Oxygen regulates ILC3 antigen presentation potential and pregnancy-related hormone actions

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Research Article

Keywords: Innate lymphoid cells, oxygen, hCG, TGF- β 1, pregnancy, tolerance, placenta

Posted Date: January 19th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1269749/v1>

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Abstract

Early pregnancy is marked by placentation and embryogenesis, which take place under physiological low oxygen concentrations. This oxygen condition is crucial for many aspects of placentation, trophoblast function, vascularization and immune function. Recently, a new family of innate lymphoid cells has been found to be expressed at the fetomaternal interface. Among these, type 3 innate lymphoid cells (ILC3) are important antigen presenting cells in the context of MHCII. The expression of MHCII on ILC3s during pregnancy is reduced. We tested the hypothesis that low oxygen concentrations reduce the potential of ILC3s to present antigens promoting fetal tolerance.

Using an *in vitro* approach, NCR⁺ ILC3s generated from cord blood stem cell precursors were incubated under different O₂ concentrations in the presence or absence of the pregnancy-related hormones hCG and TGF-β1. The expression of MHCII, accessory molecules and an activation marker were assessed by flow cytometry. We observed that 1 % O₂ reduced the expression of the MHCII molecule HLA-DR as compared to 21 % O₂ and modulated the relative effects of hCG and TGF-β1.

Our data indicate that low oxygen concentrations reduce the antigen presentation potential of NCR⁺ ILC3s and suggest that it may promote fetal tolerance during the first trimester of pregnancy.

Introduction

After implantation, several changes take place in the decidualized endometrium to assure the anchoring of the fetus and the later establishment of a fetomaternal circulation. Trophoblast cells invade into the maternal decidua, reach spiral arteries and block them temporarily. During this time, the zone between decidua and placenta remains in low oxygen conditions, which is estimated to be around 10-30 mmHg (corresponding to 1-4 % O₂) [1, 2]. Once the plugs are released, the intervillous spaces are filled with maternal blood. Thereafter, the oxygen partial pressure reaches 50-60 mmHg (7-8 % O₂) [2]. The reduced oxygen availability promotes placentation by inducing cell growth and vascular remodeling [3]. Moreover, higher O₂ concentrations are suspected to be detrimental to early pregnancy and have been recorded in missed abortions [2, 4].

The adaptations are supported by local factors as TGF-β1 and hCG [5-8]. The changes in the O₂ concentrations also influence the function of several cell types, including leukocytes that are present at the fetomaternal interface [9]. As it occurs with trophoblasts and endothelial cells, also decidual leukocytes (including macrophages and innate lymphoid cells (ILCs) as NK cells) take part in vascular remodeling are influenced by low oxygen concentrations [10].

The role of lymphocytes in vascular remodeling is not completely understood. Recently, new lymphocyte populations with angiogenic potential have been described. In particular, the helper subsets of the ILCs, which are known to be involved in tissue remodeling [11]. ILCs secrete diverse cytokines (e.g. IL-8, IL-17, IL-22, IFNγ, GM-CSF) and influence the function of other cells, including T cells. As excessive cytokine

secretion may disturb placentation, dysregulated secretory capacity of immune cells and their interactions at the fetomaternal interface are of great importance.

The interaction between ILCs and T cells is greatly dependent on cell to cell contacts. ILCs express OX40L, BAFF, DLL1, CD80 and CD86, which play an important role in the activation and modulation of adaptive immune cells [12, 13]. Therefore, ILCs represent an important link between adaptive and innate immune responses. Particularly, the antigen presentation capacity of ILCs has been found to be fundamental in immune homeostasis, as for instance in the gut [12, 14]. During pregnancy, we have observed that the antigen presentation potential of ILCs is reduced [15]. This phenomenon may represent an adaptive mechanism involved in the tolerance against fetal antigens.

The mechanisms for tolerance against fetal antigens include the absence of classical MHCI and MHCII expression by trophoblast cells [16-18]. CD4⁺ T cells, however, recognize fetal antigens indirectly, in a process mediated by antigen presenting cells (APC). APC at the fetomaternal interface include macrophages, dendritic cells, B cells and ILCs [19].

Physiological oxygen restriction is known to affect immune cells and lead, as in the case of the placenta, to tolerance-mediating effects [20]. Similar to first trimester placenta, a low oxygen concentration is characteristic of the tumor environment. The low oxygen availability of the tumor environment dampens the anti-tumoral responses of several immune cells.[10] The effects include a reduction of the expression of MHCII molecules [21, 22]. Here, we postulate that the low oxygen conditions that prevail at the beginning of pregnancy promote a tolerogenic phenotype in ILCs.

