Journal of Reproductive Immunology 132 (2019) 9–15

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

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

Estradiol impairs epithelial CXCL1 gradient in the cervix to delay neutrophil transepithelial migration during insemination

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ARTICLE INFO

Keywords:
Neutrophils
Cervix
Estradiol
Progesterone
ESR1
Sperm

ABSTRACT

Female reproductive mucosa must allow allogenic sperm survival whereas at the same time, avoid pathogen infection. To preserve sperm from neutrophil attack, neutrophils disappear from the vagina during the ovulatory phase (high estradiol); although the mechanisms that regulate neutrophil influx to the vagina during insemination remain controversial.

We investigated the sex hormone regulation of the neutrophil migration through the cervix during insemination and revealed that ovulatory estradiol dose fades the CXCL1 epithelial expression in the ectocervix and fornix; hence, retarding neutrophil migration and retaining them in the epithelium. These mechanisms spare sperm from neutrophil attack to preserve reproduction, but might compromise immunity. However, luteal progesterone dose promotes the CXCL1 gradient expression to restore neutrophil migration, to eliminate sperm and prevent sperm associated pathogen dissemination. Surprisingly, these mechanisms are hormone dependent and independent of the insemination. Thus, sex hormones orchestrate tolerance and immunity in the vaginal lumen by regulating neutrophil transepithelial migration in the fornix and ectocervix.

1. Introduction

Epithelial cells produce chemoattractants that guide neutrophils through the interstitium towards a site of infection/inflammation (Li et al., 2002; Swee et al., 2008; Lasarte et al., 2016) where they can combat invading microbes. Neutrophils are very efficient at phagocytosing and killing invaders (Nicolas-Avila et al., 2017). However, neutrophil migration into the tissues must be carefully regulated because of the potential host tissue damage resulting from uncontrolled release of toxic neutrophil metabolites (Kruger et al., 2015). Female reproductive tract (FRT) mucosa must regulate neutrophil migration to maintain the balance between the presence of commensal and opportunistic microbiota, exogenous spermatozoa and the threat of sexually transmitted pathogens.

Cyclic hormonal levels control vaginal mucosa to create a microenvironment to favor sperm survival and reproduction. During intercourse, ejaculate is pooled in the fornix and the ectocervix of the FRT. Within minutes, sperm cells start to swim and the cervix must facilitate passage of sperm if fertilization is to occur (Suarez and Pacey, 2006). In addition, during the ovulatory phase, neutrophils disappear from the vaginal lumen (Stockard and Papanicolaou, 1917; Sonoda et al., 1998; Sasaki et al., 2009; Caligioni, 2009) thus preventing sperm capture and damage (Zambrano et al., 2016). As a result, epithelial cells turn out to be unprotected from pathogens that also target the cervical regions (Ghosh, 2014; Anderson et al., 2014; Wira et al., 2015) producing an E2 and E2 receptor (ESR1) mediated window of vulnerability to infection during ovulation (Salinas-Munoz et al., 2018). In contrast, studies performed during insemination demostrate a sperm dependent wave of neutrophils after insemination (Matthijs et al., 2003; Schuberth et al., 2008; Sharkey et al., 2007, Sharkey et al., 2012). This apparently

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https://doi.org/10.1016/j.jri.2019.02.002
Received 29 October 2018; Received in revised form 24 January 2019; Accepted 17 February 2019
0165-0378/

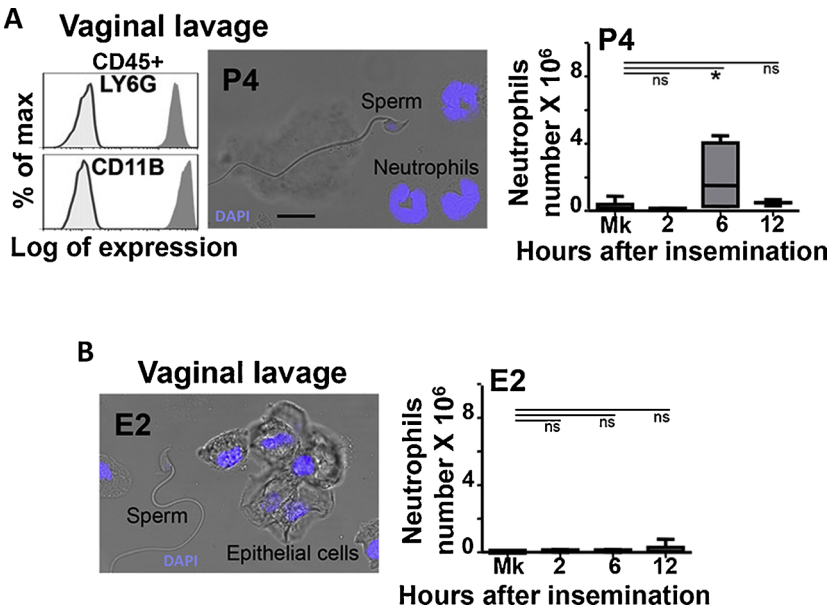

Fig. 1. Neutrophils in the vaginal lavage after insemination. Ovariectomized mice were hormone-treated and inseminated. (A) Progesterone and (B) estradiol treated mice. Representative flow cytometry plots, gating on $CD45^+$ alive cells and phase contrast microscopy of the vaginal lavage 6 h after insemination. Scale bar, 10 µm. Isotype control mAb (line) and indicated mAb (grey filled lines). Number of neutrophils in the vaginal lavage are expressed as box and whiskers 10–90 percentile (n = 8–10 mice per time points). *p < 0.05. Mann-Whitney. Mk:mock vaginal inoculated mice with vehicle without sperm. E2:estradiol; P4:progesterone, CD11B: Integrin alpha M (ITGAM) Pan-myeloid cells marker, LY6 G: (Lymphocyte antigen 6 G) Pan-neutrophils marker. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

contradictory finding highlights a gap in our knowledge about how cervical mucosa responds to sperm and the neutrophils driving forces during insemination.

