

tightly regulated process, controlled by a wide repertoire of surface receptors able to integrate different inputs from the uterine environment. In early pregnancy, the effects of obesity-linked inflammation on dNK activity are largely unknown. This study aims to examine the effects of maternal obesity on dNK activity and expression of inhibitory and activating NK cell receptors.

**Methods:** dNK from lean (BMI 20–24.9 kg/m<sup>2</sup>; n = 16) and obese (BMI ≥30 kg/m<sup>2</sup>; n = 16) women in early pregnancy (7–10 weeks gestation), were characterized by flow cytometry for differences in activity (via CD107a) and surface expression of activating (NKp30, NKp44, NKp46, NKG2D, KIR2DS1) and inhibiting (NKG2A, KIR2DL1, KIR2DL4, LILRB1) receptors. KIR2D gene levels were additionally measured by quantitative PCR (qPCR) analysis. The presence/absence of low-grade inflammation in our patient cohort was assessed in serum using a high-sensitivity CRP (C-reactive protein) ELISA.

**Results:** dNK from obese women showed elevated basal activity as defined by increased surface CD107a expression. Flow cytometry analysis revealed that the inhibiting NKG2A and activating NKp46 receptors were decreased in dNKs of obese women, while KIR2DS1/L1 levels were shown to increase. qPCR analysis demonstrated that mRNA levels of KIR2DL1 remained unchanged between lean and obese dNKs; in contrast KIR2DS1 mRNA levels were higher. Moreover, multicolour flow cytometry identified two distinct dNK populations unique in obese subjects characterized by KIR2DS1<sup>Hi</sup>/KIR2DL1<sup>+</sup> and KIR2DS1<sup>Lo</sup>/KIR2DL1<sup>+</sup>.

**Conclusion:** Our findings provide insight into how maternal obesity alters dNK function in early pregnancy through alterations in NK cell receptor expression.

#### P1.43 TROPHOBLAST TURNOVER IN HUMAN PLACENTAL CHORIONIC VILLI EXPLANTS INDUCED BY *TRYPANOSOMA CRUZI* IS MEDIATED BY TOLL LIKE RECEPTOR-2 ACTIVATION

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Congenital Chagas' disease, caused by the protozoan *Trypanosoma cruzi* (*T. cruzi*), is one of the major public health concerns in Latin America where more than one million women in fertile age are infected. During congenital transmission, the parasite is able to cross the placental barrier. It has been proposed, that the placenta present local anti-parasitic mechanisms such as an increase in trophoblast turnover. Moreover, the trophoblast expresses Pathogen Recognition Receptors such as Toll like receptors (TLRs); their activation leads to the induction of several immune and cellular responses.

**Objective:** To study TLRs expression profile against *T. cruzi* infection and its effect on trophoblast turnover.

**Methods:** Human Placental Chorionic Villi Explants (HPCVE) were challenged during 2 hours with 10<sup>5</sup> *T. cruzi* trypomastigotes (infective form) in presence and absence of blocking antibodies against TLR-2 or 4 (10 µg/ml, PAb-hTLR2 or PAb-hTLR4 Invivogen®). TLR protein expression was determined by Western blotting, the parasite infection was analysed by real time PCR. Additionally, we determined PCNA (as proliferation marker, by Western blotting), β-human chorionic gonadotropin (β-hCG, by ELISA) (as differentiation marker) and activity of caspase 3 (as apoptotic cell death markers).

**Results:** *T. cruzi* induces the protein expression of both TLR-2 (3.84 ± 0.89) and TLR-4 (2.46 ± 0.51) which is prevented by the TLR 2 and 4 inhibition. Only the inhibition of TLR-2 increases the parasitic load in HPCVE (2.67 ± 0.21 versus infected control), Interestingly, the TLR-2 inhibition also decreases the protein expression of PCNA (32.4% ± 9.9% vs infected HPCVE), the secretion of hCG (54 ± 8.3% vs infected HPCVE) and increases the caspase 3 activity (20 ± 4.9% vs infected HPCVE).

