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The Neonatal IgG Fc-Receptor (FcRn) is Expressed in the Synctiotrophoblast, Fetal Endothelial Cells and Hofbauer Cells of Human Term Placental Chorionic Villi

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Introduction

Human offspring acquire passive immunity for the first months of life by transplacental transport of maternal Immunoglobulin G (IgG). Two cellular layers, the syncytiotrophoblast (STB) and fetal endothelial cells (FEC), as well as an intervening stroma separate the maternal and fetal circulation.

It is generally accepted that in the STB IgG transcytosis is mediated by FcRn, the neonatal Fc-receptor (1,2).

The mechanism of IgG-transport across FEC remains unclear. Although FcRn is the only known IgG receptor capable of bidirectional transcytosis (3) and the only IgG receptor exhibiting a pH-related binding and release of monomeric IgG thereby enabling transcellular transport and subsequent release of intact IgG (4), the data on expression of FcRn in FEC are contradictory. FcRn was not detected in FEC in situ (1,5), but it was found in isolated and cultured human placental endothelial cells (6). As an abundant expression of the Fc-receptor FcγRIIB2 has been demonstrated in FEC (7), FcγRIIB2 was suggested to transport IgG across the FEC. However, it is unclear, how FcγRIIB2 could mediate IgG transcytosis.

For effective engineering of antibody-based therapeutics or interference with transfer of pathogenic antibodies, it is important to fully characterize the entire transplacental IgG transport route.

Aim

Due to the contradictory data concerning placental FcRn localization, we reinvestigated its expression in human term chorionic villi applying different antibodies and fixation techniques.

Summary

1) FcRn is expressed in all areas of 8 placentas investigated.
2) γsm-actin$^+$ (stem)villi and γsm-actin$^-$ (terminal)villi express FcRn.
3) STB expresses FcRn, but at a lower level than cells in the villous stroma.
4) FcRn is found in FEC (CD31$^+$). This location suggests an involvement of FcRn in IgG-transcytosis across the entire placental barrier and/or recycling/salvaging of IgG present in the fetal circulation by the FEC.
5) High expression of FcRn in (CD68$^+$) macrophages could either help to protect maternal serum IgG from catabolism (8) or facilitate processing and presentation of immune complexes.
6) HOPE-fixation improves antigen-detection compared to PFA-fixation.
7) Anti-FcRn peptide antibodies were more specific for the FcRn α-chain than a commercial antibody directed towards the extracellular domain.

Materials and Methods

Immunofluorescence microscopy
Uncomplicated term placentas were either HOPE-fixed (n=5; 9) or formalin-fixed (n=3), paraffin embedded and 4 μm sections were prepared.

Three affinity-purified rabbit antisera prepared against peptides corresponding to two extracellular and one cytoplasmic region of the α-chain as well as the H-274 antibody against the N-terminal extracellular domain (Santa Cruz Biotechnology) were used to detect FcRn.

Antibodies against cytokeratin 7, CD31 and CD68 (all Dako), γ-smooth muscle actin (γsm-actin, MP), and Alexa-488,-568,-647 fluorochrom-conjugated secondary antibodies (all Molecular Probes/Invitrogen) were used and nuclei were stained with DAPI (Roche). Images were acquired using an Axio imager epifluorescence microscope (Zeiss) equipped with TissueFAXS hard- and software (TissueGnostics GmbH) using a 20x objective. Images in Results (4) and (5) were acquired with a 63x oil objective.

Westernblotting experiments
Frozen chorionic tissue was lysed in RIPA-buffer. Placental lysates (30, 60 μg per lane) and 293T and 293T + hFcRn cell lysates (7.5 μg per lane, Santa Cruz Biotechnology) were separated on 12% reducing SDS-gels and proteins were transferred to PVDF membranes. Following membrane blocking and incubation with anti-hFcRn antibodies and secondary HRP-conjugated antibodies (Santa Cruz Biotechnology), blots were developed using a chemoluminescence substrate (Thermo Scientific).

