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Impact of *Helicobacter pylori* Infection on the Humoral Immune Response to MUC1 Peptide in Patients with Chronic Gastric Diseases and Gastric Cancer

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Many investigators have demonstrated alteration of gastric mucins in *H. pylori* infected individuals. The inflammatory environment induced by *H. pylori* leading to aberrant glycosylation of MUC1 and demasking of core peptide MUC1 epitope could enhance immune responses to MUC1. IgG and IgM immune response to MUC1 in patients with gastric cancer (n = 214) chronic gastroduodenal diseases (n = 160) and healthy blood donors (n = 91) was studied with ELISA using bovine serum albumin-MUC1 60-mer peptide as antigen. *H. pylori* serologic status was evaluated with ELISA and CagA status by immunoblotting. Gastric mucosa histology was scored according to the Sydney system. Compared to *H. pylori* seronegative individuals, higher levels of IgG antibody to MUC1 were found in *H. pylori* seropositive patients with benign gastric diseases (p < 0.01) and blood donors (p < 0.03). Higher MUC1 IgG antibody levels were associated with a higher degree of gastric corpus mucosa inflammation in patients with chronic gastroduodenal diseases (p < 0.0025). There was a positive correlation between the levels of anti-*H. pylori* IgG and MUC1 IgG antibody levels in blood donors (p = 0.03), and in patients with benign diseases (p < 0.0001). In patients with gastric cancer (n = 214) a significantly higher level of anti-MUC1 IgG than in blood donors was observed (p < 0.001) irrespective of *H. pylori* status or stage of cancer. MUC1 IgM antibody levels were not related to the *H. pylori* serology. IgG immune response to tumor-associated MUC1 is up regulated in *H. pylori* infected individuals. This increase is associated with a higher IgG immune response to *H. pylori* and with a

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higher degree of gastric mucosa inflammation. High levels of MUC1 IgG antibody irrespective of *H. pylori* serologic status characterized patients with gastric cancer. The findings suggest that, in some individuals, the *H. pylori* infection may stimulate immune response to tumor-associated MUC1 peptide antigen thus modulating tumor immunity.

**Keywords** *Helicobacter pylori*, MUC1 antibody, gastric cancer, gastric mucin, immune response.

**INTRODUCTION**

*Helicobacter pylori* (*H. pylori*) infection elicits a strong systemic and mucosal immune response, which leads to inflammation, damage and atrophy of the gastric mucosa, and enhanced risk of developing gastric adenocarcinoma. A majority of patients with gastric cancer have a current or past *H. pylori* infection (Blaser and Atherton, 2004). Both bacterial virulence factors (such as CagA, BabA and others) and host response polymorphism contribute to the degree of the host inflammatory response, *H. pylori* colonization, and precancerous histology changes (Blaser and Atherton, 2004; Crowe, 2005; Peek and Blaser, 2002; Troost et al., 2003).

Combined bacterial and host genotyping may improve the identification of patients at high risk of developing cancer (Rad et al., 2003). At the same time, some beneficial so far unexplained effects of *H. pylori* infection have also been demonstrated, such as reduced risk of esophageal and cardia adenocarcinoma (Chow et al., 1998; Henrik Siman et al., 2001; Ye et al., 2004).

Alteration of synthesis, glycosylation and expression of gastric mucins, including MUC1 mucin, has been demonstrated in *H. pylori* infected stomach (Bird and Bresalier, 2000; Slomiany and Slomiani, 2005; Tanaka et al., 2003; Teixeira et al., 2002; Vinall et al., 2002). MUC1 is a highly O-glycosylated transmembrane mucin expressed on glandular epithelia and on epithelial tumors, which is overexpressed and aberrantly glycosylated in adenocarcinomas (Karsten et al., 2005; Taylor-Papadimitriou et al., 1999). MUC1 has a large highly glycosylated extracellular domain that consists mainly of up to 120 repeated 20 amino acid units and exhibits genetic polymorphism in the number and absolute sequence of the repeats (Von Mensdorf-Pouilly et al., 2005). Deficient glycosylation leads to the exposure of a variable number of immunodominant areas on the MUC1 tandem repeat domain and to the expression of truncated carbohydrate chains that constitute tumor-associated carbohydrate antigens (*T*_N, sialyl *T*_N and TF).

