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Placental macrophage (Hofbauer cell) polarization is independent of maternal allergen-sensitization and presence of chorioamnionitis

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ABSTRACT

Background: Macrophages can polarize in which M1/classically activated and M2/alternatively activated macrophages are considered to be the extremes. M1 macrophages are involved in inflammatory reactions, while M2 macrophages are suggested to be involved in homeostasis, parasite killing, tumor promotion, tissue remodeling and in allergic reactions. We hypothesized that polarization of placental macrophages (Hofbauer cells) is influenced by the allergen-sensitization status of the mother and/or the presence of chorioamnionitis, a placental inflammation. This Hofbauer cell polarization might be associated to the intrauterine environment and influence the risk of allergy development for the child. Therefore we aimed to determine the polarization status of Hofbauer cells in health and disease.

Methods: We determined the expression of CD68, CX3CR1, IL-7R, DC-SIGN/CD209 and CD163 in placentas of sensitized versus non-sensitized mothers ($n = 17$), and placentas with or without histological chorioamnionitis ($n = 10$) by means of immunohistochemical analysis and quantitative real-time PCR (qPCR).

Results: Protein expression of the M1 markers (CX3CR1, IL-7R and CCR7) could not be detected in any of the analyzed samples while the M2 markers (DC-SIGN, CD163 and mannose receptor/CD206) were readily detected. Significant differences between non-sensitized versus sensitized mothers and uncomplicated versus chorioamnionitis complicated pregnancies were not detected at protein or at mRNA expression level.

Conclusions: These results suggest that Hofbauer cells have an M2 phenotype, and that their polarization is not affected by maternal allergen-sensitization or by presence of chorioamnionitis.

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1. Introduction

Macrophage activation has been restricted to microbicidal and tumoricidal responses, and it was only 18 years ago that an alternative activation (in response to IL-4) was recognized [1]. Since then macrophage activation has been considered to be a spectrum of polarization states in which M1/classically activated and M2/alternatively activated macrophages are considered extremes [2]. Although based on the same paradigm, different divisions with

different nomenclatures have been used: classically activated, alternatively activated and innate/acquired deactivated [3] M1, M2a, M2b and M2c [4], and classically activated, wound-healing and regulatory macrophages [5]. Although based on the same principle it is difficult to directly compare these different divisions and here we will use the term M1 and M2 macrophages. M1 macrophages are involved in inflammatory reactions and promote Th1-type immune responses, while M2 macrophages are suggested to be a continuum of functionally and phenotypically related cells, involved in homeostasis, parasite killing, tumor promotion, tissue remodeling and in allergic reactions [4,6]. The placenta contains numerous macrophages, both in the decidua and the chorion villous stroma, the latter are of fetal origin and also called Hofbauer cells. Decidual macrophages have been shown to be more of an M2 phenotype both at the level of gene and protein expression [7–11]. Also Hofbauer cells have been suggested to have an M2 phenotype

Abbreviations: qPCR, quantitative real-time PCR; Non-CA, placentas without histological chorioamnionitis; CA, placentas with histological chorioamnionitis.

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Table 1
Demographic data on the study population.

	Non-sens ^a (n = 10)	Sens ^a (n = 7)	Non-CA ^b (n = 5)	CA ^c (n = 5)
Sensitized mother (n) ^d	0	7	0	1
Acute chorioamnionitis grade 2/3 (n)	0	0	0	3/2
Age mothers in years, median (range)	32 (23–36)	31 (24–34)	34 (23–38)	32 (26–44)
Sex boy/girl	4/6	5/2	2/3	0/5
Mother smoking during pregnancy (n)	0	1	0	2
Vaginal delivery/caesarean section	6/4	6/1	5/0	4/1
Gestational age placenta in weeks, median (range)	39 (37–40)	39 (38–41)	39 (37–40)	38 (37–40)

^a Sens = sensitization.^b Non-CA = placentas without histological chorioamnionitis.^c CA = placentas with histological chorioamnionitis.^d Allergen-sensitization was defined as having a positive Phadiatop™ (Phadia AB).

since they express stabilin-1, DC-SIGN/CD209, and CD163 [12,13], however in these studies M1 markers have not been tested.

The intrauterine environment plays an important role for the risk of allergy development for the child [14], and maternal atopy has been reported to confer a higher risk for development of asthma and eczema in children than paternal atopy [15–18]. Interestingly, chorioamnionitis in combination with premature birth increases the risk for recurrent wheezing [19]. Chorioamnionitis is an acute inflammatory response of the mother and fetus, with an accumulation of polymorphonuclear leucocytes in placental tissues (notably fetal membranes). It is generally considered as a marker for intrauterine infection, most commonly bacterial, which can be clinical or subclinical [20]. Chorioamnionitis occurs in about 10–15% of all term pregnancies and are strongly associated with preterm labor, premature rupture of the membranes and significant perinatal morbidity and death [21].

