Helicobacter pylori infection and gastric MALT lymphoma

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Abstract

***Helicobacter pylori* infection is implicated in the development of two different gastric cancers: gastric adenocarcinoma and gastric MALT lymphoma. The association with the gastric MALT lymphoma is strong and causal. It is currently the only cancer which can be treated by a simple antibiotic treatment. However, the evolution of an *H. pylori* infection towards lymphoma is exceptional. Host susceptibility factors and environmental factors predisposing a patient to lymphoma have not yet been determined. The bacterial factors are currently being identified.**

Key words: lymphoma, MALT, *H. pylori*.

Abbreviations: JHP – *Helicobacter pylori* strain J99 open reading frame region; IPSID – Immuno proliferative small intestinal disease; NHL – Non-Hodgkin lymphoma; PGIL – Primitive gastrointestinal lymphoma; MALT – Mucosa associated lymphoid tissue; ORF – Open reading frame region; PCR – Polymerase chain reaction

Introduction

Twenty-five years have gone by since two Australian researchers were able to culture *Helicobacter pylori (H. pylori)* for the first time [1,2]. This bacterium which is strictly adapted to humans has unique properties. First of all, approximately half of the world population is said to be infected. Furthermore, the bacterium's adaptation to the gastric mucosa, thanks to its large production of urease which neutralizes the gastric acidity, insures its survival. Only about 10% of infected subjects develop gastroduodenal diseases, all within a very heterogeneous spectrum: gastritis, ulcers, gastric adenocarcinoma or mucosa associated lymphoid tissue (MALT)-type gastric lymphoma. *H. pylori* infection is therefore a potentially carcinogenic infection and, as such, has been recognized as a type I carcinogen (maximum level) by the International Agency In Research against Cancer [3]. The discovery of the bacterium has revolutionized one of the most important domains of gastroenterology. In fact, despite initial skepticism, ulcer treatment is now comprised of an antibiotic therapy aimed at eradicating *H. pylori*. The beneficial effect of this intervention was subsequentially applied to gastric MALT lymphoma as it is indeed possible to cure this particular cancer on a long term basis following *H. pylori* eradication [4].

For a given subject, individual factors, environmental factors and factors linked to the bacterium itself, contribute to the evolution towards a chronic infection and leads to the appearance of a lymphoma.

This article is comprised of three successive aspects: a general review of gastrointestinal lymphomas, a part dedicated especially to gastric MALT lymphoma, and finally an up-to-date on the most recent genetic data concerning *H. pylori* strains which are associated with gastric MALT lymphoma.

Primitive gastrointestinal lymphomas

The term of "lymphoma" evokes primarily a lymph node pathology. However, lymphoid tissue is also present in certain organs as the digestive tract. Indeed, 25% of all lymphomas are found elsewhere than in the lymph nodes and, amongst them, digestive lymphomas are the most frequent [5]. Work done by Isaacson and Wright led to regrouping most of the extra-gan-

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Table 1. **Histopathological classification of gastrointestinal lymphomas**

Phenotype B	
Low grade MALT B lymphoma – Western type (focalized) – Mediterranean type (extensive): IPSID (essentially alpha chain disease)	From the marginal zone of MALT
High grade MALT B lymphoma, with or without a component of weak malignancy including: – centroblast – immunoblast – large anaplasic cells	Diffuse large B cells
Centrocytic lymphoma = digestive lymphomatic polyposis	From the mantle zone
Burkitt's lymphoma or Burkitt type lymphoma	From Burkitt
Other types (equivalent to ganglionnary lymphomas)	Follicular
Phenotype T	
T lymphomas associated (EATL) with an enteropathy	T intestinal type
T lymphomas not associated with an enteropathy	

MALT – Mucosa Associated Lymphoid Tissue
IPSID – Immuno Proliferative Small Intestinal Disease
EATL – Enteropathy-Associated T Lymphoma

glionary lymphomas into one entity, the MALT lymphomas [6]. The diagnosis of gastrointestinal lymphomas should now be made following the recent classification of the World Health Organization (*Tab. 1*) [7,8].

I. Epidemiology of and predisposing factors for gastrointestinal lymphomas

Digestive lymphoma localizations represent 12.5% of all non-Hodgkin lymphomas (NHL) and are the most frequently found extraganglionary form (36%), gastric localization being the most frequent [9].

There is a male predominance in primitive gastrointestinal lymphomas (PGIL) (ratio 2:1). The average age is 57 years, but the age is much lower in Burkitt's lymphoma cases which concern young patients [10,11].

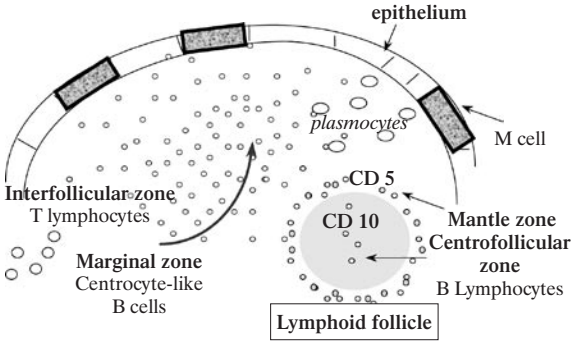
The etiology of PGIL is most often unknown. Acquired immune deficiency syndromes (AIDS) or genetic problems (chromosome X-linked deficiencies) have been associated with different kinds of lymphoma like Burkitt's lymphoma or lymphomatic polyposis syndrome. The infection hypothesis has also been considered, for example, in the case of certain T intestinal lymphomas which are associated with human T-cell leukemia retrovirus type I (HTLV-I). Recently *Campylobacter jejuni* has been suspected to play a role in the genesis of certain cases of immuno proliferative small intestinal disease (IPSID), but this hypothesis has not received unanimous approval [12,13]. *H. pylori* infection therefore represents the infectious cause of lymphoma which has been studied and characterized the most thoroughly.

