Journal of Pathology
J Pathol 2014; **234:** 329–337
Published online 8 August 2014 in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/path.4397

ORIGINAL PAPER

Targeting galectin-1-induced angiogenesis mitigates the severity of endometriosis

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Abstract

Endometriosis is characterized by the presence of endometrial tissue outside the uterus that causes severe pelvic pain and infertility in women of reproductive age. Although not completely understood, the pathophysiology of the disease involves chronic dysregulation of inflammatory and vascular signalling. In the quest for novel therapeutic targets, we investigated the involvement of galectin-1 (Gal-1), an endogenous glycan-binding protein endowed with both immunosuppressive and pro-angiogenic activities, in the pathophysiology of endometriotic lesions. Here we show that Gal-1 is selectively expressed in stromal and endothelial cells of human endometriotic lesions. Using an experimental endometriosis model induced in wild-type and Gal-1-deficient (*Lgals1*$^{-/-}$) mice, we showed that this lectin orchestrates the formation of vascular networks in endometriotic lesions *in vivo*, facilitating their ectopic growth independently of vascular endothelial growth factor (VEGF) and the keratinocyte-derived CXC-motif (CXC-KC) chemokine. Targeting Gal-1 using a specific neutralizing mAb reduced the size and vascularized area of endometriotic lesions within the peritoneal compartment. These results underline the essential role of Gal-1 during endometriosis and validate this lectin as a possible target for the treatment of disease.

Keywords: endometriosis; galectin-1; angiogenesis

Received 7 April 2014; Revised 4 June 2014; Accepted 22 June 2014

No conflicts of interest were declared.

Introduction

Endometriosis is a common gynaecological disease characterized by ectopically endometrial tissue implantation reassembled as functional endometriotic lesions, frequently disposed in ovaries and peritoneum [1]. This disease affects approximately 10% of reproductive age women and causes severe pelvic pain and infertility [2]. Patients with endometriosis often suffer from dysmenorrhoea, dyspareunia, dysuria, and chronic abdominal or pelvic pain, as well as infertility, leading to poor quality of life [3]. Endometriosis has also been associated with an elevated risk of ovarian cancer [4]. Unfortunately, current treatments are only aimed at alleviating clinical symptoms of the disease. Although the mechanisms underlying the pathogenesis of the endometriosis are poorly defined, it has been demonstrated that angiogenesis is an essential process during disease progression [5–7]. In fact, dysregulated angiogenesis and unbalanced inflammation are key components of endometriotic lesions, which recapitulate cellular and molecular networks of the tumour microenvironment [8–10].

Galectin-1 (Gal-1), an endogenous lectin that recognizes *N*-acetyllactosamine (LacNAc) structures on glycosylated receptors, coordinates a variety of physiological and pathological processes [11]. This lectin is up-regulated in tumour and inflammatory microenvironments, where it plays essential roles in angiogenesis and immune evasion mechanisms [12–15]. Interestingly, recent studies highlighted the contribution of Gal-1 to the pathogenesis of clinical settings that are

relevant in reproductive medicine, including spontaneous recurrent abortions and pre-eclampsia [16–18]. Although the expression of Gal-1 has been reported in human endometrium [19], as well as in adult mouse uterine horns [20], the pathophysiological role of this endogenous lectin in endometriosis is still uncertain.

The central role of the Gal-1–*N*-glycan axis in tumour angiogenesis [13,14] and its ability to co-opt the VEGFR2 signalling pathway to modulate endothelial cell biology [15] prompted us to evaluate the contribution of Gal-1 to vascular development and growth of autologous and heterologous endometriotic lesions experimentally induced in wild-type and *Lgals1*$^{-/-}$ female mice, and to validate the therapeutic effects of Gal-1 blockade *in vivo* using a neutralizing anti-Gal-1 mAb.

Materials and methods

Immunofluorescence and immunohistochemistry of human tissue sections

Biopsies from endometriotic lesions and eutopic endometrium from pre-menopausal women with endometriosis were taken during laparoscopy as described elsewhere [21]. Signed informed consents from all patients were included in this study, and the experimental protocol was approved by the Ethics Institutional Committee from IBYME-CONICET. The biopsies were fixed in 4% buffered formaldehyde solution, then paraffin-embedded and cut into 5 µm serial sections. Indirect immunofluorescence assays were performed on sections of eutopic endometrium ($n = 3$) and ectopic lesions ($n = 3$). Briefly, sections were dewaxed overnight in xylene and then rehydrated through graded alcohols. Antigen retrieval was carried out with 10 mM sodium citrate (pH 6.0) in a microwave oven. All sections were blocked for 2 h at room temperature with 10% normal goat serum (NGS) with 0.3% Triton X-100 in PBS. Sections were then incubated overnight at 4 °C with 1 : 500 rabbit anti-Gal-1 polyclonal Ab generated as described previously [14] and 1 : 50 mouse anti-CD31 monoclonal Ab (Dako, Carpinteria, CA, USA). Both primary Abs were diluted together in 1% NGS with 0.3% Triton X-100 in PBS. Afterwards, the specimens were extensively rinsed with PBS containing 0.3% Triton X-100 and incubated for 2 h at room temperature with the respective secondary Abs: goat AlexaFluor488-conjugated anti-rabbit IgG Ab (Life Technologies, Eugene, OR, USA) and goat AlexaFluor568-conjugated anti-mouse IgG Ab (Life Technologies), both diluted 1 : 100 in PBS with 0.15% Triton X-100. Finally, nuclei were counterstained with DAPI and slides were mounted with VectaShield mounting medium (Vector Labs, Burlingame, CA, USA) and stored at −20 °C until they were imaged with a confocal microscope (Nikon Eclipse E800/Nikon D-Eclipse C1).

