

Short communication

### CA-125 OF FETAL ORIGIN CAN ACT AS A LIGAND FOR DENDRITIC CELL-SPECIFIC ICAM-3-GRABBING NON-INTEGRIN

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**Abstract:** CA-125 (coelomic epithelium-related antigen) forms the extracellular portion of transmembrane mucin 16 (MUC16). It is shed after proteolytic degradation. Due to structural heterogeneity, CA-125 ligand capacity and biological roles are not yet understood. In this study, we assessed CA-125 as a ligand for dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN), which is a C-type lectin showing specificity for mannosylated and fucosylated structures. It plays a role as a pattern recognition molecule for viral and bacterial glycans or as an adhesion receptor. We probed a human DC-SIGN-Fc chimera with CA-125 of fetal or cancer origin using solid- or fluid-phase binding and inhibition assays. The results showed that DC-SIGN binds to CA-125 of fetal origin and that this interaction is carbohydrate-dependent. By contrast, cancer-derived CA-125 displayed negligible binding. Inhibition assays indicated differences in the potency of CA-125 to interfere with DC-SIGN binding to pathogen-related glycoconjugates, such as mannan and *Helicobacter pylori* antigens. The differences in ligand properties between CA-125 of fetal and cancer origin may be due to specificities of glycosylation. This might influence various functions of dendritic cells based on their subset diversity and maturation-related functional capacity.

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Abbreviations used: afCA-125 – CA-125 from amniotic fluid; CA-125 – coelomic-epithelium related antigen; clCA-125 – CA-125 from ovarian carcinoma OVCAR-3 cell line; ConA - concanavalin A; DC-SIGN – dendritic cell-specific ICAM-3-grabbing non-integrin; hu-FEDS – human fetoembryonic defense system; Le<sup>x</sup> – Lewis<sup>x</sup> antigen; Le<sup>y</sup> – Lewis<sup>y</sup> antigen; MUC16 – mucin 16; pCA-125 – pregnancy-associated cancer antigen 125

**Keywords:** CA-125, Mucin 16, DC-SIGN, C-type lectin, Carbohydrate binding, Pathogen-related glycoconjugates, Mannan, *Helicobacter pylori*

## INTRODUCTION

Coelomic-epithelium related antigen (CA-125) forms the extracellular portion of transmembrane mucin 16 (MUC16) and it is shed after proteolytic degradation. It is primarily expressed in embryonic tissues but also in some adult tissues, under both normal physiological and pathological conditions [1, 2]. There is a rise in the serum CA-125 level during the first trimester of pregnancy, but not during the second or third [3]. A significantly higher increase is found in subjects with ovarian cancer. Owing to this, CA-125 is widely used as a tumor marker and the available literature mostly focuses on various aspects of its diagnostic application [2].

The role of MUC16/CA-125 in the mucin-mediated pathways during the initial stages of pregnancy has not been thoroughly investigated. Generally, the regulation of mucin expression during the first trimester is necessary for successful implantation, tissue remodeling, and fetomaternal tolerance [4, 5]. It has been shown that MUC16 can act as a barrier to trophoblast adherence, i.e., that its removal during uterodome formation facilitates adhesion of the trophoblast [6]. In addition, based on the hypothesis that the human fetus is protected during development by a system of soluble and cell surface-associated glycoconjugates that utilize their carbohydrate sequences as functional groups, CA-125 was proposed to be part of the human fetoembryonic defense system (hu-FEDS) [7, 8].

Molecular function is highly contextual, so identifying binding partners is an important part of the strategy for assigning a biological role to CA-125. Several kinds of molecule have been reported to react with MUC16/CA-125 in both cell-based and solid-phase assays. Distinct types of human lectin with different specificities and binding requirements were demonstrated to recognize CA-125. The siglecs (sialic-acid Ig-like lectins), which are a subgroup of I-type lectins, and the galectins (beta-galactoside-binding lectins) were found to interact with MUC16 [9–12]. Specifically, siglec-9 was identified as a ligand for MUC16 on human NK cells, B cells, and monocytes [11]. Galectin-1 (gal-1) and galectin-3 (gal-3) were shown to be receptors for MUC16 on membrane-associated fragments of HeLa cell lysates and on the ocular epithelial cell surface [9, 10].

In addition, MUC16 expressed by metastatic pancreatic cancer cells was identified as a ligand for E- and L-selectin [13]. MUC16/CA-125 interactions with lectins are conferred by the specific glycan structures and depend on sialic acid, i.e., on the type and level of sialylation, and on lactosamine structures [11, 12, 14, 15]. In addition, recognition of high mannose glycans or blood type group Lewis<sup>x</sup> antigen (Le<sup>x</sup>) or Lewis<sup>y</sup> antigen (Le<sup>y</sup>) on the CA-125 moiety is seen as a possibility, but experimental data are still lacking [16].

Dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN; CD209) is a C-type lectin that shares common specificity for mannosylated and fucosylated glycan structures. It has a dual role in the immune system, acting as a pattern recognition molecule for viral and bacterial glycans and as an adhesion receptor [17–20].

