Role of *Helicobacter pylori* in the pathogenesis of gastric carcinoma and progression of lymphoid nodules to lymphoma

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M Guindi. Role of *Helicobacter pylori* in the pathogenesis of gastric carcinoma and progression of lymphoid nodules to lymphoma. *Can J Gastroenterol* 1999;13(3):224-227. The pathology of gastritis associated with *Helicobacter pylori* infection is summarized. The literature is reviewed regarding the role of *H pylori* in the pathogenesis of gastric carcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. The potential mechanisms of gastric carcinogenesis include transformation of the gastric mucosa by metabolic products of *H pylori*, transformation of the host cell by incorporation of *H pylori* DNA and genotoxic effects of the inflammatory response to the organism. A model for gastric carcinogenesis is proposed in which *H pylori* causes cell proliferation, and the risk of DNA damage is increased, leading to inadequate repair and malignant transformation. Investigation of early gastric carcinomas concluded that two main pathways operated in gastric carcinogenesis, both starting from *H pylori* gastritis and leading to phenotypically variable gastric or intestinal tumour growth. The histological features and molecular genetics of MALT lymphoma are briefly reviewed. There is evidence that tumour cells of low grade B cell MALT lymphoma proliferate specifically in response to *H pylori*. This response is dependent on T cell activation by *H pylori*. A proposed model for the pathogenesis of MALT lymphoma postulates that B lymphocytes with a genetic change acquire a growth advantage resulting in a monoclonal proliferation in response to *H pylori*-activated T cells. Further genetic changes may result in escape from T cell dependency.

Key Words: *Helicobacter pylori*, Gastric carcinogenesis, Gastric carcinoma, Gastritis, Mucosa-associated lymphoid tissue lymphoma

Le rôle d'*Helicobacter pylori* dans la pathogenèse du cancer de l'estomac et la progression des nodules lymphoïdes vers le lymphome

RÉSUMÉ : Le présent article résume la pathologie de la gastrite associée à une infection à *Helicobacter pylori*. On y passe en revue la littérature sur le rôle d'*H. pylori* dans la pathogenèse du cancer de l'estomac et du lymphome affectant le système lymphoïde des muqueuses (MALT). Les mécanismes potentiels de la carcinogenèse gastrique incluent la transformation de la muqueuse gastrique par les produits du métabolisme d'*H. pylori*, la transformation de la cellule hôte par l'incorporation de l'ADN d'*H. pylori* et les effets génotoxiques de la réponse inflammatoire à l'organisme pathogène. On propose un modèle de carcinogenèse dans lequel *H. pylori* provoque une prolifération cellulaire et le risque d'atteinte liée à l'ADN est accru, entraînant une réparation insuffisante et une prédisposition à la malignité. Les recherches sur les cancers gastriques au premiers stade de leur développement ont conclu à l'existence de deux mécanismes oncogènes touchant l'estomac, les deux liés à *H. pylori* et entraînant la croissance de cellules gastriques ou intestinales à phénotypes variables. On passe brièvement en revue les caractéristiques histologiques et la génétique moléculaire du lymphome du MALT. Selon certains résultats, les cellules tumorales de bas bruit du lymphome du MALT à lymphocytes B prolifèrent spécifiquement en réponse à *H. pylori*. Cette réponse est dépendante de l'activation des lymphocytes T par *H. pylori*. Selon un autre modèle avancé pour expliquer la pathogenèse des lymphomes du MALT, les lymphocytes B génétiquement modifiés acquièrent un avantage sur le plan de la croissance, ce qui entraîne une prolifération monoclonale en réponse aux lymphocytes T activés par *H. pylori*. D'autres changements génétiques peuvent éliminer la dépendance à l'endroit des lymphocytes T.

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The pathology of gastritis associated with *Helicobacter pylori* infection has been previously reviewed (1,2). Briefly, the inflammatory cell infiltrate is usually mixed, neutrophilic and mononuclear. The degree of inflammation is variable, from minimal to severe. In severe cases, neutrophils may be seen in the surface epithelium and in the gastric pits, and are concentrated in the pit regions. On average, fundic gland inflammation is usually less severe than that in the antral mucosa. There are instances when severe mucosal inflammation is accompanied by paucity of organisms. The organism is a small, curvilinear, slightly basophilic bacillus, usually seen in the mucous layer overlying the gastric surface epithelium. *H pylori* is present both overlying the gastric surface epithelium and in the pits. Intracellular invasion usually is not seen. Epithelial damage caused by *H pylori* includes mucin depletion, loss of nuclear polarity and nuclear enlargement. Mitoses are present in increased numbers. There is epithelial cell drop out and reactive hyperplasia. As a result of these epithelial changes, the gastric surface epithelium at low power is serrated at the time of microscopic examination. The organisms do not overlie the erosions or the reactive mucosa immediately adjacent to it but colonize the adjacent less reactive mucosa.