This potentially immunogenic molecule is shed into the circulation of carcinoma patients and induces humoral and, to a lesser extent, cellular immune responses. MUC1 is frequently elevated in serum of patients with adenocarcinoma, and MUC1 serum levels are used in the clinic to monitor response to treatment in breast cancer patients (Bon et al., 1997). Levels of MUC1 expression in
gastric carcinomas correlate positively with stage of disease and negatively with survival of cancer patients (Kocer et al., 2004; Lee et al., 2001). A natural humoral immune response to MUC1 is associated with a benefit in survival in early stage breast cancer patients (Von Mensdorff-Pouilly et al., 2000b), and MUC1 has attracted interest as a potential target for cancer immunotherapy.

We found that the IgG immune response to tumor-associated MUC1 peptide core epitope is up regulated in *H. pylori* infected patients with non-malignant gastric diseases and in blood donors, as well as in patients with gastric cancer irrespective of *H. pylori* status. In addition, this increase is related to the degree of gastric mucosa inflammation and associated with a higher level of IgG immune response to *H. pylori*.

**MATERIAL AND METHODS**

**Subjects and Serum Samples**

The study population (Table 1) consisted of 214 patients with histologically verified gastric carcinoma and 160 patients with non-malignant gastroduodenal diseases. In addition, a group of randomly selected blood transfusion donors (n = 91) was investigated. All patients and controls were above 40 years old.

Gastric mucosa biopsy specimens from both antrum and corpus of the stomach of patients with chronic gastroduodenal diseases were evaluated in 135 out of 160 patients according to the Sydney system for *H. pylori* density, the degree of inflammation, intestinal metaplasia and activity of the gastritis, and scored as no, mild, moderate and severe. Moderate or severe gastric mucosa atrophy in any part of the stomach was considered as atrophic gastritis.

Peptic ulcer disease was diagnosed by gastroduodenal endoscopy. Tumor staging and morphology were based on histopathological (pTNM) classification of malignant tumors (Sobin and Wittekind, 1997) and evaluated according to the system of Lauren (1965) as intestinal and diffuse type of tumor growth. Serum samples were obtained before treatment and stored at −20°C until required.

**H. pylori and CagA Status**

Serum samples were examined by enzyme-linked immunosorbent assay (ELISA) for anti-*H. pylori* IgG antibody level as described (Klaamas et al., 1996; Lelwala-Guruge et al., 1990). In brief, the plates (Maxi Sorp, Nunc, Rosklide, Denmark) were coated with a glycine cell-surface extract of *H. pylori* strain NCTC 11637 (500 ng/well) and incubated at 4°C overnight. Serum was used at dilution 1:65. Alkaline phosphatase conjugated
Table 1: Characteristics of the study population.

<table>
<thead>
<tr>
<th>Study Population</th>
<th>n</th>
<th>Age, years (range)</th>
<th>Male/female ratio</th>
<th>H. pylori status +/total (%)</th>
<th>CagA status* +/total (%)</th>
<th>Antrum H. pylori +/total (%)</th>
<th>Corpus H. pylori +/total (%)</th>
<th>Gastric mucosa morphology</th>
<th>Tumor Morphology**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood donors</td>
<td>91</td>
<td>52 (41–65)</td>
<td>1.21</td>
<td>49 (54)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Benign diseases</td>
<td>160</td>
<td>56 (40–77)</td>
<td>1.28</td>
<td>124 (78)</td>
<td>96/124 (77)</td>
<td>101/135 (75)</td>
<td>96/127 (76)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>- Non-atrophic</td>
<td>28</td>
<td>54 (40–72)</td>
<td>1.54</td>
<td>24 (86)</td>
<td>19/24 (80)</td>
<td>21/25 (84)</td>
<td>17/21 (81)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>gastritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Atrophic gastritis</td>
<td>30</td>
<td>56 (41–80)</td>
<td>1.14</td>
<td>24 (80)</td>
<td>19/24 (80)</td>
<td>34/40 (85)</td>
<td>33/39 (85)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>- Ulcus ventriculi</td>
<td>48</td>
<td>57 (42–77)</td>
<td>1.08</td>
<td>35 (73)</td>
<td>31/35 (89)</td>
<td>20/32 (63)</td>
<td>19/29 (66)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>- Ulcus duodeni</td>
<td>54</td>
<td>52 (40–76)</td>
<td>1.45</td>
<td>41 (76)</td>
<td>27/41 (66)</td>
<td>26/38 (68)</td>
<td>27/37 (71)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>214</td>
<td>67 (40–82)</td>
<td>1.22</td>
<td>170 (79)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>138</td>
<td>76</td>
</tr>
<tr>
<td>- Stage I</td>
<td>57</td>
<td>64 (40–78)</td>
<td>1.11</td>
<td>47 (82)</td>
<td>37</td>
<td>20</td>
<td>ND</td>
<td>37</td>
<td>20</td>
</tr>
<tr>
<td>- Stage II</td>
<td>39</td>
<td>63 (41–82)</td>
<td>1.16</td>
<td>31 (80)</td>
<td>26</td>
<td>13</td>
<td>ND</td>
<td>26</td>
<td>13</td>
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<tr>
<td>- Stage III</td>
<td>71</td>
<td>69 (41–81)</td>
<td>1.29</td>
<td>56 (79)</td>
<td>46</td>
<td>25</td>
<td>ND</td>
<td>46</td>
<td>25</td>
</tr>
<tr>
<td>- Stage IV</td>
<td>47</td>
<td>66 (41–79)</td>
<td>1.35</td>
<td>36 (77)</td>
<td>29</td>
<td>18</td>
<td>ND</td>
<td>29</td>
<td>18</td>
</tr>
</tbody>
</table>