Gal-1 immunohistochemistry was performed as indicated elsewhere [14] with the following modifications: the rabbit anti-Gal-1 polyclonal Ab was diluted 1 : 2000 and a commercial kit of biotinylated secondary Abs conjugated to horseradish peroxidase (Dako LSAB + System-HRP) was used. One section of each slide was incubated with a rabbit IgG isotype Ab as negative control. Sections were imaged with a standard light microscope (Nikon – Eclipse E200).

Animals

Two-month-old *Lgals1*$^{-/-}$ and wild-type C57BL/6 female mice (provided by Dr Francoise Poirier, Jacques Monod Institut, Paris, France) were housed and maintained on a 12-h light/12-h dark cycle and temperature conditions at the institutional animal facilities (IBYME-CONICET). All experimental procedures were performed according to the NIH Guidelines for the Care and the Use of Laboratory Animals, and all protocols used were approved by the Ethics Institutional Committee from IBYME-CONICET.

Surgical induction of autologous and heterologous endometriotic lesions in mice

Endometriotic lesions were experimentally induced by autologous or heterologous transplantation of pieces of uterine horns onto the bowel mesentery as previously described [7,22,23]. Briefly, mice were deeply anaesthetized with an intraperitoneal injection of ketamine and xylazine (110 mg/kg and 10 mg/kg, respectively) and immediately underwent laparotomy to expose the uterine horns and bowels. Subsequently, four experimental groups were established. In the first two autologous groups, wild-type (WT) and *Lgals1*$^{-/-}$ (KO), the right uterine horn was removed, cut longitudinally, and segmented into three equal pieces of tissue (≈4 mm^2) which were sutured onto a serosal layer from the same animal with the endometrial tissue facing the serosal layer. In the third group corresponding to heterologous WT mice (hWT), the uterine horns were removed from *Lgals1*$^{-/-}$ donor mice and transplanted onto the intestinal mesentery of syngeneic WT recipient mice. In the fourth group, heterologous Gal-1 knock-out (hKO), uterine tissue from WT donor mice was transplanted into *Lgals1*$^{-/-}$ recipient mice. In all groups, the ectopically transplanted endometrial tissue was allowed to develop for 4 weeks post-surgery, at which time the mice were euthanized by cervical dislocation.

Peritoneal Gal-1 blockade

Endometriotic lesions were surgically induced in mice as described above for the autologous WT group. Six mice received intraperitoneal injection of the neutralizing anti-Gal-1 mAb F8.G7 [14,24] on post-surgery days 7, 10, 14, 17, 21, 24, and 28. To avoid possible toxicity associated with administration of the neutralizing mAb, the first three doses were injected with the minimal effective concentration of anti-Gal-1 mAb:

7.5 mg/kg. At the second week post-surgery, when the peak of vascularization and disease severity was reached [25,26], the dose was increased to 15 mg/kg, which corresponds to the optimal dose established in other settings [14,15]. Six additional mice were randomly assigned to the control group and received the same doses of an IgG isotype-control Ab. All mice were euthanized by cervical dislocation 31 days after the endometriosis induction surgery.

Evaluation of endometriotic lesions

The abdominal wall was opened by a large ventral midline incision and the implantation sites, identified by the presence of developed lesions or by a suture alone, were counted. Subsequently, the developed lesions were measured in two perpendicular diameters ($d < D$) with a caliper and their volume was calculated with the following formula: $V = (3/4)\ \pi r^2 R$ (r and R are the radiuses, $r < R$) [23,27]. Results were expressed as the mean of the number of established lesions per mouse and the mean size of lesions per mice (in mm^3). Finally, lesions were removed and fixed in 4% buffered formaldehyde solution for 24 h at 4 °C, paraffin-embedded, and cut into 5 µm serial sections. Two non-contiguous sections from each specimen ($n = 4$ per group) were stained with haematoxylin–eosin and examined microscopically to identify the histological hallmarks of endometriotic lesions (ie glands and stroma).