Methods

Human samples

Umbilical cord blood of term cesarean sections was obtained after patient's informed consent. Mononuclear cells were isolated as previously described [15]. Briefly, blood samples were centrifuged, serum discarded and cells were isolated by density centrifugation with Lymphoprep™ (STEMCELL Technologies Inc., Vancouver, Canada), following manufacturer's instructions.

Cell culture

CD34⁺ stem cells were isolated by magnetic separation (Miltenyi, Bergisch Gladbach, Germany). Cells were then cultured in ILC3 differentiation medium (10 % FBS, 1 % Penicillin-Streptomycin (PAN-Biotech GmbH, Aidenbach, Germany), 20 ng/mL SCF, 20 ng/mL IL-7, 20 ng/mL IL-15 and 10 ng/mL Flt-3 ligand (Miltenyi, Bergisch Gladbach, Germany) in RPMI 1640 (PAN-Biotech GmbH, Aidenbach, Germany)) as previously reported [15, 23].

Treatment was performed in the presence of TGF- β 1 (2 ng/mL (R&D, Minneapolis, MN, USA)) or hCG (100 IU/mL (Ovitrelle®, Merck, Darmstadt, Germany)). Treatment under different oxygen concentrations (1 or 8 % O₂) was performed in a multigas incubator (Sanyo, Moriguchi, Osaka prefecture, Japan) supplied with 5 % CO₂ and N₂ as complementary gas. CO₂ and O₂ concentrations were measured constantly. Cells were activated with human recombinant IL-1 β and IL-23 (20 ng/mL (BioLegend, San Diego, CA, USA)) for the last 18 h of the stimulation. An increased signal of intranuclear HIF-1 α under 1 % O₂ was observed by flow cytometry after nuclei isolation and further staining with anti-HIF-1 α antibody (BD Biosciences, Franklin Lakes, NJ, USA; clone 54/HIF-1 α ; data not shown).

Flow cytometry

Cell suspensions were preincubated with Fixable Viability Dye (Thermo Fisher Scientific Inc., Waltham, MA, USA) for 30 min at 4 °C to exclude non-viable cells. For subsequent washing steps FACS buffer (1 % BSA (Sigma-Aldrich, St. Louis, MO, USA), 0.1 % NaN₃ (Carl Roth, Karlsruhe, Germany) in DPBS (PAN-Biotech GmbH, Aidenbach, Germany)) was used. Extracellular staining was performed for 30 min at 4 °C. For intracellular staining cells were permeabilized with Foxp3 staining buffer set (Thermo Fisher Scientific Inc., Waltham, MA, USA) according to manufacturer's instructions. Intracellular staining was applied for 30 min at 4 °C as well.

Antibody clones used for flow cytometry staining: Lin3 (CD3, M ϕ P9; CD14, SK7; CD19, SJ25C1; CD20, L27), CD94 (HP-3D9), NKp44 (p44-8), CD80 (L307.4), CD40 (5C3), CD69 (FN50; BD Biosciences, Franklin Lakes, NJ, USA) and NKp44 (Clone 2.29), HLA-DR (AC122; Miltenyi, Bergisch Gladbach, Germany).

Measurement was performed with FACSCanto (BD Biosciences, Franklin Lakes, NJ, USA). FMO (fluorescence minus one) controls were included. Data was analyzed with FlowJo10.4 software (FlowJo, LLC, Ashland, TN, USA).

NCR⁺ ILC3s were gated as live CD3⁻CD14⁻CD19⁻CD20⁻CD94⁻NKp44⁺ cells. After sorting and subsequent stimulation, these cells expressed CD69, HLA-DR, IL-8 and IL-22 [15]. The presence of ROR γ t, HLA-DR, CD80, CD40, CD69, IL-8 and IL-22 mRNA was also detected by qPCR and flow cytometry. Additionally, IL-8, IL-17 and IL-22 were detected in the supernatants of sorted cells by Multiplex assay (data not shown).

Statistical analysis

Data are presented as relative changes to corresponding untreated controls. Paired Student's *t*-test was used to analyze data. Graphs show mean $\pm$ SEM. For statistical analysis Microsoft Excel 2010 (Microsoft, Redmond, WA, USA) and Prism 5.01 (GraphPad Software, San Diego, CA, USA) were used. P-value $\leq$ 0.01 was considered statistically significant.

