

Research Article

Interaction of *Helicobacter pylori* with C-Type Lectin Dendritic Cell-Specific ICAM Grabbing Nonintegrin

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In this study we asked whether *Helicobacter pylori* whole cells and lipopolysaccharide (LPS) utilize sugar moieties of Lewis (Le) antigenic determinants to interact with DC-SIGN (dendritic cell specific ICAM grabbing nonintegrin) receptor on dendritic cells (DCs). For this purpose the soluble DC-SIGN/Fc adhesion assay and the THP-1 leukemia cells with induced expression of DC-SIGN were used. We showed that the binding specificity of DC-SIGN with *H. pylori* Le^{X/Y} positive whole cells and *H. pylori* LPS of Le^{X/Y} type was fucose dependent, whereas in Le^{X/Y} negative *H. pylori* strains and LPS preparations without Lewis determinants, this binding was galactose dependent. The binding of soluble synthetic Le^X and Le^Y to the DC-SIGN-like receptor on THP-1 cells was also observed. In conclusion, the Le^{X/Y} dependent as well as independent binding of *H. pylori* whole cells and *H. pylori* LPS to DC-SIGN was described. Moreover, we demonstrated that THP-1 cells may serve as an *in vitro* model for the assessment of *H. pylori*—DC-SIGN interactions mediated by Le^X and Le^Y determinants.

1. Introduction

Helicobacter pylori is a causative agent of chronic gastritis, gastroduodenal ulcers, and gastric cancers. The correlation between *H. pylori*-infection and gastroduodenal diseases was described in 1983 by Marshall and Warren [1]. The course of *H. pylori* infection depends on the host immune responses towards this pathogen, both innate and adaptive. In *H. pylori* infected individuals the gastric mucosa is massively infiltrated with immunocompetent cells, which interact in a complex way with bacterial cells. Such interactions are responsible for gastric pathologies but they are also involved in the elimination of these pathogens from the gastric mucosa [2]. During the first stages of the infection various *H. pylori* compounds, for example, urease, vacuolating cytotoxin-VacA, or cytotoxin associated gene A antigen (CagA), initiate an acute inflammatory response in the gastric epithelium, which later becomes chronic [3–5]. Long-lasting inflammation results in many pathological disorders in the mucus

layer and diminished ability of the immune cells to fight the infection [6–10].

Although a lipopolysaccharide (LPS) is an important proinflammatory compound of gram-negative bacteria [11], the structure of *H. pylori* lipid A probably evolved in the mode which promoted persistence of the infection. It was shown that *H. pylori* LPS regulates the expression of adhesins and it can diminish the secretion of inflammatory cytokines by host immune cells [12]. Recently, antiphagocytic and antiproliferative properties of *H. pylori* LPS were also detected [13, 14]. Downregulation of the natural cytotoxic capacity of lymphocytes in response to *H. pylori* LPS was correlated with the modulation of IFN- γ , interleukin 2 (IL-2) and IL-10 secretion by the immune cells [15]. It was suggested that *H. pylori* LPS, through the activation of immunocompetent cells diminish the number of bacteria in the gastric tissue and thus prolong the infection [16].

The majority of *H. pylori* strains produce LPS with Lewis (Le) blood group antigens in O-specific chains: Le^X, Le^Y,

H type 1, Le^a, Le^b, i-antigen, and sialyl Le^X [17–22]. The sugar residues in the O-specific chains, which are similar to Le determinants of the host, influence the activity of *H. pylori* LPS. The expression of Le determinants by *H. pylori* results in better attachment of the bacteria to the host epithelial cells, modulation of the inflammatory response, and evasion of the bacteria due to mimicking blood group antigens present on the gastric mucosa [23, 24]. The epitope mimicry may contribute to the pathological, autoreactive responses during *H. pylori* infections [25, 26]. The Lewis expression on *H. pylori* cells is closely related to the epithelial area and the stage of disease [27].

The interactions of LPS with host cells are mediated by both, cellular and soluble molecules involved in cell signaling via Toll-like receptor 4 (TLR 4) [28–30]. Analyses of the interactions between purified *H. pylori* LPS and TLRs revealed that, in contrast to LPSs from other gram-negative bacteria, the LPS of *H. pylori* is not effectively recognized by TLR4. The localization of TLRs on the basolateral poles of epithelial cells reduces the likelihood of *H. pylori* being recognized by these receptors [31, 32]. However, it was suggested that the phase-variable expression of Lewis antigens allows the bacteria to modulate the host adaptive immune response through interactions with DC-SIGN (dendritic cell-specific ICAM-grabbing nonintegrin) on dendritic cells (DCs) and macrophage subpopulations [33]. DCs are highly specialized antigen-presenting cells, capable of activating naive and memory T lymphocytes. A number of adhesive or cytokine receptor-mediated interactions between DCs and T lymphocytes are important for proper T lymphocyte activation [34]. DC-SIGN is a C-type lectin representing calcium-dependent carbohydrate binding molecules. DCs expressing the DC-SIGN receptor are present on all mucosal surfaces and lymphoid organs. Although no antigenic stimulation is required to induce the expression of DC-SIGN on DCs, macrophages need an environmental signal for DC-SIGN induction [35].

