REVIEWS

Gastric MALT lymphoma: a model of chronic inflammation-induced tumor development

Xavier Sagaert, Eric Van Cutsem, Gert De Hertogh, Karel Geboes and Thomas Tousseyn

Abstract | Mucosa-associated lymphoid tissue (MALT) lymphoma, or extranodal marginal zone lymphoma of MALT, is an indolent B-cell non-Hodgkin lymphoma arising in lymphoid infiltrates that are induced by chronic inflammation in extranodal sites. The stomach is the most commonly affected organ, in which MALT lymphoma pathogenesis is clearly associated with *Helicobacter pylori* gastroduodenitis. Gastric MALT lymphoma has attracted attention because of the involvement of genetic aberrations in the nuclear factor κB (NFκB) pathway, one of the most investigated pathways in the fields of immunology and oncology. This Review presents gastric MALT lymphoma as an outstanding example of the close pathogenetic link between chronic inflammation and tumor development, and describes how this information can be integrated into daily clinical practice. Gastric MALT lymphoma is considered one of the best models of how genetic events lead to oncogenesis, determine tumor biology, dictate clinical behavior and represent viable therapeutic targets. Moreover, in view of the association of gastric MALT lymphoma with dysregulation of the NFκB pathway, this signaling pathway will be discussed in depth in both normal and pathological conditions, highlighting strategies to identify new therapeutic targets in this lymphoma.

Introduction

The marginal zone of B-cell follicles is especially well developed in lymphoid organs that are continuously exposed to antigenic stimulation, such as the spleen, mesenteric lymph nodes and mucosa-associated lymphoid tissue (MALT).[1] The role of the splenic marginal zone has been well investigated. Splenic marginal zone B cells have a crucial role in T-cell-independent responses to various antigens, including polysaccharides derived from encapsulated bacteria (such as *Hemophilus influenzae*, *Neisseria meningitides* and *Streptococcus pneumoniae*).[2] As such, suboptimal functioning of the marginal zone, as seen in children under the age of 2 years or in splenectomized individuals, dramatically increases vulnerability to infections with encapsulated bacteria, and therefore vaccination against these organisms is advised in these groups.[3,4] Neoplasms of the marginal zone are termed marginal zone lymphomas, and are the third most frequent type of B-cell non-Hodgkin lymphoma (~8%) after diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma. The WHO makes a distinction between MALT lymphoma, splenic marginal zone lymphoma and nodal marginal zone lymphoma subtypes on the basis of their anatomic location.[5]

Extranodal marginal zone lymphoma of MALT, or MALT lymphoma, differs from its splenic and nodal counterparts as it arises in organs that normally lack lymphoid tissue (such as the stomach, lung and salivary and lacrimal glands) but that have accumulated B cells in response to either chronic infection or autoimmune processes.[6–13] Sustained antigenic (or autoantigenic) stimulation not only triggers polyclonal B-cell proliferation, but also attracts neutrophils to the site of inflammation. The subsequent release of reactive oxygen species (ROS) causes a wide range of genetic aberrations.[14] Moreover, the prolonged proliferation of B cells induced by chronic inflammation may also increase the risk of double-stranded DNA breaks and translocations, owing to the inherent genetic instability of B cells during somatic hypermutation and class-switching recombination.[15] Several of these mutagenic events in MALT lymphomas have been identified, including trisomy of chromosomes 3, 7, 12 or 18 and the disease-specific chromosomal translocations t(1;14)(p22;q32), t(14;18)(q32;q21), t(11;18)(q21;q21) and t(3;14)(p13;q32). Remarkably, at least two of these trisomies and at least three of these translocations affect genes with protein products that are involved in the same pathway, and result in activation of nuclear factor κB (NFκB). This transcription factor is a key mediator of the immune response and has been the focus of intense investigation over the past two decades.[16] NFκB regulates the expression of a number of genes implicated in survival and proliferation of B cells;[17,18] as such, its constitutive activation may result in uncontrolled B-cell proliferation and thus lymphoma.[19]

This article focuses on gastric MALT lymphoma, which—at 5% of all gastric neoplasms and at least 50% of all gastric lymphomas—is the most frequent lymphoma of the gastrointestinal tract. Owing to its association with

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infection by *Helicobacter pylori*, gastric MALT lymphoma provides a clear illustration of how chronic inflammation is closely linked with lymphoma pathogenesis. In this Review, we discuss current insights into the pathogenesis of gastric MALT lymphomas and describe how this information can be integrated into daily clinical practice. Strategies to identify new therapeutic targets are highlighted. Nongastric MALT lymphomas are only briefly mentioned in this article, as they are considered to be outside the scope of this Review, and have been reviewed in depth elsewhere.[20,21]

Clinical and pathological features

Endoscopic mucosal biopsy remains the gold standard technique for diagnosing gastric MALT lymphomas. Histologically, the tumor appears as a diffuse spread of neoplastic lymphoma cells that surround reactive B-cell follicles and invade epithelial structures, resulting in so-called lymphoepithelial lesions (Figure 1). Diagnosis can be difficult, especially in cases where lymphoepithelial lesions are not prominent and/or reactive B-cell follicles cannot be recognized because of neoplastic colonization. Molecular techniques such as polymerase chain reaction (PCR) can support the diagnosis of a MALT lymphoma by identifying clonal populations of B cells, which all have the same immunoglobulin gene rearrangement.

