Microenvironment and Immunology

Translocation of Helicobacter pylori CagA into Human B Lymphocytes, the Origin of Mucosa-Associated Lymphoid Tissue Lymphoma

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Abstract

Infection by cagA-positive Helicobacter pylori (H. pylori) is strongly associated with gastric carcinomas and gastric mucosa–associated lymphoid tissue (MALT) lymphomas. H. pylori translocates the bacterial protein CagA into gastric epithelial cells, and the translocated CagA deregulates intracellular signaling pathways and thereby initiates pathogenesis. This in turn raised the possibility that H. pylori is associated with the development of MALT lymphomas during persistent infection by direct interaction with B lymphocytes. In this work, we showed that CagA can be directly translocated into human B lymphoid cells by H. pylori, and the translocated CagA undergoes tyrosine phosphorylation and binds to intracellular SH-2. Meanwhile, the translocated CagA induces activation of extracellular signal-regulated kinase and p38 mitogen-activated protein kinase in human B lymphoid cells, and upregulates the expressions of Bcl-2 and Bcl-XL, which prevents apoptosis. These results provide the first direct evidence for the role of CagA as a bacterium-derived oncoprotein that acts in human B cells, and further implies that CagA is directly delivered into B cells by H. pylori and is associated with the development of MALT lymphomas.

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Introduction

Helicobacter pylori (H. pylori), a common human pathogen that infects ~50% of the world’s population, is associated with duodenal and peptic ulcer diseases (1, 2). Infection by cagA-positive H. pylori is also associated with gastric carcinomas and gastric mucosa-associated lymphoid tissue (MALT) lymphomas (1, 3). The pathogenic role of H. pylori infection in gastric MALT lymphomas was observed in in vitro experiments and clinical evaluations of the effects of eradication on the progression of gastric MALT lymphomas (4, 5). Epidemiologic studies further indicated that cagA-positive H. pylori is present in the gastric mucosa of most patients with gastric MALT lymphomas (6, 7). Clinical observations that eradication of H. pylori by antibiotic therapy can lead to the complete remission of MALT lymphomas (5) provide evidence that cagA-positive H. pylori plays an important role in the development and/or maintenance of MALT lymphomas. The development of gastric MALT lymphomas is dependent on H. pylori infection. Bacterial colonization of the gastric mucosa triggers lymphocyte infiltration (8, 9) and the formation of acquired MALTs. Bacterial infection induces and sustains an actively proliferating B-cell population through immunologic stimulation. Previous studies suggested that MALT lymphoma cells preserve B-cell properties and that their growth may be partially driven by antigenic stimulation. H. pylori stimulates lymphoma B cells through tumor-infiltrating T cells, involving CD40 and CD40L costimulatory molecules (10, 11). However, the pathogenesis and how H. pylori induces the development of B-cell MALT lymphomas are still not clear.

Much attention has been focused on the role of the cagA gene product, CagA, in the malignant transformation of cells. CagA was directly injected from bacteria into attached gastric epithelial cells by a type IV secretion system, encoded by the cag pathogenicity island (12, 13), and underwent tyrosine phosphorylation (14–17). In gastric epithelial cells, the phosphorylated CagA specifically binds to intracellular targets, including SHP-2 tyrosine phosphatase and COOH-terminal Src kinase, in a tyrosine phosphorylation–dependent manner (15, 18–20). Deregulation of intracellular signal transducers by the injected CagA is suspected of being involved in the development of gastric pathogenesis, eventually leading to a gastric adenocarcinoma. In human B lymphocytes, overexpression of cagA through transfection induces activation of extracellular signal-regulated kinase
(ERK) and their downstream apoptosis regulators, indicating that CagA has effects on the growth and survival of B lymphocytes and may play a role in the development of MALT lymphomas (21, 22).

H. pylori infection stimulates immune lymphocytes in the gastric mucosa and induces the formation of MALTs, from which MALT lymphomas of B-cell origin develop (23). Immune cells constituting MALTs migrate to and infiltrate the site of H. pylori infection in the stomach. In such circumstances, CagA may be injected into lymphocytes as well as gastric epithelial cells. Based on this notion, we examined the direct interaction between H. pylori and human B cells and tested the hypothesis that CagA may be injected into B lymphocytes after interacting with H. pylori. In this work, we showed that CagA could be directly translocated into human B cells from H. pylori. This implies the direct role and importance of CagA in the development of H. pylori–associated MALT lymphomas.