Vascular perfusion and immunofluorescence

Mice were deeply anaesthetized with an intraperitoneal injection of ketamine and xylazine (110 mg/kg and 10 mg/kg, respectively) and immediately treated by heart perfusion for 7 min with 70 µl of fluorescein-labelled *Lycopersicon esculentum* lectin (2 mg/ml; Vector Laboratories) to stain blood vessels ($n = 3$) [28]. The vascular network was then perfused with saline and subsequently fixed with 4% buffered paraformaldehyde. Afterwards, the endometriotic lesions were removed, snap-frozen in liquid nitrogen, embedded in OCT compound (Tissue-Tek), cut into 20 µm sections in a cryostat (Thermo, Cryotome E), and stored at −70 °C.

For Gal-1 immunofluorescence staining, sections were thawed for 15 min at room temperature and rinsed with PBS. All sections were blocked for 2 h at room temperature with 10% fetal bovine serum (FBS) in PBS. Sections were then incubated overnight at 4 °C with 1 : 100 rabbit anti-Gal-1 polyclonal Ab [14] diluted in PBS with 1% FBS and 0.3% Triton X-100. Then specimens were extensively rinsed with PBS containing 0.3% Triton X-100 and incubated for 2 h at room temperature with 1 : 300 goat Cy3-conjugated anti-rabbit IgG Ab (Life Technologies) diluted in PBS with 0.15% Triton X-100. Finally, slides were mounted with VectaShield mounting medium (Vector Labs) and stored at −20 °C until they were imaged with a confocal microscope (Nikon Eclipse E800/Nikon D-Eclipse C1).

Immunohistochemistry

For immunohistochemistry of endometriotic sections, we used the following primary Abs: 1 : 500 rabbit anti-Gal-1 polyclonal Ab [14], 1 : 400 rabbit anti-vWF polyclonal Ab (Dako), and 1 : 150 rabbit anti-VEGFR2 polyclonal Ab (Cell Signaling, Danvers, MA, USA). Briefly, all sections were dewaxed in xylene and rehydrated through graded alcohols. Endogenous peroxidase activity was then quenched with 3% H_2O_2 for 30 min, and antigen retrieval for Gal-1 was carried out with 10 mM sodium citrate (pH 6.0) in a microwave oven, and for vWF and VEGFR2 with successive treatments for 30 and 15 min, respectively, with 0.1% Triton X-100 followed by 20 µg/ml proteinase K in PBS. All sections were then blocked with 4% BSA in PBS for 2 h at room temperature and incubated overnight at 4 °C with the primary Ab in PBS with 1% BSA at the indicated dilutions. Subsequently, sections were incubated for 1 h at room temperature with 1 : 200 goat biotinylated anti-rabbit IgG Ab (Sigma-Aldrich, St Louis, MO, USA). Afterwards, a streptavidin–peroxidase conjugate (Dako LSAB + System) was added for 30 min at room temperature and the signal was developed with DAB as substrate (Dako DAB + Substrate Chromogen System). Finally, sections were counterstained with Gill's haematoxylin, dehydrated through graded alcohols, clarified in xylene, and properly mounted. As a negative control, one section of each slide was assayed without the primary Ab. In all cases, 4–6 random fields per section were imaged with a standard light microscope (Nikon – Eclipse E200). The relative vascularized area per mouse was determined by measuring the area of vWF-positive or VEGFR2-positive blood vessels relative to the total tissue area using ImageJ 1.42q software (NIH).

VEGF-A and CXC-KC concentrations in mouse peritoneal fluid

Peritoneal fluid was collected at the moment of mouse euthanasia and the concentrations of VEGF-A and CXC-KC were measured using commercial ELISA kits according to the manufacturer's instructions (Calbiochem QIA52 for VEGF-A and R&D DY453 for CXC-KC). All samples were assayed in duplicate and the concentration values were expressed in pg/ml.

Statistical analysis

Statistical analysis was performed using Prism software (GraphPad). Statistical comparisons of the number of established lesions among autologous and heterologous groups were carried out using the Kruskal–Wallis test followed by Dunn's multiple comparison test. Statistical analysis of the mean size of lesions, the percentage of vascularized area for vWF and VEGFR2, and the concentrations of VEGF-A and CXC-KC in peritoneal fluid among autologous and heterologous groups was performed using the one-way analysis of variance

(ANOVA) test followed by Bonferroni's multiple comparison test. Statistical comparisons of the number of established lesions and the CXC-KC concentration in peritoneal fluid between anti-Gal-1 and control groups were performed with the Mann–Whitney *U*-test. The mean size of lesions per mouse, the percentage of vascularized area for vWF and VEGFR2, and the assessment of VEGF-A concentration in peritoneal fluid were analysed with the unpaired Student's *t*-test. p values ≤ 0.05 were considered significant. Results are expressed as mean ± SEM.

Results

Gal-1 is expressed in ectopic lesions and eutopic endometrium from patients with endometriosis

To investigate the functional relevance of Gal-1 during endometriosis and its potential value as a therapeutic target, we first examined the expression of this lectin in different compartments associated with endometriotic lesions. We found substantial and selective expression of Gal-1 protein in endometriotic and eutopic endometrial stromal cells (Figures 1A and 1B), although weak expression of this lectin was found in lesion-associated endothelial cells (Figure 1A, filled white arrows). Strikingly, staining by immunohistochemistry and immunofluorescence revealed that Gal-1 was absent in both endometriotic and eutopic endometrial epithelial cells (Figures 1A and 1B).

