



Decidual cells are the initial target of polyriboinosinic–polyribocytidylic acid in a mouse model of maternal viral infection

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ABSTRACT

Background: Maternal immune activation has been implicated in the pathophysiology of neurodevelopmental disorders such as autism spectrum disorders caused by maternal infection. It has been suggested that the placental origin of inflammatory cytokines leads to neurodevelopmental disorders. However, the identity of the initial immune-activated site in the placenta, in response to maternal viral infection, is not clear.

Methods: By cross-breeding male enhanced green fluorescent protein (EGFP) transgenic mice with wild-type females, the placental tissues of maternal origin can be distinguished from those of paternal origin by EGFP expression. Using this method, at embryonic day (E) 12.5, dams were administered an intraperitoneal polyriboinosinic–polyribocytidylic acid (poly [I:C]) injection. We quantitatively analyzed the levels of phosphorylated interferon (IFN) regulatory factor 3 (pIRF3) in the placenta, and investigated the distribution of pIRF3 positive cells.

Results: We show that maternally derived decidual cells are the initial target of maternal poly (I:C) through the toll-like receptor 3/TIR-domain-containing the adapter-inducing interferon- β signaling pathway. We also show that the expression of interferon- β was upregulated in the placenta after maternal injection with poly (I:C).

Conclusion: These results suggest that maternally derived decidual cells are the initial target of maternal poly (I:C) and that this innate immune response is likely associated with a state of maternal immune activation.

1. Introduction

Epidemiological studies have suggested that maternal infection in mid-pregnancy is a risk factor for developing neurodevelopmental disorders in offspring [1–4]. A model of neurodevelopmental disorders caused by maternal infections has helped in identifying the underlying mechanisms for these developmental disorders [5–11]. Several studies have found that, the maternal interleukin-6 (IL-6)-mediated mechanism is important for understanding the pathophysiology as well as developing new strategies for preventing neurodevelopmental disorders [6, 10, 12]. Furthermore, it has been demonstrated that interleukin-17a, as a single or downstream mediator of IL-6, causes abnormal cortical structure and autism spectrum disorder (ASD)-related behavior in offspring exposed to maternal immune activation [13]. These studies have also shown that the maternal immune system is activated by injecting

polyriboinosinic–polyribocytidylic acid (poly [I:C]) and is believed to be localized in the placenta, which functions as the maternal–fetal interface. The placenta is composed of two parts—the decidua of maternal origin and trophoblasts of fetal origin. Trophoblasts express toll-like receptor 3 (TLR3) and nuclear localization of NF-kappa B after poly (I:C)-induced maternal immune activation [14]. Poly (I:C) is a ligand for TLR3 and induces an antiviral response. However, it has been suggested that the placental tissue of fetal origin does not contribute to activation of the maternal immune status [10]. Activated maternal immune cells, which comprise uterine natural killer (NK) cells, dendritic cells, and macrophages residing within the decidua, produce inflammatory cytokines, such as IL-6, after injection with poly (I:C) [10, 12]. However, there is no direct evidence indicating the site of TLR3 signaling in the placenta after the poly (I:C) injection into the dam. Understanding this will help elucidate the pathogenesis of neurodevelopmental disorders in

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maternal immune activation. In this study, we determined that the initial TLR3 signaling site of the inflammatory response is in the placenta.

2. Materials and methods

2.1. Animals

Female C57BL/6J mice aged 8–24 weeks were used in this study. To identify the origin of cells that exhibit an immune response in the placenta, we used male C57BL/6-Tg (CAG-EGFP) mice (EGFP transgenic mice) and cross-bred them with wild-type female mice to produce the placenta. The mice were maintained under standard laboratory conditions. Food and water were available *ad libitum*. A female mouse was housed with a male mouse overnight, and the day on which a vaginal plug was found in the morning was designated as embryonic day (E) 0.5. We also purchased pregnant C57BL/6J mice (SLC Japan, Inc., Tokyo, Japan). We maintained these mice for at least 1 d before sacrificing them for dissection. All procedures were performed in strict accordance with the guidelines for the Care and Use of Laboratory Animals of Kanazawa Medical University, Kanazawa, Japan. The protocol was approved by the Committee on the Ethics of Animal Experiments of Kanazawa Medical University. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

2.2. Injecting poly (I:C) and harvesting the placenta

At 12.5 days post coitum, females bred with EGFP transgenic males were administered an intraperitoneal injection of poly (I:C) (Sigma Aldrich, St. Louis, MO, USA) at 20 mg/kg based on the weight of the poly (I:C) [6]. The injection volume was unified into 10 μ L/g body weight. The control group was injected with the same volume of saline. The mice were sacrificed at 3 h post injection under anesthesia, and the placentas were collected for histochemical and molecular biology analyses.

