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ORIGINAL ARTICLE

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The involvement of the progesterone receptor in PIBF and Gal-1 expression in the mouse endometrium

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Problem: The progesterone-regulated genes, *PIBF* and *Gal-1*, are key players in the feto-maternal immunological interaction. This study aims to investigate the expression of PIBF and Gal-1 in WT and progesterone receptor KO models as well as subsequent effects of PIBF on decidualization of stromal cells.

Method of the study: PRAKO, PRBKO and PRKO BALB/c mice were used for assessing the role of PR isoforms in PIBF induction. PIBF- and Gal-1 mRNA expression in the uterus was tested by real-time PCR. The effect of PIBF on decidualization of endometrial stromal cells was verified by anti-desmin immunofluorescence. Immunohistochemistry was used for testing PIBF expression in the uterus. Gal-1, ERα and PR positive decidual NK cells were detected by immunofluorescence.

Results: PIBF mRNA was significantly increased in progesterone-treated WT mice, but not in PRKO and PRAKO mice. PIBF protein expression was reduced in the endometria of PRKO and PRAKO, but not in PRBKO mice. During a 6-day culture, PIBF induced decidual transformation of endometrial stromal cells. PIBF expression in the mouse uterus was highest during the implantation window, while Gal-1 mRNA expression continuously increased between day 2.5 and day 11.5 of gestation. Decidual NK cells express Gal-1 and ERα, but not PR at day 7.5 murine pregnancy.

Conclusion: PIBF produced via engagement of PRA, is highly expressed in the endometrium during the implantation window, and plays a role in decidualization. The concerted action of PIBF and Gal-1 might contribute to the low cytotoxic activity of decidual NK cells.

KEYWORDS
decidual NK cells, decidualization, Gal-1, PIBF, progesterone receptor isoforms

1 | INTRODUCTION

The biological activities of progesterone (P) are mainly mediated by two nuclear progesterone receptor (PR) isoforms: PRA and PRB.[1-3] Mice lacking PRA are infertile,[4,5] while engagement of PRB accounts for mammary gland development. The infertility of mice missing both isoforms (PRKO mice) is partly due to the inability of endometrial cells to undergo decidual transformation and to reach the receptive state.[4] The relative expression of PRA and PRB proteins is likely to be critical to ensure appropriate reproductive tissue responses to P.[5]

Studies on PR knockout mice revealed that PRs are required not only for endometrial receptivity and decidualization but also

for establishing an appropriate immune environment in the endometrium.[4,6] In vivo studies on the role of oestrogen (E) and P in regulating the uterine immune environment showed that the pro-inflammatory effect of E is antagonized by P in the uteri of wild-type (WT) mice, but not in those of progesterone receptor knockout (PRKO) mice.[6]

Further, PRs in the thymus are required for thymic involution during pregnancy and for normal fertility,[7] showing that PRs are involved in modulating the function of the immune system on several levels.

Progesterone regulates the genes of theprogesterone-induced blocking factor (PIBF) and of galectin-1 (Gal-1), both playing a role in the feto-maternal immunological interaction.[8] Gal-1-deficient mice show an increased rate of fetal loss. P treatment increases Gal-1 expression in myometria and deciduae of stress-challenged pregnancies at gestational day 7.5. Gal-1 treatment on the other hand prevents the stress-induced decrease of both progesterone and PIBF levels and normalizes the resorption rates.[9] These data suggest the existence of a progesterone-mediated mechanism that regulates Gal-1 expression at the feto-maternal interface.

A nucleus-associated 90 kDa PIBF isoform is constitutively expressed in immature and rapidly proliferating cells, for example, embryonic tissues and tumours.[10-13]

Smaller PIBF isoforms are expressed by various pregnancy-associated tissues. Both villous and extravillous trophoblast cells express 30, 50 and 90 kDa PIBF isoforms in first trimester.[14] The latter are localized in the cytoplasm, and after being secreted, decrease NK activity and induce Th2 cytokine production. Anti-PIBF treatment of pregnant mice results in pregnancy loss. If, however, NK cells are depleted by the use of anti-NK antibodies, PIBF deficiency does not cause an increased rate of resorptions.[15] Decidual NK also appear to be affected by PIBF, which inhibits upregulation of perforin expression in activated decidual lymphocytes as well as NK cell cytotoxicity by blocking degranulation.[16,17]

In addition to causing pregnancy loss, the lack of PIBF alters the cytokine balance. Neutralization of endogenous PIBF activity in pregnant mice by specific anti-PIBF antibody reduces the synthesis of IL-10 and increases that of IFN-γ.[18]

The PIBF receptor is a glycosylphosphatidylinositol (GPI)-anchored protein, which, for signalling, temporarily associates with the alpha chain of the IL-4 receptor,[19] thus PIBF signals via the Jak/STAT pathway.

Both P and PIBF play a role in the induction of the Th2-biased cytokine balance. IL-4 or PIBF treatment of peripheral pregnancy lymphocytes results in immediate STAT6 phosphorylation, whereas a 24-hour continuous presence of progesterone is required for the same effect, suggesting that progesterone might activate the Jak/STAT pathway indirectly, via first inducing PIBF.[19] Along that line, *PIBF1* gene was identified as P-regulated gene by cistrome analysis of the murine uterus using chromatin immunoprecipitation (ChIP) followed by massive parallel sequencing (ChIP-seq).[20]

Taken together, these data suggest that by upregulating Th2-type cytokine production and by down-regulating NK activity PIBF affects the immune response in a way, which might have an impact on the feto-maternal relationship.

Though later stages of pregnancy have been relatively well characterized in this respect, little is known about the involvement of PIBF in the embryo-maternal interactions during early pregnancy. This study was aimed at investigating the expression of PIBF and Gal-1 in wild-type and PR KO mice, and the subsequent effect of PIBF on stroma cell decidualization.

2 | MATERIALS AND METHODS

2.1 | Mice

All experimental and surgical procedures complied with the Guide of Care and Use of Laboratory Animals and were approved by the ethical committee of the University of Rijeka Faculty of Medicine and Ministry of Agriculture. BALB/c mice were housed in humidity (55 ± 10%) and temperature (22 ± 2°C) controlled rooms with a 12-hour light-dark cycle with free access to food and water.