Gal-1 plays a central role in the development of endometriotic lesions *in vivo*

We evaluated the consequences of Gal-1 deletion in experimentally induced endometriosis in *Lgals1*$^{-/-}$ (KO) and wild-type (WT) female C57BL/6 mice. Autologous (WT and KO) or heterologous (hWT and hKO) pieces of uterine tissue from donor mice were transplanted into the bowel mesentery of syngeneic recipient mice (Figure 2A). This combined experimental strategy allowed assessment of the contribution of Gal-1 to the development of mouse endometriotic lesions when this endogenous lectin was completely absent in the host (KO versus WT group) or when it was absent (hWT) or present (hKO) specifically in endometriotic tissue. Interestingly, we found that the number of established lesions decreased substantially in Gal-1 KO compared with the WT and hKO groups ($p = 0.001$, Figure 2B). Moreover, the mean size of lesions was significantly smaller in Gal-1 KO, hWT, and hKO groups compared with WT mice by 72.6%, 78.3%, and 59.7%, respectively ($p < 0.0001$, Figure 2C), thus underscoring the relevance of Gal-1 in the development of ectopic endometrial tissue. In addition, immunohistochemical analysis revealed selective expression of Gal-1 in stromal and endothelial cells, but not in epithelial cells of endometriotic lesions (Figure 2D). This expression pattern mirrored the distribution of Gal-1 in ectopic lesions from endometriosis patients (Figures 1A and 1B).

Gal-1 facilitates the development of vascular networks in endometriotic lesion *in vivo*

Because of the abundant expression of this lectin in stromal cells and its ability to co-opt the VEGFR2 pathway [15], we hypothesized that Gal-1 may control the formation of vascular networks in endometriotic lesions. To test this hypothesis, we assessed the relative vascularized areas immunostained for the endothelial cell marker von Willebrand factor (vWF). Consistent with changes in the size of endometriotic lesions, their relative vascularized areas were significantly smaller in KO, hWT, and hKO groups compared with those observed in WT mice by 63.5%, 38.0%, and 43.5%, respectively ($p < 0.0005$, Figure 3A). To investigate the contribution of vascular endothelial growth factor (VEGF) to the diminished vascularization observed in mice lacking Gal-1, the concentration of this pro-angiogenic factor, as well as the relative vascularized area positive for its canonical type-2 receptor (VEGFR2), was determined in mouse peritoneal fluid and endometriotic lesions, respectively. Strikingly, no differences were found in VEGFR2-positive vascularized areas of endometriotic lesions ($p = 0.2660$, Figure 3B) nor in VEGF-A concentrations in peritoneal fluid ($p = 0.1130$, Figure 3C) among the different groups analysed. Furthermore, no alterations in the concentration of keratinocyte-derived CXC-motif (CXC-KC) chemokine, a functional analogue of human interleukin (IL)-8 that plays a key role in angiogenesis during inflammation [29], were detected in peritoneal fluid derived from the different groups ($p = 0.6099$, Figure 3D). These results highlight the contribution of Gal-1 which influences the angiogenic phenotype of endometriotic lesions through VEGF- and CXC-KC-independent pathways.

Blockade of Gal-1 *in vivo* reduces the growth and vascularization of endometriotic lesions

In the quest for a therapeutic agent capable of mitigating the formation of vascular networks and reducing the size of endometriotic lesions, we evaluated the effects of a recently validated anti-Gal-1 (F8.G7) neutralizing mAb [14,15,24]. Intraperitoneal injection of F8.G7 mAb into mice bearing endometriotic lesions did not lead to changes in the number of lesions established per mouse compared with mice receiving an isotype control mAb ($p = 0.1004$, Figure 4A), although the mean size of the lesions was 63.2% smaller when Gal-1 was blocked using the F8.G7 mAb ($p = 0.0148$, Figure 4B). These results validated the potential therapeutic benefits of blocking Gal-1 expression to reduce the severity of endometriosis. In addition, we also found a marked reduction of vWF-positive vascularized areas (62.3%) in endometriotic lesions of mice treated with the anti-Gal-1 neutralizing mAb compared with those treated with isotype control mAb ($p = 0.0003$, Figure 5A). Similar to the