*CagA status was evaluated only in H. pylori-seropositive patients in the benign group (n = 124).

**Intestinal (IT) or diffuse type (DT) of tumor growth according to P. Lauren classification.

ND – not done.
anti-human IgG (Dako, Glostrup, Denmark) and p-nitro-phenyl-phosphate (Sigma, St. Louis, Mo) substrate was used. A pool of human IgG (Kabi, Stockholm, Sweden) was used in each ELISA plate as a positive control for 100 units.

Relative antibody activity (RAA) was calculated according to Blomberg and colleagues (1983), and RAA values equal or below 25 and above 40 were regarded as negative and positive respectively. Subjects with 'gray zone' RAA values (26–40) were not included in the study.

Determination of the CagA status was performed in patients with benign gastric diseases (n = 124) with immunoblotting as described elsewhere (Klaamas et al., 1996, 2002a). Patients with CagA +/- results were not included in this study.

**Detection of IgG and IgM Immune Response to MUC1**

IgG and IgM antibody levels to MUC1 (MUC1 Ab) were determined with ELISA as described elsewhere (Von Mensdorff-Pouilly et al., 1998, 2000b). In brief: a BSA-conjugated MUC1 60-mer non-variant (DTR) tandem-repeat peptide (250 ng per well in PBS) and 1% BSA (control) were used to coat 96-well ELISA plates (Maxisorp, Nunc, Roskilde, Denmark). After overnight incubation at 4°C, washing and blocking with 1% BSA in PBS the serum diluted 1:100 and 1:500 for IgG and IgM antibody determination, respectively, was applied and the plates were incubated overnight at 4°C. After 7 × washing with PBS-Tween20 the bound antibodies were detected with alkaline phosphatase conjugated rabbit anti-human IgG or IgM (Dako) and developed with p-nitro-phenyl-phosphate (Sigma). Absorbance values were registered at 405 nm with Labsystem Multiscan (Finland). An optical density of control wells (PBS-BSA) was subtracted from the values of the wells coated with MUC1-BSA.

To standardize the assay, a standard serum sample with levels of anti-MUC1 antibody giving about 1.0 O.D. unit was included in every plate. The tested serum value was calculated as a percentage of the value of the standard serum (100%) as described by Blomberg (4) and expressed in relative units (R.U.).

**Statistical Methods**

The IgG and IgM responses to MUC1 were not normally distributed. Comparison between groups was performed by the Mann–Whitney U-test, Pearson two-tailed correlation and χ²-test. Association of the level of MUC1 antibody with morphological criteria was evaluated by comparison of “no or mild” with “moderate or severe” subgroups. All calculations were performed using Prism version 4.0 software. Significance was defined as p < 0.05.
RESULTS

MUC1 Antibody Levels Irrespective of H. pylori Status

Gastric cancer patients showed significantly higher MUC1 IgG response (Table 2) compared to blood donors group (p = 0.0001). Such increase was found irrespective of stage of cancer or tumor morphology (data not shown). Compared to blood donors, a higher level of MUC1 IgG was also observed in the benign group (p = 0.0013). In contrast, MUC1 IgM antibody levels showed significantly lower values both in patients with gastric cancer and in the benign group, compared to blood donors.