Materials and Methods

Bacterial strains and cell lines

H. pylori bacterial strains HM-6 and HM-9 were isolated from patients with a MALT lymphoma (HM) in the National Taiwan University Hospital. The isogenic mutants HM-6ΔcagA and HM-6ΔcagE, which lacked cagA and cagE, were constructed from these H. pylori bacterial strains as previously described (24).

The human B lymphoma cell line BJAB was obtained from the American Type Culture Collection. The cell lines were grown in RPMI 1640 (Life Technologies Invitrogen) containing 10% FCS and L-glutamine in a water-saturated atmosphere at 37°C and 5% CO2.

Construction of cagA-EGFP isogenic knock-in mutant H. pylori

For construction of the cagA-EGFP isogenic knock-in vector, the cagA (HP0547) gene with upstream DNA region was amplified from chromosome DNA of H. pylori by the primer pairs cagA-4/cagA-10 (5′-ATATATCTCCAGGG-TAAAAATGGAATCGTT-3′) and plasmid EGFP-C1 and primers egfp-1/egfp2 (5′-GTGCACTAGTGAACGCGG-3′; 5′-GGATCCCTTTTTCACAGCTCAGG-3′), and plasmid EGFP-C1 and primers egfp-1/egfp2 (5′-GTGCACTAGTGAACGCGG-3′; 5′-GGATCCCTTTTTCACAGCTCAGG-3′) were used to amplify the EGFP gene. The PCR products were subcloned into the pHeL3 vector (pHeL3-AG). The cagΩ (HP0548) gene with upstream DNA region was amplified from chromosome DNA of H. pylori by the primer pairs HP0548-1/HP0548-2 (5′-AGATCTAGGATTGGAATCAAAAACGC-3′; 5′-GGATCCCTTTTTCACAGCTCAGG-3′). The PCR products were subcloned into the pHeL3-AG. The cagA-EGFP chromosomal integration vector was used for the natural transformation of H. pylori.

Analysis of CagA translocation

Analysis of CagA translocation was based on the detection of tyrosinephosphorylated CagA within host cells after the attachment of H. pylori as described in previous studies (12, 13). The human B lymphoma cell line, BJAB cells, or primary human B lymphocytes (1 × 106) were infected with H. pylori bacterial strains, incubated for 4 hours, washed five times with PBS, and lysed in 500 μL of modified radioimmunoprecipitation assay buffer [RIPA: 50 mmol/L Tris-HCl, 150 mmol/L NaCl, 1 mmol/L EDTA, 1% NP40, 0.25% Na-deoxycholate, 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 1 mmol/L orthovanadate, 1 μmol/L leupeptin, and 1 μmol/L pepstatin]. Anti-CagA monoclonal antibody (mAb) was added to a total volume of 900 μL; after 1 hour at 4°C, 40 μL of protein G-agarose were added, and the mixture was incubated for a further 2 hours at 4°C. After a short centrifugation, the beads were washed four times with 1 mL of PBS and suspended in 40 μL of sample solution.

For preparing membrane and cytoplasmic fractions of H. pylori–infected BJAB cells, H. pylori was incubated with BJAB cells at a multiplicity of infection (MOI) of 100 for 5 hours. After infection, BJAB cells were washed with ice-cold PBS with 1 mmol/L sodium vanadate, followed by lysis of the cells with 0.45-gauge needle in 200 μL of buffer [10 mmol/L Tris-HCl (pH 7.4), 1 mmol/L EDTA, 1 mmol/L sodium vanadate, and 1 mmol/L PMSF]. The membrane fractions were collected by centrifugation at 1,000 g for 10 minutes, and were resuspended in 80 μL 1× sample buffer. The cytoplasmic fractions were purified from the supernatant centrifuged at 12,000 g for 30 minutes and mixed with 50 μL of 5× sample buffer. Lysates of H. pylori–infected cells were subjected to SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes. PVDF membranes were blocked for 30 minutes with nonfat milk blocking buffer, then incubated with primary antibodies and horseradish peroxidase (HRP)–conjugated secondary antibodies. The HRP-conjugated antibodies were visualized by enhanced chemiluminescence.

 Laser scanning confocal immunofluorescence microscopy

To show the translocation of CagA proteins into B lymphocytes during H. pylori infection, BJAB cells were grown on cover glass and cultured with H. pylori (cagA-EGFP) at a MOI of 100 for 5 hours. The H. pylori–infected BJAB cells were fixed with 4% formaldehyde in PBS for 30 minutes and permeabilized with 0.1% saponin in PBS. The cultures were blocked with 1% bovine serum albumin in PBS for 20 minutes and stained with antibodies against surface IgM [anti-human IgM-Texas red (Rockland)], SHP-2 [anti-SHP-2 (C-18); Santa Cruz Biotechnology], Specimens were analyzed by confocal microscope.

