INUTERAL:
Influence of the *in utero* environment on the risk of allergy development for the child

Aim

The aim of this project is to assess if differences in the *in utero* environment can influence the risk of allergy development for the child, and whether these *in utero* differences are related to lifestyle. Since, allergic symptoms usually appear early in life, and maternal allergy is a higher risk factor than paternal allergy for the risk of the child to develop allergy, it is likely that priming occurs already *in utero* at the foetal stage. Therefore, within this project the aim is to investigate the *in utero* environment in detail in relation to lifestyle and environmental influences.

The following specific questions are addressed:

1) Does the *in utero* environment differ, with regard to microRNA expression or protein and mRNA expression of a selection of immune relevant genes, between mothers with and without allergy, as well as between those with different lifestyles?
2) If there are *in utero* mRNA expression differences do they originate from epigenetic differences?
3) Is the IgE present in the placenta specific against certain allergens or naturally occurring and does it originate from the mother or the foetus?
4) Are Hofbauer cells (placental macrophages) polarised, and are this polarisation influenced by maternal allergen-sensitisation and the presence of inflammation (histological chorioamnionitis)?

Results per question

1) mRNA levels of 17 immune relevant genes have been analyzed by means of quantitative real-time PCR in 36 placentas. These 36 placentas were selected such that 18 placentas came from families with an anthroposophic lifestyle and 18 with a conventional lifestyle. Within both these groups 9 mothers were sensitised (have allergen-specific IgE in their blood) and 9 were non-sensitised. Furthermore in total 7 families were living on a farm and 11 fathers were also sensitised. mRNA levels were measured both at the maternal and foetal side of the placenta. We could show that at the maternal side, STAT4 and GATA3 expression is related to the maternal allergen sensitisation. The expression of these genes was confirmed to be present at the protein level. Expression of IL-12(p40) was related to living on a farm on the maternal side of the placenta and to paternal sensitisation on the foetal side of the placenta. Expression of CD14 was increased at the foetal side of the placenta when the families were living on a farm.
Together these data indicate that environmental factors (lifestyle and allergen sensitisation status) can influence the *in utero* environment on the levels of gene expression. Changes in gene expression could affect the placental cytokine production, and intrauterine environment for the foetus. This could in turn influence the development of the foetal immune system and its risk to develop (allergic) diseases later in life.

These data have been published in the following article:

M. Joerink, Oortveld M., Stenius F., Rindsjö E., Alm J., Scheynius A. (2010) Lifestyle and parental allergen sensitization are reflected in the intrauterine environment at gene expression level. *Allergy* **65** (10): 1282-1289

2) The influence of the environment on the gene expression (as indicated in question 1) has been suggested to operate via epigenetic mechanisms. Epigenetic mechanisms involve changes in the DNA without changing the primary base order. DNA methylation, the addition of a methyl group

to the cytosine base, is a form of epigenetic regulation which can influence gene expression. We aimed to analyse if the DNA methylation of the promoter regions of the genes indicated in question 1 was related to the expression and or environmental differences. Therefore, we isolated DNA from the same 36 placentas and analysed them by means of high resolution melt analysis for the promoter regions of: CD14, TLR2, TLR4 (the latter two are related to CD14 and therefore interesting as well), STAT4, GATA3 and IL-12(p40).
Although the analysis is not finalised yet preliminary analysis suggest that methylation of CD14 and TLR4 is related to the expression of CD14 at the foetal and TLR4 at the maternal side of the placenta, respectively. It seems, however, that there is no relation between the environmental factors (lifestyle and allergen sensitisation of the parents) and the methylation.

3) IgE has been eluted from 12 term placentas. The total and allergen-specific IgE levels of this placental eluates have been measured and compared to the levels in both maternal blood and foetal cord blood. These analyses indicated that the levels of total IgE correlated well between placenta and maternal blood but not between placenta and foetal cord blood. Allergen-specific IgE could not be detected in the foetal cord blood, however, allergen-specific IgE detected in the placental eluate was always also represented in the maternal blood. These data suggest that the placental IgE is of maternal origin, and can be allergen-specific when the mother is sensitised.
Exposure of the foetus to the maternal IgE might increase its risk for later allergy development. We demonstrate the maternal IgE does enter the placenta, however, since the foetal cord blood does not contain any detectable levels of allergen-specific IgE, we suggest that the IgE in the placenta is taken up by macrophages (Hofbauer cells) and degraded as to protect the foetus. The uptake and degradation of IgE by Hofbauer cells has, however, not been shown and remains a speculation.

These data have been published in the following article:
M. Joerink, Rindsjö E., Stenius F., Alm J., Lilja G., Grönlund H., Scheynius A. (2009) Evidence for allergen-specific IgE of maternal origin in human placenta. *Allergy* **64** (6): 905-912

4) To analyse the polarisation status of the placental macrophages (Hofbauer cells) three M1 (CX3CR1, IL-7R and CCR7) and three M2 markers (DC-SIGN, CD163 and CD206) were selected. The protein expression of these markers was analysed by means of immunohsitochemstry. We could not detect the expression of the M1 markers while the M2 markers were readily detected, indicating that the Hofbauer cells are of a M2 phenotype. We analyzed the protein expression of two M1 markers (CX3CR1 and IL-7R) and two M2 markers (DC-SIGN and CD163) in placentas of sensitised and non-sensitised mothers and placentas with and without histological chorioamnionitis (a placental infection). M1 markers could still not be detected and for the M2 markers we could not detect any difference between the different groups. Analysis at the mRNA expression level for the same marker confirmed these results. These data indicate that the Hofbauer cells are M2 skewed and that this polarisation status is independent of maternal allergen-sensitisation or the presence of chorioamnionitis. Suggesting that, the stable polarisation of these Hofbauer cells might be necessary for a successful pregnancy.

These data are currently under revision for publication in *Placenta*
M. Joerink, Rindsjö E., van Riel B., Alm J., Papadogiannakis N. Placental macrophage (Hofbauer cell) polarization is independent of maternal allergen-sensitization and presence of chorioamnionitis.