The impact of maternal-fetal omalizumab transfer on peanut-specific responses in an ex vivo placental perfusion model

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To the Editor:

The transport of maternal IgG to the fetus is mediated by neonatal Fc receptors (FcRn) expressed on the placenta, which provides passive immunity *in utero* . Humanized monoclonal antibodies such as omalizumab bind to FcRn and are transferred to the fetus. Recently, Bundhoo et al. provided *in vitro* data of FcRn-mediated anti-IgE IgG/IgE immune complexes, suggesting a mechanism for IgE transfer across the placenta to the fetus. As IgG bound allergens are also known to cross the placenta, it is important to understand the impact of omalizumab, one of the most frequently prescribed monoclonal antibodies to treat severe asthma in reproductive age.

We report the use of a state-of-the-art *ex vivo* human placental perfusion system to investigate the impact of omalizumab on the transport of peanut allergen and IgE across the placenta (Figure 1A). We compared the transport of omalizumab (Oma), peanut extract (PE), PE/peanut allergic plasma (PA), and PE/PA/Oma. The kinetics and functionality of the transported allergen were examined *in vitro*(Table S1, see Online Repository for a detailed description of methods), by sampling perfusates collected at different time points ranging from 0 to 240 min. Ara h 2 as a proxy of peanut protein transfer was measured by ELISA (Indoor Biotechnologies, Virginia, USA; EPC-AH2-X). Basophil activation tests (BAT) were performed with sampled perfusates as reported recently, to determine the functionality of the transferred peanut allergens.

Transported Oma was detected in the fetal compartment after 15 min and relative levels steadily increased until the end of the experiment (240 min, Figure 1B), consistent with previous studies reporting linear, concentration-dependent maternal-fetal antibody transfer. In parallel, PE protein was detectable at the fetal side within the first 5 min of tissue perfusion (Figure 1C), reached the maximum concentration (0.3% of the maternal reservoir) after 90 min and remained stable thereafter.

To compare the degree to which FcRn might facilitate the transfer of allergen-IgG complexes across the placenta, we examined Ara h 2 transfer in the presence (PE/PA) or absence (PE) of plasma from peanut-allergic individuals, with (PE/PA/Oma) and without omalizumab (Figure 2A). PE/PA crossed the placenta with a moderate rise of Ara h 2 levels at the fetal side after 120 min, comparable to PE alone (1.03 ng/mL). Extending the perfusion time to 240 min resulted in a near two-fold increase (3.82 ng/mL) of PE via

PE/PA/Oma vs. PE/PA (1.97 ng/mL). The transferred peanut proteins were fully capable of crosslinking IgE as confirmed via BAT (Figure 2B). The higher degree of basophil activation matched the extent of Ara h 2 transfer. We confirmed that perfusion medium alone did not activate basophils (Figure S1). Furthermore, evidence for omalizumab-driven IgE transfer resulting in free IgE with possible functionality was assessed via incubation of stripped basophils with perfusates. We could not find evidence for IgE binding to basophils from these eluates (Figure S2).

In conclusion, functional peanut allergen is actively transported across the human placenta and this process may be enhanced by omalizumab. Active transfer of free IgE to the fetal side due to FcRn mediated complex formation was not observed. Further studies are needed to better understand how allergen-antibody complexes affect allergen-specific priming in the fetus with and without biological usage during pregnancy.

References

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Conflicts of Interest: AK, BH, JSL, BP, KS, and CW have nothing to disclose. ZS holds advisory board roles for Nutricia/Danone, Aimmune and Sanofi. TE reports to act as local PI for company sponsored trials by DBV and sub-investigator for Regeneron, holds grants from Innovation Fund Denmark, CIHR outside the submitted work. He is Co-Investigator or scientific lead in three investigator-initiated oral immunotherapy

trials supported by the Food Allergy and Anaphylaxis Program SickKids and serves as an associate editor for Allergy. He/his lab received unconditional/in-kind contributions from Macro Array Diagnostics and an unrestricted grant from ALK. He holds advisory board roles for ALK, Nutricia/Danone and Aimmune.