CA-125 might be of vital importance as a ligand for DC-SIGN. The possible involvement of CA-125 as a factor influencing the vertical transmission of various viruses can be considered in terms of data obtained for MUC6 in seminal plasma and MUC1 in breast milk, where binding to DC-SIGN leads to the inhibition of virus transfer to T cells [21, 22]. On the other hand, the presumed interactions of DC-SIGN with CA-125 as a glycosylated ligand may be directly or indirectly related to the ability of mucins to suppress alloreactivity at the maternal–fetal interface [5, 23]. The same holds true for the putative role of CA-125 in supporting anti-inflammatory and tolerogenic immune responses towards tumor cells.

In this study, we addressed the issue of CA-125 as a ligand for DC-SIGN, with the aim of gaining insight into the accessibility and capacity of its relevant glycoepitopes to bind to DC-SIGN or to interfere with its interaction with selected pathogen-derived glycans. Previously characterized CA-125 of fetal origin was probed with DC-SIGN in solid-phase binding and inhibition assays and compared to cancer-derived antigens.

## MATERIALS AND METHODS

Recombinant human DC-SIGN/CD209-Fc chimera, consisting of the extracellular portion of DC-SIGN (amino acid residues 62–404) fused at the COOH terminus to a human IgG1-Fc fragment (DC-SIGN-Fc) was purchased from R&D Systems. The biotinylated plant lectins *Lotus tetragonolobus* lectin (LTL) and concanavalin A (ConA), biotinylated goat anti-mouse IgM antibody (affinity purified, specific for the M $\mu$  chain), and the Elite Vectastain ABC Kit were obtained from Vector Laboratories. Mouse monoclonal anti-human Lewis<sup>x</sup> and Lewis<sup>y</sup> antibodies, class IgM, were obtained from Calbiochem (Merck KgaA). *Helicobacter pylori* antigen-coated plates were purchased from HUMAN GmbH. Protein A-HRPO conjugate was supplied by INEP. Mannan, fucose and bovine serum albumin (BSA) were purchased from Sigma-Aldrich. MaxiSorp microwell plates with 96 star shaped bottom wells were supplied by Nunc.

CA-125 isolated from the OVCAR-3 ovarian carcinoma cell line (clCA-125) was obtained from RayBiotech, Inc. The clCA-125 concentration was 76267 IU/ml, and had < 1% CA19-9 and < 1% CA15-3. CA-125 isolated from human adenocarcinoma (acCA-125) was from HyTest LTD. The acCA-125 concentration was 1000 kIU/ml and there was < 5% CA19-9 and CA15-3. CA-125 was also isolated from first trimester human placental extract (originally termed pregnancy-associated CA-125 or pCA-125) and human amniotic fluid

(afCA-125) as previously described, under the supervision and approval of the local ethical committee [24, 25]. The concentration of pCA-125 was 2500 IU/ml (CIS Biointernational, ELSA CA-125 II assay), and it contained < 0.2% CA19-9 (CIS Biointernational, ELSA CA19-9) and < 0.01% CA15-3 (MUC1) (CIS Biointernational, ELSA CA15-3). The afCA-125 concentration was 800 IU/ml (CIS Biointernational, ELSA CA-125 II assay), with < 0.2% CA19-9 and < 0.2% CA15-3. All of the other chemicals were of reagent grade.

#### **Detection of Le<sup>x</sup> and Le<sup>y</sup> on CA-125**

CA-125 (7-5000 IU/ml; 50 µl/well) was immobilized on microwell plates in 0.05 M carbonate buffer (pH 9.5) for 18 h at 4°C. The wells were rinsed three times with 300 µl 0.05 M PBS (pH 7.2) and, after blocking (1% BSA) and washing steps, mouse monoclonal anti-Le<sup>x</sup> or anti-Le<sup>y</sup> IgM antibody (dilution 1:500; 50 µl/well) was added. Incubation proceeded for 3 h at room temperature and, after the washing steps, bound antibody was detected using biotinylated goat anti-mouse IgM antibodies (0.5 µg/ml; 50 µl/well). Unbound conjugate was rinsed away followed by the addition of Vectastain Elite ABC reagent (50 µl/well) and incubation for 30 min. After further washing steps, TMB substrate solution (50 µl/well) was added and the reaction was stopped with 0.16 M H<sub>2</sub>SO<sub>4</sub> after 20 min. Absorbance was measured at 450 nm on a Wallac 1420 Multilabel counter (Perkin Elmer).

#### **Binding of LTL and ConA to CA-125**

The biotinylated lectins LTL or ConA (0.07 µg/ml; 50 µl/well) were added to CA-125-coated plates and allowed to react for 1 h at room temperature. Unbound lectin was removed by washing, followed by the addition of Vectastain Elite ABC reagent. Incubation, rinsing, and detection steps were performed as described for the detection of Lewis epitopes.

