European Journal of Obstetrics & Gynecology and Reproductive Biology 179 (2014) 75–82

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

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

Gonadotropin stimulation in mice leads to ovarian accumulation of immature myeloid cells and altered expression of proangiogenic genes

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ARTICLE INFO

Article history:
Received 28 September 2013
Received in revised form 25 March 2014
Accepted 22 May 2014

Keywords:
Immature myeloid cells
Dendritic cells
Ovarian stimulation
Angiogenesis

ABSTRACT

Objective: Ovarian hyperstimulation syndrome is associated with increased angiogenesis and vascular leakage. Immature myeloid cells (IMCs) and dendritic cells have been shown to be actively involved in angiogenesis in several disease models in mice and humans. Nevertheless, little is known about the role of these cells in the ovary. As such, this study sought to determine whether alterations in these ovarian myeloid cell populations are associated with gonadotropin stimulation in a mouse model.
Study design: Four-week-old pre-pubertal C57Bl/6 female mice were allocated into three groups: high-dose stimulation ($n=4$; pregnant mare serum gonadotropins (PMSG) 20 U for 2 days), low-dose stimulation ($n=5$; PMSG 5 U for 1 day) and sham-treated controls ($n=4$). Human chorionic gonadotropin 5 U was injected on Day 3, and the mice were killed on Day 5. Ovaries were analysed by flow cytometry, confocal microscopy and quantitative polymerase chain reaction.
Results: Gonadotropin stimulation increased the proportion of $CD11b^+Gr1^+$ IMCs among the ovarian myeloid cells: 22.6 ± 8.1% (high dose), 7.2 ± 1.6% (low dose) and 4.1 ± 0.3% (control) ($p=0.02$). Conversely, gonadotropin stimulation decreased the proportion of ovarian $CD11c^+MHCII^+$ dendritic cells: 15.1 ± 1.9% (high dose), 20.7 ± 4.8% (low dose) and 27.3 ± 8.2% (control) ($p=0.02$). IMCs, unlike dendritic cells, were localized adjacent to $PECAM1^+$ endothelial cells. Finally, gonadotropin stimulation was associated with increased expression of S100A8, S100A9, Vcan and Dmbt1, and decreased expression of MMP12.
Conclusions: Gonadotropin stimulation is associated with proangiogenic myeloid cell alterations, reflected by a dose-dependent increase in ovarian IMCs and a parallel decrease in dendritic cells. Recruited IMCs localize strategically at sites of angiogenesis. These changes are associated with differential expression of key proangiogenic genes.

Introduction

Ovarian hyperstimulation syndrome (OHSS) is a rare iatrogenic disorder that may occur when heavily stimulated ovaries, containing numerous follicles with high oestradiol production, are exposed to exogenous human chorionic gonadotropin (hCG) [1,2]. OHSS is characterized by ovarian enlargement, increased vascular permeability and fluid shifting into the third space. This results in abdominal distension and increased intra-abdominal pressure concomitant with haemoconcentration [3]. The pathogenesis of OHSS is unclear. However, several factors that are induced by exogenous hCG and affect vascular permeability and angiogenesis, such as vascular endothelial growth factor (VEGF), transforming growth factor, platelet-derived growth factor, angiopoietins, prostaglandins and others, have been shown to play a role in this acute process [4–9].

Immature myeloid cells (IMCs), also known as myeloid-derived suppressor cells, have been shown to play an active role in several biological processes that involve angiogenesis. These cells have been shown to infiltrate placentas of pregnant mice and humans, and actively promote angiogenesis. Their presence is correlated with placental weight and birth weight [10,11]. IMCs have been shown to promote tumour growth and metastasis by modulating the cytokine environment and promoting angiogenesis [12–14]. Dendritic cells are specialized antigen-presenting cells that play key roles in the initiation and modulation of the adaptive immune response [15]. Tumour-infiltrating dendritic cells have been shown

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http://dx.doi.org/10.1016/j.ejogrb.2014.05.025
0301-2115/

to promote tumour growth and angiogenesis by secreting pro-angiogenic molecules, assembling into tumour neovessels, and/or transdifferentiating into endothelial-like cells [16–18]. The authors have recently shown that with striking similarity to their role in tumour growth and angiogenesis, supplementation of dendritic cells augmented the growth of endometriosis lesions and intra-lesion angiogenesis [19].

IMCs and dendritic cells are at different stages of myeloid development, as IMCs represent cells in early phases of development while dendritic cells are mature, fully differentiated cells.