Results

Oxygen restriction reduces ILC3 antigen presentation potential

Recently, we showed that antigen presentation capacity by ILC3s is reduced in uterine and decidual compared to peripheral samples [15].

To test the effect of oxygen-restricted conditions on ILC3s, *in vitro*-generated NCR⁺ ILC3s were incubated in the presence of atmospheric oxygen concentration (21 % O₂), 8 % O₂ or 1 % O₂ (Figure 1).

Compared to 21 % O₂, the incubation in 1 % O₂ significantly reduced the percentage of HLA-DR⁺ ILC3s (by 41 %) and their antigen presentation capacity (MFI; by 35 %). Costimulatory molecules CD40 and CD80 were contrarily affected by reduced O₂ concentration. Under 8 % O₂, the proportion of CD80⁺ ILC3s and the CD80 MFI were significantly decreased (by 11 and 9 %, respectively) compared to 21 % O₂. In contrast, the percentage of CD40⁺ ILC3s was significantly increased (by 17 %) under 1 % O₂ compared to 21 % O₂.

The activation marker CD69 was not significantly affected by oxygen restriction.

Oxygen modulates hCG-mediated effects on ILC3s

Recently, we showed that pregnancy-related hormones hCG and TGF-β1 reduce ILC3 antigen presentation potential [15]. Since lower oxygen concentration also reduced antigen presentation capacity, synergistic effects were assessed (Figure 2).

HCG significantly reduced the percentage of HLA-DR⁺ ILC3s and HLA-DR expression (MFI) under 21 % O₂ (by 26 % and 12 %, respectively) and 8 % O₂ (by 24 % and 19 %, respectively). The effect under 1 % O₂ was not statistically significant.

Oxygen also modulated hCG-mediated effects on CD80 expression, leading to a decrease in

CD80 expression after treatment with hCG under 1 % O₂ (by 5 %). However, CD40 expression was not additionally affected by hCG treatment. The expression of CD69 was significantly reduced by hCG under 21 % O₂, but only by 1 %.

ILC3s under 1 % O₂ are more sensitive to TGF-β1-mediated reduction of CD40 and CD69

Similarly to hCG, we previously showed a significant decreasing effect of TGF-β1 on antigen presentation potential of ILC3s [15]. Additive effects of O₂ restriction and TGF-β1 were investigated (Figure 3).

TGF- β 1 reduced the proportion of HLA-DR-expressing ILC3s under 21 % (by 27 %) and under 8 % O₂ (by 46 %), but not significantly under 1 % O₂. HLA-DR expression (MFI) of ILC3s was significantly reduced through TGF- β 1 only under 8 % O₂ (by 29 %).

CD80 expression and proportion of CD80⁺ ILC3s was significantly decreased by TGF- β 1 under all oxygen conditions (percentage: by 12 % each; MFI: by 22, 26, 20 %, respectively).

Although oxygen restriction to 1 % O₂ increased the proportion of CD40⁺ ILC3s, stimulation with TGF- β 1 significantly reduced CD40 expression and the proportion of CD40-expressing ILC3s under 21 % (by 18 %; 12 %) and 1 % O₂ (by 22 %, 18 %).

CD69 expression became more sensitive to TGF- β 1-mediated regulation with decreasing O₂ concentrations. TGF- β 1 did not significantly change CD69 expression under 21 or 8 % O₂, but significantly reduced CD69 expression by ILC3s under 1 % O₂ (by 14 %).

Discussion

The role of oxygen on ILC biology has not been studied extensively [24]. In several tissues, oxygen modulates diverse biological processes, especially angiogenic events [10]. During placentation, the trophoblastic plug in the spiral arteries leads to a temporary state of low oxygen availability in the placenta and the contact zone of the decidua [1]. At this time, important modifications of the vasculature and the extracellular matrix take place [25] and the interplay between trophoblast cells and several immune cell subsets is crucial [26].

