Advances in Medical Sciences 63 (2018) 205–211

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Original research article

Lotus tetragonolobus and *Maackia amurensis* lectins influence phospho-IκBα, IL-8, Lewis b and H type 1 glycoforms levels in *H. pylori* infected CRL-1739 gastric cancer cells

Iwona Radziejewska[a,*], Małgorzata Borzym-Kluczyk[b], Katarzyna Leszczyńska[c], Joanna Wosek[a], Anna Bielawska[d]

[a] Department of Medical Chemistry, Medical University of Białystok, Białystok, Poland
[b] Department of Pharmaceutical Biochemistry, Medical University of Białystok, Białystok, Poland
[c] Department of Microbiology, Medical University of Białystok, Białystok, Poland
[d] Department of Biotechnology, Medical University of Białystok, Białystok, Poland

ARTICLE INFO

Article history:
Received 2 March 2017
Accepted 13 June 2017
Available online 1 December 2017

Keywords:
H. pylori
Lectins
Mucins
N-acetylglucosamine

ABSTRACT

Purpose: Attachment of *Helicobacter pylori* to the mucous epithelial cells and the mucous layer is said to be a crucial step for infection development. Sugar antigens of gastric mucins (MUC5AC, MUC1) can act as receptors for bacterial adhesins. The aim of the study was to investigate if *Lotus tetragonolobus* and *Maackia amurensis* lectins influence the level of MUC1, MUC5AC, Lewis b, H type 1, sialyl Lewis x, phospho-IκBα and interleukin 8 in *Helicobacter pylori* infected gastric cancer cells.
Materials and methods: The study was performed with one clinical *H. pylori* strain and CRL-1739 gastric cancer cells. To assess the levels of mentioned factors immunosorbent ELISA assays were used.
Results: Coculture of cells with bacteria had no clear effect on almost all examined structures. After coculture with *H. pylori* and lectins, a decrease of the level of both mucins, Lewis b and H type 1 antigens was observed. Lectins addition had no effect on sialyl Lewis x. *Maackia amurensis* caused slight increase of phospho-IκBα while interleukin 8 level was decreased.
Conclusions: Lotus tetragonolobus and *Maackia amurensis* lectins can mediate in binding of *Helicobacter pylori* to gastric epithelium.

1. Introduction

Helicobacter pylori, a bacterium isolated first in 1982 [1], is responsible for chronic active gastritis, peptic ulcer disease and gastric cancer [2,3]. It is estimated that approximately 50% of the world's population is infected [4]. It is interesting that the vast majority of infected individuals (80–90%) are asymptomatic despite histologic gastritis [5]. The ability of the bacterium to cause disease depends on a variety of host, environmental and bacterial factors [6]. The mucus layer of the gastrointestinal tract is said to interact with *H. pylori* [7,8]. The attachment of the bacteria to the mucous epithelial cells and the mucous layer lining the gastric epithelium seems to be a crucial step for the pathogenesis of *H. pylori* [9,10]. Secreted oligomeric mucins MUC5AC and MUC6 are dominating components of mucous layer. There is also a membrane-tethered MUC1 mucin which extracellular domain can be released and is present in mucous [4,11]. Mucins are glycoproteins containing highly diverse carbohydrates, receptors for *H. pylori* adhesins [8]. The best characterized adhesins are the blood group antigen binding adhesin (BabA) that binds to Lewis b and H type 1 structures [12] and the sialic acid binding adhesin (SabA) that recognizes sialylated oligosaccharides [13,14].

During the course of bacterial infection and inflammation mucins glycosylation can change. Some of these alterations can be considered as a form of defense against *H. pylori* infection [13,15,16]. However, the importance of these changes as well as involvement of mucins in the inflammation development are still not fully understood.

MUC1 seems to play a special role in the course of *H. pylori* infection because of epithelial-membrane binding and possible participation in intracellular signaling cascades leading to the activation of transcription factors (e.g. nuclear factor kappa-light-

* Corresponding author at: Department of Medical Chemistry, Medical University of Bialystok, Mickiewicza str. 2a, Białystok, Poland, Tel.: +48 85 748 5673; fax: +48 85 748 5669.
E-mail address: iwona@umb.edu.pl (I. Radziejewska).

http://dx.doi.org/10.1016/j.advms.2017.06.003
1896-1126/

chain-enchancer of activated B cells) that induce the production of proinflammatory chemokines (e.g. interleukin 8) [17–19]. Phosphorylation of IκBα (inhibitor of NF-κB) inhibitor and its subsequent degradation in the cytoplasm are required for the translocation of NF-κB to the nucleus [20,21]. It is suggested that MUC1 can inhibit IκBα phosphorylation and NF-κB activation by binding to IKK kinase [22]. Other mechanisms of MUC1 participation in intracellular signaling and *H. pylori* inflammation development are not excluded.

