIV/FLORENCE CONSENSUS

Gut 2012 May;61(5):646-64.

L'eradicazione di *Helicobacter pylori* è fortemente raccomandata:

- nei pazienti con ulcera peptica
- nei pazienti con linfoma gastrico MALT a basso grado di malignità
- nei pazienti con gastrite atrofica
- nei pazienti operati di resezione gastrica per cancro
- nei parenti di primo grado di pazienti con cancro gastrico

MAASTRICHTIV/FLORENCE CONSENSUS

Gut 2012 May;61(5):646-64.

L'eradicazione di *Helicobacter pylori* è consigliata:

- nei pazienti con dispepsia
- nei pazienti che devono fare terapia anti secretoria per lungo termine

***Helicobacter pylori* eradication treatment.**

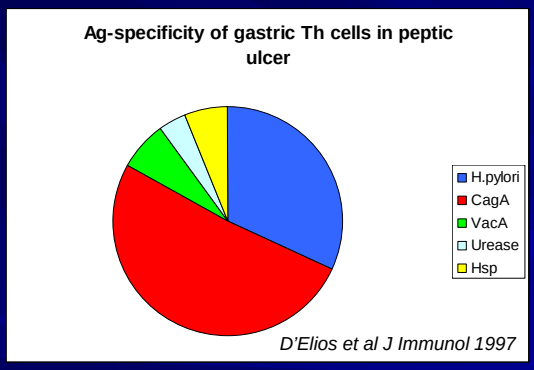
First line treatment
- PPI double dose
+ clarithromycin 500mg bid
+ amoxicillin 1g bid
(or metronidazole 500mg bid) for 7-14 days
Second line treatment
- PPI double dose bid
+ levofloxacin
+ amoxicillin 1g bid for 10 days or
- PPI double dose morning and evening
+ metronidazole 500mg bid
+ amoxicillin 1g bid for 14 days or
Bismuth-based quadruple therapy

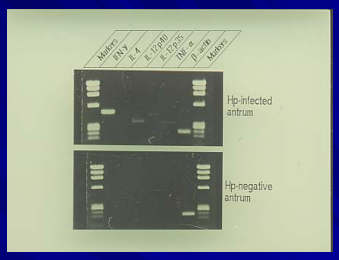
D'Elios et al Expert Rev Anti Infect Ther 2010

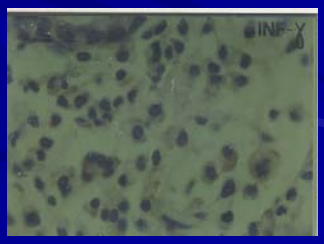
H. pylori, host response, and related clinical outcomes

- Bacterial factors (different strains, pathogenicity island)
- Host factors (genetics, cytokine / chemokine network, gastrin, somatostatin, pepsinogen, regulation of acid secretion)
- Inflammation (site, type, etc)

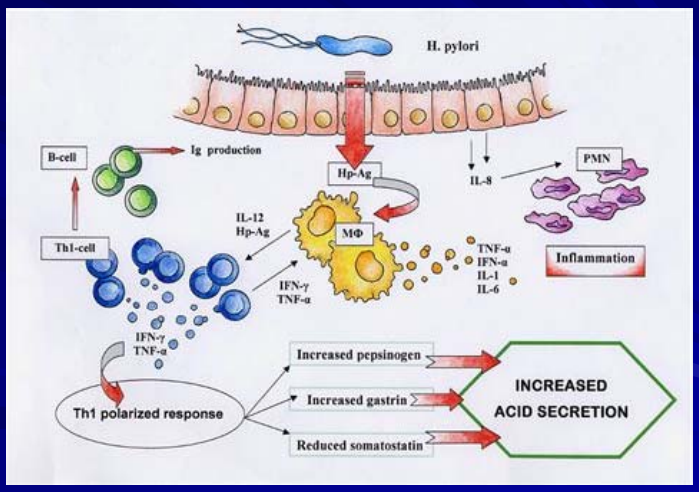
Th1 polarization in *H. pylori* peptic ulcer



