Primary Gastric MALT-Type Lymphoma
Pathogenesis

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ABSTRACT Gastric lymphoma is considered to be primary when the initial symptoms of the disease are located in the stomach or when the tumour mass is located in the stomach. MALT lymphoma represents 35-40% of primary gastric lymphomas. If the involvement of Helicobacter pylori in the primary gastric lymphoma etiopathogenesis and the favourable response of the disease to the eradication therapy tend to become postulated, much less known is the role of the host’s immune response in pathogenesis, as long as only a small proportion of infected patients develop lymphoma. Besides the tumour specific T cells, it is necessary for Helicobacter pylori that malignant B cells have also certain features allowing their uncontrolled proliferation at their stimulation by T lymphocytes. There are probably some genetic abnormalities that confer them growth advantage or abnormal biological properties such as the ability to recognize autoantigens. Gastric lymphomas are characterized by microsatellite instability (MALT lymphoma) and chromosomal instability, involving loss or gain of large chromosomal regions or even chromosomes. In MALT lymphoma there are found at least three recurrent chromosomal translocations in most cases (65%); t(11;18)(q21;q21) that results in the appearance of a chimerical fusion gene API2-MALT; t(1;14)(p22;q32) that alters the expression bcl10; and t(14;18)(q32;q21) that causes the alteration of the MALT1 expression. Concerning different genes, all these translocations result in the activation of nuclear factor kB (NFkB), which has an important regulator role of numerous genes involved in immunity and apoptosis, and consequently in the progression of lymphoma.

KEY WORDS MALT lymphoma, Helicobacter pylori, nuclear factor kB

Introduction

Mucosa associated lymphoid tissue lymphomas represent 7-8% of B-cell lymphomas [9]. They originate in the lymphatic tissue of the stomach lining and they often occur after a chronic infection with Helicobacter pylori. Normally, the stomach has no lymphatic tissue. The first published studies have shown the presence of bacteria in 90% of cases [40]. In a Japanese study, Helicobacter pylori was detected in 61% of patients with primary gastric lymphoma. The incidence of infection was higher in patients with MALT lymphoma (72%) compared with those with primary gastric diffuse large-cell lymphoma (55%). Moreover, the appearances of Helicobacter pylori positive were significantly fewer in the cases of primary gastric lymphoma compared to cases of chronic active gastritis (100%) or peptic ulcer (91%) [21]. Helicobacter pylori is a spiral germ revealed for the first time in 1983 by Warren and Marshall, at the level of the gastric mucosa in patients with peptic ulcer. At that time, it was thought that it could have an etiologic role in the development of ulcer disease. The presence of Helicobacter pylori was revealed in approximately 70% of the patients with gastric ulcer and in up to 90% of the patients with duodenal ulcer. Subsequently, there was defined the role of Helicobacter pylori in the pathogenesis of a variety of gastric diseases: chronic gastritis, peptic ulcer, gastric carcinoma. Besides the tumour specific T cells, it is necessary for Helicobacter pylori that malignant B cells have also certain features allowing their uncontrolled proliferation at their stimulation by T lymphocytes. There are probably some genetic abnormalities that confer them growth advantage or abnormal biological properties such as the ability to recognize autoantigens. Malignant cells from MALT lymphoma present genetic instability and a series of genetic abnormalities. The consequence of all these genetic alterations is the appearance of the imbalance between cell proliferation and programmed cell death (apoptosis).

