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Mucosal humoral immune response to CagA shows a high prevalence in patients with gastric MALT-type lymphoma

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Abstract In the pathogenesis of gastric mucosa-associated lymphoid tissue (MALT)-type lymphoma, CagA-positive *Helicobacter pylori* strains have been suspected of making a significant contribution. To investigate this hypothesis in more detail, the mucosal humoral immune response of 15 patients with gastric MALT-type lymphoma was examined in the tumor and in the tumor-free gastritis of the same patient. Mononuclear cells from different sites (antrum, corpus, lymphoma) were cultured. Culture supernatant and serum of the same patient were used for immunodetection of CagA. All patients displayed an immune response to CagA in the tissue-culture supernatants. Although the humoral immune response in the tumor was restricted to a very few *H. pylori* antigens, antibodies directed against CagA protein were found in most patients. The immune response to CagA in nearly all lymphoma patients – not only in the serum, but also in the mucosa, including the tumor site – support the hypothesis that CagA is involved in the pathogenesis of gastric MALT-type lymphoma.

Key words *Helicobacter pylori* · CagA · MALT-type lymphoma · Mucosal immune response

Introduction

Helicobacter pylori (*H. pylori*) is associated with the development of primary gastric Non-Hodgkin lymphoma of mucosa-associated lymphatic tissue type (MALT-type lymphoma). A strong epidemiological link has been reported [7], and a concomitant infection with *H. pylori* is found in 92–98% of patients with MALT-type lymphoma [11]. Furthermore, remission of low grade MALT-type lymphoma has been reported after *H. pylori* eradication [12]. Although *H. pylori* infection is very common, only a few patients develop a primary gastric MALT-type lymphoma. In addition to previously unknown factors, this may be due to a difference in bacterial strains. *H. pylori* strains were subdivided into type-1 strains bearing CagA protein and type-2 strains that do not [13]. CagA protein is considered a marker of more aggressive *H. pylori* strains, as it is associated with peptic ulcer disease [2, 9] and also regarded as an increased risk of the development of gastric adenocarcinoma [1, 8].

In a recent study, a high percentage of CagA-positive *H. pylori* strains in patients with gastric MALT-type lymphoma was found when testing serum immunoglobulin G (IgG) antibodies against CagA protein [5]. However, serological detection of CagA-positive *H. pylori* strains does not necessarily reflect their colonizing of the gastric mucosa at the present time, since antibodies represent an immune response in the past. Mucosa-derived IgA antibodies play the major role in the mucosal immune response, but are represented in serum only at a low level.

Therefore, the process of analysis focused on CagA-specific mucosal IgA and IgG antibodies produced by plasma cells from different sites of the chronically inflamed tumor-free gastric mucosa, as well as tumor-infiltrated gastric mucosa from patients with gastric MALT-type lymphoma.

Materials and methods

Patients and microculture of gastric mucosal tissue

Gastrectomy specimens of 15 patients with gastric MALT-type lymphoma (six low grade, nine high grade, isotype IgM), which were infected with *H. pylori* as tested by serology, were investigated. MALT-type lymphoma was diagnosed according to established criteria [6]. Mucosal specimens from each patient were collected from different sites of chronically inflamed tumor-free mucosa and from lymphoma-infiltrated gastric mucosa as determined by histology. Corpus and antrum of six patients were examined separately. Sera from all patients were collected during surgery.

Gastric tissue specimens were disrupted with a scalpel immediately after surgery in order to isolate single mononuclear cells

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for culturing. To remove potential contamination by small amounts of serum in the tissue capillaries, samples were washed three times in RPMI 1640 medium. Purified cells (4×10^5/ml) were cultured in RPMI 1640 medium containing 10% fetal calf serum (FCS) and 40 µg gentamicin, at 37°C in a humidified 5% carbon dioxide/ 95% air incubator. After 7 days, supernatant was collected by centrifugation at 500 *g* for 10 min to remove cell debris and was stored at –70°C until beginning of the assay.

Immunodetection of CagA-specific mucosal and serum antibodies

CagA-specific mucosal IgA and IgG antibodies were identified using a *H. pylori* Western-blot kit (Biermann, Bad Nauheim, Germany). Specificity and sensitivity were 100% (IgG and IgA), as determined by the manufacturer. The supernatant from microcultured gastric mucosal tissue was used in its undiluted form. Serum was diluted 1:50.