Fucose and sialic acid are of widespread occurrence as components of the oligosaccharide chains of glycoconjugates present in animal cells and tissues and appear to play a role in many important biological recognition mechanisms [23]. LTA lectin purified from seeds of *Lotus tetragonolobus* was shown to agglutinate human O(H) red blood cells. It is able to recognize Fucα1-3GlcNAc (N-acetylglucosamine) antigen on carbohydrate chains [24]. *Lotus tetragonolobus* seeds are edible, which are one of vegetables known as asparagus pea or winged pea. MAL (*Maackia Amurensis* leukoagglutinin) is one of two lectins isolated from *Maackia amurensis* seeds. It strongly binds carbohydrate antigens containing sialic acid, particularly Neu5Acα2-3Galβ1-4GlcNAc/Glc (Gal - galactose) sequence and reveals lymphocyte mitogenic activity [25]. The above lectins are able to recognize and bind carbohydrate antigens which can be potentially involved in binding of gastric mucins with *H. pylori* adhesins. Because of this we assumed that if examined lectins are able to influence the expression of mucins, specific carbohydrate antigens or other factors taking part in interactions with bacteria, they can have impact on *H. pylori* infection development.

2. Materials and methods

2.1. Cell culture and bacteria

Gastric adenocarcinoma cells (CRL-1739, ATCC, Manassas, VA, USA) were cultured according to standard procedures in F-12 medium containing 10% heat inactivated FBS (fetal bovine serum) (Life Technologies, Carlsbad, CA, USA), 100 U/mL penicillin and 100 μg/mL streptomycin (Sigma, St. Louis, MO, USA). Cells were seeded in six-well plates. *H. pylori* was laboratory strain from Department of Microbiology (Medical University of Białystok, Poland). It was isolated from gastric epithelial cells from patient suffering from gastritis. The scrapings were collected before the beginning of the treatment, under endoscopic examination, from the prepyloric area and the body of the stomach. Immediately the scrapings were carried into the transport medium *Portagerm pylori* (bioMerieux, Saint-Vulbas, France). Then, after homogenization, the bacteria were cultured on Pylori Agar and Columbia Agar supplemented with 5% sheep blood (bioMerieux, Saint-Vulbas, France) for 7 days at 37 °C under microaerophilic conditions using Genbag microaer (bioMerieux, Saint-Vulbas, France). Microorganisms were identified upon the colony morphology, by the Gram method. Additionally, the activity of the bacterial urease, catalase and oxidase were also determined. To prove *H. pylori* species, ELISA test (enzyme-linked immunosorbent assay) (HpAg48; EQUIPAR, Spain) was used. Then bacteria were subcultured in the same conditions, suspended at 1.2×10^9 bacteria/ml in PBS and added to growing gastric cancer cells at a multiplicity of infection (MOI) of 10 for the indicated below period of time.

24 h prior *H. pylori* treatment, the cell medium was changed to antibiotics-free F-12 medium, supplemented or not with unlabeled LTA (*Lotus Tetragonolobus* agglutinin) or MAL lectins (Vector, Burlingame, CA, USA) (0.08 μg of proper lectin/1 μg protein in lysed gastric cancer cells; conditions were established previously). After bacteria addition the cells were cultured for 24 h. Culture media were collected for IL-8 determinations. The cells were washed with PBS and lysed at 4 °C with RIPA buffer (Sigma, St. Louis, MO, USA) supplemented with protease inhibitors (Sigma, St. Louis, MO, USA), diluted 1: 200 in RIPA buffer. Culture media and lysates were aliquoted and stored in −70 °C. The study was approved by the Institutional Ethical Committee with the principles of the Declaration of Helsinki and informed consent was obtained from the patient.

2.2. Lectin-based ELISA for sugar antigens recognized by LTA and MAL lectins

50 μL of cell lysates with protein concentration 100 μg/mL (dilution was performed with PBS buffer) were coated on microtiter plates and incubated overnight at room temperature (RT). The protein content in the samples was measured using bicinchoninic method [26]. The plates were washed three times (100 μL) in PBS, 0.05% Tween (PBS-T) between all ensuring steps. Unbound sites were blocked with 100 μL of 1% blocking reagent for ELISA (Roche Diagnostics, Mannheim, Germany) for 1 h. The plates were incubated for 1 h with 100 μL of biotynylated *Lotus tetragonolobus* (LTA) recognizing Fucα1-3GlcNAc (Glc - glucose) and *Maackia amurensis* (MAL) lectin specific for Neu5Acα2-3Galβ1-4GlcNAc/Glc antigen (Vector, Burlingame, CA, USA) diluted to 0.5 μg/mL in washing buffer with 1% of BSA (bovine serum albumin) (Sigma, St Luis, MO, USA). LTA lectin solution was supplemented with 0.1 mmol/L $CaCl_2$ and MAL with 0.1 mmol/L $CaCl_2$ and 0.01 mmol/L $MnCl_2$. Plates were then incubated with 100 μL of horseradish peroxidase avidin D solution (Vector, Burlingame, CA, USA) for 1 h. After washing 4 times in PBS, the coloured reaction was developed by incubation with 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) – liquid substrate for horseradish peroxidase (Sigma, St Luis, MO, USA). Absorbance at 405 nm was measured after about 30–40 min. All samples were analyzed in triplicate in three independent tests. As a negative control the wells with PBS instead of cell lysates were used. The

Table 1
Source of antibodies and their dilutions.