MUC1 Antibody in Relation to H. pylori and CagA Serology

MUC1 IgM levels did not differ between H. pylori-seronegative and -seropositive subgroups in all groups studied (Table 2). Anti-MUC1 IgG antibody levels ranked significantly higher in H. pylori-seropositive blood donors (p = 0.03) and patients with benign gastric diseases (p = 0.013) than in the corresponding seronegative group (Figure 1, Table 2). This common trend was observed in all benign subgroups tested, but the differences were not significant in some groups (atrophic and chronic gastritis) possibly due to the small number of H. pylori seronegative individuals among them.

In contrast, anti-MUC1 IgG antibody levels did not differ between H. pylori seronegative and seropositive gastric cancer patients. H. pylori seronegative and seropositive patients with gastric cancer showed each a significantly higher IgG immune response to MUC1 compared to blood donors (p < 0.001 and p = 0.01 for H. pylori-seronegative and seropositive subgroups, respectively).

A positive correlation between the levels of anti-H. pylori IgG and anti-MUC1 IgG was found in blood donors (r = 0.23; p = 0.032), and in patients with chronic gastroduodenal diseases (r = 0.33, p < 0.0001 for the whole benign group) (Figure 2). Patients with peptic ulcer disease showed the most pronounced correlation (r = 0.41; p < 0.0001, n = 102). Such association was not observed in patients with gastric cancer or for MUC1 IgM antibody levels (data not shown).

Among 124 H. pylori seropositive patients with chronic gastroduodenal diseases tested for CagA status 96 were CagA seropositive. CagA status was not related to MUC1 IgG or IgM antibody levels: Median (range) anti-MUC1 antibody levels (R.U.) in CagA seropositive and seronegative groups were 69 (3-497) and 65 (26-605) for MUC1 IgG, and 33 (1-198) and 32 (3-123) for IgM antibody levels (p = 0.85 and p = 0.74 for MUC1 IgG and IgM, respectively).
Table 2: MUC1 antibody levels in the study groups and in relation to *H. pylori* serology.

<table>
<thead>
<tr>
<th>Study population</th>
<th>n</th>
<th>H. pylori Seronegative/seropositive</th>
<th>Total group</th>
<th>H. pylori seronegative</th>
<th>H. pylori seropositive</th>
<th>Total group</th>
<th>H. pylori seronegative</th>
<th>H. pylori seropositive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood donors</td>
<td>91</td>
<td>42/49</td>
<td>63 (6-241)</td>
<td>52.5 (6-127)</td>
<td>66 (14-241)</td>
<td>40 (4-177)</td>
<td>39 (7-94)</td>
<td>40 (4-177)</td>
</tr>
<tr>
<td>Benign diseases</td>
<td>160</td>
<td>36/124</td>
<td>75 (22-605)</td>
<td>64.0 (22-150)</td>
<td>82 (6-605)</td>
<td>32 (3-198)</td>
<td>32 (3-123)</td>
<td>34 (5-198)</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>214</td>
<td>44/170</td>
<td>85 (14-599)</td>
<td>82.0 (16-529)</td>
<td>89 (14-599)</td>
<td>32 (1-353)</td>
<td>33 (11-153)</td>
<td>32 (1-353)</td>
</tr>
</tbody>
</table>

*p* values are shown for statistically significant differences between subgroups as calculated by Mann–Whitney U-test: *Compared to blood donors; **compared to *H. pylori* seronegative within the group.
MUC1 Antibody in Relation to Tumor Histology and Gastric Mucosa Inflammation

MUC1 IgG levels showed no significant difference between patients with intestinal or diffuse type of gastric tumors at any stage of disease in both *H. pylori* seropositive and negative subgroups. However, a significantly higher MUC1 IgM antibody level was found in *H. pylori* seropositive patients with diffuse type tumors and stage I (n = 18, \( p < 0.0001 \)), and stage II disease (n = 10, \( p = 0.018 \)), compared to seronegative diffuse type subgroups.

In the benign group, a higher degree of gastric mucosa inflammation in the corpus of the stomach was associated with a higher density of *H. pylori* colonization. Four of 44 patients with “no + mild” versus 15 of 46 patients with “moderate + severe” inflammation had higher density of *H. pylori* colonization (\( \chi^2 = 6.12; \text{df} = 1; \ p = 0.013 \)).