The fact that fetal cells (expressing paternal antigens) are tolerated by maternal immune system represents a significant challenge to our understanding of immune response mechanisms. Nevertheless, it is well-accepted that immune cells adapt during pregnancy to avoid fetal rejection [27, 28]. The tolerance to fetal antigens comprises both, active and passive mechanisms. Active mechanisms of fetal tolerance include cellular and humoral components which are triggered after paternal antigen recognition. In 1-5 % of pregnancies, nonetheless, early spontaneous recurrent pregnancy loss can be observed. In the absence of miscarriage inducing factors, idiopathic recurrent pregnancy losses are believed to be caused mainly by immune factors; particularly, the impaired recognition of paternal antigens and the subsequent loss of control over the inflammatory response [29].

Trophoblasts are invasive fetal cells that establish close contact with maternal immune cells [26]. In order to avoid immune rejection, trophoblasts do not express most polymorphic classical MHC I and MHC II molecules. MHC II molecules, however, can be found in intracellular deposits of trophoblast cells [30]. During placentation, trophoblasts undergo cycles of growth and apoptosis [31]. Antigen presenting cells clean up the debris and can in this context present fetal antigens to CD4⁺ T cells [32]. In order to induce tolerance, instead of fetal rejection, the antigen presentation should occur in a manner that induces T cell tolerance instead of a pro-inflammatory phenotype.

ILC3s are central components of immune homeostasis in mucosal barriers, where they collaborate with the tolerance against commensal bacteria [33]. ILC3s can intake and process antigens, to later present them in the context of MHCII to CD4⁺ T cells [34]. This interaction plays a fundamental role in the induction of intestinal commensal bacteria tolerance [35]. The role of ILCs in fetal tolerance has not been studied extensively yet. Our group has previously observed that ILCs reduce their antigen presentation potential during pregnancy, and that uterine ILCs retain a low presentation potential [15]. Additionally, local soluble factors can modulate antigen presentation potential. Here we observed that low oxygen concentrations, as measured in the contact zone between decidua and placenta, induce a reduction of the MHCII expression. This can be interpreted as induction of tolerance, which may facilitate the invasion of fetal cells into the maternal decidua.

Furthermore, we observed that the effects of factors that regulate ILC3 function, such as hCG or TGF- β 1, are also dependent on the oxygen availability. The drastic reduction of MHCII expression in the culture at 1 % O₂ seemed to mask the relative effects of hCG and TGF- β 1. These, on the other hand, may play an important role later on in pregnancy, when the blood flow into the fetal circulation has been established and the oxygen concentration increases.

The modulatory role of oxygen could be appreciated in the expression of co-stimulatory molecules as well. The culture under 1 % O₂ had an additive effect on the hCG modulation of the expression of CD80 and improved the regulatory effect of TGF- β 1 on CD40 and CD69.

The maintenance of fetal tolerance is fundamental for pregnancy success. The loss of fetal tolerance can induce pathological conditions as in villitis of unknown etiology and secondary recurrent miscarriage.[36] It has been observed that alterations of the oxygenation at the beginning of pregnancy impact negatively on pregnancy outcome. Premature or excessive entry of maternal blood into the intervillous spaces is observed in miscarriage [4, 37]. The deleterious effects on fetal cell function of the resulting over oxygenation are partially mediated by free oxygen radicals, which impair early pregnancy events [38, 39]. It is likely that the downregulation of the antigen presentation potential of ILC3s by low oxygen contributes to early tolerance against fetal-derived antigens.

During the first trimester of pregnancy, which is characterized by physiological low oxygen concentrations in the placenta, trophoblasts possess an invasive phenotype. There is, as a consequence, an increased cell replication which is balanced by a high apoptotic rate [40]. It has been proposed that antigen presenting cells clean up apoptotic trophoblasts [41]. For this reason, it is tempting to speculate that a reduced fetal antigen presentation would positively influence the balanced immune responses at the fetomaternal interface, by preventing excessive immune reactions. On the contrary, as observed in hyperoxic placentas, pregnancy problems may arise.

Abbreviations

ILCs: innate lymphoid cells, MHC: major histocompatibility complex, NCR: natural cytotoxicity receptor, hCG: human chorionic gonadotropin, TGF- β 1 transforming growth factor β 1, HLA: human leukocyte antigen, NK: natural killer, BAFF: B-cell activating factor, DLL1: delta like canonical notch ligand 1, CD: cluster of differentiation, IL: interleukin, SCF: stem cell factor, Flt-1: Fms-Like Tyrosine Kinase 1, HIF-1 α : Hypoxia-inducible factor 1-alpha, FACS: fluorescence-activated cell sorting, BSA: bovine serum albumin, DPBS: Dulbecco's phosphate-buffered saline, FMO: fluorescence minus one, ROR γ t: RAR-related orphan receptor gamma, SEM: standar error of mean, MFI: mean fluorescence intensity.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the University Medicine Greifswald (BB160/17a). Each participant provided informed written consent for their participation in the study.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interest.