DCs and macrophages are the main targets for LPS, thus participating in the immune response to gram-negative bacteria. It is possible that the Le epitope mimicry may contribute to a different effectiveness of IL-8 and tumor necrosis factor (TNF) secretion by peripheral blood mononuclear leukocytes in response to *H. pylori* LPS. It was shown that macrophages stimulated with *H. pylori* LPS of Le^X or Le^Y type produce cytokines more effectively than those cultured in the presence of *H. pylori* LPS without these determinants [36]. The presence of Le^X or Le^Y moieties in *H. pylori* LPS promotes a production of potentially self-destructive anti-Le^X and anti-Le^Y IgG in patients with coronary heart disease, seropositive for anti-*H. pylori* antibodies [26]. Appelmek et al. reported that the epitope which was most commonly recognized by anti-LPS antibodies in the sera from *H. pylori*-infected patients was the Le^X blood group antigen [25].

The interaction between *H. pylori* LPS and DCs is poorly understood. In this study, we asked whether *H. pylori* targets DC-SIGN using its LPS and whether this binding occurs via Le^X and Le^Y determinants or not. The binding of *H. pylori* to DC-SIGN may result in functional consequences especially regarding the ability of DCs to produce and secrete

cytokines. This could be of great importance for the control of *H. pylori* infections since both direct and indirect stimulation of T lymphocytes by *H. pylori* LPS is very likely. Furthermore, activated lymphocytes more effectively control the bacterial growth and might diminish gastric mucosa inflammation by releasing cytokines, possibly of Th2 type. Weak inflammatory response helps the bacteria to survive in the host tissue.

In this study, we estimated the binding of *H. pylori* whole cells and *H. pylori* LPS preparations with or without Le^{X/Y} determinants to the DC-SIGN receptor in a solid-phase binding assay. We have also made an attempt to examine whether Le^X and Le^Y antigens are able to associate with native, endogenously expressed DC-SIGN. For this purpose we adopted the THP-1 monocyte-macrophage leukemia cell model pretreated with PMA, GM-CSF, and IL-4 to induce the expression of DC-SIGN.

2. Materials and Methods

2.1. Bacterial Strains. The structure of O-antigen chain and core oligosaccharide regions of *H. pylori* strains with or without Le^X, Le^Y, and Le^{XY} determinants was analysed as previously described [17, 18]. The bacteria were stored at -70°C in tryptic soy broth containing 10% glycerol. Before being used in experiments the bacteria were cultured for 48 h, at 37°C in microaerophilic conditions in the GasPack EZ Campy Container System (Becton, Dickinson and Company, Sparks, USA) on Columbia blood agar with 10% heat inactivated foetal calf serum and washed in phosphate buffered saline (PBS), pH 7.2. The expression of Le determinants on *H. pylori* cells was confirmed by ELISA (enzyme-linked immunosorbent assay) using mouse monoclonal anti-Le^X (mAb anti-Le^X) and anti-Le^Y (mAb anti-Le^Y) antibodies (Merck, Darmstadt, Germany), and control antigens: synthetic Le^X and Le^Y determinants (Merck) or Le^X-BSA (Dextra Laboratories Ltd, Reading, UK), as previously described [37].

2.2. *H. pylori* Lipopolysaccharides. The *H. pylori* LPS preparations were classified chemically and serologically as Le^{X(+)}, Le^{Y(+)}, Le^{XY(+)}, or Le^{XY(-)} [17, 18]. The *H. pylori* LPS was obtained by the hot phenol-water extraction method after pretreatment of bacterial biomass with protease. The LPS crude preparation was then treated with RNase, DNase, protein kinase, and ultracentrifugation, as previously described [38].

2.3. The Estimation of *H. pylori* Whole Cells and *H. pylori* LPS Binding to Soluble DC-SIGN in the Solid-Phase Assay. The binding of *H. pylori* whole cells and *H. pylori* LPS preparations to DC-SIGN was estimated by ELISA, as recommended by Geijtenbeek et al. [34]. The ELISA plates were coated in triplicate with antigens: 1×10^7 bacterial cells/well, $2 \mu\text{g}/\text{mL}$ of LPS, and $1 \mu\text{g}/\text{mL}$ of synthetic Le^X-BSA determinants (Dextra Laboratories Ltd) in the carbonate buffer pH 9.6, 18 h, 4°C . After blocking (3% bovine serum albumin in PBS supplemented with 0.05% Tween 80-PBS/BSA/Tween, 2 h at 20°C) and washing (Tris-saline-magnesium buffer-TSM,

pH 7.4–8.0), the plates were incubated with 1 μg /well of the recombinant DC-SIGN/Fc chimera—rhDC-SIGN/Fc (R&D Systems, Minneapolis, USA) in TSM, 1 h, 37°C, and washed again. The immunocomplexes were detected using anti-human IgG antibodies conjugated with HRP—horseradish peroxidase (Dako, Glostrup, Denmark). For color development, chromogen *o*-phenylenediamine dihydrochloride (Sigma, St. Louis, USA) was added in the concentration of 1 mg/mL in the citric phosphate buffer, pH 5.0, with 0.005 mL of 30% H_2O_2 /mL. The results were expressed as OD₄₅₀ values. In each ELISA test the control wells were included for the evaluation of unspecific reactions. The ELISA cut-off value was defined as twice mean OD for the control wells coated with only one type of antigen or rhDC-SIGN/Fc, incubated with an HRP-conjugated secondary antibody and chromogen-substrate detection solution. The cut-off OD values were in the range of 0.1–0.2.