Most MALT lymphomas present as extranodal disease limited to the site of origin (Ann Arbor stage IE disease). The only prospective study to undertake histopathological staging of gastric MALT lymphomas from over 200 patients showed involvement of the regional lymph nodes (stage IIE) in 32% of cases, and secondary tumors in multiple extranodal sites (for example, the Waldeyer ring, intestine and spleen) in 11% of cases at the time of presentation.[22] Consequently, thorough tumor staging should be performed at diagnosis, especially of disease at the above-mentioned sites. Gastric MALT lymphoma is remarkably indolent and tends to remain localized for several years. The coexistence of gastric MALT lymphoma and DLBCL in some patients, as well as evidence provided by transcriptional profiling, indicates that MALT lymphomas can go on to develop into aggressive DLBCLs, and some authors suggest the designation 'blastic MALT lymphoma' for this DLBCL subtype.[23] Patients with gastric MALT lymphoma have a 10-year survival of ~90% and disease-free survival of ~70%.[24,25] Once progression to DLBCL occurs, the data on survival are conflicting: in the early 1990s, two retrospective studies reported 5-year survival of 42% and 56%, respectively,[24,26] while prospective studies conducted 10 years later showed no or only a marginal difference in overall survival between gastric MALT lymphoma and gastric DLBCL.[27,28]

Pathogenesis

The pathogenesis of MALT lymphoma involves several distinct steps that result in transformation from a reactive, polyclonal lymphoproliferation to a neoplastic, monoclonal lymphoproliferation (Figure 2). Increasing evidence suggests that chronic antigen stimulation

Key points

- Diagnosis of gastric mucosa-associated lymphoid tissue (MALT) lymphoma is made by morphologic analysis of endoscopic mucosal biopsy samples and supported by molecular genetic analysis

- Gastric MALT lymphoma is caused by *Helicobacter pylori* infection; therefore, every diagnosis of this lymphoma should prompt a thorough investigation for the presence of *H. pylori*

- To date, gastric MALT lymphoma is the only malignancy for which antibiotics are the first choice of therapy with curative intent

- General screening for the chromosomal translocation t(11;18)(q21;q21) is not recommended; however, its presence can aid diagnosis of gastric MALT lymphoma and predict resistance to *H. pylori* eradication treatment

- A subgroup of gastric MALT lymphomas is characterized by chromosomal translocations that affect genes encoding molecules involved in activation of nuclear factor κB, which might represent attractive therapeutic targets

- A watch-and-wait policy is now considered adequate management for patients with minimal residual disease after successful *H. pylori* eradication therapy
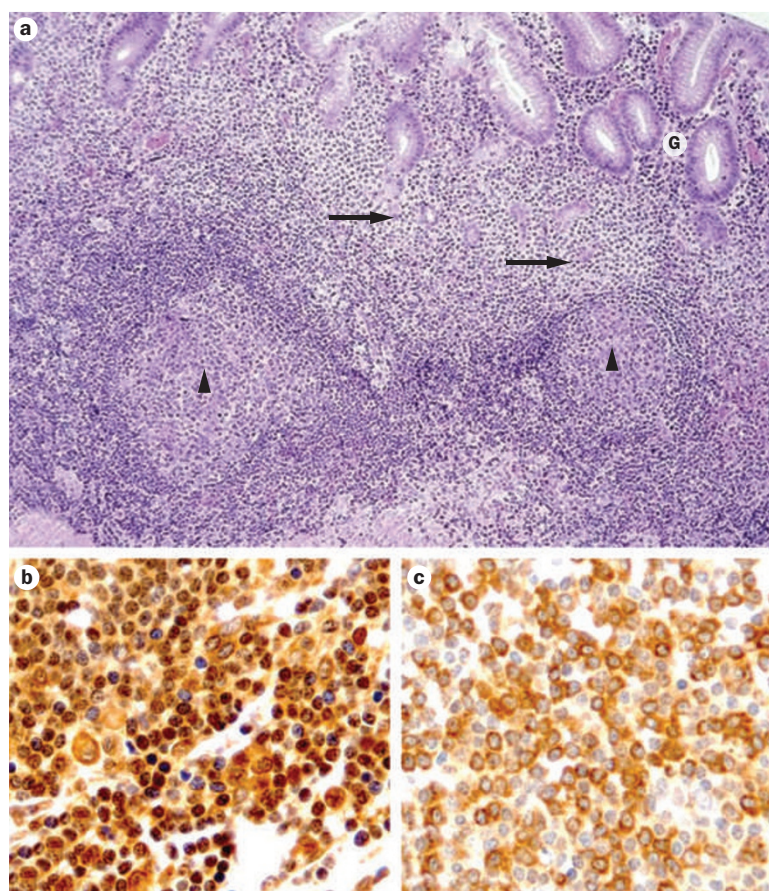

Figure 1 | Morphologic and immunohistochemical features of gastric MALT lymphoma. **a** | Gastric MALT lymphoma cells surround reactive B-cell follicles (arrowheads) and invade the gastric glandular epithelium (G), resulting in so-called lymphoepithelial lesions (arrows). Hematoxylin and eosin stain, original magnification ×10. **b** | Nuclear Bcl-10 expression is a typical feature of t(11;18)(q21;q21)-positive and (1;14)(p22;q32)-positive MALT lymphomas. **c** | t(14;18)(q32;q21)-positive MALT lymphomas are characterized by perinuclear Bcl-10 expression. Panels b and c both utilized Bcl-10 staining with original magnification ×400. Abbreviation: Bcl-10, B-cell lymphoma 10.

