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ARTICLE

# The development of endometriosis in a murine model is dependent on the presence of dendritic cells

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**Abstract** Endometriosis is a common condition associated with pelvic pain and infertility. This study group has previously shown that supplementation of dendritic cells led to enhancement of endometriosis lesion growth and angiogenesis. This study determined whether endometriosis is dependent on the presence of endogenous dendritic cells. Surgical induction of endometriosis was performed in $CD11c^{+}$ DTR/GFP transgenic (Tg) female mice in which dendritic cells were ablated upon injection of diphtheria toxin (DT). Mice were allocated into four groups ($n = 5$ each): group I, wild-type mice treated with vehicle; group II, wild-type mice treated with DT; group III, Tg mice treated with DT; and group IV, Tg mice treated with vehicle. After 10 days, mice were killed and endometriosis lesions were analysed by flow cytometry. DT treatment led to ablation of dendritic cells in spleens and endometriosis lesions in Tg mice while no ablation was observed in controls. Corresponding to dentritic cell ablation, endometriosis lesions in group III were ~5-fold smaller than in the control groups (ANOVA $P < 0.0001$). This study suggests that endometriosis development is dependent on the presence of endogenous dendritic cells. Therapies designed to inhibit dentritic cell infiltration as possible treatments for endometriosis warrant further study.



**KEYWORDS:** angiogenesis, dendritic cells, endometriosis, mouse model, infertility, myeloid cells

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## Introduction

Endometriosis, characterized by abnormal growth of endometrial tissue in locations other than the uterine lining, is a common disorder affecting 10—15% of women of reproductive age and is associated with pelvic pain and infertility (Cramer and Missmer, 2002; Giudice and Kao, 2004). Although highly investigated, the cause of endometriosis is yet unknown. The leading theories include retrograde menstruation resulting in implantation of viable fragments of endometrium on peritoneal surfaces, metaplasia of coelomic epithelium, haematogenic and lymphogenic spread of endometrial tissue, and remnants of the Müllerian duct (Osteen et al., 2005; Stefansson et al., 2002; Witz, 1999). Another potential pathophysiology is the decreased ability to recognize and clear ectopic endometrial tissue in abnormal locations due to impaired immune surveillance. Additionally, increased concentrations of leukocytes in the ectopic tissues and peritoneal cavity, secreting various growth factors and cytokines, may lead to increased proliferation and survival of the endometriotic implants (Dmowski et al., 1981; Oosterlynck et al., 1991; Osteen et al., 2005).

Multiple lines of evidence suggest that angiogenesis (the process involving the growth of new blood vessels from pre-existing ones) is a major prerequisite for the initiation and progression of endometriosis (Donnez et al., 1998; Taylor et al., 2002). Similar to tumour growth and metastasis, endometriosis implants also require neovascularization in order to provide them with an adequate supply of oxygen and essential nutrients (Folkman, 2002; Groothuis et al., 2005). Various proangiogenic factors, both in the peritoneal fluid and in the endometriosis tissue itself, have been shown to take part in the development of these lesions (Laschke and Menger, 2007; McLaren et al., 1996; Suzumori et al., 2004). Moreover, antiangiogenic therapy proved effective in suppressing the development of endometriotic lesions in rodent models (Becker et al., 2006a; Dabrosin et al., 2002; Hull et al., 2003).

Dendritic cells are specialized antigen-presenting cells that play a pivotal role in the instigation and modulation of the adaptive immune response (Banchereau and Steinman, 1998). Recently, tumour-infiltrating dendritic cells were shown to promote tumour growth and angiogenesis by various mechanisms. Dendritic cells have been shown to secrete proangiogenic cytokines, assemble into tumour neovessels and even to transdifferentiate into endothelial- like cells (Fainaru et al., 2010; Murdoch et al., 2008; Sozzani et al., 2007). This study group has previously shown that, with striking similarity to their role in tumour growth and angiogenesis, dentritic cell supplementation leads to enhancement of endometriosis lesion growth and of intralesion angiogenesis in a mouse model (Fainaru et al., 2008). Nevertheless, the question whether the development of endometriosis is dependent on the presence of endogenous dendritic cells remains unanswered. To explore this notion, the current study utilized $CD11c^+$ DTR/GFP transgenic (Tg) mice, in which dentritic cells can be ablated by administration of diphtheria toxin (DT). This allowed an investigation *in vivo* whether dendritic cells are indeed an inherent part of endometriosis growth.

## Materials and methods

### In-vivo ablation of dentritic cells

This study used $CD11c^+$ DTR/GFP Tg mice (B6.FVB-Tg (Itgax-DTR/EGFP)57LAn/J; Jung et al., 2002), in which a transgene was designed to place a simian diphtheria toxin receptor (DTR) together with a green-fluorescence protein (GFP) reporter under the control of the Itgax (CD11c) promoter. Dendritic cells in these mice, which normally express the CD11c gene, also express GFP, which can be easily detected by flow cytometry. Additionally, upon exposure to a single dose (2—4 ng/g mouse) of DT (Sigma, St Louis, USA), these mice are transiently depleted of all dendritic cells for 2—3 days, whereas DT administration has no effect on $CD11c^+$ cells in wild-type mice that do not express DTR. DT (3 ng/g) or vehicle was therefore administered on days 2, 5 and 9 of the experiment.

The mice were allocated into four groups ($n = 5$ each): group I, WT mice treated with vehicle injections; group II, WT mice treated with DT injections, to control for possible nonspecific effects of DT that are not related to DTR expression; group III, $CD11c^+$ DTR/GFP Tg mice treated with DT; and group IV, $CD11c^+$ DTR/GFP Tg mice treated with vehicle injections, to control for possible nonspecific effects of the $CD11c^-$ DTR/GFP transgene on the endometriosis model.

All animal procedures were performed in compliance with Boston Children's Hospital guidelines and protocols approved by the Institutional Animal Care and Use Committee (protocol number A05-09-082R, approved 21 September 2005).

### Surgical model of endometriosis

The surgical model of endometriosis was based on Becker et al. (2006b). The procedure was performed on 6—8-week-old female C57Bl/6 wild-type (WT) mice (Jackson Laboratories, Bar Harbor, ME, USA) and $CD11c^+$ DTR Tg mice generated on the same C57Bl/6 background. Under isoflurane anaesthesia, a midline abdominal incision was performed and the uterine horns were removed, leaving the ovaries *in situ*. In a Petri dish containing Dulbecco's modified Eagle medium F-12 (Gibco, Grand Island, NY, USA) supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco), the uterine horns were opened longitudinally with scissors. Tissue samples were obtained using a 2-mm dermal biopsy punch (Miltex, Bethpage, NY, USA). Four biopsies were sutured to the peritoneal wall using a 7-0 braided silk suture (Ethicon, Somerville, NJ, USA). The abdominal incision was closed with a continuous 5-0 braided silk suture (Ethicon). Mice were monitored daily after surgery for general appearance and food and water consumption. Most of the animals showed normal symptoms of recovery after surgery (18 of 20 mice). One mouse in group IV died 1 day after surgery and one mouse in group III died 8 days after surgery; all mice in groups I and II survived. Mouse weights were monitored before surgery and at the day of experiment termination. The average change in mouse weight was +11.5%, +8.1%, −1.6% and +10.3% in groups I, II, III and IV, respectively. It is possible that systemic effects from a DT-induced generalized dentritic cell