2.3. Histology and immunohistochemistry

Paraffin-embedded placentas were sectioned (10 μ m thick) and adhered to glass slides. Dewaxed and rehydrated sections were stained with hematoxylin and eosin for standard morphological observation. Immunohistochemical analysis using 3,3'-diaminobenzidine (DAB) staining were then conducted on these tissues. Heat-mediated antigens were retrieved using 10 mM citrate buffer (pH 6.0) under microwave irradiation for 5 min. Endogenous peroxidase activity was quenched using 3% hydrogen peroxide in methanol for 10 min. The slides were blocked with 1% bovine serum albumin in phosphate-buffered saline (PBS) containing 0.1% Triton-100 (PBS with Tween, PBST). After washing with PBST, the slides were incubated at 4 °C overnight with the primary antibodies, rabbit anti-TLR3 pAb (1:2000; Abcam plc, Cambridge, UK), anti-TIR-domain-containing adapter-inducing interferon- β (TRIF) pAb (1:5000; Abcam), and rabbit anti-phospho-IRF3 (Ser396) mAb (1:200; Cell Signaling Technology, Danvers, MA, USA). Non-immune rabbit immunoglobulin G (Sigma Aldrich) was used as the negative control. After washing with PBST, the sections were reacted with horseradish peroxidase (HRP)-conjugated anti-rabbit antibody using the EnVision™ system, according to the manufacturer's instructions (Dako). The slides were visualized using the DAB substrate (Dako) and counterstained with hematoxylin before dehydration. The sections were subsequently mounted and captured using NanoZoomer C9600-03 (Hamamatsu Photonics K.K., Hamamatsu, Japan). For immunofluorescence staining, 30- μ m frozen sections were used. Specific staining was obtained using the Opal™ 4-Color Manual IHC Kit (PerkinElmer Japan Co., Ltd., Yokohama, Japan), following the manufacturer's instructions. Primary and secondary antibodies as previously mentioned were used. Rabbit anti-GFP pAb (1:200; Abcam) was used in detecting EGFP. Images were visualized using the LSM 710 confocal

microscope (ZEISS, Oberkochen, Germany). All sections used were chosen from areas near the midsagittal section of the body, where the centrally located spiral arterioles and central canal in the placenta were observed. Quantifications of the pIRF3 signal were performed on three representative images from each group using ImageJ software (<https://imagej.nih.gov/ij/>). Three rectangular areas (50 \times 50 μ m) of each image were chosen, and the percentage of pIRF3 signals within each area was calculated.

2.4. Western blot analysis

Dissected placentas were quickly stored at –80 °C, and western blotting was performed to analyze specific proteins. Using PRO-PREP™ (iNtRON Biotechnology, Inc., Gyeonggi-do, Korea), proteins were extracted from placentas following the manufacturer's instructions. The protein contents were determined using the DS-11 NanoPad (DeNovix, Wilmington, DE, USA). Samples were boiled for 10 min in sample buffer (Wako Pure Chemical Industries, Osaka, Japan), separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and electroblotted onto a polyvinylidene difluoride (PVDF) membrane using the iBlot Device (Invitrogen). To analyze pIRF3 and IRF3, the samples were separated using Phos-tag® SDS-PAGE (Wako Pure Chemical Industries) and electroblotted onto a PVDF membrane after incubating with transfer buffer that included 10 mmol ethylenediaminetetraacetic acid (EDTA). The membrane was incubated with the indicated antibody after blocking in Starting Block blocking buffer (Pierce). Rabbit anti-TLR3 pAb (1:500; Abcam), rabbit anti-phospho-IRF3 (Ser396) mAb (1:500; Cell Signaling Technology), mouse anti-IRF3 mAb (1:200; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), and mouse anti- β -actin pAb (1:1000; Santa Cruz Biotechnology, Inc.) were used as the primary antibodies. The membrane was washed with Tris-buffered saline with Tween 20 and incubated at room temperature with HRP-conjugated anti-rabbit or anti-mouse secondary antibody (MBL International Corporation, Woburn, MA, USA). The signal was visualized using an enhanced western blot chemiluminescent substrate (Thermo Fisher Scientific). To reblot the membrane with either of the primary antibodies, the membrane was stripped using Restore™ Plus Western Blot Stripping Buffer (Thermo Fisher Scientific). The band densitometries from each blot membrane were quantified using ImageJ software (<https://imagej.nih.gov/ij/>), and the relative expression was evaluated.

