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Implication of the enteric glia in the IBS-like colonic inflammation associated with endometriosis

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Abstract

Background Endometriosis is a complex gynecological disorder characterized by the ectopic growth of endometrial tissue. Symptoms of endometriosis are known to impair the quality of life of patients, and among these are found dysmenorrhea, chronic pelvic pain, and gastrointestinal (GI) issues. GI issues such as painful bowel movements, bloating and constipation or diarrhea, are one of the common reasons for misdiagnosis with irritable bowel syndrome (IBS). Enteric glial cells (EGC) are known to play a role in pain associated with IBS, and reactive gliosis has been reported in patients with IBS, but the role of EGC in endometriosis has yet to be elucidated. We hypothesized that endometriosis will induce reactive gliosis, with increased expression of the glial fibrillary acidic protein (GFAP) and S100B, in the myenteric plexus of colonic sections in an animal model of endometriosis.

Methods In the present study animal experiments were employed to explore the impact of endometriosis on the gastrointestinal tract. Using a surgically induced endometriosis rat model, we collected ileal and colonic segments for analysis. We used H&E to assess microscopic damage in colon and ileum, immunofluorescence to measure GFAP and S100B expression in the colon, and toluidine blue staining to measure mast cell infiltration in colon and ileum. Immunofluorescence images were captured using confocal microscope and analyzed using ImageJ software.

Results All endometriosis animals developed vesicles. These animals had a significant increase in the colonic macroscopic damage compared to Sham and Naïve controls. Colonic and ileal sections didn't show statistical differences in microscopic damage between groups, yet endometriosis ileum had significantly increased mast cell infiltration compared to Naïve. GFAP immunostaining showed significantly increased integrated density in endometriosis when compared to Sham or Naïve, while no statistical difference was found in S100B integrated density between groups.

Conclusions We conclude that endometriosis can alter GI homeostasis by inducing colon inflammation, reactive gliosis, and ileal mast cell infiltration. Taken together this suggests endometriosis can mimic IBS histopathology beyond the symptomatology, reinforcing this disease's complexity and the need to treat it beyond the gynecological setting.

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Keywords Endometriosis, Enteric glia, Inflammation, Colon

Background

Endometriosis is a complex gynecological disease that affects around 10% of women during their reproductive age. This disease is characterized by the ectopic growth of endometrial tissue in the peritoneal cavity, and symptoms such as dysmenorrhea, chronic pelvic pain, as well as gastrointestinal (GI) issues that hinder the quality of life of patients [1–3]. Among gastrointestinal symptoms, women with endometriosis have reported abdominal pain, painful bowel movements, diarrhea, constipation, and bloating, independently of lesion localization leading to misdiagnosis with irritable bowel syndrome (IBS) [4, 5]. IBS is a gastrointestinal disorder characterized by abdominal pain accompanied by painful bowel movements, bloating, diarrhea and/or constipation that can severely impact patient lives and every day activities [6]. IBS prevalence is estimated to be 8–20% of the worldwide population, and alarmingly women are diagnosed twice as much with IBS than men [7].

Interestingly, a systemic review and meta-analysis aimed at identifying if there was an association between endometriosis and IBS, found that women with endometriosis are three times more likely to have IBS than women without [3]. Further studies have hypothesized that comorbidity of endometriosis and IBS exists given women with endometriosis have a higher risk of having IBS than women without the condition [8]. A recent systemic review, and meta-analysis [9], tried to determine whether endometriosis and IBS are comorbid through systematic analysis of published literature, but the lack of studies, and multifactorial nature of the conditions, didn't allow the authors to come to a proper conclusion. The authors therefore proposed that endometriosis could be inducing an IBS-like syndrome, rather than representing a comorbidity due to the inability of the Rome criteria to differentiate between the two conditions. Hence, there is still a clear need to understand how endometriosis impacts the gastrointestinal system and elucidate the pathophysiological relationship between endometriosis and IBS.

As it stands right now, to our knowledge, there are no studies regarding how endometriosis might be affecting the gastrointestinal system. And while the etiology of IBS remains elusive, studies aiming to resolve this have proposed an interplay between the brain-gut axis, microflora and immune cells, resulting in low-grade inflammation [6]. Increasing evidence puts the enteric nervous system at the center of IBS pathophysiology, specifically the enteric glia cells (EGCs) [10]. Clinical studies have shown increased inflammation within the enteric nervous system, with increased expression of glial fibrillary acidic protein (GFAP), resulting in enteric reactive gliosis [11]. EGCs are known to have a bidirectional relationship with immune cells during physiological states, and when presented with an immune challenge, EGCs enter in a reactive gliosis state with increased expression of GFAP and S100B [12], leading to pain sensitization [13].

Based on the strong association between endometriosis and IBS, we therefore hypothesized that endometriosis would induce colonic inflammation and enteric reactive gliosis. To address this hypothesis, we used a well validated auto-transplantation rat model of endometriosis, allowing us to collect functional and histological data. This is the first study to explore the changes endometriosis induces to the gastrointestinal system considering the question as to whether endometriosis and IBS are comorbid. Understanding the effects of endometriosis in the enteric glia could open a new path for treatments, especially in women with persistent gastrointestinal symptoms that continue hindering their quality of life. This could also shed light on new therapeutic targets beyond the gynecological setting.