#### **Binding of DC-SIGN-Fc to immobilized CA-125**

CA-125 (7-5000 IU/ml; 50 µl/well) in 0.05 M carbonate buffer (pH 9.5) was immobilized on microwell plates for 18 h at 4°C. Unadsorbed antigens were removed by aspiration, the wells were rinsed three times with 300 µl 0.05 M PBS (pH 7.2) and then blocked with 1% BSA (200 µl/well) in 20 mM TSM (Tris-HCl buffer; pH 7.4) containing 150 mM NaCl, 1 mM CaCl<sub>2</sub> and 2 mM MgCl<sub>2</sub>. After the washing steps, DC-SIGN-Fc (5 µg/ml; 50 µl/well) was added followed by incubation for 3 h at room temperature. Unbound lectin was aspirated and the wells were rinsed, followed by the addition of protein A-HRPO conjugate (0.3 µg/ml; 50 µl/well). After incubation for 2 h and further washing steps, TMB-substrate solution was added (50 µl/well). The reaction was stopped after 20 min with 0.16 M H<sub>2</sub>SO<sub>4</sub> (50 µl/well). Absorbance was measured at 450 nm. The inhibition assay involved preincubation of DC-SIGN-Fc (5 µg/ml) with either mannan (25 µg/ml) or fucose (50 mM) alone, or with their mixed solutions (final concentration adjusted to 25 µg/ml mannan and 50 mM fucose), followed by binding to CA-125 at one selected concentration (250 IU/ml). The

incubation, rinsing, and detection steps were performed as described above. Binding of DC-SIGN-Fc in the presence of inhibitor was expressed as a percentage of the DC-SIGN-Fc binding alone (control value). Statistical analysis was performed using two tailed unpaired t-tests; p values < 0.05 were considered statistically significant.

In addition, a mutual inhibition assay was performed using lectins and monoclonal antibodies. Thus, before the addition of DC-SIGN-Fc (5 µg/ml), the CA-125-coated plate was preincubated with: ConA (50 µg/ml; 50 µl/well), LTL (2 µg/ml; 50 µl/well), and anti-Le<sup>x</sup> and anti-Le<sup>y</sup> (dilution 1:50; 50 µl/well) for 2 h at room temperature, with specific binding of DC-SIGN-Fc detected as described above.

#### **Inhibition of DC-SIGN-Fc binding to pathogen-related glycoconjugates using CA-125**

Mannan (20 µg/ml; 50 µl/well) in 0.05 M carbonate buffer (pH 9.5) was adsorbed on a microwell plate for 18 h at 4°C. The wells were rinsed three times with 300 µl 0.05 M PBS (pH 7.2) and blocked with 1% BSA (200 µl/well) in TSM (pH 7.4). After the washing steps, we added DC-SIGN-Fc (at the selected concentration of 10 µg/ml; 50 µl/well) in TSM, or DC-SIGN-Fc preincubated (for 1 h at room temperature) with pCA-125 and afCA-125 (300 IU/ml) to immobilized mannan and incubated for 3 h at room temperature. Unbound lectin was removed and protein A-HRPO conjugate added (0.3 µg/ml; 50 µl/well). After incubation for 1 h and more washing steps, TMB substrate solution was added (50 µl/well). The reaction was stopped with 0.16 M H<sub>2</sub>SO<sub>4</sub> and absorbance was measured at 450 nm. Binding of DC-SIGN-Fc to mannan in the presence of inhibitor was expressed as a percentage of the DC-SIGN-Fc binding to mannan alone.

DC-SIGN-Fc (50 µl/well) was allowed to react with *Helicobacter pylori* antigen-coated plates from a commercially available kit for detection of antibodies against *H. pylori* in human blood serum (HUMAN GmbH). These antigens were derived from a detergent extract of a *H. pylori* type I preparation rich in Le<sup>x</sup>-lipopolysaccharides. Binding assays were performed using the procedure described for mannan-coated plates. Statistical analysis was performed using two tailed unpaired t-tests; p values < 0.05 were considered statistically significant.

## **RESULTS**

### **CA-125 has carbohydrate structures that DC-SIGN recognizes specifically**

The accessibility of mannosylated and fucosylated glycans on immobilized CA-125, as possible binding determinants for DC-SIGN, were tested using plant lectins (Fig. 1A and B) or carbohydrate-specific antibodies (Fig. 1C and D). ConA, specific for high mannose and complex type N-glycans, and anti-Le<sup>x</sup> or -Le<sup>y</sup> antibodies showed concentration-dependent binding to pCA-125 and to

a lesser extent to afCA-125. In addition, LTL, which recognizes fucosylated structures (alpha-linked L-fucose), bound weakly only pCA-125.

Compared to the antigens of fetal origin, cancer-derived antigens generally reacted at higher concentrations. clCA-125 was recognized by ConA and anti-Le<sup>x</sup>, while its reaction with anti-Le<sup>y</sup> was very poor, and LTL showed no binding. Only ConA gave a faint measurable reaction for acCA-125.

