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The mucin MUC16 (CA125) binds to NK cells and monocytes from peripheral blood of women with healthy pregnancy and preeclampsia

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Abstract

Problem—MUC16 (CA125) released from ovarian tumors binds to NK cells and monocytes via the inhibitory receptor Siglec-9. Here, we investigate if MUC16 also binds to circulating immune cells during pregnancy and in women with preeclampsia.

Method of study—MUC16 binding was monitored by flow cytometry and immunoprecipitation and RT-PCR was used to monitor indigenous expression in immune cells. Serum CA125 levels were measured by a clinical assay.

Results—MUC16 was equally distributed on Siglec-9^{pos} CD16^{pos}/CD56^{dim} and CD16^{neg}/CD56^{br} NK cells in the healthy pregnant and preeclampsia groups. While serum CA125 levels and number of NK and monocytes were similar, increased binding of MUC16 was observed on these immune cells in the preeclampsia cohort as compared to the healthy pregnant samples.

Conclusion—MUC16 binding to NK cells and monocytes likely contributes to tolerance of the fetal allograft from maternal responses and may also serve as a novel biomarker for preeclampsia.

Keywords

Immune cell subsets; MUC16; CA125; Siglec-9; Biomarker

Introduction

CA125, is a tumor biomarker used extensively to monitor epithelial ovarian cancer ¹⁻³. CA125 is a repeating peptide epitope present in the tandem repeat region of MUC16, a 3-5 million Da heavily glycosylated mucin overexpressed by epithelial ovarian tumors ⁴⁻⁶. We have previously demonstrated that ovarian tumors utilize MUC16 to attenuate the cytolytic responses of NK cells ^{7, 8}. The large molecular weight and high negative charge of this heavily glycosylated mucin also acts as a barrier that prevents the NK cells from forming activating immunologic synapses with the ovarian tumor targets ^{6, 7, 9}.

MUC16 is a membrane-spanning mucin that is initially expressed on the surface of epithelial cells and especially on the epithelial ovarian tumor cells ^{6, 10}. Proteolytic cleavage results in release of the mucin from the cell surface. The shed mucin, (sMUC16) molecules traverse to the peripheral blood from the extracellular milieu, where they can be detected using the

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clinical serum CA125 test. In our analysis of peripheral blood mononuclear cells (PBMC) isolated from ovarian cancer patients and the immune cells isolated from their peritoneal fluid (PFMC) we observed that approximately 10-15% of B cells, 30-40% of NK cells and >90% of monocytes were positive for sMUC16^{11, 12}. Several lines of evidence indicate that the PBMC and PFMC do not express sMUC16 but instead specifically bind to the mucin released from ovarian tumors^{11, 12}.

We have now also demonstrated that sMUC16 predominantly binds to immune cells via Siglec-9¹¹, a α 2-3-linked sialic acid binding I-type lectin known to serve as an inhibitory immune cell receptor¹³⁻¹⁷. Siglec-9 is expressed on approximately 30-40% of the CD16^{pos}/CD56^{dim} NK cell subsets and in ovarian cancer patients these cells are double positive for sMUC16¹¹. High expression of Siglec-9 is observed on >90% of the monocytes and a correspondingly high level of sMUC16 binding is observed on these immune cells in ovarian cancer patients.

MUC16 is expressed by endometrial epithelial cells and also in the decidua¹⁸⁻²¹. Indeed, serum CA125 levels increase during pregnancy²², which is one of the reasons why serum CA125 cannot be used as an early diagnostic test exclusively for ovarian cancer. Considering our previous work on sMUC16 binding to PBMC and PFMC of ovarian cancer patients^{11, 12}, we investigated if the mucin is also present on specific subsets of immune cells of pregnant women.