In this study, we aimed to determine the polarization status of Hofbauer cells in health and disease. We hypothesized that Hofbauer cells have an M2 phenotype in healthy term placenta and that this phenotype can be influenced by the allergen-sensitization status of the mother and/or the presence of placental inflammation (chorioamnionitis). This Hofbauer cell polarization might in turn influence the intrauterine environment and the risk of allergy development for the child. Therefore we determined the polarization of Hofbauer cells in placentas of sensitized versus non-sensitized mothers, and in placentas with or without histological chorioamnionitis by means of immunohistochemical analysis and quantitative real-time PCR (qPCR). CD68 was used as a general Hofbauer cell marker while CX3CR1, IL-7R and CCR7 were used as M1 markers and DC-SIGN, CD163, and mannose receptor/CD206 as M2 markers [2,4,22,23].

2. Materials and methods

2.1. Subjects and placenta samples

Pregnant women (n = 330) were recruited within the ALADDIN (Assessment of Lifestyle and Allergic Disease During Infancy) study, from anthroposophic and conventional maternal-child health centers in the Stockholm area, Sweden. A questionnaire filled out by the parents two months after the birth of the child, was

used to determine the lifestyle of the families as described before [24]. Placenta biopsies (n = 282) from both maternal and fetal side of the placenta were taken by midwives, treated with RNA later (Ambion, Applied Biosystems, Foster City, CA, USA), and stored as described before [24]. Remaining placentas were transported on ice to the laboratory and 2 placenta biopsies, about 0.5 cm thick and spanning the whole thickness of the placenta, a piece of the membranes, and of the umbilical cord were washed with PBS, fixed in formalin and paraffin-embedded. This study was approved by the local Ethical Committee and all parents gave their written informed consent.

2.2. Blood samples and IgE analysis

Parental blood samples were collected and plasma was used to determine allergen-sensitization by means of the Phadiatop™ test (Phadia AB, Uppsala, Sweden) as described before [24].

2.3. Chorioamnionitis

All placenta samples were submitted to routine histopathological examination for clinical purposes. The presence of chorioamnionitis was evaluated on hematoxylin and eosin-stained formalin-fixed sections using a three-grade scale, as follows: grade 1: presence of polymorphonuclear leucocytes at the subchorionic plate and/or lower third of chorion; grade 2: at least two separate foci of leucocyte infiltration on chorion and amnion; and grade 3: extensive leucocyte infiltration in chorion and amnion, together with necrosis of amniotic epithelium and/or micro-abscess formation [25].

2.4. Sample selection

For the analysis of the influence of maternal allergen-sensitization on the polarization of Hofbauer cells placentas were selected from families with a conventional lifestyle, of which the father was non-sensitized and the placentas had no signs of chorioamnionitis (n = 26). From these 26 families, 19 had a non-sensitized mother and 7 had a sensitized mother. Ten placentas were randomly selected from the 19 non-sensitized mothers while all 7 placentas from the sensitized mothers were analyzed (Table 1).

For the analysis of the influence of chorioamnionitis on the polarization of Hofbauer cells, placentas were randomly selected from all 282 placenta samples. Five samples without signs of chorioamnionitis and five samples with grade 2 or 3 of chorioamnionitis, with five and four non-sensitized mothers respectively, were selected (Table 1).

2.5. Immunostaining

Six µm sections were cut from formalin-fixed paraffin-embedded placenta biopsies, slides were deparaffinized and antigen retrieval was performed in a pressure cooker with Antigen Decloaker (Histolab Products AB, Sweden). For

Table 2
Antibodies used.

Antibody	Clone/isotype	Final conc. (µg/ml)	Source
Anti-CD68	PG-M1/Mouse IgG ₃ κ	0.75	Abcam
Anti-CX3CR1	2A9-1/Rat IgG _{2b} κ	4.0	MBL Int. Co.
Anti-IL-7Rα (CD127)	Polyclonal/Goat IgG	2.0	SIGMA
Anti-CCR7	Y59/Rabbit IgG	0.25	Abcam
Anti-DC-SIGN (CD209)	DCN46/Mouse IgG _{2b} κ	0.31	BD Pharmingen
Anti-CD163	10D6/Mouse IgG ₁	0.7	Leica Biosystems Novocastra
Anti-mannose receptor (CD206)	15-2/Mouse IgG ₁	2.0	Abcam