Antibody	Catalogue number	Clone	Source	Dilution
Anti-MUC1 (IgG)	ab89492	BC2	Abcam	1:400
Anti-MUC5AC (IgG)	M5293	45M1	Sigma	1:400
Anti-Lewis b (IgG)	MA1-90886	LWB01	Thermo Scientific	1:400
Anti-H type 1 (IgG)	ab3355	17-206	Abcam	1:400
Anti-silayl Lewis x (IgM)	MAB2096	MAB2096	Millipore	1:200
Anti-phospho-IκBα (IgG)	5210	12C2	Cell Signalling	1:100
Anti-mouse IgG peroxidase conjugated	A9044		Sigma	1:2000
Anti-mouse IgM peroxidase conjugated	B9265		Sigma	1:2000

absorbance of negative control (less than 0.1) was subtracted from absorbances of the samples.

2.3. ELISA for MUC1, MUC5AC, Le b, H type 1, sialyl Le x and phospho-IκBα

50 μL of cell lysates with protein concentration 200 μg/mL (for phospho-IκBα determination) or 100 μg/mL (for MUC1, MUC5AC, Le b Lewis b antigen, H type 1 and sialyl Le x determination sialylated Lewis x antigen) were coated on microtiter plates and incubated overnight at RT. Blocking and washing steps were as described above. Then the plates were incubated (2 h at RT) with 100 μL of monoclonal antibodies diluted in 1% bovine serum albumin (BSA) in PBS-T (for specifications and dilutions of antibodies see Table 1). Then the plates were incubated with secondary antibody, horseradish peroxidase conjugated rabbit anti-mouse IgG or anti-mouse IgM and the coloured reaction was performed as described above. All samples were analyzed in triplicate in three independent tests. The negative control was used as described above.

2.4. IL-8 ELISA

IL-8 was quantitatively determined by ELISA test using commercially available capture and detection antibodies (BD Biosciences, San Diego, CA, USA) according to the manufacturer's instructions. Briefly, microwells of 96-well plate (NUNC F96; Maxisorp, Roskilde, Denmark) were coated with 100 μL of capture antibody diluted in 0.1 M bicarbonate buffer, pH 9.5 and incubated overnight at 4 °C. The plates were washed three times (200 μL) in PBS-T between all ensuring steps. Unbound sites were blocked with 200 μL PBS, 10% FBS. Then the cell culture media were added and incubated for 2 h at RT followed by incubation with biotinylated detection antibody, peroxidase-labeled streptavidin and tetramethylbenzidine substrate. Absorbance at 450 nm was measured after 30 min and IL-8 levels were determined from standard curve prepared with serial dilutions of purified chemokine. All samples were analyzed in triplicate or quadruplicate in three independent tests and standard curves were performed on each plate.

2.5. Statistics

Experimental data were presented as mean ± standard deviation SD from at least three independent experiments using the STATISTICA 10 StatSoft program. Statistical differences were determined by one-way ANOVA followed by the Duncan's multiple range post hock test, $p < 0.05$ was considered statistically significant.

3. Results

The presence of carbohydrate antigens revealed by LTA and MAL lectins in CRL-1739 cell lysates was determined. In Fig. 1 it is seen that cancer cells possess Fucα1-3GlcNAc (recognized by LTA lectin) and Neu5Acα2-3Galβ1-4GlcNAc/Glc (recognized by MAL lectin). These antigens, as part of specific sugar structures, can be potentially considered as receptors for bacterial adhesins. However, coculture of cells with *H. pylori* had no effect on the expression of these antigens.

In our preliminary results it was revealed that lectins LTA and MAL had no influence for mucins expression in gastric cancer cells CRL-1739 (results are no shown). MUC1 and MUC5AC are mucins which are said to be potentially involved in *H. pylori* induced inflammation process especially because of interactions between mucins sugar antigens and bacterial adhesins. In Fig. 2 we can

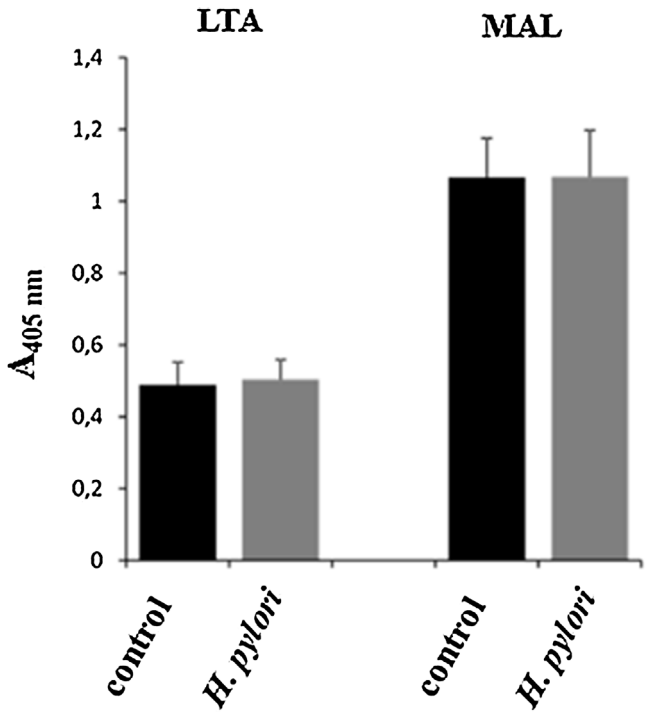

Fig. 1. Relative levels of carbohydrate antigens Fucα1-3GlcNAc recognized by *Lotus tetragonolobus* (LTA) and Neu5Acα2-3Galβ1-4GlcNAc/Glc detected by *Maackia amurensis* (MAL) lectin in CRL-1739 cell lysates (as absorbance at 405 nm after reactivity with proper lectins in ELISA tests). CRL-1739 cells were cultured for 24 h without or with *H. pylori*. Values ± SD are the mean of triplicate cultures.