2.5. Statistical analysis

Data are presented as the mean \pm standard deviation. Statistical analyses were performed using the XLSTAT software, using Student's *t*-test for comparing two groups or one-way analysis of variance for comparing more than two groups. The differences were considered significant at $p < 0.05$.

3. Results

3.1. Expression and distribution of TLR3 in the mouse placenta

Although TLR3, a poly (I:C) receptor, has been observed to be expressed *in vitro* in trophoblast cells during the first trimester of pregnancy in humans [15], *in vivo* evidence for TLR3 expression in the mouse placenta is unclear. We first investigated the protein expression levels of TLR3 from embryonic day (E) 10.5 to E14.0 using western blotting, and determined the localization of TLR3 in the normal placenta using immunostaining. The protein expression of TLR3 was confirmed in the placenta, and the relative expression levels of TLR3 were not significantly different among E10.5, E12.5, and E14.0 (Fig. 1A and B). Immunoreactivity to anti-TLR3 antibody was observed in the decidua and trophoblast cells (Fig. 1C).

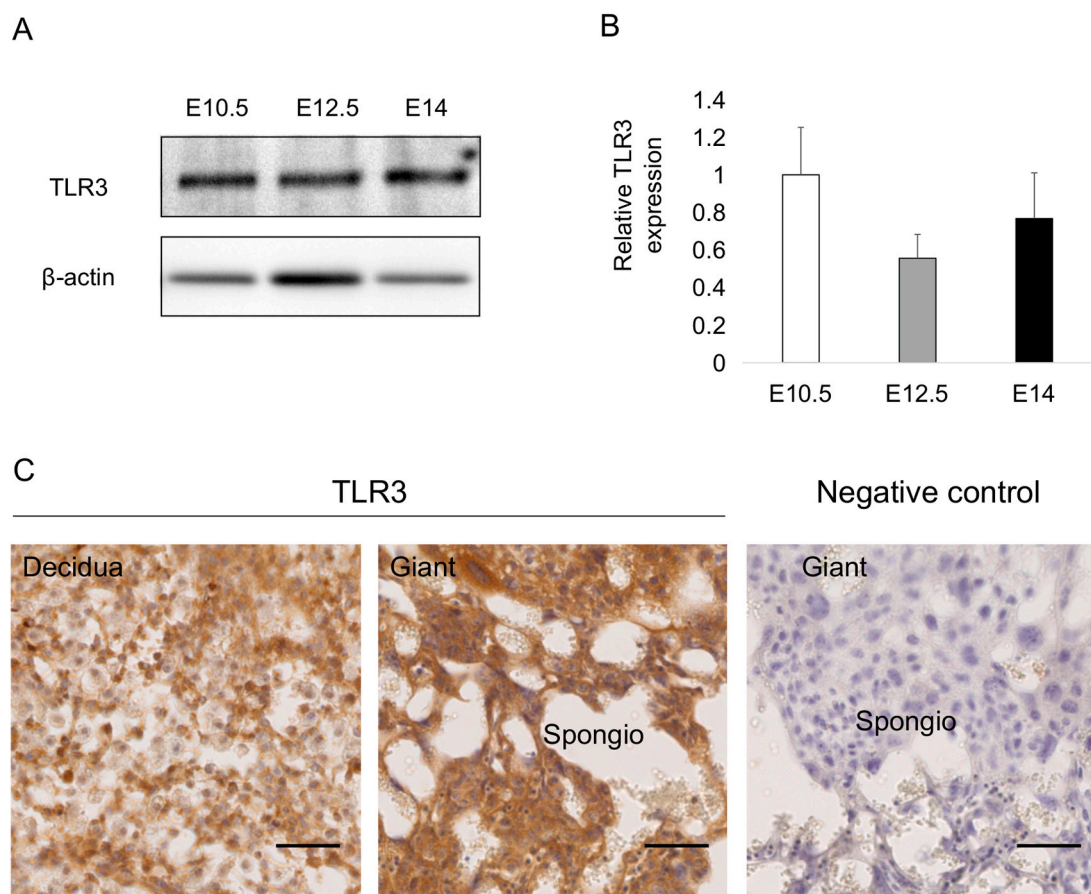


Fig. 1. Protein expression levels and distribution of toll-like receptor 3 (TLR3) in the mouse placenta. (A and B) Western blot analysis of TLR3 in the control placenta at embryonic day (E) 10.5, E12.5, and E14. β -Actin served as the internal control. The protein expression levels of TLR3 are not significantly different among the three groups ($n = 3$ for each group). Data represent the mean \pm standard deviation. (C) Paraffin-embedded sections of the control placenta at E12.5. Immunohistochemical staining of TLR3, visualized using 3,3'-diaminobenzidine (DAB). The image on the right shows the negative control. Notes: Giant, giant trophoblast cells; spongio, spongiotrophoblast cells. Images were taken using a $40\times$ lens. The bar = $50\ \mu\text{m}$.