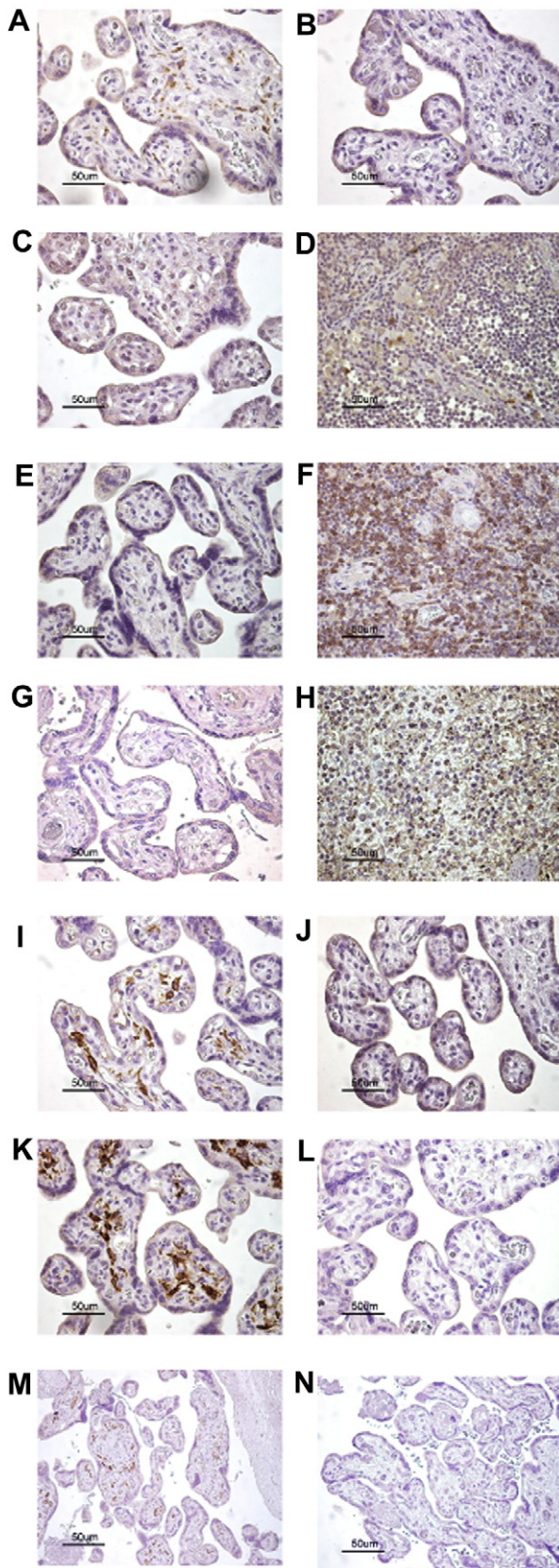


Fig. 1. Immunostaining for macrophage markers in placenta and thymus. Formalin-fixed paraffin-embedded placenta (A–C, E, G, I–N), thymus (D, F) and spleen (H) sections were stained with the ABC ELITE method and DAB. (A) CD68, (B) Mouse IgG₃K, (C–D) CX3CR1, (E–F) IL-7R, (G–H) CCR7, (I) DC-SIGN, (J) Mouse IgG_{2b}K, (K) CD163, (L, N) Mouse IgG₁, (M) CD206. In case of positive placental staining the negative isotype

immunohistochemical staining primary and biotinylated secondary antibodies (Table 2) were diluted in REAL™ Antibody Diluent (Dako, Glostrup, Denmark). The ABC-ELITE method and diaminobenzidine substrate (DAB, Vector Laboratories) were used to visualize bound antibodies. Slides were incubated with normal serum (species depending on the secondary antibody) and 3% H₂O₂ in methanol prior to antibody incubations to reduce background staining. The appropriate isotype controls were included for all immunohistochemical analysis and revealed to be negative. Thymus or spleen sections were used as positive control, hereto thymus and spleen biopsies were obtained from the archives of Division of Pathology at Karolinska University Hospital, Huddinge, Stockholm, Sweden.

2.6. Quantitative real-time PCR (qPCR)

Total RNA was isolated from RNA later preserved placenta biopsies from the fetal side and first-strand cDNA was generated as described before [24]. All qPCR reactions were performed in duplicate in a volume of 25 μl: 12.5 μl Rotor-Gene SYBR Green PCR Kit (Qiagen, Hilden, Germany), 5 μl cDNA, 2.5 μl primermix (1× QuantiTect Primer Assay, Qiagen, diluted in TE buffer), and 5 μl nuclease-free water. The reaction was performed in a Rotor-Gene Q (Qiagen). The following PCR program was used: 95 °C for 5 min, followed by 40 cycles of 5 s at 95 °C, and 10 s at 60 °C. Nuclease-free water and controls without reverse transcriptase in the cDNA reaction were used as negative controls. QuantiTect Primer Assays (for CD68, CX3CR1, IL-7R, DC-SIGN, CD163, EEF1A1, GAPDH, and YWHAZ) were confirmed to have a specific melting curve and temperature, and only one band of the expected size on a 1.5% agarose gel. To calculate primer pair specific threshold and efficiency, a standard curve was run on a purified PCR product. Detailed information about RNA, primers and PCR products are available upon request.