observe that bacterium (while cocultured with cancer cells for 24 h) had no influence on expression of both mucins. However, coculture of cells with *H. pylori* and lectins resulted in statistically significant decrease of MUC1 expression (by 18.7% for LTA and by 19% for MAL). For MUC5AC, statistically significant decrease of expression (by 14.28%) was observed after coincubation with MAL lectin. In case of LTA lectin the decrease (by 7.35%) was not statistically significant.

As it is seen in Fig. 3, coculture of *H. pylori* with examined cells had no influence on Lewis b antigen expression. By contrast, addition of bacterium and lectins to the coculture of cancer cells caused statistically significant inhibition of this antigen expression, by 31.3% for LTA and 27.5% for MAL. In case of H type 1 structure, similar relations can be observed with the exception that *H. pylori* addition caused slight non-significant increase of the expression of this antigen. LTA lectin caused 22.5% decrease of the antigen and for MAL the decrease was by 12.9%. There is almost no effect of bacterium and both lectins on sialyl Lewis x expression.

NF-κB normally resides in a form of inactive complex with IκBα. After phosphorylation of IκBα, NF-κB is released allowing the activated transcription factor to translocate into the nucleus and activate proinflammatory (e.g. IL-8) gene expression. As shown in Fig. 4, *H. pylori* treated CRL-1739 cells had only slightly lower (by 7%) non significant level of phospho IκBα in cells lysates in comparison to non-infected cells. LTA addition to the culture had no effect on phospho IκBα expression. Almost no effect of this lectin addition was also observed on IL-8 released to the culture medium (Fig. 5). By contrast, MAL pretreatment caused statistically significant decrease (by 59%) of IL-8 production. However, in these conditions, phospho IκBα was slightly increased (without statistical significance) (Fig. 4).

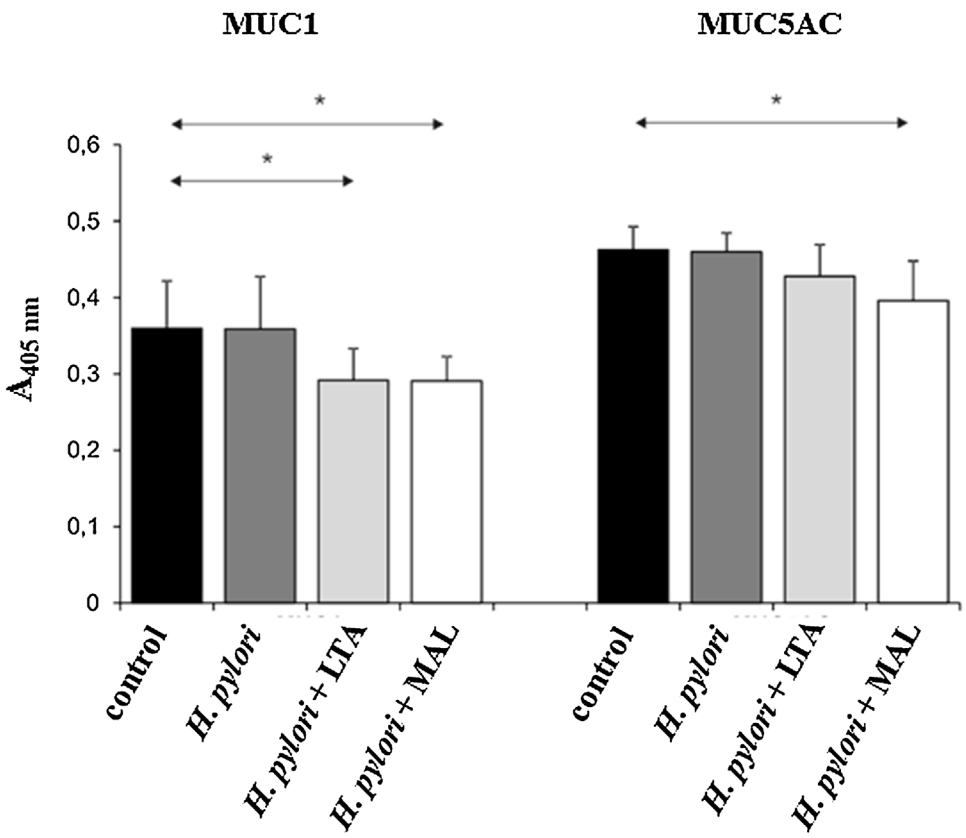

Fig. 2. Relative levels of MUC1 and MUC5AC mucins in CRL-1739 cell lysates (as absorbance at 405 nm after reactivity with monoclonal anti-MUC1 and anti-MUC5AC antibodies in ELISA tests). 24 h prior *H. pylori* treatment, the cell medium was changed to antibiotics-free medium supplemented or not with LTA and MAL lectins. After bacteria addition the cells were cultured for next 24 h. Values ± SD are the mean of triplicate cultures. * $p < 0.05$.