Our results indicate that the binding pattern of sMUC16 to NK cells and monocytes from peripheral blood of pregnant women closely matches the expression of Siglec-9 on these immune cells. Important differences were observed in the subsets of NK cells from normal pregnant women and preeclampsia patients that bind to sMUC16 and express Siglec-9. Data presented in this study lays the groundwork for future studies on the potential biological significance of sMUC16 binding to immune cells in healthy pregnant women and preeclampsia patients. In addition, differences in the binding patterns of sMUC16 to NK cells and the expression of Siglec-9 on these cells may also be exploited for the development of a novel diagnostic test for the detection of preeclampsia.

Methods

Cell lines and reagents

Siglec-9 expressing Jurkat cells were a kind gift from Dr. Jim Paulson (Scripps Research Institute, Ca). The Jurkat cells were cultured in RPMI-1640 media supplemented with 10% fetal calf serum. All other reagents were commercially obtained. ECC-1 and OVCAR-3 cells were purchased from ATCC and were cultured in RPMI 1640 media containing 10% fetal Bovine serum. Fluorophore conjugated Anti-CD14 (PerCP-CY5.5, clone: M5E2), CD3 (APC-Cy7, clone SK7), CD56 (Alexa 700, clone B159), CD16 (PE-Cy7, clone 3G8), CD19 (PE, clone HIB19), Siglec-9 (CDw329; FITC, clone E10-286) were from BD Biosciences and secondary antibodies were purchased from Jackson ImmunoResearch. All other reagents were from Sigma or Invitrogen.

Subjects

All subjects signed an informed consent and the study was approved by the Institutional Review Boards of the University of Wisconsin-Madison and Meriter Hospital. The women were recruited at the time of admission to Labor and Delivery. Subjects were considered eligible controls if they had completed 37 weeks of gestation, had an uncomplicated prenatal course, and had no preexisting co-morbidities. Eligible preeclamptic subjects were identified using strict diagnostic criteria. Prenatal records were reviewed to ensure that there was no evidence of hypertension prior to twenty weeks of gestation. Hypertension was defined as

systolic blood pressures of at least 140 mm Hg or diastolic blood pressures of at least 90 mm Hg two times measured 6 hours apart. The definition of proteinuria is the excretion of 300 mg of protein or greater in a 24 hour urine collection²³. Urine dip analysis alone without confirmatory 24 hour urine protein excretion was not considered sufficient to enroll a subject into the study. Subjects were excluded from the study if they carried the diagnosis of chronic hypertension, diabetes, anti-phospholipid lipid antibody syndrome, or systemic lupus erythematosus. Subjects were also excluded if given the diagnosis of abruption or had meconium stained amniotic fluid. Of the 26 women included in the study there was one African American and all others were Caucasian. Table 1 shows the baseline characteristics of the groups. The groups showed significant difference in age as well as gestational age which is expected when considering the disease process.

Isolation of peripheral blood lymphocytes

Blood samples were obtained upon delivery from full term uncomplicated pregnancies as well as pregnancies complicated by preeclampsia. Mononuclear cells were isolated under sterile conditions using Histopaque (Sigma Aldrich, St Louis, MO). The mononuclear layer was retained and washed once with PBS-BSA before being cryopreserved in 90% fetal bovine serum (FBS; Hyclone) containing 10% dimethyl sulphoxide (DMSO). Serum samples were layered over Histopaque with the mononuclear layer being isolated and washed and cryopreserved.

Analysis of Siglec-9 and MUC16 on NK cells by flow cytometry

Cryopreserved cells were thawed and washed (15 min at 300Xg in PBS-BSA). Incubation of cells with primary and secondary antibodies was performed for 30 min on ice. After incubation with each antibody, cells were washed with PBS-BSA at 300Xg for 10 min at 4°C. Cells were blocked with goat IgG prior to staining with anti-MUC16 antibody VK8. The cells were washed and Allophycocyanin (APC)-conjugated goat anti-mouse (GAM) IgG, antibody was added. The cells were then washed and incubated with mouse IgG for 20 min to bind any additional Fab sites on the GAM secondary. The cells were then incubated with a cocktail of fluorophore-conjugated antibodies to stain for CD3, CD45, CD56, CD16, and Siglec-9^{11, 12}.