II. Comparison of morphological characteristics of MALT lymphomas and Peyer patches

MALT lymphomas share morphologic characteristics which bring them close to Peyer patches and allow them to be differentiated from lymph node lymphomas, as follows:

– the constant presence of lymphoid follicles with a clear center;
– lymphoepithelial lesions formed by invasion of individuals glands by aggregates of lymphoma cells.

Figure 1. **Schematic representation of lymphoid tissue from the digestive tract (MALT)** [60]



Peyer patches are indeed comprised of lymphoid follicles with a clear center which, when activated, are surrounded by a mantle zone and a marginal zone. The T lymphocytes are arranged along the venules and histocytes. The follicles bulge towards the intestinal lumen and establish a zone called "the dome", found between the follicle and the epithelium and comprised of B lymphocytes from the marginal zone (*Fig. 1*). Immunohistochemical studies show that only at the dome level are there intraepithelial B lymphocytes (CD20+, CD79a+) in formations resembling miniature lymphoepithelial lesions.

Gastric MALT lymphoma

Gastric lymphoma is considered to be the classic lymphoma of MALT-type of the digestive tract. It is a B cell lymphoma with a very unusual pathogenesis and evolution which slowly progresses and stays localized in the stomach for a long time. The development of the lymphoma is directly linked to the *H. pylori* infection although it is not known why this evolution is present in only a very small number of infected subjects.

I. Epidemiology of gastric MALT lymphoma

Epidemiological data on gastric MALT lymphoma are very heterogeneous. An epidemiological study carried out in Germany reported on the detection of 94 cases in a total population of 3.5 million inhabitants over a 3 year period, and estimated the incidence to be 0.7-0.8 per 100000, with an average age of 62.1 years and a sex ratio slightly in favor of the male gender [9]. This incidence seems to be comparable to that of other European countries with the exception of England where the incidence is lower (0.2 per 100000). Amongst the North African countries, in Tunisia, the incidence is estimated at 6.3 per 100000 for men and 3.8 per 100000 for women [14].

II. Relationship between *H. pylori* infection and gastric MALT lymphoma

In order to analyze the relationship between *H. pylori* infection and gastric MALT lymphoma, it is interesting to review the Bradford Hill criteria which were first used to show the causal link between lung cancer and tobacco smoking [15]. These criteria include: 1) an association and a temporal relationship between the two situations, 2) the biological plausability, i.e. the pathophysiological mechanisms underlying this association, and 3) the efficacity of an intervention. The existence of an animal model is another argument in favour of a causal relationship.

2-1. Association and temporal relationship

Numerous epidemiological arguments back the fact that an association exists between gastric MALT lymphoma and *H. pylori* infection.

First, the prevalence of *H. pylori* infection in patients suffering with gastric MALT lymphoma, based on several studies, is between 80 and 90%, whereas the prevalence in the adult population in France for example, ranges from 25 to 30% [16]. Parsonnet et al. [17] offered a major epidemiological argument implicating *H. pylori* infection in MALT lymphoma. These authors showed in a case-control study nested in two large cohorts that the relative risk of developing this type of lymphoma was six times higher in the presence of an *H. pylori* infection. Furthermore, this increase was noted only for the gastric lymphomas; there was no increase in the relative risk for nodal lymphomas. They found that 88% of the patients had anti-*H. pylori* antibodies in blood samples collected fifteen years before the diagnosis of lymphoma, which clearly indicates that the *H. pylori* infection preceded the appearance of the gastric MALT lymphoma.

For certain cases, another species of the *Helicobacter* genus has been incriminated: *Helicobacter heilmannii* [18]. However, these cases are very rare, especially since the prevalence of *H. heilmannii* infection in humans in very low: 0.5% compared to an average of 20 to 25% in France for *H. pylori* [19].

2-2. Pathophysiological mechanism

The stomach normally is lacking in lymphoid tissue. After an *H. pylori* infection, a lymphoid infiltrate appears, which constitute a chronic gastritis. In certain cases the lympoid tissue can be organized as lymphoid follicles. MALT lymphoma emerges from these lymphoid structures [20]. Therefore, these lymphoid follicles appear after an antigenic stimulation by *H. pylori*. This hypothesis was validated *in vitro* by showing that T lymphocytes sensitized for *H. pylori* produce cytokines which stimulate B lymphoid proliferation [21-23]. The remaining question is whether the activation of the B cells requires the presence of a continuous antigenic stimulation by *H. pylori* or whether it is the consequence of an autoimmune mechanism [24]. In fact, the neoplastic B cells frequently produce antibodies directed toward autoantigens. Furthermore, the cells need to be in contact with the intratumoral T cells in order to proliferate, a CD40 and CD40 ligand interaction occurs [25]. This would explain the tendency for lymphomas with a weak degree of malignancy to remain localized and to regress following an *H. pylori* eradication. The existence of a clonal lymphocytic proliferation over several years would favour the occurrence of genetic alterations and the lymphoma would progressively proliferate independently of *H. pylori* [26]. Even if the mechanism for the evolution of a *H. pylori* infection to the gastritis stage and then to MALT lymphoma remains unknown, the role played by the bacterium seems to be likely.