4. Discussion

It is known that gastric mucins are involved in interactions with *H. pylori* adhesins during infection development. Secreted MUC5AC and epithelial MUC1 are said to interact directly with bacterium by specific carbohydrate receptors, while MUC6 reveals antimicrobial activity [9,27–29]. The adhesion of *H. pylori* to gastric epithelial cells plays a crucial role in the origin of gastroduodenal diseases [10,30]. It is said that *H. pylori* is among major activators of NF-κB pathway through IκBα phosphorylation [31]. As a result, IL-8, one of the important chemotactic factors for granulocytes, secreted mainly by epithelial cells, can be produced. It can have a significant implication for the infection pathogenesis [32].

In our study we assumed that LTA and MAL lectins, recognizing specific sugar antigens can influence the interactions between bacterial adhesins and epithelial carbohydrate structures. LTA lectin isolated from edible *Lotus tetragonolobus* seeds recognizes especially Fucα1-3GlcNAc antigen [24]. MAL lectin isolated from *Maackia amurensis* seeds is commonly used to detect Neu5Acα2-3Galβ1-4GlcNAc/Glc antigen. This lectin can also bind unsialylated, sulfonated SO_4-3-Galβ structure. To be sure about binding specificity, sialidase treatment could be performed what would reduce or eliminate the lectin-binding signal in case of lack of sulfated structures [25]. However this treatment was not used in our study so we assume that examined lectin detects especially sialylated antigens. As potential effects of our experiments we expected the changes in the expression of some factors participating in the mentioned interplay of pathogen with the "host". Surprisingly, upon our results we observed almost no influence of *H. pylori* (cocultured with examined cells for 24 h) on the level of carbohydrate antigens recognized by examined lectins as well as on MUC1, MUC5AC mucins, Le b, H type 1 and sialyl Le x structures. These results are not in accordance with the suggestions of other authors about alterations of epithelial carbohydrate glycosylation during the course of infection [13,15,16,33,34]. Our determinations of mentioned structures were done with cell lysates, so we suggest that main alterations can occur within structures located in the culture medium. However, in this study such determinations were not performed. A lack of adhesive properties of examined strain to mentioned structures shouldn't be also excluded. The study was done with one clinical strain in which specific adhesive properties were not characterized. By contrast, both used lectins, while cocultured together with *H. pylori,* caused a decrease of levels of MUC1, MUC5AC mucins and also Le b and H type 1 antigens. One of possible explanations of these results can be that substantial amounts of mucins (with Le b and H type 1 on them) were bound to bacteria and were removed by centrifugation prior to determination in cell lysates (by ELISA tests). So we can suggest that lectins mediate in binding of *H. pylori* with specific carbohydrate antigens present on mucins. We can also assume that lectins can interact with carbohydrates on *H. pylori* surface as sugar antigens are commonly expressed on the O-specific chain of the bacterial lipopolysaccharide (LPS) [35]. Our findings are similar to those obtained with human gastric epithelial cell line MKN7 by Linden et al. [17]. The authors suggest that mucins, especially MUC1 with its extracellular long domain, can act as releasable decoy, because bacterium, after binding to mucin can be released together with extracellular domain of this mucin. However our results are from cell lysates and the culture media were not examined. Interestingly almost no effect of *H.* pylori and lectins addition is observed in case