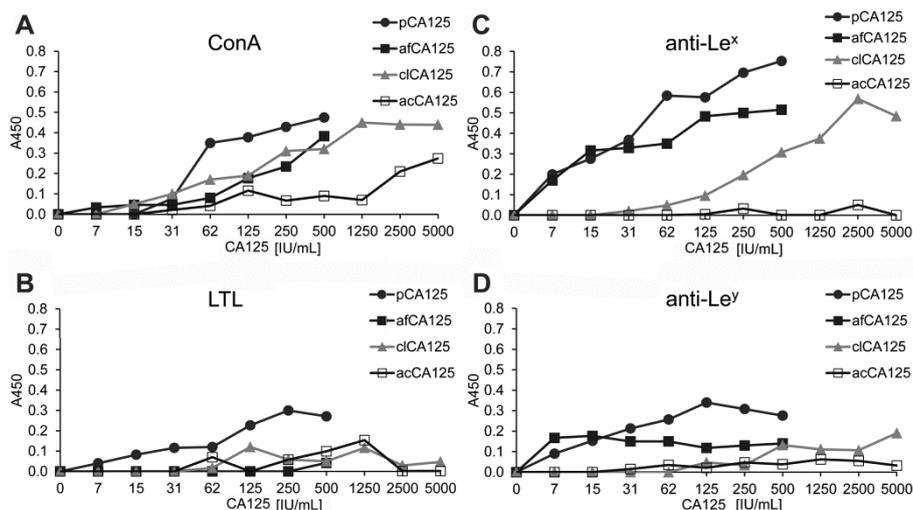


Fig. 1. Mannosylated and fucosylated glycans on CA-125 of fetal and cancer origin. Immobilized CA-125 was allowed to react with biotinylated plant lectins or carbohydrate-binding antibodies at room temperature. The unbound material was washed out followed by specific detection using Vectastain ABC reagent for lectins or biotinylated anti-mouse IgM antibody and Vectastain ABC for antibodies. Absorbance was measured at 450 nm. Typical binding curves are presented. A – Binding of concanavalin A (ConA). B – Binding of *Lotus tetragonolobus* lectin (LTL). C – Binding of monoclonal anti-human Lewis<sup>x</sup> IgM (anti-Le<sup>x</sup>). D – Binding of monoclonal anti-human Lewis<sup>y</sup> IgM (anti-Le<sup>y</sup>). pCA-125 – pregnancy-associated CA-125; afCA-125 – CA-125 from amniotic fluid; clCA-125 – CA-125 from ovarian carcinoma OVCAR-3 cell line; acCA-125 – CA-125 from adenocarcinoma.

#### DC-SIGN binds to CA-125 in a carbohydrate-dependent mode

The interaction of DC-SIGN with immobilized CA-125 of different origins is shown in Fig. 2A. Dose-dependent binding was observed with pCA-125 and afCA-125, whereas clCA-125 and acCA-125 exhibited negligible reactions. When mannan and fucose were applied alone as inhibitors, they showed partial but considerable inhibition of both pCA-125 and afCA-125 binding to DC-SIGN (Fig. 2B). A statistically significant difference between mannan and fucose in terms of inhibitory potential was observed for both pCA-125 ( $p \leq 0.0473$ ) and afCA-125 ( $p \leq 1.0387E-06$ ). Mannan inhibited the binding of DC-SIGN to pCA-125 and afCA-125 by 43% and 58% of the control value, respectively, while fucose inhibited binding by 29% and 18% of the control value, respectively. Combined

inhibition with both sugars inhibited the binding of DC-SIGN to pCA-125 and afCA-125 by 81% and 73%, respectively.

The blocking of fucosylated structures on pCA-125 using LTL or anti-Le<sup>x</sup> had little to no effect on DC-SIGN binding, whereas anti-Le<sup>y</sup> showed significant inhibition, decreasing the binding by 15% of the control value (Fig. 2C). In contrast to this, ConA increased the binding of DC-SIGN to pCA-125 by 20%.