3.2. Poly (I:C) injection induces TLR3 signaling in the decidua

TLR3 signaling can occur through phosphorylation of type 1 interferon (IFN) regulatory factor 3 (pIRF3) as well as the pathway for nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappa B). In addition, activation of the NF-kappa B pathway is triggered by stimulation of another cytokine, such as tumor necrosis factor α (TNF α). A previous study showed that poly (I:C) injection into the doe causes NF-kappa B signaling in trophoblast cells [14]. To elucidate the initial site and identify a more specific signaling pathway activated by poly (I:C), we quantitatively analyzed the pIRF3 levels in the whole placenta and investigated the distribution of pIRF3-positive cells after poly (I:C) injection. We quantitatively compared the level of pIRF3 in poly (I:C) groups to control groups by western blot analysis and observed that pIRF3 levels increased after poly (I:C) injection (Fig. 2A and B). Six hours after the poly (I:C) injection, we observed a decrease in nuclear pIRF3 levels by western blotting (data not shown). Three hours after poly (I:C) injection, IL-6 in the maternal serum increased to $1642.4 \pm 208.6\ \text{pg/mL}$ (S1 Fig). We mainly analyzed the pIRF3 levels in the placenta 3 h after the poly (I:C) injection. The timing of the initial immune response via the TLR3 signaling pathway positively correlated with incremental increases in maternal IL-6. Using immunofluorescent staining, pIRF3 was detected in the decidua of both groups, and pIRF3 signals appeared to increase in the poly (I:C) group 3 h after the injection (Fig. 2C–F). Theoretically, preceding phosphorylation of IRF3 is necessary for inducing IRF3 signaling prior to nuclear translocation of IRF3. Accordingly, pIRF3 was detected around the nucleus (Fig. 2G and H).

The percentage of pIRF3 signal in the area significantly increased in the poly (I:C) group (Fig. 2I). This quantification of pIRF3 expression through immunofluorescence supports the results of the western blot analysis of pIRF3 levels in whole placenta. To determine the TLR3 signals in the placenta, localization of TLR3 and TIR-domain-containing adapter-inducing interferon- β (TRIF) were determined using immunofluorescence staining. In the decidua, TLR3 and TRIF staining showed a cytoplasmic pattern (Fig. 2J–O).

3.3. The decidua is composed of cells derived from different origins with varying characteristics

We analyzed the origin of the decidual cells that were pIRF3-positive using hybrid mice obtained by cross-breeding EGFP transgenic male mice with wild-type female mice. The offspring exhibited EGFP expression in the paternally derived cells but not in those that were maternally derived (Fig. 3A). The placenta is composed of maternally derived decidua and paternally derived trophoblasts. At the cell layer between the decidua and giant trophoblasts, trophoblast cells invade the decidua up to $150\text{--}300\ \mu\text{m}$ [16]. In the placenta of this EGFP-heterozygous offspring, it is easy to distinguish whether the origin of decidual cells is maternal or paternal (Fig. 3B). As expected, EGFP-expressing cells derived from fetal origins were detected directly, and were distributed in the decidua (Fig. 3B). There were no apparent differences between the control and poly (I:C) groups in the distribution and number of EGFP-expressing cells. Next, we attempted to determine the characteristics of the cells that were EGFP-negative or positive. We