Antibodies

Anti-PY99, anti-CagA (A-10), anti-urease, and anti–SHP-2 (C-18) mAbs were from Santa Cruz Biotechnology (use in the concentration of 0.2 μg/mL). Anti–Bcl-xl, anti–Bcl-2, anti–Mcl-1, anti-ERK, anti–phospho-ERK, anti–p38 MPK, and anti–phospho-p38MPK mAbs were from Cell Signaling Technology (used in the concentration of 1:1,000 dilution).
Anti–hepatocyte growth factor receptor (HGFR) and anti–α tubulin mAbs were from GeneTex (used in the concentration of 1 μg/mL). The anti–human IgM-Texas red mAb used in immunofluorescence studies were from Rockland (used in the concentration of 10 μg/mL).

Etoposide-induced apoptosis in B lymphocyte

BJAB cells were cocultured with *H. pylori* strain HM-9 and its isogenic knockout mutant in cagA (HM-9ΔcagA) for 12 hours. After treatment, cells were treated with etoposide (100 μmol/L) for 24 hours, and the cell survival was measured with MTT assay. Cells were incubated with 0.5 mg/mL MTT for 6 hours. The medium was discarded, and DMSO solution was added to dissolve crystal product, then absorbance was measured at 570 nm.

**Results**

Direct translocation of CagA protein into the human B lymphocyte BJAB cell line after infection with *H. pylori*

Immune cells constituting MALT migrate to and infiltrate the site of *H. pylori* infection in the stomach. Furthermore, following extensive destruction of the gastric mucosa by severe gastritis or ulceration, the likelihood that lymphocytes will come into direct contact with cagA-positive *H. pylori* dramatically increases. In such circumstances, CagA may be injected into lymphocytes as well as gastric epithelial cells. Based on this notion, we examined the possibility of the direct translocation of CagA into nongastric epithelial cells, particularly human B cells. To verify this hypothesis, we subjected a lysate of the human B lymphocyte cell line, BJAB
cells, infected with *H. pylori* MALT lymphoma strains (HM-6, HM-9) to immunoprecipitation with antiserum to CagA, and then the precipitated protein were blotted with both anti-CagA and anti-phosphotyrosine (anti-PY99) mAbs. The results in Fig. 1 show that the precipitated protein reacted with both anti-CagA and anti-phosphotyrosine (Fig. 1). Thus, CagA is the tyrosine-phosphorylated protein that is induced by *H. pylori* attachment, named CagAP-Tyr. In human B lymphocyte cell lines (BJAB), CagAP-Tyr was identified upon attachment of *H. pylori* HM-6 and HM-9 but not its isogenic knockout mutant in cagA or cagE, which has a defect in the type IV secretion apparatus. B, stability of CagA within B lymphoid cells. BJAB cells were incubated with HM-6 for 5 h and then were treated with antibiotics Gentamycin in the concentration of 200 μg/mL to eradicate the extracellular bacteria. The cell lysates were collected at different time points as indicated in the figure and were subjected for immunoprecipitation and Western blot using anti-PY99 and CagA antibodies.

Figure 2. Translocation of CagA into the human B-cell line, BJAB cells, is dependent on the type IV secretion system. A, BJAB cells were incubated with HM-6 and HM-9, and its isogenic knockout mutant in cagA (HM-6ΔcagA, HM-9ΔcagA) and cagE (HM-6ΔcagE, HM-9ΔcagE). Lysates were subjected for immunoprecipitation and Western blot using anti-PY99 and CagA antibodies. Immunoblots showing tyrosine phosphorylation of CagA upon attachment of *H. pylori* HM-6, HM-9 but not its isogenic knockout mutant in cagA or cagE, which has a defect in the type IV secretion apparatus. B, stability of CagA within B lymphoid cells. BJAB cells were incubated with HM-6 for 5 h and then were treated with antibiotics Gentamycin in the concentration of 200 μg/mL to eradicate the extracellular bacteria. The cell lysates were collected at different time points as indicated in the figure and were subjected for immunoprecipitation and Western blot using anti-PY99 and CagA antibodies.
infection with *H. pylori*, SHP-2 was immunoprecipitated from lysates by BJAB cells infected with *H. pylori*. The immunoprecipitates and total cell lysates were immunoblotted with anti-CagA, anti-PY99, and anti-SHP-2 mAbs. Results in Fig. 1C show that CagA communoprecipitated endogenous SHP-2 (Fig. 1C). Thus, our results indicate that CagA is delivered into host cells and binds SHP-2 in human B lymphoid cells.

