Accepted Manuscript

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PII: S0143-4004(16)30511-2

DOI: 10.1016/j.placenta.2016.09.004

Reference: YPLAC 3466

To appear in: *Placenta*

Received Date: 26 October 2015

Revised Date: 2 August 2016

Accepted Date: 7 September 2016

Please cite this article as: Buckley RJ, Whitley GS, Dumitriu IE, Cartwright JE, Macrophage polarisation affects their regulation of trophoblast behaviour, *Placenta* (2016), doi: 10.1016/j.placenta.2016.09.004.

Macrophage polarisation affects their regulation of trophoblast behaviour

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Abstract

Introduction: During the first trimester of human pregnancy, fetally-derived extravillous trophoblast (EVT) cells invade into uterine decidua and remodel the uterine spiral arteries to ensure that sufficient blood reaches the maternal-fetal interface. Decidual macrophages have been implicated in the regulation of decidual remodelling and aberrant activation of these immune cells is associated with pre-eclampsia.

Methods: The monocytic cell line THP-1 was activated to induce an M1 or M2 phenotype and the conditioned media was used to treat the EVT cell line SGHPL-4 in order to determine the effect of macrophage polarisation on trophoblast behaviour *in-vitro*. SGHPL-4 cell functions were assessed using time-lapse microscopy, endothelial-like tube formation assays and western blot.

Results: The polarisation state of the THP-1 cells was found to differentially alter the behaviour of trophoblast cells *in-vitro* with pro-inflammatory M1 conditioned media significantly inhibiting trophoblast motility, impeding trophoblast tube formation, and inducing trophoblast expression of caspase 3, when compared to anti-inflammatory M2 conditioned media.

Discussion: Macrophages can regulate trophoblast functions that are critical during decidual remodelling in early pregnancy. Importantly, there is differential regulation of trophoblast function in response to the polarisation state of these cells. Our studies indicate that the balance between a pro- and anti-inflammatory environment is important in regulating the cellular interactions at the maternal-fetal interface and that disturbances in this balance likely contribute to pregnancy disorders associated with poor trophoblast invasion and vessel remodelling.

Keywords: decidua; macrophage; extravillous trophoblast; polarisation; THP-1

INTRODUCTION

Human pregnancy represents a unique immunological paradigm; requiring tolerance of the semi-allogeneic fetus, regulation of placentation, and maintenance of host-defence against pathogens. During the first trimester of pregnancy, the uterine decidua changes significantly as fetally-derived extravillous trophoblast (EVT) cells invade and remodel the uterine spiral arteries, ensuring a sufficient blood supply to facilitate the transfer of nutrients across the maternal-fetal interface (1). Pre-eclampsia is a complication of pregnancy typically characterised by gestational hypertension and proteinuria, and clinically diagnosed after the 20th week of gestation (2). Pre-eclampsia is estimated to affect 2-8% of pregnant women worldwide and is a leading cause of maternal and fetal morbidity and mortality (3). Although the pathophysiology of pre-eclampsia is yet to be fully elucidated, inadequate spiral artery remodelling and shallow trophoblast invasion during the first trimester are associated with the condition (4-6).

Macrophages are large mononuclear phagocytic cells that predominantly function to clear extraneous cellular material from the interstitial environment but also have a central role in innate and adaptive immunity (7). Given the array of macrophage functions, considerable macrophage diversity and plasticity exists (8). The extremes of activation state are represented by classically activated (CA) macrophages which act as effector cells in immune responses, and alternatively activated (AA) macrophages which are involved in immunosuppression and wound healing/tissue repair. However, specific differentiation depends on the local tissue environment, with evidence that macrophages can switch between activation states when exposed to pro- or anti-inflammatory cytokines (9).

During the first-trimester of pregnancy, approximately 40% of all decidual cells are leukocytes, of which 70% are decidual natural killer cells and 20-30% are decidual macrophages (10, 11). Histological studies have shown that the population of decidual macrophages remains relatively stable throughout pregnancy as opposed to the population of decidual natural killer cells, which declines as pregnancy progresses (11). Furthermore, decidual macrophages are found in abundance at the site of implantation, clustered around spiral arteries and in close proximity to invading EVT (12-14), suggestive of an important role at the maternal-fetal interface.

Decidual macrophages have not been extensively characterised though microarray studies have shown that they have a unique phenotype with expression of genes associated with both classical and alternative activation. When compared to peripheral blood monocytes, the majority of upregulated genes are found to be implicated in immune modulation and tissue remodelling, reflecting the phenotype of an AA macrophages (15). DNA methylation profiling of decidual macrophages has demonstrated hypermethylation of genes encoding classical markers of macrophage activation and hypermethylation of genes encoding alternative activation (16).

However, decidual macrophages also express some genes associated with immune activation, and secrete pro-inflammatory cytokines such as TNF-alpha, in addition to potent anti-inflammatory cytokines such as IL-10 (17, 18). Recent studies have suggested that there may be sub-sets of decidual macrophages characterised by CD11c expression, with a high CD11c expression associated

with lipid metabolism and inflammation, and low CD11c expression associated with extracellular matrix formation, muscle homeostasis, and tissue development (19). ICAM-3 expression has also been correlated with the CD11c expressing sub-populations (20). The expression of genes associated with alternative activation in addition to some genes associated with immune activation likely reflects the need for a tolerogeneic environment to support successful pregnancy while maintaining the potential for an effective inflammatory response against pathogens.