Association with Helicobacter pylori positive chronic gastritis

Helicobacter pylori is a germ adapted to acid environment and to the particularities of gastric mucosa. Gastric mucosal inflammation occurs because of the enzymes it releases (urease, phospholipase A, cytotoxin …) and it is maintained by inflammatory mediators such as platelet activating factor (FAP), leukotriene B4 and phospholipase A2 [24]. It was demonstrated that gastric infection with Helicobacter pylori causes the accumulation of lymphoid tissue in gastric mucosa in which it was noted the presence of lymphoid follicles. In 1984, Issacson et al. showed that the presence of lymphoid follicles in gastric mucosa is pathognomonic of Helicobacter pylori infection. Moreover, besides the lymphoid follicles, it also appears the lymphoepitheloid lesions, a defining feature of MALT. Epidemiological and experimental data support the hypothesis that the microorganism can be an antigenic
stimulus able to support the development of gastric lymphoma [43, 3, 17]. The aetipathogenetical correlation between Helicobacter pylori and primary gastric lymphoma was confirmed biologically and molecularly, both on gastric biopsies made on patients with long history of chronic gastritis. Their histological and molecular study revealed the presence of clonal B lymphocytes that later will be at the origin of the tumour [44]. There are studies that support the role of trigger of Helicobacter pylori in the development of MALT tissue and the presence in almost all cases of MALT-type lymphomas of the anti-Helicobacter pylori antibodies [12]. However, epidemiological studies have not shown a statistically significant association between the infection with Helicobacter pylori and the development of a primary gastric lymphoma, neither in the case of MALT lymphomas nor in the case of primary gastric diffuse large-cell lymphoma [26]. Although the Helicobacter pylori infection is present in more than 50% of the world population, most people who are infected do not develop gastric lymphomas; so, it is widely accepted that an additional factor (environmental, infectious or genetic) is necessary to initiate lymphomogenesis. The Cag A Helicobacter pylori infection (cytotoxin-associated antigen A) is involved in gastric carcinoma [1] and it was speculated that the Cag A-positive Helicobacter pylori infection could be involved in lymphomogenesis. If the involvement of Helicobacter pylori in primary gastric lymphoma aetipathogenesis and the favourable response of the disease to the eradication therapy tend to become postulated, much less known is the role of the host’s immune response in pathogenesis, as long as only a small proportion of infected patients develop lymphoma. The difference between MALT incidence and the infection incidence can be correlated with the differences between the host’s inflammatory response mediated by cytokines and the HLA polymorphism. For example, there was discovered a significant decrease of the HLA-B35 expression in the case of gastric MALT lymphoma patients [31]. However, none of them is sufficient to cause lymphoma, one or both requiring the presence of genetic abnormalities that lead to irreversible progression to MALT lymphoma [8]. Gastric lymphoma originates in the lymphoid tissue called "acquired" MALT. Lingering Helicobacter pylori infections lead to the accumulation of lymph follicles (reagents) in the gastric mucosa, surrounded by B lymphocytes, as in the MALT tissue. The mechanism of action of Helicobacter pylori is indirect: initially it causes a gastritis development that is followed by the aggregation of T CD4+ lymphocytes and B lymphocytes in gastric lamina propria [35]. T lymphocytes activated by the presence of the antigen determine the B lymphocytes proliferation and the appearance of lymphoid follicles, similar to those from the structure of the Peyer plaques from the ileum. Arguments in favour of the association between Helicobacter pylori infection and MALT-type gastric lymphomas:

- in 92% of the cases of MALT gastric lymphomas, it was demonstrated the presence of Helicobacter pylori infection on the anathomopathological samples that were analysed [10].
- the serological test is positive for Helicobacter pylori in 85% of patients with gastric lymphoma [35].
- the titer of anti-Helicobacter pylori antibodies determined by months or years before the appearance of malignant lymphoproliferation revealed their presence in 90.9% of cases [40].
- in vitro studies – proliferating cells in a gastric lymphoma with low malignancy B cells in culture, survive 5 days in standard conditions. Adding different types of Helicobacter pylori destroyed by heat resulted in aggregation and proliferation of tumour cells. This phenomenon was associated with the expression of receptors for IL2 and the IL2 release by tumour cells. In the same experiment, performed using proliferating cells of other types of lymphomas of low malignancy, there was no response in relation to the bacteria. Removal of all T lymphocytes from the gastric lymphoma cell suspension before the cell cultivation determined the absence of Helicobacter pylori-induced activation. These findings are likely to show that Helicobacter pylori stimulates the intra-tumoural T cells, which in turn promote tumour cell proliferation. Therefore, B tumour cells are not stimulated directly by Helicobacter pylori [12].
- the gastric MALT lymphoma regression of low malignancy after the antibiotic therapy of eradication of Helicobacter pylori.