Neutrophils recruit to sites of injury (Foxman et al., 1997) or infection (da Silva et al., 2012) by CXCR2-, CCR1- and CXCR4-ligands. Because CXCR2 depletion in vaginal neutrophils abolishes neutrophil transepithelial migration (TEM) (Lasarte et al., 2016) and CXCR2-ligands have been found in vaginal secretions (Lindqvist et al., 2009; Hickey et al., 2013; Deruaz and Luster, 2015), we studied the role of CXCR2/CXCR2-ligands axis to drive neutrophils to the vaginal lumen during insemination and its female sex hormone regulation to prevent neutrophil capture and damage to sperm during ovulation. We set up a mouse model of insemination in the presence of estradiol (E2 ovulatory phase) or progesterone (P4 luteal phase) to study the role of hormones on the neutrophil TEM regulation during insemination. We analyzed the CXCL1 expression regulation and neutrophil TEM at the cervical area, which is the main entrance for neutrophils to the vaginal lumen and the sperm to the uterus. We discovered that E2-treatment induces epithelial down-regulation of CXCL1 expression and it could play a role in the retardation of neutrophils at the ectocervix and fornix mucosa, and the associated lack of neutrophils in the vaginal lumen during ovulation.

2. Materials and methods

2.1. Ethical approval

Animal experiments comply with the ARRIVE guidelines and in accordance with the EU Directive 2010/63/EU for animal experiments. The IiSGM Animal Care and Use Committee approved all the procedures (PROEX 147/14).

2.2. In vivo hormonal treatment, vaginal inoculation and lavage

Female BALB/c (H-2d), C57BL/6 Wt and $ESR1^{-/-}$ [B6N(Cg)-Esr1tm4.2Ksk/J JAX stock #026176] were maintained under specific pathogen-free, 12 h light/dark, with environmental enrichment and temperature and humidity controlled in the Animal Facility of IiSGM. $ESR1^{-/-}$ mice were unfertile and without cyclic changes (Dupont et al., 2000). Four-week-old mice (13–15 g) were ovariectomized under sevoflurane anesthesia (Lasarte et al., 2013). After 2 weeks of recovery, females were randomly assigned to the groups and injected subcutaneously with 0.02 mg of 17β-E2 or and 0.2 mg of P4 (Calbiochem, Germany) dissolved in 100 µl of sesame oil (Sigma-Aldrich, USA) every 4 days. Hormone treatment was sufficient to maintain hormone concentrations (Lasarte et al., 2016). Seventy-two hours after first hormonal treatment, 5×10^6 sperm in 20 µl of PBS or PBS alone (mock) were inoculated into the vagina. Vaginal secretions were collected by gently flushing 50 µl of sterile PBS the vaginal vault four times (Salinas-Munoz et al., 2018).

2.3. Sperm collection

Sperm was collected from CD1 Crl:CD1(ICR) (outbred) vasa deferentia. Briefly, vasa deferentia were removed and sperm was expelled carefully and washed in PBS (Lasarte et al., 2013).

2.4. Flow cytometry

Direct membrane immunofluorescence was performed using: rat anti-mouse CD45 (eBioscence, USA), rat anti-mouse LY6 G (eBioscience, USA), rat anti-mouse CD11B (eBioscience, USA) and rat anti-human and mouse CDH1 (Biolegend, USA). CXCL1 (rabbit, Abcam, UK) expression was determined by intracellular staining using the BD Cytofix/Cytoperm (BD bioscience, USA) following manufacturer's instructions in the presence of 50 µg/ml mouse IgG. We used the same isotype control antibody as a negative control and dead cells were excluded by 7-amino-actinomycin-D staining (Sigma, USA). Samples were analyzed in Gallios device (Beckman Coulter, USA) and data were analyzed using Flowjo software (Tree Star, Inc, USA). Cells were counted using Flow-Count fluorospheres (Beckman Coulter, USA) following the manufacturer's instructions.