Aberrant decidual macrophage activation towards a more CA phenotype has previously been associated with the pathology of pre-eclampsia. Term decidua from pre-eclamptic pregnancies has significantly more pro-inflammatory CD86+ macrophages when compared with normal pregnancies (21). In addition, a study of first trimester decidual tissue from chorionic villus sampling found a lower ratio of regulatory CD206/CD86+ macrophages in the decidua of women who subsequently developed pre-eclampsia compared to those with a normal pregnancy outcome. Moreover, there is an increase in decidual macrophage mRNA expression of the pro-inflammatory cytokine IL-6 prior to clinical signs of pre-eclampsia (22), and excess TNF-α production has been postulated to inhibit normal EVT invasion in pre-eclampsia (23).

The aim of our study was to model the effects of differential macrophage polarisation on trophoblast behaviour. The human acute monocytic leukemia cell line (THP-1) can be polarised to generate macrophage phenotypes at the extreme ends of the polarisation spectrum and were used to generate CA and AA macrophage-like cells. The effect of factors secreted by these cells on a trophoblast cell line was analysed with respect to the motility, proliferation, apoptosis, and formation of network structures.

MATERIALS AND METHODS

Macrophage Differentiation

THP-1 cells were differentiated into macrophage-like cells by adapting a previously described method (24). Briefly, cells were treated with 100nM of phorbol 12-myristate 13-acetate (Sigma-Aldrich, Dorset, UK) in phenol red free Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen, Paisley, UK) supplemented with 10% (v/v) fetal bovine serum (FBS), containing 2mmol/L L-glutamine, 100 IU/mL penicillin, 100mg/mL streptomycin and 2.5µg/mL amphotericin B (THP-1 medium). After 6h 100ng/mL of lipopolysaccharide (LPS) and 20ng/mL of IFN-γ or 20ng/mL of IL-4 (PeproTech, Rocky Hill, NJ) and 20ng/mL of IL-13 (PeproTech) was added to generate an CA or AA phenotype, respectively. The cells were cultured in the polarising media for 3 days and washed thoroughly three times with PBS. The cells were then treated with fresh THP-1 medium containing 10% (v/v) FBS or no serum. The conditioned media (CM) was collected after 24h, centrifuged to remove cellular debris, and stored at -80°C until used.

Characterisation of Macrophage Polarisation

To assess polarisation, the pro-inflammatory cytokines TNF-α and IL-6 (CA markers), or the anti-inflammatory cytokine transforming growth factor beta (TGF-β) (AA marker) were quantified in the CM. For this purpose, Human Duo-set enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Abingdon, UK) were used according to the manufacturer's instructions.

Trophoblast Cell Line culture

SGHPL-4 cells were derived from primary human first trimester trophoblast and have been used extensively as a model for EVT (25, 26). SGHPL-4 cells express HLA-G and have been shown to respond in a manner similar to primary EVT (Cartwright et al 2002, Harris et al 2006). SGHPL-4 cells were cultured in Hams F10 media supplemented with 2mM L-glutamine, 100 units/ml penicillin, 0.1mg/ml streptomycin and 10% (v/v) FBS (SGHPL-4 medium).

SGHPL-4 Survival, Proliferation and Motility Assay

To determine whether macrophage polarisation impacts on EVT behaviour, SGHPL-4 cells were treated with polarised THP-1 CM and assessed using time-lapse microscopy. The SGHPL-4 cells were serum-starved overnight (SGHPL-4 medium containing 0.5% (v/v) FBS) prior to treatment with polarised THP-1 CM ($1x10^4$ cells/ml). An Olympus 1x70 inverted microscope (Olympus, Southend-on-Sea, UK) with a Hamamatsu C4742-95 digital camera and motorised stage (Hamamatsu Protonics) and Image-Pro Plus software (MediaCybernetics, Version 4.5) was used to image two positions in each well every 15min for 48h. Forty cells from each treatment were chosen at random and tracked using Image-Pro Insight software. To determine apoptotic cell death, the time frame at which apoptotic morphology became apparent was recorded (a phase bright appearance followed by membrane blebs or blisters (27)). To determine cell proliferation, the time frame at which a cell divided was recorded.

SGHPL-4 motility was assessed using Image-Pro Insight software to track the individual trajectory of 20 cells chosen at random for each treatment. To explore a possible role for macrophage secreted TNF-α in regulating trophoblast motility, TNF-α was neutralised in the CM using 5μg/ml of Mouse Anti-Human TNF monoclonal antibody with mouse IgG1κ used as an isotype control (BD Pharmingen, Oxford). Previous studies have shown that Mouse Anti-Human TNF monoclonal antibody is capable of neutralising the bioactivity of TNF in CM when used as per the manufacturer's instructions (28).

SGHPL-4 Western Blot Analysis

A western blot analysis was undertaken to assess whether SGHPL-4 treatment with THP-1 CM affects the levels of apoptotic marker cleaved caspase 3. SGHPL-4 cells were cultured in polarised THP-1 CM for 24h ($1x10^5$ cells/ml). After 24h the cell lysate was collected and a western blot analysis performed, using 1:1000 rabbit anti-cleaved caspase 3 (Cell Signaling, UK) and 1:10000 mouse anti-α-tubulin (Abcam, Cambridge, UK). Western blots were scanned using an Odyssey Scanner and the

density of each band determined. Results are expressed as a ratio to the loading control within each sample.