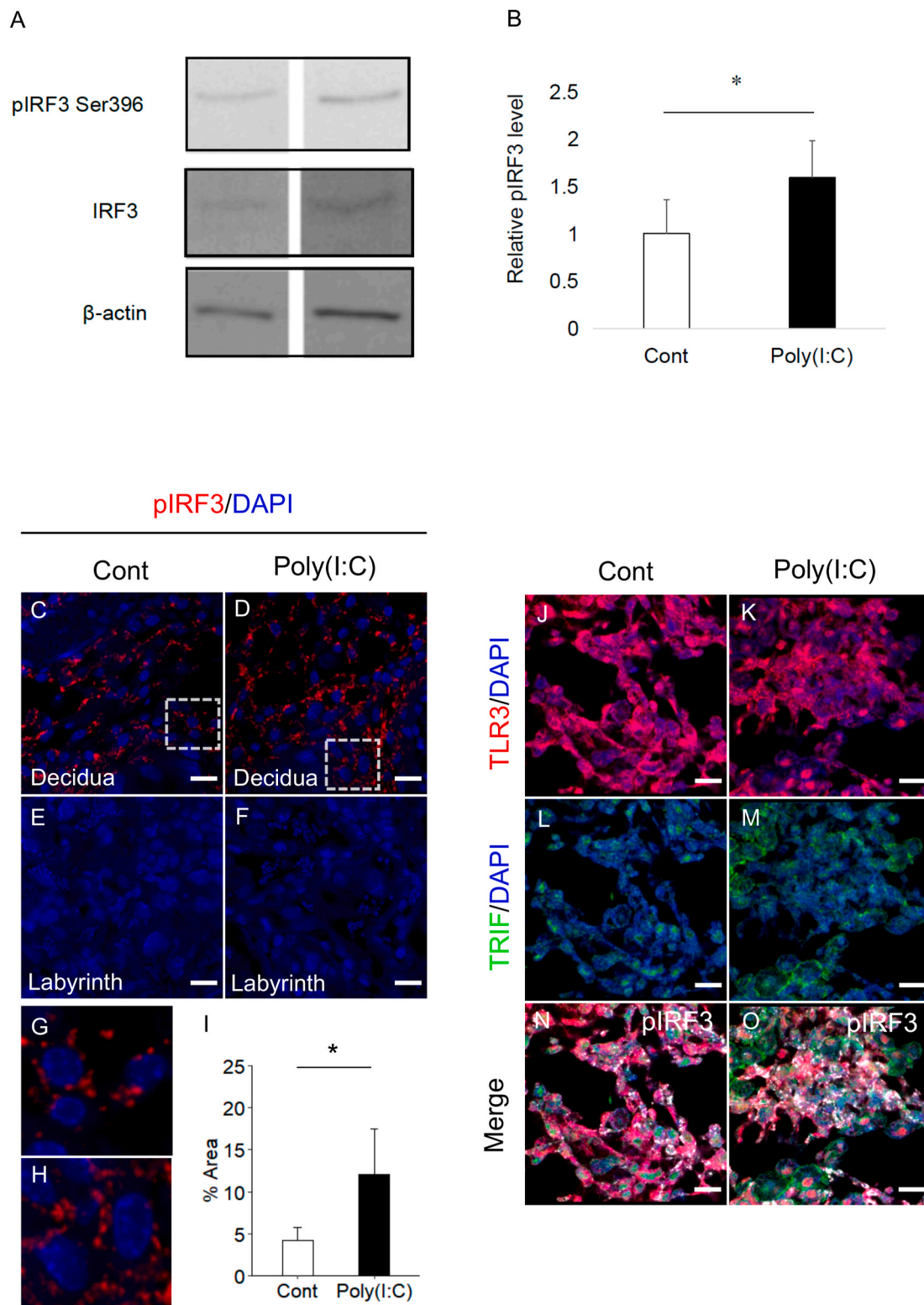


Fig. 2. Polyriboinosinic–polyribocytidylic acid (poly [I:C]) injection induces phosphorylated type 1 interferon (IFN) regulatory factor 3 (pIRF3) through toll-like receptor 3 (TLR3) signaling in the decidua. (A) Western blot analysis of pIRF3 in the placenta at embryonic day (E) 12.5 between the control and poly (I: C) groups. Representative bands of the control and poly (I:C) groups are shown. (B) Relative pIRF3 expression levels are shown as the mean \pm SD ($n = 6$ for each group; $*p < 0.05$). (C–F) Frozen placenta sections were incubated with anti-pIRF3 antibody and stained using the immunofluorescence technique. (G, H) Magnified images of the rectangular area in Fig. 2C and D are shown. The percentage of pIRF3 signals significantly increased in the poly (I:C) group. Calculation of the area was performed on a representative of three independent images. (J–O) Frozen sections of placenta were incubated with anti-TLR3, TIR-domain-containing adapter-inducing interferon- β (TRIF), and pIRF3 antibodies and stained by immunofluorescence. $n = 3$ for each group. The full-length blots are included in a Supplementary Information file (S4 Fig). The grouping of blots was cropped as representatives.