2.7. Counting and statistical analysis

The number of positive cells, defined as clear brown staining surrounding a blue nucleus located in the chorionic villi, was evaluated under coded conditions by three independent investigators (MJ, ER, and BvR). To estimate the consistency between the different investigators all three counted the same 9 photographs (3 of CD68, 3 of DC-SIGN and 3 of CD163). A Spearman's rank correlation test indicated highly significant correlations between the results of the different investigators. To estimate the influence of maternal allergen-sensitization or the presence of histological chorioamnionitis on the polarization of Hofbauer cells six representative photographs of every staining (CD68, DC-SIGN, and CD163) were taken of all 27 placentas. Every investigator counted two of the six pictures, and the mean number of positive cells from all six pictures was used for further analysis. A two-tailed Student's *t*-test was used to compare the mean number of positive cells between non-sensitized and sensitized mothers. A Mann–Whitney *U* test was used to compare the mean number of positive cells between placentas without histological chorioamnionitis and with histological chorioamnionitis.

The relative mRNA expression was calculated for the different samples using the mean Ct value of the duplicate measurements and the Pfaffl method [26] using the geometric mean expression of three reference genes (EEF1A1, GAPDH, and YWHAZ) as the reference [27]. A Log2 transformation was performed to normalize the data and a two-tailed Student's *t*-test to compare the mean relative expression between two groups. Statistical analysis was conducted using PASW Statistics 18 (SPSS Inc., Chicago IL, USA). *P* ≤ 0.05 was accepted as being significant.

3. Results

3.1. Hofbauer cells express M2 but not M1 markers

Immunohistochemical staining clearly demonstrated the presence of CD68 (Fig. 1A) and the M2 markers DC-SIGN, CD163 and CD206 in term placenta (Fig. 1I, K and M respectively). Protein expression of the M1 markers CX3CR1, IL-7R and CCR7 was, however, not detected in term placenta (Fig. 1C, E and G respectively) despite their detection in thymus or spleen (Fig. 1D, F and H). These data suggest that Hofbauer cells have an M2 phenotype. qPCR was performed to determine the mRNA expression of CD68, CX3CR1, IL-7R, DC-SIGN, and CD163 in placental biopsies. Transcripts of all markers could be readily detected in these mixed cell biopsies; with Ct values (at a threshold of 0.05) around 26 for CX3CR1, IL-7R, and DC-SIGN and around 22 for CD68 and CD163.

control is presented and in case of negative placental staining the positive control, thymus or spleen, is presented. Sections were counterstained with hematoxylin (blue), positive staining is brown. Original magnification 40×. Scale bars: 50 μm.

These data suggest that the mRNA expression levels of CX3CR1, IL-7R, and DC-SIGN are about 16 times lower than those of CD68 and CD163.

3.2. Expression of CD68, DC-SIGN and CD163 is independent of maternal allergen-sensitization status

The number of CD68⁺, DC-SIGN⁺ and CD163⁺ cells within the chorionic villi was determined in a term placenta from a non-sensitized mother following an uncomplicated pregnancy, by three independent investigators by counting three photographs of 0.66 mm² each. The correlation between the results of the different investigators, as calculated by a Spearman's rank correlation test, was highly significant (MJ-ER $R = 0.77$ $P = 0.015$; MJ-BvR $R = 0.92$ $P = 0.0007$; ER-BvR $R = 0.75$ $P = 0.021$) (data not shown) and the counting results in subsequent analysis were averaged between the different investigators. To estimate the influence of maternal allergen-sensitization on the polarization of Hofbauer cells, 17 placentas (10 from non-sensitized and 7 from sensitized mothers) were immunohistochemically stained for CD68, DC-SIGN and CD163. Six representative photographs were taken of every staining and counted by the three investigators (2 photographs each). The mean number of positive cells from all six photographs was used for statistical analysis using a two-tailed Student's *t*-test. The mean number of CD68⁺, DC-SIGN⁺ and CD163⁺ cells in placenta was not significantly different between the placentas of non-sensitized and sensitized mothers (Fig. 2A–C) ($P = 0.21$, $P = 0.77$, and $P = 0.47$, respectively). Since all 5 macrophage markers could be detected at the level of mRNA expression we determined their levels and the levels of the three reference genes in the same 17 placentas. The relative mRNA expression levels were Log2 transformed to normalize the data and a two-tailed Student's *t*-test was used to compare the mean relative expression levels between the two groups. This revealed that the mRNA expression levels of CD68, CX3CR1, IL-7R, DC-SIGN, and CD163 were not significantly different between the placentas of non-sensitized and sensitized mothers (Fig. 3A) ($P = 0.33$, $P = 0.83$, $P = 0.79$, $P = 0.61$, and $P = 0.89$, respectively). Together these data suggest that maternal allergen-