Table 2. **Summary of the main studies performed from 1993 à 2002 evaluating the impact of *H. pylori* eradication on the regression of low grade gastric MALT lymphoma**

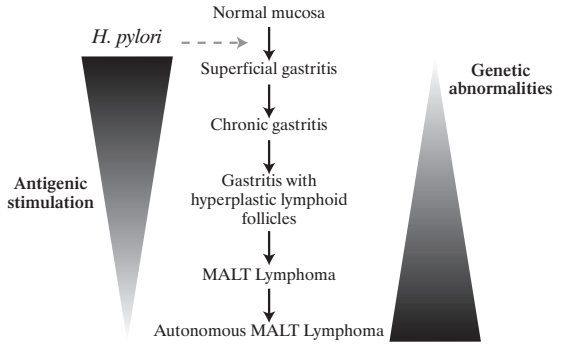
Author	Reference	Year	Number of patients	% remission
Wotherspoon et al.	[27]	1993	6	83
Bayerdörffer et al.	[61]	1995	33	69
Savio et al.	[62]	1996	12	84
Roggero et al.	[63]	1995	25	60
Fischbach et al.	[64]	1996	15	93
Montalban et al.	[65]	1997	9	88
Pinotti et al.	[66]	1997	45	68
Neubauer et al.	[67]	1997	50	80
Nobre-Leitao et al.	[68]	1998	17	100
Steinbach et al.	[69]	1999	28	50
Thiede et al.	[70]	2000	84	81
Fischbach et al.	[71]	2000	36	89
Ruskone-Fourmestraux et al.	[72]	2001	34	56
De Jong et al.	[73]	2001	23	56
Matsushima et al.	[74]	2002	14	71
Diz-Lois Palomares et al.	[75]	2002	14	71
Levy et al.	[30]	2002	48	69
Liu et al.	[44]	2002	111	43
Accumulated data	**1993-2002**		**604**	**72.8**

2-3. Effect of an intervention

The possibility of obtaining a regression of the lymphoma by taking an *H. pylori* eradication treatment constitutes the definitive proof of the causal role of the infection.

Wotherspoon et al. [27] showed in a pilot study including six patients that it was possible to obtain a regression of a low grade gastric MALT lymphoma in five of these patients, 22 months after the eradication of *H. pylori*. Several studies have determined that the tumor regression is 70 to 80%, with extremes of 50 to 100%, within a minimum delay of 4 to 6 months and after a maximum of 18 months, and after a post-treatment lapse greater than 6 years [4,28]. The variety of results obtained in the different studies stems mainly from the heterogeneity of the patients studied and the differences related to the locoregional extension of the lymphoma. Indeed, if only patients at stage EI were included in the different studies, the regression rate would be close to 80%. Furthermore, the time interval chosen between the eradication treatment and the lymphoma control may have a slight effect on the disparity of the results obtained (*Tab. 2*).

Figure 2. **Hypothetical cascade from the appearance of a MALT lymphoma at the gastric mucus level after infection with *H. pylori***



In France, the most commonly used eradication treatment combines amoxicillin (1 g bid) and clarithromycin (500 mg bid) with a double dose of proton pump inhibitor (PPI), compared to the usual dose, for 7 to 14 days. A control, based on *H. pylori* culture of gastric biopsies, should be carried out at least one month after the end of the treatment to verify *H. pylori* eradication and eventually modify the treatment depending on the antibiotic susceptibility results. The slow evolution of the disease allows a delay of at least 6 months before checking the histological and endoscopic evolution during the first two years [29,30]. In the case of a lymphoma with a high degree of malignancy, the chances of healing due to an *H. pylori* eradication are smaller [31]. Although it is now well accepted that a *H. pylori* infection contributes to the appearance of lymphoid tissue in the stomach and to its evolution towards a malignant proliferation, the *H. pylori*-dependent characteristics of the lymphoma disappears as genetic abnormalities accumulate (*Fig. 2*).

Regarding lymphomas linked to *H. heilmannii*, a tumoral regression has also been obtained after an eradication treatment identical to that of *H. pylori* [18].

The *H. pylori* eradication treatment seems to have a positive effect on extragastric MALT lymphomas (salivary gland, duodenum, colon, bladder, lung) even though it is often impossible to detect *H. pylori* in these extra-abdominal sites [32,33].

Lastly, treatment of *H. pylori* positive lymphomas which do not respond to eradication treatment, i.e. most of those of high grade of malignancy or, certain of those of low grade malignancy, as well as treatment of *H. pylori* negative gastric MALT lymphomas, is based on more classic therapeutic approaches for lymphomas. Gastric resection is the oldest treatment still being used. This approach can be helpful in removing big tumors but an average survival rate of 63% at most after 5 years can be expected for lymphomas at the EI2 stage [11]. Radiotherapy is currently preferred to surgery [34]. Finally chemotherapy using alkylant agents like chlorambucil or cyclophosphamide can be used successfully on patients diagnosed at an advanced stage of lymphoma or after a treatment failure [34].

2-4. Existence of an animal model

A prolonged gastric *H. pylori* infection in the BALB/c mouse constitutes a lymphogenesis model [35,36]. In these experimental lymphomas, one finds the characteristics of centrocyte-like cells, lymphoepithelial lesions, glandular destruction and consequently an aspect which is quite similar to that of human lymphomas. However, in the BALB/c model, lymphoepithelial lesions which evoke a gastric MALT lymphoma appear only in certain animals infected orally with *H. pylori* (an average of 40%) and approximately 20 months post-infection [36]. In some cases lymphoma can also evolve toward high grade gastric MALT lymphoma. One should note that in this model an eradication treatment induces the regression of the lymphoma.

III. Associated molecular abnormalities

Several translocations have been identified in the tumoral cells of gastric MALT lymphomas. Their significance is becoming more and more clear; in fact, they cause an antiapoptotic effect which is strictly related to a malignant expansion [37].

3-1. The translocation t(1;14)(p22;q32)

The translocation t(1;14) is found in approximately 5% of gastric MALT lymphomas. It leads to the overexpression of Bcl-10 protein whose gene is put under the control of the promotor gene of the immunoglobulin heavy chains. Bcl-10 possesses a CARD amino-terminal domain ("caspase recruitment domain") and can activate the NF-κB transcription factor [38]. In the lymphomatous cells, the Bcl-10 gene is overexpressed but it is also mutated, provoking the synthesis of a truncated protein capable of activating NF-κB and cell proliferation, but its overexpression does not induce apoptosis [39]. The t(1;14) translocation is also frequently associated with other supernumerary genetic abnormalities like those at the 3, 8 and 12 chromosome level. Trisomy 3 is found in 30% of gastric MALT lymphomas even though its role in the progression of the disease has not been well established [40].