In *H. pylori* seropositive patients with chronic gastroduodenal diseases, higher MUC1 IgG antibody levels were associated with a higher degree of

Figure 1: IgG immune response to MUC1 in relation to *H. pylori* serology in the study population. Box-and-whiskers plots show median, range and quartiles of MUC1 IgG antibody; open and closed boxes depict *H. pylori* seronegative and seropositive subjects, respectively. The \( p \) values for differences between *H. pylori* seronegative and -positive subgroups are calculated by the Mann–Whitney U-test.
Figure 2: Linear regression analysis showing the correlation between the levels of anti-\textit{H. pylori} IgG and anti-MUC1 IgG antibodies. (A) blood donors (n = 91); (B) patients with benign disease (n = 160); (C) patients with peptic ulcer disease (n = 102).
inflammation of the gastric corpus mucosa (p = 0.0025) but not of the antrum (p = 0.67) (Figure 3). Regarding the density of \textit{H. pylori} colonization, no significant difference (p = 0.084) in MUC1 IgG antibody levels was found in patients with moderate + severe vs. no + mild density of \textit{H. pylori} colonization in the corpus mucosa of the stomach. In \textit{H. pylori} seropositive patients with chronic gastroduodenal diseases, no relation between MUC1 IgM antibody levels and gastric mucosa inflammation was found. MUC1 IgG and IgM antibody levels did not differ in \textit{H. pylori} seronegative patients in relation to gastric mucosa inflammation, possibly due to the small number of patients in these

![Box plot showing IgG immune response to MUC1 in relation to degree of inflammation in corpus and antrum.](image)

**Figure 3:** IgG immune response to MUC1 in relation to the degree of inflammation of the gastric mucosa in \textit{H. pylori} seropositive patients with chronic gastroduodenal diseases (combined group). Medians, quartiles and ranges are shown. The p values are calculated by the Mann-Whitney U-test.
subgroups. Other histological criteria (the activity of gastritis, intestinal metaplasia) were not related to MUC1 antibody levels (data not shown).

DISCUSSION

All patients and controls had detectable levels of anti-MUC1 antibodies of both IgG and IgM isotype in serum. However, antibody levels varied appreciably in all groups studied. A common trend for the up regulation of anti-MUC1 IgG immune response in *H. pylori*-positive patients with chronic gastric diseases and blood donors was demonstrated. This increase was more pronounced in patients with peptic ulcer disease and non-atrophic gastritis. The increase of MUC1 antibody levels in *H. pylori* seropositive individuals was mostly restricted to the IgG isotype, whereas the level of IgM MUC1 antibody was decreased in patients compared to blood donors, it was not related to *H. pylori* status and was rather similar in all groups studied (Table 2).

The only exception was the higher MUC1 IgM levels in *H. pylori* seropositive patients with early stages of diffuse type gastric tumors but this needs further confirmation due to the small number of *H. pylori* seronegative patients in this subgroup (5 of 33). A significant association between MUC1 IgG antibody levels and the degree of inflammation in the corpus gastric mucosa was revealed in *H. pylori*-seropositive patients. In addition, a positive correlation is present between anti-*H. pylori* IgG, which indirectly reflects the degree of inflammation of gastric mucosa (Bhat et al., 2005; Macarthur et al., 2004; Sheu et al., 1997), and IgG immune responses to MUC1 (Figure 2). The association between inflammatory conditions induced by *H. pylori* and IgG immune responses to MUC1 could be due to the changes (aberrant glycosylation) of the MUC1 molecule induced by inflammation. MUC1 antibodies directed to the peptide core of the molecule have also been described in ulcerative colitis (Hinoda et al., 1993).

The well-documented overexpression and altered glycosylation of MUC1 associated to carcinomas may explain the lack of correlation between *H. pylori* serology and MUC1 IgG in patients with gastric cancer (Karsten et al., 2005; Taylor-Papadimitriou et al., 1999; Von Mensdorff-Pouilly et al., 2000a). Upregulation of humoral immune responses to MUC1 associated to a benefit in survival has been shown in patients with various cancers (Hamanaka et al., 2003, Hirasawa et al., 2000; Von Mensdorff-Pouilly et al., 2000b). Furthermore, underglycosylation leads to the appearance on the MUC1 molecule of tumor-associated glycotopes, such as the Thomsen-Friedenreich (TF) antigen (Galβ1–3GalNAc). We have shown earlier that IgG immune responses to TF antigen is significantly increased in *H. pylori* infected individuals (Klaamas et al., 2002b).