After the final wash, cells were resuspended in approximately 300 µl of PBS-BSA. Immediately prior to data acquisition on the LSRII flow cytometer (Beckton Dickinson, San Jose, CA), DAPI, a viability indicator was applied to each sample. Each day, quality control procedures were run on the LSRII prior to data acquisition. Beckton Dickinson Cytometry Setup and Tracking (CST) beads were run daily to reproducibly set up the cytometer. Use of these beads allows for determination of cytometer baseline, the standard deviation of electronic noise, assessment of photomultiplier tube (PMT) drift, and adjusts cytometer settings to maximize population resolution in each detector. These measurements also verify that data is acquired within the linear range of each PMT. Spherotech Rainbow Beads, both mid-range and ultra, were run each day to determine that for a given PMT voltage, nearly identical target values (mean fluorescence intensity (MFI), were obtained for each detector of interest. Setting the instrument up in this manner, with these controls, allowed for the negative controls from each patient for each day to give rise to comparable MFIs in each fluorescence channel. From here, compensation controls were generated and acquired for experiments done on individual days to adjust for spectral overlap between PMTs. Flow Jo software was used to analyze the flow cytometry data.

CA-125 Assay

Serum was separated and the number of units of CA125 in the sample was determined by using the standard clinical radioimmunoassay (Abbott AxSYM, Abbott Park, IL). CA125

measurements were conducted in the clinical pathology laboratory of the University of Wisconsin-Madison Hospital and Clinics.

Immunoprecipitation of sMUC16 from immune cell lysates

The PBMC's from healthy donor, ovarian cancer patient and OVCAR-3 ovarian cancer cell line were lysed in RIPA buffer containing protease inhibitors. After calculating the protein concentration (BCA) lysate equivalent to 500 µg of total protein was mixed with 100 µg of VK-8 antibody and tubes were rotated overnight at 4°C. Protein G-agarose beads (100 µl) were added to the mixture and the tubes were rotated for 1 h at room temperature. Suspensions were centrifuged (400Xg for 1 min), supernatants were removed, and the beads were washed three times with RIPA buffer (500 µl) containing protease inhibitors. Beads were then resuspended in 30 µL Laemmli buffer, boiled for 5 min and loaded on 7.5% SDS-PAGE gel. Proteins from the gel were transferred to PVDF membranes and MUC16 was detected using the VK-8 as the primary antibody and a HRP-conjugated goat anti-mouse as the secondary antibody.

MUC16 RT-PCR

OVCAR-3, ECC-1, and PBMC samples were homogenized in Trizol and RNA was isolated according to the manufacturer's instructions (Invitrogen). The RNA was reverse transcribed into cDNA using Omniscript RT kit from Qiagen (Cat.No.205111). The MUC16 and GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) were amplified with the respective primers (MUC16 forward: 5'GCCTCTACCTTAACGGTTACAATGAA3'; reverse: 5'GGTACCCCATGGCTGTTGTG-3; and GAPDH forward: GAGTCAACGGATTTGGTCGT, reverse: TTGATTTTGGAGGGATCTCG) using fast cycling PCR kit from Qiagen as described in our earlier studies^{12, 24}. MUC 16 was amplified using the initial activation for 10 minutes at 95°C, followed by 35 cycles of 15 seconds at 95°C and 1 minute at 60°C. The cycling conditions used for amplifying GAPDH were, initial activation at 95°C for 5min, followed by 30 cycles of the denaturation at 96°C for 5seconds, annealing at 60°C for 5seconds and extension at 68°C for 10seconds, with final extension at 72°C for 1min. GAPDH was used as an endogenous reference to determine the integrity of the mRNA in each sample. The PCR product was run on 2.5% agarose gel at 100V for 2 hr and bands were visualized using Fluorchem8900 ultraviolet transilluminator.