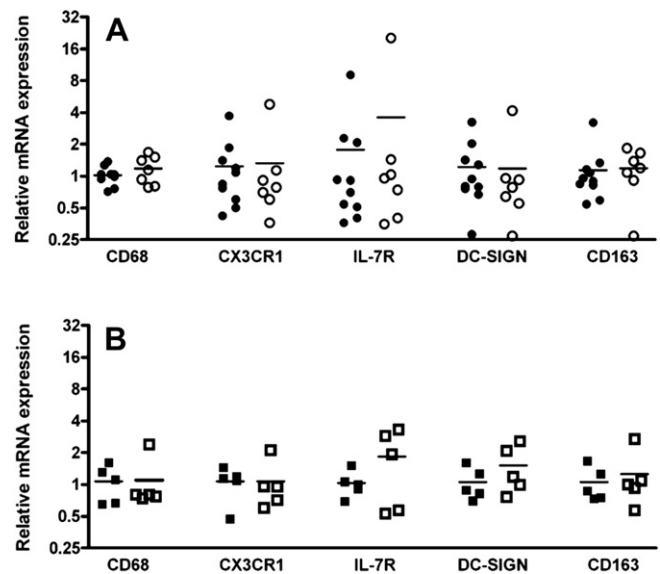


Fig. 3. mRNA expression at the fetal side of the placenta of (A) non-sensitized (●) and sensitized (○) mothers and (B) of placentas without (■) and with (□) histological chorioamnionitis. mRNA levels were determined by qPCR and relative gene expression was calculated (see Materials and methods). Dots (●○) and squares (■□) indicate the relative mRNA expression of individual placentas before transformation and horizontal lines indicate the mean. Note the Log2 scale. Data were Log2 transformed to normalize the data and a Student's *t*-test was used to evaluate differences between groups. No significant differences were identified.

sensitization neither influences the number of CD68⁺ (Hofbauer) cells nor the polarization status of these Hofbauer cells in term placenta.

3.3. Expression of CD68, CX3CR1, IL-7R, DC-SIGN and CD163 is independent of the presence of chorioamnionitis

To identify if Hofbauer cell polarization is associated with placental inflammation we selected 5 placentas from pregnancies

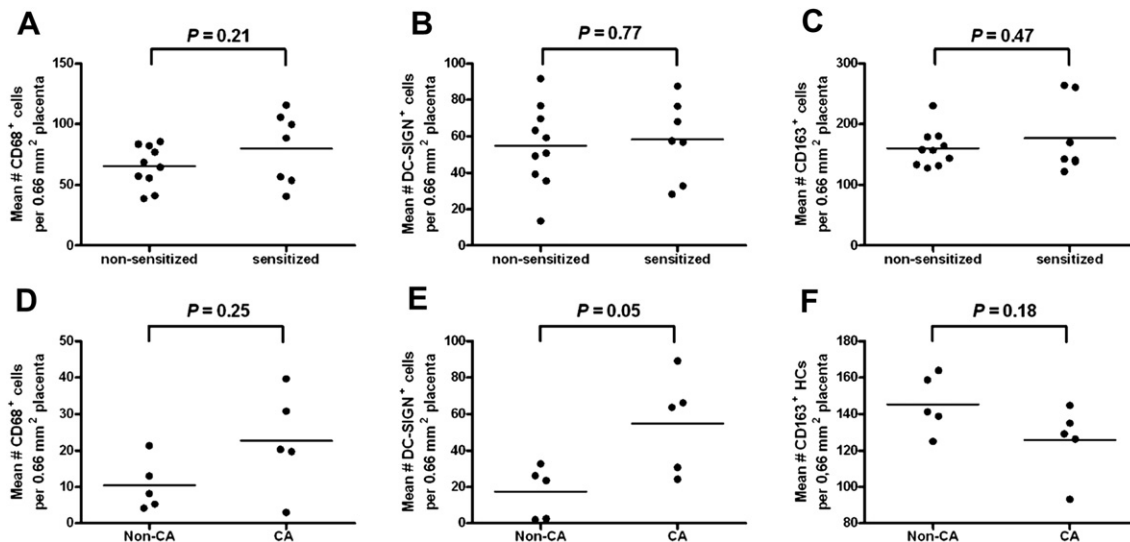


Fig. 2. Number of CD68⁺, DC-SIGN⁺ and CD163⁺ cells in term placenta. Formalin-fixed paraffin-embedded placenta sections from non-sensitized ($n = 10$) and sensitized ($n = 7$) mothers (A–C) and from placentas without ($n = 5$) and with ($n = 5$) histological chorioamnionitis (D–F) were stained with the ABC ELITE method and DAB for the expression of CD68, DC-SIGN and CD163. Dots (●) indicate the average number of positive cells counted by three independent investigators (see M&M) and horizontal lines indicate the mean. A two-tailed Student's *t*-test was used to compare the mean number of positive cells for (A) CD68, (B) DC-SIGN, and (C) CD163, between non-sensitized and sensitized mothers. A Mann–Whitney *U* test was used to compare the mean number of positive cells for (D) CD68, (E) DC-SIGN, and (F) CD163, between placentas without (Non-CA) and with (CA) histological chorioamnionitis. A significant difference was identified for the number of DC-SIGN⁺ cells ($P = 0.05$), however, when correcting for the number of CD68⁺ cells this significant difference was lost ($P = 0.12$).