3-2. The t(11;18) translocation

The t(11;18) translocation has been implicated in 21 to 60% of gastric MALT lymphomas. This translocation is also found in the marginal zone of other mucus sites in lymphomas [41]. It involves two genes: a human para-capsase, MLT1 (MALT lymphoma associated translocation-1); and c-IAP2 or API, a function of which is to inhibit the capsases by interacting directly with them. The translocation produces an IAP2-MLT1 fusion which has the capacity of activating the NF-κB pathway and therefore the cell protection against apoptosis [42]. It is associated with the most advanced stages, in particular with tumors having started to invade the submucosa, and also with the absence of regression in EI and EII stage lymphomas undergoing *H. pylori* eradication treatment [43-45]. The translocation should therefore be identified because its detection is predictive of the efficiency of an eradication treatment.

3-3. The t(14;18)(q32;q21) translocation

A third translocation was reported, t(14;18)(q32;q21), initially associated with a MALT type lymphoma of the skin and the liver, and then found in different localizations like the salivary glands [46]. Its identification implies a third pathway of carcinogenesis. However, for the moment this translocation does not appear to be associated with gastric lymphomas.

Studies on *H. pylori* strains associated with gastric MALT lymphoma

H. pylori was the first bacterium to be classified as a type I carcinogen (maximum level) by the International Agency of Research against Cancer [3]. Since its discovery, many research projects have focused on virulence factors or genetic markers but few studies have included *H. pylori* strains associated with gastric MALT lymphoma.

I. Study of the major virulence factors associated with *H. pylori*

H. pylori is perfectly adapted to the human stomach thanks to factors which allow it to: 1) resist against gastric acidity, 2) move around in the gastric mucus, and 3) escape from the immune response of the host. The major virulence factors found in *H. pylori* are those stimulating inflammation and the cell damage which results thereof, and in particular the products of the cag pathogenicity island and other proinflammatory proteins. Eight virulence factors were recently evaluated by studying a large collection of French strains issued from patients with gastric MALT lymphoma or gastritis [47]. Four factors involved in gastric inflammation and tissue lesions (CagA, CagE, OipA and IceA) as well as the vacuolizing cytotoxin VacA were tested. CagA is the virulence factor which has been most thoroughly studied along with VacA, and it has been associated in particular with duodenal ulcer and gastric adenocarcinoma. Four external membrane proteins were also studied: BabA, SabA, HopZ and HopQ. BabA, SabA and HopZ are adherence factors, BabA and SabA recognize in particular Lewis type antigens [48-50]. None of these factors tested individually could be significantly associated with strains from low grade gastric MALT lymphoma whereas three of them (IceA1, SabA and HopZ) showed a tendency to be associated. These strains have probably not a proinflammatory potential which distinguishes them from strains associated with ulcers or gastric adenocarcinoma [47].

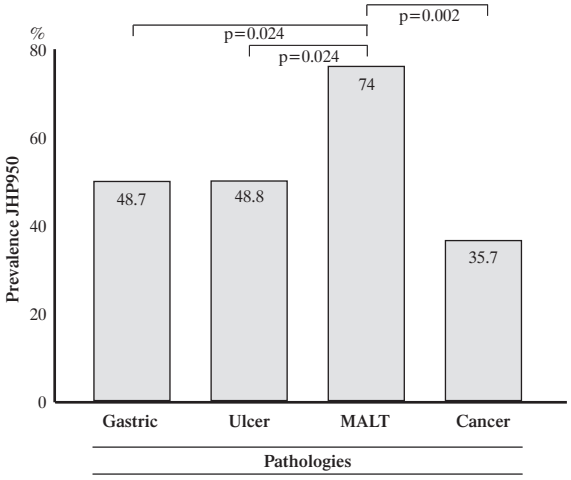
II. Study of the genetic characteristics of *H. pylori* strains associated with gastric MALT lymphoma

The development of a low grade gastric MALT lymphoma is most likely the consequence of an infection with an *H. pylori* strain, which harbours a particular set of genes or a particular gene expression, in a host with a particular genetic susceptibility. Within *H. pylori* species a considerable genomic as well as phenotypic diversity do exist. It is therefore possible that some strains have evolved towards a capacity to induce disease. Since known pathogenicity factors have not be implicated, the genetic material from these strains must be analyzed in order to determine either specific genetic markers or new virulence factors.

The new method for studying the genetic material in bacteria is comprised of DNA chips which detect the presence of ORFs in a set of strains compared to the genome of the strains for which the whole genomic sequence is known [51]. As no *H. pylori* strain associated with MALT lymphoma has ever been sequenced, a subtractive hybridization technique was used [52]. This technique has the advantage of extracting from the genome of a particular strain what is specific to it when compared to a control strain. Indeed, it is within the variable part of the genome of strains associated with gastric MALT lymphoma that new virulence factors are expected to be found. A specific marker for low grade gastric MALT lymphoma strains, the ORF JHP950, has reinforced the hypothesis that these MALT strains share a common genetic profile. Indeed, the prevalence of the JHP950 ORF in gastric MALT lymphoma strains was significantly higher than in strains isolated from duodenal ulcer and gastric adenocarcinoma (*Fig. 3*) [53]. This ORF belongs to the so-called plasticity zone of *H. pylori*. This zone is not considered to be a pathogenicity island per se but more likely a large sized genomic island [52]. However, ORF JHP950 is a part of the predicted operon containing ORF JHP947 which has been associated with strains isolated from patients with gastric adenocarcinoma [54]. ORF JHP950 is associated with gastric MALT lymphoma on the same level as the cagA gene is associated with ulcer strains [55].