2.4. The Binding Specificity of *H. pylori* Whole Cells and *H. pylori* LPS to Soluble DC-SIGN Detected in the Solid-Phase Inhibition Assay. In order to identify the specificity of DC-SIGN binding, the inhibition of a solid-phase binding assay was performed using various blocking agents: mouse monoclonal antibodies against Le^X and Le^Y determinants, 2 μg /well (Merck), lipopolysaccharide binding protein (LBP), 2 μg /well (HyCult Biotechnology, Uden, Holland), rabbit antibodies against the LPS core region 1 : 500, and fucose or galactose, 25 μg /well (Sigma). These inhibitors were added into the selected wells coated with *H. pylori* LPSs or Lewis^X-BSA and the plates were incubated for 30 min, 37°C. After washing, the ELISA assay was performed as previously described. Specificity of DC-SIGN binding to *H. pylori* whole cells was established by pretreatment of bacterial cells (30 min, 37°C) with monoclonal anti-Le^X and anti-Le^Y antibodies, LBP and fucose or galactose. The binding specificity was estimated by comparing OD values for the ELISA assay without inhibitors to the results obtained in the assay developed using inhibitory agents.

2.5. THP-1 Cell Culture. The acute monocytic leukemia cell line THP-1 was obtained from the American Type Culture Collection (ATCC, Rockville, MD). Cells were cultured in RPMI 1640 medium with 10% foetal calf serum and maintained at 5×10^5 cell/mL. THP-1 cell differentiation was induced by the addition of phorbol myristate acetate (PMA) in the final concentration of 50 nM. After 24 h incubation, a granulocyte-macrophage colony-stimulating factor—GM-CSF (25 ng/mL)—either alone or in combination with interleukin 4-IL-4 (10 ng/mL) was added, and the cell cultures were continued for 5 days, at 37°C, 5% CO_2 . For subsequent analysis, differentiated THP-1 cells were detached from culture plates by incubation with PBS 5 mM EDTA on ice.

2.6. The Immunofluorescence Assay for the Detection of DC-SIGN Expression on THP-1 Cells and Its Binding to Soluble Le^X or Le^Y Determinants. The phenotypic analysis of the cells was carried out by an indirect immunofluorescence assay using

mouse monoclonal anti-DC-SIGN antibodies and FITC-conjugated polyclonal sheep antibodies against mouse immunoglobulins. The first incubation was performed in the presence of 1% inactivated sheep serum solution in PBS. The fluorescence intensity was estimated using the Victor 2 reader (Wallac, Oy, Turku, Finland) and cell imaging was performed using JuLI Smart fluorescent cell analyzer (Digital Bio Technology, Boston, USA). To verify whether DC-SIGN on THP-1 cells binds to Le^X or Le^Y determinants, the cells were incubated with soluble, synthetic Le^X or Le^Y, 20 μg /mL (Merck), and then treated with mouse monoclonal antibodies against DC-SIGN, followed by the treatment with FITC-conjugated polyclonal sheep antibodies to mouse immunoglobulins.

The interaction of soluble DC-SIGN (R&D Systems) with Le^X or Le^Y determinants expressed on the surface of THP-1 cells was also estimated. The fluorescence values for the THP-1 cells incubated with mouse mAb anti-Le^X or anti-Le^Y detected with FITC-labeled sheep anti-mouse IgG were compared to the fluorescence intensity values obtained in the inhibition assay where DC-SIGN was used before the incubation with mouse mAb anti-Le^X or anti-Le^Y and then with FITC-labeled sheep anti-mouse IgG.

3. Results

3.1. Binding of *H. pylori* Whole Cells to rhDC-SIGN/Fc. The solid-phase binding assay results presented in Figure 1 show that *H. pylori* whole cells containing LPS with Le^X, Le^Y or Le^{XY} determinants bound to rhDC-SIGN/Fc. The binding of *H. pylori* whole cells expressing Le^X to rhDC-SIGN was inhibited by mAb anti-Le^X and fucose but not with anti-Le^Y mAb. By comparison the binding of *H. pylori* whole cells with Le^Y expression was inhibited by anti-Le^Y mAb and fucose but not with anti-Le^X mAb. The binding of *H. pylori* Lewis^{XY} positive whole cells to DC-SIGN was partially decreased when anti-Le^X mAb and anti-Le^Y mAb were used separately and almost completely when anti-Le^X and anti-Le^Y mAb were used simultaneously. The binding was successfully inhibited by fucose moieties as well. In addition we showed that bacteria bearing LPS without Le^{XY} determinants were also able to bind rhDC-SIGN. The binding affinity of *H. pylori* whole cells which do not carry Le^X and Le^Y determinants to rhDC-SIGN was inhibited neither with mAb anti-Le^X and mAb anti-Le^Y nor with polyclonal antibodies to the core region of LPS. Similarly, the LBP molecule which binds LPS via lipid A had no inhibitory effect. The binding of *H. pylori* whole cells producing LPS without Le^{XY} determinants to rhDC-SIGN/Fc was successfully inhibited by galactose (Figure 2).