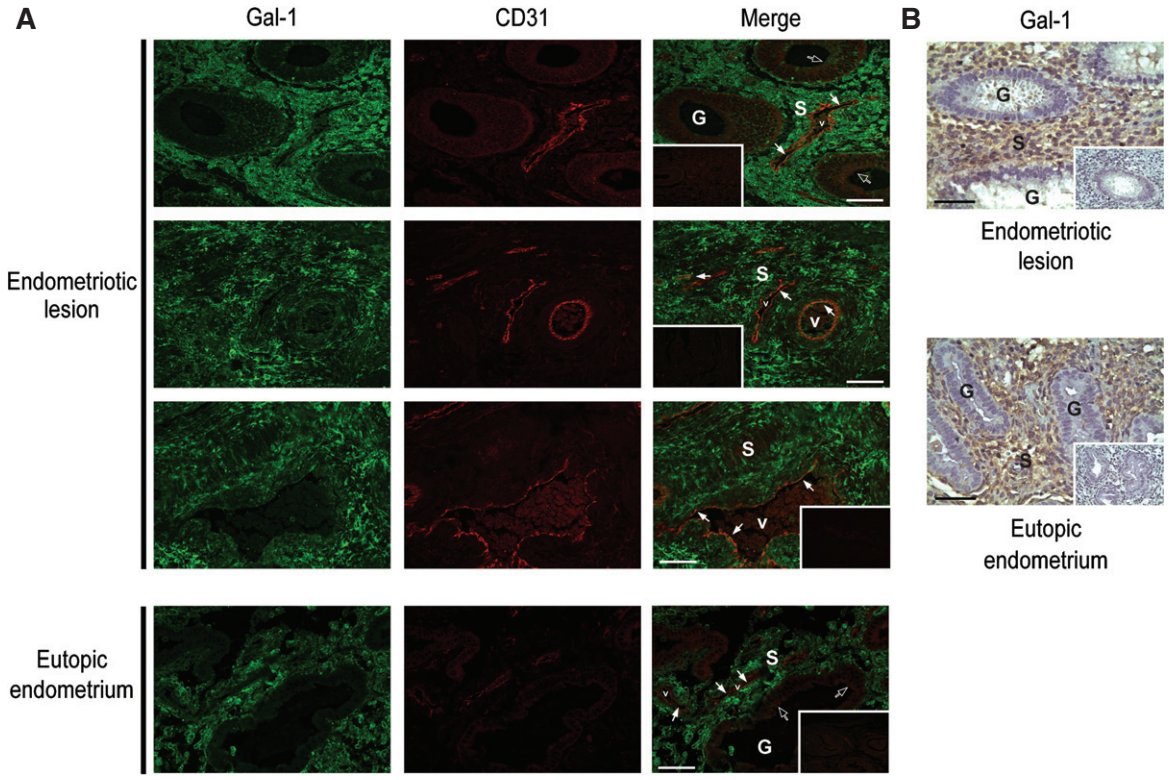

Figure 1. Gal-1 expression in endometriotic lesions (defined as endometrial stroma and glands developed outside the uterus) and eutopic endometrium (the inner membrane lining the uterus cavity) from patients with endometriosis is restricted to the stromal and endothelial compartments. (A) Representative microphotographs of indirect immunofluorescence (IFI) staining for Gal-1 (green) and the endothelial cell marker CD31 (red) was performed on tissue sections of ectopic lesions and eutopic endometrium from patients with endometriosis ($n = 3$ per group). Scale bar = 50 μm. Inset: negative control without primary antibody. G: gland; S: stroma; v: blood vessels. Filled white arrows indicate representative endothelial cells immunostained for Gal-1 and CD31, and open white arrows indicate non-specific background in epithelial cells, similarly to negative control. (B) Immunohistochemistry (IHC) assay for Gal-1 detection was performed on tissue sections of eutopic endometrium and ectopic lesions from patients with endometriosis ($n = 5$ per group). Scale bar = 50 μm. Inset: negative control without primary antibody. G: gland; S: stroma.