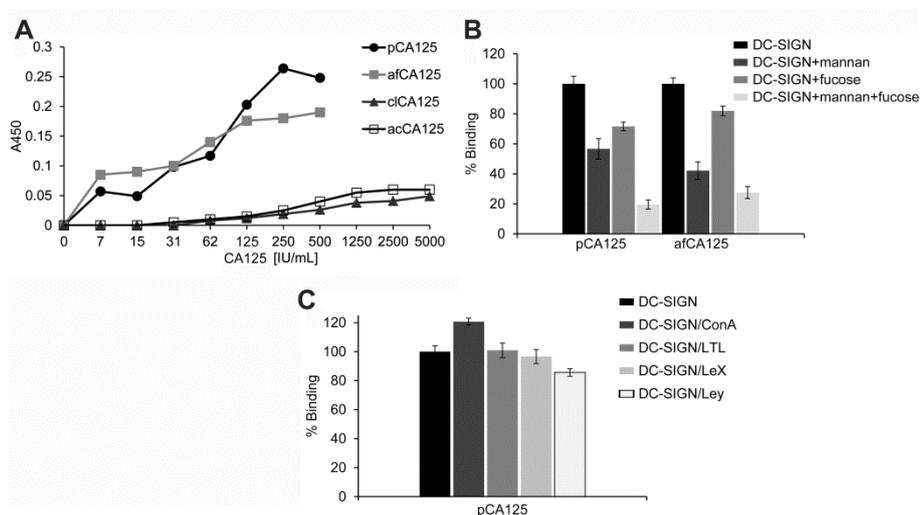


Fig. 2. Binding of DC-SIGN to immobilized CA-125. A – Immobilized CA-125 of different origins were incubated with DC-SIGN for 3 h at room temperature. The unbound material was washed out followed by the addition of protein A-HRPO and TMB substrate solution. Absorbance was measured at 450 nm. B – DC-SIGN was allowed to react with inhibitors alone or combined: mannan (25  $\mu$ g/ml), fucose (50 mM), and their mixed solutions (final concentration adjusted to 25  $\mu$ g/ml mannan and 50 mM fucose), prior to incubation with immobilized CA-125. The binding of DC-SIGN to CA-125 in the presence of the inhibitors was expressed as a percentage of the binding to CA-125 alone. Inhibition was tested at the selected concentration of CA-125 of 250 IU/ml. The presented results are the mean values of two independent experiments. The standard error ranged from 3–11%. C – Immobilized pCA-125 (250 IU/ml) was preincubated with ConA (50  $\mu$ g/ml; 50  $\mu$ l/well), LTL (2  $\mu$ g/ml; 50  $\mu$ l/well), or anti-Le<sup>x</sup> or anti-Le<sup>y</sup> (dilution 1:50; 50  $\mu$ l/well) for 2 h at room temperature, followed by incubation with DC-SIGN. The presented results are the mean values of two independent experiments. The standard error ranged from 3–6%. pCA-125 – pregnancy-associated CA-125; afCA-125 – CA-125 from amniotic fluid; clCA-125 – CA-125 from ovarian carcinoma OVCAR-3 cell line; acCA-125 – CA-125 from adenocarcinoma; concanavalin A (ConA); *Lotus tetragonolobus* lectin (LTL); monoclonal anti-human Lewis<sup>x</sup> IgM (anti-Le<sup>x</sup>); monoclonal anti-human Lewis<sup>y</sup> IgM (anti-Le<sup>y</sup>).

### CA-125 inhibition of DC-SIGN binding to pathogen-related glycoconjugates

The potency of CA-125 of fetal origin to inhibit DC-SIGN binding to pathogen-related glycoconjugates was probed on mannan and *Helicobacter pylori* antigens. Inhibition was carried out at constant concentrations of immobilized

glycoconjugates and DC-SIGN, selected after the examination of different experimental parameters to yield optimal settings. The results obtained indicated that pCA-125 and afCA-125 inhibited DC-SIGN binding to mannan by 26% and 52%, respectively (Fig. 3A). A statistically significant difference between the two antigens as inhibitors of DC-SIGN binding to mannan was found ( $p \leq 0.0173$ ). Moreover, pCA-125 statistically significantly decreased DC-SIGN binding to *H. pylori* antigens by 56% ( $p \leq 0.0058$ ), whereas binding was preserved in the presence of afCA-125 (Fig. 3B).

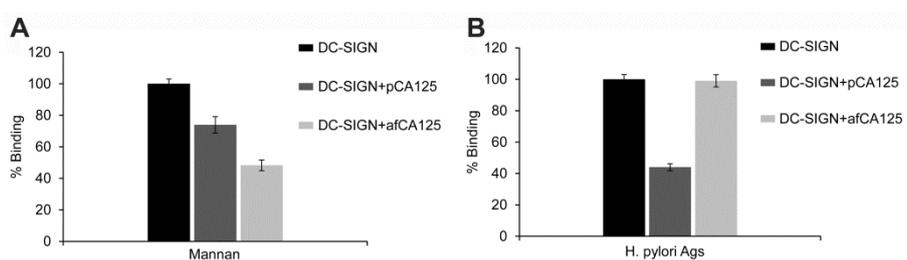


Fig. 3. Inhibition of DC-SIGN binding to pathogen-related glycoconjugates. DC-SIGN was allowed to react with immobilized mannan (A) or *H. pylori* antigens (B) alone or preincubated with CA-125 for 3 h at room temperature. Bound lectin was detected with protein A-HRP0 and absorbance was measured at 450 nm. Binding of DC-SIGN to pathogen-related glycoconjugates in the presence of CA-125 was expressed as a percentage of the DC-SIGN binding without the addition of the corresponding antigens. The presented results are the mean values of two independent experiments. The standard error ranged from 1–8%. pCA-125 – pregnancy-associated CA-125; afCA-125 – CA-125 from amniotic fluid; *H. pylori* Ags – *Helicobacter pylori* antigens.

## DISCUSSION

DC-SIGN is in the lectin-like receptor class sharing a C-type lectin fold that is a ligand-binding motif not only for carbohydrates but also for some proteins, nucleic acids, lipids, and inorganic molecules [17, 26]. A soluble chimeric DC-SIGN-Fc fusion protein has been used in different types of assay to analyze lectin specificity and binding mode. Although limitations with respect to the oligomerization state of lectin exist, this experimental design was demonstrated to be relevant and allowed identification of various interacting glyco-structures [27, 28].

In this study, comparable test formats were applied to probe the binding of human DC-SIGN-Fc chimera to pregnancy- and cancer-associated CA-125, previously characterized to be heterogeneous in protein and carbohydrate composition. Thus, the main differences between pregnancy- and cancer-associated CA-125 were attributed to N-glycan-associated structures reactive with ConA and PHA-E (phytohemagglutinin-E), as well as glyco-structures reactive with SBA (*Glycine max* agglutinin), SNA (*Sambucus nigra* agglutinin) and WFA (*Wisteria floribunda* agglutinin), as evaluated by their lectin-binding

patterns [24, 25, 29]. Generally, the existence of various CA-125 forms was also supported by recent data obtained using microarray glycoprofiling, mass spectrometry, and HPLC [30, 31]. Thus, under the experimental conditions applied, CA-125 heterogeneity was also observed concerning glyco-structures that are supposed to react with DC-SIGN. The possible influence of both abundance and accessibility was most expressed in the case of fucosylated structures and Lewis antigens. These results were in contrast to those obtained with mannose-containing structures. Moreover, compared to CA-125 of fetal origin, differences between the results for cancer-derived CA-125 were more pronounced, and such antigens were generally less reactive in the same concentration range.

