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Short communication

Stress increases VCAM-1 expression at the fetomaternal interface in an abortion-prone mouse model

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ABSTRACT

Sound stress exposure increases fetal loss via inflammatory pathways. Inflammation is known to up-regulate cell adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), which mediates the adhesion of leukocytes to the vascular endothelium. In this work, we studied the frequency of VCAM-1$^+$ vessels at the fetomaternal interface in stressed and non-stressed pregnant CBA/J female mice mated with DBA/2J (high fetal loss model) or BALB/c (low fetal loss model) males. The high fetal loss model had fewer large vessels on gestation day 6.5, and stress reduced the frequency of large vessels to a similar number in both high and low fetal loss models. In the high fetal loss model, however, the frequency of VCAM-1+ vessels was dramatically increased. This study shows that VCAM-1 expression is modulated by stress at the fetomaternal interface in abortion-prone cross-breeding.

1. Introduction

During human and mouse decidualization, maternal immune cells are recruited to the decidua to exert an immunomodulatory effect on the placenta. Leukocyte homing into the decidua is regulated through a specialized mechanism at the maternal endothelium of local vessels. This mechanism allows the entry of only specific leukocyte subsets. Vascular cell adhesion molecule-1 (VCAM-1) is expressed on the endothelium of some vessels in the decidua basalis of early human and mouse pregnancy (Kruse et al., 2002). VCAM-1 is an immunoglobulin-like adhesion molecule expressed on the surface of activated endothelial cells that binds to $\alpha_4\beta_1$ integrin, an integrin constitutively expressed on lymphocytes, monocytes, and eosinophils. VCAM-1 can mediate both rolling-type adhesion and firm adhesion, depending on the avidity status of $\alpha_4\beta_1$ integrin. It was observed that the specialized uterine NK cells, which are $\alpha_4\beta_1$-positive, constitute a defined cluster located around the VCAM-1 positive vessels at the implantation site (Burrows et al., 1993; Kruse et al., 2002). VCAM-1 also enhances extravasation of polymorphonuclear leukocytes that play a role in subsequent resorptions/abortions (Clark et al., 1998; Lee et al., 2008; Waugh and Lomakina, 2009).

Pregnancy failure usually takes place as spontaneous abortion during the first trimester in humans; it is known that stress increases fetal loss during the peri-implantation period. Over the last few years, the mechanisms involved in fetal loss were studied employing the murine model of DBA/2J-mated CBA/J females, which provides an established experimental approach, particularly in pregnancies challenged by stress (Arck et al., 1995; Blois et al., 2004a). These allogenically pregnant mice subjected to ultrasonic sound stress early in gestation showed a significant

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increase in the abortion rate (Arck et al., 1995). Adhesion molecules participate in stress-triggered abortion in this model by facilitating the recruitment of inflammatory cells to the fetomaternal interface (Blois et al., 2005).

In this work, we studied the expression pattern of VCAM-1 in implantation sites from gestation day 6.5 from stressed and non-stressed pregnant CBA/J mice. We compared the results from DBA/2J (high fetal loss model) and BALB/c mating (low fetal loss model).

2. Materials and methods

2.1. Animals

Two sets of experiments were carried out. Mice were purchased from Charles River Breeding Laboratories (Germany) and from Comisión Nacional de Energía Atómica (Argentina). Animal care and experimental procedures were followed according to each institution's guidelines (LaGeSo in Germany and CICUAL, Facultad de Medicina, Universidad de Buenos Aires in Argentina). DBA/2J- and BALB/c-mated CBA/J female mice were randomized into a control and a stressed group (each one $n = 10$). The observation of a vaginal plug was denoted on 0.5 day of pregnancy. The mice were sacrificed on gestation day (gd) 6.5; implantation sites were removed, counted and cryo-sectioned. By this gd, viable and resorbing sites cannot be distinguished and the implantation sites are easily visible externally as spherical swellings measuring 3×4 mm approximately. Three implantation sites were analyzed per mouse. Simultaneously, additional control and stressed groups of both cross-breeding (each one, $n = 10$) were sacrificed at day 13.5 to record the abortion rate, which was calculated as reported previously (Blois et al., 2004a).