Genetically modified BALB/c PRKO, PRAKO and PRBKO mice were generously provided by John P. Lydon and Orla M. Conneely (Baylor College of Medicine Houston, TX, USA). Six- to eight-week old WT, PRAKO, PRBKO and PRKO BALB/c mice[4] were used for assessing the role of different PR isoforms in PIBF induction. The mice were ovariectomized and allowed to rest for 2 weeks. Mice were then treated with 1 mg of P subcutaneously, or sesame oil as a control, and killed 6 hours later. Uterine tissues were collected for RNA isolation and immunohistology.

For investigating pregnancy-induced mRNA and proteins expression, wild-type BALB/c female mice (10-12 weeks old) were housed with fertile males. The day, the vaginal plug was observed, was considered day 0.5 of pregnancy. The animals were killed by cervical dislocation on specific days of pregnancy (dpc), and uterine tissue was used for mRNA isolation and immunohistochemical analyses.

2.2 | Primary stromal cell culture

Isolation of primary stromal cells was performed as described previously.[21,22]

In order to standardize the phase of the oestrous cycle, 6- to 8-week-old mice were subcutaneously injected for three consecutive days with 100 ng/100 μL of E, prior to stromal cell isolation. On the 4th day, uterine horns of E treated mice were dissected longitudinally and cut into 3-5mm pieces. After washing with Hank's balanced salt solution (HBSS), uterine tissues were digested with HBSS containing 6 mg/mL of dispase (Invitrogen, Carlsbad, CA, USA), 25 mg/mL of pancreatin, 100 units/L of penicillin, 0.1 mg/mL of streptomycin and 1.25 mg/L of fungizone (Invitrogen). Tissue suspension was kept on ice for 1 hour, followed by 1 hour incubation at room temperature and then 10 minute incubation at 37°C. The reaction was terminated by addition of 10% fetal bovine serum (FBS), and the supernatant containing the epithelial cell aggregates was discarded. The partially digested tissues were then washed twice in HBSS and then placed in HBSS

containing 0.5 mg/mL of collagenase. After incubation for 45 minute at 37°C, the tubes were vortexed for 10-12 second. Cell suspensions were then passed through a 70-µm gauze filter (Millipore). Cells were resuspended in Dulbecco's modified Eagle's Medium-F12 medium (DMEM-F12; with 100 unit/L of penicillin, 0.1 mg/mL of streptomycin, 1.25 mg/L of fungizone) containing 2% heat-inactivated fetal calf serum. Cell viability was assessed by trypan blue staining.

Four hundred thousand cells were seeded in 6-well cell culture plates containing collagen-coated coverslips (collagen I from rat tail, 50 µg/mL in 0.02 mol/L acetic acid, BD biosciences) and incubated at 37°C and 5% CO_2. The unattached cells were removed by washing several times with HBSS, and fresh medium (control) or media supplemented with 2 µg/mL of PIBF was added. The culture medium was changed every 2 or 3 days with continuous supplementation with PIBF.

The level of desmin expression was used as a marker for decidualization.[23]

2.3 | RNA isolation and real-time RT-PCR analysis

Total RNA was isolated from uterine tissues (n = 3 per pool) using Trizol (Invitrogen) according to the manufacturer's protocol. The RNA was reverse transcribed, and gene levels were quantified by quantitative real-time PCR (qRT-PCR) using the ABI Prism 7300 detection system (Applied Biosystems, Foster City, CA, USA). This procedure was independently repeated three times.

PIBF expression was evaluated using TaqMan Assay Mm00547083 (Applied Biosystems). Relative expression levels of *Gal-1* mRNA were determined by real-time PCR analysis using SYBR Green PCR Universal Mastemix (Applied Biosystems) according to manufacturer's instructions. Oligonucleotide primers for mouse *Gal-1* forward (5'-AAC CTG GGG AAT GTC TCA AAG T-3′) and reverse (5'-GGT GAT GCA CAC CTC TGT GA-3') were obtained from Metabion, Steinkirschen, Germany. Relative mRNA levels were determined after normalization to mouse *GAPDH* expression.[24]

2.4 | Immunohistochemistry

PIBF expression in the endometria of pregnant mice and desmin expression in decidualized stromal cells were detected by immunohistochemistry.

Five micrometre paraffin-embedded sections were deparaffinized, rehydrated and revealed with DAKO Target Retrieval Solution, (DAKO, Glostrup, Denmark), pH 6.0 in a microwave oven. Endogenous peroxidase activity was inhibited by treatment with 3% H_2O_2, and non-specific binding was blocked with 3% bovine serum albumin (BSA; Affymetrix, Santa Clara) in PBS. The slides were than reacted with 1:25 diluted biotinylated monoclonal anti-PIBF antibody produced in our laboratory,[25] or biotinylated mouse IgG2a as a control for 1 hour in a humidified chamber. After the incubation, the slides were washed for 3 × 5 minute, and sections that had been incubated with anti-PIBF antibody were reacted with 1:100 diluted streptavidin-horseradish-peroxidase (GE Healthcare, Little Chalfont, UK), for 30 minute in a humidified chamber. The reaction was developed with diamino-benzidine (DAKO). Nuclei were counterstained with haematoxylin (DAKO) for 3 minute, and the slides were mounted with entellan (Merck, Kenilworth, NJ, USA).

2.5 | Immunofluorescence for detecting decidualization as well as Gal-1, ERα and PR expression in decidual NK cells

Decidual transformation of endometrial stromal cells was detected by desmin reactivity. Cultured cells fixed with 4% PFA were incubated overnight at 4°C with 1:200 diluted rabbit anti-desmin antibody (Abcam, Cambridge, UK). To visualize immune complexes, Alexa donkey anti-rabbit 488 nm (1:300 dilution; Molecular Probes, Eugene, OR, USA) was used. Nuclei were visualized with DAPI (Vector Laboratories, Burlingame, CA, USA).

For immunofluorescent analyses of Gal-1, PR, ERα expression in NK cells, uterine sections (5 µm) were prepared from tissues that were fixed in 4% PFA and paraffin-embedded. The sections were deparaffinized, rehydrated in graded concentrations of ethanol and subjected to heat-induced antigen retrieval (10 mmol/L sodium citrate, pH 6.0). The slides were then washed in PBS and pre-incubated in 1% BSA in PBS containing 0.001% NaN_3 for 1 hour at room temperature.