3.2. Specificity of *H. pylori* LPS Binding to rhDC-SIGN. The results presented in Figure 2 show that *H. pylori*-rhDC-SIGN/Fc binding occurs via LPS. The inhibition of LPS-rhDC-SIGN/Fc interaction with mAb anti-Le^X and/or mAb anti-Le^Y or fucose indicated that rhDC-SIGN/Fc bound to Le determinants in LPS molecules: Le^X, Le^Y, or both in fucose-dependent manner. The role of Le^X determinants in the interaction with DC-SIGN was confirmed in the solid-phase

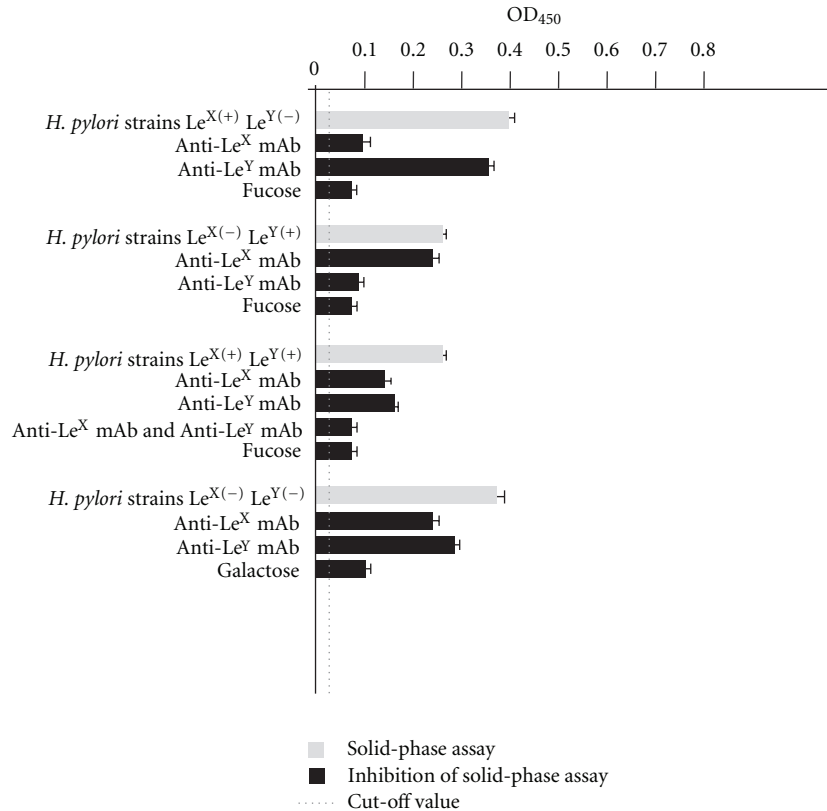


FIGURE 1: The binding of *H. pylori* whole cells positive or negative for Le^X, Le^Y, or Le^{XY} determinants with a soluble DC-SIGN receptor. The binding estimated by the solid-phase assay (grey bars). The specificity of binding estimated by the solid-phase inhibition assay (black bars).

binding and binding inhibition assays using the plates coated with synthetic Le^X-BSA and several blocking agents: anti-Le^X mAb, anti-Le^Y mAb, or fucose. We showed that the LPSs without Le^X or Le^Y determinants also effectively bound to rhDC-SIGN/Fc. The interaction via the core region or lipid A of the LPS was rather excluded due to the lack of the inhibitory effect when anti-LPS core region antibodies or LBP were used, respectively. The binding of purified *H. pylori* LPS without Le^X and Le^Y determinants was blocked by galactose, indicating that this sugar moiety in *H. pylori* LPS lacking Le^X or Le^Y determinants mediated the binding of Le^{X/Y} negative LPS to rhDC-SIGN.

3.3. Detection of DC-SIGN on the Surface of THP-1 Cells and Its Binding to Soluble Le^X and Le^Y Determinants in the Indirect Immunofluorescence Assay. The surface expression of DC-SIGN on THP-1 cells was induced by PMA, GM-CSF, and IL-4, treatment. We verified this by quantitative fluorescence measurement of THP-1 cells labeled with mouse anti-DC-SIGN mAb and then with FITC-conjugated sheep antibodies against mouse Igs before and after PMA, GM-CSF, and IL-4 treatment. The unspecific fluorescence intensity of control cells, which were not treated with PMA, GM-CSF and IL-4 reached the value of 240 RFU, whereas the unspecific fluorescence intensity of PMA, GM-CSF and IL-4 treated cells was equal to 545 RFU (Figures 3(a), 3(b)). The fluorescence

of nondifferentiated THP-1 cells labeled with anti-DC-SIGN mAb reached 900 RFU versus 4400 RFU for differentiated THP-1 cells (Figure 3(c), 3(d)). Pretreatment of differentiated THP-1 cells with synthetic Le^X, before the incubation with anti-DC-SIGN antibodies, diminished the fluorescence emission of the cells to 1270 RFU (Figure 3(e)) which accounted for 70% decrease in fluorescence intensity. Similarly, pretreatment of differentiated THP-1 cells with synthetic Le^Y before the incubation of the cells with anti-DC-SIGN mAb, diminished the fluorescence emission to 2800 RFU which constitute for 36% inhibition (Figure 3(f)).