H PYLORI AND GASTRIC CARCINOMA

H pylori is associated with both the intestinal and diffuse types of gastric cancer, as well as with tumours involving the gastric antrum and corpus (3,4). A proposal for the pathogenesis of gastric carcinoma of the intestinal type has been delineated by Correa (5). In summary, chronic gastritis progresses to chronic atrophic gastritis and subsequently to intestinal metaplasia. Dysplasia develops within the latter, leading to carcinoma of the intestinal type. A decrease in acid secretion, abnormal synthesis of acid mucins and excessive levels of nitrite in the gastric lumen accompany the changes in gastric epithelium.

The role of *H pylori* in the pathogenesis of gastric cancer was reviewed by Parsonnet (6), who postulated that there are several potential mechanisms of gastric carcinogenesis. First, metabolic products of the organism may transform the gastric mucosa. Second, *H pylori* DNA may incorporate into the host cells causing transformation analogous to viral carcinogenesis. Third, *H pylori* may induce an inflammatory response that is genotoxic. Parsonnet points out that of these potential mechanisms, the latter is most consistent with currently available data. Several studies show that *H pylori* carcinogenesis may be mediated by the increased rate of gastric epithelium proliferation and the decreased secretion of ascorbic acid, a known antioxidant, caused by *H pylori* infection. *H pylori* causes a hyperproliferative state in the gastric epithelium. Jankowski (7) suggested this is caused by increased production by epithelial cells of growth regulatory peptides, such as epidermal growth factor, in response to cytokines. Cell proliferation studies have shown an increased number of proliferating cells in chronic gastritis (8,9). One study (10) examined pre- and posteradication indexes of proliferating cell nuclear antigen (PCNA) in gastric mucosa.

PCNA is a nuclear antigen that functions as a cofactor during the DNA synthesis phase of the cell cycle (11). The study (10) found a marked reduction of PCNA-labelled cells (which indicates cell replication) after eradication of *H pylori*. The postclearance indexes were very close to those observed in uninflamed gastric mucosa. The authors postulated that, given the longstanding nature of the inflammatory response with abundant polymorphonuclear leukocytes and macrophages, it is probable that hyperproliferation is present for many years in most patients. In a hyperproliferative state, spontaneous mutations can accumulate, thereby leading to neoplastic transformation (12,13).

H pylori may play a part in mutagenesis. In *H pylori*-induced gastritis, polymorphonuclear leukocytes migrate from the capillaries into the lamina propria and the gastric glands. Polymorphonuclear leukocytes give rise to oxidative bursts when challenged in vitro by *H pylori* and its products (14). Free oxygen radicals are known to cause DNA damage and may induce genetic mutations. Parsonnet (6) proposed a model for gastric carcinogenesis in which *H pylori* causes cell proliferation, increasing the risk for DNA damage by replication error, endogenous inflammation-related mutagens and exogenous dietary mutagens. Some DNA damage induced in epithelial cells accumulates with time. The longer the duration of infection, the higher the likelihood of inadequate repair and malignant transformation is.

Solcia et al (15) studied early gastric carcinomas in an attempt to obtain more information on the early steps of tumour development. In addition, they attempted to delineate the sequence of morphological and molecular events leading from *H pylori* gastritis to cancer of different histological types. *H pylori* colonization was detected in the nontumoural mucosa of 76% of intestinal type carcinoma, 100% of diffuse type and 87% of mixed cancers. At all mucosal sites and at all stages of neoplastic disease, the extension of intestinal metaplasia as a whole, as well as of its type I or III variants, was higher in intestinal than in diffuse cancer cases. Dysplastic lesions were detected only in the nontumoural mucosa associated with intestinal or mixed cancers. All dysplastic lesions diffusely expressed the CAR-5 antigen, which is a colorectal and immature enterocyte antigen widely present in embryonic and early fetal gut epithelia (16). On mucin and immunoperoxidase staining, most tumours display complex admixtures of cellular immunophenotypes (that is a mixture of gastric and intestinal features). CAR-5 immunoreactivity is prevalent largely in microglandular growth of the early diffuse cancers but is rather poorly expressed in dispersed signet ring cells. The latter are more reactive with gastric type markers, such as the M1 foveolar mucin. Both laminin immunohistochemistry and transmission electron microscopy showed that the basement membrane, which is well developed around normal and well differentiated malignant glands, was partly attenuated or focally interrupted around poorly differentiated glands, and lost completely around isolated dispersed cells (15). The diffuse dispersed cancers showed loss of B1 integrin expression. Of note, the rate of

p53 mutation was 41% (15). The authors (15) proposed that this finding suggests a role for p53 in the pathogenesis of intestinal type gastric carcinomas similar to that played by the same gene in colon cancer (17). The authors proposed that p53 mutations may be caused by nitrosative deamination resulting from the combined action of nitric oxide and oxygen free radicals released by the inflammatory response to *H pylori* infection. Based on these findings, the authors proposed a model of indirect carcinogenesis due to *H pylori*-activated mucosal inflammation comprised of two pathways (15). The common starting point of these different carcinogenesis pathways is *H pylori*-induced chronic active gastritis, which operates early in the development of carcinogenesis in both subtypes of gastric cancer. In the first pathway, differentiation and proliferation mechanisms are primarily affected in the long lasting process that leads from chronic active gastritis to the intestinal subtype of carcinoma. This pathway recapitulates the events of colorectal carcinogenesis (17-19). In the second pathway that leads to the diffuse subtype of gastric cancer, there is an impairment of cell to cell joining systems, such as mutation in the genes involved in the synthesis of basement membrane proteins. Such events result in a more rapid process leading directly from non-neoplastic, somewhat atypical mucosal renewal zones in the gland neck regions to cancer.