Methods

Animal welfare & IACUC

All animal studies were approved by the Institutional Animal Care and Use Committee at Ponce Health Sciences University (PHSU). Female Sprague Dawley rats of approximately 2 months old (Animal Research Facilities, PHSU, PR), weighing approximately 190–200 g, were randomized by a third party to one of three groups: Naïve ($n=8$), Sham Control ($n=8$) and Endometriosis ($n=8$). Treatment assignment was kept at all times from the researcher to reduce bias at the moment of behavior assessment. The sample size was calculated a priori using the means and deviations of preliminary data with 95% confidence (type I error = 5%), and a power of 80% using G*Power Software (G*Power, RRID: SCR_013726). Animals were housed single caged in a room maintained at 23 °C with light/dark cycle alternating every 12 h. Animals had access to normal chow and water *ad libitum*. A series of vaginal smears were collected one week before surgery, at time of surgery, one week after surgery, and at time of sacrifice to assess reproductive cyclicity. Smears were analyzed by staining with Wright's stain following the method outlined in Cuevas et al. [14]. This study complies with ARRIVE guidelines for animal studies reporting.

Endometriosis induction

Endometriosis was induced following the Vernon and Wilson model (1985) [15] and as described by our group

previously [16]. Animals were weighed and anesthetized with pentobarbital (10 mg/kg i.p.) and a small laparotomy was performed to access the uterine and intestinal mesentery. The distal 2 cm of right uterine horn were surgically removed and immersed in warm (37 °C) sterile culture medium. The endometrium was exposed by opening the uterine horn lengthwise with sterile scissors. Four pieces of the autologous uterine tissue were cut in 2 mm pieces and implanted near the mesenteric vessels of the small intestine with the serosal surface facing the vessels. Sham surgery animals received mechanical manipulation on the right uterine horn for two minutes and four sutures without implants on the intestinal mesentery. The incision was closed, and animals were allowed to recover for 21 days.

Pain behavior assessment

Pain behavior using Electronic Von Fey and Hot Plate was assessed at three different timepoints: before surgery at day −1 (baseline), two weeks after surgery, and before sacrifice, as described below.

Electronic von frey

Animals were acclimatized to the chambers of the elevated grid for 15 min, then an additional 15 min with the researcher in the room so as to acclimate them to their presence and decrease any stress effects. After this, using the non-bending filament attached to the Electronic Von Frey apparatus (Ugo Basile, Italy), we pushed each hind paw three times and collected the force measurement necessary to elicit paw withdrawal of each animal in the Data Collection Application software (Ugo Basile, Italy) [17]. Recorded data was then exported and organized in an excel sheet.

Hot plate

Animals were acclimatized 10 min to the Hot Plate (Colombus Instruments, Columbus, OH) (not heated) the day before the test to reduce the stress of manipulation and enclosure. The day of the Hot Plate animals were brought to the testing area where the Hot Plate was heated to 52 degrees Celsius [18]. We tested each animal for 40 s and video recorded the behavior, using a Logitech Webcam (Logitech, San Jose, CA) connected to computer, to properly measure the time animals took to respond to the heat stimulus. The Hot Plate was cleaned between animals.

Endometriosis assessment

At time of sacrifice a laparotomy was performed and the peritoneal cavity was examined for the presence of developed endometriosis as described before [19]. Once identified, endometriotic vesicles were carefully removed and their longest length and width measured with a digital caliper to calculate vesicle area. The vesicles were weighed individually, collected and stored in a -80 degrees Celsius freezer for future experiments.

Colon assessment and collection

The distal colon was excised and opened along the mesentery line. The tissue was assessed visually and scored for the presence of adhesions (zero to two for none to major, respectively), hyperemia (zero to ten, for no damage to major damage), diarrhea (zero to one, absence or presence of watery feces), and bowel wall thickness (measured in mm with a caliper) as described by Appleyard and Wallace (1995) [20]. The individual parameters were summed to give a macroscopic score. After assessment, around 2.5 cm were collected in cold Hanks' Balanced Salt Solution, washed in phosphate-buffered saline (PBS) and fixed with paraformaldehyde (PFA) 4% for 3 h at room temperature for whole mount preparation.

Approximately 1.5 cm long sections of full thickness medial colon and distal ileum were collected and fixed in 10% buffered formalin for 72 h. Later, tissues were dehydrated in a series of ascending alcohols, methyl salicylate and then embedded in paraffin. Embedded tissue was cut on a microtome at 4 µm and set on charged slides for subsequent staining and analyses.

Microscopic damage assessment

Microscopic damage was assessed by two observers, blinded to treatments, as described elsewhere [21]. We assessed loss of mucosal architecture (zero to three for mild to severe, respectively), cell infiltration (zero for absence of infiltration, and one to three to indicate the location: muscularis mucosae, lamina propria and serosa, respectively), muscle thickening (zero to three, indicating less than half of muscle thickening, half to three fourths of muscle thickness, mucosal thickness and all muscle, respectively), goblet cell depletion (zero to one, for absence or presence, respectively) and crypt abscess (zero to one, for absence or presence, respectively). The total sum of these scores produced the microscopic score.

Mast cell analysis

Colonic and ileal mast cells were stained with toluidine blue as described previously [14, 19]. Mast cells were counted in whole tissue by two observers blinded to treatments, and the counts averaged. Whole tissue area was measured (mm^2), and mast cell count was normalized to area.