In the next step, uterine sections were reacted with 1:400 diluted rabbit polyclonal antibody to Gal-1 (Abcam) followed by donkey anti-rabbit Alexa Fluor 594 nm (1:500 dilution; Molecular Probes) as a second antibody.

Rabbit monoclonal anti ERα antibody (Abcam) was used at 1:200 dilutions. Polyclonal antibody to PR (DAKO) was diluted 1:100. Immunocomplexes of primary antibodies with PR or ERα were visualized with donkey anti-rabbit Alexa Flour 594 antibody (1:500 dilution; Molecular Probes). To identify uNK cells, tissue sections were incubated with fluorescein-labelled Dolichos biflorus lectin (DBA; Vector Laboratories) at 1:200 dilution for 30 minute. Nuclei were visualized with DAPI (Vector Laboratories).

All Images were captured on Olympus imaging system BX51 equipped with DP71CCD camera (Olympus, Tokyo, Japan), and CellF imaging software was used. Images were edited using Photoshop CS6 (Adobe, San Jose, CA, USA).

2.6 | Statistical analysis

All data are reported as mean ± SEM and were analysed by a Student's *t*-test and one-way ANOVA followed by the Scheffe post hoc method. $P \leq 0.05$ was considered significant. All statistical analyses were performed using STATISTICA 10 (StatSoft Inc, Tulsa, OK, USA).

3 | RESULTS

3.1 | In the mouse uterus, PIBF is produced via activation of progesterone receptor A

To test whether activation of the classical PRs was involved in PIBF production, 6- to 8-week old ovariectomized BALB/c mice were treated with

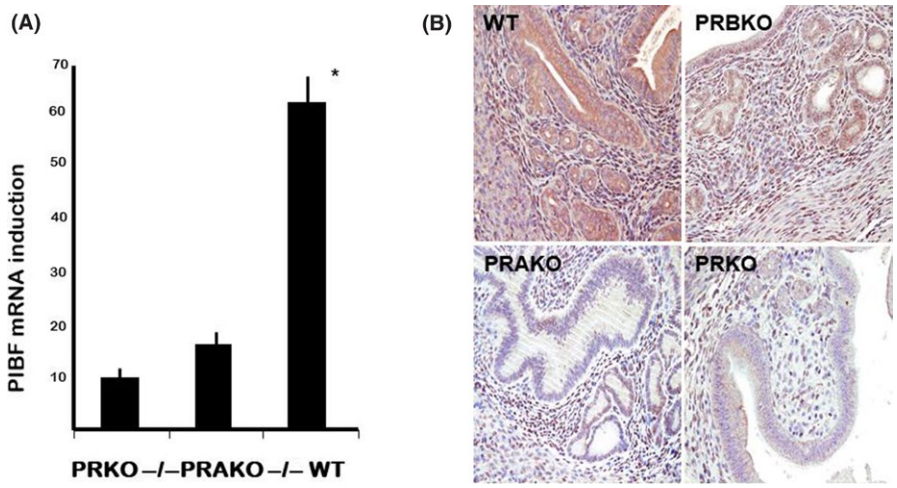

FIGURE 1 PIBF expression in the uterus of ovariectomized, P-treated mice. Six- to eight-wk-old BALB/c mice were ovariectomized and allowed to rest for 2 wk. Mice were then treated subcutaneously with 1 mg of P, or sesame oil as a control, and killed 6 h later, when uterine tissues were collected for RNA isolation and immunohistology. (A) Real-time reverse-transcription PCR was performed for quantification of gene expression and normalized to expression level in PRKO mice. The *Pibf1* mRNA was increased in ovariectomized and progesterone-treated WT mice, but not in PRKO and PRAKO animals. The bars represent the mean ± SEM of three experiments. $^*P \leq 0.05$ (Student's *t*-test). (B) Immunohistochemical analysis of P-induced expression of PIBF in the endometria of WT, PRAKO and PRBKO mice. Five-micrometre paraffin sections were reacted with 1:25 diluted biotinylated monoclonal anti-PIBF antibody. PIBF protein expression was reduced in the endometria of PRKO and PRAKO, but not in PRBKO mice. Images were captured on an Olympus imaging system equipped with a DP71CCD camera with 40× objective. This experiment was performed three times with identical results

1 μ g of P, or sesame oil as a control, for 6 hours. Uterine tissues were collected for RNA isolation and immunohistology. Real-time reverse-transcription PCR was performed for quantification of gene expression.

PIBF mRNA was significantly increased in ovariectomized and progesterone (P)-treated wild-type (WT) mice, but not in PRKO and PRAKO mice under the same conditions (Figure 1A). PIBF expression in the uterus was checked by immunohistochemistry. PIBF protein expression was reduced in the endometria of PRKO and PRAKO, but not in PRBKO mice (Figure 1B).

These data suggest that PIBF transcription depends on the activation of PR-A.

3.2 | PIBF induces decidual transformation of mouse endometrial stromal cells

To investigate the possible role of PIBF in decidualization, endometrial stromal cells were isolated from the uterus of non-pregnant mice. The cells were cultured to confluence and incubated with medium