Furthermore, by comparing the data obtained with this marker to those in the study on *H. pylori* major virulence factors, a significant association between this ORF and the genes, IceA1 and SabA, was shown in gastric MALT lymphoma strains [53]. Studies on the genetic diversity of *H. pylori*, such as the one performed by Salama et al. [51], showed a cluster of genes, in particular cag pathogenicity island ORFs associated to certain genes like *babA* or *hopQ* which were consequently identified as virulence factors [56,57]. The gene cluster identified in MALT strains could be a result of the phylogenetic evolution of lymphoma strains; i.e., they would have been selected during evolution because they offer an advantage, which remains to be determined, for the strains that contain them. ORF JHP950 codes for a protein with an undetermined function like 33% of the ORFs in the *H. pylori* genome, and therefore its role cannot be integrated into the pathophysiology of lymphoma [58]. Only

Figure 3. **Prevalence of ORF JHP950 in *H. pylori* strains associated with low grade gastric MALT lymphoma (MALT) (n=43) in comparison to strains associated with chronic gastritis (Gastritis) (n=39), duodenal ulcer (Ulcer) (n=41) or gastric adenocarcinoma (Cancer) (n=28)** [53]



p= Fisher exact test

complementary approaches such as reverse genetics, proteomics or others will allow us to answer this question. This study therefore allowed us to show the presence of genetic markers in certain *H. pylori* strains associated with gastric MALT lymphoma which could be used in the screening of strains with a high risk of causing a lymphoma.

II. Recent data obtained by comparative genomics

H. pylori strains associated with gastric MALT lymphoma therefore apparently share common genes. This hypothesis was recently confirmed by results obtained by comparative genomic studies. DNA from the collection of 43 lymphoma strains previously mentioned were hybridized on high density membranes containing a selection of 248 non-ubiquitous genes (the variable part of the *H. pylori* genome) and 50 ubiquitous genes (the stable part of the *H. pylori* genome). A statistical analysis carried out using the normalized values of these hybridizations revealed that 80% of the strains associated with lymphoma could be grouped together in the same cluster, distinguishing them from strains associated with other pathologies linked to *H. pylori* (gastritis, ulcer and intestinal metaplasia) [59].

These recent data do not allow us yet to understand the pathophysiological mechanisms of these particular strains but incite us strongly to look at the genetic material of these strains from every angle. It is from this perspective that a complete genome sequencing of a strain originating from a low grade gastric MALT lymphoma was conducted, in order to be able to compare it to the genomes of other *H. pylori* strains issued from other diseases.

Conclusions

The implication of *H. pylori* in the genesis of gastric MALT lymphoma has been clearly established. This discovery has revolutionized the treatment of this lymphoma because a *H. pylori* eradication treatment is capable of curing the lymphoma in most instances. This infection portrays a magnificent model of carcinogenesis induced by a chronic bacterial infection. The pathophysiological mechanisms leading to the occurrence of the lymphoma are beginning to be understood. The most recent molecular data indicates that strains associated with this cancer share common genetic characteristics. The identification of a first genetic marker for these strains and the results which are expected from the complete sequencing of a MALT lymphoma strain should lead to a screening method at the precancerous stage of strains with a high risk of causing lymphoma, and therefore, to propose a targeted antibiotic treatment. However, all of the elements involved in lymphogenesis are probably not limited only to bacteria and therefore a joint research effort involving individual susceptibility factors should be carried out.

