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Expression of Lewis^b blood group antigen in *Helicobacter pylori* does not interfere with bacterial adhesion property

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Abstract

AIM: The finding that some *Helicobacter pylori* strains express Lewis b (Le^b) blood group antigen casts a doubt on the role of Le^b of human gastric epithelium being a receptor for *H. pylori*. The aim of this study was to determine if expression of Le^b in *H. pylori* interferes with bacterial adhesion property.

METHODS: Bacterial adhesion to immobilized Le^b on microtitre plate was performed in 63 *H. pylori* strains obtained from Singapore using *in vitro* adherence assay. Expression of Lewis blood group antigens was determined by ELISA assay.

RESULTS: Among 63 *H. pylori* strains, 28 expressed Le^b antigen. *In vitro* adhesion assay showed that 78.6 % (22/28) of Le^b-positive and 74.3 % (26/35) of Le^b-negative *H. pylori* isolates were positive for adhesion to immobilized Le^b coated on microtitre plate (P=0.772). In addition, blocking of *H. pylori* Le^b by prior incubation with anti-Le^b monoclonal antibody did not alter the binding of the bacteria to solid-phase coated Le^b.

CONCLUSION: The present study suggests that expression of Le^b in *H. pylori* does not interfere with the bacterial adhesion property. This result supports the notion that Le^b present on human gastric epithelial cells is capable of being a receptor for *H. pylori*.

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INTRODUCTION

Helicobacter pylori is the major etiologic agent of chronic active gastritis, and is generally accepted as a causative factor in the pathogenesis of gastritis, peptic ulcer (PU) disease and gastric adenocarcinoma[1-3]. It is estimated that over 50 % of the world' s population are infected with *H. pylori*.

The bacterium shows a strict tropism for gastric epithelium and is usually isolated from gastric epithelium and duodenal mucosa with gastric metaplasia. The presence of specific receptors for *H. pylori* on the gastric mucosa may explain its gastric tropism. The Lewis^b (Le^b) blood group antigen has been reported to be a receptor of *H. pylori*, and the blood-group antigen-binding adhesin, BabA, has been shown to mediate binding of *H. pylori* to human Le^b on gastric epithelium[4]. Following the finding that Le^b was expressed in some strains of *H. pylori*[5], the role of host Le^b as a receptor for *H. pylori* has been questioned considering the *H. pylori* lipopolysaccharide (LPS) Le^b may interfere with the interaction between the bacteria BabA and Le^b on gastric epithelium[5, 6]. This may be more important for the Asian strains where there is a higher frequency of *H. pylori* strains expressing type 1 blood-group antigens (Le^a, Le^b) (43.5 % for Le^b)[7, 8] comparing with Western strains (<10 % for Le^b). The high frequency of Le^b expression in *H. pylori* strains in our population offers a unique opportunity to investigate the potential influence of Le^b in *H. pylori* on the bacterial adhesion property.

MATERIALS AND METHODS

Patients and H. pylori isolates

H. pylori strains were isolated from the gastric biopsies of 108 patients undergoing upper gastrointestinal endoscopy for dyspepsia at the National University Hospital, Singapore. Informed consent was obtained from all the patients for gastroscopy and biopsies. A subset of 63 *H. pylori* strains from these 108 strains which were performed in our previous study[8] was randomly chosen for the *in vitro* adherence assay. Of these, 36 were isolated from male patients and 27 were from female patients. The average age of the patients was 43 years (16-78 years). Based on endoscopic and histologic examination, the patients were classified into the following groups: peptic ulcer (n=33), and chronic gastritis (n=30). The bacteria were isolated and identified as described previously[8]. Each strain was cultured on chocolate agar for 3-4 days at 37 ℃ in a humid incubator (Forma Scientific, Mountain View, USA) supplemented with 5 % CO_2.

In vitro adherence assay

The adherence assay was performed according to Gerhard *et al*[9] with minor modifications. Briefly, for each of the 63 *H. pylori* strains, the 4-day-old culture was harvested and washed twice in 0.05 M carbonate buffer (pH 9.6) before resuspending in 1 ml of the same buffer. A 10 μl of 10 mg· ml^{-1}of digoxigenin (Roche Diagnostics, Mannheim, Germany) solution was added to the bacterial suspension and incubated for 60 min at RT. Polysorb 96-well- microtiter plates (Nunc, Rochester, USA) were coated with 50 ng per well of Le^a, Le^b, Le^x and Le^y, (IsoSep, Tullinge, Sweden) in 50 μl of 0.05 M carbonate buffer (pH 9.6) while 50 μl of the same buffer was added as negative control. Following overnight incubation at RT, the solution was decanted without washing, and 100 μl of blocking buffer (0.5 % non-fat milk/0.2 %Tween-20) was added. After the plate was further incubated at RT for 1 hour, the solution was decanted without washing, and then 50 μl of digoxigenin

labeled bacteria diluted to an OD of 0.5 at 600 nm were added to each well of the plates and incubated for another 1 hour at RT with gentle agitation. After washing with PBS, 50 µl of 150 mU· ml^{-1} of anti-digoxigenin-HRP antibody (Roche Diagnostics, Mannheim, Germany) was added and incubated for 1 hour at RT. The plates were washed 3 times with PBS before adding 50 µl of o-phenylenediamine dihydrochloride (Sigma, Louis, USA) (0.4 mg· ml^{-1} in citric acid buffer with 0.025 % H_2O_2). The reaction was stopped with the addition of 2.5 M sulfuric acid. The OD value was read at 490 nm in an ELISA reader (Bio-Tek, Houston, USA). The strains were considered positive for adhesion to the antigen if the ratio of $OD_{Ag}/OD_{control}$ was >2.0[9]. The assay was carried out in duplicate for all the strains tested.