precedes MALT lymphoma pathogenesis.[21] MALT lymphomas of the stomach and lung, which are exposed to an abundant influx of exogenous environmental antigens,
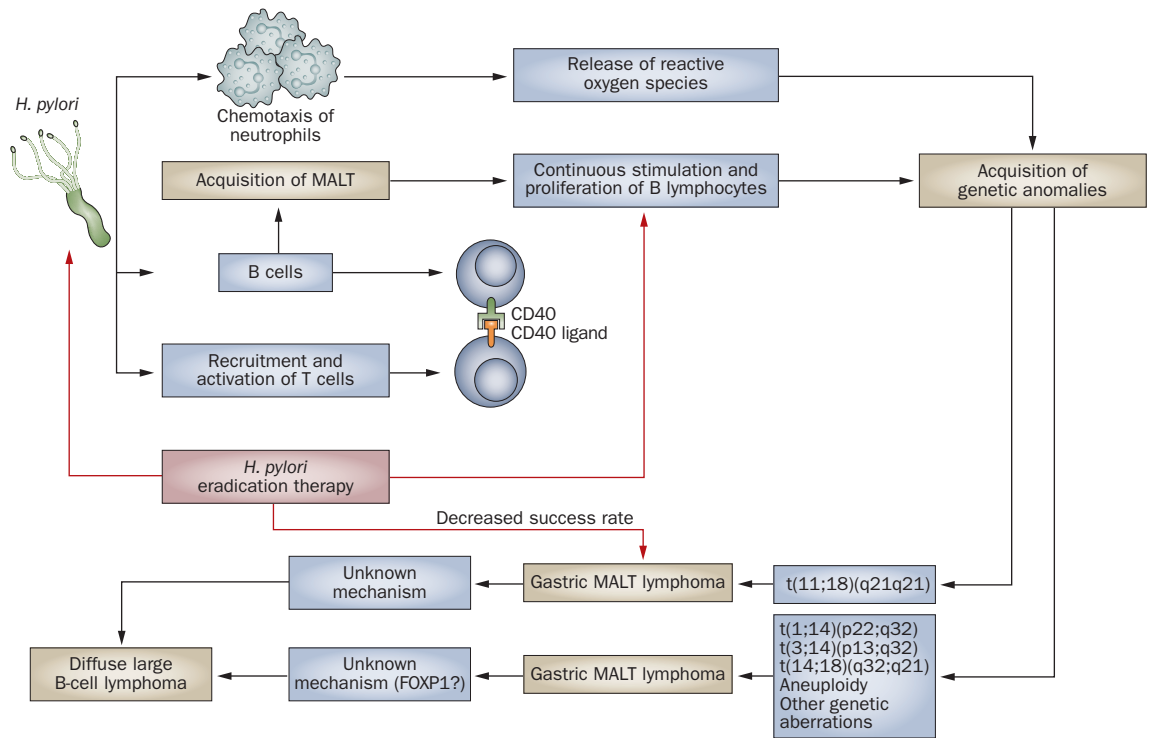
Figure 2 | Hypothetical model of gastric MALT lymphoma pathogenesis. *H. pylori* infection attracts B cells, T cells and neutrophils to the gastric mucosa. B-cell proliferation is driven by CD40–CD40 ligand interaction with *H. pylori*-activated, reactive T cells, as well as by cytokines. The chronic proliferative state of these B cells, as well as neutrophil-mediated release of reactive oxygen species in areas of chronic inflammation, induces additional oncogenic events that eventually make lymphoproliferation independent of antigenic stimulation. *H. pylori* eradication therapy inhibits (red arrows) these tumor-promoting processes, although t(11;18)(q21;q21)-positive MALT lymphomas are less likely to respond to this therapy than are tumors without this translocation. Additional genetic alterations in t(11;18)(q21;q21)-negative MALT lymphomas can ultimately result in transformation to clinically aggressive diffuse large B-cell lymphoma.

are thought to be associated with a known or unknown infectious agent, whereas MALT lymphomas in the salivary glands and ocular adnexa are often linked to autoimmune disease.[21]

In contrast to MALT lymphomas at other sites, however, only two microbial species are currently associated with gastric MALT lymphoma: *H. pylori* and *Helicobacter heilmannii*. Gastric MALT lymphomas constitute a distinct category of infection-related lymphomas, in which these infectious agents induce chronic activation of the immune system and maintain a protracted proliferative status of lymphocytes. Eventually neoplastic lymphoid transformation occurs. This transformation process is in contrast to that associated with the lymphotropic oncogenic viruses (human herpesvirus 8, human T-lymphotropic virus 1, and Epstein–Barr virus), which directly infect and transform lymphoid cells into tumor cells.

First step: gastric acquisition of MALT
Unlike the rest of the gastrointestinal tract, the stomach lacks MALT under physiological conditions, because the low pH prevents the survival of lymphocytes in the gastric wall. However, infection with either *H. pylori* or *H. heilmannii* results in buffering of the gastric pH owing to the secretion of bacterial urease. The decreased acidity of stomach secretions, combined with the presence of

a bacterial infection, triggers lymphoid infiltration and thus gastric acquisition of MALT.[21]

The role of antigen stimulation
Evidence for the role of an antigen (or autoantigen) in gastric MALT lymphoma pathogenesis is also supported by sequence analysis of the immunoglobulin heavy chain locus (*IGH@*) in DNA from MALT lymphomas.[29] This study revealed the occurrence of somatic hypermutation in the variable regions of *IGH* with a pattern indicative of antigen selection.[29] The *IGH* genes from MALT lymphoma samples frequently included sequence variants implicated in autoantibody production.[29] In addition, ~50% of the tumors showed intraclonal variation in the *IGH* locus, which suggests that continued antigenic stimulation is also important in clonal B-cell expansion.[29] As both somatic hypermutation and intraclonal variation are antigen-driven processes, their occurrence in gastric MALT lymphoma strongly indicates a role for antigens or autoantigens during both initiation and progression of this neoplasm. However, the ongoing mutation rate decreases as these tumors progress, which indicates that direct antigenic stimulation becomes steadily less important in gastric MALT lymphoma pathogenesis. This change is probably due to accumulation of ROS-induced genetic anomalies, which make lymphoproliferation progressively less dependent on antigenic stimulation over time.

H. pylori infection

The infectious etiology of gastric MALT lymphoma is the best documented among all marginal-zone lymphomas. In healthy individuals, a thick mucus layer and gastric acid limit bacterial colonization of the stomach. However, *H. pylori* can survive in this environment. Gastric MALT lymphoma (as well as peptic ulcers and gastric carcinoma) has now been proven to be caused by *H. pylori* infection.[30] First, the prevalence of *H. pylori* bacteria in the gastric mucosa and of *H. pylori* seropositivity in patients with gastric MALT lymphoma is well above that in other populations, with one study revealing *H. pylori* seropositivity in up to 98% of patients with gastric MALT lymphomas.[12,30,31] Second, gastric MALT lymphoma has the highest incidence in regions of endemic *H. pylori* infection.[32] Third, *H. pylori* triggers T-cell-mediated, clonal B-cell expansion *in vitro* by activating the CD40 pathway.[33] Fourth, *H. pylori* eradication therapy leads to complete lymphoma regression in about 80% of patients with early stage disease.[34,35] Finally, gastric MALT lymphomas can be induced *in vivo* in mouse models by chronic *H. pylori* infection.[36] Although the causal link between *H. pylori* infection and gastric MALT lymphoma is indisputable, the reasons why some *H. pylori*-infected patients develop gastric MALT lymphomas whereas others do not are still unclear. The virulence of different *H. pylori* strains has been ruled out as a possible explanation.[37] The potential role of the host's genetic background as a risk factor for gastric MALT lymphoma was investigated in one study that documented polymorphisms in *MALT1* (which encodes MALT lymphoma translocation protein 1) in a large cohort of patients with this neoplasm, but no evidence of such a link was found.[38]