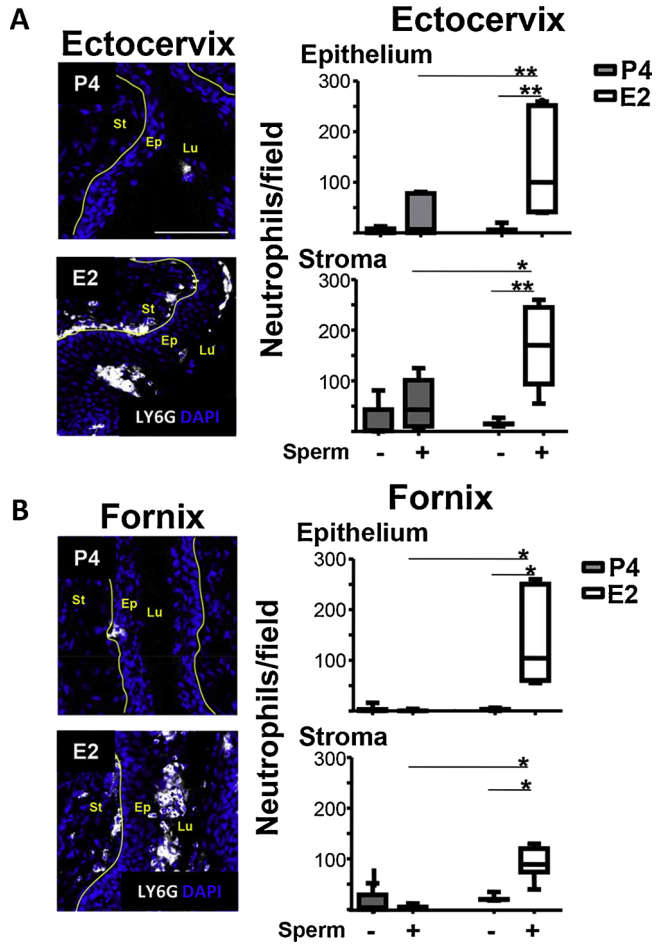

Fig. 2. Neutrophils in the ectocervix and fornix after insemination. Hormone-treated ovariectomized mice were mock or inseminated in the vagina for 6 h. Photomicrograph of the (A) ectocervix and (B) fornix with LY6 G-stained neutrophils (white). Scale bar, 200 µm. LY6 G + cells/field in the lower female reproductive tract epithelium and stroma. Data were calculated in at least 3 different sections of each sample (n = 5) and expressed as box and whiskers 10–90 percentile. *p < 0.05 and **p < 0.01 Mann-Whitney. Yellow line indicates the lamina propia. E2:estradiol; P4:progesterone; Lu: lumen; Ep: epithelium; St: Stroma. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

2.5. Confocal microscopy

FRT tissues were embedded in Tissue-Tek OCT (Sakura, Netherlands). Eight-micrometer sections were fixed with acetone, blocked (50 µg/ml mouse IgG and 10% BSA) and stained with: rat anti-mouse LY6 G (eBioscence, USA), rabbit anti-mouse CXCL1 (abcam, USA), rabbit anti-mouse CXCL2 (AbD serotec, USA), rabbit anti-mouse CXCL5 (eBioscience, USA), rabbit anti-mouse CXCR2 (Santa Cruz Biotech, USA) and rat anti-mouse CDH1 (Biolegend, USA). For in vivo protein expression quantification, tissues were imaged using the glycerol ACS APO 20x NA 0.60 objective of a confocal fluorescence microscope (SPE, Leica Microsystems), maintaining the acquisition settings all over the process for each sample and among samples, as previously described (Samaniego et al., 2013; Lasarte et al., 2016). Mean fluorescence intensities (MFI) were assessed at multiple regions of interest (ROIs) by field, randomly depicted at specific areas of the FRT stroma and epithelium. For CXCL1, sub-epithelial stromal vs epithelial ROIs were depicted, as previously described (Samaniego et al., 2013; Lasarte et al., 2016). For single-cell quantification, LY6 G + stained neutrophils were segmented and MFI of different markers quantified at matched cells. All quantifications, including line-profiles, were performed using the FIJI (Fiji Is Just ImageJ) software (NIH).

2.6. Statistical analysis

The tests used in each experiment are in the figure legends. GraphPad Prism 5 (GraphPad Software, Inc, USA) was used to determine statistical significance. A p value < 0.05 considered significant.

3. Results

3.1. Estradiol impairs neutrophil recruitment into the vaginal lumen, inducing their retention at the ectocervix and fornix

To study the role of sperm in the neutrophil influx into the vaginal lumen, we set up a model of insemination in hormone-treated mice. Inseminated P4-treated mice showed a significant increase of neutrophils (˜15-fold) in the vaginal lavage 6 h after insemination compared with non-inseminated mice. However, neutrophil infiltration was insignificant in E2-treated mice (Fig. 1A and B).

Neutrophil influx inhibition to the vaginal lumen in inseminated E2-treated mice could be due to a diminished extravasation or an impeded TEM. To sort out these possibilities, we excised the lower FRT after vaginal lavage and quantified the neutrophil content at the ectocervix and fornix by confocal microscopy. We detected a higher density of neutrophils in E2-treated compared with P4-treated mice in the stroma and the epithelium, both in the ectocervix and fornix after the vaginal lavage (Fig. 2A, and B). These E2-dependent neutrophil accumulations may explain the absence of neutrophil in the vaginal lavage of E2-treated mice (Fig. 1B). Whereas P4-treatment promotes TEM into the vaginal lumen, E2-treatment arrests neutrophils in the ectocervix and fornix.