Table 1 CagA-specific immunoglobulin G (IgG) and IgA immune response in the gastric mucosa and in the corresponding serum

Patient no.	Tumor grading	Mucosal immune response				Serum immune response	
		Chronic gastritis IgG	Chronic gastritis IgA	Tumor IgG	Tumor IgA	IgG	IgA
1	Low	–	+	–	–	–	–
2	Low	+	+	+	+	+	+
3	Low	+	–	–	–	+	–
4	Low	–	+	+	–	+	–
5	Low	–	+	–	+	+	–
6	Low	+	+	+	+	+	+
7	High	+	+	+	+	+	–
8	High	+	+	+	+	+	+
9	High	+	+	–	–	+	+
10	High	+	+	+	+	+	+
11	High	+	+	+	+	+	+
12	High	+	+	+	+	+	+
13	High	+	+	+	+	+	+
14	High	+	+	+	–	+	+
15	High	+	+	+	+	+	+
CagA positivity		80%	93%	73%	67%	93%	67%
CagA positivity (IgG or IgA)		100%		80%		93%	

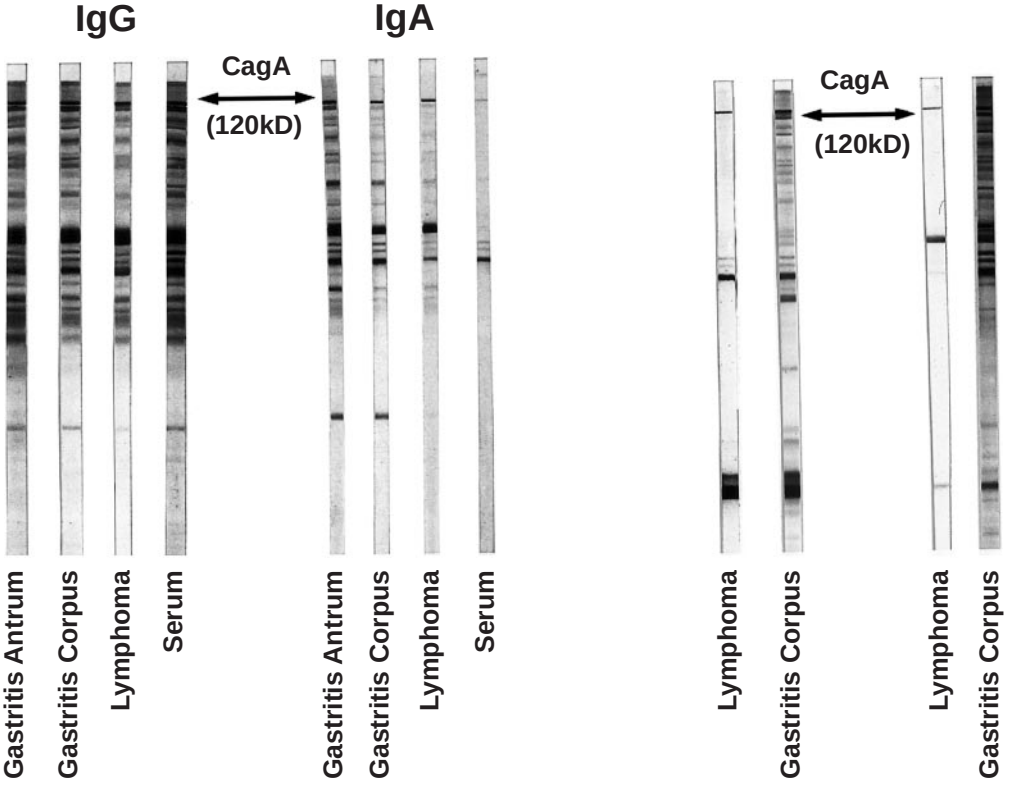

Fig. 1 **a** Mucosal immunoglobulin G (IgG) and IgA immune response to *Helicobacter pylori* CagA antigen in the chronic tumor-free gastritis (antrum, corpus), lymphoma, and corresponding serum representatively shown in a patient with a high-grade gastric mucosa-associated lymphoid tissue (MALT)-type lymphoma (patient 8 from Table 1). **b** IgG immune response in the microcultured lymphoma tissue and corresponding chronically inflamed tumor-free corpus tissue representatively shown in two patients with gastric MALT-type lymphoma (patients 6 and 7 from Table 1). In contrast to chronic gastritis (corpus), only very few *H. pylori* antigens, including CagA, were recognizable by the humoral immune response in the lymphoma tissue

Results

All 15 patients with gastric MALT-type lymphoma registered an immune response to *H. pylori*, as demonstrated by *H. pylori* immunoblotting. In all patients, CagA-specific antibodies (12/15 CagA-specific IgG, 14/15 CagA-specific IgA) were found in the gastric mucosa exhibiting chronic gastritis. In the tumor-infiltrated tissue of 80% of the patients, CagA-specific antibodies were detected (11/15 CagA-specific IgG, 10/15 CagA-specific IgA). No differences were found between low- and high-grade gastric MALT-type lymphoma.