Remarkably, despite proliferation of antibody-producing gastric MALT lymphoma cells after *H. pylori* stimulation, tumor-derived immunoglobulins recognize various autoantigens instead of *H. pylori*.[39] One possible hypothesis is that gastric MALT lymphoma arises from *H. pylori*-stimulated but autoreactive B cells. Conversely, 5–10% of patients with gastric MALT lymphoma are *H. pylori*-negative.[12,30,31] In some of these cases, *H. pylori* infection may be present, but undiagnosed. This situation is particularly likely if *H. pylori* testing is performed on only the biopsy sample, as some lymphomas arise in atrophic mucosa where the *H. pylori* bacterial load is low. Negative *H. pylori* test results in a patient with gastric MALT lymphoma should, therefore, prompt further investigation by means of a ^{13}C-urea breath test, serological antibody test, immunostaining of biopsy tissue samples and/or stool culture. Some *H. pylori*-negative gastric MALT lymphomas seem to be associated with *H. heilmannii* infections and, interestingly, these lymphomas also respond to treatment with the same antibiotics used in *H. pylori* eradication therapy.[40]

Second step: acquisition of genetic abnormalities

Some genetic abnormalities are strongly associated with MALT lymphomas arising in specific locations, and this polarization is considered to reflect a distinct underlying pathogenesis. For example, genetic differences might reflect different exposure to various inflammatory agents associated with MALT lymphomas in disparate locations. Besides trisomy of chromosomes 3, 7, 12 and 18, the chromosomal translocations t(11;18)(q21;q21), t(1;14)(p22;q32), t(14;18)(q32;q21) and t(3;14)(p13;q32), which result in *BIRC3–MALT1*, *IGH–BCL10*, *IGH–MALT1* and *IGH–FOXP1* rearrangements, respectively, are well known to occur with variable frequencies in gastric (and also nongastric) MALT lymphomas.[41–44] These translocations can be detected in either fresh frozen or paraffin-embedded tumor tissue by reverse-transcription PCR and fluorescence *in situ* hybridization (FISH). One or both of these techniques are now routinely performed in most laboratories as screening tests for various cancers. Aneuploidy can be identified by karyotyping or FISH (using special probes), but these two techniques are only available in specialized academic laboratories.

t(11;18)(q21;q21)

The translocation t(11;18)(q21;q21) is the most common structural chromosomal abnormality in gastric MALT lymphomas. Remarkably, this translocation is not found in other lymphoma types and its presence in gastric MALT lymphoma correlates with the absence of any further genetic aberrations.[45,46] Although the presence of t(11;18)(q21;q21) may facilitate and/or confirm the diagnosis of MALT lymphoma, current guidelines do not recommend routine screening for this translocation once the diagnosis of a gastric MALT lymphoma has been established.[47] Depending upon the study performed, t(11;18)(q21;q21) is present in 10–50% of gastric MALT lymphomas, whereas this translocation is rare in nongastric MALT lymphomas other than pulmonary MALT lymphomas.[41,48–50] The translocation t(11;18)(q21;q21) fuses the amino (N)-terminus of the *BIRC3* gene (formerly termed *API2*, on chromosome 11q21) to the carboxyl (C)-terminus of the *MALT1* gene (located on chromosome 18q21), which creates the fusion gene *BIRC3–MALT1* (Figure 3).[51]

BIRC3 (baculovirus inhibitor of apoptosis protein repeat-containing protein 3) is a member of the inhibitor of apoptosis family and inhibits the biological activity of certain caspases.[52] It contains three baculovirus inhibitor of apoptosis repeat (BIR) domains, a CARD (caspase recruitment domain) motif and one RING finger motif. MALT1 (also known as paracaspase) is a key mediator of the antigen-receptor signaling pathway that leads to NFκB activation.[53] This protein comprises an N-terminus death domain, two immunoglobulin-like domains and a C-terminus caspase-like domain.[53] The break points in both *BIRC3* and *MALT1* are well characterized.[41,50,51,54–57] All break points result in *BIRC3* being fused in-frame to *MALT1* and generate a total of four variants of the BIRC3–MALT1 fusion protein, all of which contain the three intact BIR domains of the N-terminus BIRC3 portion and the intact caspase-like domain of the C-terminus MALT1 portion. The location of the *MALT1* break point seems to be associated with specific gene expression profiles in t(11;18)(q21;q21)-positive gastric MALT lymphomas.[58]
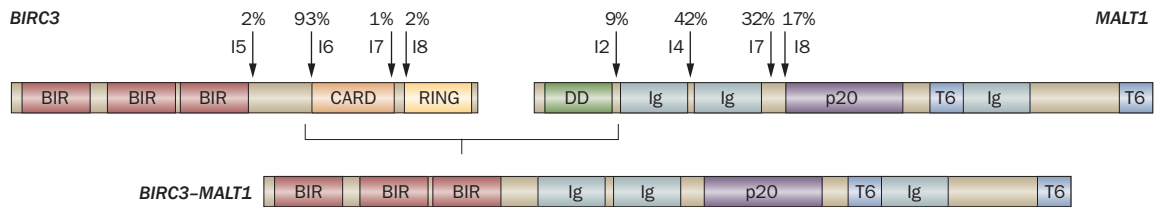
Figure 3 | The *BIRC3–MALT1* fusion gene. Known break points (arrows) in *BIRC3* and *MALT1* are shown with their frequencies. The break points within *BIRC3* almost always occur in I6 (according to Ensembl Gene ENSG00000023445), whereas those within *MALT1* are located in I2, I4, I7 and I8, which result in four possible versions of the *BIRC3–MALT1* fusion gene: *BIRC3*(I6)–*MALT1*(I2), *BIRC3*(I6)–*MALT1*(I4), *BIRC3*(I6)–*MALT1*(I7) and *BIRC3*(I6)–*MALT1*(I8). The fusion gene depicted is the *BIRC3*(I6)–*MALT1*(I4) version. Abbreviations: BIR, baculovirus inhibitor of apoptosis repeat; CARD, caspase recruitment domain; DD, death domain; I, intron; Ig, immunoglobulin-like; p20, caspase-like p20 domain; RING, really interesting new gene; T6, tumor necrosis factor receptor associated factor 6 binding site.