Lewis antigen expression

The expression of Lewis blood group antigens (Le^a, Le^b, Le^x and Le^y) was determined by enzyme-linked immunosorbent assay (ELISA) as described previously[8, 10]. The following murine monoclonal antibodies (mAb) were used: mAb 54-1F6A, specific for $Lewis^x$ (Le^x); mAb 1E52, specific for Le^y; mAb 7Le, specific for Le^a and mAb 225Le, specific for Le^b. Bacterial whole cells from 3-day cultures (7.5×10^6 ml^{-1}) were suspended in 1 ml phosphate buffered saline (PBS) at pH 7.4. One hundred ml of suspension was added to each well of 96-well microtitre plate (Nunc, Rochester, USA) and incubated overnight at room temperature (RT). Plates were then washed three times with PBS containing 0.05 % Tween-20 (PBST). Subsequently, an aliquot of 100 ml of mAbs (100 ng· ml^{-1}) was added and incubated overnight at RT. After washing three times with PBST, a 100 ml solution of 1:1 000 horseradish peroxidase-labelled goat anti-mouse immunoglobulin (DAKO, Glostrup, Denmark) diluted in PBST with 0.5 % goat serum was added. Color development occurred with the addition of H_2O_2 and o-phenylenediamine dihydrochloride (Sigma, St. Louis, USA) in phosphate citrate buffer (pH 5.4) in the dark for 30 min at RT. A 50 ml of 2.5 M H_2SO_4 solution was added to each well to stop the reaction. The optical density (OD) was read at 490 nm. OD of 0.2 was chosen as the cut-off value because the sum of non-specific background binding value for mAbs never exceeded an OD of 0.1. Synthetic protein-linked Lewis antigens, i.e., Le^a, Le^b, Le^x and Le^y (IsoSep, Tullinge, Sweden) were used as positive controls for the mAbs.

Adherence blocking assay by anti-Le^b mAb

To test if blocking LPS Le^b would alter the binding of the bacteria to Le^b on ELISA plate, two chemically characterized strains with Le^b (H428 and H507)[7] were subjected to adherence blocking assay by incubation with mAb 225Le, specific for Le^b[8, 11]. The bacteria were incubated with 225Le mAb (1.0, 10.0, and 100.0 µg· ml^{-1}) for 1 hour at RT, and then washed twice with PBST (PBS + 0.05 % Tween-20). The adherence assay was performed as described earlier.

Statistical analysis

Frequencies were compared using 2-tailed Fisher' s exact test (SPSS 9.0, Chicago, USA). The rations of OD values were expressed as means ± standard deviations, and the distributions of the ratios were compared by using Student' s *t* test for comparison of means of independent samples. A *P* value <0.05 was considered statistically significant.

RESULTS

In vitro adherence assay

Of the 63 *H. pylori* strains tested, 48 (76.2 %) were positive for adhesion to immobilized Le^b antigen on microtitre plate. The positive *H. pylori* strains showed ratios of $OD_{Ag}/OD_{control}$ between 2.0-4.1 while the negative strains exhibited values between 0.89-1.67. None of the *H. pylori* strains bound to Le^a, Le^x or Le^y.

Effect of Le^b expression in H. pylori on bacterial adhesion property

In the test for expression of Lewis antigen in *H. pylori*, 28 out of the 63 *H. pylori* strains expressed Le^b while 35 did not express Le^b based on ELISA. Among these 28 Le^b-positive strains, 22 (78.6 %) were positive for adhesion to immobilized Le^b antigen coated on plate as compared to 26 (74.3 %) of 35 Le^b-negative strains (*P*=0.772). Furthermore, the ratio of $OD_{Ag}/OD_{control}$ was not significant difference between LPS Le^b-positive strains and Le^b-negative strains (2.3±0.7 *vs* 2.3±0.7, *P*=0.988).

Adherence blocking assay by anti-Le^b mAb

Two chemically characterized Le^b positive *H. pylori* strains H428 and H507[7] were subjected to adherence blocking assay by prior incubation with the bacteria with specific mAb 225Le (100.0 µg· ml^{-1}). The ratio of $OD_{Ag}/OD_{control}$ for strain H428 was changed from 2.7 to 2.6 after incubation with mAb 225Le. The value of adhesion assay for strain H507 was showed minimal change from 1.1 to 1.0. No difference of the ratio of $OD_{Ag}/OD_{control}$ was observed when the bacteria were incubated with different concentrations (1.0, 10.0, and 100.0 µg· ml^{-1}) of mAb 225Le, which suggested that Le^b expression in *H. pylori* did not interfere with the bacterial adhesion to Le^b.