3.2. Estradiol down-regulates CXCL1 epithelial expression

Because chemokine gradients guide neutrophils to cross the epithelium (Li et al., 2002; Swee et al., 2008) and neutrophil CXCR2 expression is 90% responsible for neutrophil TEM to the vagina (Lasarte et al., 2016), we focused on the neutrophil CXCR2 expression at the ectocervix and fornix. E2-treatment did not significantly change neutrophil CXCR2 expression (Fig. 3A), in accordance with our previously published ex vivo data (Lasarte et al., 2016). Then, we concluded that neutrophil CXCR2 expression might not be a critical factor for neutrophil accumulation in the fornix and ectocervix of E2- and P4-treated mice. Therefore, we searched for CXCR2-ligand expression

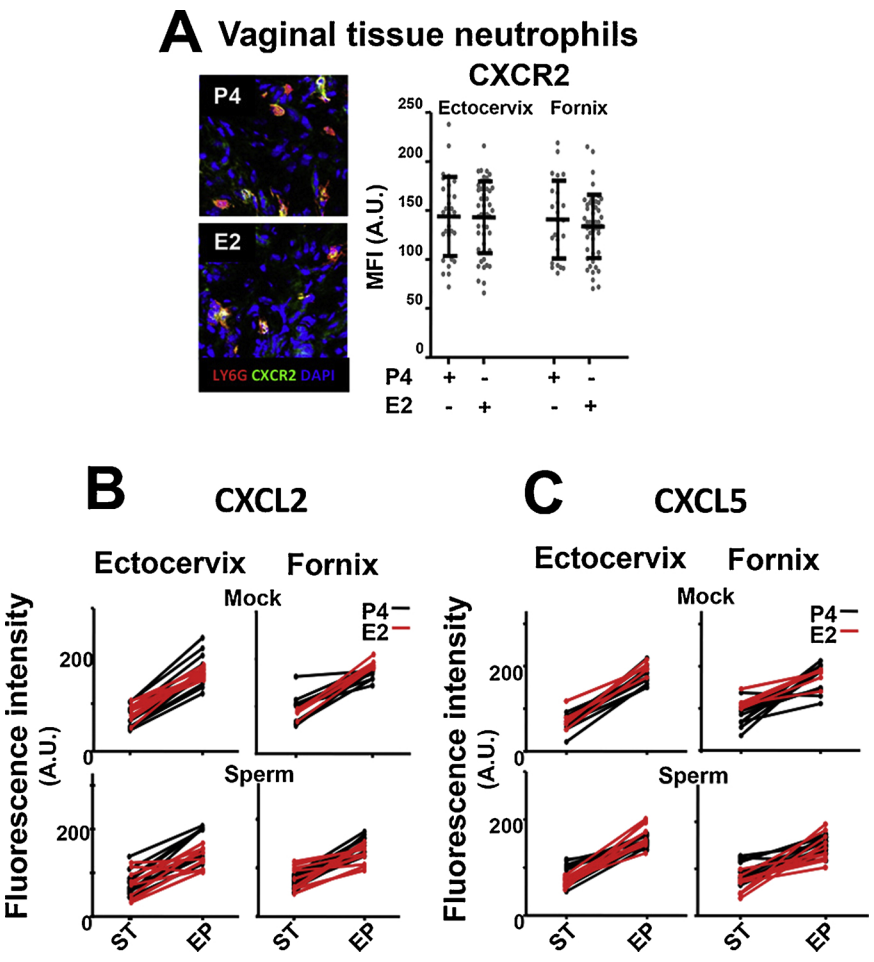

Fig. 3. CXCR2 neutrophil expression and CXCL2 and CXCL5 gradient expression in the ectocervix and fornix. Hormone-treated ovariectomized mice. (A) Representative pictures of sections assayed for CXCR2 (green) and LY6 G + (red). Quantification of CXCR2 expression in LY6 G + neutrophils in the ectocervix and fornix. Data were calculated in at least 3 different sections of each sample (n = 3) and are expressed as CXCR2 mean intensity ± SD. Hormone-treated ovariectomized mice were mock or inseminated. Vaginal sections were (B) CXCL2 and (C) CXCL5-stained. Chemokines gradient at the stroma/epithelium. Data were calculated in at least 3 different points in 3 different sections of each sample. n = 5. Mock: vaginal inoculated mice with vehicle without sperm. E2: estradiol; P4: progesterone. A.U.:arbitrary units; Ep:epithelium; St: Stroma. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

alterations induced by E2-treatment that might account for its inhibitory effects.

CXCR2-ligands (CXCL1, CXCL2 and CXCL5) have been found in vaginal secretions (Lindqvist et al., 2009; Hickey et al., 2013; Deruaz and Luster, 2015). Therefore, we analyzed the chemokine expression and we did not detect differences in the CXCL2 and CXCL5 expression after the hormonal-treatments (Fig. 3B and C). However, we detected lower CXCL1 (murine functional homolog of human CXCL8) expression in the lower FRT epithelial cells from E2- compared to P4-treated mice by flow cytometry (Fig. 4A) and confocal microscopy. We detected a strong CXCL1 expression in the P4-treated ectocervix (˜5-fold) and fornix (˜4-fold) epithelium compared with E2-treated mice (Fig. 4B, C and D), which explains CXCL1 gradient variation during the ovarian cycle along the lower FRT (vagina, ectocervix and fornix) (Lasarte et al., 2016). Indeed, E2-teated ESR1-/- mice showed strong CXCL1 expression in the ectocervix and fornix, verifying the key role of E2 in the CXCL1 repression (Fig. 5A). Therefore, the CXCL1 gradient could be one of the factors that might explain the E2-dependent lack of neutrophils in the vaginal lavage and the accumulation of neutrophils in the ectocervix and fornix epithelium.