**Translocation of cagA into human B lymphocytes is dependent on the type IV secretion system**

It was shown that *H. pylori* can translocate the CagA protein into human gastric epithelial cells through the type IV secretion system (12). To further prove that the translocation of CagA protein into human B lymphocytes is also through a similar bacterial secretion system, we constructed isogenic cagA and cagE knockout mutants of *H. pylori* strains HM-6, −ΔcagA and HM-6, −ΔcagE. Neither mutant strain was able to induce tyrosine phosphorylation, whereas the wild-type (WT) strain was (Fig. 2). Knockout mutants in cagE, which have a defect in the secretion apparatus, still produced CagA, but lost the tyrosine phosphorylation capability (Fig. 2). This assay showed that a functional type IV secretion system is necessary for the translocation of CagA into BJAB cells, where it is converted into CagA<sup>P-Tyr</sup>. Therefore, CagA might be the observed tyrosine-phosphorylated protein translocated into BJAB cells by the type IV secretion system.

To obtain further biochemical evidence for the residence and stability of CagA<sup>P-Tyr</sup> in the eukaryotic cell after CagA protein is delivered into host cells, we prepared cell lysate by *H. pylori* attached and removed the attached *H. pylori* by treatment with antibiotics Gentamycin to eradicate the extracellular bacteria. The results in Fig. 2B showed that residence of CagA<sup>P-Tyr</sup> in the human B lymphocyte cell line was identified upon attachment of *H. pylori*, and it could last for as long as 72 hours after delivery even in the absence of the attached *H. pylori*.

To further show the direct delivery of CagA into B cells by *H. pylori*, we construct a cagA-EGFP knock-in isogenic *H. pylori* mutant strain, which express the CagA-GFP fusion protein with green florescence. The results in Fig. 3 showed that in cultured cagA-EGFP *H. pylori* was cultured with BJAB cells, most of the *H. pylori*-associated CagA was accumulated around the cell. Successful translocation of CagA into BJAB cells was revealed by staining with antibodies against cell surface IgM (red) and was analyzed by confocal microscope (Fig. 3). This phenomenon could be observed as early as 1 hour (2.3%), and the positive cells increased to 12.3% (2 h) and up to 31.67% (5 h) in the time course analysis. Therefore, our results indicate that CagA is directly delivered into host cells by *H. pylori* in human B lymphoid cells.

**CagA translocation induces activation of mitogen-activated protein kinase and upregulation of the antiapoptotic proteins, Bcl-2 and Bcl-X, in human B lymphocytes**

To investigate whether CagA may exert its effects on signal pathways in human B lymphocytes, we investigated whether CagA modulates the activation of mitogen-activated protein kinase (MAPK) and its downstream apoptosis regulators in B lymphocytes. Results in Fig. 4 show that translocation of CagA induced the phosphorylation of ERK and P38 MAPK in the BJAB human B cell line, and the activation of ERK and P38 MAPK by *H. pylori* was significantly reduced in the cagA knockout *H. pylori* mutant when compared with the WT *H. pylori* strain (Fig. 4A), indicating that translocation of the *H. pylori* CagA protein induces the activation of ERK and P38 MAPK in a human B-cell line.

A hallmark of cancer cells is the acquisition of resistance to apoptosis. It would be interesting to know whether CagA prevents apoptosis and promotes accumulation of genetically abnormal cells that would be eliminated from the tissue. To address this possibility, we examined the effect of CagA on the expression of the antiapoptotic proteins, Bcl-2 and Bcl-XL, which are important regulators of apoptosis. The results in Fig. 4B show that the expression of Bcl-2 family proteins, Bcl-2 and Bcl-XL, increased after interaction with the *H. pylori* WT strain (HM-9); however, upregulation was not seen in the cagA deletion mutant, HM−ΔcagA (Fig. 4B), indicating that the CagA protein could be delivered into human B lymphoid cells through *H. pylori* to upregulate the expressions of Bcl-2 and Bcl-XL.