Several studies have revealed that t(11;18)(q21;q21)-positive gastric MALT lymphomas are more often resistant to *H. pylori* eradication treatment than are tumors lacking this translocation.[50,59–61] Nevertheless, complete lymphoma regression can still be obtained in 20% of patients with (11;18)(q21;q21)-positive disease after *H. pylori* eradication. Consequently, all patients who have *H. pylori*-positive gastric MALT lymphoma should undergo eradication therapy, regardless of their t(11;18)(q21;q21) status.[62] For a decade, clinicians assumed that t(11;18)(q21;q21)-positive gastric MALT lymphomas rarely, if ever, evolved to DLBCL,[50,59–61] but new data have shown that this translocation can be found at approximately equivalent frequencies in both gastric MALT lymphomas and gastric DLBCLs.[63] Thus, the presence of t(11;18)(q21;q21) in gastric MALT lymphoma does not exclude progression to DLBCL.

The presence of t(11;18)(q21;q21) in gastric MALT lymphomas is also strongly associated with infection by CagA-positive strains of *H. pylori*.[31,50] These strains generate a strong inflammatory response characterized by release of interleukin (IL)-8, a powerful chemokine involved in neutrophil activation and subsequent ROS secretion.[50] A tempting hypothesis, therefore, is that the genetic abnormality t(11;18)(q21;q21) is a consequence of the increased oxidative stress associated with inflammatory responses in premalignant MALT-like lesions, specifically in the mucosa of organs exposed to exogenous antigens.

t(14;18)(q32;q21)

Depending upon the study performed, the translocation t(14;18)(q32;q21), which generates the fusion gene *IGH–MALT1*, is demonstrated to be present in 2–20% of gastric MALT lymphomas.[64,65] As a consequence of this translocation, the *MALT1* gene located at 18q21 becomes juxtaposed to the *IGH* promoter located at 14q32, which results in overexpression of MALT1 protein. In contrast to t(11;18)(q21;q21)-positive tumors, however, t(14;18)(q32;q21)-positive MALT lymphomas mainly occur in nongastric tissues, and as such are not discussed further in this article.[65,66]

t(1;14)(p22;q32) and t(1;2)(p22;p12)

One of either t(1;14)(p22;q32) or its variant t(1;2)(p22;p12) is present in approximately 5% of gastric MALT lymphomas.[64,67] These two translocations are grouped together because they involve the *IGH* gene on chromosome 14 and the *IGK* gene on chromosome 2, which encode immunoglobulin heavy chains and light chains respectively. Patients with t(1;14)(p22;q32) or t(1;2)(p22;p12) tend to present with advanced stages of gastric MALT lymphoma and their tumors typically have additional genetic aberrations, such as other structural chromosomal anomalies or alterations in chromosomal number.[43,68] Analogous to the t(14;18)(q32;q21) translocation described above, the *BCL10* gene (located at chromosome 1p22) is brought under the control of either the *IGH* transcriptional enhancer located at 14q32 or the *IGK* immunoglobulin light-chain κ gene enhancer at 2p12, both of which result in overexpression of *BCL10*.[69] This gene encodes a CARD-containing protein that has a key role in antigen-receptor signaling to NFκB.[70]

t(3;14)(p13;q32) and FOXP1 overexpression

In 2005, *FOXP1* (forkhead box P1, located at chromosome 3p13) was identified as a new translocation partner of *IGH*, not only in MALT lymphomas, but also in DLBCLs of a mainly extranodal location.[44,65] In both these lymphoma subtypes, the overall frequency of t(3;14)(p13;q32) is low (not more than 10%).[44,65,71,72]

Remarkably, a considerable number of t(3;14)(p13;q32)-negative MALT lymphomas and t(3;14)(p13;q32)-negative DLBCLs have strong nuclear FOXP1 expression, which suggests that mechanisms other than *FOXP1* rearrangements (such as trisomy of chromosome 3) might underlie the upregulation of FOXP1 expression in tumors. The implications of this nuclear FOXP1 overexpression are still debated: two studies found FOXP1 overexpression to be associated with reduced survival in patients with DLBCL,[73,74] whereas a third study could not confirm this finding.[75] Also, two studies found strong nuclear FOXP1 expression in gastric MALT lymphomas to be confined to a subgroup of tumors that were at risk of transforming into a DLBCL subtype with a poor clinical outcome.[71,76]

The *FOXP1* gene encodes a member of the FOX family of transcription factors, which are characterized by a common DNA-binding winged helix or forkhead domain.[77] FOXP1 is known to be essential for B-cell maturation in the bone marrow, since FOXP1-deficient

mice showed an arrest at the pro-B-cell to pre-B-cell transition, which is probably related to a lack of expression of the recombination-activating genes *Rag1* and *Rag2*.[78] However, how FOXP1 mediates signaling in the mature, peripheral B-cell pool and how this protein could contribute to MALT lymphoma pathogenesis remain unclear.

Other genetic aberrations
The above-described translocations were all identified in the 1990s using cytogenetic and FISH analysis. In addition, these techniques also revealed the occurrence of trisomy of chromosomes 3, 7, 12 and 18 in samples from t(11;18)(q21;q21)-negative gastric MALT lymphomas. Trisomy of chromosomes 3 or 18 is the most frequent type of aneuploidy; remarkably, these two chromosomes each contain a key gene in MALT lymphoma pathogenesis—*FOXP1* and *MALT1*, respectively. The biological effect of these trisomies is still unknown.

In the past 10 years, genetic data obtained by comparative genomic hybridization (CGH) studies[46,79–81] have confirmed that chromosomal gains and losses occur at a higher frequency in t(11;18)(q21;q21)-negative than in t(11;18)(q21;q21)-positive gastric MALT lymphomas. Array-based CGH showed that chromosomal imbalances (namely, regions of recurrent gain on chromosomes 3, 1p36.2 and 18q, and regions of recurrent loss on 1p36.3 and 7q31–q3) in t(11;18)(q21;q21)-negative gastric MALT lymphomas may be linked with nonresponsiveness to *H. pylori* eradication therapy.[82]