Initially, the neoplastic proliferation of B cells is induced antigenically and it depends on the Th cells activation. In a recently published study (2011), it is demonstrated the presence, in the gastric mucosal cells, of some elevated levels of cytokines, particularly of a cytokine called APRIL, which belongs to the tumour necrosis factors family and which has already a demonstrated role in B cells maturation and survival. APRIL seems to be produced in excessive amounts by macrophages present in the lymphomatous gastric infiltrate, located close to the neoplastic cells. They produce APRIL both at the direct stimulation of Helicobacter pylori and also while being stimulated by T lymphocytes which are activated in turn by T lymphocytes. It is the first study publishing the APRIL involvement in MALT lymphoma aetiopathogenesis. In repeated biopsies of the gastric mucosa of patients with Helicobacter pylori-positive chronic gastritis and with primary gastric
lymphoma, there were revealed numerous macrophages producing APRIL, which are extremely rare in normal gastric mucosa. A further proof is represented by the in vitro studies which have demonstrated the ability of Helicobacter pylori to induce the APRIL expression in monocytes from which arise macrophages, these ones indicating a primary role of bacteria in the induction of APRIL production by cells derived from monocytes. A series of experimental and clinical arguments support the idea that APRIL supports the B cells transformation and the progression to the diffuse large B-cell lymphoma. Different forms of B lymphomas contain APRIL-positive cells, which are represented either by neoplastic cells or by immune cells that accompany the neoplastic cells, including macrophages [30, 5, 33]. Macrophages are also abundant in the case of primary gastric diffuse large B-cell lymphoma, in the absence of Helicobacter pylori. This fact demonstrates that the role of APRIL in lymphoma induction reflects only partially the activation of macrophages associated with tumour proliferation. APRIL expression was also demonstrated in macrophages-like cells, the monocytes from which macrophages are derived, and in inflammatory macrophages [16]. The role of Helicobacter pylori in the gastric MALT lymphoma is well established, considering that chronic antigenic stimulation induced by Helicobacter pylori is essential for the appearance of the lymphoma. The survival and transformation of B-cells in lymphomatous cells requires additional signals, coming either from the T lymphocytes either directly by the antigenic autostimulation of neoplastic cells [13]. Gastric inflammation causes the appearance of a large number of macrophages, which, under a Helicobacter pylori infection, release large amounts of APRIL. This mechanism may be enhanced and maintained by the activated T lymphocytes. As a further evidence of this involvement in the lymphomogenesis, it is the fact that APRIL-producing macrophages are very few in the gastric mucosa of patients with MALT lymphoma who are in complete remission of the disease obtained after the eradication therapy for the infection [20]. Based on this new evidence concerning lymphomogenesis, a new effective therapy, targeted, could be based on blocking the production of APRIL, the target of this therapy being represented by macrophages and by the cytokines secreted by them [36]. The MALT tissue development and the appearance of the primary gastric lymphomas have been correlated with other Helicobacter species, for example, Helicobacter heilmannii. Helicobacter heilmannii infection is 300 times rarer than the Helicobacter pylori one [19, 22]. There have been reported 5 cases of gastric lymphoma patients infected with this Helicobacter pylori strain, who after the eradication of the infection after 2 years of follow up there was no lymphoma relapse or recurrence of infection, which would be an argument in favour of the link between the Helicobacter heilmannii infection and the appearance of a primary gastric lymphoma [19]. Data provided by the low frequency of Helicobacter pylori in large-cell lymphomas without MALT component in comparison with the MALT lymphoma and the large B-cell lymphoma with MALT component, say that Helicobacter pylori has a minor role in high malignancy lymphomas without the MALT component [24, 40]. In these cases, it was revealed the presence of a non-Helicobacter pylori flora, which could have an important role in the development of these lymphomas. Therefore, although it has a proven important role, Helicobacter pylori may not be the only etiologic factor in the development of gastritis, of MALT tissue and of primary gastric lymphomas [10, 12, 27]. Available data up to now remain contradictory. The way of developing of a MALT lymphoma could be outlined as follows: the antigen presenting cell presents the antigen to the T CD4+ lymphocyte that activates and induces the proliferation of B lymphocytes. Besides the tumour specific T cells, it is necessary for Helicobacter pylori that malignant B cells have also certain features allowing their uncontrolled proliferation at their stimulation by T lymphocytes. There are probably some genetic abnormalities that confer them growth advantage or abnormal biological properties such as the ability to recognize autoantigens. Chromosomal abnormalities are present in lymphomas in a percentage ranging from 76 to 100% in various statistics, a percent which is higher than in acute leukemia, but their prognostic importance seems to not exceed that of abnormalities in acute leukemias [39]. Their role in the pathogenesis of malignant lymphoproliferations is indubitable, creating a favourable terrain, the accumulation of new cytogenetic abnormalities during the clonal evolution being a contributing factor for the turning of a lymphoma of low malignancy into one of high malignancy that is resistant to therapy. The consequence of genetic changes in lymphomas is represented by breaking the balance between cell proliferation and programmed cell death (apoptosis). The increasing of cell proliferation rate and/or the decreasing of cell death rate are involved in the aetopathogenesis of gastric lymphomas [38]. Gastric lymphomas are characterized by instability in the microsatellite (the MALT lymphoma) – somatic mutations consisting in insertions or deletions of repetitive sequences in the tumour DNA, these sequences being called microsatellites – and chromosomal instability [7]. In MALT lymphomas there have not been described translocations involving the c-myc gene, but in 20% of patients there were identified point mutations [23, 29]. C-myc gene expression disorder has as consequence the activation of the apoptotic process [23]. The c-myc activation in