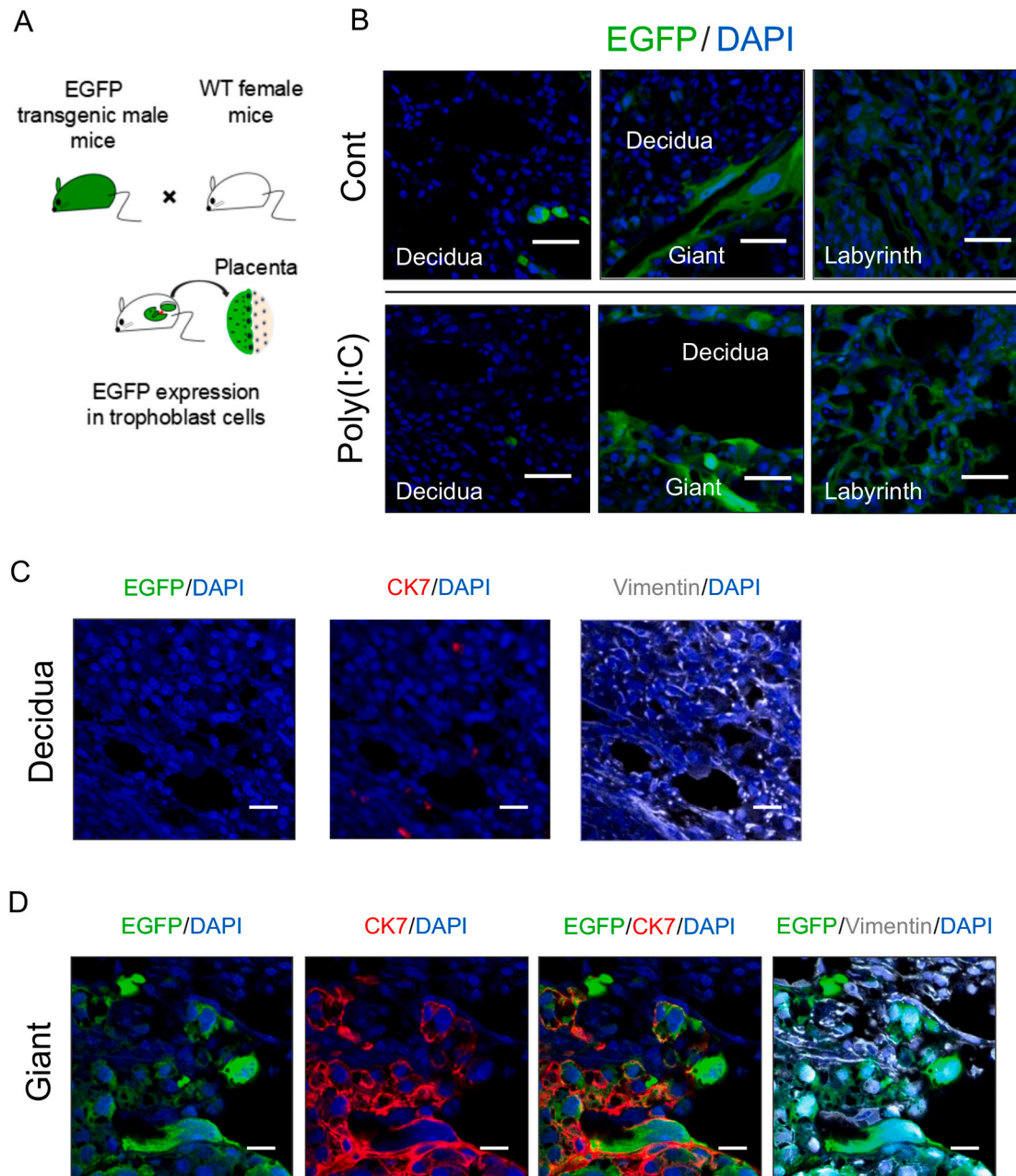


Fig. 3. Origin and characteristics of cells in the decidua. (A) The enhanced green fluorescent protein (EGFP) transgenic male mice were mated with wild-type female mice. In this cross-breeding, the offspring exhibited EGFP expression only in the paternally derived cells. (B) Distribution of EGFP-expressing cells in the placenta. (C, D) Frozen sections of placenta were incubated with anti-GFP, CK7, and vimentin antibodies and stained via immunofluorescent staining in the control group. Notes: Images are representative of data taken using a $40\times$ oil lens; $n = 2$ for each group. The bar = $50\ \mu\text{m}$ in A and $20\ \mu\text{m}$ in B. Giant, giant trophoblast cells; labyrinth, labyrinth layer. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

investigated by double labeling with antibodies against vimentin and cytokeratin 7 (CK7) to distinguish mesenchymal cells from trophoblast cells, respectively. EGFP-negative cells were stained with vimentin but not CK7 (Fig. 3C and D); and the EGFP-positive cells were stained with both CK7 and vimentin (Fig. 3C and D).