3.3. CXCL1 expression decreases during ovarian cycle

In order to mimic the ovarian cycle sequence and validate the hormone's effect on CXCL1 expression during insemination, we pretreated mice with E2 for 72 h. Later, we inseminated and treated them with E2 or P4 and we analyzed CXCL1 expression at the stroma and epithelium. We detected a strong CXCL1 expression in the E2/P4-treated ectocervix and fornix (˜3-fold) compared with E2/E2-treated mice (Fig. 5B), which shows P4 would be able to restore CXCL1 gradient after ovulation (E2 peak) during luteal phase. This alteration in CXCL1 gradient could be one of the factors that explain the lack of neutrophils in the vaginal lavage and the accumulation of neutrophils in the ectocervix and fornix epithelium upon E2-treatment, to spare sperm from neutrophil attack and preserve reproduction during ovulation.

4. Discussion

We suggest an E2-dependent molecular mechanism that retards neutrophil migration to the vaginal lumen during ovulation. E2-treatment reduces cervical epithelial cell CXCL1 gradient formation,

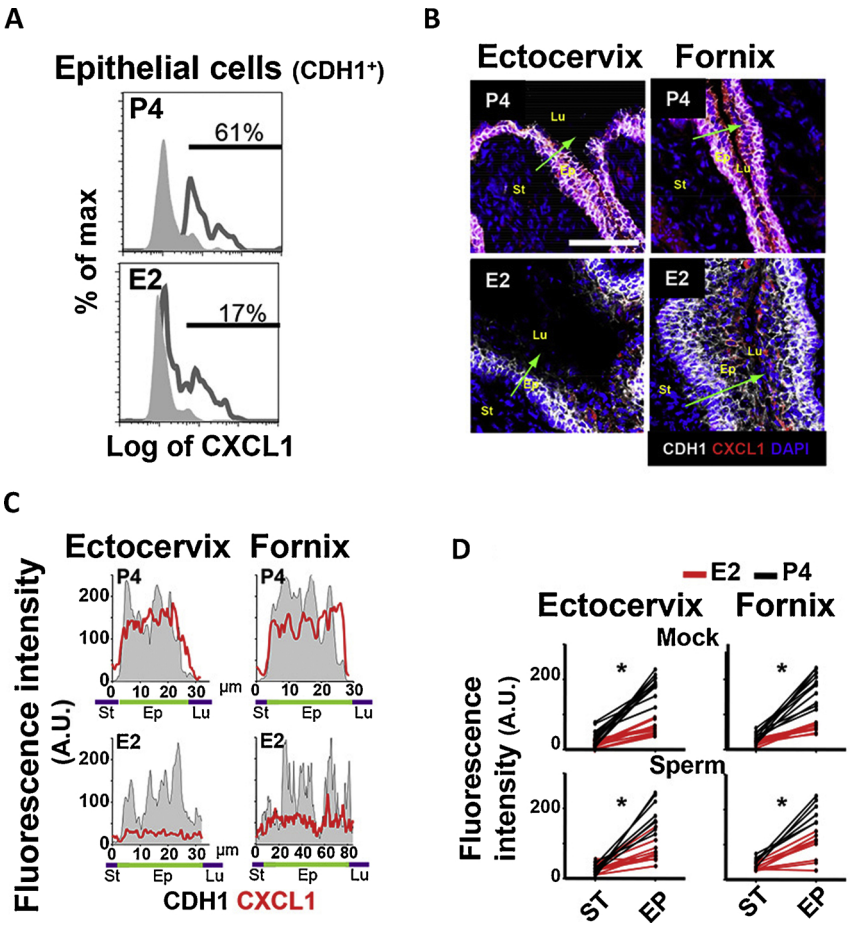

Fig. 4. Female sex hormones regulates the CXCL1 gradient in the ectocervix and fornix after insemination. Hormone-treated ovariectomized mice were mock or inseminated. (A) Representative flow cytometry plots of the CXCL1 expression in the ectocervix and fornix epithelial cells (CDH1^{+}). Numbers indicate the percentage of cells in the gate. (B) Photomicrograph of the ectocervix and fornix stained for CXCL1 (red) and CDH1 (white). Scale bar, 200 µm. (C) CXCL1 expression in the epithelium (CDH1 +) cross section at the indicated green arrow in B. (D) Cxcl1 gradient in the stroma/epithelium. Data were calculated from at least 3 different points of samples (n = 6) stained in 3 different sections. Data are expressed as box and whiskers 10–90 percentile. *p < 0.05 Mann-Whitney. Isotype control mAb (grey filled lines) and indicated mAb (lines). Yellow line:- lamina propia. Scale bar, 200 µm. Mock: vaginal inoculated mice with vehicle without sperm. E2:estradiol; P4:progesterone. Lu:lumen; Ep:epithelium; St:Stroma; A.U.:arbitrary units (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

which retard neutrophil migration. Consequently, neutrophils accumulate in the ectocervix and vaginal fornix. In contrast, P4-treatment promotes cervical CXCL1 gradient formation and neutrophil TEM. These data help explain how FRT mucosa conciliates reproduction with immunity.