In agreement with this, our results indicated that DC-SIGN recognizes CA-125 of fetal and cancer origin in a very different way. This can be related to data indicating that subtle differences in the arrangement of carbohydrate residues and their branching and spacing considerably affects the recognition profile of C-type lectins, including DC-SIGN [32, 33].

Indeed, a previous investigation of CA-125 of fetal and cancer origin showed that their molecular topology, being dependent on the molecular mass and degree of glycosylation, was clearly different [34]. Thus, DC-SIGN binding to immobilized CA-125 of fetal origin was carbohydrate-dependent and no striking difference was observed when CA-125 isolated from trophoblasts was compared with that from amniotic fluid. In contrast to this, cancer-derived CA-125 from an ovarian carcinoma cell line and adenocarcinoma displayed negligible binding.

The results of DC-SIGN inhibition assays using fetal CA-125 in the solid or fluid phase indicated that both mannosylated and fucosylated structures contributed to the observed lectin binding, but not in the same way. In light of the statistical analysis, the former seem to be preferential targets for DC-SIGN. Of the fucosylated epitopes, Le<sup>y</sup> was found to be the most distinguished. However, DC-SIGN and ConA, as examples of mannose-binding lectin-type polyspecificity, recognize different structural features on CA-125 glycans, as indicated by the lack of mutual inhibition. It seems that increased binding of DC-SIGN is due to ConA-induced clustering of glycans, which is known to increase C-type lectin affinity [35].

This finding was complemented with the results obtained for two different types of pathogen-related glycans using fluid-phase CA-125 as the competitor. Thus, probes included mannan, as the primordial ligand for C-type lectins mimicking a variety of mannosylated structures bound by DC-SIGN, and a complex mixture of *H. pylori* antigens mimicking fucosylated structures including Lewis<sup>x/y</sup> glycotopes. Generally, mammalian glycoproteins are considered to be poor ligands for C-type lectins in comparison to pathogen-related glycoconjugates, due to the distribution and spacing between carbohydrate residues involved in recognition.

Our results indicated that DC-SIGN binding to mannan was not blocked to the background level using either pCA-125 or afCA-125, suggesting that the two

antigens share similar but complex determinants to mannan. The inhibition potency of pCA-125 was lower, while afCA-125 had no effect on DC-SIGN binding to *H. pylori* antigens. This could be related to their observed reactivities with LTL lectin and anti-Lewis antibodies. Thus, the composition of fucosylated glycans seems to be more critical for DC-SIGN recognition of CA-125, possibly involving distinct or partially overlapping glycotopes to *H. pylori* antigens.

The heterogeneity of the MUC16/CA-125 structure makes it a complex ligand, the capacity of which is still elusive. Thus, our results could be considered in the light of previously reported data indicating that CA-125 shares some properties with pathogen ligands, exhibiting diverse interactions with DC-SIGN. The N-glycosylation profile for CA-125 glycans is supposed to be very similar to that of HIV-1 glycoprotein gp120, which is a known ligand for DC-SIGN [16]. As for the protein backbone, CA-125 homology to conserved domains from the outer envelope protein of the *Herpesviridae* family was found. This is the major antigen responsible for the production of neutralizing antibodies *in vivo* [36].

Moreover, it is known that DC-SIGN has the capacity to bind various viral envelope proteins, but these interactions are not strictly defined. Thus, binding of HIV-1 gp120 is defined as carbohydrate-independent, since the deglycosylated molecule is still recognized by DC-SIGN [26, 33]. On the other hand, its interaction with envelope glycoproteins from different members of the *Herpesviridae* family is supposed to be mediated by carbohydrates [37, 38].

The available data raise the question of the biological significance and position of MUC16/CA-125 among mucins and molecules sharing similar carbohydrate and peptide epitopes and competing for diverse receptors sharing overlapping ligand specificities. The limitation of the experimental system applied in this study is that it could not mimic the situation *in vivo*, where local microenvironments directly influence DC function and phenotype, resulting in considerable heterogeneity [39]. However, the results obtained, together with previously published data, point to the possible selectivity of DC-SIGN to CA-125 of fetal origin and siglec(s) to CA-125 of cancer-origin [12]. This may be a clue for designing a strategy to examine the mucin network in immune recognition mediated by this distinct group of C-type lectins.

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**Conflict of interest.** None of the authors declare any conflict of interest.

## REFERENCES

1. Montz, F.J. CA125 in: **Serological Cancer Markers** (Sell, S. Ed.), The Humana Press Totowa, NJ, 1992, 417–425.
2. Scholler, N. and Urban, N. CA125 in ovarian cancer. **Biomark. Med.** 1 (2007) 513–523. DOI: 10.2217/17520363.1.4.513.

