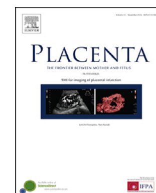
Placenta 47 (2016) 73–80

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta



Macrophage polarisation affects their regulation of trophoblast behaviour

R.J. Buckley, G.S. Whitley, I.E. Dumitriu, J.E. Cartwright*

Cardiovascular and Cell Sciences Research Institute, St. George's, University of London, Cranmer Terrace, London, SW17 0RE, UK

A R T I C L E I N F O

Article history:
Received 26 October 2015
Received in revised form
2 August 2016
Accepted 7 September 2016

Keywords:
Decidua
Macrophage
Extravillous trophoblast
Polarisation
THP-1

A B S T R A C T

Introduction: During the first trimester of human pregnancy, fetally-derived extravillous trophoblast (EVT) invade into the 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 a classically- or alternatively-activated macrophage 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 classically-activated macrophage conditioned media significantly inhibiting trophoblast motility, impeding trophoblast tube formation, and inducing trophoblast expression of cleaved caspase 3, when compared to anti-inflammatory alternatively-activated macrophage 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.

© 2016 Elsevier Ltd. All rights reserved.

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].

* Corresponding author.
E-mail address: jcartwri@sgul.ac.uk (J.E. Cartwright).

http://dx.doi.org/10.1016/j.placenta.2016.09.004
0143-4004/© 2016 Elsevier Ltd. All rights reserved.

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 macrophage [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].

Decidual macrophages also express 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.

2. Materials and methods

2.1. Macrophage differentiation

THP-1 cells were differentiated into macrophage-like cells by adapting a previously described method [24]. Briefly, cells were treated with 100 nM of phorbol 12-myristate 13-acetate (PMA) (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 2 mmol/L L-glutamine, 100 IU/mL penicillin, 100 mg/mL streptomycin, and 2.5 μg/mL amphotericin B (THP-1 medium). After 6 h, 100 ng/mL of lipopolysaccharide (LPS) and 20 ng/mL of IFN-γ, or 20 ng/mL of IL-4 (PeproTech, Rocky Hill, NJ) and 20 ng/mL of IL-13 (PeproTech), were added to generate a 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 either 10% (v/v) FBS or no serum. The conditioned media (CM) was collected after 24 h, centrifuged to remove cellular debris, and stored at −80 °C until used.

2.2. 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.

2.3. 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 medium supplemented with 2 mM L-glutamine, 100 units/ml penicillin, 0.1 mg/ml streptomycin, and 10% (v/v) FBS (SGHPL-4 medium).

2.4. 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 (1×10^4 cells/ml). An Olympus 1 × 70 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 15 min for 48 h. 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 5 μg/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].

Download English Version:

https://daneshyari.com/en/article/5894105

Download Persian Version:

https://daneshyari.com/article/5894105

Daneshyari.com