Female reproductive mucosa recognizes semen as a foreign material and induces a wave of leukocyte recruitment in the human cervix after coitus, (Sharkey et al., 2012) and a Th17 immune response against sperm that produces vaginal neutrophil infiltration (Lasarte et al., 2013; Veldhoen, 2017). This anti-sperm immune reaction could abolish reproduction (Zambrano et al., 2016), however during the ovulatory phase (high E2 levels) neutrophils disappear from the vaginal lumen (Stockard and Papanicolaou, 1917) and accumulate in the ectocervix and fornix (Salinas-Munoz et al., 2018). Here, we show evidence of an ESR1-dependent mechanism by which E2-treatment accumulates neutrophils at the ectocervix and fornix, which could avoid sperm damage during ovulation. Therefore, epithelial CXCL1 E2-dependent repression regulates unwanted neutrophil responses during ovulation.

Neutrophil infiltration is contingent on the ovarian cycle timing in the FRT (Salinas-Munoz et al., 2018). However, in the intestine, lung and bladder, inflammatory signals up-regulate the epithelial expression of CXCL1 to mediate neutrophil TEM (Buanne et al., 2007; Becker et al., 1994; O'Brien et al., 2016). Here, we show an independent of sperm mechanism to withdraw neutrophils from the vaginal lumen to allow sperm survival during ovulation, although it might compromise immunity. In contrast, P4 during the luteal phase promotes neutrophil's quick invasion of the vaginal lumen (Sonoda et al., 1998; Lasarte et al., 2016; Salinas-Munoz et al., 2018) to restore immunity, clear and trap sperm or sperm-associated microbes (Friberg et al., 1987; Alghamdi and Foster, 2005). Therefore, we suggest that the 'window of vulnerability' to pathogens in the lower FRT could last during the E2 peak (day ˜12 in human ovarian cycle) until the P4 peak (day ˜17 in human ovarian cycle). Because, P4 restores neutrophil infiltration and immunity after ovulation (Sonoda et al., 1998; Lasarte et al., 2016; Salinas-Munoz et al., 2018).

Here we describe how sex hormones regulate CXCL1 epithelial expression in response to insemination in similar magnitude than in response to pathogens (Lasarte et al., 2016) to preserve reproduction. Our data reveal that E2 reduces neutrophil passage into the vaginal lumen by causing neutrophil accumulation in the sub- epithelial spaces of the fornix and ectocervix in response to sperm. In the case of E2 over exposure, opportunistic microbiota or STDs pathogens could grow and promote infection; however, uncontrolled neutrophil response during ovulation could lead to sperm attack and infertility.