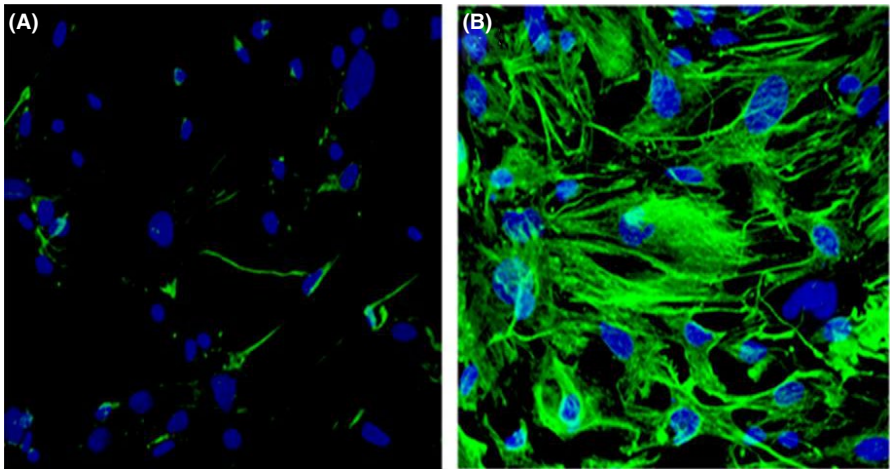

FIGURE 2 PIBF induces decidualization of mouse endometrial stromal cells. Endometrial stromal cells were isolated from the uterus of non-pregnant mice. Decidualization was induced by incubating confluent cells for 6 d in a (A) medium without PIBF and (B) with PIBF (2 μg/mL). The level of desmin expression was used as a marker for the decidualization. The cells were incubated overnight at 4°C with 1:200 diluted rabbit anti-desmin antibody, and Alexa 1:300 diluted donkey anti-rabbit 488 nm was used to visualize immune complexes. Images were captured on an Olympus imaging system equipped with a DP71CCD camera with 100× objective. This experiment was performed three times with identical results. Desmin reactivity appears as green fluorescence. Nuclei were counterstained with DAPI (blue)

containing 2 μg/mL PIBF for 6 days. Control cell cultures were incubated in medium without PIBF. Desmin was used as a marker for decidualization.[23] During a 6-day culture, PIBF induced significantly elevated desmin expression in mouse decidual stromal cells (Figure 2), which indicates that PIBF might be one of the factors that contribute to decidua formation, and possibly endometrial receptivity.

3.3 | PIBF expression in the uterus corresponds with the implantation window

To assess the expression profile during the peri-implantation period, PIBF expression in the mouse uterus at days 2.5, 3.5, 4.5, 5.5 and 7.5 after sighting of the vaginal plug was tested by immunohistochemistry. Paraffin-embedded uterine sections were reacted with anti-PIBF monoclonal antibody. At the implantation site at gestation day 4.5, immunostaining indicative of PIBF is present in nuclei and cytoplasm of uterine stromal and epithelial cells. Furthermore, PIBF expression in the uterus was highest at days 4.5 and 5.5, during the implantation window (Figure 3), suggesting that PIBF might have a role in implantation.

3.4 | The expression of Gal-1 mRNA and Gal-1 in the uteri of BALB/c mice during early pregnancy

Previous findings suggest that Gal-1 expression is required for implantation and the maintenance of early pregnancy. We tested Gal-1 expression at the implantation sites in early pregnancy.

Pregnant BALB/c mice were killed at the indicated time points, uterine horn tissue was removed, and the conceptuses on day 7.5 and 11.5 of pregnancy were carefully dissected out of the implantation site. Real-time reverse-transcription PCR was performed for quantification of gene expression (Figure 4A). Gal-1 expression in the endometria was detected by immunofluorescence, using 1:400 diluted rabbit polyclonal antibody to Gal-1 (Abcam) followed by donkey anti-rabbit Alexa Fluor 594 nm (1:500 dilution; Molecular Probes) as a second antibody (Figure 4B).

Both Gal-1 mRNA and Gal-1 expression continuously increased in the mouse uterus between day 2.5 and day 11.5 of gestation.

3.5 | ERα, PR and Gal-1 expression in decidual NK cells

Earlier data show that murine decidual NK cells express PIBF in their cytoplasmic granules,[26] and that there is a cross-regulation between progesterone and Gal-1 at the feto-maternal interface.[9]

Therefore, we tested whether decidual NK cells express PRs and Gal-1.

Decidual NK cells do not express PRs (Figure 5A), but most of them do express ERα (Figure 5B) and Gal-1 at day 7.5 murine pregnancy at the mesometrial area of the endometrium (Figure 6).

These findings confirm that PRs are absent from decidual NK cells. Therefore, it is unlikely that either PIBF or Gal-1, localized in the cytoplasmic granules of these cells, is produced by the NK cells themselves, rather taken up from the surrounding environment.

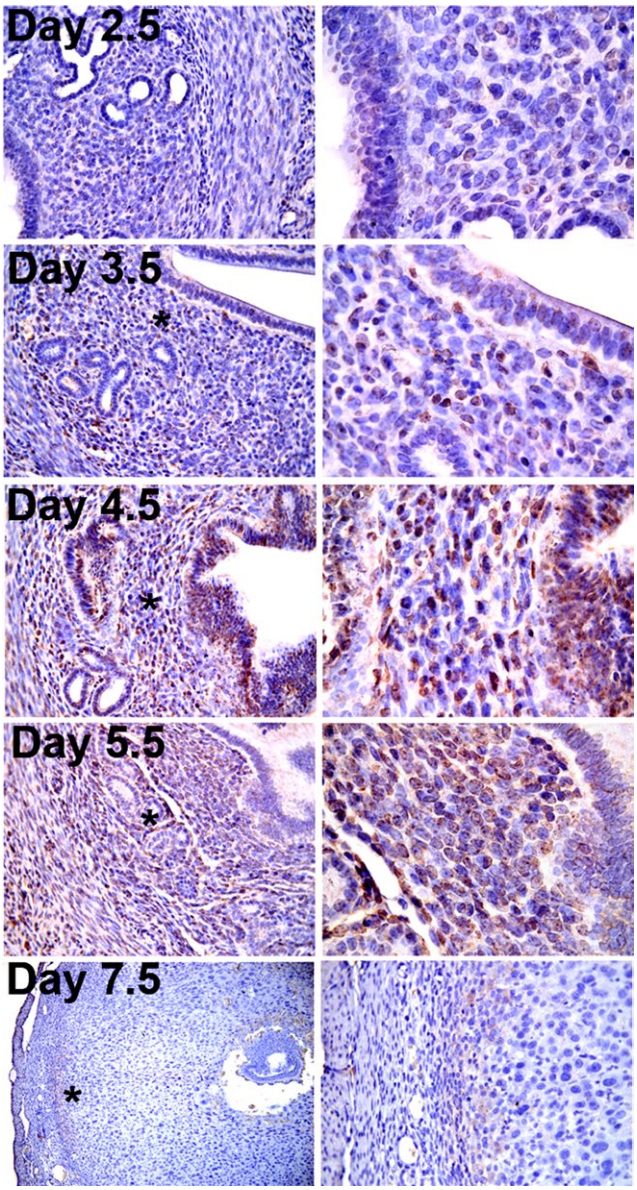

FIGURE 3 PIBF expression in the endometrium of wild-type mice during the peri-implantation period. BALB/c mice were mated overnight. Sighting of the vaginal plug was considered day 0.5 of pregnancy. Animals were killed at the indicated time points. Uterine horns were removed, incubated with 4% PFA, paraffin-embedded sections (5 μm) were prepared, and the slides were reacted with 1:25 diluted anti-PIBF monoclonal antibody. Images were captured on an Olympus imaging system equipped with a DP71CCD camera with 10× (left panels) and 40× objective (right panel). This experiment was performed three times with identical results

4 | DISCUSSION

The *Pibf1* gene contains a progesterone response element[20] and is activated following engagement of PRA in the mouse uterus.