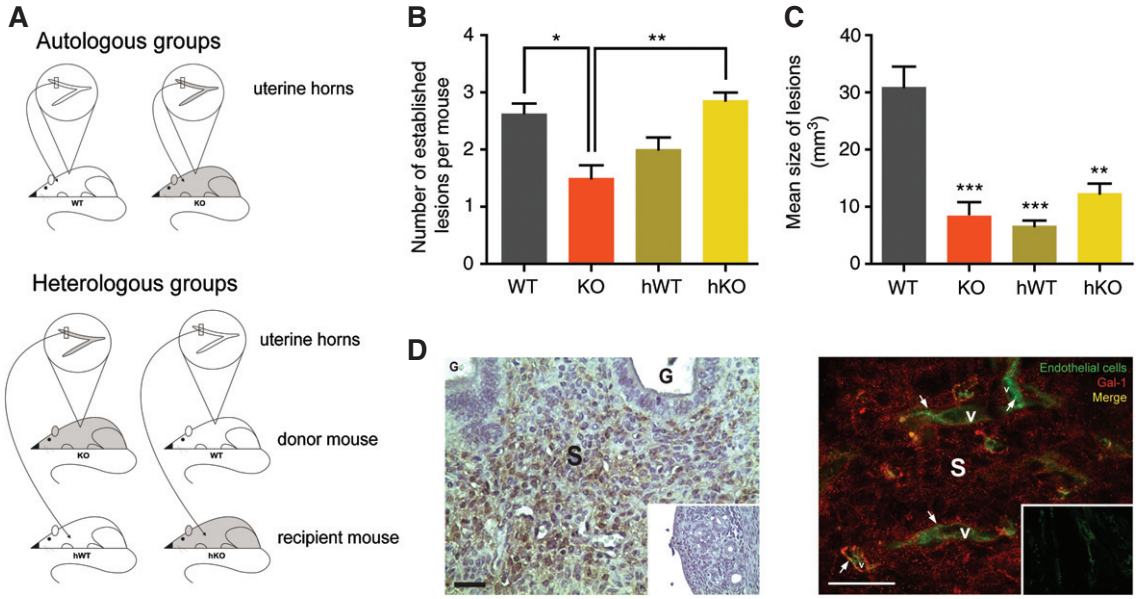

Figure 2. Lack of endogenous Gal-1 suppresses growth of endometriotic lesions in mice. (A) Representative scheme of experimental mice groups (see the Materials and methods section). (B) Mean number of established lesions and (C) the mean size of lesions are shown in graphs ($n = 10$ for WT, KO, and hWT groups, and $n = 7$ for hKO group). (D) IHC and IFI assays of Gal-1 in endometriotic lesions induced in WT mice ($n = 3$). Scale bar = 30 μm for IHC and 75 μm for IFI. Inset: negative control without anti-Gal-1 antibody. G: gland; S: stroma; v: blood vessel. Arrows denote Gal-1 expression in endothelial cells immunostained with FITC-conjugated *Lycopersicon esculentum* lectin. $^{*}p < 0.05$; $^{**}p < 0.01$ versus KO group for the mean number of established lesions, and $^{**}p < 0.01$; $^{***}p < 0.001$ versus WT group for the mean size of lesions.

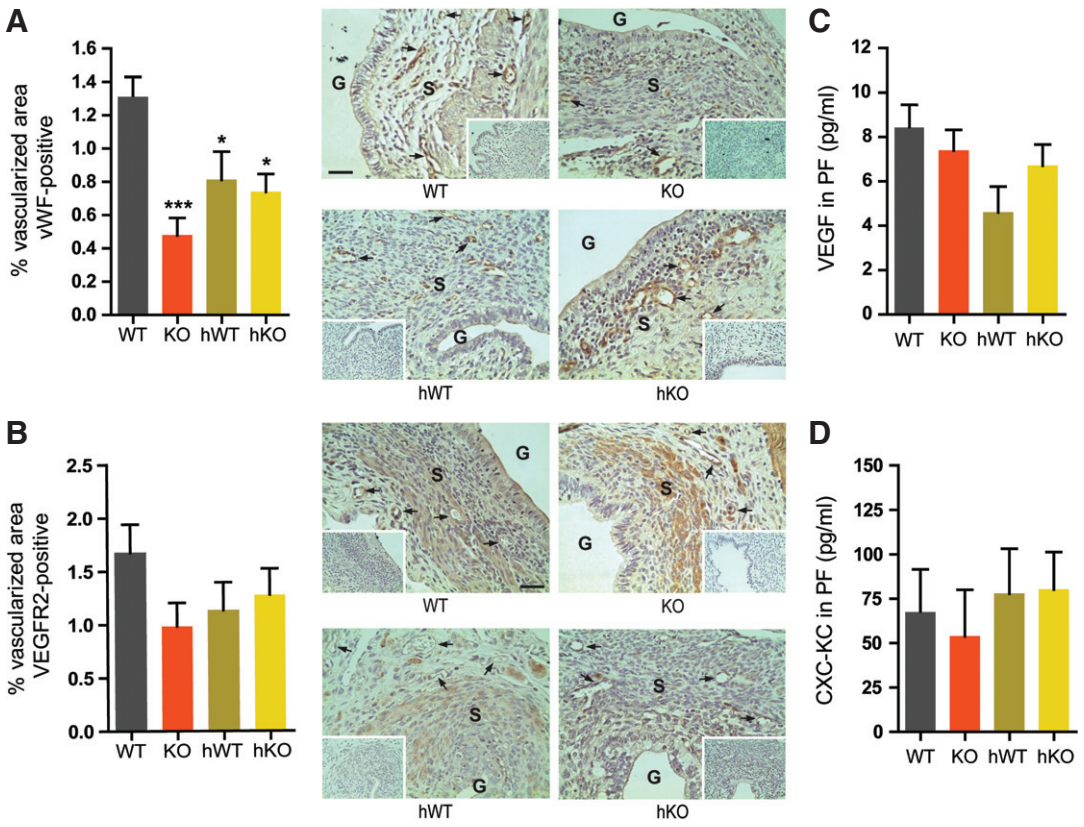

Figure 3. Gal-1 promotes the vascular network development of endometriotic lesions in mice. (A) Relative vascularized area stained for vWF ($n = 6–8$ animals per group) and (B) the relative vascularized area stained for VEGFR2 ($n = 6–8$ animals per group) were evaluated by IHC. Scale bar = 50 µm. Inset: negative control without primary antibody. G: gland; S: stroma. Arrows denote vWF- or VEGFR2-positive blood vessels. (C) VEGF-A concentration in mouse peritoneal fluid ($n = 7–10$ animals per group) and (D) CXC-KC concentration in peritoneal fluid ($n = 6–7$ animals per group) were measured by ELISA. $^{*}p < 0.05$; $^{***}p < 0.001$ versus WT group.