Statistics

The Flo Jo flow cytometry software was used to obtain the raw data on the percentage of immune cells positive for sMUC16, Siglec-9 and other immune markers. The data was plotted using the Graph Pad statistical software. Statistical significance of the data was determined using the non-parametric Mann Whitney U test.

Results

sMUC16 binds to immune cells isolated from the peripheral blood of pregnant women

Immune cells from healthy individuals and ovarian cancer patients do not express endogenous MUC16 but instead bind the mucin that is present in the serum and the peritoneal fluid of ovarian cancer patients¹¹. Higher levels of sMUC16 (measured as CA125) are also observed in the serum of pregnant women as compared to the healthy non-pregnant cohort^{21, 25}. We therefore tested if similar to our observation with immune cells from ovarian cancer patients, sMUC16 also binds to immune cells of pregnant women.

MUC16 was immunoprecipitated from the lysates of PBMCs isolated at the time of delivery from healthy pregnant women. However, this mucin was not detected in the

immunoprecipitates from the lysates of healthy donor derived PBMCs (Fig. 1a). RT-PCR studies demonstrated that similar to ovarian cancer ¹², the PBMCs from healthy pregnant women did not express endogenous MUC16 (Fig. 1b).

We have previously shown that sMUC16 binds to immune cells via Siglec-9 ¹¹. Binding of sMUC16 to Siglec-9 is rapid as shown by in vitro studies with Jurkat cells expressing this receptor (Fig. 2). The lack of MUC16 synthesis by immune cells of pregnant women suggested that similar to our observations in ovarian cancer, MUC16 is also captured on the surface of immune cells in pregnancy via Siglec-9. In subsequent experiments we therefore simultaneously monitored sMUC16 binding and Siglec-9 expression on the immune cells from pregnant women and preeclampsia patients by using multi-color flow cytometry.

sMUC16 binding pattern to immune cell subsets in pregnancy

All of our flow cytometry experiments require careful standardization of the instruments with calibration beads. We are therefore able to compare the binding pattern of sMUC16 and Siglec-9 expression in immune cells from pregnant subjects. Analysis of PBMC from healthy pregnant women indicated sMUC16 binding to B cells, NK cells and monocytes (Fig. 3). In the case of NK cells, equal levels of the mucin were observed on the CD16^{pos}/CD56^{dim} and CD16^{neg}/CD56^{br} subsets (Fig. 3). The sMUC16 binding pattern in pregnancy samples matched the expression of Siglec-9 on the two NK cell subsets, further suggesting that Siglec-9 is the predominant receptor for sMUC16 on these immune cells (Fig. 3).

Low level of sMUC16 was detected on CD3^{pos} T cells that have previously been shown to express only low levels of Siglec-9 ¹⁷. On the other hand, monocytes express high levels of Siglec-9 and were also strongly positive for sMUC16 (Fig. 3). In the case of B cells from pregnant women only 10% of the cells were double positive for sMUC16 and Siglec-9 even though approximately 80% of the Siglec-9^{neg} B cells were positive for sMUC16 (Fig. 3). These data suggest that at least on the B cells, sMUC16 binding is Siglec-9-independent. Galectin-1 is another reported binding partner of MUC16 ²⁶. We have not found any correlation between Galectin-1 expression on B cells and sMUC16 (data not shown).

NK cell bound sMUC16 is detected even at low serum CA125 levels

Maximum serum CA125 levels are observed in the first trimester of pregnancy ^{22, 25}. We therefore monitored serum CA125 levels and immune cell bound sMUC16 in three women before and at 9 and 18 weeks of pregnancy. A gradual increase in serum CA125 was observed at 9 weeks of pregnancy ¹². Continued monitoring of these three patients at 18 weeks of the pregnancy (same gestation as that monitored in our previous study ¹²) showed a trend toward lower serum CA125 levels (Fig. 4). However, levels of sMUC16 bound to immune cells continued to show an upward trend at 18 weeks of pregnancy even as the serum CA125 levels decreased (Fig. 4).