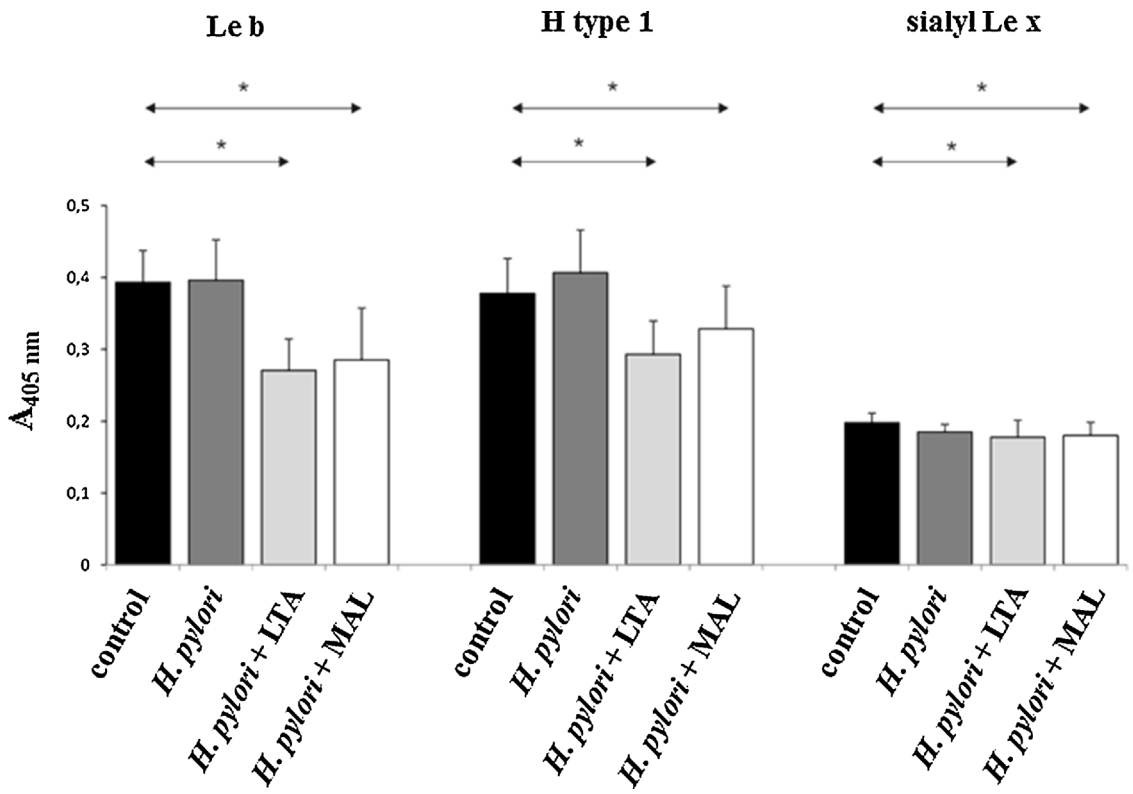

Fig. 3. Relative levels of Lewis b, H type 1 and sialyl Lewis x antigens in CRL-1739 cell lysates (as absorbance at 405 nm after reactivity with monoclonal anti-Lewis b, anti-H type 1 and anti-sialyl Lewis x antibodies in ELISA tests). 24 h prior *H. pylori* treatment, the cell medium was changed to antibiotics-free medium supplemented or not with LTA and MAL lectins. After bacteria addition the cells were cultured for next 24 h. Values ± SD are the mean of triplicate cultures; * $p < 0.05$.

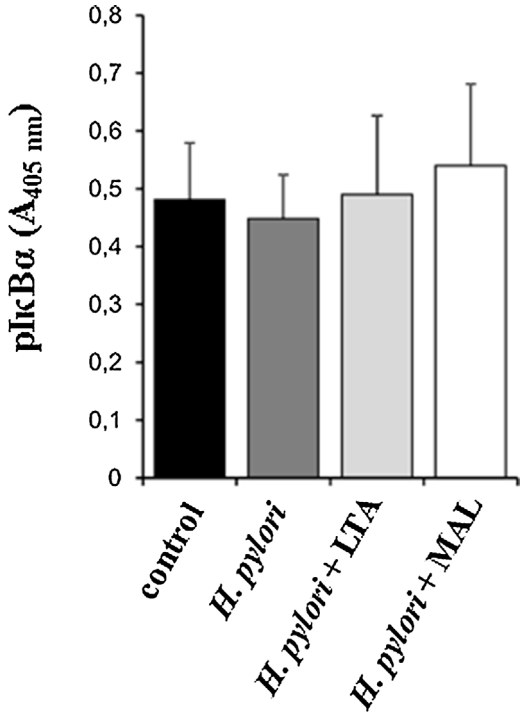

Fig. 4. Relative level of phospho IκBα in CRL-1739 cell lysates (as absorbance at 405 nm after reactivity with monoclonal anti-phospho-IκBα antibody in ELISA test). 24 h prior *H. pylori* treatment, the cell medium was changed to antibiotics-free medium supplemented or not with LTA and MAL lectins. After bacteria addition the cells were cultured for next 24 h. Values ± SD are the mean of triplicate cultures.

of sialyl Le x antigen. It is known that sialylated Le structures are common in infected and inflamed gastric mucous. Upon our results we can suggest that the bacterial strain used in our study has no adhesive properties to sialylated sugar antigens and LTA and MAL lectins don't participate in *H. pylori* binding to these carbohydrate structures.

As it was mentioned above, phosphorylated form of IκBα and IL-8 levels can be influenced by *H. pylori* during infection development. However such impact was not observed after 24 h coincubation of bacterium with examined, gastric cancer cells or after coincubation with *H. pylori* and LTA lectin. This can be explained by possible degradation of phospho-IκBα in peroxisomes shortly after activation. 24 h incubation time used in our experiments can be too long period for proper assessment of the factor. Interestingly, clear inhibitory effect on IL-8 level in culture media was observed after coincubation of cells with *H. pylori* and MAL. We assume that in these conditions bacterial infection is not able to induce signal transduction leading to increased IL-8 production. Slightly increased level of phospho-IκBα after coculture of gastric cancer cells with the bacterium and MAL lectin can suggest other than described above pathway of IL-8 expression, where both factors act independently.