without histological chorioamnionitis and 5 with histological chorioamnionitis and performed immunohistochemical staining for CD68, CX3CR1, IL-7R, DC-SIGN, and CD163. No clear positive staining was detected for the M1 markers CX3CR1 and IL-7R. Staining with CD68 and the M2 markers DC-SIGN and CD163 were, however, positive and the counting strategy as described above was used to determine the number of positive cells. A Mann–Whitney *U* test indicated that the mean number of DC-SIGN⁺ cells were significantly different between placentas without histological chorioamnionitis (Non-CA) and placentas with histological chorioamnionitis (CA) (Fig. 2E, $P = 0.05$). However, when correcting for the number of CD68⁺ cells the significant difference for DC-SIGN was lost ($P = 0.12$), suggesting that the number of DC-SIGN⁺ cells might be different but that the polarization per CD68⁺ cell is not different. To verify that chorioamnionitis does not influence the Hofbauer cell polarization the mRNA expression levels of 5 macrophage markers and three reference genes were determined by qPCR in the same 10 placentas. The relative mRNA expression levels were Log2 transformed to normalize the data and a two-tailed Student's *t*-test was used to compare the mean relative expression levels between the two groups. This revealed that the mRNA expression levels of CD68, CX3CR1, IL-7R, DC-SIGN, and CD163 were not significantly different between placentas with and without histological chorioamnionitis (Fig. 3B) ($P = 0.92$, $P = 0.89$, $P = 0.43$, $P = 0.29$, and $P = 0.77$, respectively). Together these data suggest that chorioamnionitis, a placental inflammation, does not influence the polarization status of the Hofbauer cells in term placenta.

4. Discussion

Here we report that placental macrophages of fetal origin, often referred to as Hofbauer cells, have an M2 phenotype and that this polarization status is not influenced by maternal allergen-sensitization or the presence of chorioamnionitis.

In term placenta we could readily detect the protein expression of the M2 markers DC-SIGN, CD163 and CD206, confirming previously published data for DC-SIGN and CD163 [12,13]. In contrast, we could not detect the M1 markers CX3CR1, IL-7R and CCR7 (Fig. 1). qPCR analysis indicated that the expression levels of CX3CR1, IL-7R, and DC-SIGN were about 16 times lower than those of CD68 and CD163. Although the lower mRNA expression levels of CX3CR1 and IL-7R are in agreement with the protein data, the low expression of DC-SIGN is somewhat surprising and could indicate that this protein has a low turnover rate. Together these data indicate that Hofbauer cells in term placenta have a true M2 phenotype, which is in agreement with the notion that established pregnancy is considered a Th2-skewed phenomenon [28] even though this paradigm might be an oversimplification [29].

M2 macrophages are suggested to be involved in allergic reactions. IL-10 producing monocytes, which preferentially differentiate into alternatively activated macrophages, have been shown to be increased in the peripheral blood of atopic patients [23]. In addition, monocyte derived dendritic cells from allergic asthmatics preferentially prime naïve T cells toward Th2 cell development [30]. Therefore we hypothesized that Hofbauer cells would polarize toward an M2 phenotype when the mother is sensitized. The number of DC-SIGN⁺ and CD163⁺ cells in placentas was however not significantly different between non-sensitized and sensitized mothers (Fig. 2A–C). Furthermore, no gene expression differences for any of the analyzed genes (CD68, CX3CR1, IL-7R, DC-SIGN and CD163) were detected (Fig. 3A). The biopsies used for the mRNA expression analysis contain a mixture of tissues and cells: chorionic villi (including, e.g. Hofbauer cells, trophoblasts, endothelial cells and other stromal cells), the chorionic and amniotic membranes, and small amounts of residual maternal blood. In order to estimate

the expression relative to the number of Hofbauer cells only, we also calculated the mRNA expression relative to CD68 mRNA expression. This did not reveal any significant differences either (data not shown), confirming that Hofbauer cell polarization is independent of maternal allergen-sensitization. The number of CD68⁺ and DC-SIGN⁺ cells was comparable suggesting that these two markers are expressed on the same cells as reported previously [13,31]. The number of CD163⁺ cells was however about 2 times higher than the number of CD68⁺ cells indicating that CD163⁺ is expressed on other cells as well. DC-SIGN⁺CD163⁺ cells have been reported [13], however a part of the CD163⁺ cells might represent an intermediate phenotype between myofibroblast and macrophage since they co-express procollagen and desmin [32]. Interestingly the CD163⁺ cells could also be a subpopulation of hematopoietic stem/progenitor cells as suggested by Matthews et al. [33]. Double immunofluorescence labeling for CD34 and CD163 should be performed to determine if these types of progenitor cells are present in human term placenta.