The expression of TF epitope in *H. pylori* cell surface membrane glycoconjugates was demonstrated immunochemically (Klaamas et al., 2002a),
suggesting that this may be one of the reasons for the up-regulation of TF specific immune response in *H. pylori* infected individuals. Recently we demonstrated a positive correlation between the levels of MUC1 IgG and TF epitope specific IgG antibodies in patients with early gastric cancer. Moreover, higher responses to both epitopes were related to a better survival of surgically treated patients with gastric cancer (Kurtenkov et al., 2007). This implies that altered glycosylation of MUC1 is associated with both exposure of MUC1 core peptide epitopes and TF antigen expression, which in turn leads to the induction of immune response to these epitopes.

To our knowledge the present findings are the first evidence that *H. pylori* infection is associated with an enhanced IgG immune response to the cancer-related MUC1 peptide epitope providing further evidence that *H. pylori* infection may modulate an immune response to tumor-associated antigens. This systemic immune response to MUC1 may be protective against cancer, or delay its onset when present in *H. pylori*-infected individuals. The anti-tumor potential of MUC1 antibodies has been demonstrated (Hirasawa et al., 2000; Snijdewint et al., 2001; Von Mensdorff-Pouilly et al., 2005b). It is not unreasonable to assume that this mechanism may also operate in the stomach of individuals whose immune response to MUC1 tumor-associated epitopes is up regulated due to the *H. pylori* infection in comparison to infected individuals that do not show such a response.

The reason why only a part of the infected individuals show up-regulation of the IgG immune response to MUC1 needs further study. Possibly this is related to the proinflammatory genotype of the host, for instance to the IL1beta, and TNF promoter gene polymorphisms, in “strong MUC1 responders” or to a synergistic effect of the host polymorphism and several *H. pylori* virulence factors (Macarthur et al., 2004; Rad et al., 2003). Another possibility is that *H. pylori* infection leads to the expression of glycoforms of MUC1, such as clustered or with reduced glycosylation density, that may induce antibodies with a variable reactivity to the non-glycosylated non-variant triple tandem repeat MUC1 peptide used in this study. Such variations in reactivity have been reported recently for differentially glycosylated MUC1 and murine monoclonal antibodies (Karsten et al., 2004) and for variant sequences of the MUC1 peptide core and patients sera (Von Mensdorff-Pouilly et al., 2005).

Whereas these variations in reactivity are significant for immunotherapy, they do not affect the validity of the results obtained with the MUC1 peptide used in the assay. Aberrant and diminished glycosylation of MUC1 leads to the exposure of the mucin peptide core normally covered by the branching sugars. Immune responses to the MUC1 core peptide recognize more than one minimal epitope within the same individual, and serum samples from cancer patients bind effectively to GalNAc glycosylated MUC1 peptides (Von Mensdorff-Pouilly et al., 2000c). Linear regression analysis shows a high
correlation between responses to the naked peptide and to GalNAc glycopeptides, so that measurement of responses to the naked peptide is valid to evaluate immune responses to the mucin.

Natural antibodies to MUC1 show a strong cross-reactivity with variant (ESR) and non-variant (DTR) sequences of the MUC1 repeat domain (Von Mensdorff-Pouilly et al., 2005); therefore, screening for MUC1 immune responses with a non-variant (DTR) tandem repeat peptide, the classical MUC1 peptide, does not affect the validity of the results. However, whereas in cancer patients MUC1 antibodies have a higher frequency of preferential binding to the non-variant DTR sequence, sera from non-malignant control subjects are preferentially directed to variant repeat clusters (Von Mensdorff-Pouilly et al., 2005). A study investigating a possible protective effect against gastric cancer of MUC1 IgG antibodies in \textit{H. pylori} seropositive patients with benign gastric diseases should test in parallel responses to ESR variant and DTR non-variant sequences of MUC1.

In conclusion, the IgG immune response to tumor-related MUC1 peptide is up regulated in \textit{H. pylori} infected individuals. This increase is associated with a higher IgG immune response to \textit{H. pylori} and with a higher degree of gastric mucosa inflammation. Patients with gastric cancer present high levels of MUC1 IgG antibody irrespective of \textit{H. pylori} serology. A possible protection against or delay in onset of gastric cancer \textit{via} modulation of tumor immunity in \textit{H. pylori} seropositive individuals that have an immune response to MUC1 compared to seropositive individuals with no immune response to MUC1 merits further study.

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