Whole-mount immunofluorescence

Distal colon fixed with PFA 4%, was pinned down on a Sylgard plate (World Precision Instruments, Inc, #SYLG184) with mucosa facing up. Using a dissection microscope (LEICA EZ4 W, Leica Microsystems),

the inner layers were dissected leaving only the longitudinal muscle with myenteric plexus. After, tissue was cut in two 4mm^2 squares. Double immunostaining was performed using whole-mount immunofluorescence technique as described elsewhere [22]. Tissues were incubated overnight with mouse anti-GFAP (1:200, BioLegend, cat#644702) and rabbit anti-S100B (1:100, Abcam, cat#abEP1576Y). Next day tissues were incubated for 2 hours with secondary antibodies Alexa Fluor 488 goat anti-mouse (1:500, Invitrogen, cat#A-11029, RRID: AB_2534088) and Alexa Flour 555 goat anti-rabbit (1:500, Invitrogen, cat#A-21428, RRID: AB_141784). Finally, tissues were incubated for 5 min with DAPI (Invitrogen, cat#R37606), washed twice in PBS for 5 min and mounted on charged slides (VWR, cat#48311-703) with Prolong Gold Antifade Mounting Media (Invitrogen, cat#P36934). Fluorescence was assessed using the HC PL APO 60x/1.40 oil immersion objective of an Eclipse Ti2 Nikon Confocal Microscope. DAPI staining was excited using 405 nm emission laser, Alexa Fluor 488 was excited using 488 nm emission laser, and Alexa Fluor 555 was excited using 561 nm emission laser; all located in LU-N4 laser unit. Images were captured using NiS Elements AR Software (Ver. 5.30.05). All microscope equipment and parts are from Nikon Instruments, Inc. (Melville, NY). Three high power fields per animal were quantified for fluorescence integrated density using ImageJ Software (ImageJ, RRID: SCR_003070).

Immunohistochemistry

Colonic tissue sections were deparaffinized in xylene substitute for 30 min and rehydrated in a descending series of alcohols to water. Tissue sections were prepared for immunohistochemistry as explained elsewhere [19]. Protein block was performed using normal goat serum for 15 min (Cat#HK112-9KE, BioGenex), followed by an overnight incubation with α-7 receptor primary antibody (1/50 dil. Cat#SC-58607, Santa Cruz Biotechnology). A negative control with PBS instead of primary antibody was run in each slide. A Multi-Link was used as the secondary antibody for 20 min. The slides were placed in the Streptavidin Peroxidase for 20 min (Super Sensitive Link-Label IHC Detection System, Cat#LP000-UCLE, BioGenex), followed by PBS wash for 5 min. For development, one drop of 3,3′ Diaminobenzidine (DAB) (Cat#HK542-XAKE, BioGenex) was used on each tissue and the exposure was monitored for 2:30 min under a light microscope. Then, the slides were dipped in distilled water, washed with running water for 5 min, dehydrated, cleared with xylene and mounted with Cytoseal 60 (Cat#8310-4, Thermo Scientific). Three representative HPF per tissue were photographed using a Nikon Confocal Microscope A1 (Ver.4.10) with NIS Elements Software (AR 2.22.15 and analyzed blindly for α-7 receptor expression by an observer using Image J software.

Statistical analysis

Data was graphed and analysed by using GraphPad Prism version 9.5.1 (GraphPad Prism, San Diego, CA, RRID: SCR_002798). A $p<0.05$ was considered to represent a statistical significance difference. The mean difference ± the standard deviation (S.D.) was used to assess the differences among treatment groups. ROUT test was used to identify and exclude outliers. In order to assess the statistical significance of the mean differences, a parametric one-way ANOVA was used for normally distributed variables, and post-hoc comparisons were done using the Student-Newman-Keuls. A non-parametric Kruskal-Wallis H test was used for variables that did not follow a normal distribution. To assess the statistical significance of pain behavior we used a paired Two-Way ANOVA with repeated measures and post-hoc comparisons were done using the Dunnett's test.

Results

Endometriosis induces allodynia but not hyperalgesia

Two-way ANOVA analysis revealed that endometriosis animals developed allodynia by Day 19 (11.53 ± 6.66 g) compared to baseline (22.96 ± 8.27 g; $p<0.05$), whereas sham animals developed allodynia by day 12 (11.93 ± 8.56 g) (after surgery) compared to baseline (17.06 ± 6.58 g; $p<0.05$), but reverted by day 19. Naïve animals remained normal throughout protocol (Fig. 1a). No difference was found across groups for hot plate latency time (Fig. 1b)

Endometriosis development

At time of sacrifice endometriosis animals had 96.88% of the implanted autologous tissue develop into endometriotic vesicles. The average vesicle weight per animal was 55.98 ± 22.67 mg, with an average area of 15.78 ± 5.42mm^2. Animals that underwent sham surgery didn't have any vesicles develop.

Endometriosis induces low level colonic inflammation

After ordinary one-way ANOVA analysis, the total colonic macroscopic score was found to be significantly increased in animals with endometriosis (Fig. 2a; 3.09 ± 0.39; $p<0.0001$) when compared to Naïve (0.28 ± 0.23) and Sham (0.47 ± 0.38) groups. Animals with endometriosis had significantly increased presence of adhesions (Fig. 2b), hyperemia (Fig. 2c) and colon thickness (Fig. 2d) when compared to control groups. Diarrhea was not observed in any group.

Microscopic examination of colonic and ileal regions revealed no damage in animals with endometriosis as compared to control group (data not shown).

