**Rapid Communication**

**Relevance of MUC1 mucin variable number of tandem repeats polymorphism in *H pylori* adhesion to gastric epithelial cells**

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In this work we tested the hypothesis that MUC1 VNTR (variable number of tandem repeats) domain variability affects the adhesion of different *H pylori* strains to gastric carcinoma cell lines.

**Aim:** To evaluate the influence of MUC1 mucin variable number of tandem repeats (VNTR) variability on *H pylori* adhesion to gastric cells.

**Methods:** Enzyme linked immunosorbent assay (ELISA)-based adhesion assays were performed to measure the adhesion of different *H pylori* strains (HP26695 and HPTx30a) to gastric carcinoma cell lines (GP202 and MKN45) and GP202 clones expressing recombinant MUC1 with different VNTR lengths.

**Results:** Evaluation of adhesion results shows that *H pylori* pathogenic strain HP26695 has a significantly higher (P < 0.05) adhesion to all the cell lines and clones tested, compared to the non-pathogenic strain HPTx30a. Bacteria showed a significantly higher (P < 0.05) adhesion to the GP202 cell line, when compared to the MKN45 cell line. Furthermore, both strains showed a significantly higher (P < 0.05) adhesion to GP202 clones with larger MUC1 VNTR domains.

**Conclusion:** This work shows that MUC1 mucin variability conditions *H pylori* binding to gastric cells. The extent of bacterial adhesion depends on the size of the MUC1 VNTR domain. The adhesion is further dependent on bacterial pathogenicity and the gastric cell line. MUC1 mucin variability may contribute to determine *H pylori* colonization of the gastric mucosa.

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**Key words:** *H pylori*; MUC1; Variable number of tandem repeats; Polymorphism; Adhesion; Mucin; Gastric; Infection

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**Introduction**

The Gram negative bacterium *H pylori* is involved in the pathogenesis of several gastrointestinal diseases, ultimately leading to gastric carcinoma. In the gastric mucosa, the majority of the bacteria is found within the mucus layer, but can also be attached to gastric epithelial cells, a crucial step for the maintenance, spreading and severity of the infection.

This attachment is mediated by the interaction of bacterial molecules, such as adhesins and LPS, with gastric cell surface ligands such as glycolipids and glycoproteins. MUC1 is a membrane glycoprotein that protects epithelial surfaces and has been recently identified as an *H pylori* binding target. Extracellular MUC1 variable number of tandem repeats (VNTR) domain is highly glycosylated, presenting carbohydrate structures (e.g. Lewis b carbohydrate antigen) involved in the binding of *H pylori* through its adhesins BabA and SabA. Furthermore this repetitive region shows extensive allelic variation ranging from 25-125 repeat units. The relevance of MUC1 VNTR variability for *H pylori* adhesion to gastric cells remains to be clarified.

In this work we tested the hypothesis that MUC1 VNTR polymorphism affects the *H pylori* adhesion to gastric cells and thus plays an important role in the colonization

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of gastric mucosa. We used *H. pylori* strains with different pathogenicity (strain HP26695 and strain HPTx30a) co-cultured with gastric cell lines GP202 and MKN45, and GP202 clones expressing recombinant MUC1 with different VNTR lengths. Adhesion was evaluated by an enzyme linked immunosorbent assay (ELISA)-based adhesion assay.

The results showed that MUC1 VNTR polymorphism influences the binding of *H. pylori* to gastric cells. Furthermore, higher adhesion was observed in co-cultures with the pathogenic strain (HP26695) when compared to the non-pathogenic strain (HPTx30a) and GP202 cell line when compared to the MKN45 cell line. This work contributes to the understanding of the interplay between host and bacterial factors in *H. pylori* infection pathogenesis.

**MATERIALS AND METHODS**

**Cell lines**

We used two gastric carcinoma cell lines: GP202, previously established in our laboratory,[13] from a signet ring cell gastric carcinoma that constitutively expresses MUC1 and MKN45 [Japan Health Sciences Foundation].