MUC16 binding to immune cells of patients with preeclampsia

Impairment of immune responses, especially those mediated by decidual NK cells, have been associated with the pathogenesis of preeclampsia ^{27, 28}. Given the demonstrated immunomodulatory roles of sMUC16 ^{7, 8}, we next determined if there were any differences in the binding pattern of this mucin to immune cells in preeclamptic patients compared to healthy pregnant women. First, we confirmed the results of previous studies ^{22, 29, 30} that the serum CA125 levels were not significantly different between healthy pregnant women and preeclampsia patients (Fig. 5a). Similarly, there was no statistically significant difference in the percentage of monocytes (Fig. 5b) and NK cells (data not shown) in the PBMC of healthy pregnant women and preeclampsia patients.

Next we compared the binding of sMUC16 and expression of Siglec-9 on NK cells and monocytes from PBMC of preeclampsia patients and healthy pregnant subjects. Samples from healthy pregnant women described in Table 1 were analyzed in these experiments but did not include samples from patients who were already studied for experiments described in Fig. 3. The preeclampsia cohort included patients with both mild as well as severe disease, however a subgroup analysis was not performed in this initial study. Similar to our observations in healthy pregnant women, sMUC16 was also detected on NK cells and monocytes isolated from the PBMC of patients with preeclampsia (Fig. 6 and 7). The pattern of sMUC16 binding was distinct between the NK cell subsets of healthy pregnant women and preeclamptic women. Siglec-9 expression was comparable on the CD16^{pos}/CD56^{dim} and CD16^{neg}/CD56^{br} NK cells of the healthy pregnant women and the preeclampsia patients (Fig. 6a and b). However, a statistically significant increase in sMUC16 binding was observed on the CD16^{pos}/CD56^{dim} and CD16^{neg}/CD56^{br} NK cells of preeclampsia patients as compared to the healthy pregnant group (Fig. 6a and b). Significant differences were also observed in the case of monocytes isolated from healthy pregnant women and patients with preeclampsia. Increased sMUC16 binding was observed on monocytes of preeclampsia patients (Fig. 7). A trend for increased expression of Siglec-9 on the monocytes of preeclampsia patients was observed. However, the differences in expression levels of Siglec-9 between the two cohorts were not statistically significant ($p=0.649$; Fig. 7).

Discussion

Mucins are high molecular weight glycoproteins that exhibit important biological roles via their carbohydrate as well as protein epitopes^{10, 31}. Probably the best characterized roles of the protein epitopes of mucins are the importance of the C-terminal domains of mucins such as MUC1 and MUC4 in intracellular signaling³². Oligosaccharides attached to the extracellular domains of mucins have also been shown to interact with different lectin receptors and influencing functions such as cell-cell adhesion and regulating immune responses³³⁻³⁵. MUC16 is, to date, the largest mucin identified in the human genome and similar to other mucins of its class plays a major biological role in normal as well as pathologic tissues via its extensive N-linked and O-linked oligosaccharide chains and its approximately 24,000 amino acid protein backbone^{7, 10, 36-38}.

Highlights of the biological activities of MUC16 reported at this point include its role in (a) facilitating ovarian tumor metastasis via its interactions with mesothelin, a GPI-anchored glycoprotein expressed on mesothelial cells and cancer cells³⁷⁻³⁹, (b) allowing ovarian tumor cells to escape NK cell immune synapse formation and directly inhibiting the ability of NK cells to cytolyse cancer targets^{7, 8}, (c) imparting resistance to chemotherapy in ovarian cancer cells⁴⁰, (d) contributing to cell survival in cancer cells^{41, 42}, and (d) serving as a barrier to adhesion of trophoblasts to the endometrial epithelium and of bacteria to the corneal epithelia⁴³. Considering this background, our observations that sMUC16 binds to immune cells during pregnancy and shows higher level of binding to the CD16^{neg}/CD56^{br} NK cell subset and monocytes from preeclamptic patients are likely to have important physiologic consequences. First, sMUC16 binding to monocytes and CD16^{pos}/CD56^{dim} NK cells is likely to inhibit the cytolytic activities of these immune cells, as shown in our previous study thereby ensuring suppression of maternal cytotoxic immune responses against the fetal tissues⁸.