3. Seki, K., Kikuchi, Y., Uesato, T. and Kato, K. Increased serum CA 125 levels during the first trimester of pregnancy. **Acta Obstet. Gynecol. Scand.** 65 (1986) 583–585.
4. Carson, D.D., DeSouza, M.M., Kardon, R., Zhou, X., Lagow, E. and Julian, J. Mucin expression and function in the female reproductive tract. **Hum. Reprod. Update** 4 (1998) 459–464.
5. Redžović, A., Laškarin, G., Dominović, M., Haller, H. and Rukavina, D. Mucins help to avoid alloreactivity at the maternal fetal interface. **Clin. Dev. Immunol.** (2013) 542152. DOI: 10.1155/2013/542152.
6. Gipson, I.K., Blalock, T., Tisdale, A., Spurr-Michaud, S., Allcorn, S., Stavreus-Evers, A. and Gemzell, K. MUC16 is lost from the uterodome (pinopode) surface of the receptive human endometrium: in vitro evidence that MUC16 is a barrier to trophoblast adherence. **Biol. Reprod.** 78 (2008) 134–142.
7. Clark, G.F., Dell, A., Morris, H.R., Patankar, M.S. and Easton, R.L. The species recognition system: a new corollary for the human fetoe embryonic defense system hypothesis. **Cells Tissues Organs** 168 (2001) 113–121.
8. Clark, G.F. and Patankar, M.S. Opinion: Hu-FEDS: in search of 'universal self'. **Mol. Hum. Reprod.** 3 (1997) 985–987.
9. Seelenmeyer, C., Wegehingel, S., Lechner, J. and Nickel, W. The cancer antigen CA125 represents a novel counter receptor for galectin-1. **J. Cell. Sci.** 116 (2003) 1305–1318.
10. Argüeso, P., Guzman-Aranguez, A., Mantelli, F., Cao, Z., Ricciuto, J. and Panjwani, N. Association of cell surface mucins with galectin-3 contributes to the ocular surface epithelial barrier. **J. Biol. Chem.** 284 (2009) 23037–23045. DOI: 10.1074/jbc.M109.033332.
11. Belisle, J.A., Horibata, S., Jennifer, G.A., Petrie, S., Kapur, A., André, S., Gabius, H.J., Rancourt, C., Connor, J., Paulson, J. C. and Patankar, M.S. Identification of Siglec-9 as the receptor for MUC16 on human NK cells, B cells, and monocytes. **Mol. Cancer** 9 (2010) 118. DOI: 10.1186/1476-4598-9-118.
12. Mitić N.M., Milutinović B.S. and Janković M.M. Assessment of sialic acid diversity in cancer- and non-cancer related CA125 antigen using sialic acid-binding Ig-like lectins (Siglecs). **Dis. Markers** 32 (2012) 187–194. DOI: 10.3233/DMA-2011-0872.
13. Chen, S.H., Dallas, M.R., Balzer, E.M. and Konstantopoulos, K. Mucin 16 is a functional selectin ligand on pancreatic cancer cells. **FASEB J.** 26 (2012) 1349–1359. DOI: 10.1096/fj.11-195669.
14. Rump, A., Morikawa, Y., Tanaka, M., Minami, S., Umesaki, N., Takeuchi, M. and Miyajima, A. Binding of ovarian cancer antigen CA125/MUC16 to mesothelin mediates cell adhesion. **J. Biol. Chem.** 279 (2004) 9190–9198.
15. Patankar, M.S., Jing, Y., Morrison, J.C., Belisle, J.A., Lattanzio, F.A., Deng, Y., Wong, N.K., Morris, H.R., Dell, A. and Clark, G.F. Potent suppression of

- natural killer cell response mediated by the ovarian tumor marker CA125. **Gynecol. Oncol.** 99 (2005) 704–713.
16. Kui Wong, N., Easton, R.L., Panico, M., Sutton-Smith, M., Morrison, J.C., Lattanzio, F.A., Morris, H.R., Clark, G.F., Dell, A. and Patankar, M.S. Characterization of the oligosaccharides associated with the human ovarian tumor marker CA125. **J. Biol. Chem.** 278 (2003) 28619–28634.
  17. Geijtenbeek, T.B., Torensma, R., van Vliet, S.J., van Duijnhoven, G.C., Adema, G.J., van Kooyk, Y. and Figdor, C.G. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. **Cell** 100 (2000) 575–585.
  18. Engering, A., Geijtenbeek, T.B., van Vliet, S.J., Wijers, M., van Liempt, E., Demaurex, N., Lanzavecchia, A., Fransen, J., Figdor, C.G., Piguet, V. and van Kooyk, Y. The dendritic cell-specific adhesion receptor DC-SIGN internalizes antigen for presentation to T cells. **J. Immunol.** 168 (2002) 2118–2126.
  19. Figdor, C.G., van Kooyk, Y. and Adema, G.J. C-type lectin receptors on dendritic cells and Langerhans cells. **Nat. Rev. Immunol.** 2 (2002) 77–84.
  20. Cambi, A. and Figdor, C.G. Dual function of C-type lectin-like receptors in the immune system. **Curr. Opin. Cell. Biol.** 15 (2003) 539–546.
  21. Saeland, E., de Jong, M.A., Nabatov, A.A., Kalay, H., Geijtenbeek, T.B. and van Kooyk, Y. MUC1 in human milk blocks transmission of human immunodeficiency virus from dendritic cells to T cells. **Mol. Immunol.** 46 (2009) 2309–2316. DOI: 10.1016/j.molimm.2009.03.025.
  22. Stax, M.J., van Montfort, T., Sprenger, R.R., Melchers, M., Sanders, R.W., van Leeuwen, E., Repping, S., Pollakis, G., Speijer, D. and Paxton, W.A. Mucin 6 in seminal plasma binds DC-SIGN and potently blocks dendritic cell mediated transfer of HIV-1 to CD4(+) T-lymphocytes. **Virology** 391 (2009) 203–211. DOI: 10.1016/j.virol.2009.06.011.
  23. Laškarin, G., Medančić, S.S., Redžović, A., Durić, D. and Rukavina, D. Specific decidual CD14(+) cells hamper cognate NK cell proliferation and cytolytic mediator expression after mucin 1 treatment in vitro. **J. Reprod. Immunol.** 95 (2012) 36–45. DOI: 10.1016/j.jri.2012.06.002.
  24. Janković, M.M. and Tapušević, B.S. Molecular forms and microheterogeneity of oligosaccharide chains of pregnancy-associated CA125. **Hum. Reprod.** 20 (2005) 2632–2638.
  25. Milutinović, B.S. and Janković, M.M. Analysis of the protein and glycan parts of CA125 antigen from human amniotic fluid. **Arch. Biol. Sci.** 59 (2007) 97–103.
  26. García-Vallejo, J.J. and van Kooyk, Y. Endogenous ligands for C-type lectin receptors: the true regulators of immune homeostasis. **Immunol. Rev.** 230 (2009) 22–37. DOI: 10.1111/j.1600-065X.2009.00786.x.
  27. Mitchell, D.A., Fadden, A.J. and Drickamer, K. A novel mechanism of carbohydrate recognition by the C-type lectins DC-SIGN and DC-SIGNR.