Funding

This work was supported by the Deutsche Forschungsgemeinschaft (DFG), awarded to DM (MU 4404/3-1) and intramural funding from Greifswald University.

Author's contributions

RE performed experiments, analyzed data and contributed to the elaboration of the manuscript. JE performed experiments. MZ contributed with the design of experiments, the writing of the manuscript and reagents. DM conceived and designed the experiments, analyzed data, wrote the paper and supervised the work.

Acknowledgements

We thank the state graduate funding (Landesgraduiertenförderung Mecklenburg - Vorpommern) for the financial support of RE.

References

1. Huppertz B, Weiss G, Moser G: Trophoblast invasion and oxygenation of the placenta: measurements versus presumptions. *J Reprod Immunol* 2014, 101-102:74-79.
2. Jauniaux E, Watson AL, Hempstock J, Bao YP, Skepper JN, Burton GJ: Onset of maternal arterial blood flow and placental oxidative stress. A possible factor in human early pregnancy failure. *Am J Pathol* 2000, 157:2111-2122.
3. Burton GJ, Cindrova-Davies T, Yung HW, Jauniaux E: Oxygen and development of the human placenta. *Reproduction* 2020.
4. Jauniaux E, Zaidi J, Jurkovic D, Campbell S, Hustin J: Comparison of colour Doppler features and pathological findings in complicated early pregnancy. *Hum Reprod* 1994, 9:2432-2437.
5. Zygmunt M, Hahn D, Munstedt K, Bischof P, Lang U: Invasion of cytotrophoblastic JEG-3 cells is stimulated by hCG in vitro. *Placenta* 1998, 19:587-593.
6. Zygmunt M, Herr F, Keller-Schoenwetter S, Kunzi-Rapp K, Münstedt K, Rao CV, Lang U, Preissner KT: Characterization of human chorionic gonadotropin as a novel angiogenic factor. *J Clin Endocrinol Metab* 2002, 87:5290-5296.
7. Maynard SE, Karumanchi SA: Angiogenic factors and preeclampsia. *Semin Nephrol* 2011, 31:33-46.
8. Ferrari G, Cook BD, Terushkin V, Pintucci G, Mignatti P: Transforming growth factor-beta 1 (TGF-beta1) induces angiogenesis through vascular endothelial growth factor (VEGF)-mediated apoptosis. *J Cell Physiol* 2009, 219:449-458.
9. Lima PD, Zhang J, Dunk C, Lye SJ, Croy BA: Leukocyte driven-decidual angiogenesis in early pregnancy. *Cell Mol Immunol* 2014, 11:522-537.
10. Palazon A, Aragonés J, Morales-Kastresana A, de Landazuri MO, Melero I: Molecular pathways: hypoxia response in immune cells fighting or promoting cancer. *Clin Cancer Res* 2012, 18:1207-1213.
11. Klose CS, Artis D: Innate lymphoid cells as regulators of immunity, inflammation and tissue homeostasis. *Nat Immunol* 2016, 17:765-774.
12. Castellanos JG, Longman RS: Innate lymphoid cells link gut microbes with mucosal T cell immunity. *Gut Microbes* 2020, 11:231-236.