3.4. Detection of Le^X and Le^Y Determinants on the Surface of THP-1 Cells and Their Binding to Soluble DC-SIGN in the Indirect Immunofluorescence Assay. The unspecific fluorescence intensity of nondifferentiated and differentiated THP-1 cells is shown on Figures 4(a) and 4(b), respectively. The presence of Le^X determinants on the surface of THP-1 cells was verified by the preincubation of the cells with mouse mAb anti-Le^X followed by the treatment of the cells with FITC-conjugated antibodies against mouse Igs. The fluorescence intensity of the cells treated in this way was equal to 2388 RFU (Figure 4(c)). By comparison, the pretreatment of the cells with soluble DC-SIGN before the incubation with a mouse mAb anti-Le^X and with FITC-conjugated antibodies against mouse Igs caused a decrease in the fluorescence

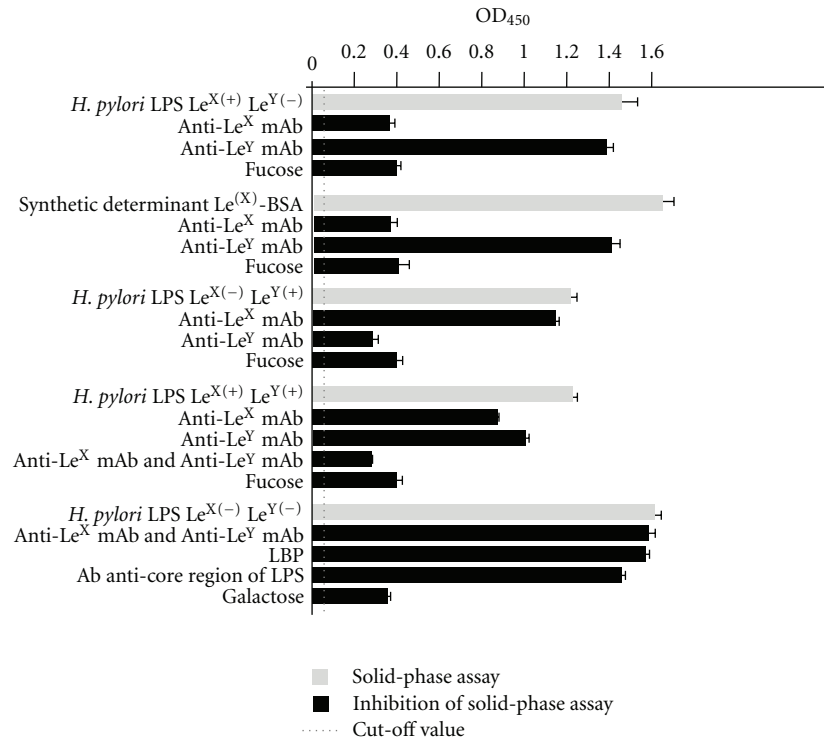


FIGURE 2: The binding of *H. pylori* LPS with or without expression of Le^{XY} determinants and synthetic Le^X-BSA with a soluble DC-SIGN receptor. The binding estimated by the solid-phase assay (grey bars). The specificity of binding estimated by the solid-phase inhibition assay (black bars).

intensity from 2388 RFU to 1282 RFU (46% reduction) (Figure 4(d)). The Le^Y exposition on the surface of THP1 was visualized by treatment of the cells with anti-Le^Y mAb followed by the incubation with secondary antibody conjugated with FITC. The fluorescence intensity of THP-1 cells stained with anti-Le^Y mAb was equal to 913 RFU (Figure 4(e)) and was not diminished (971 RFU) after treatment of the cells with soluble DC-SIGN (Figure 4(f)).

4. Discussion

Geijtenbeek et al. demonstrated that DC-SIGN is exclusively expressed on DCs and it mediates the interaction between DCs and resting T cells via ICAM-3, which is required for DCs-T-cell clustering and for DC-induced proliferation of resting T cells [34]. Several pathogens are able to target DC-SIGN and modulate DC functions and, due to this, escape immune response of the host [39–42]. Little attention was paid to the role of DCs in *H. pylori* infection, and how this pathogen is able to persistently colonize so many hosts is poorly understood. DC-SIGN recognizes both internal branched mannose residues and terminal di-mannoses, α -1-3 and α -1-4 fucosylated glycan structures as well as certain N-acetylglucosamine containing molecules on self proteins/or pathogens [43–46]. It was suggested that *H. pylori* binds to DC-SIGN via common pathogen recognition patterns such as high mannose and/or Lewis carbohydrates [33]. The presence of Lewis epitopes in the O-specific region of LPS possibly enables these bacteria to omit the immune responses,

resulting in the phenomenon of antigenic mimicry. It was assumed that the differences in *H. pylori* binding affinity to the DC-SIGN receptor is determined by the Le phase variation. This ability of *H. pylori* might allow the bacteria to modulate the host immune responses and in consequence contributes to the persistent character of this infection [33].

The results obtained in this study indicate that *H. pylori* whole cells with antigenic Le^X and/or Le^Y determinants bind to the recombinant DC-SIGN receptor. Similarly, *H. pylori* LPS of Le^X, Le^Y, Le^{XY} type was involved in rhDC-SIGN/Fc binding. The fucose residues present in the structure of Le^X and Le^Y determinants were responsible for binding specificity of *H. pylori* cells containing LPS of Le^X, Le^Y, or Le^{XY} to DC-SIGN receptor. This was shown in binding inhibition assay with monoclonal anti-Le^X and/or anti-Le^Y antibodies, and fucose. Bergman et al. demonstrated that DC-SIGN recognizes *H. pylori* and the binding of *H. pylori* whole cells or purified LPS to DC-SIGN was blocked with anti-DC-SIGN antibodies, mannan, or by ethylene glycol tetraacetic acid (EGTA)-Ca²⁺ chelating agent. *H. pylori* expressing Le^Y or short chains of Le^X (monomeric Le^X up to (Le^X)₄), but not polymeric Le^X, were able to bind DC-SIGN. Double knockout *H. pylori* mutants without α -3-fucosyltransferases FutA and FutB unable to produce Le^Y and monomeric Le^X did not bind DC-SIGN. Also steric organization of the sugar residues of Le antigens influences their binding affinity to DC-SIGN [33].