References

1. Warren JR, Marshall B. Unientified curved bacilli on gastric epithelium in active chronic gastritis. Lancet, 1983; 1: 1273-5.
2. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet, 1984; 1: 1311-5.
3. IARC. Schistosomes, liver flukes and *Helicobacter pylori*. in "Monographs on the evaluation of carcinogenic risks to humans" IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994; 61: 1-241.
4. Stolte M, Bayerdörffer E, Morgner A, Alpen B, Wundisch T, Thiede C, Neubauer A. *Helicobacter* and gastric MALT lymphoma. Gut, 2002; 50 (Suppl 3): 19-24.
5. Freeman C, Berg JW, Cutler SJ. Occurrence and prognosis of extranodal lymphomas. Cancer, 1972; 29: 252-60.
6. Isaacson PG, Wright DH. Malignant lymphoma of mucosa-associated lymphoid tissue. A distinctive type of B-cell lymphoma. Cancer, 1983; 52: 1410-6.
7. Isaacson PG, Spencer J, Wright DH. Classifying primary gut lymphomas. Lancet, 1988; 2: 1148-9.
8. Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J, Lister TA, Bloomfield CD. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting-Airlie House, Virginia, November 1997. J Clin Oncol, 1999; 17: 3835-49.
9. Ullrich A, Fischbach W, Blettner M. Incidence of gastric B-cell lymphomas: a population-based study in Germany. Ann Oncol, 2002; 13: 1120-7.
10. Fischbach W, Kestel W, Kirchner T, Mossner J, Wilms K. Malignant lymphomas of the upper gastrointestinal tract. Results of a prospective study in 103 patients. Cancer, 1992; 70: 1075-80.
11. Radaszkiewicz T, Dragosics B, Bauer P. Gastrointestinal malignant lymphomas of the mucosa-associated lymphoid tissue: factors relevant to prognosis. Gastroenterology, 1992; 102: 1628-38.
12. Peterson MC. Immunoproliferative small intestinal disease associated with *Campylobacter jejuni*. N Engl J Med, 2004; 350: 1685-6.
13. Lecuit M, Abachin E, Martin A, Poyart C, Pochart P, Suarez F, Bengoufa D, Feuillard J, Lavergne A, Gordon JI, Berche P, Guillevin L, Lortholary O. Immunoproliferative small intestinal disease associated with *Campylobacter jejuni*. N Engl J Med, 2004; 350: 239-48.
14. Ben-Khelifa H. Gastric lymphoma: is the worldwide incidence rising? Gastrointest Endosc, 2002; 56: 955.
15. Hill AB. The environment and disease: association or causation? Proc R Soc Med, 1965; 295-300.
16. Eck M, Greiner A, Schmausser B, Eck H, Kolve M, Fischbach W, Strecker P, Muller-Hermelink HK. Evaluation of *Helicobacter pylori* in gastric MALT-type lymphoma: Differences between histologic and serologic diagnosis. Mod Pathol, 1999; 12: 1148-51.
17. Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, Orentreich N, Vogelman JH, Friedman GD. *Helicobacter pylori* infection and gastric lymphoma. N Engl J Med, 1994; 330: 1267-71.
18. Morgner A, Lehn N, Andersen LP, Thiede C, Bennedsen M, Trebesius K, Neubauer B, Neubauer A, Stolte M, Bayerdorffer E. *Helicobacter heilmannii*-associated primary gastric low-grade MALT lymphoma: Complete remission after curing the infection. Gastroenterology, 2000; 118: 821-8.
19. Broutet N, Mégraud F. Epidemiology of *Helicobacter pylori* infection. 1996 Epidemiology of *Helicobacter pylori* infection. *Helicobacter pylori* (Mégraud F, and Lamouliatte H, Eds.), 1 Epidémiologie, Pathogénie, Diagnostic, Paris.
20. Miyamoto M, Haruma K, Hiyama T, et al. High incidence of B-cell monoclonality in follicular gastritis: a possible association between follicular gastritis and MALT lymphoma. Virchows Arch, 2002; 440: 376-80.
21. Hussell T, Issacson PG, Crabtree JE, Spencer J. The response of cells from low-grade B-cell gastric lymphomas of mucosa-associated lymphoid tissue to *Helicobacter pylori*. Lancet, 1993; 342: 571-4.
22. Hussell T, Isaacson PG, Crabtree JE, Spencer J. *Helicobacter pylori*-specific tumour-infiltrating T cells provide contact dependent help for the growth of malignant B cells in low-grade gastric lymphoma of mucosa-associated lymphoid tissue. J Pathol, 1996; 178: 122-7.
23. D'Elios MM, Amedei A, Manghetti M. Impaired T-cell regulation of B-cell growth in *Helicobacter pylori*-related gastric low-grade MALT lymphoma. Gastroenterology, 1999; 117: 1105-12.
24. Kawahara Y, Yokota K, Mizuno M, Yunoki N, Uesu T, Okada H, Kobayashi K, Hirai Y, Oguma K, Tsuji T. Antibodies to human gastric epithelial cells and heat shock protein 60 in *Helicobacter pylori* positive mucosa associated lymphoid tissue lymphoma. Gut, 1999; 45: 20-3.
25. Guindi M. Role of activated host T cells in the promotion of MALT lymphoma growth. Semin Cancer Biol, 2000; 10: 341-4.
26. Dierlamm J, Baens M, Wlodarska I, Stefanova-Ouzounova M, Hernandez JM, Hossfeld DK, De Wolf-Peeters C, Hagemeijer A, Van den Berghe H, Marynen P. The apoptosis inhibitor gene API2 and a novel

18q gene, MLT, are recurrently rearranged in the t(11;18)(q21;q21) associated with mucosa-associated lymphoid tissue lymphomas. Blood, 1999; 93: 3601-9.

27. Wotherspoon AC, Doglioni C, Diss TC, Pan L, Moschini A, de Boni M, Isaacson PG. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. Lancet, 1993; 342: 575-7.

28. Savio A, Zamboni G, Capelli P, Negrini R, Santandrea G, Scarpa A, Fuini A, Pasini F, Ambrosetti A, Paterlini A, Buffoli F, Angelini GP, Cesari P, Rolfi F, Graffeo M, Pascarella A, Valli M, Mombello A, Ederle A, Franzin G. Relapse of low-grade gastric MALT lymphoma after *Helicobacter pylori* eradication: true relapse or persistence? Long-term post-treatment follow-up of a multicenter trial in the north-east of Italy and evaluation of the diagnostic protocol's adequacy. Recent Results Cancer Res, 2000; 156: 116-24.

29. Sandmeier D, Benhattar J, Bouzourene H. The natural history of a gastric low grade B cell MALT lymphoma followed during 11 years without treatment. J Clin Pathol, 2002; 55: 548-50.

30. Levy M, Copie Bergman C, Traulle C. Conservative treatment of primary gastric low-grade B-cell lymphoma of mucosa-associated lymphoid tissue: Predictive factors of response and outcome. Am J Gastroenterol, 2002; 97: 292-7.

31. Ng WW, Lam CP, Chau WK, Fen-Yau Li A, Huang CC, Chang FY, Lee SD. Regression of high-grade gastric mucosa-associated lymphoid tissue lymphoma with *Helicobacter pylori* after triple antibiotic therapy. Gastrointest Endosc, 2000; 51: 93-6.

32. Raderer M, Pfeffel F, Pohl G, Mannhalter C, Valencak J, Chott A. Regression of colonic low grade B cell lymphoma of the mucosa associated lymphoid tissue type after eradication of *Helicobacter pylori*. Gut, 2000; 46: 133-5.

33. van den Bosch J, Kropman RF, Blok P. Disappearance of a mucosa-associated lymphoid tissue (MALT) lymphoma of the urinary bladder after treatment for *Helicobacter pylori*. Eur J Haematol, 2002; 68: 187-8.

34. Schechter NR, Portlock CS, Yahalom J. Treatment of mucosa-associated lymphoid tissue lymphoma of the stomach with radiation alone. J Clin Oncol, 1998; 16: 1916-21.

35. Lee A, O'Rourke J, Enno A. Gastric mucosa-associated lymphoid tissue lymphoma: implications of animal models on pathogenic and therapeutic considerations-mouse models of gastric lymphoma. Recent Results Cancer Res, 2000; 156: 42-51.

36. Mueller A, O'Rourke J, Grimm J. Distinct gene expression profiles characterize the histopathological stages of disease in *Helicobacter*-induced mucosa-associated lymphoid tissue lymphoma. Proc Natl Acad Sci USA, 2003; 100: 1292-7.