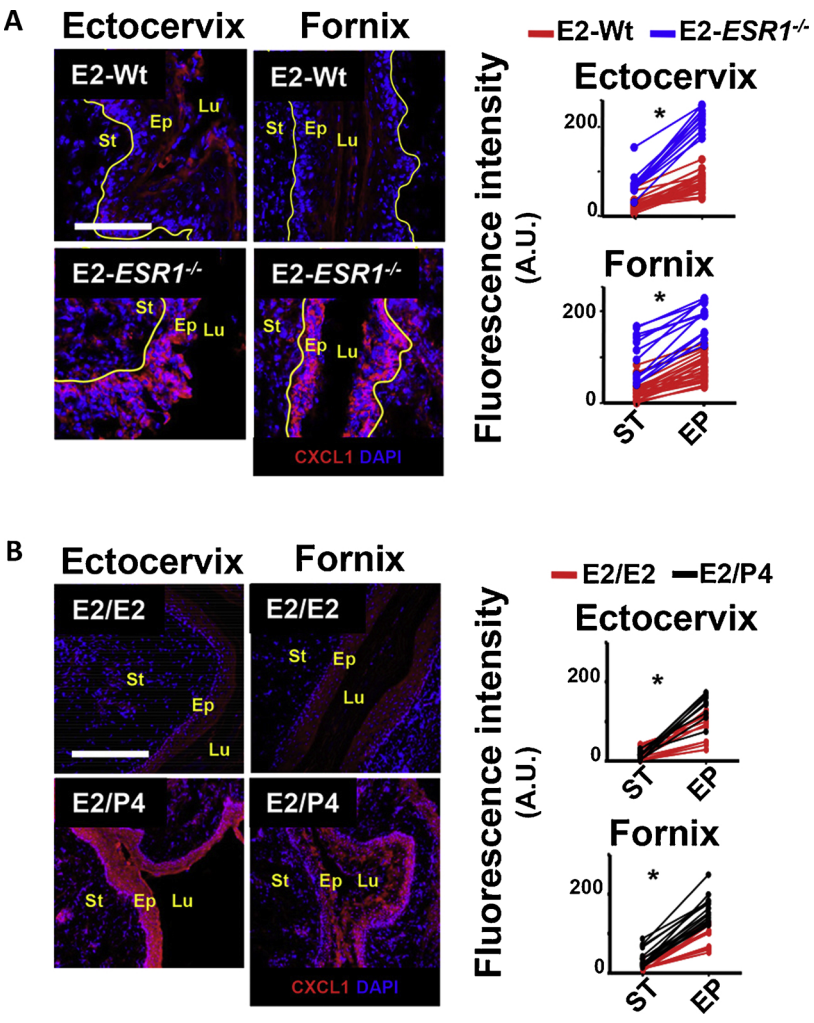

Fig. 5. Estradiol down-regulates the CXCL1 gradient in the ectocervix and fornix after insemination. Hormone-treated ovariectomized mice were inseminated. (A) Photomicrograph of the ectocervix and fornix stained for CXCL1 (red) from estradiol-treated *ESR1*$^{-/-}$ and Wt mice. CXCL1 gradient in the stroma/epithelium (n = 5). Ovariectomized mice were estradiol-treated and inoculated with sperm in the vagina. After the insemination, mice were treated with estradiol or progesterone to mimic the ovarian cycle. (B) Photomicrograph of the ectocervix and fornix stained for CXCL1 (red). CXCL1 gradient in the stroma/epithelium (n = 6). Data were calculated in at least 3 different points in 3 different sections of each sample. *p < 0.05 Mann-Whitney. Isotype control mAb (grey filled lines) and indicated mAb (lines). Scale bar, 200 µm. E2:estradiol; P4:progesterone. Lu:lumen; Ep:epithelium; St:Stroma; A.U.:arbitrary units (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Funding and conflicts of interest

This work was partially supported by Fundacion Mutua Madrileña and the Ministry of Economy and Competitiveness ISCIII-FIS grants, PI16/00050, and PI17/01324, co-financed by ERDF (FEDER) Funds from the European Commission, "A way of making Europe". MR holds a Miguel Servet II contract (CPII14/00009). LS holds a IiSGM intramural contract. The authors declare no conflicts of interest.

Declarations of interest

None.

Acknowledgement

Authors thank the units of flow cytometry, cell culture and statistical analysis. We are grateful J. Villarejo, F. Asensio and F. Sanchez-Cobos, for expert help and support.

References

Alghamdi, A.S., Foster, D.N., 2005. Seminal DNase frees spermatozoa entangled in neutrophil extracellular traps. Biol. Reprod. 73, 1174–1181.
Anderson, D.J., Marathe, J., Pudney, J., 2014. The structure of the human vaginal stratum corneum and its role in immune defense. Am. J. Reprod. Immunol. 71, 618–623.
Becker, S., Quay, J., Koren, H.S., Haskill, J.S., 1994. Constitutive and stimulated MCP-1, GRO alpha, beta, and gamma expression in human airway epithelium and bronchoalveolar macrophages. Am. J. Physiol. 266, L278–86.
Buanne, P., Di Carlo, E., Caputi, l., Brandolini, l., Mosca, M., Cattani, F., Pellegrini, l., Biordi, l., Coletti, G., Sorrentino, C., Fedele, G., Colotta, F., Melillo, G., Bertini, R., 2007. Crucial pathophysiological role of CXCR2 in experimental ulcerative colitis in mice. J. Leukoc. Biol. 82, 1239–1246.
Caligioni, C.S., 2009. Assessing reproductive status/stages in mice. Curr. Protoc. Neurosci Appendix 4, Appendix 4I.
Da Silva, J.B., Carvalho, E., Covarrubias, A.E., Ching, A.T., Mattaraia, V.G., Paiva, D., De Franco, M., Favaro, R.D., Pereira, M.M., Vasconcellos, S., Zorn, T.T., Ho, Pl., Martins, E.A., 2012. Induction of TNF-alfa and CXCL-2 mRNAs in different organs of mice infected with pathogenic Leptospira. Microb. Pathog. 52, 206–216.
Deruaz, M., Luster, A.D., 2015. Chemokine-mediated immune responses in the female genital tract mucosa. Immunol. Cell Biol. 93, 347–354.
Dupont, S., Krust, A., Gansmuller, A., Dierich, A., Chambon, P., Mark, M., 2000. Effect of single and compound knockouts of estrogen receptors alpha (ERalpha) and beta (ERbeta) on mouse reproductive phenotypes. Development 127, 4277–4291.
Foxman, E.F., Campbell, J.J., Butcher, E.C., 1997. Multistep navigation and the combinatorial control of leukocyte chemotaxis. J. Cell Biol. 139, 1349–1360.
Friberg, J., Confino, E., Suarez, M., Gleicher, N., 1987. Chlamydia trachomatis attached to spermatozoa recovered from the peritoneal cavity of patients with salpingitis. J. Reprod. Med. 32, 120–122.
Ghosh, M., 2014. Secreted mucosal antimicrobials in the female reproductive tract that are important to consider for HIV prevention. Am. J. Reprod. Immunol. 71, 575–588.
Hickey, D.K., Fahey, J.V., Wira, C.R., 2013. Mouse estrous cycle regulation of vaginal versus uterine cytokines, chemokines, alpha-/beta-defensins and TLRs. Innate Immun. 19, 121–131.
Kruger, P., Saffarzadeh, M., Weber, A.N., Rieber, N., Radsak, M., Von Bernuth, H., Benarafa, C., Roos, D., Skokowa, J., Hartl, D., 2015. Neutrophils: Between host defence, immune modulation, and tissue injury. PLoS Pathog. 11, e1004651.
Lasarte, S., Elsner, D., Guia-Gonzalez, M., Ramos-Medina, R., Sanchez-Ramon, S., Esponda, P., Munoz-Fernandez, M.A., Relloso, M., 2013. Female sex hormones regulate the Th17 immune response to sperm and Candida albicans. Hum. Reprod.
Lasarte, S., Samaniego, R., Salinas-Munoz, l., Guia-Gonzalez, M.A., Weiss, l.A., Mercader, E., Ceballos-Garcia, E., Navarro-Gonzalez, T., Moreno-Ochoa, l., Perez-Millan, F., Pion, M., Sanchez-Mateos, P., Hidalgo, A., Munoz-Fernandez, M.A., Relloso, M., 2016. Sex hormones coordinate neutrophil immunity in the Vagina by controlling chemokine gradients. J. Infect. Dis. 213, 476–484.
Li, Q., Park, P.W., Wilson, C.L., Parks, W.C., 2002. Matrilysin shedding of syndecan-1 regulates chemokine mobilization and transepithelial efflux of neutrophils in acute