In this study *H. pylori* whole cells and LPS preparations without Le^X and Le^Y determinants were also found to be able

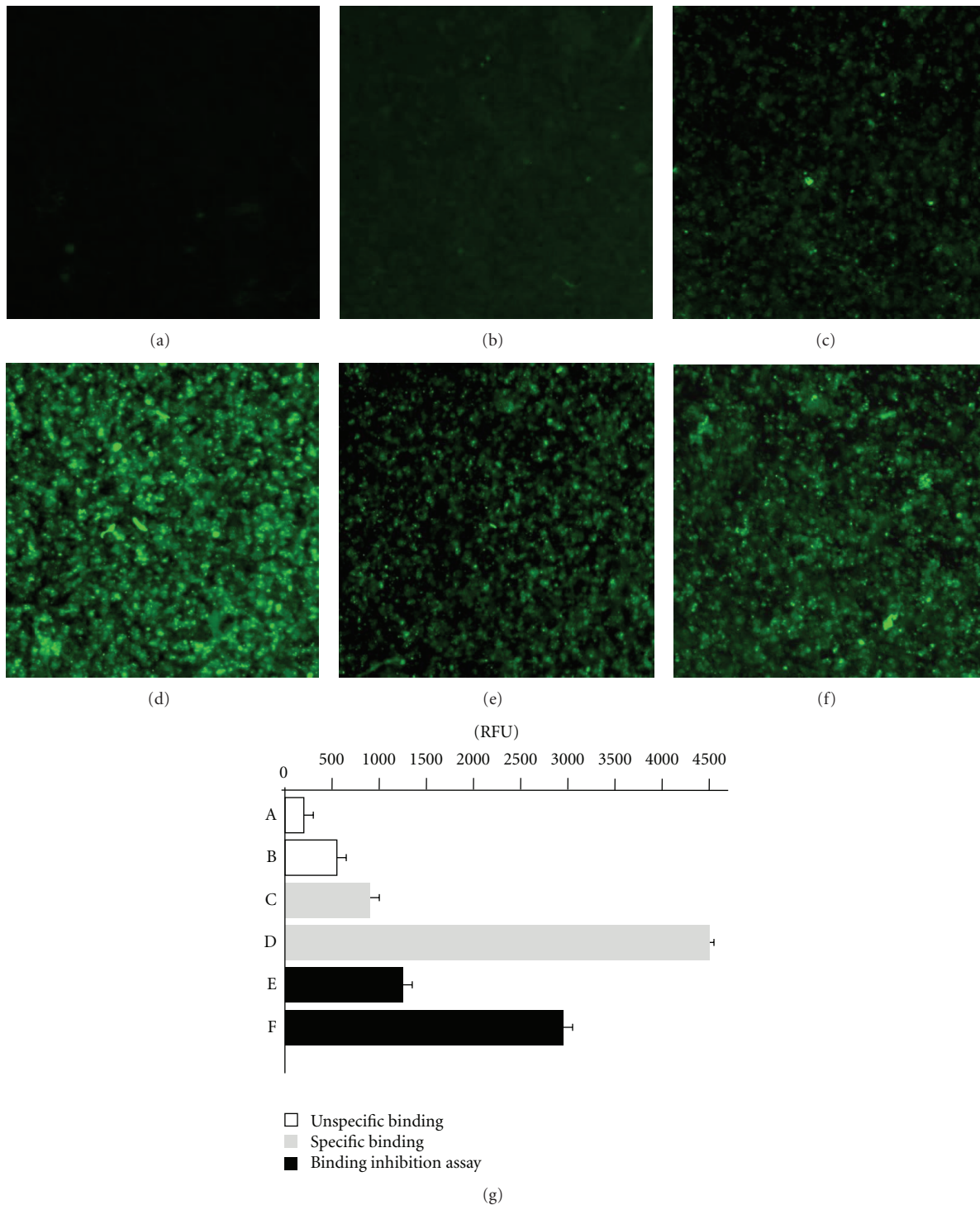


FIGURE 3: The cell imaging and fluorescence intensity reflecting the interactions between soluble synthetic Le^X and Le^Y determinants with DC-SIGN receptor on THP-1 leukemia cells, in the immunofluorescence assay. Control THP-1 cells, nondifferentiated (a) or differentiated (b) with PMA, GM-CSF and IL-4 stained with FITC-conjugated sheep antibodies against mouse Igs (secondary Ab). Nondifferentiated (c) and differentiated (d) THP-1 cells stained with mouse anti-DC-SIGN monoclonal antibodies (mAb) and with FITC-conjugated secondary Ab. Differentiated THP-1 cells preincubated with synthetic Le^X (e) or Le^Y (f) determinants before the staining of the cells with anti-DC-SIGN mAb followed by the treatment with FITC-conjugated secondary Ab.