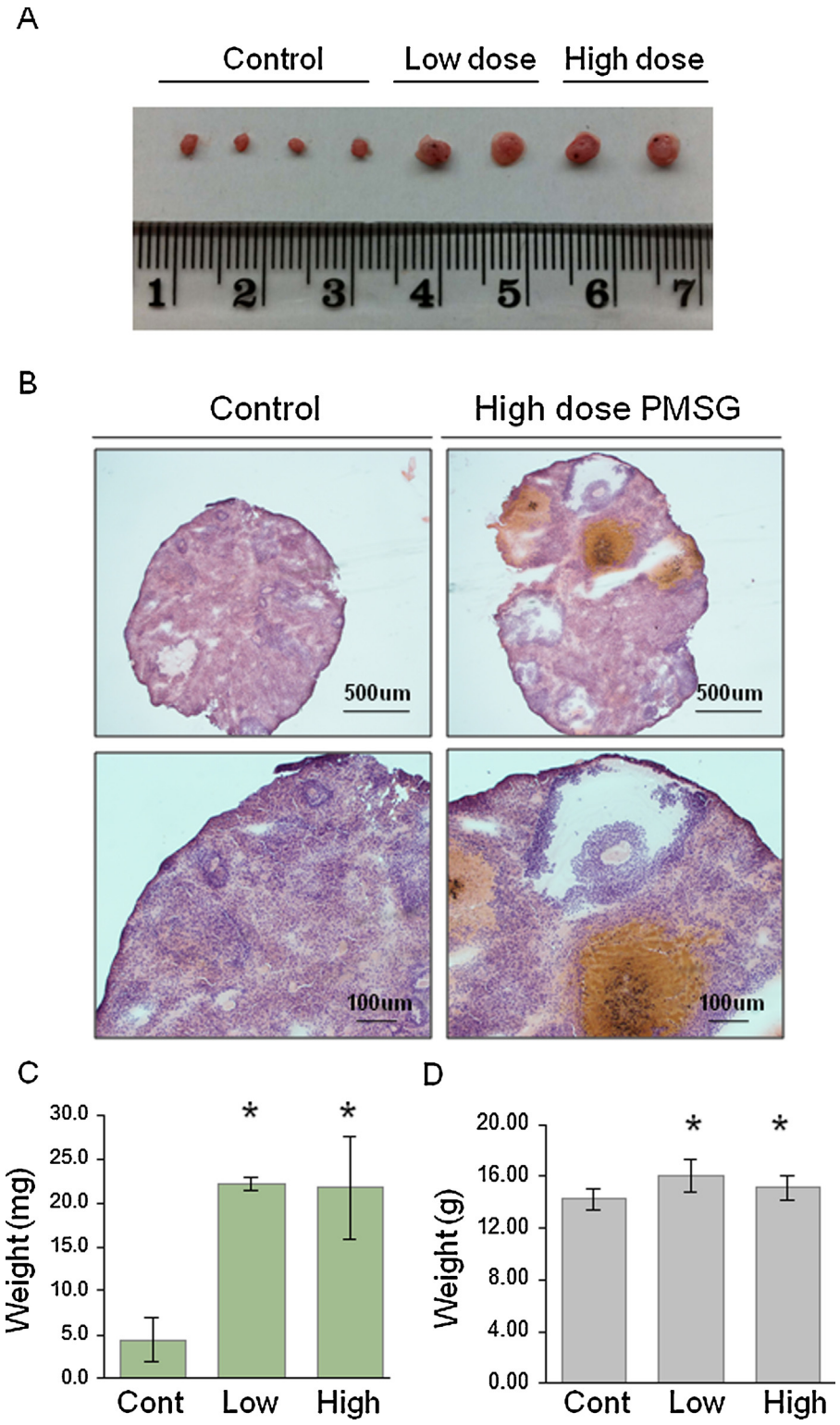

Fig. 1. Mouse model of ovarian stimulation. (A) Representative images of harvested murine ovaries 48 h after human chorionic gonadotropin, following intraperitoneal administration of low and high doses of pregnant mare serum gonadotropins (PMSG). (B) Representative images of haematoxylin and eosin staining of 10 μm histological sections of a control ovary vs a high-dose hyperstimulated ovary. Images were viewed with a Nikon Eclipse 800 microscope, captured using a Nikon DXM1200 digital camera, and processed with Nikon ACT-1 2.63 software. (C) Bar histograms depicting average gross weights of mice (right) and ovaries (two per mouse) (left) harvested from control mice (cont, $n = 4$), low-dose hyperstimulated mice (low, $n = 5$) and high-dose hyperstimulated mice (high, $n = 4$). $^{*}p < 0.05$ (Mann–Whitney test).