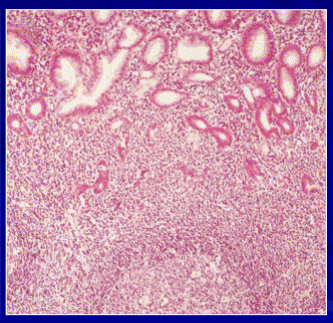




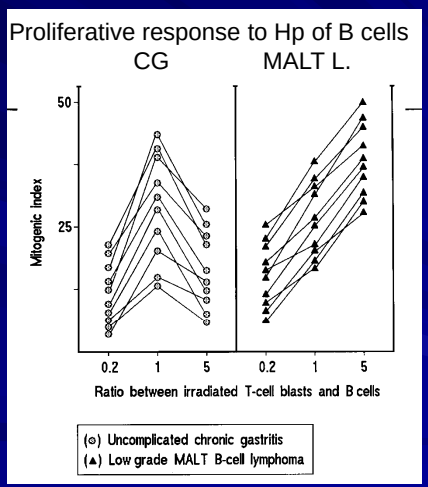
H. pylori, Th1 polarization and peptic ulcer



H. pylori and gastric low grade MALT Lymphoma

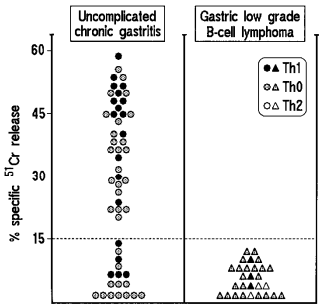


Th-dependent enhanced B cell help in gastric low grade MALT Lymphoma

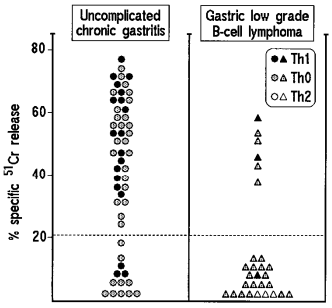


Defective T cell killing in gastric low grade MALT Lymphoma

Failure of Hp-reactive T-cell clones derived from gastric low grade MALToma to express Ag-induced cytotoxicity against Ag-presenting autologous B cells

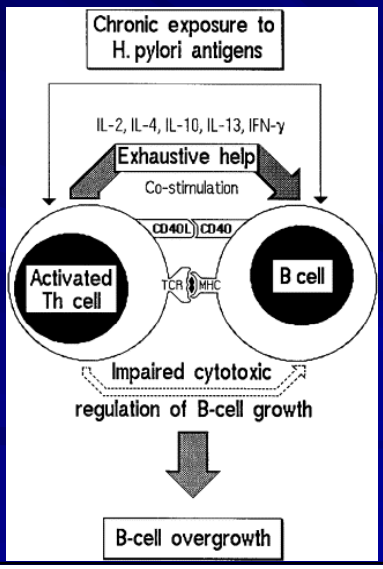


Failure of Hp-reactive T-cell clones derived from gastric low grade MALTomas to induce Fas-FasL mediated apoptosis of Jurkat cells

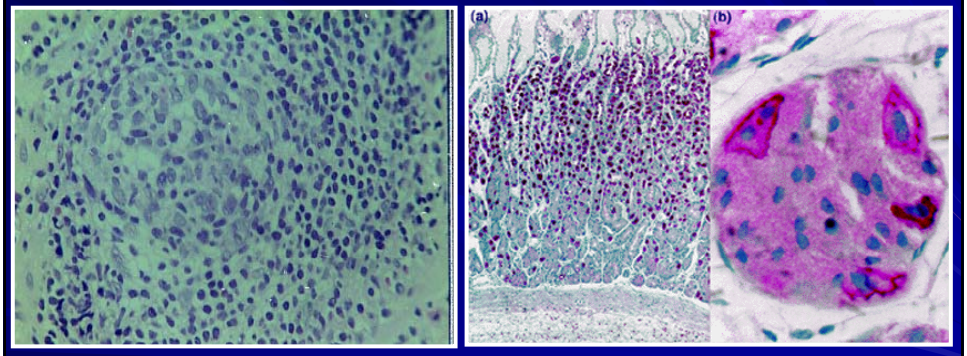


D'Elios et al Gastroenterology 1999

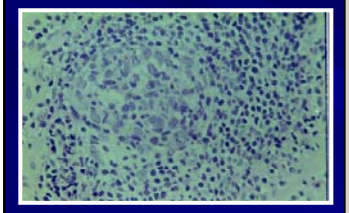
H. pylori-induced Th-mediated helper activity contributes to B-cell growth in gastric low grade MALT lymphoma