**Conclusion:** Our results suggest, that TLR-2 activation by *T. cruzi* leads to an increase of the epithelial trophoblast turnover, which could be a local antiparasitic mechanism of the human placenta.

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#### P1.44 THE EX VIVO INFECTION OF HUMAN PLACENTAL CHORIONIC VILLI EXPLANTS WITH *TRYPANOSOMA CRUZI* AND *TOXOPLASMA GONDII* IS MEDIATED BY DIFFERENT TOLL-LIKE RECEPTORS

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*Trypanosoma cruzi* (*T. cruzi*), the causative agent of Chagas disease, and *Toxoplasma gondii* (*T. gondii*), responsible for Toxoplasmosis are two parasites that can cross the placental barrier. *T. cruzi* and *T. gondii* present low and high congenital transmission rates, respectively. Toll-like receptors (TLR) -2, -4 and -9, expressed by the human placenta, recognize both parasites. Therefore, *T. cruzi* and *T. gondii* induced activation of those placental TLRs may be involved in the defense mechanism against both parasites and might determine the probability of transmission.

**Objectives:** To study the protein expression of TLR-2, -4 and -9, part of the associated cytokine profile and their possible role during parasite infection

**Methods:** Human placental chorionic villi explants (HPCVE) were incubated, during 2 hours, in presence and absence of 10<sup>5</sup> *T. cruzi* trypomastigotes or 10<sup>5</sup> *T. gondii* tachyzoites and in the presence or absence of blocking antibodies (TLR-2, -4) or suppressive oligonucleotides (TLR-9). LPS and CpG-DNA were used as positive controls. TLRs protein expression was determined by Western blotting and parasite infection was analyzed by real time PCR. The presence of IL-1, IL-4, IL-6, IL-8, IL-10, IFN-γ and TNF-α in the culture media was determined by ELISA.

**Results:** *T. cruzi* and *T. gondii* induce both a statistical significant increase of the protein expression of TLR-2 and TLR-4, but not of TLR-9. On the other hand, *T. cruzi* induces higher levels of pro-inflammatory and immunomodulating cytokines than *T. gondii*. Interestingly, the inhibition of TLR-2 increases the *T. cruzi* DNA load and the inhibition of TLR-4 and -9 increases the *T. gondii* DNA content in HPCVE.

**Conclusion:** The infection of HPCVE with both parasites is mediated by different TLRs and elicits different cytokine profiles. Our results might explain, at least partially, the different transmission rates of *T. cruzi* and *T. gondii*.

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#### P1.45 SHARED IMMUNOREGULATORY PROPERTIES OF GALECTIN-9 AND PD-L1 IN PREGNANT WOMEN AND PATIENTS WITH STAGE IV MELANOMA

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**Objectives:** There are striking similarities between trophoblasts and tumors, specifically when it comes to immune regulation. We studied two molecules, PD-L1 and galectin-9, which are important in promoting immunosuppressive effects of tumors, to test if they have any relevance in human pregnancy.

**Methods:** Maternal plasma samples were collected monthly from healthy, primigravid women throughout pregnancy and compared to non-pregnant controls and patients with stage IV melanoma. Plasma levels of galectin-9 and PD-L1 were measured by ELISA and compared by a student t-test. Immunohistochemistry (IHC) for galectin-9 and PD-L1 staining was completed using healthy, term placentas and stage IV melanoma tumors.

**Results:** Galectin-9 was significantly increased in the pregnant mother's plasma (2524pg/mL) as well as cancer patient plasma (3969pg/mL), compared to non-pregnant/healthy controls (997pg/mL; p<0.001). When we grouped mothers into those having a male and those having a female child, we observed significantly more soluble galectin-9 in the plasma of women carrying a male (3013 vs. 1360pg/mL; p<0.0001). Although galectin-9 levels were elevated throughout pregnancy, soluble PD-L1 levels increased with gestation, dropping dramatically 6 weeks post-partum. IHC demonstrated high galectin-9 and PD-L1 staining around the trophoblastic cells of the placenta and around the tumor edge.

**Conclusions:** Similar to the phenomenon seen in cancer biology, pregnancy supports the down-regulation of immunity by both systemic