37. Isaacson PG, Du MQ. MALT lymphoma: from morphology to molecules. Nat Rev Cancer, 2004; 4: 644-53.

38. Zhang Q, Siebert R, Yan M, Hinzmann B, Cui X, Xue L, Rakestraw KM, Naeve CW, Beckmann G, Weisenburger DD, Sanger WG, Nowotny H, Vesely M, Callet-Bauchu E, Salles G, Dixit VM, Rosenthal A, Schlegelberger B, Morris SW. Inactivating mutations and overexpression of BCL10, a caspase recruitment domain-containing gene, in MALT lymphoma with t(1;14)(p22;q32). Nat Genet, 1999; 22: 63-8.

39. Ruland J, Duncan GS, Elia A, del Barco Barrantes I, Nguyen L, Plyte S, Millar DG, Bouchard D, Wakeham A, Ohashi PS, Mak TW. Bcl10 is a positive regulator of antigen receptor-induced activation of NF-kappaB and neural tube closure. Cell, 2001; 104: 33-42.

40. Wotherspoon AC, Finn TM, Isaacson PG. Trisomy 3 in low-grade B-cell lymphomas of mucosa-associated lymphoid tissue. Blood, 1995; 85: 2000-4.

41. Baens M, Maes B, Steyls A. The product of the t(11;18), an API2-MLT fusion, marks nearly half of gastric MALT type lymphomas without large cell proliferation. Am J Pathol, 2000; 156: 1433-9.

42. Uren AG, O'Rourke K, Aravind LA, Pisabarro MT, Seshagiri S, Koonin EV, Dixit VM. Identification of paracaspases and metacaspases: two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma. Mol Cell, 2000; 6: 961-7.

43. Liu HX, Ruskone-Fourmestraux A, LavergneSlove A. Resistance of t(11;18) positive gastric mucosa-associated lymphoid tissue lymphoma to *Helicobacter pylori* eradication therapy. Lancet, 2001; 357: 39-40.

44. Liu HoX, Ye HT, Ruskone Fourmestraux A, De Jong D, Pileri S, Thiede C, Lavergne A, Boot H, Caletti G, Wundisch T, Molina T, Taal BG, Elena S, Thomas T, Zinzani PL, Neubauer A, Stolte M, Hamoudi RA, Dogan A, Isaacson PG, Du MQ. T(11;18) is a marker for all stage gastric MALT lymphomas that will not respond to *H. pylori* eradication. Gastroenterology, 2002; 122: 1286-94.

45. Nakamura S, Matsumoto T, Jo Y, Fujisawa K, Suekane H, Yao T, Tsuneyoshi M, Iida M. Chromosomal translocation t(11;18)(q21;q21) in gastrointestinal mucosa associated lymphoid tissue lymphoma. J Clin Pathol, 2003; 56: 36-42.

46. Streubel B, Lamprecht A, Dierlamm J, Cerroni L, Stolte M, Ott G, Raderer M, Chott A. T(14;18)(q32;q21) involving IGH and MALT1 is a frequent chromosomal aberration in MALT lymphoma. Blood, 2003; 101: 2335-9.

47. Lehours P, Ménard A, Dupouy S, Bergey B, Richy F, Zerbib F, Ruskone-Fourmestraux A, Delchier JC, Megraud F. Evaluation of the association of nine *Helicobacter pylori* virulence factors with strains involved in low grade gastric MALT lymphoma. Infect Immun, 2004; 74: 880-8.

48. Ilver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, Berg DE, Covacci A, Engstrand L, Boren T. *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science, 1998; 279: 373-7.

49. Mahdavi J, Sonden B, Hurtig M, Olfat FO, Forsberg L, Roche N, Angstrom J, Larsson T, Teneberg S, Karlsson KA, Altraja S, Wadstrom T, Kersulyte D, Berg DE, Dubois A, Petersson C, Magnusson KE, Norberg T, Lindh F, Lundskog BB, Arnqvist A, Hammarstrom L, Boren T. *Helicobacter pylori* SabA adhesin in persistent infection and chronic inflammation. Science, 2002; 297: 573-8.

50. Peck B, Ortkamp M, Diehl KD, Hundt E, Knapp B. Conservation, localization and expression of HopZ, a protein involved in adhesion of *Helicobacter pylori*. Nucl Acids Research, 1999; 27: 3325-33.

51. Salama N, Guillemin K, McDaniel TK, Sherlock G, Tompkins L, Falkow S. A whole-genome microarray reveals genetic diversity among *Helicobacter pylori* strains. Proc Natl Acad Sci USA, 2000; 97: 14668-73.

52. Occhialini A, Marais A, Alm R, Garcia F, Sierra R, Megraud F. Distribution of open reading frames of plasticity region of strain J99 in *Helicobacter pylori* strains isolated from gastric carcinoma and gastritis patients in Costa Rica. Infect Immun, 2000; 68: 6240-9.

53. Lehours P, Dupouy S, Bergey B, Ruskone-Foumestraux A, Delchier JC, Rad R, Richy F, Tankovic J, Zerbib F, Megraud F, Menard A. Identification of a genetic marker of *Helicobacter pylori* strains involved in gastric extranodal marginal zone B cell lymphoma of the MALT-type. Gut, 2004; 53(7): 931-7.

54. Santos A, Queiroz DM, Ménard A, Marais A, Rocha GA, Oliveira CA, Nogueira AM, Uzeda M, Megraud F. New pathogenicity marker found in the plasticity region of the *Helicobacter pylori* genome. J Clin Microbiol, 2003; 41: 1651-5.

55. Labigne A, Lamouliatte H, Birac C. Distribution of the *cagA* gene among *Helicobacter pylori* strains associated with peptic ulcer. Am J Gastroenterol, 1994; 89: 1326.

56. Gerhard M, Lehn N, Neumayer N, Boren T, Rad R, Schepp W, Miehlke S, Classen M, Prinz C. Clinical relevance of the *Helicobacter pylori* gene for blood-group antigen-binding adhesin. Proc Natl Acad Sci USA, 1999; 96: 12778-83.