CagA-specific serum antibodies were detected in 93% of the patients (14/15 CagA-specific IgG, 10/15 CagA-specific IgA). In one patient, only mucosal CagA-specific IgA antibodies were found, whereas mucosal IgG, serum IgA, and IgG findings were negative. Table 1 summarizes the data.

Figure 1a demonstrates that, in the case of a representative patient from our collective, the immune response to *H. pylori* and CagA in the antrum and corpus gastritis displayed no qualitative differences when investigated separately.

In contrast to the broad immune response to *H. pylori* in the chronic gastritis, immune response to *H. pylori* in the tumor tissue was restricted only to a few *H. pylori* antigens in most patients (12 of 15). However, in all of these 12 patients (80%), immunoreactivity to CagA was clearly detectable (Fig. 1b).

Discussion

In all patients with gastric MALT-type lymphoma, mucosal antibody production directed against CagA was discovered in the gastric mucosa. These findings demonstrate a direct immune response to CagA-bearing *H. pylori* strains in patients with MALT-type lymphoma, thus supporting our views on the role of CagA in lymphomagenesis. In the tumor-free mucosa, 93% of the patients registered an IgA immune response to CagA, whereas, in the serum, CagA-specific IgA antibodies were found only in 67% of patients. Therefore, CagA-specific IgA in the serum is not representative of the corresponding mucosal immune response.

The findings of CagA-specific IgA antibodies in the gastric mucosa emphasize the importance of CagA protein for the mucosal immune response, as IgA is responsible for the first-line defense against *H. pylori*, and, as opposed to IgG, locally produced and secreted into the gastric lumen.

The local immune reactivity to *H. pylori*, in particular to CagA antigen, was nearly identical in the tumor-free tissue, irrespective of its biopsy site (antrum, corpus). This indicates that immune response to *H. pylori* and to CagA protein is a multifocal or diffuse process involving the whole stomach, and that it is not restricted to distinct areas of the stomach. This immunological finding has parallels with histopathological findings, in which chronic gastritis and lymph follicles were found to become distributed all over the stomach during the course of *H. pylori* infection.

At first sight, the presence of antibodies directed against *H. pylori*, in particular that of CagA-specific IgA and IgG antibodies in the tumor tissue, is astonishing because the tumor immunoglobulin was IgM in all cases (data not shown). However, it appears logical with regard to the MALT concept, in which all gastric MALT-type lymphomas evolve from a *H. pylori*-associated chronic gastritis. Therefore, IgA and IgG immune response to *H. pylori* in the tumor tissue reflects the residual chronically inflammatory plasmacellular infiltrate accompanying the tumor, which is a diagnostic feature in MALT-type lymphoma. However, the residual plasmacellular infiltrate does not necessarily explain the restricted antibody pattern. Further studies are required to investigate this phenomenon.

Although in many patients the immune response in the lymphoma was restricted only to a few *H. pylori* antigens, as opposed to a broad immune response in the chronic gastritis, CagA as an immunodominant antigen was found among these restricted antigens in the tumor tissue. These data additionally emphasize the importance of CagA in the pathogenesis of gastric MALT-type lymphoma.

The role of CagA-positive *H. pylori* strains in the pathogenesis of MALT-type lymphoma is discussed controversially. In the study performed by Witherell et al., in which CagA-specific serum IgG was investigated, a lower association of CagA-positive *H. pylori* strains was found in patients with gastric MALT-type lymphoma [10]. The discrepancy may be due to the different methods used in each study. In the study of Witherell et al., only CagA-specific IgG serum antibodies were examined using the enzyme-linked immunosorbent assay (ELISA) technique, whereas, in our study, CagA was detected by both mucosal and serum IgA and IgG antibodies, using a sensitive Western-blot technique. In another study, the CagA status of patients with gastric MALT-type lymphoma was investigated on the genome level, using *H. pylori* strains cultured from gastric biopsies. In this study, CagA-positive *H. pylori* strains were found only in 58% of the patients [3]. However, culturing of *H. pylori* strains may lead to a strain selection and does, therefore, not necessarily reflect the in vivo situation.

That CagA antigen is crucial in *H. pylori*-associated inflammation and may, therefore, be important for the pathogenesis of MALT-type lymphoma, was substantiated by recent findings of Elios and coworkers [4]. They found a high percentage of CagA-positive T-cell clones in the mucosa of patients with *H. pylori* gastritis, indicating that CagA is an immunodominant antigen during a *H. pylori* infection. They have also shown that these T-cell clones act as potent helpers for B-cell proliferation. This might denote an important mechanism, which initiates uncontrolled B-cell proliferation and neoplastic transformation, and which has been considered as the major pathomechanism for gastric MALT-type lympho-

ma. The functional data and the humoral immune response to CagA presented in this study indicate that CagA is not only a marker for an increased risk, but may together with currently unknown factors play a role in the development of gastric MALT-type lymphoma.

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