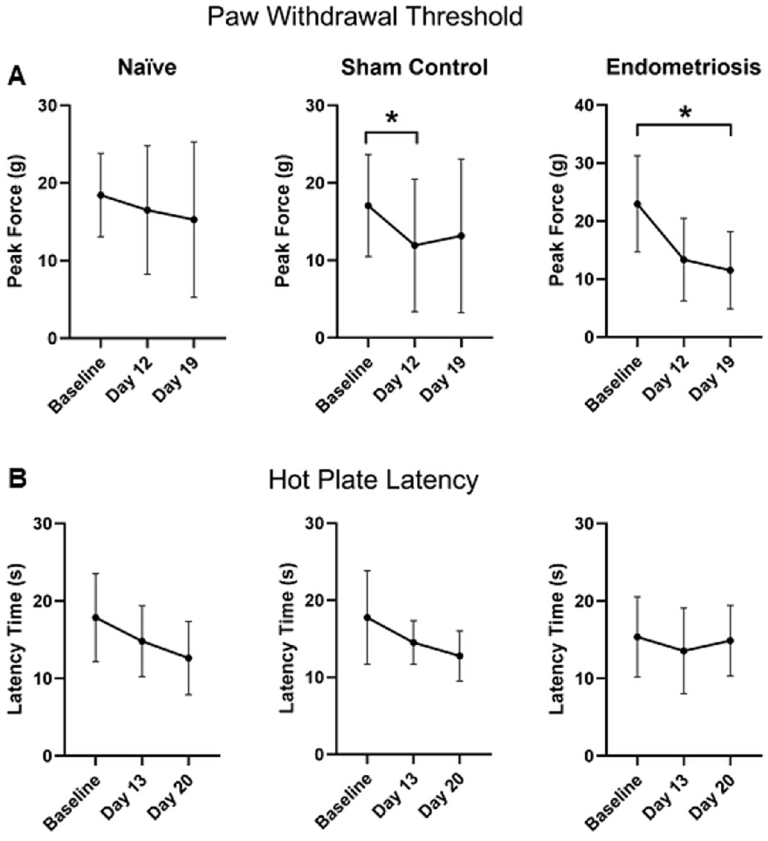

Fig. 1 Pain associated with endometriosis. **A**) Paw withdrawal threshold of Sham Control decreased significantly by Day 12 (after surgery) but returned to normal by Day 19. Endometriosis animals paw withdrawal decreased significantly by Day 19. **B**) No difference in hot plate latency time was found across groups ($n = 8$ animals/per group ± s.d. $^*p < 0.05$)

Examination of mast cells in the colon of endometriosis animals (Fig. 3a) shows a tendency towards increased mast cell infiltration, with a 63.1% increase of the mean compared to Sham and 34.4% increase when compared to Naïve, but this did not reach statistical significance. Ordinary one-way ANOVA revealed ileal mast cell count (Fig. 3b) to be significantly increased in endometriosis ($8.51\times10^{-6} \pm 4.11\times10^{-6}$ /μm^2; $p<0.05$) group when compared to naïve ($3.89\times10^{-6} \pm 1.66\times10^{-6}$ /μm^2).

Endometriosis induces colonic reactive gliosis

After Kruskal-Wallis test, GFAP (Fig. 4a) immunoreactivity was found to be significantly increased in the endometriosis group (25,655 FU±5355; $p<0.0001$) when compared to Naïve (9,035 FU±2725) and Sham (13,650 FU±1542) groups, while no significant difference was found for S100B (Fig. 4b).

No significant changes were observed in α-7 receptor expression in colon tissue (Fig. 5).

Discussion

Over the last several years there have been great advancements in the understanding of endometriosis and its pathophysiology, but there is still a lack of research on its extra-gynecological manifestations and comorbidities. Among them is the development of IBS-like symptoms that often leads to an IBS diagnosis before the correct endometriosis one due to the gastrointestinal symptoms being clinically impossible to differentiate [23]. This adds to the delay in diagnosis for endometriosis that can be up to 7 years on average, which is further precipitated by the frequent dismissal of symptoms by clinicians [24]. Recent systemic reviews and meta-analyses aimed to estimate the prevalence of IBS in endometriosis patients and found that women with endometriosis have an approximately threefold increased risk of developing IBS [3]. One theory to explain this finding proposes that the peritoneal inflammatory environment created by endometriosis might induce changes in the near non-inflamed organs [25], however the question still remains whether endometriosis induces IBS-like symptoms, or the diseases are comorbid [9]. Thus, we aimed to elucidate whether induction of endometriosis could be enough to induce low-grade gastrointestinal inflammation with IBS features such as mast cell infiltration and reactive gliosis.

The most prominent shared hallmark of both endometriosis and IBS is abdominopelvic pain [26, 27]. In the present study, we observed that animals with