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Statement of Author Contribution: AK, BH, JSL, and KS contributed to data acquisition. TE, BH and CW contributed to experimental designs. AK, BH, JSL, BF, KS, CW, ZS, and TE contributed to data interpretation and preparation of the manuscript. The final version of the manuscript was approved by all authors.

Keywords : omalizumab, placental transport, *ex vivo*placental perfusion system, peanut allergy, basophil activation test

Abbreviations : FcRn; neonatal Fc receptors; BAT, basophil activation test; Oma, omalizumab

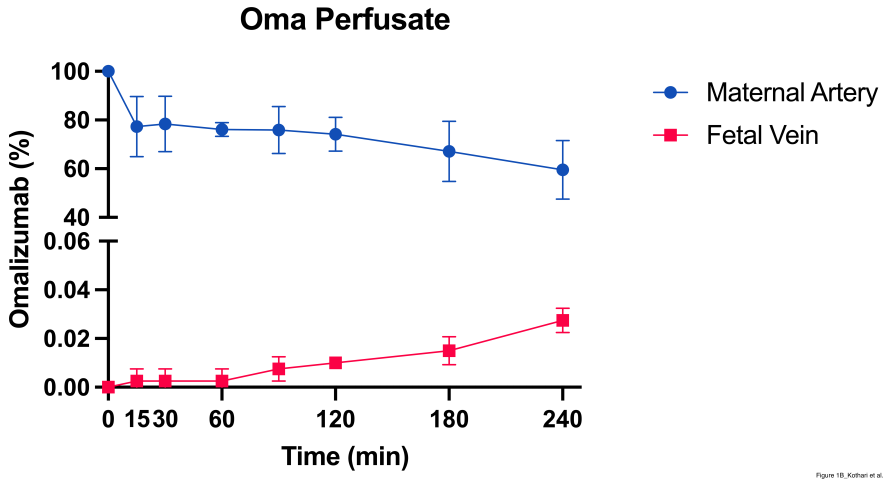
Word Count: 529

Figure Legends:

Figure 1. Transfer of Omalizumab and Peanut protein across the placental barrier.

(A) In the human placental *ex vivo* model sample in-flow (artery) and out-flow (vein) are recorded to and from the placental tissue. (B) Omalizumab and (C) peanut extract transfer kinetics as percent change from maternal artery to fetal vein detected via ELISA.

Figure 2 . Placental peanut allergen transfer in the context of plasma from allergic individual and omalizumab . (A) Ara h 2 concentrations (ng/mL) in fetal vein and artery samples were determined by ELISA in PE alone (dashed line, 1.03 ng/mL) and PE/PA compared to PE/PA/Oma experiments. (B) Basophil activation tests were conducted by flow cytometry to assess the functionality of transferred allergen. Activation levels are expressed as %CD63^{+} basophils in PE, PE/PA and PE/PA/Oma experiments.