SGHPL-4 Network-Formation Assay

As EVT invade towards uterine spiral arteries, they alter their phenotype to become more endothelial-like (29). This can be modelled by assessing the ability of SGHPL-4 to form endothelial-like network structures (30). The effect of the polarised THP-1 CM on the ability of SGHPL-4 cells to form endothelial-like networks on Matrigel™ (BD, Oxford, UK) was assessed using a µ-slide Angiogenesis Assay (Ibidi, Planegg, Germany) according to the manufacturer's instructions. Images were captured as above, and network formation was assessed by measuring the average branch length and counting the total number of individual branches using Image-Pro Insight software.

Statistics

Statistical analysis was carried out using GraphPad Prism (Version 6.01). Paired T-tests or repeated measures one-way ANOVAs with post-test multiple comparisons were carried out as stated and statistical significance was assumed at $p<0.05$.

RESULTS

THP-1 cells can be polarised to reflect an CA or AA phenotype

To confirm THP-1 polarisation, secretion of pro- and anti-inflammatory cytokines by the CA and AA-polarised THP-1 cells was assessed by ELISA (**Figure 1**). The CA CM had a significantly higher concentration of the pro-inflammatory cytokines IL-6 and TNF-α than the AA cells ($p<0.05$). The CA CM contained approximately 60-fold more IL-6 than the AA CM, where levels were only just within the assay detection limit. TNF-α production was over two-fold higher in the CA CM than the AA CM. Conversely, the AA CM contained approximately 5-fold more of the anti-inflammatory cytokine TGF-β ($p<0.001$) than the CA CM.

CA macrophages reduce SGHPL-4 motility

SGHPL-4 motility in response to the polarised THP-1 CM was assessed by tracking individual SGHPL-4 cells over 48h (**Figure 2**). Cells treated with the CA CM had significantly lower cell motility when compared to the media control and the AA CM ($p<0.01$ and $p<0.01$, respectively). Motility was unaffected by the AA CM when compared with the control. In order to assess whether TNF-α secretion was responsible for the differential effect of the CA and AA CM on the motility of the trophoblast cell line, TNF-α was blocked from the CM but this had no effect when compared to the control IgG1κ.

Both CA and AA macrophages inhibit SGHPL-4 proliferation

To determine whether THP-1 polarisation state altered SGHPL-4 proliferation, SGHPL-4 cells were monitored by time-lapse microscopy following treatment with CA and AA CM. Treatment with both CA and AA CM almost completely abolished SGHPL-4 cell proliferation compared with the control ($p<0.001$) (**Figure 3A, B**), with an average of 49.5% of the SGHPL-4 cells proliferating in the control compared with 5.5 and 6% of the SGHPL-4 proliferating in the CA and AA CM, respectively.

Secreted factors from CA polarised macrophages induce SGHPL-4 apoptosis

Trophoblast apoptosis in response to polarised THP-1 CM was assessed by culturing SGHPL-4 cells with CA or AA CM and monitoring morphological changes by time-lapse microscopy. A significant difference in trophoblast apoptosis was not observed with either treatment ($p>0.05$) (**Figure 3C, D**). However, upon visual inspection morphological differences were consistently observed between the CA and AA treatments. Consequently, a western blot for the apoptotic protein cleaved PARP was undertaken. The CA CM was found to significantly increase the levels of cleaved caspase 3 in SGHPL-4 cells, over that of the control and the AA CM ($p<0.05$) (**Figure 4**).

AA macrophages are more able to promote SGHPL-4 network-formation than CA macrophages

The ability of SGHPL-4 to form endothelial-like networks in response to polarised THP-1 CM was assessed. The AA CM promoted the formation of long tube-like structures that appeared to contain many trophoblast cells, whereas the CA CM resulted in the formation of significantly shorter tube-like structures containing fewer cells ($p<0.05$). Branching of the tube-like structures was observed with both the CA and AA CM, although branching was more frequent with the CA CM resulting in the formation of shorter but more numerous and branching tube-like structures ($p<0.01$), and a network resembling a fine mesh of cells with a total length significantly higher than that of the control or AA treatment ($p<0.001$). No, or very few, tube-like structures were observed when SGHPL-4 were culture in the absence of THP-1 CM (**Figure 5**).

DISCUSSION

Impaired trophoblast invasion and spiral artery remodelling are associated with complications of pregnancy such as pre-eclampsia. The aim of this study was to determine whether the polarisation status of macrophages can impact upon trophoblast behaviour *in-vitro*. This was achieved by activating and polarising the human leukemic cell line THP-1 to reflect either a mature CA or AA macrophage phenotype and treating a trophoblast cell line with conditioned media (CM) from these cells. The results of this study suggest that macrophages can alter the behaviour of trophoblast cells *in-vitro* and that this behaviour may be differentially affected by polarisation state.