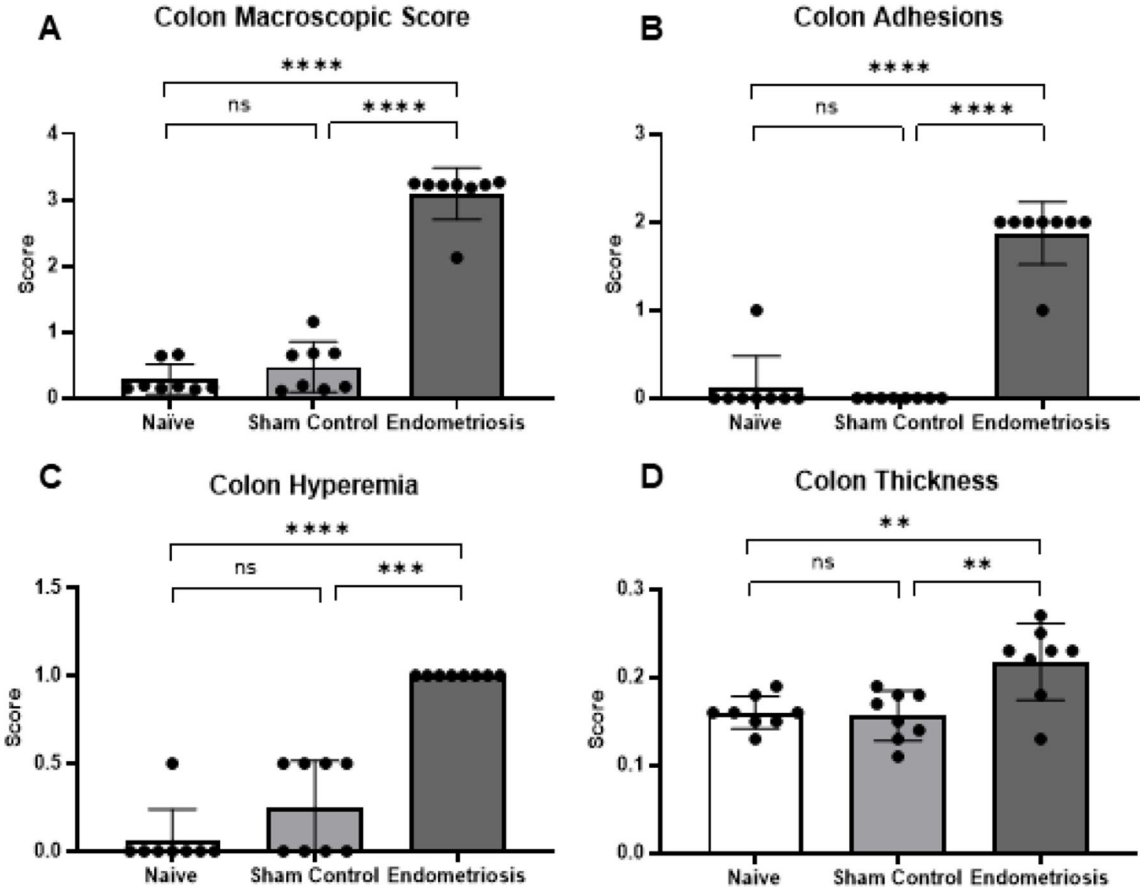

Fig. 2 Colonic damage associated with endometriosis. **A**) Colonic damage was significantly increased in endometriosis animals compared to Naïve and Sham Control. Analyzed parameters show endometriosis has increased **B**) adhesions, **C**) hyperemia and **D**) full thickness ($n = 8$ animals/group ± s.d.**$p < 0.01$ ***$p < 0.001$ ****$p < 0.0001$)

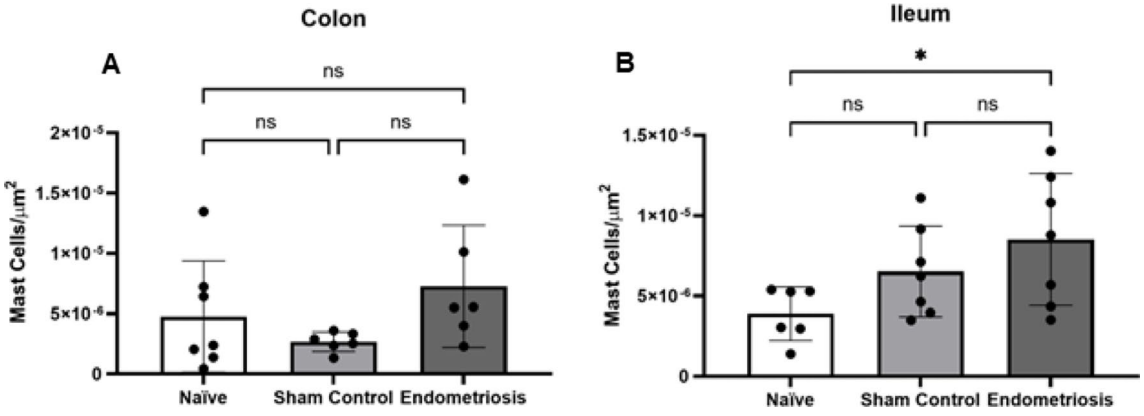

Fig. 3 Endometriosis increases ileal mast cell infiltration. **A**) No significant differences were found in colonic mast cell count among groups. **B**) Endometriosis animals showed a statistically significant increase in ileal mast cell infiltration when compared to Naïve ($n = 6$–8 animals/group ± s.d. *$p < 0.05$)

endometriosis had increased mechanical allodynia by day 19 post-induction, suggesting increased peripheral somatic pain. While visceral hypersensitivity is widely regarded as the underlying cause of IBS-associated pain [28], there is evidence from animal models suggesting IBS also increases peripheral somatic pain [as reviewed by [29]]. These findings are consistent with our observations. The link between endometriosis and IBS-associated pain may be explained by central sensitization [30]. A clinical study found women with IBS had abnormal endogenous pain modulation and somatic hypersensitivity, pointing to central sensitization as the driver [31].