To address whether upregulation of Bcl-2 and Bcl-XL by CagA promotes survival of human B lymphoid cells, we examined the effect of *H. pylori* on apoptosis induced by etoposide, and results in Fig. 4C show that B cells were more...
resistant to epotoside-induced apoptosis after interaction with the WT *H. pylori* strain (HM-9), but not the cagA deletion mutant, HM-9ΔcagA (Fig. 4C), indicating that translocation of CagA into B lymphoid cells induced upregulation of Bcl-2 and Bcl-XL, and thereby enhanced the survival of human B lymphoid cells against apoptosis.

**Direct translocation of the CagA protein into primary human B lymphocytes after infection with *H. pylori***

To obtain further, direct evidence for the translocation of CagA protein into human B lymphocytes by *H. pylori in vivo*, we isolated primary human B lymphocytes from peripheral blood and cocultured them with *H. pylori*. Similar to the human B lymphoma cell line, phospho-CagA was identified upon attachment of *H. pylori* with primary human B lymphocytes (Fig. 5). The translocation of CagA protein was seen in the WT *H. pylori* strain but not the cagA- or cagE-deficient *H. pylori* mutant strains (Fig. 5A). Moreover, an interaction with *H. pylori* induced upregulation of the activation markers CD69, CD80, CD86, and CD25 in human B lymphocytes, indicating *H. pylori* induces B-cell activation (Fig. 5B).

To further directly support the notion that the translocation of CagA protein into human B lymphocytes induces activation of B cells leading to the development of MALT...
lymphomas, we further used immunohistochemistry assay to detect CagA proteins in tumor cells within human MALT lymphoma tissues. The results in Fig. 6 show that the H. pylori CagA protein was detected in tumor cells within MALT lymphoma tumor tissues in the pathology specimens. We have examined 36 H. pylori–positive and 7 H. pylori–negative cases, and among these MALT lymphoma tumor tissues, CagA protein was detected in 19 of 36 H. pylori–positive cases, whereas no CagA expression was detected in the gastric mucosa in all of the seven H. pylori–negative cases. Taken together, our results indicate that CagA, a bacterial oncoprotein, can be translocated into human B lymphocytes after infection with H. pylori.

Discussion

H. pylori is associated with gastric carcinomas and gastric MALT lymphomas. The H. pylori virulence factor CagA is causally linked to gastric MALT lymphomas. However, it is unclear how CagA promotes the development of gastric MALT lymphomas. Previous studies established that the cagA gene product CagA can be directly injected into bacterium-attached host gastric epithelial cells through the bacterial type IV secretion system (12). The translocated CagA deregulates intracellular signaling pathways and thereby initiates pathogenesis (14, 16). In this work, we further obtained direct evidence that CagA, a bacterial oncoprotein, could be translocated into human B lymphocytes through the bacterial type IV secretion system. The identification of CagA as an oncoprotein that is translocated into human B lymphocytes may have important biological consequences for the host. This implies that H. pylori is associated with the development of MALT lymphomas during persistent infection by direct interaction with B lymphocytes.

H. pylori colonization induces systemic and mucosal immune responses (25). Bacterial colonization of the gastric mucosa triggers lymphoid infiltration and the formation of acquired MALTs. Previous studies suggested that H. pylori infection induces and sustains an actively proliferating B-cell population through direct and indirect immunologic stimulation, and infiltrating T cells seem to play an important role in the development of MALT lymphomas (4, 10). However, in this report, we provide direct evidence that H. pylori can directly translocate CagA oncoprotein into B lymphocytes and trigger its activation, and it also confers a direct role for CagA in inducing the formation of MALT lymphomas. The role of the cagA gene product, CagA, in malignant transformation of cells is well established. CagA is directly injected from the bacteria into attached gastric epithelial cells (12) and undergoes tyrosine phosphorylation (14, 16). Deregulation of intracellular signal transducers by the injected CagA has been suspected to be involved in the development of gastric pathogenesis, eventually leading to gastric adenocarcinomas. In gastric epithelial cells, phosphorylated CagA specifically binds to intracellular targets, including SHP-2 tyrosine phosphatase, in a tyrosine phosphorylation–dependent manner (18, 19). In our results, we also showed that similar to gastric epithelial cells, phosphorylated CagA specifically binds to intracellular targets, including SHP-2 tyrosine phosphatase, in a tyrosine phosphorylation–dependent manner (18, 19). In our results, we also showed that similar to gastric epithelial cells, phosphorylated CagA specifically binds to intracellular targets, including SHP-2 tyrosine phosphatase, in a tyrosine phosphorylation–dependent manner (18, 19). In our results, we also showed that similar to gastric epithelial cells, phosphorylated CagA specifically binds to intracellular targets, including SHP-2 tyrosine phosphatase, in a tyrosine phosphorylation–dependent manner (18, 19). In our results, we also showed that similar to gastric epithelial cells, phosphorylated CagA specifically binds to intracellular targets, including SHP-2 tyrosine phosphatase, in a tyrosine phosphorylation–dependent manner (18, 19). In our results, we also showed that similar to gastric epithelial cells, phosphorylated CagA specifically binds to intracellular targets, including SHP-2 tyrosine phosphatase, in a tyrosine phosphorylation–dependent manner (18, 19). In our results, we also showed that similar to gastric epithelial cells, phosphorylated CagA specifically binds to intracellular targets, including SHP-2 tyrosine phosphatase, in a tyrosine phosphorylation–dependent manner (18, 19).
BCl-2 and BCl-XL in human B lymphoid cells, thereby enhancing the survival of human B lymphoid cells against apoptosis. Our results showed that the residence of CagAP-Tyr in the human B lymphocyte cell line was identified upon attachment of *H. pylori*. The delivered CagAP-Tyr could induce dysregulation of intracellular signaling, and this effect would persist and could maintain as long as the *H. pylori* continue attached. The delivered CagA induces the activation and stimulation of B cells, and could initiate the first step of transformation. It is possible that transformation of B lymphocytes would occur in the presence of other oncogenic events, such as chromosome translocation or oncoprotein activation, to trigger transformation of B lymphocytes and development of MALT lymphoma.