The mechanisms involved in the regulation of trophoblast invasion and survival are poorly understood. However, when these important functions are impaired, inadequate placentation and remodelling of spiral arteries can occur and are implicated in complications of pregnancy such as pre-eclampsia (4-6). An inverse relationship between reduced trophoblast invasion and macrophage infiltration of the spiral arteries has been reported in pre-eclampsia (14, 21), however, the polarisation status of these macrophages was unknown. In this study, when we exposed a trophoblast cell line to CA CM a significant inhibition of motility was observed when compared to the AA CM or control medium. This suggests that decidual macrophage polarisation towards a more pro-inflammatory phenotype may contribute to impaired migration of trophoblast impacting on their ability to interact with cells of the spiral artery.

Macrophages are primary producers of TNF-α, a pro-inflammatory cytokine belonging to a superfamily of soluble TNF ligands with diverse functionality (31). TNF-α is known to have a role in cellular apoptosis, proliferation, and motility (32) and is implicated in the pathology of pre-eclampsia. For example, studies have shown that serum concentrations of TNF-α are significantly elevated in women with pre-eclampsia compared to normotensive controls (33). Furthermore, TNF-α has previously been shown to inhibit EVT migration and invasion as a result of plasminogen activator inhibitor (PAI)-1 induction (18, 34). Therefore, we investigated whether TNF-α was the factor responsible for the differential effect of the CA and AA CM on EVT motility. However, blocking TNF-α was not found to alter the CA effect suggesting that additional mechanisms may influence trophoblast motility in response to macrophage polarisation.

Decidual macrophages secrete a plethora of additional soluble factors some of which are known to influence trophoblast invasion and migration. For example, they are known to secrete vascular endothelial growth factor (VEGF) and IL-10 both of which are thought to have an inhibitory effect on trophoblast motility (35, 36). They also produce a number of soluble factors that have been shown to promote trophoblast motility such as IL-1β and IL-8 (37, 38). It is likely that a combination of inhibitory and promotional factors is responsible for macrophage regulation of trophoblast motility, and it is possible that the balance is tipped towards inhibition with CA polarisation.

Apoptosis is an important mechanism in normal placental development and decidual remodelling, although excessive apoptosis may play a role in the pathology of pre-eclampsia (39). When we investigated the effect of macrophage polarisation on EVT apoptosis by time-lapse microscopy no significant difference above basal levels was observed with either the CA or AA treatment. However, differences in SGHPL-4 morphology between the treatments were observed. When SGHPL-4 protein levels of cleaved caspase 3 were investigated, the CA treatment was found to significantly up-regulate this apoptotic marker. It is likely that these results reflect the stage at which apoptosis was assessed; caspase 3 is an intracellular protein with a central role in the apoptotic cascade whereas morphological analysis of membrane integrity through time-lapse assesses the terminal stage of apoptosis. This observation correlates with previous studies that have shown an association between the number of activated macrophages and apoptosis in other EVT cell lines (40).

During the first trimester of pregnancy, EVT migrate in columns from the placental villi into the decidua. EVT at the tips of these columns subsequently detach and differentiate to become either interstitial or endovascular trophoblast which, as they differentiate, exit the cell cycle and cease proliferating (41, 42). In our study treatment with both the CA and AA CM was found to abolish

SGHPL-4 proliferation. Given these observations, it is possible that macrophages within the decidua are capable of influencing EVT differentiation towards a less proliferative, more invasive, endovascular phenotype, irrespective of macrophage phenotype. It will be important to confirm this with isolated primary first trimester trophoblast cells.

Spiral artery remodelling occurs during the first half of human pregnancy and results in replacement of the endothelium and vascular smooth muscle cells by invasive endovascular EVT (41). This requires endovascular EVT to adopt a vascular phenotype (29) and SGHPL-4 cells have previously been shown to form tube-like networks when seeded on Matrigel^{TM} (30, 43). Decidual macrophages secrete a range of angiogenic growth factors including angiogenin, keratinocyte growth factor, fibroblast growth factor B, vascular endothelial growth factor A, and angiopoietin-1 and -2 (44). Both the CA and AA macrophage CMs were found to induce network formation however the AA CM induced formation of significantly longer tube-like structures comprised of many cells in close contact, whereas the CA CM produced shorter, branching structures, which were morphologically less organised and contained fewer cells. AA macrophages have been shown to express fewer angio-inhibitory cytokines than classically activated MØ (45). Our results may reflect a requirement for tissue remodelling AA-like decidual macrophages during the co-ordinated changes to spiral arteries, and suggests that the presence of inflammatory CA-like decidual macrophages may lead to inefficient remodelling.

By using THP-1 cells to model the extremes of macrophage polarisation we have clearly shown that macrophage phenotype can affect trophoblast behaviour. THP-1 cells are widely used to model human monocytes and macrophages, and have been well characterised (46). PMA is commonly used to activate THP-1 cells (47-49) and LPS and IFN-γ, and IL-13 and IL-4, frequently used to generate CA- and AA-like cells, respectively, in order to mimic the extremes of macrophage polarisation (24, 50, 51). In future studies it would be interesting to determine the effect of different stimulatory conditions on primary decidual macrophages and their interactions with trophoblast.