5. Conclusions

Because of observed changes in the expression of some factors potentially participating in the development of *Helicobacter pylori* infection we suggest that *Lotus tetragonolobus* and *Maackia amurensis* lectins can mediate in binding of bacterium to gastric epithelium. As *H. pylori* uses the epithelial carbohydrates as receptors for adhesion and infection, a strategy of glycan related inhibition of bacterial colonization using carbohydrate-binding structures could have therapeutic effect on infection. The

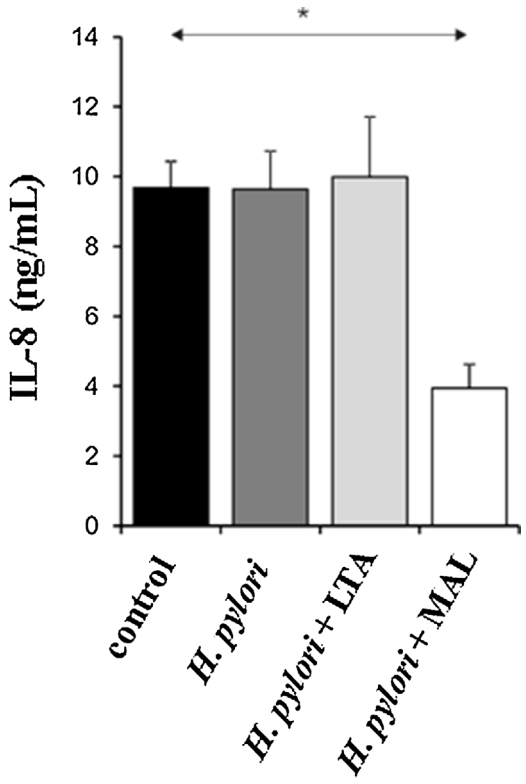

Fig. 5. Interleukin-8 levels secreted into the CRL-1739 cell culture supernatant (determined in ELISA test). 24 h prior *H. pylori* treatment, the cell medium was changed to antibiotics-free medium supplemented or not with LTA and MAL lectins. After bacteria addition the cells were cultured for next 24 h. Values ± SD are the mean of triplicate cultures; * $p < 0.05$.

increasing antibiotic resistance creates the need to look for this kind of new, alternative therapeutic strategies.

Our work is preliminary one with some limitations, including especially usage of only one *H. pylori* strain without specific characterization of its adhesive properties. The subject is planned to be explored more thoroughly with different times of cocultures of gastric cells with some bacterial strains and lectins. We are going to check if there would be any correlation between bacterial adhesins genotypes and gastric cells response related to changes in factors taking part in interactions concerning bacteria and epithelium.

Conflict of interest

The authors declare no conflict of interests.

Financial disclosure

This work was supported by Medical University of Białystok grant 143-03753F.

This study was conducted with the use of equipment purchased by the Medical University of Bialystok as part of the OP DEP 2007–2013, Priority Axis I.3, contract No POPW.01.03.00-20-008/09.