Earlier data revealed that anti-PIBF treatment of pregnant mice at day 10 results in an increased resorption rate, indicating that PIBF is required for the maintenance of pregnancy.[27]

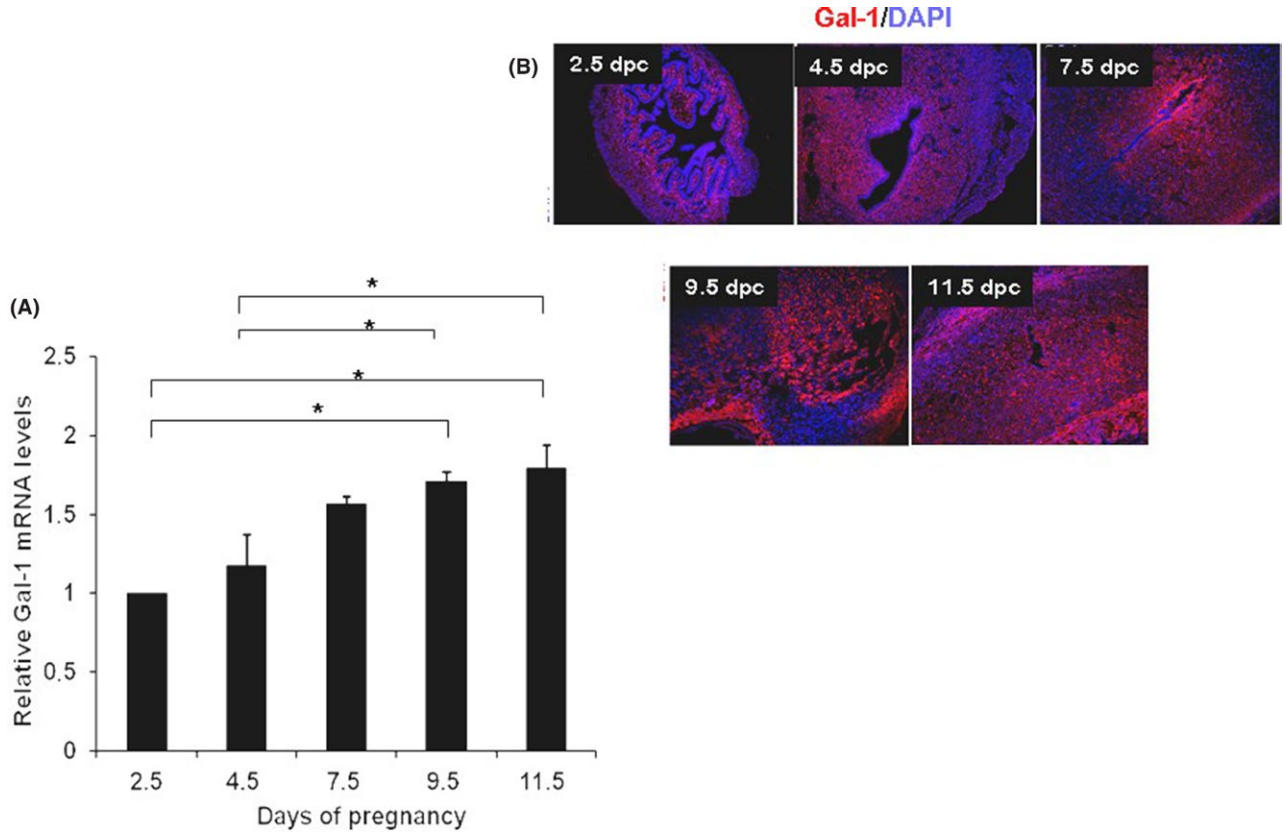

FIGURE 4 *Gal-1* mRNA and Gal-1 expression in uteri of wild-type mice during early pregnancy. BALB/c mice were mated overnight. Sighting of the vaginal plug was considered day 0.5 of pregnancy. Animals were killed at the indicated time points. Uterine horn tissue was removed and was collected for RNA isolation or immunofluorescent staining. (A) Real-time reverse-transcription PCR was performed for quantification of gene expression and normalized to expression level at 2.5 dpc. Bars represent mean ± SEM (N = 6) *$P \leq 0.05$ (ANOVA followed by the Scheffe post hoc method). (B) Uterine sections were reacted with 1:400 diluted rabbit polyclonal antibody to Gal-1 followed by 1:500 diluted donkey anti-rabbit Alexa Fluor 594 nm as a second antibody. Images were captured on an Olympus imaging system equipped with a DP71CCD camera with 10× objective. This experiment was performed three times with identical results

Recently, we showed that extracellular vesicles produced by the pre-implantation mouse embryo express PIBF, and that PIBF containing extracellular vesicles can alter the cytokine profile of murine spleen cells.[28] These data suggest that PIBF might play a role in the feto-maternal interaction during the peri-implantation period.

PIBF induces decidualization of mouse endometrial stromal cells, and most importantly, its expression in the mouse uterus corresponds with the implantation window.It is well documented that P activity is required for the establishment and maintenance of pregnancy,[29,30] and the present observations suggest that not only some of the pro-gestational effects of P are mediated by PIBF but also that PIBF might indeed play a role in the process of implantation.