GP202 clones expressing recombinant MUC1 with different VNTR lengths[14] were previously established by stable transfection with an eukaryotic expression vector pHb-APr1-neo containing subcloned epitope-tagged MUC1 (FLAG-MUC1) cDNAs with different number of TR units (0, 3, 9 and 42 repeats, respectively GP202-dTR, GP202-3TR, GP202-9TR and GP202-42TR)[15]. GP202-Neo was obtained by transfection with empty vector.

The parental cell lines and transfectants were cultured in 150 cm² flasks at 37°C in a humidified 5% CO₂ incubator and maintained in RPMI 1640 medium (with Glutamax and 25 mmol/L Heps) supplemented with 10% fetal bovine serum and 50 µg/mL gentamicin. Media was changed every 3 to 4 days, and the cell lines were passaged when they reached 80% to 90% confluence using 0.05% trypsin-0.53 mmol/L ethylenediamine tetra-acetic acid in PBS, and allowed to form confluent monolayers. Cells were washed and fixed at 4°C with 8% paraformaldehyde for 60 min. Endogenous peroxidase was inactivated by addition of 1% H₂O₂ in methanol. After washing with PBS, anti-*H. pylori* monoclonal antibody MAB922 (Chemicon, USA) was added overnight, 4°C, followed by addition of peroxidase-conjugated goat anti-mouse immunoglobulins (Santa Cruz Biotechnology) 30 min, RT. Tetramethylbenzidine (TMB) (Sigma, USA) was added and reaction stopped with 1 mol/L HCl. Plates were read in a 680 ELISA microplate reader (Bio-Rad, USA) at 450 nm. OD values were used as the index of the number of *H. pylori* adhering to cells[16]. Two sets of triplicates were made for each assay.

**Statistical analysis**

Statistical analysis was performed using the Mann-Whitney test, StatView Software version 5.0 (SAS Institute). A *P* value of less than 0.05 was accepted as statistically significant.

**RESULTS**

Evaluation of *H. pylori* adhesion shows that pathogenic strain HP26695 has significantly (*P* < 0.05) higher adhesion values for both GP202 and MKN45 cell lines (1.97 ± 0.10 and 1.47 ± 0.06) when compared with the non-pathogenic strain HPTx30a (1.40 ± 0.15 and 0.85 ± 0.15; Figure 1). This statistically significant association between pathogenicity and higher adhesion (strain HP26695 vs HPTx30a) is also observed for the GP202 MUC1 recombinant clones (GP202-Neo 1.1 ± 0.10 vs 0.72 ± 0.06; GP202-3TR 1.32 ± 0.09 vs 1.0 ± 0.10; GP202-42TR 1.45 ± 0.08 vs 1.18 ± 0.05; GP202-9TR 2.2 ± 0.12 vs 1.96 ± 0.12; and GP202-42TR 2.3 ± 0.07 vs 1.89 ± 0.11; Figure 2). Furthermore, GP202 cell line shows higher adhesion levels than MKN45 cell line for both bacteria strains (HP26695 strain 1.97 ± 0.10 vs 1.47 ± 0.06; HPTx30a strain 1.40 ± 0.15 and 0.85 ± 0.15; Figure 1).

Adhesion of both *H. pylori* strains (HP26695 and HPTx30a) is significantly higher in all the GP202-MUC1 transfectants over-expressing MUC1 (GP202-dTR 1.32 ± 0.09 and 1.0 ± 0.10; GP202-3TR 1.45 ± 0.08 and 1.18 ± 0.05; GP202-9TR 2.2 ± 0.12 and 1.96 ± 0.12; GP202-42TR 2.3 ± 0.07 and 1.89 ± 0.11) when compared with the control, GP202 Neo (1.1 ± 0.10 and 0.72 ± 0.06, Figure 2). There is also an association between the increased number of Tandem Repeats (GP202-9TR and GP202-42TR) and the increased adhesion, for both strains (Figure 2).
DISCUSSION

Epidemiological studies and animal models have shown that *H. pylori* chronic infection is associated with several gastric pathologies, ranging from asymptomatic gastritis to gastric adenocarcinoma and MALT lymphoma[1,2]. The different consequences of the infection suggest that several factors from the host and the bacteria are involved in the bacteria-host interactions, being the pathogenic potential dependent upon the molecular context of the colonization of gastric mucosa. To date several factors involved in the *H. pylori* infection have already been identified (e.g. bacterial adhesins, host mucins and pro-inflammatory cytokines) however the complete mechanism remains to be clarified[8-11,20-26].