Parsonnet et al (20) found a decreased risk of gastric carcinoma in a series of *H pylori*-infected patients with duodenal ulcer. Duodenal ulcer is associated with chronic antral gastritis, which also involves neutrophils. Yet, the risk of gastric carcinoma was not increased. This issue was the subject of an editorial by Correa (21) who pointed out that this incongruity can be explained by the presence of other causal factors that interact with *H pylori* infection in the process of carcinogenesis. Such factors may include diets deficient in fresh fruits and vegetables (sources of ascorbate) and those resulting in excessive salt intake.

H PYLORI AND MUCOSA-ASSOCIATED LYMPHOID TISSUE LYMPHOMA

Mucosa-associated lymphoid tissue (MALT) lymphomas are extranodal lymphomas arising from mucosa-associated lymphoid tissue (22,23). The histological features of low grade MALT lymphoma were previously described (24,25), and recent developments were updated by Isaacson (26). The lymphoma infiltrates around and between reactive follicles in the marginal zone. It spreads diffusely into the surrounding mucosa. The tumour cells are polymorphous. They are small to medium sized with moderately abundant cytoplasm and irregular nuclei resembling nuclei of centrocytes. The tumour cell population also includes small lymphocytes, small cells that resemble nodal monocytoid B cells, and neoplastic plasma cells often distributed in subepithelial or interfollicular zones. An important feature of low grade MALT lymphomas is the presence of lymphoepithelial lesions formed by the invasion of individual gastric glands by aggregates of tumour cells, which ultimately leads to destruction of the glandular epithelium. Reactive follicles may be selectively colo-

nized by the lymphoma. In this case, the follicle centre is replaced by tumour cells that can exhibit blast transformation or plasma cell differentiation (27).

H pylori is identified in more than 90% of MALT lymphomas of the stomach (28). *H pylori* infection has been epidemiologically linked to MALT lymphoma in many studies (29,30). Several studies demonstrate that lymphoid follicles are not a normal finding in the gastric mucosa (31,33). Genta et al (33) studied mapped gastric biopsies from normal volunteers, *H pylori*-infected asymptomatic patients, and *H pylori*-infected patients with gastric and duodenal ulcers. They showed convincingly that the normal stomach does not contain MALT, that its development is the result of *H pylori* infection and that *H pylori* appears to be a precursor in the development of primary gastric lymphoma (33).

Immunohistochemistry staining and genotype investigations of MALT lymphoma using the Southern blot technique have revealed clonal rearrangements of immunoglobulin genes (34). Monoclonality can also be demonstrated by using polymerase chain reaction (PCR) technology. Trisomy 3 and, less frequently, trisomy 18 can be detected in the majority of MALT lymphomas (35,36). Hussell et al (37) demonstrated that the tumour cells of B cell low grade MALT lymphoma proliferate specifically in response to *H pylori*. This B cell response is dependent on T cell-specific activation by *H pylori*. Cells were teased from resection specimens of low grade MALT lymphoma and cocultured with different strains of *H pylori*. The organisms also induce interleukin-2 receptor expression by tumour cells and secretion of interleukin-2 by T cells. Removal of T cells from the medium abolishes all responses. The study by Wotherspoon et al (38) further substantiates the theory that MALT lymphomas are antigen-responsive. Five of six patients with biopsy-proven B cell low grade MALT lymphoma showed full histological regression of the lymphoma following *H pylori* eradication with disappearance of the neoplastic B cell clone.

On the basis of the above observations, a model for the pathogenesis has been proposed (26,34). B and T cells are recruited to the gastric mucosa as part of the immune response to *H pylori*. In rare cases, the lymphoid infiltrate contains B cells with a genetic change (such as trisomy) that confers a proliferation advantage to affected cells. This results in monoclonal proliferation in response to *H pylori*-activated T cells. Further genetic changes that have not been characterized may result in escape from T cell dependency. This may account for the dissemination of low grade gastric MALT lymphoma. Subsequent additional genetic events, such as mutations, then would result in high grade transformation.

It appears that MALT lymphomas can be either biclonal or oligoclonal. One of two patients reported by Zucca et al (39) with biopsy-proven B cell low grade MALT lymphoma had two major lymphoma clones that, on DNA sequencing analysis, apparently arose from a biclonal proliferation of B cells. One clone disappeared after several years following antibiotic treatment, whereas the other did not. The authors postulated that the persistent clone may have progressed to a state of independence from *H pylori*. This may be analogous

to lymphomas induced by Epstein-Barr virus in the setting of organ transplantation where withdrawal of immunosuppression stops lymphoma progression. In both patients, the gastric MALT lymphoma was demonstrated to arise from a B cell clone at the site of the chronic gastritis that had been documented by biopsies obtained years before the development of lymphoma.

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