We demonstrate a continuous increase in the mRNA expression of another P-regulated gene Gal-1 between days 2.5 and 11.5 of gestation in mice. *Gal-1* gene expression in the mouse uterine tissues is regulated by ovarian steroids during implantation[8]; furthermore, a cross-regulation between Gal-1 and P has been demonstrated in stress-challenged murine pregnancies.[9]

Although Gal-1 expression in the female reproductive system was first reported in the 1990s, many functional aspects of this lectin during pregnancy have recently been discovered.[31-33] Gal-1 is present on trophoblast cells in the placental bed in haemochorial placentation and decidualization.[34,35] Proteomic studies showed that Gal-1 expression is reduced on placental villous tissues from patients with spontaneous miscarriages.[36] In mice, Gal-1 deficiency results in a higher frequency of fetal loss in allogeneic matings compared to syngeneic matings.[37]

In our hands, a subpopulation of decidual NK cells expressed Gal-1. Though all NK cell types express Gal-1,[38] Gal-1 + NK cells are enriched in the decidua, compared to peripheral blood,[38] and Gal-1 is endowed with an immune-regulatory function.[39]

In spite of their high perforin content, spontaneous cytotoxic activity of decidual NK cells is moderate.[40] Though there is no evidence that NK cells directly attack the trophoblast, recurrent miscarriage is often accompanied by increased decidual NK activity, suggesting that this mechanism might be a component of the underlying pathology.[41-45]

In mice, PIBF protects pregnancy by controlling NK activity.[24] Earlier data[16] show that PIBF inhibits degranulation of NK cells. Decidual NK cells contain PIBF, which co-localizes with perforin in the cytoplasmic granules. In day 12.5 normal mouse pregnancy, only 54% of the PIBF+ decidual NK cells are perforin positive, whereas in

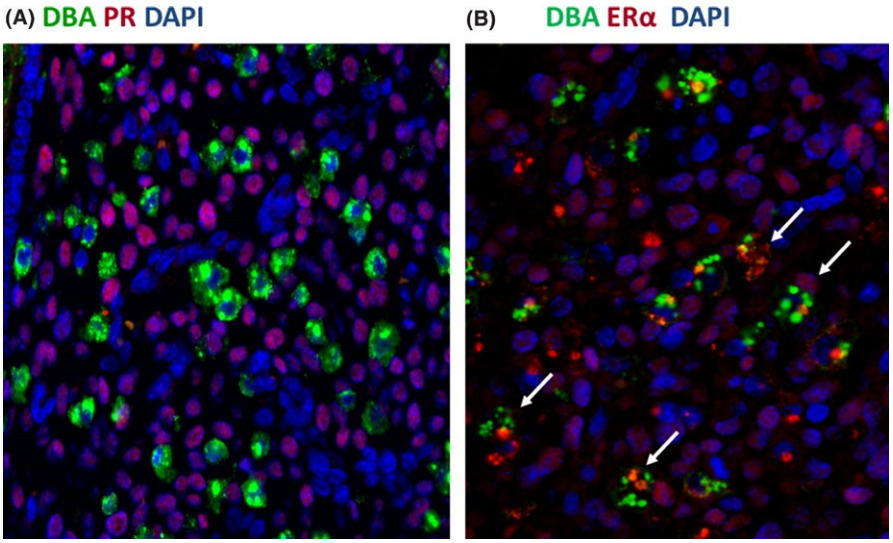

FIGURE 5 Expression of PR and ERα in decidual NK cells. BALB/c mice were mated overnight. Sighting of the vaginal plug was considered day 0.5 of pregnancy. Animals were killed at 7.5 dpc. Immunofluorescent staining was performed with 1:100 diluted rabbit polyclonal to PR (A) and 1:200 diluted rabbit monoclonal antibody to ERα (B). Immunocomplexes were detected with anti-rabbit Alexa Fluor 594 (red). Fluorescein-labelled DBA lectin was used to identify uNK cells (green). Nuclei were counterstained with DAPI (blue). Images were captured on an Olympus imaging system equipped with a DP71CCD camera with 40× objective. The asterix in panel B depicts the region shown at a higher magnification in the insert. This experiment was performed three times with identical results

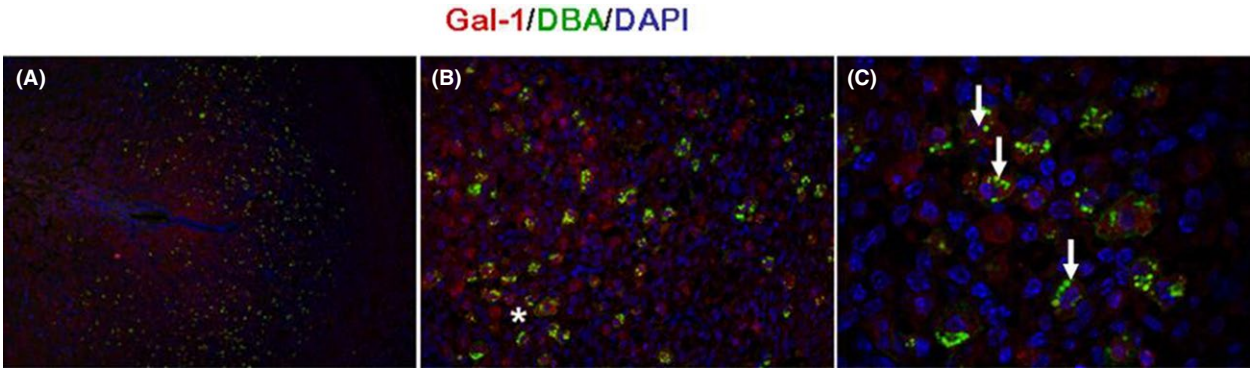

FIGURE 6 Gal-1 expression in mouse decidual NK cells. BALB/c mice were mated overnight. Sighting of the vaginal plug was considered day 0.5 of pregnancy. Animals were killed at 7.5 dpc. For analyses of Gal-1 expression in decidual NK cells, immunofluorescent staining was performed with 1:400 diluted rabbit polyclonal antibody to Gal-1, followed by 1:500 diluted donkey anti-rabbit Alexa fluor 594 (red). Fluorescein-labelled DBA lectin (diluted 1:200) was used to identify uNK cells (green). Nuclei were counterstained with DAPI (blue). Images were captured on an Olympus imaging system equipped with a DP71CCD camera with 10× (A), 40× (B) and 100× (C) objectives, respectively. The asterix in panel B depicts the region shown in panel C at a higher magnification. This experiment was performed three times with identical results

RU486 treated PIBF deficient mice of the same gestational age, not only do most of the PIBF + NK cells disappear, but all of the remaining ones contain perforin.[26]

Here, we show that mouse decidual NK cells do not express nuclear PRs, but they do express ERα. RU486 treatment of pregnant mice results in diminished transcriptional PR-A and PR-B activity and impaired PIBF synthesis by PR positive endometrial cells that could also affect the activity of neighbouring uNK cells via a paracrine mechanism. PIBF does not contain ERα response element while perforin does.[46] A possible mechanism for reduced cytotoxic activity of decidual NK cells might be that while perforin expression is upregulated via an ER-mediated mechanism, PIBF produced by PR+ endometrial cells is taken up by NK cells and inhibits perforin release.