Adhesion of *H. pylori* to gastric mucosa is a fundamental step for epithelium colonization. Different adhesion mechanisms, commonly targeting carbohydrate structures present on gastric cells surface, have been identified[20] with *H. pylori* ligands including, among others, blood group antigens on mucins and glycolipids[8,11,20-26].

The best-characterized *H. pylori* adhesin is BabA, that mediates a strong adhesion between the bacteria and Le^b^ blood group antigen expressed on the surface of epithelial cells[8,27]. This work showed that adhesion is a relevant feature of *H. pylori* pathogenicity potential, with significantly higher adhesion levels observed for the HP26695 (pathogenic strain) when compared to the HPTx30a (non-pathogenic strain) in both cell lines. Considering that both strains don’t express BabA adhesin[20], the observed differences can not be explained through the BabA binding model, what suggests that other bacterial molecules are involved in the adhesion process.

Another important observation is that there is a higher adhesion of HP26695 and HPTx30a strains to GP202 cell line when compared to MKN45 cell line. This reflects different expression levels and availability of ligands at the cells surface. Previous characterization of mucins and carbohydrate expression on GP202 and MKN45 cell lines showed that Le^b^ has a significantly higher expression in GP202 cell line[29]. Still, this difference might not be relevant since BabA is not present in both bacterial strains[29]. In addition, the MUC1 expression is identical for both cell lines[29] and therefore can not be held responsible for the observed differences. GP202 has a higher expression of other carbohydrate antigens (Le^a^ and Le^y^)[29,30] compared to MKN45, that might be involved in *H. pylori* binding interactions. Moreover, additional ligands/interactions that are not yet explored may also exist that can explain this difference in adhesion levels between cell lines.

In order to study the influence of MUC1 VNTR variability in *H. pylori* binding, we used GP202, the cell line that showed higher bacteria adhesion and we analyzed GP202 transfected clones expressing recombinant MUC1 with a different number of repeats. These clones overexpress similar levels of recombinant MUC1[14]. We observed that MUC1 VNTR polymorphism has influence in the extent of *H. pylori* binding to gastric cells, with the higher adhesion levels observed in clones with larger VNTR regions. This may be due to the fact that MUC1 with larger Tandem Repeat regions contains more potential glycan receptors, thus potentially providing more bacterial binding sites. Moreover, we have previously shown that differences in VNTR length lead to glycosylation changes in the MUC1 Tandem Repeat[14], which may also contribute to the altered adhesion observed. Detailed evaluation of the results showed a small increase between the adhesion of GP202-Neo (control) and GP202-dTR that may be explained by the overexpression of MUC1 in recombinant clone GP202-dTR[14] and by the potential presence of O-glycosylated binding sites outside the VNTR region. No significant difference was observed between the adhesion of the bacteria to GP202-9TR and to GP202-42TR clones. We have previously observed the overexpression of MUC1 underglycosylated forms in GP202-42TR[14], which might explain why the adhesion levels are not proportional to VNTR size.

All these observations are important for understanding the bacterial and host molecular context of the colonization of gastric mucosa. Identification of a pathogenesis background, based upon host susceptibility traits like MUC1 VNTR polymorphism, will help to identify candidates more prone to bacterial colonization and patients more resilient to eradication strategies.
Background
More than half of the world population is persistently infected by H pylori. Adhesion of the bacteria to the gastric mucosa is essential for attachment and infection. Therefore it is important to know host and bacterial factors that condition the adhesion.

Innovations and breakthroughs
The study of host factors that influence the binding of H pylori to gastric cells may help to identify candidates more prone to bacterial colonization and patients more resilient to eradication strategies.

Applications
These findings may help to develop screening methods to identify candidates more prone to bacterial colonization and to develop more efficient eradication strategies, as well as to develop strategies to prevent or minimize H pylori binding to the gastric mucosa.

Peer review
This is a good study designed to elucidate that MUC1 VNTR polymorphism affects H pylori adhesion to gastric cells. The results are informative and potentially helpful for prevention of H pylori binding to the gastric mucosa.

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