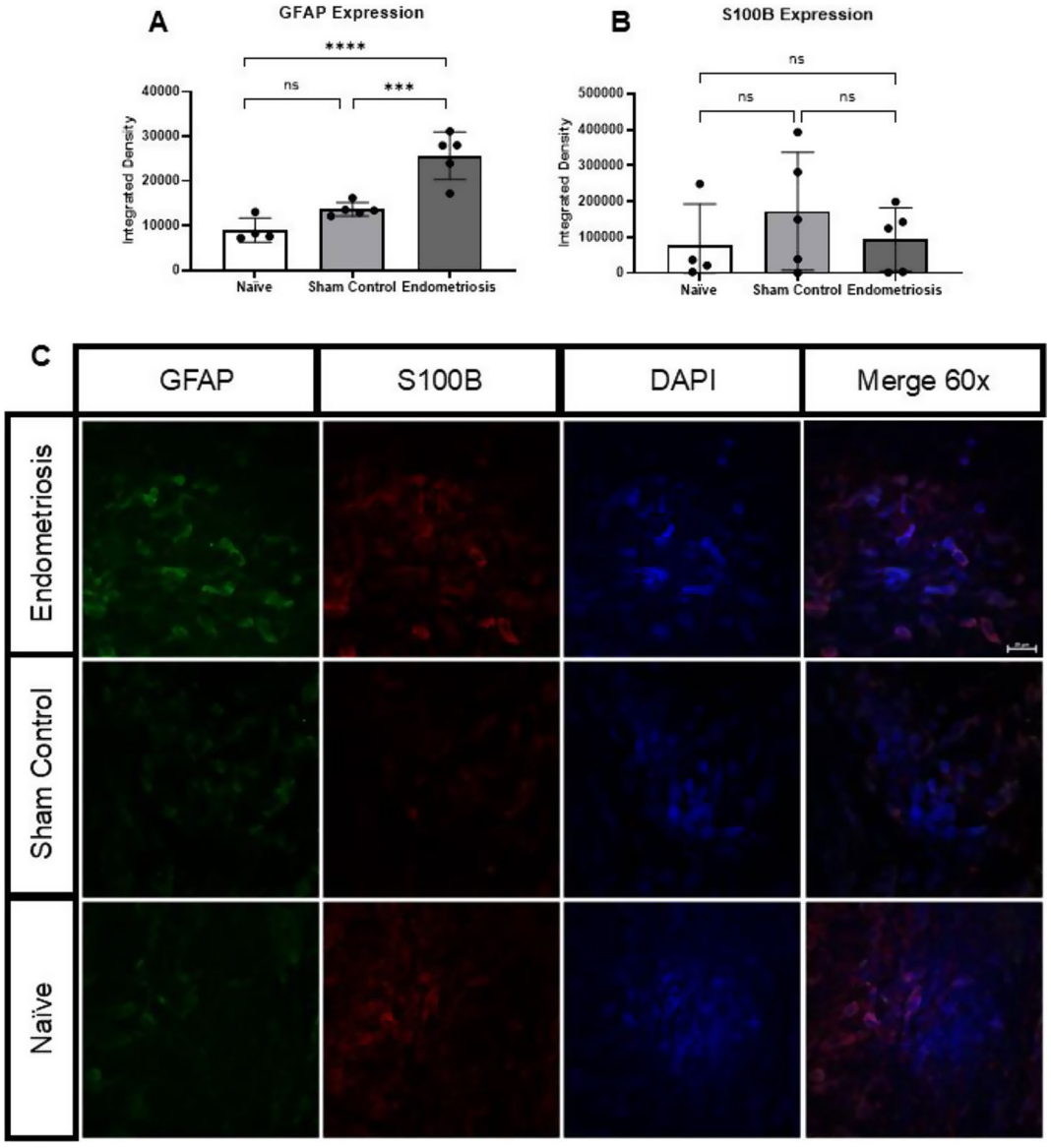

Fig. 4 Endometriosis induces reactive gliosis. (**A**) Endometriosis animals showed an increase of GFAP expression when compared to Sham Control and Naïve animals. (**B**) No significant differences were found among groups for S100B expression. (**C**) Representative pictures of GFAP, S100B, DAPI and Merged for colonic tissues (4–5 animals/group ± s.d. ***$p < 0.001$ ****$p < 0.0001$)

Similarly, a meta-analysis of clinical research on endometriosis-associated pain concluded that the pain experienced by women with endometriosis is related to central sensitization [32].

A recent study found that patients with IBS showed reactive gliosis in colonic biopsies, with increased GFAP expression but no differences in S100B [11]. Our results show that endometriosis animals have higher colonic damage, demonstrate reactive gliosis as seen by the higher expression of GFAP, and have increased ileal mast cell infiltration, all of which are characteristic of IBS. The presented results align with clinical studies reporting that endometriosis patients present gastrointestinal symptoms, like those in IBS, suggesting an association between the two conditions [9]. Therefore, the original hypothesis that endometriosis would induce colonic inflammation and enteric reactive gliosis is partially validated by the results presented here and opens a series of subsequent questions that could be addressed in future experiments.

Our group have previously reported the effects of peritoneal endometriosis on colonic tissue, showing increased colonic damage at 60 days after endometriosis induction surgery [16]. The data presented here show that the damage can be developed as early as 21 days after the induction of endometriosis, similar to what has been observed in another prior study from our lab [18]. Interestingly, colonic damage was not accompanied by