In-vivo, it is unlikely that decidual macrophages can be defined as either CA or AA with increasing evidence that they have a unique phenotype. For instance, gene expression profiling has demonstrated that first trimester decidual macrophages resemble AA macrophages when compared to peripheral blood $CD14^+$ monocytes, with up-regulation of genes implicated in immune modulation and tissue remodelling, but also upregulate genes corresponding to an CA phenotype (15). Furthermore, it is possible that there are different macrophage populations within the decidua, in response to the local microenvironment. For example, two distinct subsets of $CD14^+$ decidual macrophages have been characterised by their level of CD11c expression (19) and intercellular adhesion molecule 3 (ICAM-3) expression (20). Therefore, in future studies it will be important to investigate whether decidual macrophage activation is location-specific and to determine how this contributes to the regulation of neighbouring cells, in addition to addressing whether this is different in pre-eclamptic pregnancies.

Although further *in-vivo* studies are required, our study demonstrates that macrophage polarisation can affect the behaviour of a trophoblast cell line *in-vitro* and is likely to have an important role in the regulation of placental development. Furthermore, macrophage polarisation towards a more pro-inflammatory phenotype may be one of the mechanisms responsible for the shallow placentation and impaired spiral artery remodelling observed in pre-eclampsia.

Author's roles

R.JB., I.E.D., G.S.W. and J.E.C. designed the experiments. R.B. carried out all of the experiments. The manuscript was prepared by R.B. and J.E.C. and all authors critically revised the manuscript and approved the final version.

Acknowledgements

The authors acknowledge Dr. Androulla Elia for providing the THP-1 cells.

Funding

R.B. was the recipient of a St. George's, University of London PhD studentship.

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Figure 1. Quantification of IL-6, TNF-α and TGF-β1 in the CM of THP-1 cells exposed to PMA and polarising factors. THP-1 cells ($4x10^6$) were activated in 6ml of THP-1 medium with 100nM PMA and classically or alternatively activated with 100ng/ml of LPS and 20ng/ml of IFN-γ or 20ng/ml of IL-4 and 20ng/ml of IL-13, respectively. After 72h the polarising media was replaced with fresh THP-1 medium without polarising factors. The CM was collected after 24h and the concentration of IL-6, TNF-α and TGF-β assessed by ELISA and the treatments compared statistically using a paired T-test. **A)** The concentration of IL-6 (pg/ml), n=9, *p<0.05. **B)** The concentration of TNF-α (pg/ml), n=9, *p<0.05. **C)** The concentration of TGF-β1 (pg/ml), n=6, ***p<0.001. Data is presented as mean + SEM.

Figure 2. SGHPL-4 motility when treated with polarised THP-1 CM. SGHPL-4 cells ($1x10^4$/ml) were serum-starved with SGHPL-4 medium containing 0.5% (v/v) FBS for 18h and subsequently treated with 1ml of CA or AA CM, or THP-1 medium as a control. The cells were imaged using time-lapse microscopy for 48h. Motility is presented as an average of 20 cells per treatment in arbitrary units. The treatments were compared statistically using a repeated measures ANOVA and Tukey's multiple comparisons test**. A)** CA CM significantly decreased the motility of trophoblast, n=5 experimental repeats, **p<0.01. **B)** THP-1 CM was treated with a neutralising antibody against TNF-α (anti-TNF) or a control IgG1κ. TNF-a neutralisation had no effect on trophoblast motility, n=4 experimental repeats, *p<0.05. Data is presented as mean + SEM.

Figure 3. SGHPL-4 proliferation and cell death when treated with polarised THP-1 CM. SGHPL-4 cells ($1x10^4$/ml) were serum-starved with SGHPL-1 medium for 18h and subsequently treated with 1ml of CA or AA CM, or THP-1 medium as a control. The cells were imaged using time-lapse microscopy for 48h and cell morphology observed for division or apoptotic changes. The treatments were compared statistically using a repeated measures ANOVA and Tukey's multiple comparisons test, n=5 experimental repeats. **A)** A significant difference in proliferation was found between the treatments and the control, ***p<0.001. **B)** Proliferation over time. **C)** No significant differences in apoptosis were found between the treatments, p>0.05. **D)** Apoptosis over time. Data is presented as mean + SEM.

Figure 4. SGHPL-4 expression of cleaved caspase 3 (17kDa) when treated with polarised THP-1 CM. SGHPL-4 cells were incubated with CA CM, AA CM or control media for 24h, total protein collected, and western blot analysis of cleaved caspase 3 undertaken. The treatments were compared statistically using a repeated measures ANOVA and Tukey's multiple comparisons test, n=6 experimental repeats. **A)** Representative western blot analysis of cleaved caspase 3 and tubulin. **B)** SGHPL-4 cell incubation with CA CM significantly upregulates expression of cleaved caspase 3 (17kDa) when compared with AA CM or THP-1 control medium, **p<0.01. Data is presented as mean + SEM. **C)** Representative morphology of SGHPL-4 cells treated with CA CM, AA CM or control media. Original magnification: 10X.

Figure 5. SGHPL-4 endothelial-like network formation when treated with polarised THP-1 CM. SGHPL-4 cells were cultured on Matrigel™ in the presence of polarised THP-1 CM, or THP-1 medium as a control, to induce endothelial-like network formation, and imaged after 24h. The treatments were compared statistically using a repeated measures ANOVA and Tukey's multiple comparisons test, n=4 experimental repeats. **A)** The total number of branches was significantly increased in the CA CM compared with the AA CM and control (**p<0.01). **B)** The average length of branch structures was significantly increased in response to the AA CM when compared with the CA CM and the control (**p<0.01). Original magnification: 4X. Data is presented as mean + SEM.