2.2. Stress induction

Stress induction was performed according to Blois et al. (2004a). The CBA/J mice were exposed to sound stress for a single 24-h period on day 5.5 of pregnancy. The sound was emitted by a rodent repellent device (Conrad Electronics, Germany) at 300 Hz in intervals of 15 s. The device was placed into the mice's cage so that they could not escape the sound perception.

2.3. Immunohistochemistry

Acetone fixed-cryo sections (8-μm thickness) from implantation sites were rinsed in PBS and then incubated for 30 min at room temperature with 0.3% H_2O_2 in MeOH. Sections were blocked with 10% goat normal serum for 15 min at 37 °C and incubated afterwards overnight at 4 °C with rat anti-VCAM-1 (BD Biosciences), anti-PECAM (platelet endothelial cell adhesion molecule, BD Biosciences) or the respective control rat isotypes. A biotin-conjugated secondary antibody was then added for 30 min at 37 °C. The Vectastain® ABC peroxidase kit (Vector Labs) was used for PECAM staining while the alkaline phosphatase one was used for VCAM-1 detection. Diaminobenzidine and H_2O_2 were employed as a substrate for PECAM, while VCAM-1 was detected with New-fuchsin. Finally, the sections were counterstained with Mayer's hematoxylin and mounted using Crystal/Mount™ (Biomeda).

2.4. Vascular detection

PECAM staining was used for determining the number of vessels in cryosections (Fong et al., 1995). The mean number of blood vessels in each implantation site was calculated from three non-serial cryosections. Vessels were counted in the entire section and classified according to their size in: small ($<55 \times 55$ μm); medium (55×55 μm–110×110 μm) and large ($>110 \times 110$ μm).

2.5. Statistical analysis

The histochemical labeling was assessed in a double-blinded manner by two independent observers. Data were transformed with square root function for PECAM (number of vessels per section) and number of implantation sites and with arcsine function for VCAM-1 prior to statistical analysis. One-way analysis of variance (ANOVA) and Newman–Keuls multiple comparison test were performed to compare mean differences using GraphPad Prism 5-Graphics Software. Differences at $p < 0.05$ were considered statistically significant.

3. Results and discussion

In order to check the effect of sound stress on the resorption rate of the high fetal loss and low fetal loss mouse models (CBA/J × DBA/2J and CBA/J × BALB/c respectively), female pregnant mice exposed or not exposed to sound stress were sacrificed at gd 13.5. This work was carried out in two different facilities. In Germany (GE), the abortion rate of the CBA/J × DBA/2J cross-breeding was around $10 \pm 2\%$ ($n = 10$), as previously reported (Blois et al., 2004a) and in Argentina (AR) this value increased to $23 \pm 2\%$ ($n = 10$), in agreement with previous results (Miranda et al., 1998; Blois et al., 2004b). Flora and endogenous stress levels appear to explain baseline loss rate differences among different animal colonies (Clark et al., 2004, 2008). Nevertheless, both in the GE and in the AR colonies, sound stress increased this value to $44 \pm 3\%$ ($n = 10$). On the other hand, independently of the animal facility the abortion rate of the low fetal loss model was around 5–8% and was not affected by stress, in agreement with Arck et al., 1995. Therefore, DBA/2J-mated CBA/J females are more susceptible to stress and microenvironment conditions than BALB/c-mated CBA/J females.

Events leading to spontaneous abortion/resorption in the CBA/J × DBA/2J model occur prior to gestation day 9.5 (Clark et al., 1999, 2001; Girardi et al., 2006). In the present study, we focused on changes in the maternal uterine lining at the implantation site on gd 6.5, 2 days after implantation, with or without sound stress delivered on gd 5.5. In the implantation sites, endothelial cells were identified by PECAM immunohistochemistry and vessels were classified according to their size into small, medium or large vessels (Fig. 1A). It can be seen that the number of small and medium-sized vessels was similar in both models (Fig. 1B), but the number of large sized vessels was reduced