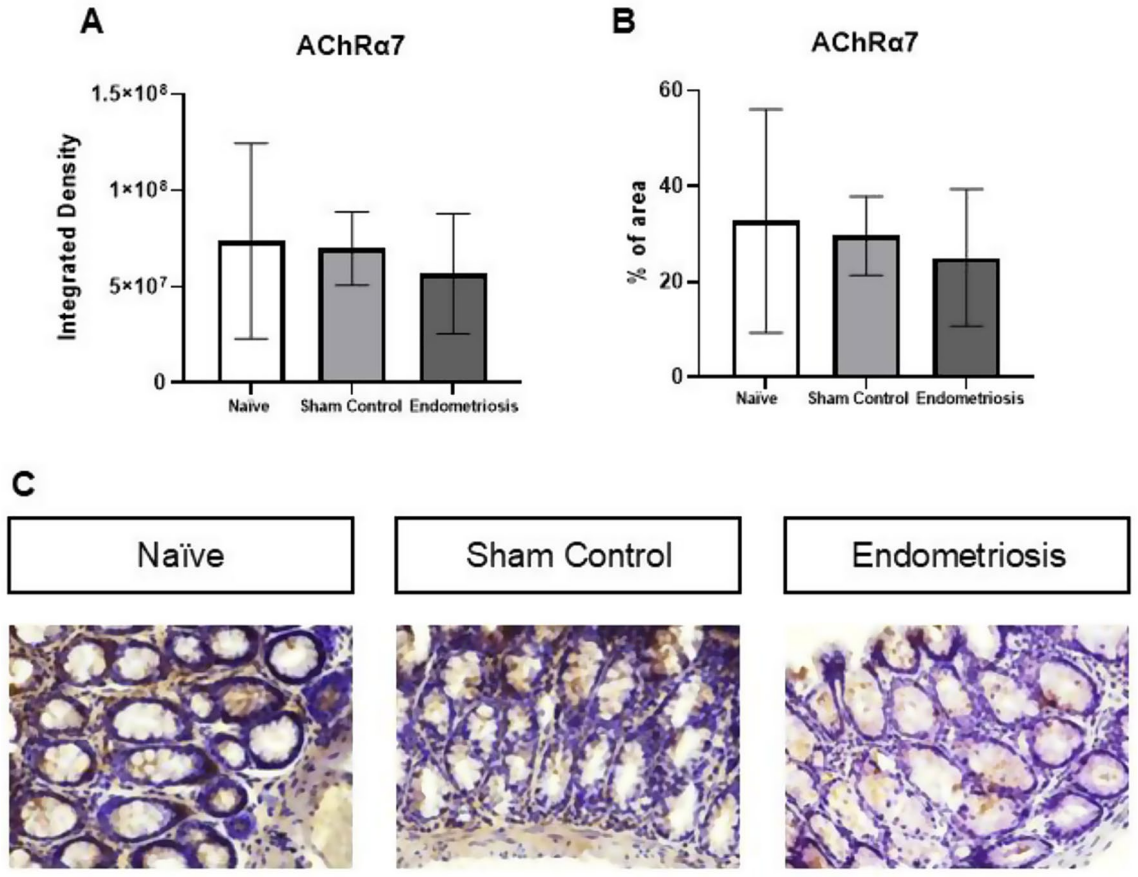

Fig. 5 Endometriosis induces no changes in α-7 receptor expression. **A**) Endometriosis animals showed no statistically significant difference in α-7 receptor integrated density or **B**) percentage of area compared to Naïve and Sham Control. **C**) Representative pictures of Naïve, Sham Control, and Endometriosis colonic tissues ($n = 6–8$ animals/per group ± s.d.)

microscopic damage, parallel to what has been reported in an IBS rat model of water avoidance stress [33]. It should be noted that the colonic damage we observe is on the low end compared to that found in colitis animal models [21], and this is further supported by IBS research that points to low level mucosal inflammation as the cause for symptoms [34]. Importantly, our sham animals didn't develop colonic damage, despite the nature of the surgery, leading us to conclude that the damage is most likely induced by the endometriosis and not the surgical procedure *per se*.

We also looked at the presence of mast cells in the colon and ileum. Despite the lack of organic disease in IBS, a common immunological hallmark observed both clinically and in animal models, is the increased infiltration of mast cells in the gastrointestinal tract compared to healthy controls [35]. Mast cells are known to infiltrate and contribute to IBS low-level mucosal inflammation [36] and their proximity to enteric nerves is known to correlate with abdominal pain in IBS [37]. Studies in ileum biopsies of patients with IBS have also found increased mast cells [38] and decreased serotonin cells that correlate with visceral pain [39]. Similarly, various studies have reported increased mast cell infiltration in lesions of women, and in an animal model, with endometriosis [40], and it is thought that mast cell density in the endometriotic microenvironment is responsible for the abdominal pain which occurs in patients [41]. Previous studies by our group, which investigated the impact of stress on the endometriosis animal model, found increased colonic mast cells in endometriosis animals which had been exposed to stress compared to non-stress controls, whereas no differences were found in non-stressed animals whether or not they had endometriosis [42]. Similarly, the current data presented here found no differences between groups for colonic mast cell infiltration, but did find increased mast cell infiltration in the ileum of endometriosis animals compared to naïve. Several studies have shown that collected ileum biopsies from IBS patients do show increased mast cell infiltration compared to healthy individuals [43–45], further pointing to the relevance of our results in better understanding the pathophysiological link between endometriosis and IBS.

Mast cells are known to interact with EGCs in the process of pain sensitization within the gastrointestinal tract. While EGCs were originally depicted as support cells, these are known to modulate immune responses in the enteric nervous system and support gastrointestinal homeostasis [46]. Among the different molecules involved in EGC processes, GFAP and S100B stand out. GFAP is known to help in maintaining the intestinal barrier, while secretion of S100B at low levels helps with neuronal survival. When presented with inflammation, these two molecules overexpress leading to gastrointestinal and neuro inflammation [12]. Recently, EGCs have been implicated as an important factor in the pain sensitization process in IBS [13], while a clinical study found that colon biopsies of patients with IBS had increased expression of GFAP, but no differences were observed for S100B expression. Similarly, our data shows that endometriosis animals have increased expression of GFAP when compared to control animals, while no differences are observed in S100B (Fig. 6**).** We also examined whether endometriosis altered the expression levels of the acetylcholine nicotinic receptor α-7, known to be expressed in EGCs [12]. Acetylcholine is the principal neurotransmitter in the vagus nerve, controlling immune cell functions via the α-7 receptor [47]. A recent study showed that induction of colitis in mice led to increased expression of α-7 in colonic tissue [48], possibly as a protective mechanism to attenuate the inflammation intrinsically. To our surprise however, we didn't observe any statistically significant difference in the colonic sections of our endometriosis animal model, despite the increased GFAP expression and macroscopic scoring. A possible explanation for these results may be that endometriosis is considered low-grade inflammation versus the acute inflammation of colitis, and changes in the parasympathetic response to low-grade inflammation might not be observed until a longer timeline.