PIBF inhibits NK cell degranulation, while recent studies revealed the ability of Gal-1 to control the non-secretory lytic pathway of cytotoxic cells, by influencing Fas-Fas ligand interactions.[47]

Based on the above data, it cannot be ruled out that the concerted action of PIBF and Gal-1 in decidual NK cells contribute to the low cytotoxic activity of decidual NK cells.

Furthermore; Galectin-1 and PIBF have pleiotropic physiological roles in cell adhesion, migration, proliferation, apoptosis,

mRNA splicing, angiogenesis and regulation of immune response. Interaction of these proteins in decidual NK cells could be of importance for downstream events in pregnancy progression.

5 | CONCLUSION

Our results show that activation of PRA results in PIBF production. PIBF induces decidual transformation of stromal cells, and its expression in the mouse endometrium peeks during the implantation window, suggesting that and that PIBF might be involved in the implantation process.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Li X, Lonard DM, O'Malley BW. A contemporary understanding of progesterone receptor function. *Mech Ageing Dev*. 2004;125:669-78.
2. Mulac-Jericevic B, Conneely OM. Reproductive tissue selective actions of progesterone receptors. *Reproduction*. 2004;128:139-146.
3. Li X, O'Malley BW. Unfolding the action of progesterone receptor. *J Biol Chem*. 2003;278:39261-39264.
4. Lydon JP, DeMayo FJ, Funk CR, Mani SK, Hughes AR, Montgomery CA, et al. Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev*. 1995;9:2266.
5. Fernandez-Valdivia R, Mukherjee A, Mulac-Jericevic B, Conneely OM, DeMayo FJ, Amato P, et al. Revealing progesterone's role in uterine and mammary gland biology: insights from the mouse. *Semin Reprod Med*. 2005;23:22-37.
6. Tibbetts TA, Conneely OM, O'Malley BW. Progesterone via its receptor antagonizes the pro-inflammatory activity of estrogen in the mouse uterus. *Biol Reprod*. 1999;60:1158-65.
7. Tibbetts TA, DeMayo F, Rich S, Conneely OM. O'Malley BW Progesterone receptors in the thymus are required for thymic involution during pregnancy and for normal fertility. *Proc Natl Acad Sci U S A*. 1999;96:12021-6.
8. Choe YS, Shim C, Choi D, Lee CS, Lee KK, Kim K. Expression of galectin-1 mRNA in the mouse uterus is under the control of ovarian steroids during blastocyst implantation. *Mol Reprod Dev*. 1997;48:261-6.
9. Blois S, Ilarregui JM, Tometten M, Garcia M, Orsal AF, Toscano M, et al. A pivotal role for galectin-1 in fetal tolerance Nat. *Med*. 2007;13:1450-7.
10. Miko E, Halasz M, Jericevic-Mulac B, Wicherek L, Arck P, Arató G. Progesterone-induced blocking factor (PIBF) and trophoblast invasiveness. *J Reprod Immunol*. 2011;90:50-7.
11. Halasz M, Polgar B, Berta G, Czimbalek L, Szekeres-Bartho J. Progesterone-induced blocking factor differentially regulates trophoblast and tumor invasion by altering matrix metalloproteinase activity. *Cell Mol Life Sci*. 2013;70:4617-30.
12. Balassa T, Berta G, Jakab L, Bohonyi N, Szekeres-Bartho J. The effect of the Progesterone-Induced Blocking Factor (PIBF) on E-cadherin expression, cell motility and invasion of primary tumour cell lines. *J Reprod Immunol*. 2018;125:8-15.
13. Lachmann M, Gelbmann D, Kálmán E, Polgár B, Buschle M, Von Gabain A, et al. PIBF (progesterone induced blocking factor) is overexpressed in highly proliferating cells and associated with the centrosome. *Int J Cancer*. 2004;112:51-60.
14. Anderle C, Hammer A, Polgar B, Hartmann M, Wintersteiger R, Blaschitz A, et al. Human trophoblast cells express the immunomodulator progesterone-induced blocking factor. *J Reprod Immunol*. 2008;79:26-36.
15. Szekeres-Bartho J, Par G, Dombay Gy, Smart YC, Volgyi Z. The anti-abortive effect of progesterone-induced blocking factor in mice is manifested by modulating NK ctivity. *Cell Immunol*. 1997; 177: 194-9.
16. Faust Z, Laskarin G, Rukavina D, Szekeres-Bartho J. Progesterone-induced blocking factor inhibits degranulation of natural killer cells. *Am J Reprod Immunol*. 1999;42:71-5.
17. Laskarin G, Strbo N, Sotosek V, Rukavina D, Faust Z, Szekeres-Bartho J, et al. Progesterone directly and indirectly affects perforin expression in cytolytic cells. *Am J Reprod Immunol*. 1999;42:312-20.
18. Szekeres-Bartho J, Faust Z, Varga P, Szereday L, Kelemen K. The immunological pregnancy protective effect of progesterone is manifested via controlling cytokine production. *Am J Reprod Immunol*. 1996;35:348-51.
19. Kozma N, Halasz M, Polgar B, Poehlmann TG, Markert UR, Palkovics T, et al. Progesterone-induced blocking factor activates STAT6 via binding to a novel IL-4 receptor. *J Immunol*. 2006;176:819-26.
20. Rubel CA, Lanz RB, Kommagani R, Franco HL, Lydon JP, DeMayo FJ. Research resource: Genome-wide profiling of progesterone receptor binding in the mouse uterus. *Mol Endocrinol*. 2012;26:1428-42.
21. Li Q, Kannan A, Wang W, Demayo FJ, Taylor RN, Bagchi MK, et al. Bone morphogenetic protein 2 functions via a conserved signaling pathway involving Wnt4 to regulate uterine decidualization in the mouse and the human. *J Biol Chem*. 2007;43:31725-31732.
22. De Clercq K, Hennes A, Vriens J. Isolation of Mouse Endometrial Epithelial and Stromal Cells for In Vitro Decidualization. *J Vis Exp*. 2017; 121. https://doi.org/10.3791/55168.
23. Glasser SR, Julian J. Intermediate filament protein as a marker of uterine stromal cell decidualization. *Biol Reprod*. 1986;35:463-74.
24. Polesskaya A, Naguibneva I, Fritsch L, Duquet A, Ait-Si-Ali S, Robin P, et al. CBP/p300 and muscle differentiation: no HAT, no muscle. *EMBO J*. 2001;20:6816-6825.
25. Polgar B, Kispal G, Lachmann M, Paar C, Nagy E, Csere P, et al. Molecular cloning and immunologic characterization of a novel cDNA coding for progesterone-induced blocking factor. *Journal of Immunology*. 2003;171:5956-5963.
26. Bogdan A, Berta G, Szekeres-Bartho J. PIBF positive uterine NK cells in the mouse decidua. *J Reprod Immunol*. 2017;119:38-43.
27. Szekeres-Bartho J, Par G, Szereday L, Smart CY, Achatz I. Progesterone and non-specific immunologic mechanisms in pregnancy. *Am J Reprod Immunol*. 1997;38:176-82.
28. Pallinger E, Bognar Z, Bogdan A, Csabai T, Abraham H, Szekeres-Bartho J. PIBF+ extracellular vesicles from mouse embryos affect IL-10 production by CD8+ cells. *Sci Rep*. 2018;8:4662.
29. Virgo BB, Bellward GD. Serum progesterone levels in the pregnant and postpartum laboratory mouse. *Endocrinology*. 1974;95:1486-90.