In 2009, new data also shed light on an epigenetic phenomenon in MALT lymphomas: hypermethylation of CpG islands within gene promoter regions, which inhibits transcription and leads to subsequent gene silencing. CpG island hypermethylation of genes such as *CDKN*2A, *DAPK1*, *APBA1*, *APBA2* and MINT 31 (a 'methylated in tumors' locus mapped to chromosome 17q21) has been described in gastric MALT lymphomas.[83,84] Moreover, the CpG island methylator phenotype was associated with both *H. pylori* infection and disease progression.[83] Finally, attention has been focused on microRNAs, which are an epigenetic mechanism of post-transcriptional gene silencing.[85] These noncoding RNAs bind to and induce degradation of target messenger RNA molecules. The observed increase in expression of the microRNAs miR-155 in B-cell malignancies and miR-21 in *H. pylori*-infected cells hint that microRNAs could potentially be involved in MALT lymphoma pathogenesis, although their precise role remains to be investigated.[86,87]

Third step: dysregulation of NFκB signaling
NFκB was first described in B lymphocytes as a transcription factor binding to the κB site of the immunoglobulin κ light-chain enhancer. Soon afterwards, NFκB activity was demonstrated to be inducible in all cell types. To date, five members of the NFκB transcription factor family have been identified: RelA (p65), RelB, c-Rel, NFκB1 (p50) and NFκB2 (p52).[18] All five NFκB family members share a conserved REL homology domain for DNA binding and they are all essential for lymphocyte

survival and activation. Two distinct NFκB signaling pathways have been identified: the canonical and noncanonical pathways.[88,89]

The noncanonical pathway
The noncanonical pathway engages RelB–p52 dimers and is induced by a limited number of stimuli, including tumor necrosis factor (TNF) ligand superfamily member 13B (also known as BAFF or BLyS), lymphotoxin β and CD40 ligand. This pathway does not require NFκB essential modulator (NEMO, also known as IκB [inhibitor of NFκB] kinase [IKK] subunit γ) for phosphorylation of p100 (the precursor of p52) by IKKα and the subsequent degradation of its C-terminus half, which results in nuclear translocation of the RelB–p52 dimers. The noncanonical pathway switches on a large number of genes with products that mediate distinct regulatory functions in adaptive immunity, including *CXCL13* (CXC-motif chemokine 13, formerly known as B-lymphocyte chemoattractant) and *TNFSF13B*, as well as genes that are directly involved in lymphoid organogenesis, such as *NTAN1* (which encodes protein N-terminus asparagine aminohydrolase).[16] Although all current knowledge of gastric MALT lymphomas is based on experiments studying the aberrant activation of the canonical pathway, gastric MALT lymphoma pathogenesis might plausibly also be modulated by the noncanonical NFκB signaling pathway. *In vitro* experiments have shown that *H. pylori* activates the noncanonical NFκB pathway in B lymphocytes.[90]

The canonical pathway
The canonical signaling pathway engages RelA–p50 dimers and is triggered by viruses, bacterial lipopolysaccharides and proinflammatory cytokines such as TNF and IL-1. In unstimulated B-cells, IκB proteins bind to RelA and p50 to form latent complexes that are present in the cytoplasm. Activation of this pathway induces polyubiquitinylation and activation of NEMO, which results in the phosphorylation and subsequent proteasomal degradation of IκB by the IKK catalytic subunit IKKα (Figure 4). RelA–p50 dimers then translocate to the nucleus and mediate transcription of a large number of genes, the products of which are mostly involved in regulation of innate immunity, including various cytokines, chemokines, adhesion molecules and enzymes, as well as the antiapoptotic proteins Bcl-2, Bcl-2-related protein A1 and Bcl-2-like protein 1, as well as proliferation-promoting proteins, such as cyclin D2.[16]

Bcl-10 and MALT1
In the past decade, identification of MALT1 and Bcl-10 (two key proteins that function downstream of the antigen receptor and upstream of the IKK complex) led to an important advance in our understanding of the canonical pathway. Studies of knockout mice established that both Bcl-10 and MALT1 are essential transducers of antigen-receptor signals that activate the canonical NFκB pathway.[17,91,92] Also, Bcl-10-induced NFκB activation is reduced in MALT1-deficient cells, an effect that
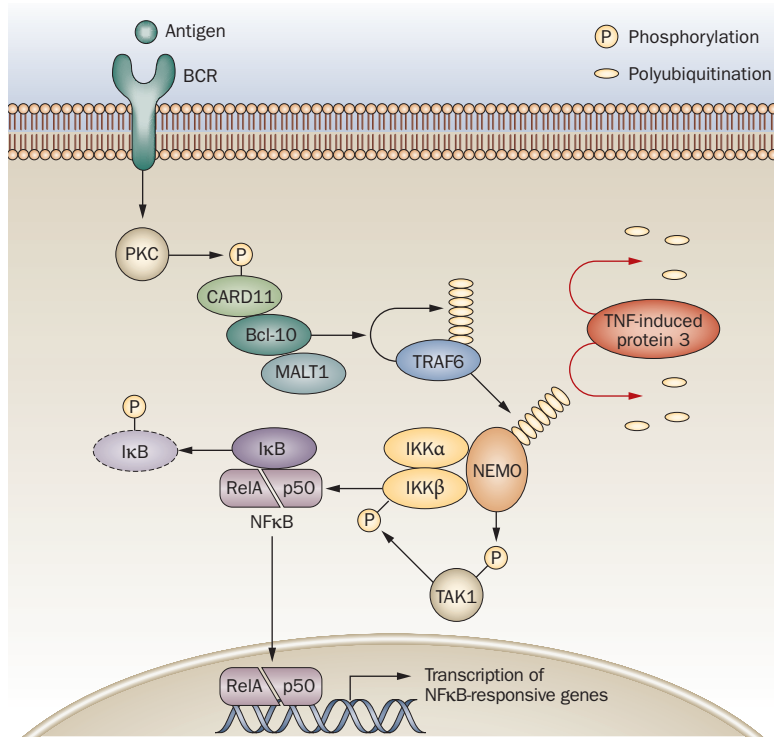
Figure 4 | The canonical NFκB signaling pathway. Antigen binding to the BCR activates a signaling pathway in cell membrane lipid rafts, leading to PKC-induced phosphorylation of CARD11. The latter leads to oligomerization of the downstream effectors Bcl-10 and MALT1 with TRAF6. As a consequence, TRAF6 induces polyubiquitinylation of the γ subunit of NEMO, which in turn induces TAK1-induced phosphorylation of its β subunit. As a result, IκB is targeted for phosphorylation and proteasomal degradation. This event allows RelA–p50 heterodimers to enter the nucleus and mediate transcription of NFκB-responsive genes. TNF-induced protein 3 acts as a negative regulator of NFκB activation by reversing ubiquitinylation of key molecules in the canonical pathway. Abbreviations: Bcl-10, B-cell lymphoma 10; BCR, B-cell receptor; CARD11, caspase recruitment domain family member 11; IκB, inhibitor of nuclear factor κB kinase; IKK, inhibitor of nuclear factor κB kinase; MALT1, MALT lymphoma translocation protein 1; NEMO, nuclear factor κB essential modulator; PKC, protein kinase C; TAK1, transforming growth factor β activating kinase; TNF, tumor necrosis factor; TRAF6, TNF-receptor associated factor 6.