- Subunit organization and binding to multivalent ligands. **J. Biol. Chem.** 276 (2001) 28939–28945.
28. Appelmelk, B.J., van Die, I., van Vliet, S.J., Vandenbroucke-Grauls, C.M., Geijtenbeek, T.B. and van Kooyk, Y. Cutting edge: carbohydrate profiling identifies new pathogens that interact with dendritic cell-specific ICAM-3-grabbing nonintegrin on dendritic cells. **J. Immunol.** 170 (2003) 1635–1639.
  29. Janković, M.M. and Milutinović, B.S. Glycoforms of CA125 antigen as a possible cancer marker. **Cancer Biomark.** 4 (2008) 35–42.
  30. Chen, K., Gentry-Maharaj, A., Burnell, M., Steentoft, C., Marcos-Silva, L., Mandel, U., Jacobs, I., Dawnay, A., Menon, U. and Blixt, O. Microarray glycoprofiling of CA125 improves differential diagnosis of ovarian cancer. **J. Proteome Res.** 12 (2013) 1408–1418. DOI: 10.1021/pr3010474.
  31. Saldova, R., Struwe, W.B., Wynne, K., Elia, G., Duffy, M.J. and Rudd, P.M. Exploring the glycosylation of serum CA125. **Int. J. Mol. Sci.** 14 (2013) 15636–15654. DOI: 10.3390/ijms140815636.
  32. Feinberg, H., Mitchell, D.A., Drickamer, K. and Weis, W.I. Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. **Science** 294 (2001) 2163–2166.
  33. Geijtenbeek, T.B., van Duijnhoven, G.C., van Vliet, S.J., Krieger, E., Vriend, G., Figdor, C.G. and van Kooyk, Y. Identification of different binding sites in the dendritic cell-specific receptor DC-SIGN for intercellular adhesion molecule 3 and HIV-1. **J. Biol. Chem.** 277 (2002) 11314–11320.
  34. Janković, M.M. and Milutinović, B.S. Pregnancy-associated CA125 antigen as mucin: evaluation of ferning morphology. **Mol. Hum. Reprod.** 13 (2007) 405–408.
  35. Varki, A. Selectin ligands. **Proc. Natl. Acad. Sci. USA** 91 (1994) 7390–7397.
  36. Janković, M.M. and Mitić, N.M. MUC16/CA125 in the context of modular proteins with an annotated role in adhesion-related processes: in silico analysis. **Int. J. Mol. Sci.** 13 (2012) 10387–10400. DOI: 10.3390/ijms130810387.
  37. Halary, F., Amara, A., Lortat-Jacob, H., Messerle, M., Delaunay, T., Houlès, C., Fieschi, F., Arenzana-Seisdedos, F., Moreau, J.F. and Déchanet-Merville, J. Human cytomegalovirus binding to DC-SIGN is required for dendritic cell infection and target cell trans-infection. **Immunity** 17 (2002) 653–664.
  38. de Jong, M.A., de Witte, L., Bolmstedt, A., van Kooyk, Y. and Geijtenbeek, T.B. Dendritic cells mediate herpes simplex virus infection and transmission through the C-type lectin DC-SIGN. **J. Gen. Virol.** 89 (2008) 2398–2409. DOI: 10.1099/vir.0.2008/003129-0.
  39. Kämmerer, U., Rieger, L., Honig, A. and Kämpgen, E. Characterization of human dendritic cells at the materno-fetal interface. In: **Immunology of Pregnancy**, (Mor, G., Ed.), Springer, New York, 2006, 122–129.