30. Bhurke AS, Bagchi IC. Bagchi MK progesterone-regulated endometrial factors controlling mmplantation. *Am J Reprod Immunol*. 2016;75:237-45.
31. Phillips B, Knisley K, Weitlauf KD, Dorsett J, Lee V, Weitlauf H. Differential expression of two beta-galactoside-binding lectins in the reproductive tracts of pregnant mice. *Biol Reprod*. 1996;55:548-58.
32. Maquoi E, van den Brûle FA, Castronovo V, Foidart JM. Changes in the distribution pattern of galectin-1 and galectin-3 in human placenta correlates with the differentiation pathways of trophoblasts. *Placenta*. 1997;18:433-9.
33. Barrientos G, Freitag N, Tirado-González I, Unverdorben L, Jeschke U, Thijssen VL, et al. Involvement of galectin-1 in reproduction: past, present and future. *Hum Reprod Update*. 2014;20:175-93.
34. Vicovac L, Jankovic M, Cuperlovic M. Galectin-1 and -3 in cells of the first trimester placental bed. *Hum. Reprod*. 1998;13:730-735.
35. von Wolff M, Wang X, Gabius HJ, Strowitzki T. Galectin fingerprinting in human endometrium and decidua during the menstrual cycle and in early gestation. *Mol. Hum. Reprod*. 2005;11:189-194.
36. Liu AX, Jin F, Zhang WW, Zhou TH, Zhou CY, Yao WM, et al. Proteomic analysis on the alteration of protein expression in the placental villous tissue of early pregnancy loss. *Biol Reprod*. 2006;75:414-20.
37. Blois SM, Kammerer U, Alba Soto C, Tometten MC, Shaikly V, Barrientos G, et al. Dendritic cells: key to fetal tolerance? *Biol. Reprod*. 2007;77:590-598.
38. Koopman LA, Kopcow HD, Rybalov B, Boyson JE, Orange JS, Schatz F, et al. Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. *J. Exp. Med*. 2003;198:1201-1212.
39. Rabinovich GA, Toscano MA. Turning 'sweet' on immunity: galectin–glycan interactions in immune tolerance and inflammation. *Nat. Rev. Immunol*. 2009;9:338-352.
40. Crncic TB, Laskarin G, Frankovic KJ, Tokmadzic VS, Strobo N, Bedenicki I, et al. Early pregnancy decidual lymphocytes beside perforin use Fas ligand (FasL) mediated cytotoxicity. *J. Reprod. Immunol*. 2007;73:108-17.
41. Quenby S, Farquharson R. Uterine natural killer cells, implantation failure and recurrent miscarriage. *Reprod Biomed Online*. 2006;13:24-8.
42. Veljkovic Vujaklija D, Dominovic M, Gulic T, Mahmutefendic H, Haller H, Saito S, et al. Granulysin expression and the interplay of granulysin and perforin at the maternal-fetal interface. *J Reprod Immunol*. 2013;97:186-96.
43. Yamada H, Kato EH, Kobashi G, Ebina Y, Shimada S, Morikawa M, et al. High NK cell activity in early pregnancy correlates with subsequent abortion with normal chromosomes in women with recurrent abortion. *Am. J. Reprod. Immunol*. 2001;46:132-6.
44. Quenby S, Nik H, Innes B, Lash G, Turner M, Drury J, et al. Uterine natural killer cells and angiogenesis in recurrent reproductive failure. *Hum Reprod*. 2009;24:45-54.
45. Lachapelle MH, Miron P, Hemmings R, Roy DC. Endometrial T, B, and NK cells in patients with recurrent spontaneous abortion. Altered profile and pregnancy outcome. *J Immunol* 1996; 156: 4027-4034.
46. Hewitt SC, Li L, Grimm SA, Chen Y, Liu L, Yin Li Y, et al. Research Resource: Whole-Genome Estrogen Receptor - Binding in Mouse Uterine Tissue Revealed by ChIP-Seq. *Mol Endocrinol*. 2012;26:887-898.
47. Clemente T, Vieira NJ, Cerliani JP, Adrain C, Luthi A, Dominguez MR, et al. Proteomic and functional analysis identifies galectin-1 as a novel regulatory component of the cytotoxic granule machinery. *Cell Death and Disease*. 2017;8:e3176.

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