Effect of mixed Lewis expression on bacterial adhesion property

Of the 63 *H. pylori* strains tested, 55 expressed 2 or more Lewis antigens (Le^a, Le^b, Le^x or Le^y), and the remaining 8 strains expressed 1 Lewis antigen. Among the 55 strains with expression of 2 or more Lewis antigens, 42 (76.4 %) were positive for adhesion to immobilized Le^b compared with 6 (75 %) of 8 strains with 1 Lewis antigen expression (*P*=1.000). Furthermore, the ratio of $OD_{Ag}/OD_{control}$ was not significant difference between the strains with expression of 2 or more Lewis antigen and strains with 1 Lewis antigen (2.3±0.7 *vs* 2.2±0.8, *P*=0.836).

DISCUSSION

Attachment of *H. pylori* to gastric epithelium is important for its colonization and survival. This adhesion property protects the bacteria from the displacement from the stomach by gastric emptying and peristalsis[12]. A recent study has demonstrated that the *H. pylori* blood-group antigen-binding adhesin, BabA, facilitates bacterial colonization and augments a nonspecific immune response[13]. The fucosylated blood group antigens of Le^b and H type 1 have been proposed as receptors of *H. pylori*[4]. In addition, a study using transgenic mice expressing the human Le^b epitope in gastric epithelial cells indicated that Le^b antigen functioned as a receptor for *H. pylori* adhesin and mediated its attachment to gastric pit and surface mucous cells. However, following the observation[5] that some *H. pylori* strains also express Le^b, the question on the possible role of host Le^b as a receptor for *H. pylori* has been raised by Wirth *et al*[5] as well as Clyne and Drumm[6]. Furthermore, if host Le^b is the principle receptor for *H. pylori*, one might then expect a decreased prevalence of *H. pylori* in the secretor subjects because Le^b present in saliva or gastric mucus in secretor individuals would competitively inhibit the binding of *H. pylori*. However, numerous studies have shown that there is no association between prevalence of *H. pylori* and host secretor status[14], which indicates that competitive inhibition by Le^b in gastric mucus in secretor individuals may not be able to effectively prevent the colonization of *H. pylori*.

In this study, it was shown that there was no significant difference between Le^b-positive and Le^b-negative *H. pylori* strains with respect to their ability to adhere to immobilized Le^b, mimicking the epithelial cell Le^b antigen. This suggests that expression of Le^b in *H. pylori* strains in our study population does not interfere with the bacterial adhesion to immobilized Le^b *in vitro*. Furthermore, the blocking experiment using anti-Le^b mAb on two Le^b expressing strains (H428 and H507) showed that the expression of Le^b in *H. pylori* had no effect on the adhesion property of *H. pylori*. Additionally, our study population are mainly of Chinese origin which are predominantly Le (b+) phenotype[8], and *H. pylori* strains isolated from Asian population have a tendency to express type 1 Lewis antigen (Le^a and Le^b)[7, 8]. However, a high prevalence of *H. pylori* infection has been found in this population. These data further support our observation that expression of Le^b in *H. pylori* does not affect the bacterial adhesion.

Our previous study found that increased expression of Lewis antigens in *H. pylori* was associated with peptic ulceration in our population[8]. We, therefore,attempted to determine whether strains with Lewis antigen expression have advantage for binding to immobilized Le^b, but only observed that the increased expression of a combination of Le antigens in *H. pylori* had no influence on bacterial adhesion.

The chemical structures of Le^a, Le^b, Le^x, Le^y, iantigen, H type 1 and blood group A antigens expressed by *H. pylori* have been elucidated[7,15,16]. These antigens are also expressed on gastric mucosa[14]. The possible interaction between Lewis antigens expressed by *H. pylori* and gastric epithelium is intriguing. Le^x-Le^x homotypic interaction has been found to be important in eukaryotic cell interaction, and Le^x structure has recently been proven to be involved in the formation of adhesion pedestal between *H. pylori* and gastric epithelium[14]. More recently, Le^x structure in *H. pylori* has been demonstrated to promote the bacterial adhesion to gastric epithelium[17]. However, Le^b-Le^b homotypic interaction has not been proven, and our *in vitro* study does not support such an interaction of *H. pylori* LPS Le^b with epithelial Le^b. The next question is how a *H. pylori* population can manage to exist as single bacterium *in vivo* but not autoaggregative, when bacterium expresses both BabA adhesin and Le^b? *H. pylori* lectins such as BabA may evolve an ability to distinguish between host and bacterial ligands based on differences in their core structure, and thus avoid bacterial autoaggregation[18, 19].

In conclusion, expression of Le^b blood group antigen in *H. pylori* strains in our Asian population does not interfere with the bacterial adhesion to immobilized Le^b on microtitre plate. This result supports the notion that host Le^b present on the gastric epithelium is capable of being a receptor for *H. pylori*.

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