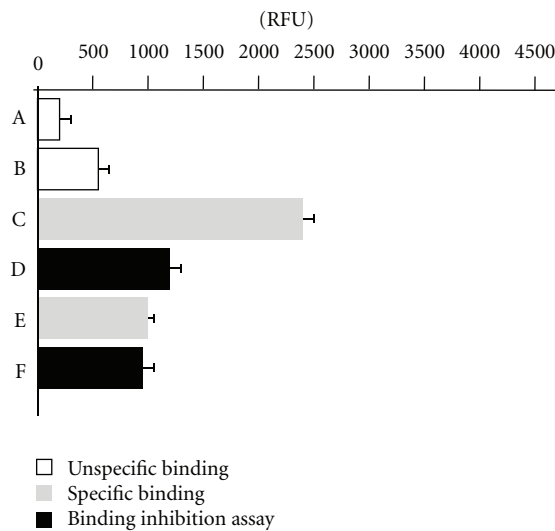
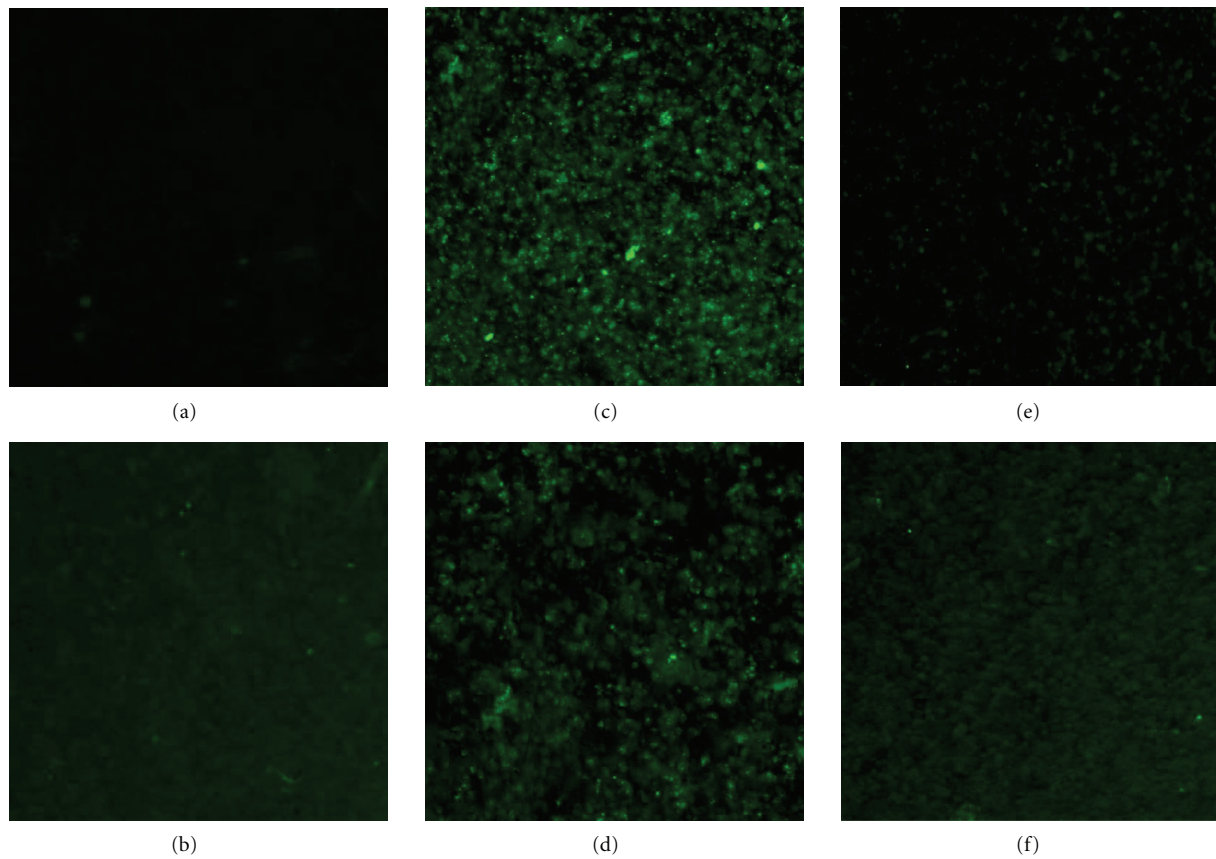


FIGURE 4: The cell imaging and fluorescence intensity reflecting the interactions between soluble DC-SIGN receptor with the Le^X and Le^Y determinants exposed on the surface THP-1 leukemia cells, in the immunofluorescence assay. Control THP-1 cells, nondifferentiated (a) or differentiated (b) with PMA, GM-CSF and IL-4 stained with FITC-conjugated sheep antibodies against mouse Igs (secondary Ab). Differentiated THP-1 cells treated with anti-Le^X mAb and FITC-conjugated secondary Ab (c) or firstly treated with soluble DC-SIGN followed by treatment as above (d). Differentiated THP-1 cells treated with anti-Le^Y mAb and FITC-conjugated secondary Ab (e) or firstly treated with soluble DC-SIGN followed by the treatment as above (f).