Figure 1

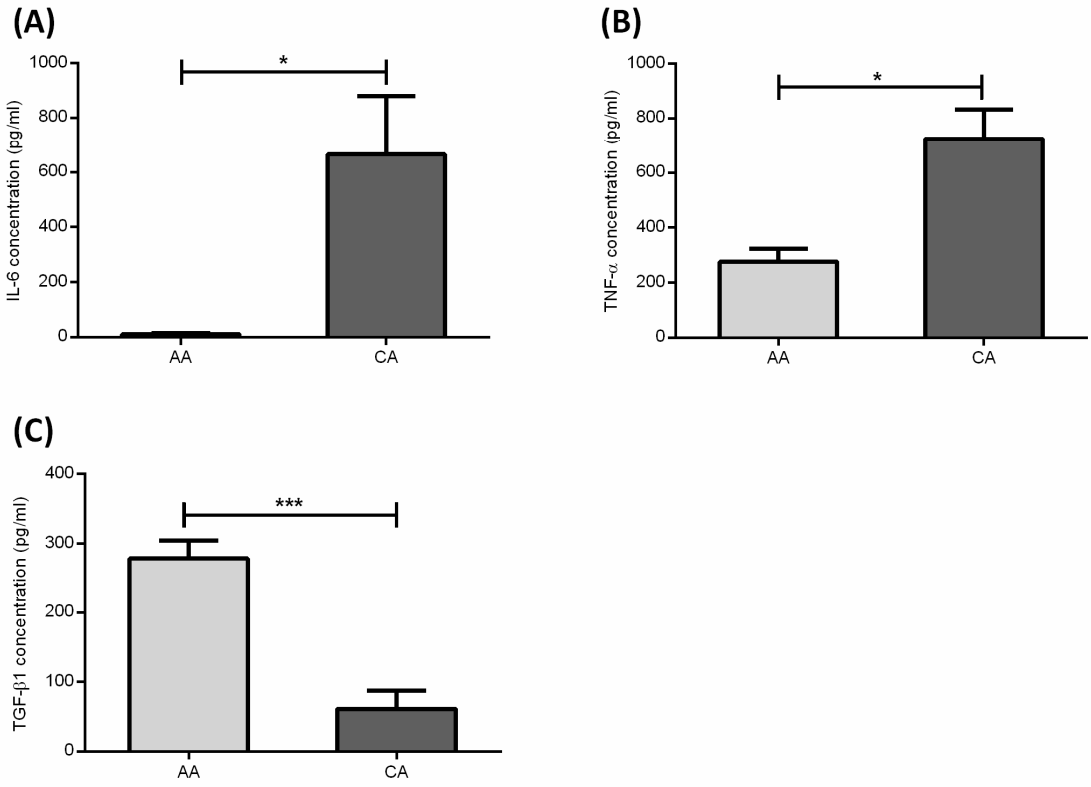


Figure 2

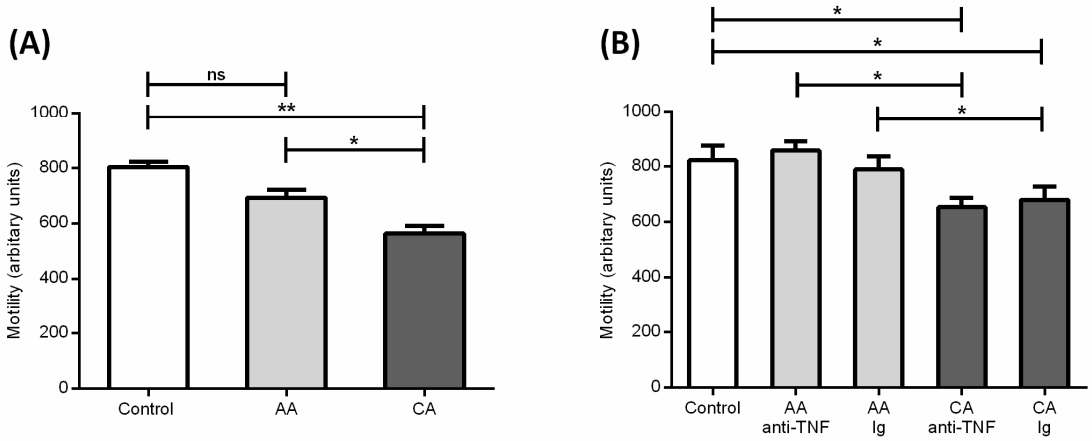


Figure 3

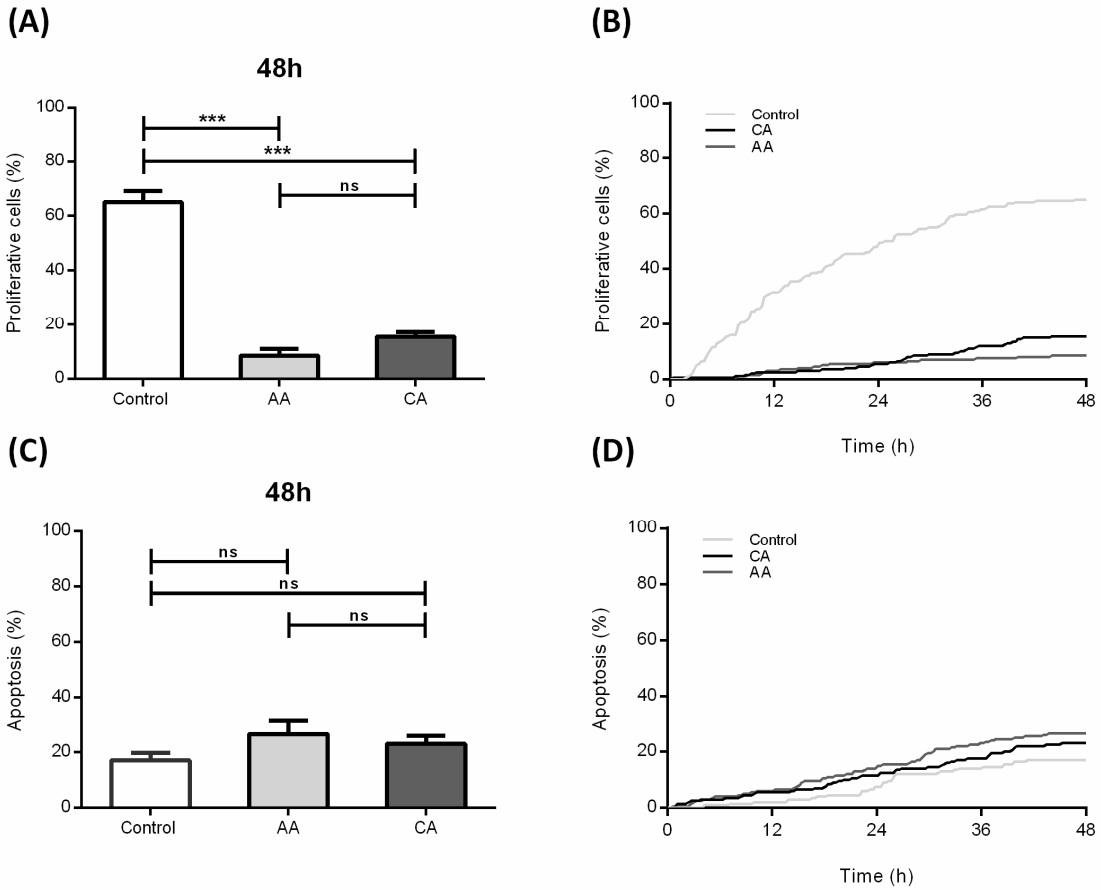
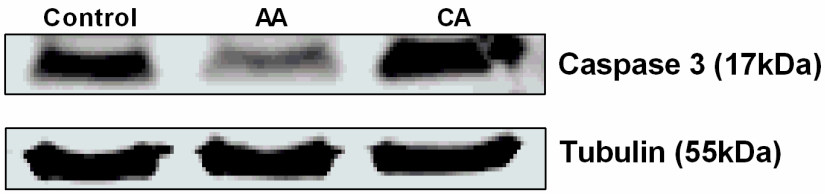