References

[1] Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984;1:1311–5, doi:http://dx.doi.org/10.1016/S0140-6736(84)91816-6.
[2] Boltin D, Niv Y. Mucins in gastric cancer – an update. J Gastrointest Dig Syst 2013;3:15519.
[3] McLean MH, El-omar EM. Genetics of inflammation in the gastrointestinal tract and how it can cause cancer. Resent Results Cancer Res 2011;185:173–83.
[4] Guang W, Czinn SJ, Blanchard TG, Kim KCH, Lillehoj EP. Genetic regulation of MUC1 expression by *Helicobacter pylori* in gastric cancer cells. Biochem Biophys Res Commun 2014;445:145–50.
[5] Kusters JG, Van Vliet AH, Kuipers EJ. Pathogenesis of *Helicobacter pylori* infection. Clin Microbiol Rev 2006;19:449–90.
[6] Rossez Y, Maes E, Darroman TL, Gosset P, Ecobichon C, Curt MJC, et al. Almost all human gastric mucin O-glycans harbor blood group A, B, or H antigens and are potential binding sites for *Helicobacter pylori*. Glycobiology 2012;22:1193–206.
[7] Linden SK, Wickstrom C, Lindell G, Gilshenan K, Carlstedt I. Four models of adhesion are used during *Helicobacter pylori* binding to human mucins in the oral and gastric niches. Helicobacter 2008;13:81–93.
[8] Kenny DT, Skoog EC, Linden SK, Struwe WB, Rudd PM, Karlsson NG. Presence of terminal N-acetylgalactosamineβ1-4N-acetylglucosamine residues on O-linked oligosaccharides from gastric MUC5AC: involvement in *Helicobacter pylori* colonization? Glycobiology 2012;22:1077–85.
[9] Linden SK, Sutton P, Karlsson NG, Korolik V, McGuckin MA. Mucins in the mucosal barrier to infection. Mucosal Immunol 2008;1:183–97.
[10] McGuckin MA, Linden SK, Sutton P, Florin TH. Mucin dynamics and enteric pathogens. Nat Rev Microbiol 2011;94:265–78.
[11] Linden SK, Driessen KM, Mcguckin MA. Improved in vitro model systems for gastrointestinal infection by choice of cell line, pH, microaerobic conditions, and optimization of culture conditions. Helicobacter 2007;12:341–53.
[12] Ilver D, Arnqvist A, Ögren J, Frick IM, Kersulyte D, Incecik ET, et al. *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science 1998;279:373–7.
[13] Mahdavi J, Sonden B, Hurtig M, Olfat FO, Forsberg L, Roche N, et al. Helicobacter pylori SabA adhesin in persistent infection and chronic inflammation. Science 2002;297:573–8.
[14] Magnusson EK, Norberg T, Lindh F, Lundskog BB, Arnqvist A, Hammarstrom L, et al. *Helicobacter pylori* SabA adhesin in persistent infection and chronic inflammation. Science 2002;297:573–8.
[15] Linden S, Nordman H, Hedenbro J, Hurtig M, Borén T, Carlstedt I. Strain and blood group-dependent binding of *Helicobacter pylori* to human gastric MUC5AC glycoforms. Gastroenterology 2002;123:1923–30.
[16] Skoog EC, Sjoling A, Navabi N, Holgersson J, Lundin SB, Linden SK. Human gastric mucins differently regulate *Helicobacter pylori* proliferation, gene expression and interactions with host cells. PLoS One 2012;7:e36378 10.1371.
[17] Linden SK, Sheng YH, Every AL, Miles KM, Skoog EC, Florin THJ, et al. MUC1 limits *Helicobacter pylori* infection both by steric hindrance and by acting as a releasable decoy. PLoS Pathog 2009;5:e1000617 10.1371.
[18] Park YS, Guang W, Blanchard TG, Kim KC, Lillehoj EP. Suppression of IL-8 production in gastric cells by MUC1 mucin and peroxisome proliferator-associated receptor-γ. Am J Physiol Gastrointest Liver Physiol 2012;303:G765–74.
[19] Lillehoy EP, Guang W, Ding H, Czinn SJ, Blanchard TG. Helicobacter pylori and gastric inflammation: role of MUC1 mucin. J Pediatr Biochem 2012;2:125–32.
[20] Choi IJ, Fujimoto S, Yamauchi K, Graham DY, Yamaoka Y. *Helicobacter pylori* environmental interactions: effect of acidic conditions on *H. pylori*-induced gastric mucosal interleukin-8 production. Cell Microb 2007;9:2457–69.
[21] Sokolova O, Borgmann M, Rieke C, Schweitzer K, Rothkotter HJ, Naumann M. *Helicobacter pylori* induces type 4 secretion system-dependent, but CagA-independent activation of IκBs and NF-κB/RelA at early time points. Int J Med Microb 2013;303:548–52.
[22] Guang W, Ding H, Czinn SJ, Kim KC, Blanchard TG, Lillehoj EP. Muc1 cell surface mucin attenuates epithelial inflammation in response to a common mucosal pathogen. J Biol Chem 2010;285:20547–57.
[23] Knibbs RN, Goldstein IJ, Ratcliffe RM, Shibuya N. Characterization of the carbohydrate binding specificity of the leukoagglutinating lectin from *Maackia amurensis*. J Biol Chem 1991;266:83–8.
[24] Yan L, Wilkins PP, Alvarez-Manilla G, Do S, Smith DF. Immobilized *Lotus tetragonolobus* agglutinin binds oligosaccharides containing the Le^x determinant. Glycoconjugate J 1997;14:45–55.
[25] Geisler C, Jarvis DL. Effective glycoanalysis with *Maackia amurensis* lectins requires a clear understanding of their binding specificities. Glycobiology 2011;21:988–93.
[26] Smith PK, Krohn RJ, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, et al. Measurement of protein using bicinchoninic acid. Anal Biochem 1985;150:76–85 [Erratum: Anal Biochem 1987;163:279.].
[27] Van Den Brink GR, Tytgat KM, Van Der Hulst RW, Van Der Loos CM, Einerhand AW, Buller HA, et al. *H. pylori* colocalises with MUC5AC in the human stomach. Gut 2000;46:601–7.
[28] Kawakubo M, Ito Y, Okimura Y, Kobayashi M, Sakura K, Kasama S, et al. Natural antibiotic function of a human gastric mucin against *Helicobacter pylori* infection. Science 2004;305:1003–6.
[29] Lee H, Wang P, Hoshino H, Ito Y, Kobayashi M, Nakayama J, et al. α1,4GlcNAc-capped mucin-type O-glycan inhibits cholesterol α-glucosyltransferase from *Helicobacter pylori* and suppresses *H. pylori* growth. Glycobiology 2008;18:549–58.
[30] Kaji T, Ishihara S, Ashizawa N, Hamamoto N, Endo H, Fukuda R, et al. Adherence of *Helicobacter pylori* to gastric epithelial cells and mucosal inflammation. J Lab Clin Med 2002;139:244–50.

[31] Ebrahimi-Askari R, Behmanesh M, Ghalandary M, Soleimani M. Nuclear factor-κB1 expression levels in human gastric adenocarcinoma. Mol Biol Res Commun 2012;1:57–64.
[32] Rad R, Gerhard M, Lang R, Schoniger M, Rosch T, Schepp W, et al. The *Helicobacter pylori* blood group antigen-binding adhesin facilitates bacterial colonization and augments a nonspecific immune response. J Immun 2002;168:3033–41.
[33] Byrd JC, Yunker CK, Xu QS, Sternberg LR, Bresalier RS. Inhibition of gastric mucin synthesis by *Helicobacter pylori*. Gastroenterology 2000;118:1072–9.
[34] Radziejewska I, Borzym-Kluczyk M, Namiot Z, Stefańska E. Glycosylation of mucins present in gastric juice: the effect of *Helicobacter pylori* eradication treatment. Clin Exp Med 2011;11:81–8.
[35] Sheu SM, Sheu BS, Yang HB, Lei HY, Wu JJ. Anti-Lewis x antibody promotes *Helicobacter pylori* adhesion to gastric epithelial cells. Infect Immun 2007;75:2661–7.