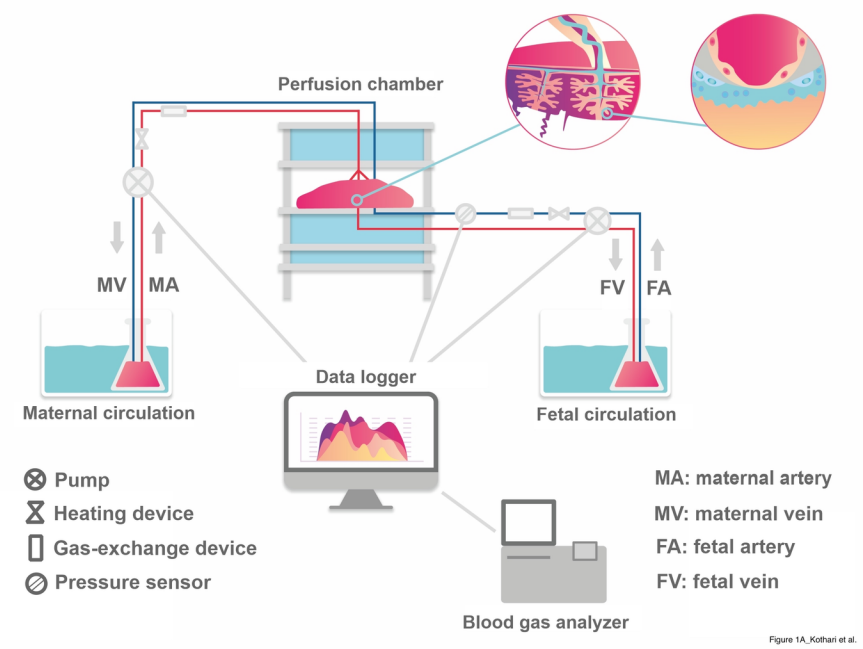
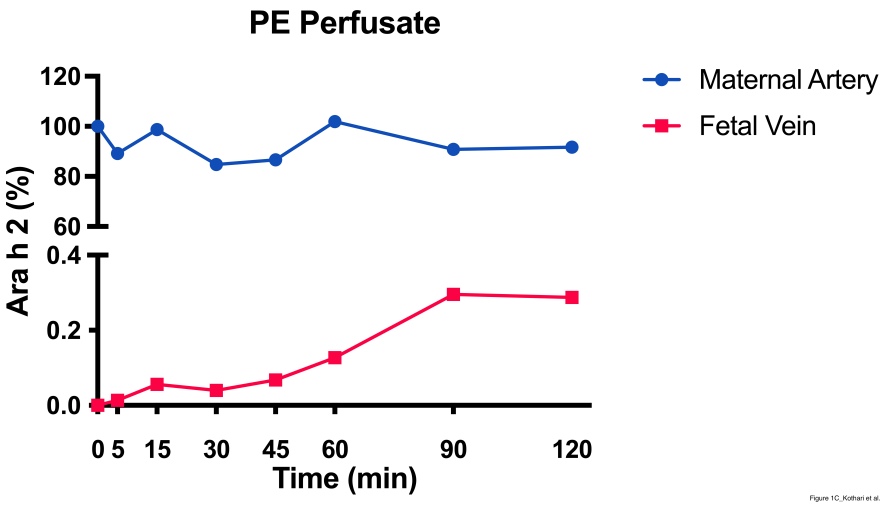


Figure 1A_Kothari et al.



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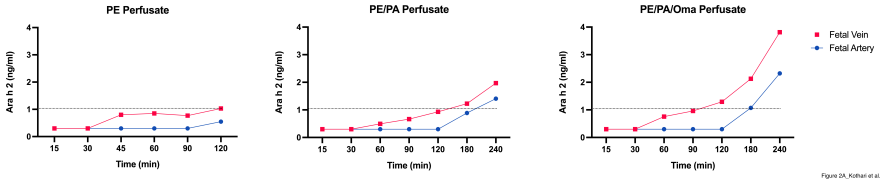

Figure 2A_Kothari et al.

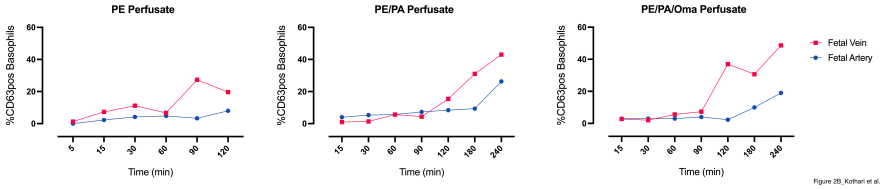

Figure 2B_Kothari et al.