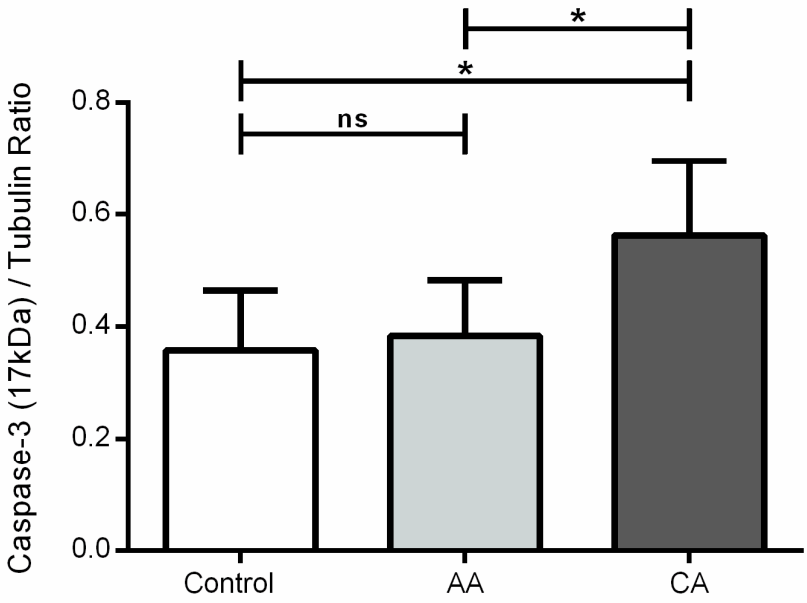


Figure 4

(A)


Control
AA
CA
Caspase 3 (17kDa)
Tubulin (55kDa)

(B)


Caspase-3 (17kDa) / Tubulin Ratio
0.0
0.2
0.4
0.6
0.8
ns
*
*
Control
AA
CA

(C)