Second, the CD16^{neg}/CD56^{br} NK cells exhibit low cytotoxicity and instead are high producer of cytokines⁴⁴. This NK cell subset has been conclusively implicated as performing a pro-fetal growth function by expressing angiogenic cytokines that enhance trophoblastic invasion of the uterine spiral arterioles²⁸.

Recent data emerging from experiments with monocytic cell lines indicates that engagement of Siglec-9 induces these cells to increase expression of the immunosuppressive cytokine IL-10⁴⁵. This cytokine is known to promote placental angiogenesis. Thus sMUC16 binding to monocytes and NK cells may likely promote fetal development. Increased binding of sMUC16 to CD16^{neg}/CD56^{br} NK cells and monocytes in preeclampsia may be a corrective maneuver to increase VEGF levels to promote angiogenesis in pregnancy.

Preeclampsia is a hypertensive disorder unique to pregnancy and affects 2-7% of pregnancies^{23, 46}. It is a major cause of both maternal and fetal morbidity and mortality. Considered a leading cause of iatrogenic prematurity, preeclampsia is characterized by poor perfusion to multiple vital fetal organs which is reversed upon delivery. A primary pathological feature of preeclampsia is insufficient invasion of the spiral arterioles by the fetal trophoblast with subsequent widespread systemic endothelial dysfunction. CD16^{neg}/CD56^{br} NK cells present in the decidua are implicated in successful trophoblastic invasion of the uterine spiral arterioles and the regulation of uterine blood flow²⁸.

Maternal hypertension is the primary mode of detection of preeclampsia. Increase in maternal blood pressure however occurs at a later stage in pregnancy. Attempts are therefore underway to identify biomarkers that can detect the onset of preeclampsia at an early stage⁴⁷⁻⁴⁹. Past reports have studied potential increases in the serum levels of CA125 and demonstrated no significant change in the levels of this marker between women with healthy pregnancies and those with preeclampsia^{22, 29}. The studies presented here suggest a novel method for the identification of biomarkers that can predict preeclampsia. Based on this method we predict that binding of sMUC16 bound to circulating CD16^{neg}/CD56^{br} NK cells and monocytes above a threshold value may serve as an important method for detection and monitoring of preeclampsia. Additionally, since sMUC16 also binds to CD16^{pos}/CD56^{dim} NK cells and monocytes in ovarian cancer patients monitoring the levels of this mucin on immune cells may also prove important in detecting and monitoring ovarian cancer^{11, 12}. Immune cell bound sMUC16 provides an advantage over the conventional serum CA125 assay as it does not primarily rely on the absolute concentration of the biomarker in the serum; a major obstacle since the concentration of the biomarker may vary based on its rapid turnover by the liver⁵⁰; but instead relies on the measurement of the captured antigen on specific immune cell subsets.

Analysis of a large cohort of healthy donors, healthy pregnant women, and patients with ovarian cancer or preeclampsia will be necessary to establish threshold values for sMUC16 binding to NK cell subsets and monocytes to distinguish between healthy pregnant women and patients with preeclampsia. These studies are currently underway in our laboratories. Sequential samples obtained from ovarian cancer patients undergoing chemotherapy and pregnant diabetic women (a high risk group for preeclampsia) are being monitored for sMUC16 binding and Siglec-9 expression on immune cell subsets. These studies will be very useful in providing a profile of sMUC16 binding and Siglec-9 expression on immune cells and its potential in early identification and monitoring of ovarian cancer and preeclampsia.