References

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Acknowledgements

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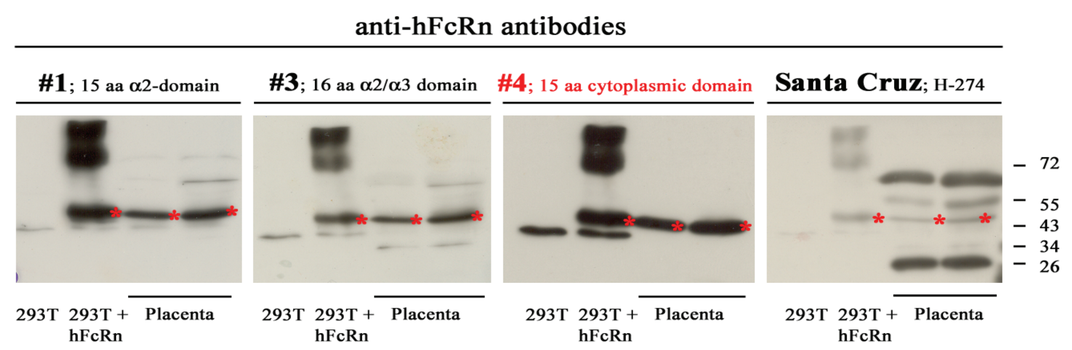
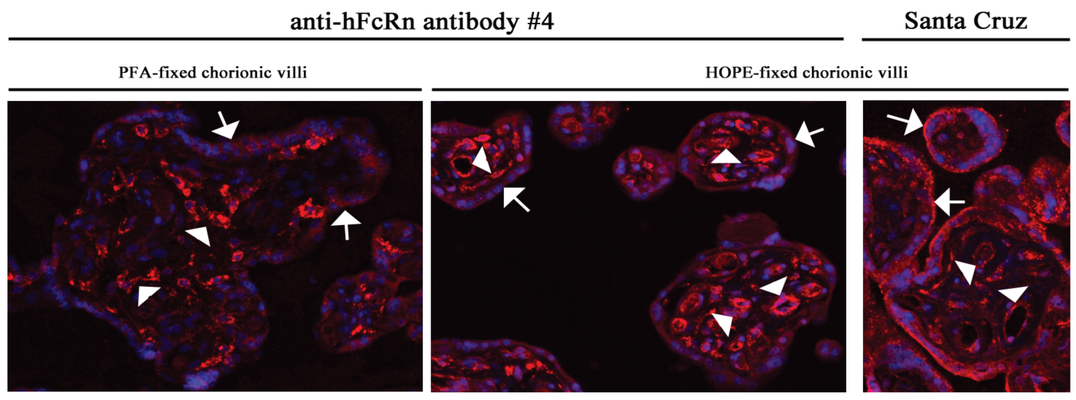
Results

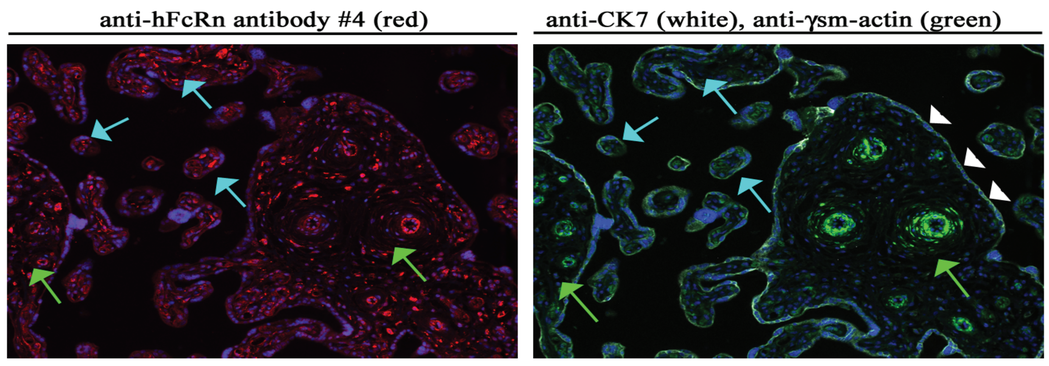
1. Specificity of 4 anti-hFcRn antibodies (red star: FcRn α-chain)



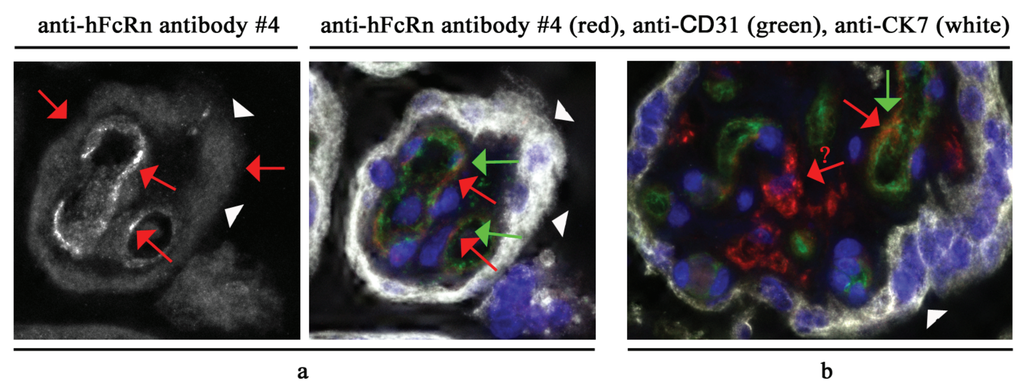
2. FcRn is expressed in the STB (arrow), but also stromal cells (arrowhead)



3. FcRn is expressed in terminal (blue arrow) and stem villi (green arrow)



4. FcRn locates to FEC (CD31$^+$, green arrow, a/b), and CD31$^-$ cells (? in b)



5. FcRn is expressed in placental macrophages (CD68$^+$, green arrow)