Previous studies (4, 10) and the histologic features of MALT lymphomas suggest that MALT lymphoma cells preserve B-cell properties and that their growth may be partially driven by antigenic stimulation. Removal of tumor-infiltrating T cells before the experiment abolished all the effects of *H. pylori* on tumor B cells *in vitro* (4). The active role of tumor-infiltrating T cells in the growth of tumor B cells is still to be determined. However, in this study, our results clearly showed that *H. pylori* delivers the CagA oncoprotein into B lymphocytes, triggering its activation. In addition to translocated CagA, activation of B lymphocytes can also be induced by contact with whole bacteria. This could be due to the direct interaction between bacteria and host cells through Toll-like receptor and/or other non–Toll-like receptor innate receptors. Our results showed both the WT *H. pylori* and CagA mutants significantly induced the upregulation of the activation markers CD69, CD80, CD86, and CD25 in human B lymphocytes when compared with the control groups. However, there was more increase in the intensity of fluorescence (mean fluorescence intensity) in WT *H. pylori* than the CagA mutants, suggesting that in addition to the interaction with whole bacteria, the translocated CagA could further promote the activation of human B lymphocytes. Thus, our results indicate that *H. pylori* can directly deliver the CagA oncoprotein into human B lymphocytes and therefore promote their proliferation and resistance to apoptosis. This confers a direct role for CagA in inducing the formation of MALT lymphomas. Nevertheless, our results still cannot exclude the active role of tumor-infiltrating T cells in the development of MALT lymphomas. Tumor-infiltrating T cells may respond to antigen stimulation through *H. pylori* and trigger the proliferation of tumor B cells either directly through CD40/CD40L interacting with B cells or indirectly by secretion of cytokines or growth factors, leading to the proliferation of B cells and eventually to the development of MALT lymphomas. Therefore, both CagA and infiltrating T lymphocytes may synergistically contribute to the development of MALT lymphoma during persistent *H. pylori* infection.

The direct role of CagA in inducing the formation of MALT lymphomas was also supported by a recent cagA transgenic mice study (26). Ohnishi and colleagues (26) generated cagA transgenic mice and showed that the expression of CagA is sufficient for the development of gastrointestinal and hematopoietic neoplasms. The finding provides evidence for the hyperactivation of SHP-2 in cagA transgenic mice and suggests a role of CagA of deregulating SHP-2 in leukemogenesis. This in turn raises the possibility that CagA can be delivered into B cells that
migrate to the $H.\text{ pylori}$–infected stomach, leading to the development of MALT lymphomas. Our results support this notion by providing the first direct evidence that CagA, a bacterial oncoprotein, can be translocated into human B lymphocytes through the bacterial type IV secretion system. Taken together, our study establishes a causal relationship between $H.\text{ pylori}$ CagA and MALT lymphomas, and suggests that CagA is a critical molecular target for therapeutic application to $H.\text{ pylori}$–associated neoplasms.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


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