3.4. Poly (I:C)-induced TLR3 signaling occurs in the maternally derived mesenchymal cells in the decidua

We then investigated the cells by double staining using anti-GFP Ab and anti-pIRF3 Ab. Phosphorylated IRF3 was detected in the EGFP-negative cells in the decidua of both groups (Fig. 4A–D). The localization pattern of pIRF3-positive cells was not noticeably different between

the control and poly (I:C) groups. We also determined the immunohistochemical localization using antibodies against vimentin, CK7, and pIRF3. Phosphorylated IRF3-positive cells were colocalized with vimentin but not with CK7 (Fig. 4E–J). However, pIRF3 cells were detected in EGFP-negative and CK7-negative as well as vimentin-positive cells. Hsiao and Patterson [10] demonstrated that the number of activated immune cells that were CD69-positive increased in the decidua after poly (I:C) injection into the doe. Since it is well known that immune cells express TLR3, they potentially show a direct response, thereby increasing the phosphorylation of IRF3 in the maternally derived cells. To clarify this, we analyzed the distribution of activated immune cells in the placenta after poly (I:C) injection using anti-CD69 antibody. CD69-positive cells were observed within the intervillous

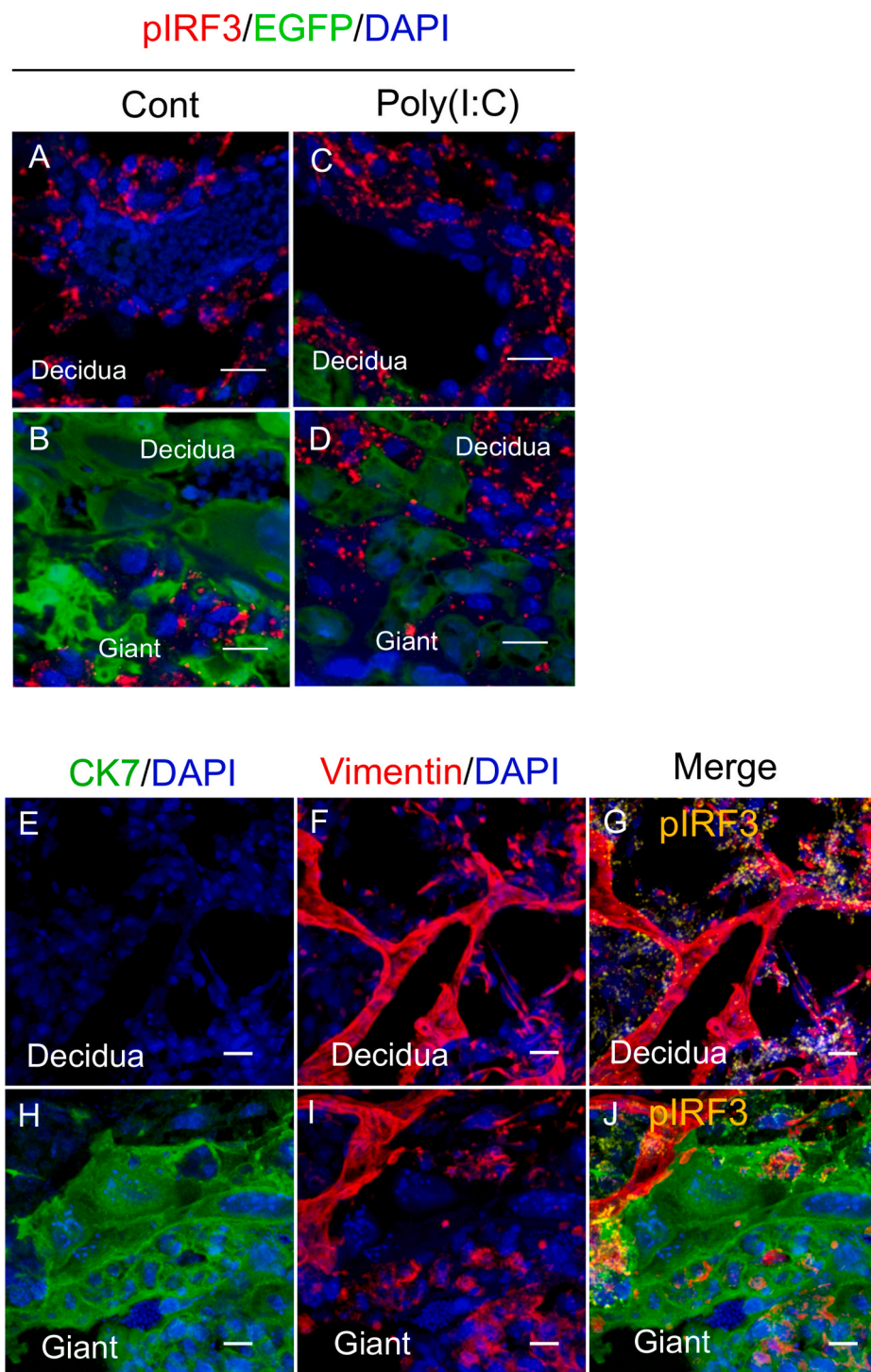


Fig. 4. Polyriboninosinic–polyribocytidylic acid (poly [I:C]) injection induced phosphorylated type 1 interferon (IFN) regulatory factor 3 (pIRF3)-positive cells are mainly maternally derived mesenchymal cells. (A–D) Double staining using anti-green fluorescent protein (GFP) and anti-pIRF3 antibodies. (E–J) Multiple staining using anti-cytokeratin 7 (CK7), vimentin, and pIRF3 antibodies in the poly (I:C) group. From the confocal images, maximum intensity projections of z-sections were created using the ImageJ software (<https://imagej.nih.gov/ij/>). Notes: Representative images are taken using a 40 × oil × lens; n = 2 (A–D) and n = 3 (E–J). The bar = 20 μm.

space filled with maternal blood (S2 Fig). The distribution pattern of these cells was quite different from that of pIRF3-positive cells, which were localized in the stroma of the decidua (Fig. 4E–G). Our findings so far suggest that the IRF3 signal in response to maternal poly (I:C) injection was restricted in decidua but not in activated immune cells. To determine the functional role of the IRF3 signal through TLR3 after maternal immune activation, we analyzed the expression of type I interferons (IFNs) using quantitative real-time polymerase chain reaction. Maternal poly (I:C) injection induced a large increase in IFN β transcription in placental tissue compared to the control ($p < 0.05$, S3 Fig A). We did not observe any differences in the transcription level of IFN α 4

(S3 Fig B).

4. Discussion