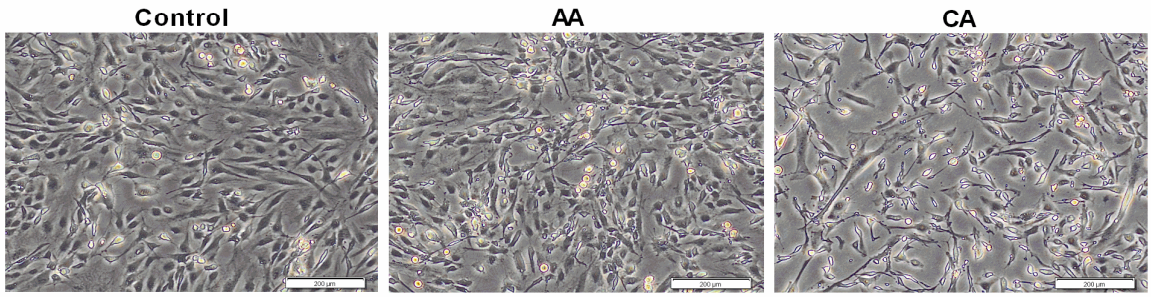
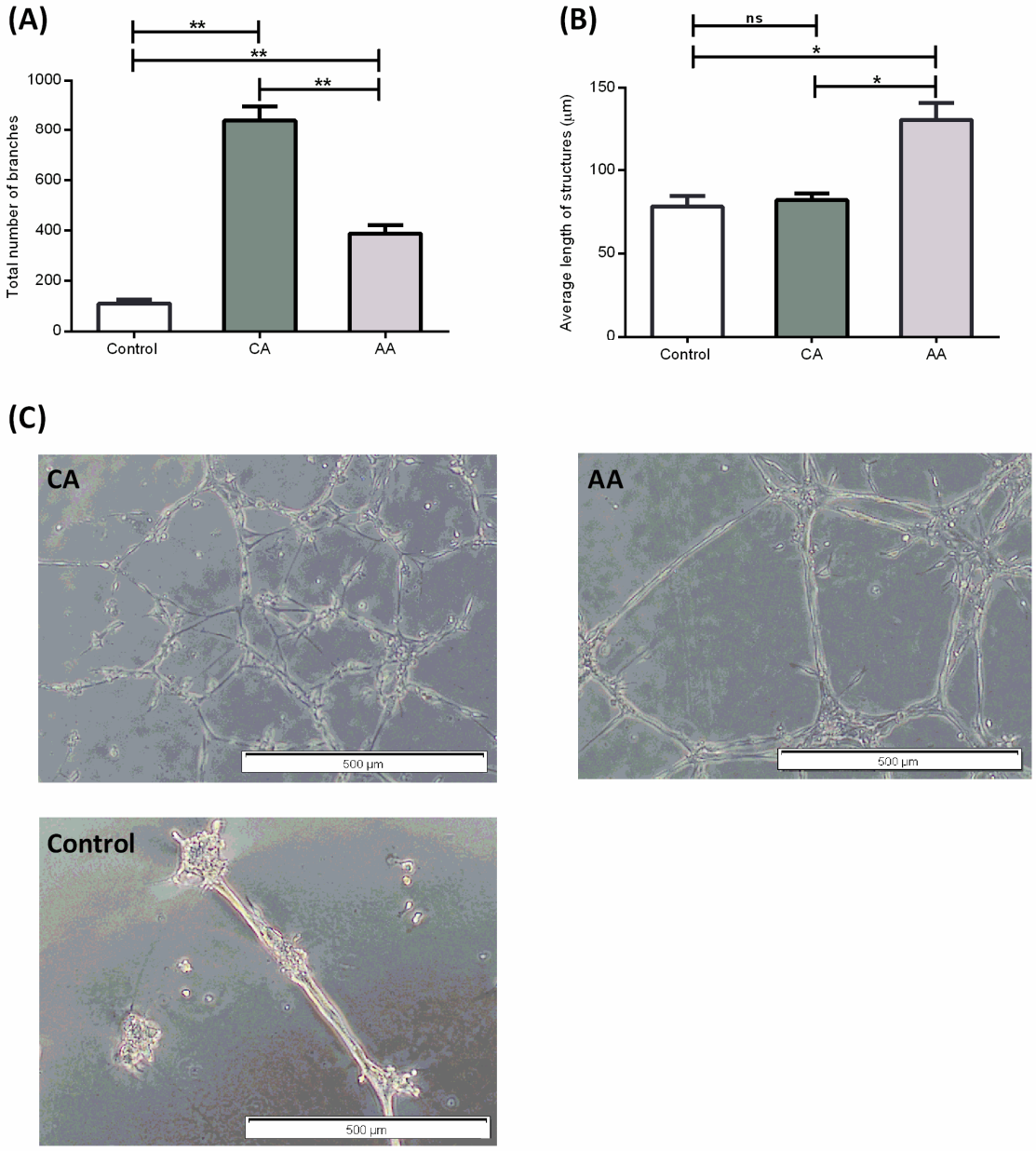

Control
AA
CA

Figure 5


(A)
**
**
**
Total number of branches
1000
800
600
400
200
0
Control
CA
AA
(B)
ns
*
*
Average length of structures (µm)
150
100
50
0
Control
CA
AA
(C)
CA
500 µm
AA
500 µm
Control
500 µm

Highlights

- Extravillous trophoblast motility is inhibited by classically activated macrophages.
- Alternatively activated macrophages support trophoblast network-formation.
- Macrophage polarisation can affect important trophoblast functions.
- Alterations in macrophage phenotype may impair trophoblast decidual remodelling.