findings observed in *Lgals1*$^{-/-}$ mice, we found no differences in the vascularized areas of VEGFR2-positive vessels in endometriotic lesions of anti-Gal-1-treated mice versus the isotype-treated control group ($p = 0.3235$, Figure 5B). Moreover, no fluctuations were detected in the concentrations of VEGF-A or CXC-KC in peritoneal fluid from the different groups analysed ($p = 0.1574$, Figure 5C and $p = 0.1526$, Figure 5D, respectively). These results emphasize the direct involvement of Gal-1 in the vascularization of ectopic endometriotic lesions.

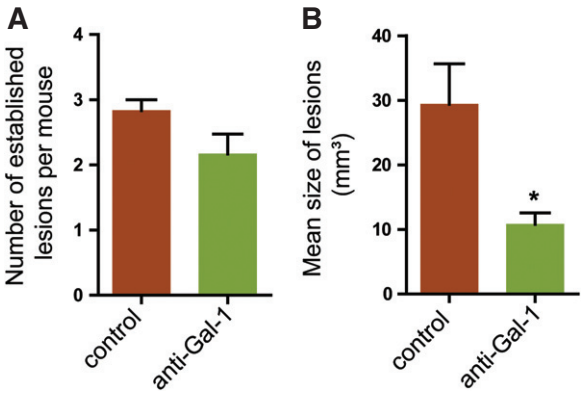

Figure 4. Gal-1 blockade in the peritoneal cavity reduces the growth of endometriotic lesions in wild-type mice. (A) Mean number of established lesions and (B) the mean size of lesions are shown in graphs ($n = 6$ per group). $^{*}p < 0.05$.

Discussion

Increased incidence of endometriosis and lack of effective therapeutic modalities urge a more in-depth understanding of the mechanisms and molecular pathways that influence the development and severity of the disease. Similar to neoplastic and retinal diseases, selective interruption of vascular signalling networks represent a major challenge for disease treatment and management. Thus, an increasing number of studies have focused on investigating anti-angiogenic strategies for the treatment of endometriosis. These include selective blockade of growth factor signalling, administration of statins, cyclo-oxygenase-2 inhibitors, dopamine agonists, and peroxisome proliferator-activated receptor agonists, among others [7,27,30–33].

Recent efforts involving the genetic manipulation of *N*- and *O*-glycosylation pathways, as well as blockade of endogenous galectins, have illuminated essential roles of lectin–glycan interactions in regulatory circuits that critically influence inflammatory and vascular pathologies [11,34]. The present study highlights the central role of endogenous stromal-derived Gal-1 in