M1 macrophages are suggested to be involved in inflammatory reactions. G6PD activity and phagocytosis were shown to be increased in Hofbauer cells from second trimester placentas complicated by chorioamnionitis [34]. Therefore we hypothesized that Hofbauer cells would polarize toward an M1 phenotype in the presence of chorioamnionitis. Protein expression of the M1 markers CX3CR1 and IL-7R could however not be detected in placentas with histological chorioamnionitis. CD68, DC-SIGN and CD163 could be detected in all placentas; however, the number of positive cells was only significantly different for DC-SIGN (Fig. 2D–F), this difference was however lost when correcting for the number of CD68⁺ cells indicating that the number of DC-SIGN⁺ cells might be different but that the polarization per CD68⁺ cell is not influenced by the presence of histological chorioamnionitis. Recently we have demonstrated that the number of CD68⁺ cells is significantly lower in placentas with histological chorioamnionitis as compared to gestational age matched controls [35]. Here, we could not confirm this earlier finding, possibly because of the difference in quantification method and the low number of placentas used in this study. No mRNA expression differences for any of the analyzed genes (CD68, CX3CR1, IL-7R, DC-SIGN and CD163) were detected (Fig. 3B), confirming the protein data. mRNA expression levels were calculated relative to the CD68 mRNA expression as well, however, this did not reveal any significant differences either (data not shown). Together these data indicate that Hofbauer cell polarization is independent of the presence of chorioamnionitis.

The role of the apparent M2 polarization of Hofbauer cells described here is unclear. In fact, the precise function of Hofbauer cells in general is still incompletely understood [36]. It is feasible to speculate that, in parallel to decidual macrophages, which show a similar M2 polarization [11], Hofbauer cells may actively participate in fetal immunological mechanisms, such as tolerance and fetal protection. Therefore it might be that Hofbauer cell polarization is not at all influenced by maternal or environmental factors. An immunological role for Hofbauer cells is indirectly suggested by their abundant numbers (especially during early and mid-pregnancy), which diminish toward term [35] when the fetal immune system is further developed. Whether alterations in number, polarization, and function of Hofbauer cells can be implicated in pregnancy disorders such as preeclampsia or early pregnancy loss remains to be clarified in further studies, preferably using isolated Hofbauer cells and cell cultures.

Contributors

M. Joerink conceptualized the project, interpreted the results and wrote the manuscript. E. Rindsjö and B. van Riel performed the

IHC and edited the manuscript. J. Alm is the principal investigator within ALADDIN and edited the manuscript. N. Papadogiannakis conceptualized the project and edited the manuscript.

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Conflict of interest

The authors declare no financial or commercial conflict of interest.