folicular lymphomas with a primary digestive inception in the absence of t(8; 14), t(2; 8) or t(8; 22), as well as the activation of the bcl 2 oncogene in the folicular lymphomas of the lymph nodes are arguments of different pathogenic mechanisms and of the different origin of the proliferating cell [23]. Chromosomal instability is the most common type of genetic abnormality found in gastric lymphomas, involving losses or gains of large chromosomal regions or even chromosomes. Genes that encode the synthesis of surface immunoglobulin (B lymphocyte antigen receptor) present somatic hypermutations and intrachromosomal variations, which suggests that there is an antigenic selection in the germinal center, followed by a continuous clonal expansion, conducted directly (at least partially) by the antigenic stimulation [4]. One of the mechanisms that would explain the occurrence of B lymphomas translocations and their particular character would be represented by the illegitimate recombinations at the level of VDJ fragments that encode the variable portion of the antigen receptor of B lymphocyte, respectively, the surface Ig. Illegitimate recombination means the participation at the genetic rearrangement process of some genic loci that normally do not enter the structure of immunoglobulins, such as bcl 2 and c-myc genes [14; 23]. In MALT lymphoma there are at least three recurrent chromosomal translocations in the most cases (65%): t(11;18)(q21;q21) that results in the appearance of a chimerical fusion gene API2-MALT1; t(1;14)(p22;q32) that alters the expression bcl10; and t(14;18)(q32;q21) that causes the alteration of the MALT1 expression. Concerning different genes, all these translocations result in the activation of nuclear transcription factor NFkB (nuclear factor kB), which has an important regulator role of numerous genes involved in immunity and apoptosis [6, 13, 42]. Consequently, the activation of NFkB has an important role in the development and progression of lymphoma. Other genetic abnormalities identified in MALT lymphoma are trisomy 3, p53 abnormalities and p16 deletions [42]. t(11;18)(q21;q21) is the most common chromosomal abnormality found in MALT lymphoma (13-35% of cases), not being encountered in the marginal zone lymphoma with lymph nodes or splenic inception. Translocation was also detected exceptionally in the de novo diffuse large B-cell lymphoma with gastric location [2]. From the translocation it results a fusion between the API2 gene (inhibitor of apoptosis) located on chromosome 11 and the MALT1 gene located on chromosome 18. API2 which is excessively expressed in lymphoid cells is part of the inhibitor of apoptosis (IAP) family of genes. The API2-MALT1 fusion gene synthesizes the protein called API2-MALT1 that causes a marked inhibition of apoptosis, thus favouring the survival of malignant cells and their antigen-dependent proliferation [11]. MALT lymphomas with t(11;18) are genetically stable and they rarely accumulate new genetic abnormalities, unlike the negative ones for this translocation in which there are signalled many genetic abnormalities [2]. MALT and t(11;18)(q21; q21) lymphoma patients do not respond at the eradication therapy of the Helicobacter pylori infection and they are usually diagnosed in advanced stages. t(14; 18)(q32; q21) involves the gene that encodes the surface immunoglobulin heavy chain located on chromosome 14 (Fig. 2) and the MALT1 gene located on chromosome 18. It is present in approximately 15-20% of the cases of MALT.
lymphoma. It appears extremely rare in the primary gastric lymphoma, being found most commonly in the location at the level of the salivary glands or of the MALT lymphoma ocular annexes [34, 37]. t(1,14)(p22;q32) causes disruption of BCL10 expression in neoplastic B cell nucleus, causing the activation of NFkB [13]. The molecular consequences of these cytogenetic abnormalities have as final target the NFkB. In physiological conditions, the binding between bcl10 and MALT1 is crucial in activating MALT1, leading to activation of NFkB. Unlike the normal protein MALT1, API2-MALT1 fusion protein is able to activate NFkB independently of BCL10 [13, 42]. It is very likely that the bacterial infection, by interacting with the host’s individual factors, lead to the appearance of lymphoma. Polymorphism interacting with the host’s individual factors, lead to the appearance of lymphoma.

Conclusions

Although the involvement of Helicobacter pylori in MALT lymphoma etiopathogenesis is proven and widely accepted, the infection alone cannot lead to lymphoma. Lymphomogenesis is a multistep process, attended by a number of factors (genetic, cytokines, infectious, environmental factors), leading eventually to the selection of B lymphocytes that become neoplastic, acquiring growth and survival advantage compared to normal lymphocytes. It is important to decipher the exact mechanism of neoplastic transformation, because this is important in the discovery of new therapeutic approaches with curative character, regardless of the stage of the disease.

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