13. Magri G, Cerutti A: Role of group 3 innate lymphoid cells in antibody production. *Curr Opin Immunol* 2015, 33:36-42.
14. Farkas AM, Ivanov, II: Escaping Negative Selection: ILC You in the Gut. *Immunity* 2015, 43:12-14.
15. Eienkel R, Ehrhardt J, Hartmann K, Kruger D, Muzzio DO, Zygmunt M: Hormonally controlled ILC antigen presentation potential is reduced during pregnancy. *Reproduction* 2020, 160:155-169.
16. Blaschitz A, Hutter H, Dohr G: HLA Class I protein expression in the human placenta. *Early Pregnancy* 2001, 5:67-69.
17. Murphy SP, Tomasi TB: Absence of MHC class II antigen expression in trophoblast cells results from a lack of class II transactivator (CIITA) gene expression. *Mol Reprod Dev* 1998, 51:1-12.
18. Mattsson R: The non-expression of MHC class II in trophoblast cells. *Am J Reprod Immunol* 1998, 40:383-384.
19. Tong M, Abrahams VM: Immunology of the Placenta. *Obstet Gynecol Clin North Am* 2020, 47:49-63.
20. Taylor CT, Colgan SP: Regulation of immunity and inflammation by hypoxia in immunological niches. *Nat Rev Immunol* 2017, 17:774-785.
21. Yang M, Ma C, Liu S, Sun J, Shao Q, Gao W, Zhang Y, Li Z, Xie Q, Dong Z, Qu X: Hypoxia skews dendritic cells to a T helper type 2-stimulating phenotype and promotes tumour cell migration by dendritic cell-derived osteopontin. *Immunology* 2009, 128:e237-249.
22. Wu X, Kong X, Chen D, Li H, Zhao Y, Xia M, Fang M, Li P, Fang F, Sun L, et al: SIRT1 links CIITA deacetylation to MHC II activation. *Nucleic Acids Res* 2011, 39:9549-9558.
23. Croxatto D, Micheletti A, Montaldo E, Orecchia P, Loiacono F, Canegallo F, Calzetti F, Fulcheri E, Munari E, Zamo A, et al: Group 3 innate lymphoid cells regulate neutrophil migration and function in human decidua. *Mucosal Immunol* 2016, 9:1372-1383.
24. Li Q, Li D, Zhang X, Wan Q, Zhang W, Zheng M, Zou L, Elly C, Lee JH, Liu YC: E3 Ligase VHL Promotes Group 2 Innate Lymphoid Cell Maturation and Function via Glycolysis Inhibition and Induction of Interleukin-33 Receptor. *Immunity* 2018, 48:258-270 e255.
25. Zygmunt M, Herr F, Münstedt K, Lang U, Liang OD: Angiogenesis and vasculogenesis in pregnancy. *Eur J Obstet Gynecol Reprod Biol* 2003, 110 Suppl 1:S10-18.
26. Pollheimer J, Vondra S, Baltayeva J, Beristain AG, Knofler M: Regulation of Placental Extravillous Trophoblasts by the Maternal Uterine Environment. *Front Immunol* 2018, 9:2597.
27. Taglauer ES, Adams Waldorf KM, Petroff MG: The hidden maternal-fetal interface: events involving the lymphoid organs in maternal-fetal tolerance. *Int J Dev Biol* 2010, 54:421-430.

28. Erlebacher A, Vencato D, Price KA, Zhang D, Glimcher LH: Constraints in antigen presentation severely restrict T cell recognition of the allogeneic fetus. *J Clin Invest* 2007, 117:1399-1411.
29. Krechetova LV, Vanko LV, Vtorushina VV, Nikolaeva MA, Inviyaeva EV, Tetrushvili NK: Lymphocyte Activation in the Development of Immune Tolerance in Women with Recurrent Pregnancy Loss. *Biochemistry (Mosc)* 2020, 85:583-593.
30. Ranella A, Vassiliadis S, Mastora C, Valentina M, Dionyssopoulou E, Athanassakis I: Constitutive intracellular expression of human leukocyte antigen (HLA)-DO and HLA-DR but not HLA-DM in trophoblast cells. *Hum Immunol* 2005, 66:43-55.
31. Mor G, Abrahams VM: Potential role of macrophages as immunoregulators of pregnancy. *Reprod Biol Endocrinol* 2003, 1:119.
32. Linscheid C, Petroff MG: Minor histocompatibility antigens and the maternal immune response to the fetus during pregnancy. *Am J Reprod Immunol* 2013, 69:304-314.
33. Penny HA, Hodge SH, Hepworth MR: Orchestration of intestinal homeostasis and tolerance by group 3 innate lymphoid cells. *Semin Immunopathol* 2018, 40:357-370.
34. von Burg N, Chappaz S, Baerenwaldt A, Horvath E, Bose Dasgupta S, Ashok D, Pieters J, Tacchini-Cottier F, Rolink A, Acha-Orbea H, Finke D: Activated group 3 innate lymphoid cells promote T-cell-mediated immune responses. *Proc Natl Acad Sci U S A* 2014, 111:12835-12840.
35. Hepworth MR, Monticelli LA, Fung TC, Ziegler CG, Grunberg S, Sinha R, Mantegazza AR, Ma HL, Crawford A, Angelosanto JM, et al: Innate lymphoid cells regulate CD4⁺ T-cell responses to intestinal commensal bacteria. *Nature* 2013, 498:113-117.
36. Petroff MG: Review: Fetal antigens—identity, origins, and influences on the maternal immune system. *Placenta* 2011, 32 Suppl 2:S176-181.
37. Schwarzler P, Holden D, Nielsen S, Hahlin M, Sladkevicius P, Bourne TH: The conservative management of first trimester miscarriages and the use of colour Doppler sonography for patient selection. *Hum Reprod* 1999, 14:1341-1345.
38. Burton GJ, Jauniaux E: Placental oxidative stress: from miscarriage to preeclampsia. *J Soc Gynecol Investig* 2004, 11:342-352.
39. Agarwal A, Majzoub A: Role of Antioxidants in Assisted Reproductive Techniques. *World J Mens Health* 2017, 35:77-93.
40. Straszewski-Chavez SL, Abrahams VM, Mor G: The role of apoptosis in the regulation of trophoblast survival and differentiation during pregnancy. *Endocr Rev* 2005, 26:877-897.