References

- [1] Stein M, Keshav S, Harris N, Gordon S. Interleukin 4 p.tently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *J Exp Med* 1992;176:287–92.
- [2] Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol* 2002;23:549–55.
- [3] Gordon S. Alternative activation of macrophages. *Nat Rev Immunol* 2003;3:23–35.
- [4] Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* 2004;25:677–86.
- [5] Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* 2008;8:958–69.
- [6] Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. *Front Biosci* 2008;13:453–61.
- [7] Gustafsson C, Mjösberg J, Matussek A, Geffers R, Matthiesen L, Berg G, et al. Gene expression profiling of human decidual macrophages: evidence for immunosuppressive phenotype. *PLoS ONE* 2008;3:e2078.
- [8] Cupurdija K, Azzola D, Hainz U, Gratchev A, Heitger A, Takikawa O, et al. Macrophages of human first trimester decidua express markers associated to alternative activation. *Am J Reprod Immunol* 2004;51:117–22.
- [9] Heikkinen J, Möttönen M, Komi J, Alanen A, Lassila O. Phenotypic characterization of human decidual macrophages. *Clin Exp Immunol* 2003;131:498–505.
- [10] Lidström C, Matthiesen L, Berg G, Sharma S, Ernerudh J, Ekerfelt C. Cytokine secretion patterns of NK cells and macrophages in early human pregnancy decidua and blood: implications for suppressor macrophages in decidua. *Am J Reprod Immunol* 2003;50:444–52.
- [11] Nagamatsu T, Schust DJ. The contribution of macrophages to normal and pathological pregnancies. *Am J Reprod Immunol* 2010;63:460–71.
- [12] Kzyshkowska J, Gratchev A, Schmutzmaier C, Brundiers H, Krusell L, Mamidi S, et al. Alternatively activated macrophages regulate extracellular levels of the hormone placental lactogen via receptor-mediated uptake and transcytosis. *J Immunol* 2008;180:3028–37.
- [13] Böckle BC, Sölder E, Kind S, Romani N, Sepp NT. DC-sign+ CD163+ macrophages expressing hyaluronan receptor LYVE-1 are located within chorion villi of the placenta. *Placenta* 2008;29:187–92.
- [14] Kumar R. Prenatal factors and the development of asthma. *Curr Opin Pediatr* 2008;20:682–7.
- [15] Bergmann RL, Edenharter G, Bergmann KE, Guggenmoos-Holzmann I, Forster J, Bauer CP, et al. Predictability of early atopy by cord blood-IgE and parental history. *Clin Exp Allergy* 1997;27:752–60.
- [16] Litonjua AA, Carey VJ, Burge HA, Weiss ST, Gold DR. Parental history and the risk for childhood asthma. Does mother confer more risk than father? *Am J Respir Crit Care Med* 1998;158:176–81.
- [17] Liu CA, Wang CL, Chuang H, Ou CY, Hsu TY, Yang KD. Prenatal prediction of infant atopy by maternal but not paternal total IgE levels. *J Allergy Clin Immunol* 2003;112:899–904.
- [18] Moore MM, Rifas-Shiman SL, Rich-Edwards JW, Kleinman KP, Camargo Jr CA, Gold DR, et al. Perinatal predictors of atopic dermatitis occurring in the first six months of life. *Pediatrics* 2004;113:468–74.
- [19] Kumar R, Yu Y, Story RE, Pongracic JA, Gupta R, Pearson C, et al. Prematurity, chorioamnionitis, and the development of recurrent wheezing: a prospective birth cohort study. *J Allergy Clin Immunol* 2008;121:878–84. e6.
- [20] Redline RW. Placental inflammation. *Semin Neonatol* 2004;9:265–74.
- [21] Redline RW. Inflammatory responses in the placenta and umbilical cord. *Semin Fetal Neonatal Med* 2006;11:296–301.
- [22] Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *J Immunol* 2006;177:7303–11.
- [23] Prasse A, Germann M, Pechkovsky DV, Markert A, Verres T, Stahl M, et al. IL-10-producing monocytes differentiate to alternatively activated macrophages and are increased in atopic patients. *J Allergy Clin Immunol* 2007;119:464–71.
- [24] Joerink M, Oortveld MA, Stenius F, Rindsjö E, Alm J, Scheynius A. Lifestyle and parental allergen sensitization are reflected in the intrauterine environment at gene expression level. *Allergy* 2010;65:1282–9.
- [25] Rindsjö E, Hulthen Varli I, Ofori MF, Lundquist M, Holmlund U, Papadogiannakis N, et al. Presence of IgE cells in human placenta is independent of malaria infection or chorioamnionitis. *Clin Exp Immunol* 2006;144:204–11.
- [26] Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2001;29:e45.
- [27] Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol* 2002;3:RESEARCH0034.
- [28] Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal–fetal relationship: is successful pregnancy a Th2 phenomenon? *Immunol Today* 1993;14:353–6.
- [29] Chaouat G. The Th1/Th2 paradigm: still important in pregnancy? *Semin Immunopathol* 2007;29:95–113.
- [30] Chen XQ, Yang J, Hu SP, Nie HX, Mao GY, Chen HB. Increased expression of CD86 and reduced production of IL-12 and IL-10 by monocyte-derived dendritic cells from allergic asthmatics and their effects on Th1- and Th2-type cytokine balance. *Respiration* 2006;73:34–40.
- [31] Soilleux EJ, Morris LS, Lee B, Pöhlmann S, Trowsdale J, Doms RW, et al. Placental expression of DC-SIGN may mediate intrauterine vertical transmission of HIV. *J Pathol* 2001;195:586–92.
- [32] Kim SS, Romero R, Kim JS, Abbas A, Espinoza J, Kusanovic JP, et al. Coexpression of myofibroblast and macrophage markers: novel evidence for an in vivo plasticity of chorioamniotic mesodermal cells of the human placenta. *Lab Invest* 2008;88:365–74.
- [33] Matthews KE, Mueller SG, Woods C, Bell DN. Expression of the hemoglobin–haptoglobin receptor CD163 on hematopoietic progenitors. *Stem Cells Dev* 2006;15:40–8.
- [34] Matsubara S, Takayama T, Yamada T, Usui R, Izumi A, Watanabe T, et al. Hofbauer cell activation and its increased glucose-6-phosphate dehydrogenase activity in second trimester-spontaneous abortion: an ultrastructural dual staining enzyme-cytochemical study. *Am J Reprod Immunol* 2003;49:202–9.
- [35] Vinnars MT, Rindsjö E, Ghazi S, Sundberg A, Papadogiannakis N. The number of CD68+ (Hofbauer) cells is decreased in placentas with chorioamnionitis and with advancing gestational age. *Pediatr Dev Pathol* 2010;13:300–4.
- [36] Ingman K, Cookson VJ, Jones CJ, Aplin JD. Characterisation of Hofbauer cells in first and second trimester placenta: incidence, phenotype, survival in vitro and motility. *Placenta* 2010;31:535–44.