57. Cao P, Cover TL. Two different families of *hopQ* alleles in *Helicobacter pylori*. J Clin Microbiol, 2002; 40: 4504-11.

58. Boneca IG, de Reuse H, Epinat JC, Pupin M, Labigne A, Moszer I. A revised annotation and comparative analysis of *Helicobacter pylori* genomes. Nucleic Acids Res, 2003; 31: 1704-14.

59. Thiberge JM, Dillies MA, Coppée JY. Analyse comparative du génome de 120 souches de *H. pylori* associées à différentes pathologies. 2004. Abstract. Journées Francophones de Pathologie Digestive 2004.

60. Ruskone-Fourmestraux A. MALT gastric lymphomas. Rev Med Interne, 2004; 25: 573-81.

61. Bayerdorffer E, Neubauer A, Rudolph B, Thiede C, Lehn N, Eidt S, Stolte M. Regression of primary gastric lymphoma of mucosa-associated lymphoid tissue type after cure of *Helicobacter pylori* infection. MALT Lymphoma Study Group. Lancet, 1995; 345: 1591-4.

62. Savio A, Franzin G, Wotherspoon AC, Zamboni G, Negrini R, Buffoli F, Diss TC, Pan L, Isaacson PG. Diagnosis and posttreatment follow-up of *Helicobacter pylori*-positive gastric lymphoma of mucosa-associated lymphoid tissue: Histology, polymerase chain reaction, or both? Blood, 1996; 87: 1255-60.

63. Roggero E, Zucca E, Pinotti G. Eradication of *Helicobacter pylori* infection in primary low-grade gastric lymphoma of mucosa-associated lymphoid tissue. Ann Intern Med, 1995; 122: 767-9.

64. Fischbach W, Kolve M, Engemann R. Unexpected success of *Helicobacter pylori* eradication in low-grade lymphoma. Gastroenterology, 1996; 110.

65. Montalban C, Manzanal A, Boixeda D, Redondo C, Alvarez I, Calleja JL, Bellas C. *Helicobacter pylori* eradication for the treatment of low-grade gastric MALT lymphoma: Follow-up together with sequential molecular studies. Ann Oncol, 1997; 8: 37-9.

66. Pinotti G, Zucca E, Roggero E, Pascarella A, Bertoni F, Savio A, Savio E, Capella C, Pedrinis E, Saletti P, Morandi E, Santandrea G, Cavalli F. Clinical features, treatment and outcome in a series of 93 patients with low-grade gastric MALT lymphoma. Leuk Lymphoma, 1997; 26: 527-37.

67. Neubauer A, Thiede C, Morgner N, Alpen B, Ritter M, Neubauer B, Wundisch T, Ehninger G, Stolte M, Bayerdorffer E. Cure of *Helicobacter pylori* infection and duration of remission of low-grade gastric mucosa-associated lymphoid tissue lymphoma. J Natl Cancer Inst, 1997; 89: 1350-5.

68. Nobre-Leitao C, Lage P, Cravo M, Cabecadas J, Chaves P, Alberto-Santos A, Correia J, Soares J, Costa-Mira F. Treatment of gastric MALT lymphoma by *Helicobacter pylori* eradication: a study controlled by endoscopic ultrasonography. Am J Gastroenterol, 1998; 93: 732-6.

69. Steinbach G, Ford R, Glober G, Sample D, Hagemeister FB, Lynch PM, McLaughlin PW, Rodriguez MA, Romaguera JE, Sarris AH, Younes A, Luthra R, Manning JT, Johnson CM, Lahoti S, Shen Y, Lee JE, Winn RJ, Genta RM, Graham DY, Cabanillas FF. Antibiotic treatment of gastric lymphoma of mucosa-associated lymphoid tissue. An uncontrolled trial. Ann Intern Med, 1999; 131: 88-95.

70. Thiede C, Wundisch T, Neubauer B, Alpen B, Morgner A, Ritter M, Ehninger G, Stolte M, Bayerdorffer E, Neubauer A. Eradication of Helicobacter pylori and stability of remissions in low-grade gastric B-cell lymphomas of the mucosa-associated lymphoid tissue: results of an ongoing multicenter trial. Recent Results Cancer Res, 2000; 156: 125-33.

71. Fischbach W, Dragosics B, KolveGoebeler ME. Primary gastric B-cell lymphoma: Results of a prospective multicenter study. Gastroenterology, 2000; 119: 1191-202.

72. Ruskoné Fourmestraux A, Lavergne A, Aegerter PH, Megraud F, Palazzo L, de Mascarel A, Molina T, Rambaud JL. Predictive factors for regression of gastric MALT lymphoma after anti-*Helicobacter pylori* treatment. Gut, 2001; 48: 297-303.

73. de Jong D, Vyth Dreese F, Dellemijn T, Verra N, Ruskone-Fourmestraux A, Lavergne-Slove A, Hart G, Boot H. Histological and immunological parameters to predict treatment outcome of *Helicobacter pylori* eradication in low-grade gastric MALT lymphoma. J Pathol, 2001; 193: 318-24.

74. Matsushima Y, Kinoshita Y, Fukui H, Maekawa T, Yazumi S, Okada A, Nakase H, Kawanami C, Iwano M, Hashimoto K, Takeda Z, Okazaki K, Chiba T. Immunological and molecular analysis of B lymphocytes in low-grade MALT lymphoma of the stomach. Are there any useful markers for predicting outcome after *Helicobacter pylori* eradication? J Gastroenterol, 2002; 37: 428-33.

75. Diz-Lois Palomares MT, Souto Ruzo J, Yanez Lopez JA, Alvarez Garcia A, Arnal Monreal F, Vazquez-Iglesias JL. Early-stage gastric MALT lymphomas: eradication of *H. pylori* and outcome. Rev Esp Enferm Di, 2002; 94: 669-78.