lung injury. Cell 111, 635–646.
Lindqvist, M., Navabi, N., Jansson, M., Samuelson, E., Sjoling, A., Orndal, C., Harandi, A.M., 2009. Local cytokine and inflammatory responses to candidate vaginal adjuvants in mice. Vaccine 28, 270–278.
Matthijs, A., Engel, B., Woelders, H., 2003. Neutrophil recruitment and phagocytosis of boar spermatozoa after artificial insemination of sows, and the effects of inseminate volume, sperm dose and specific additives in the extender. Reproduction 125, 357–367.
Nicolas-Avila, J.A., Adrover, J.M., Hidalgo, A., 2017. Neutrophils in Homeostasis, Immunity, and Cancer. Immunity 46, 15–28.
O'brien, V.P., Hannan, T.J., Yu, l., Livny, J., Roberson, E.D., Schwartz, D.J., Souza, S., Mendelsohn, C.L., Colonna, M., lewis, A.L., Hultgren, S.J., 2016. A mucosal imprint left by prior Escherichia coli bladder infection sensitizes to recurrent disease. Nat. Microbiol. 2, 16196.
Salinas-Munoz, l., Campos-Fernandez, R., Mercader, E., Olivera-Valle, I., Fernandez-Pacheco, C., Matilla, l., Garcia-Bordas, J., Brazil, J.C., Parkos, C.A., Asensio, F., Munoz-Fernandez, M.A., Hidalgo, A., Sanchez-Mateos, P., Samaniego, R., Relloso, M., 2018. Estrogen Receptor-Alpha (ESR1) Governs the Lower Female Reproductive Tract Vulnerability to Candida albicans. Front. Immunol. 9, 1033.
Samaniego, R., Estecha, A., Relloso, M., Longo, N., J, l. E, Longo-Imedio, I., J, A. A., M, A. D. P, Puig-kroger, A., Sanchez-Mateos, P., 2013. Mesenchymal contribution to recruitment, infiltration, and positioning of leukocytes in human melanoma tissues. J. Invest. Dermatol.
Sasaki, S., Nagata, K., Kobayashi, Y., 2009. Regulation of the estrous cycle by neutrophil infiltration into the vagina. Biochem. Biophys. Res. Commun. 382, 35–40.
Schuberth, H.J., Taylor, U., Zerbe, H., Waberski, D., Hunter, R., Rath, D., 2008. Immunological responses to semen in the female genital tract. Theriogenology 70, 1174–1181.
Sharkey, D.J., Macpherson, A.M., Tremellen, K.P., Robertson, S.A., 2007. Seminal plasma differentially regulates inflammatory cytokine gene expression in human cervical and vaginal epithelial cells. Mol. Hum. Reprod. 13, 491–501.
Sharkey, D.J., Tremellen, K.P., Jasper, M.J., Gemzell-Danielsson, K., Robertson, S.A., 2012. Seminal fluid induces leukocyte recruitment and cytokine and chemokine mRNA expression in the human cervix after coitus. J. Immunol. 188, 2445–2454.
Sonoda, Y., Mukaida, N., Wang, J.B., Shimada-Hiratsuka, M., Naito, M., Kasahara, T., Harada, A., Inoue, M., Matsushima, K., 1998. Physiologic regulation of postovulatory neutrophil migration into vagina in mice by a C-X-C chemokine(s). J. Immunol. 160, 6159–6165.
Stockard, C.R., Papanicolaou, G.N., 1917. A rhythmical "Heat period" in the guinea-pig. Science 46, 42–44.
Suarez, S.S., Pacey, A.A., 2006. Sperm transport in the female reproductive tract. Hum. Reprod. Update 12, 23–37.
Swee, M., Wilson, C.L., Wang, Y., Mcguire, J.K., Parks, W.C., 2008. Matrix metalloproteinase-7 (matrilysin) controls neutrophil egress by generating chemokine gradients. J. Leukoc. Biol. 83, 1404–1412.
Veldhoen, M., 2017. Interleukin 17 is a chief orchestrator of immunity. Nat. Immunol. 18, 612–621.
Wira, C.R., Rodriguez-Garcia, M., Patel, M.V., 2015. The role of sex hormones in immune protection of the female reproductive tract. Nat. Rev. Immunol.
Zambrano, F., Carrau, T., Gartner, U., Seipp, A., Taubert, A., Felmer, R., Sanchez, R., Hermosilla, C., 2016. Leukocytes coincubated with human sperm trigger classic neutrophil extracellular traps formation, reducing sperm motility. Fertil. Steril. 106, 1053–1060 e1.