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Fig. 1. sMUC16 is captured but not expressed by immune cells of pregnant women. a, PBMC isolated from healthy pregnant women (lane 1), and a non-pregnant individual were lysed and immunoprecipitated with the anti-MUC16 antibody, VK8⁵¹. The immunoprecipitated material (5 μ g/lane) was analyzed by western blotting and VK8 was used for detection of the mucin. Lysate of ovarian cancer cell line OVCAR-3 was used as positive control (lane 3). b, mRNA was isolated from PBMCs from women with healthy pregnancy and MUC16 was detected by qPCR. mRNA from OVCAR-3 and ECC-1 cells were used as positive controls.

Fig. 2.
Rapid binding of sMUC16 to immune cells via Siglec-9. Jurkat cells transfected with Siglec-9 were incubated with sMUC16 partially purified from the conditioned media of OVCAR-3 cells. Binding of the mucin to the Siglec-9 expressing Jurkat cells was determined by flow cytometry.

Fig. 3. sMUC16 predominantly binds to Siglec-9 expressing cells isolated from the peripheral blood of healthy pregnant women. sMUC16 binding to T cells, B cells, NK cells, and monocytes from PBMC of healthy pregnant women was determined by flow cytometry. Siglec-9 positive events were gated and the binding of sMUC16 to these immune cell subsets was determined. Immune cells from each patient were analyzed by flow cytometry in duplicates and the data for each reading is shown.

Fig. 4.

Increasing levels of immune cell bound sMUC16 during pregnancy. Three healthy pregnant women (HP#1, HP#2, and HP#3) were analyzed for serum CA125 levels (Units/ml shown below bar graph) and sMUC16 bound to peripheral blood immune cells (bar graph) isolated at weeks 9 and 18 of pregnancy. Immune cells from these three donors prior to their pregnancy were also available and were also tested for bound sMUC16. Bar graphs show the mean fluorescence intensity (MFI) for the total sMUC16 positive events in each sample used in this analysis.

Fig. 5.

Healthy pregnant women and preeclampsia patients have comparable levels of serum CA125 and monocytes. A, Serum samples were drawn from healthy pregnant (n=27) and preeclampsia patients (n=9) and CA125 levels in each sample were determined using a clinical assay. b, Healthy pregnant women and preeclampsia patients have comparable levels of peripheral blood monocytes. Comparable levels of immune cells were present in the peripheral blood of healthy pregnant women and preeclampsia patients. Representative data for peripheral blood monocytes is shown here. Data shows the total number of CD3^{neg}/CD16^{pos}/CD14^{pos} monocyte events as determined by flow cytometry in each blood sample.

Fig. 6.

Differential binding to sMUC16 to NK cell subsets of preeclamptic patients. Peripheral blood mononuclear cells isolated from healthy pregnant women and preeclampsia patients at the time of delivery were subjected to multi-color flow cytometry. The CD16^{pos}/CD56^{dim} and CD16^{neg}/CD56^{br} NK cell subsets were identified as described in our previous work ^{11, 12}. sMUC16 was detected using the VK-8 antibody and percentage of sMUC16 and Siglec-9 positive cells were identified. The flow cytometer was calibrated using fluorescent bead standards allowing us to compare the data obtained from all samples from experiments conducted on different days. Duplicate readings for each patient are shown.

Fig. 7.

Differential binding to sMUC16 to monocytes of preeclamptic patients. Peripheral blood mononuclear cells isolated from healthy pregnant women and preeclampsia patients at the time of delivery were subjected to multi-color flow cytometry. Monocytes were identified as described ^{11, 12} and percent of sMUC16 (detected using VK-8 antibody) and Siglec-9 positive events were identified. The flow cytometer was calibrated using fluorescent bead standards allowing us to compare the data obtained from all samples from experiments conducted on different days. Duplicate readings for each patient are shown.

Table 1

Characteristics	Controls (n=17)	Preeclampsics (n=9)	pValue
Age	33.45 (22-42)	27.4 (19-40)	*0.0095
Body Mass Index (BMI)	32.45 (26-47)	37.2 (27-51)	0.0555
Gestational Age at Delivery (GA)	38.75 (37.2-41)	35.89 (28.1-38.3)	*0.0009