Table 1 | Frequent translocations in gastric MALT lymphomas

Translocation	Fusion transcript	Bcl-10 staining pattern	Mechanism of upregulated NFκB activation
t(11;18)(q21;q21)	BIRC3–MALT1	Moderately nuclear	Polyubiquitinylation of NEMO
t(14;18)(q32;q21)	IGH–MALT1	Perinuclear	MALT1 oligomerization (Bcl-10 dependent)
t(1;14)(p22;q32)	BCL10–IGH	Strongly nuclear	MALT1 oligomerization
t(3;14)(p13;q32)	FOXP1–IGH	Negative	Unknown

Abbreviations: Bcl-10, B-cell lymphoma 10; NEMO, nuclear factor κB essential modulator; MALT1, MALT lymphoma translocation protein 1.

activation, as endogenous Bcl-10 and MALT1 can be co-immunoprecipitated from lysates of nonstimulated B cells and T cells.[53]

A remarkable feature of gastric MALT lymphomas is the aberrant subcellular localization of Bcl-10 in tumor cells, which can be detected by immunohistochemistry (Figure 1b,c, Table 1). In normal marginal-zone lymphoid tissue, Bcl-10 is weakly expressed in the cytoplasm of B cells.[69] By contrast, in gastric MALT lymphomas, t(11;18)(q21;q21)-positive tumors show moderate nuclear Bcl-10 expression, t(1;14)(p22;q32)-positive tumors demonstrate strong nuclear Bcl-10 expression, and t(14;18)(q32;q21)-positive tumors are characterized by perinuclear Bcl-10 localization.[64,69,93] The biological consequences of this aberration remain unknown, although one possibility is that an altered Bcl-10 distribution might reflect impaired intracellular transport of Bcl-10 between the nucleus and cytoplasm, which is regulated by MALT1.[94] In the presence of t(1;14)(p22;q32), overexpression of Bcl-10 results in its nuclear retention owing to a relative shortage of MALT1 compared with Bcl-10. In t(14;18)(q32;q21)-positive MALT lymphomas, Bcl-10 remains cytoplasmic because all nuclear Bcl-10 is exported by MALT1. A relative shortage of MALT1, due to the loss of one allele in t(11;18)(q21;q21)-positive MALT lymphomas, might be responsible for the nuclear retention of Bcl-10 as the associated BIRC3–MALT1 fusion protein is unable to export Bcl-10. Thus, a nuclear Bcl-10 staining pattern can indirectly indicate the presence of t(1;14)(q32;q21) or t(11;18)(q21;q21).

CARD11

In 2001, the upstream activator of the Bcl-10–MALT1 complex was identified as CARD11 (caspase recruitment domain family, member 11), a 130 kDa CARD-containing protein that is only associated with lipid rafts in cell membranes.[95–97] The following model of B-cell receptor (BCR)-induced activation of the canonical NFκB pathway fits the best with all currently available data (Figure 4). Binding of an antigen to the BCR initiates a tyrosine-phosphorylation signaling cascade that culminates in the generation of second messengers that activate isoforms of PKC (protein kinase C).[98] PKCβ then phosphorylates and structurally reconfigures CARD11, which exposes the CARD motifs of CARD11 and allows them to interact with downstream components.[98] Phosphorylated CARD11 recruits Bcl-10 and MALT1 (which are believed to form Bcl-10–MALT1 heterodimers in the absence of BCR stimulation) to the lipid rafts and also binds to Bcl-10 through a CARD–CARD interaction.[99,100] As a result, the Bcl-10–MALT1 heterodimers oligomerize with CARD11 to form high-molecular-weight CARD11–Bcl-10–MALT1 complexes that interact with and induce oligomerization of TRAF6 (TNF-receptor associated factor 6) via the C-terminus of MALT1.[101] As a result, TRAF6 polyubiquitinylates various target proteins, including NEMO and TRAF6 itself, via the E3 ubiquitin ligase activity of its RING E3 domain. In contrast to Lys48-linked polyubiquitinylation, which induces proteasomal

can only be reversed after restoration of MALT1 expression.[17] Taken together, these data suggest that the physical interaction of functional Bcl-10 and MALT1 proteins is essential for optimal NFκB activation. The physical association between Bcl-10 and MALT1 involves a short region downstream of the CARD motif of Bcl-10 and the immunoglobulin-like domain of MALT1. This interaction probably still occurs in the absence of NFκB pathway

degradation, the Lys63-linked polyubiquitinylation of TRAF6 facilitates the interaction of NEMO and TRAF6 with TAK1 (transforming growth factor β activating kinase). This interaction activates TAK1, which subsequently fully activates the IKK complex via phosphorylation of its β subunit, which results in IκB phosphorylation and degradation, as well as nuclear translocation of p65–p50 dimers.

Aberrant NFκB activation

NFκB regulates the transcription of proliferation-promoting and antiapoptotic genes in B cells, and thus its constitutive activation might result in lymphoma pathogenesis. Mounting evidence links the oncogenic phenotype associated with t(11;18)(q12;q21), t(1;14)(p22;q32) and t(14;18)(q32;q21) to aberrant NFκB activation by BIRC3–MALT1, Bcl-10 and MALT1, respectively (Table 1). In cells with the translocations t(1;14)(p22;q32) and t(14;18)(q32;q21), fusion of the *IGH* enhancer with *BCL10* and *MALT1*, respectively, leads to overexpression of Bcl-10 and MALT1 proteins, which hints at a role for dysregulation of NFκB signaling in MALT lymphoma pathogenesis.