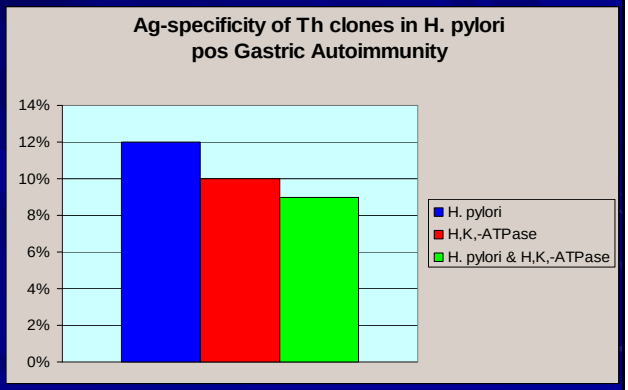


H. pylori and gastric autoimmunity



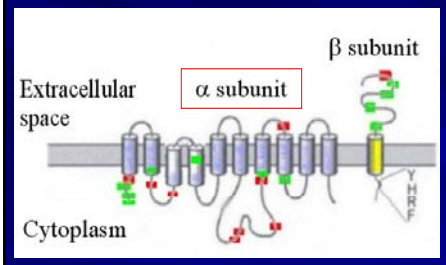
Proliferative response of gastric Th clones to *H. pylori* or to H^+K^+ATPase in gastric autoimmunity





Amedei et al JEM 2003

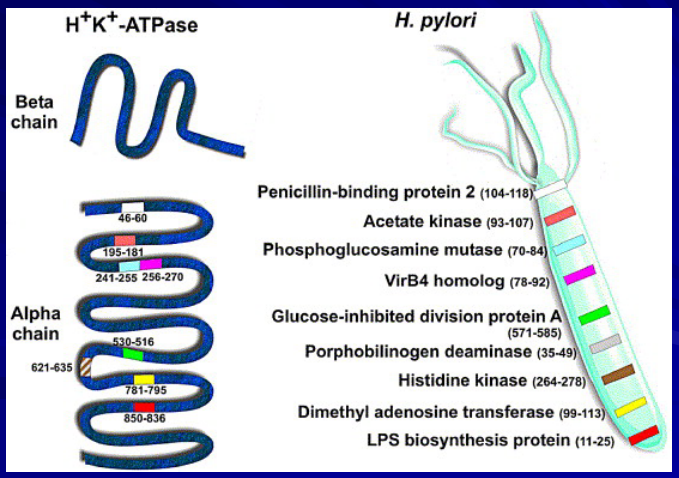
H^+,K^+-ATPase & *H. pylori* peptides recognized by cross-reactive gastric Th clones



T-cell clones (epitope)	Amino acid sequence recognized: H^+,K^+-ATPase Bacterial peptide	(MI ± SD)	*H. pylori* protein including the crossreactive peptide (position)
1.C31 (α621-635)	IRVIMVTGDHPITAK	(79 ± 9)	
	VRVDVRRLDHLMNLI	(66 ± 5)	Histidine kinase (264-278)
1.A04 (α781-795)	NLKKSIAVLTHNIP	(194 ± 16)	
	ISNLPYYIATRLVLN	(108 ± 12)	Dimethyl adenosine transferase (99-113)
2.P24 (α46-60)	KKEMEINDHCLSVAE	(23 ± 3)	
	LNNYQKENSLYNHNL	(27 ± 2)	Penicillin-binding protein 2 (104-118)
2.R37 (α836-850)	KAESDIMHLSPNPK	(50 ± 7)	
	NMRVFIHLSPKTCK	(19 ± 2)	LPS biosynthesis protein (11-25)
3.A30 (α836-850)	KAESDIMHLSPNPK	(49 ± 6)	
	NMRVFIHLSPKTCK	(16 ± 1)	LPS biosynthesis protein (11-25)
4.A15 (α181-195)	VIRDGDKFQINADQL	(39 ± 2)	
	VVQGGDKFHAPVLVD	(20 ± 1)	Acetate kinase (93-107)
4.C32 (α241-255)	CTHESPLETRNIAFP	(87 ± 9)	
	VIQIGPMPTPAIAFL	(75 ± 8)	Phosphoglucosamine mutase (70-84)
4.C27 (α256-270)	STMCLEGTAQGLVVN	(137 ± 17)	
	ALDSLEKVVARLVVK	(34 ± 2)	VirB4 homolog (78-92)
4.C26 (α516-530)	VVKGAPERVLERCSS	(104 ± 9)	
	VFKGIPGLSLEAVEK	(47 ± 4)	GidA (571-585)
4.A05 (α621-635)	IRVIMVTGDHPITAK	(99 ± 12)	
	IRIVKTTGDKILDAP	(51 ± 6)	Porphobilinogen deaminase (35-49)

