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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

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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.
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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-

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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

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