To our knowledge this is the first study to observe the impact of non-intestinal endometriosis on colonic enteric glia and ileal mast cells. These observations are clinically relevant based on the high prevalence of gastrointestinal symptoms found in women with endometriosis, and the detrimental effects these have on their quality of life. However, there are some limitations. The present study uses a rat animal model of endometriosis to investigate the effects on the gastrointestinal system. While there is translational value in the results, they may not be directly comparable to humans due to potential species-specific differences in the response to endometriosis. However, this model has been well validated by our group and others and has shown homologous immunological responses to human endometriosis [49, 50]. Further, Sprague-Dawley rats are frequently used for gastrointestinal research due to homologous morphology and physiology to humans [51], making our findings valuable for understanding the relationship between IBS and endometriosis

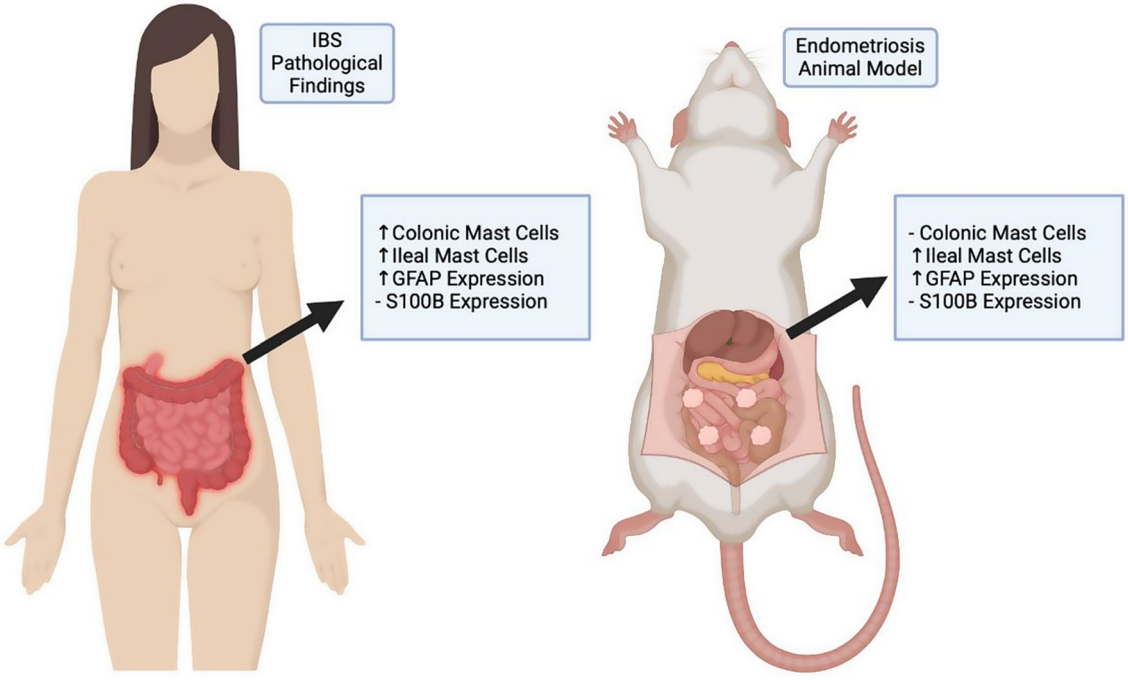

Fig. 6 Translational assessment. Visual comparison between findings in the clinical scenario and our animal model. Increased numbers of mast cells in the ileum and the higher expression of GFAP with no change in S100B in our endometriosis animal model echo findings in IBS patients suggesting similarities in underlying mechanisms for gastrointestinal disturbances. ↑ means increased, **-** means no difference

in patients. The short-term observation of 21 days may not allow us to capture the long-term effects of endometriosis on the gastrointestinal system. Thus, follow up studies with a longer timeline (e.g. 60 days) should be done next to find out if reactive gliosis persists beyond the initial acute phase of endometriosis development. Also, a longer timeline would help differentiate surgery specific effects that may still be present at 21 days. While the present study provides evidence of colonic inflammation, ileal mast cell infiltration and reactive gliosis in the rat model of endometriosis, it does not investigate the underlying molecular mechanisms responsible for these changes. Future research could focus on enteric glia and macrophage interactions since macrophages are known to play an important role in endometriosis inflammation and may be closely involved with the gastrointestinal inflammation observed in our study [52]. Further research addressing these limitations could enhance our understanding of the pathophysiological relationship between endometriosis and irritable bowel syndrome.

Conclusion

Our results reinforce the complex crosstalk between the different organ systems that is significantly altered by the presence of endometriosis. In this case, the gastrointestinal tract being one of the most affected systems as strongly documented by clinical reports. We found that endometriosis inflammation causes alterations to intestinal homeostasis in an IBS-like manner even without direct gastrointestinal involvement. This is the first step towards understanding the clinical relationship between endometriosis and IBS, providing evidence that endometriosis induces gastrointestinal alterations in a non-IBS animal model. This suggests that IBS symptoms in patients with endometriosis is not necessarily comorbid, but rather an effect of the inflammation caused by the endometriosis. We recognize the possibility of full comorbidity in some patients, but additional mechanistic and clinical research is certainly needed to understand the full spectrum in light of the diverse symptomatology of both conditions.

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Author contributions
LR-A and CA designed the study. LR-A, MC, JT and UR-C performed the experiments. LR-A, MC, JT, UR-C and CA analyzed the data. LR-A and CA drafted the manuscript. All authors have carefully revised the manuscript for important intellectual content and have read and approved the final manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All research involving animals was approved by the Institutional Animal Care and Use Committee at Ponce Health Sciences University. Consent to participate declaration: not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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