D'Elios et al Trends Mol Med 2004

9 H. pylori proteins harbour cross-reactive epitopes to human gastric proton pump



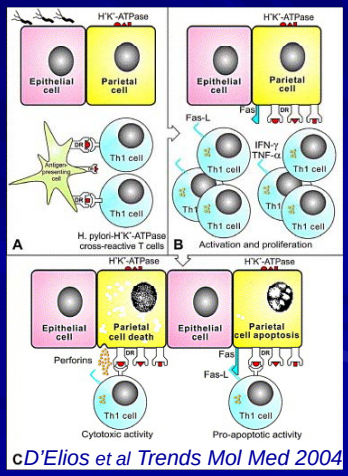
D'Elios et al Trends Mol Med 2004

H. pylori and autoimmune gastritis - *molecular mimicry* -

- In genetically predisposed individuals *H. pylori* infection can induce gastric autoimmunity.
- Gastric T cells specific for epitopes of some *H. pylori* antigens can also recognize cross-reactive epitopes of the gastric H^+K^+-ATPase of parietal cells, shifting a defence mechanism into autoimmunity.
- The results obtained suggest that molecular mimicry is a relevant pathogenic mechanism in gastric autoimmunity.

H. pylori, T cells, gastric autoimmunity and atrophy

- The cross-reactive T cells display a cytotoxic profile and provide help for the synthesis of antibodies.
- Cross-reactive T cells are able to induce apoptosis, thus contributing to the development of gastric atrophy .



c*D'Elios et al Trends Mol Med 2004*

H. pylori constituents associated with gastric cancer

H. pylori disease	Host factor(s)	Hp genotype associated with
CagA	Src kinases, SHP-2, ERK, ZO-1, c-Met	*cagA*$^{+}$
VacA	RPTP-β	vac*A*s1m1
BabA	Lewis^{b}	*babA2*$^{+}$
SabA	Sialyl-Lewis^{x}	*sabA*+

Inflammation, Gastrin and Gastric Adenocarcinoma

- Gastrin promotes gastric hyperproliferation

  (Dockray et al, Annu Rev Physiol 2005)

- In gerbils cag+ strains able to increase gastrin plasma level induce gastric adenocarcinoma

  (Rieder et al, Gastroenterology 2005; Watanabe et al, Gastroenterology 1998; Honda et al, Cancer Res 1998; Zheng et al, J Gastroenterol Hepatol 2004)

- Hypergastrinemic transgenic mice develop cancer after 2 ys. The development of cancer is accelerated by *H. pylori* infection.

  (Wang et al, Gastroenterology 2000; Fox et al, Cancer Res 2003)

Pro- vs anti- inflammatory cytokines and gastric adenocarcinoma

- Polymorphisms that increased pro-inflammatory cytokines (IL-1β and TNF-α) increased risk of gastric cancer

  (Machado et al, Gastroenterology 2001, Gastroenterology 2003; Garza-Gonzales, Int J Cancer, 2005)
- Polymorphisms that decrease anti-inflammatory cytokines (IL-10) associate with increased risk of gastric cancer

  (El-Omar, Gastroenterology 2003)

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