In vitro experiments have shown that overexpression of Bcl-10 directly activates NFκB signaling, whereas overexpression of MALT1 only activates NFκB signaling after Bcl-10-induced oligomerization of the Bcl-10–MALT1 construct described above.[102] In t(1;14)(p22;q32)-positive MALT lymphomas, which are characterized by Bcl-10 overexpression, Bcl-10 is thought to form oligomers through linkage of its CARD motif without the need for upstream signaling, thus triggering MALT1 oligomerization and aberrant NFκB activation. Conversely, in t(14;18)(q32;q21)-positive MALT lymphomas, which overexpress MALT1, the oligomerization of MALT1 (and, therefore, subsequent NFκB activation) is believed to be dependent on Bcl-10, as MALT1 does not contain a structural domain that would enable self-oligomerization.[63]

In t(11;18)(q21;q21)-positive MALT lymphomas, the fusion protein BIRC3–MALT1 is believed to activate NFκB directly by increasing NEMO polyubiquitinylation, as has been demonstrated *in vitro* and in *BIRC3–MALT1* transgenic mice.[103,104] This process requires the presence of the first BIR domain in BIRC3.[103,104] The BIRC3–MALT1 fusion protein has been detected in the lipid rafts of human B-cell lymphoma BJAB cells, in association with increased constitutive NFκB activity and resistance to Fas-induced apoptosis.[105] In fact, the presence within lipid rafts of only the MALT C-terminus, which is derived from the BIRC3 portion of the fusion protein, is sufficient to trigger NFκB activation via enhanced NEMO polyubiquitinylation.[104] In addition, oligomerization of BIRC3–MALT1 and/or association of its MALT1 domains with proteins involved in downstream signaling (such as TRAF6) might be facilitated by the association of these proteins with lipid rafts, which could result in the normal process of antigen-induced oligomerization of MALT1–TRAF6 being bypassed, and thus ensuring constitutive activation of the NFκB pathway.

Furthermore, BIRC3–MALT1 induces transactivation of the *BIRC3* gene through NFκB activation, which creates a positive-feedback loop in which self-activation due to BIRC3–MALT1 upregulates *BIRC3* expression in t(11;18)(q21;q21)-positive MALT lymphomas.[106]

In vitro, both wild-type MALT1 and the BIRC3–MALT1 fusion protein mediate proteolytic cleavage of TNF-induced protein 3, which impairs its NFκB inhibitory function.[107] TNF-induced protein 3 inhibits NFκB activation by catalyzing the removal of polyubiquitin chains, not only from TRAF6 and NEMO (Figure 4) but also from receptor-interacting protein (RIP), which is a key mediator of TNF-induced NEMO activation.[108] Deletion of the *TNFAIP3* gene has been implicated in the pathogenesis of nongastric MALT lymphomas, as well as DLBCL;[109–111] however, whether constitutive proteolysis of TNF-induced protein 3 leading to uncontrolled NFκB activation may act as a pathogenetic mechanism *in vivo* in patients who have gastric MALT lymphomas characterized by either *IGH–MALT1* or *BIRC3–MALT1* rearrangements remains to be investigated.

Treatment and follow-up

H. pylori eradication with antibiotics is now the generally accepted first-choice therapy for patients with localized, *H. pylori*-positive gastric MALT lymphoma.[112–114] If patients with gastric MALT lymphomas do respond to *H. pylori* eradication therapy, an important point to remember is that the time to complete remission can range from about 2 weeks up to more than 1 year. When antibiotic therapy fails to eradicate *H. pylori* or in patients with a localized, *H. pylori*-negative gastric MALT lymphoma, modest doses of involved-field radiotherapy (30–40 Gy over 4 weeks) can be applied to the stomach and perigastric nodes with excellent results.[113] Disseminated disease is usually considered an indication for immunotherapy with anti-CD20 monoclonal antibodies and/or chemotherapy.[113] Only a few chemotherapy regimens have been tested specifically in MALT lymphomas. Oral alkylating agents (either chlorambucil or cyclophosphamide) or purine nucleoside analogs (fludarabine or cladribine) can result in a high rate of disease control. Surgery only has a role in the treatment of gastric MALT lymphomas if local complications (such as gastric perforation) occur.

Serial gastric biopsies are essential for the surveillance of patients with gastric MALT lymphoma to enable a histological evaluation of the tumor response to treatment. A first endoscopic examination is recommended 2–3 months after the cessation of antibiotic treatment, to confirm *H. pylori* eradication, followed by endoscopy every 6 months for 2 years.[113] In the past 3 years, two studies reported that a conservative 'watchful waiting' approach seemed to be safe in patients who demonstrate persistence of histological gastric MALT lymphoma infiltrates despite successful *H. pylori* eradication and normalization of endoscopic findings.[115,116] Such patients are considered to have minimal residual disease,[35] also designated 'responding residual disease' by the Groupe d'Etude des Lymphomes de l'Adulte.[117]

Conclusions

Gastric MALT lymphoma pathogenesis is a multistep process initiated by infection with *H. pylori*, which induces genetic abnormalities and subsequent malignant transformation. The diagnosis of a gastric MALT lymphoma, made by endoscopic mucosal biopsy, should prompt investigations to identify the presence of *H. pylori*. In all *H. pylori*-positive patients who have localized disease, triple antibiotic therapy should be the sole initial treatment, although the treating physician should bear in mind that the presence of t(11;18)(q21;q21) is associated with a reduced likelihood of successful lymphoma regression. An important aim in the treatment of gastric MALT lymphomas is to prevent transformation into an aggressive DLBCL, although the underlying mechanisms of that transformation are not yet known. Gene alterations associated with gastric MALT lymphoma, including *BIRC3–MALT*, *IGH–BCL10* and *IGH–MALT*, result in constitutive activation of the NFκB pathway. As such, pharmaceutical interventions that target members of this pathway may represent an attractive treatment strategy in the future.

Review criteria

The MEDLINE database was searched in September 2009 to identify full-text publications in English that contained the terms "marginal zone", "MALT lymphoma", "diffuse large B-cell lymphoma", "Helicobacter pylori", "Helicobacter heilmannii", "hepatitis C virus", "histology", "genetics", "NFκB", "MALT1", "BCL10", "CARD11" and "FOXP1". The reference lists from retrieved articles were also examined to look for further relevant papers.

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