41. Abrahams VM, Kim YM, Straszewski SL, Romero R, Mor G: Macrophages and apoptotic cell clearance during pregnancy. *Am J Reprod Immunol* 2004, 51:275-282.

Figures

Figure 1

Oxygen restriction reduces ILC3 antigen presentation potential.

In vitro-generated ILC3s were incubated under indicated oxic conditions for 72 h. Cells were activated with 20 ng/mL IL-1 β and IL-23 for 18 h prior flow cytometry staining. **(A)** Representative gating strategies for identification of NCR⁺ ILC3s are shown. The overlapping histograms depict the expression of extracellular markers on NCR⁺ ILC3s under 21 % O₂ (stripped line) or reduced oxygen concentration (filled line). FMO controls are displayed as a filled curve. **(B)** Graphs show percentage of marker-expressing ILC3s (left) and the mean fluorescence intensity (MFI; right) of HLA-DR, CD80, CD40 and CD69. Data of 8 or 1 % O₂ (grey) was normalized to 21 % O₂ (outlined bar). Data is shown as mean $\pm$ SEM and was analyzed by paired Student's *t*-test. **p* < 0.05. Experiment was performed for each condition 5 times in duplicates.

Figure 2

hCG addition complements antigen presentation potential reduction in ILC3s.

Additionally to oxic conditions, *in vitro*-generated ILC3s were stimulated with 100 IU/mL hCG for 72 h. 18 h before flow cytometry staining cells were activated with 20 ng/mL IL-1 β and IL-23. **(A)** Representative gating strategies for identification of NCR⁺ ILC3s are shown. The overlapping histograms depict the expression of extracellular markers on NCR⁺ ILC3s under 21 % O₂ (stripped line) or reduced oxygen concentration (filled line). FMO controls are displayed as a filled curve. **(B)** The proportion of marker-expressing ILC3s (left) and the MFI of HLA-DR, CD80, CD40 and CD69 were analyzed (right). Effects of hCG (grey bars) were normalized to untreated cells (outlined bars) under the same oxic conditions. Bars show mean $\pm$ SEM. Data was analyzed by paired Student's *t*-test. **p* < 0.05, ***p* < 0.01. The experiment was repeated 5 (8 and 1 % O₂) or 10 times (21 % O₂) in duplicates.

Figure 3

Reduced oxygen availability enhances TGF- β 1-mediated reduction in ILC3 accessory molecules.

Defined oxic conditions were combined with the addition of 2 ng/mL TGF- β 1 to treat *in vitro*-generated ILC3s for 72 h. Cells were activated by 20 ng/mL IL-1 β and IL-23 18 h before flow cytometry staining. **(A)** Representative gating strategies for identification of NCR⁺ ILC3s are shown. The overlapping histograms depict the expression of extracellular markers on NCR⁺ ILC3s under 21 % O₂ (stripped line) or reduced oxygen concentration (filled line). FMO controls are displayed as a filled curve. **(B)** The proportion of ILC3s expressing the indicated marker (left) and its MFI (right) were analyzed. Effect of TGF- β 1 (grey bars) is shown relative to controls with the same oxic condition (outlined bars). Bars show mean $\pm$ SEM. Data was analyzed by paired Student's *t*-test. **p* < 0.05, ***p* < 0.01, ****p* < 0.001. The experiment was repeated 5 (8 and 1 % O₂) or 10 times (21 % O₂) in duplicates.