to bind DC-SIGN. The binding was successfully inhibited with galactose but not with anti-Le^{XY} or anti-LPS core region antibodies. Guo et al. showed that Le^X oligosaccharide primarily binds to DC-SIGN through the fucose moieties. However, galactose is involved in the stabilization of this interaction [44]. The *H. pylori* Le^{XY} negative strain used in this study contained a *galE* gene encoding UDP-galactose 4-epimerase, which mediates the incorporation of galactose in the O-side chain of LPS. To elucidate the role of galactose in *H. pylori* LPS binding to DC-SIGN, the construction of *gale*-deficient mutant would be of major benefit. Recently, Klena et al. demonstrated that only *E. coli* strains bearing the complete core region (without O-antigen) are able to mediate binding to DC-SIGN and, in consequence, further phagocytosis of bacteria by macrophages [47]. Previous studies demonstrated that DC-SIGN, besides binding to mannose and fucose, can also interact with glucose, α -1,4-diglucosyl (maltose) structures, and galactose [48]. The binding of galactose to mannose-specific C-type carbohydrate recognition domains (CRDs) can occur through interactions with the C-1 and C-2 hydroxyl groups of the free sugar [49]. Recently α -glucans were identified as new ligands for DC-SIGN [50].

DC-SIGN expression is induced *de novo* during the generation of monocyte-derived cells and is normally considered as a DC-specific phenotypic marker. However, DC-SIGN was also detected on synovial, placenta, and alveolar macrophages [34]. The expression of DC-SIGN depends on IL-4 which drives monocyte/macrophages into the “alternative activation” pathway or results in the generation of monocyte-derived dendritic cells if combined with GM-CSF. It was shown that leukemic THP-1 cells, widely used as a model for monocyte-macrophage differentiation, express very low, basal amounts of DC-SIGN and that expression of DC-SIGN in THP-1 cells is regulated during differentiation induced by PMA, GM-CSF, and IL-4 [51]. These results demonstrate that DC-SIGN is a marker for both DCs and alternatively activated macrophages and is present on THP-1 cells, which may be considered a useful cellular system to characterize the pathogen binding capacities. In this study, we asked whether THP-1 cells with the surface expression of DC-SIGN induced by PMA, GM-CSF, and IL-4 can bind to synthetic Le^X and Le^Y determinants. Considering the fact that the role of DCs and DC-SIGN receptors in the course of *H. pylori* infections is poorly understood, the detection of interactions between *H. pylori* antigens and DC-SIGN, especially in the context of Le^X and Le^Y determinants in *H. pylori* LPS structure, could be of great importance. This study was focused on the development of a cellular model to estimate the interactions between *H. pylori* expressing Le^X and/or Le^Y antigens and the DC-SIGN receptor in a solid-phase assay. The upregulation of the DC-SIGN receptor on the surface of THP-1 cells was induced by the stimulation of the cells with PMA, GM-CSF, and IL-4 as recommended by Puig-Kröger et al. and assessed quantitatively by an immunofluorescence assay, using monoclonal anti-DC-SIGN antibodies and FITC-conjugated anti-mouse Igs [51]. Our results demonstrated that DC-SIGN-like receptor is present on the surface of THP-1 cells and that this receptor is able to bind synthetic Le^X and Le^Y determinants. This enables the assessment of molecular effects of the

H. pylori-DC-SIGN interactions via Le determinants using the THP-1 cellular model. Previously, Perez-Perez et al., using PMA differentiated THP-1 cells, showed that the lipid A from *H. pylori* LPS has a low ability to mediate macrophage activation [52]. We expect that the *in vitro* THP-1 cellular model could help to elucidate how leukemic cells, especially of monocytic lineage, respond to *H. pylori* polysaccharide stimuli, regarding the expression of HLA-DR, CD40, CD80 and CD86, as well as the antigen uptake ability and potency in inducing allogenic T-cell proliferation. This cellular tool could reveal new facts regarding immune response elicited by *H. pylori* compounds, especially in the context of its activity as a bacterial carcinogen classified to IInd group. Recently, Chan et al. showed that *Ganoderma lucidum* polysaccharides can induce transformation of THP-1 cells into dendritic cells with an immune-stimulatory function [53]. It was also demonstrated that, apart from $\alpha_1\beta_2$ integrin, DC-SIGN on macrophages and DCs is an additional ligand for the lymphocyte ICAM-3 molecule which mediates the contact between T cells and antigen-presenting cells. ICAM-3 also assists in the interaction of granulocytes with DC-SIGN of DCs [54].

We also asked whether the expression of Le^X and Le^Y determinants on the surface of THP-1 cells makes this model suitable for the estimation of interactions with soluble DC-SIGN.

In this study we demonstrated binding of soluble DC-SIGN receptor with Le^X determinants on THP-1 cells. By comparison using this model we could not show successful binding of soluble DC-SIGN with Le^Y due to low expression of such determinants on THP-1 cells. In this case the THP-1 cellular model may selectively mimic the interactions between surface-exposed Le^X determinants and DC-SIGN molecule thus it opens new experimental possibilities. On the other hand, the results demonstrating the presence of a DC-SIGN-like receptor on the surface of THP-1 cells show that this cellular model can be used for the assessment of molecular effects due to *H. pylori*-DC-SIGN interactions via surface exposed or soluble Lewis compounds, which can be released during bacterial cell lysis in the inflammatory milieu of *H. pylori* colonized gastric mucosa. This idea correlates with the results obtained in the solid-phase assay showing that Le^X, Le^Y, and Le^{XY} determinants on *H. pylori* whole cells and *H. pylori* LPS preparations are involved in binding with DC-SIGN.

Conflict of Interests

The authors have no conflicting financial interests.

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