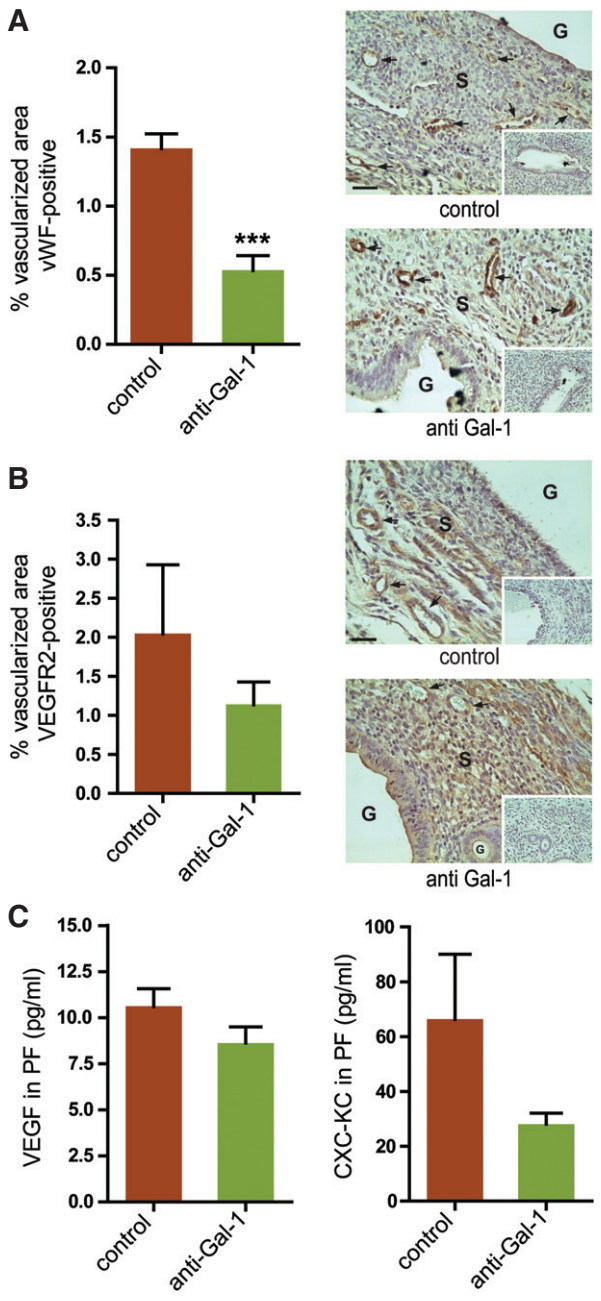

Figure 5. Peritoneal blockade of Gal-1 impairs vascular development of endometriotic lesions in wild-type mice. (A) Relative vascularized area stained for vWF ($n = 6$ in anti-Gal-1 group and $n = 5$ in control group) and (B) the relative vascularized area stained for VEGFR2 ($n = 5$ in anti-Gal-1 group and $n = 4$ in control group) were evaluated by IHC. Scale bar = 50 μm. Inset: negative control without primary antibody. G: gland; S: stroma. Arrows denote vWF- or VEGFR2-positive blood vessels. (C) VEGF-A concentration in mouse peritoneal fluid ($n = 6$ per group) and (D) CXC-KC concentration in peritoneal fluid ($n = 6$ per group) were measured by ELISA. $^{***}p < 0.001$.

promoting the formation of vascular networks during endometriosis. Accordingly, in recent studies we have demonstrated that Gal-1 links tumour hypoxia to angiogenesis in viral-associated tumours [14] and co-opts the VEGFR2 signalling pathway to preserve angiogenesis in tumours refractory to anti-VEGF therapy [15]. This pathway involves selective remodelling of glycosylated receptors in tumour-associated vessels in immunosuppressive versus pro-inflammatory microenvironments. In this study we found that Gal-1 is selectively expressed in the stromal and endothelial cell compartments of human and mouse endometriotic tissue and contributes to vascularization and growth of endometriotic lesions independently of VEGF and CXC-KC. Lack of Gal-1 in the lesions themselves or in the abdominal cavity of mice harbouring endometriotic lesions resulted in a marked reduction of lesion size.

Given the broad immunoregulatory activities of Gal-1, including promotion of T-cell apoptosis, induction of tolerogenic dendritic cells, expansion of T regulatory cells, and polarization of macrophages towards an alternative M2 phenotype [11], we cannot rule out possible consequences of Gal-1 blockade not only in the regulation of the angiogenic phenotype but also in the control of the associated inflammatory response. In this regard, different members of the galectin family can modulate endothelial cell biology through different mechanisms. While Gal-1 binds directly to VEGFR2 [15] or neuropilin-1 on endothelial cells [35] and promotes H-Ras signalling to the Raf/extracellular signal-regulated kinase (ERK) kinase cascade [13], Gal-3 induces endothelial cell morphogenesis through binding to *N*-glycans on $\alpha_v\beta_3$ integrin [36]. On the other hand, Gal-8 triggers endothelial cells signalling through binding to the activated leukocyte cell adhesion molecule (ALCAM; CD166) [37]. As Gal-3 is also up-regulated in endometriotic tissue compared with eutopic endometrium [38] and Gal-8 is abundantly expressed in the endometrium during the luteal phase [39], the contribution of other members of the galectin family to disease severity and progression should be explored.

As current therapies for endometriosis, including surgery and/or prolonged hormonal manipulation [40,41], are aimed at ameliorating the symptoms of the disease but none of them can promote regression of endometriotic lesions or can successfully cure disease, our findings highlight a novel molecular target to reduce vascularization and growth of endometriotic lesions. In this regard, we have validated the potential therapeutic benefits of an anti-Gal-1 monoclonal Ab for the treatment of the disease. In this regard, galectin inhibitors that block the carbohydrate recognition domain have been recently developed [42–44], although they may have broad inhibitory capacity and lack of specificity for individual members of the galectin family. Interestingly, anginex, a synthetic peptide that was originally modelled to reproduce the β-sheet structure of anti-angiogenic proteins, efficiently binds to Gal-1 [45] and could also be considered for the treatment of endometriosis. In summary, our results emphasize the central role of Gal-1 in sustaining angiogenesis in endometriosis and validate this lectin as a possible target for the treatment of disease.

Acknowledgments

We thank G Sequeira, MS Bianchi, M Buffone, and M Ferrero for helpful advice. We are also grateful to M Shipp and J Ouyang for sharing the F8.G7 mAb that was generated and validated in collaboration with GAR and DOC. This work was supported by grants from the National Agency for Promotion of Science and Technology (ANPCYT) BID-PICT 1632, CONICET PIP 1223 (to RIB and GFM), PICT 2012–2440 (to GAR), and Roemmers Foundation (to GFM).

Author contribution statement

JIB, GAR, and GFM designed experiments and wrote the manuscript. JIB, AGR, MAB, CNO, and JCS performed the experiments. RIB and DOC participated in the analysis and interpretation of the data. JJS and AMG selected patients and provided biopsies.

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