We show here that maternal poly (I:C) injection induced a TLR3-mediated immune response in maternally derived mesenchymal cells but not in immune cells. In this study, we examined the TLR3-mediated immune response in the placenta using mice by cross-breeding EGFP transgenic male mice with wild-type female mice. In the offspring, the paternally derived cells exhibit EGFP, which made it easy to clearly distinguish these from the maternally derived EGFP-negative cells in the

placenta (Fig. 3A). A previous study demonstrated that activated immune cells, such as dendritic cells, macrophages, and NK cells, are candidates for inducing inflammatory cytokines and IL-6, and that these immune cells are known to express TLR3 [10]. However, it remains unclear whether TLR3 signaling occurs in the activated immune cells. In contrast, it has been demonstrated by immunostaining nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) p65 that trophoblast cells respond to poly (I:C) injection into the doe [14]. TLR3 signaling was transduced by NF- κ B p65 as well as by pIRF3. NF- κ B p65 is induced by various stimuli, including the TLR3 specific agonist. Although we examined TLR3 signaling using poly (I:C) injection into the doe, we did not determine the location of initial immune activation. By revealing the initial activation site and the type of cells that actually respond to stimuli, we can determine the exact pathogenesis of the maternal infection mouse model for neurodevelopmental disorders, and search for new therapeutic strategies. Within this context, we examined the pIRF3-positive cells and the pIRF3 levels in the placenta induced by the poly (I:C) injection into the doe. The characteristics and distribution of pIRF3-positive cells indicated that the maternally derived mesenchymal cells in the decidua reacted to poly (I:C) injection. However, it was unclear how the immune cells were activated by this treatment. pIRF3 functions as a transcription factor to induce IFNs; therefore, we examined IFN β expression levels and observed that they increased after poly (I:C) injection. Consequently, we considered that induced IFN β activates immune cells, which then secrete cytokines such as IL-6. Cultured endometrial cells can secrete cytokines such as IL-6, as well as chemokines involved in the immune response [17,18]. Therefore, we cannot exclude the possibility that decidual cells may secrete cytokines *in vivo*. Because of the nonimmune cells, it is doubtful that the response of maternally derived decidual mesenchymal cells to poly (I:C) actually occurred. A study on implantation failure has shown that early endometrial cells responded to poly (I:C) administration *in vitro* [19], which is consistent with our results. In addition, after the administration of poly (I:C) on decidual stromal cells, necroptosis of these cells was induced by TLR3/TRIF signaling [20]. Yu et al. [20] showed through *in vivo* analysis that poly (I:C) administration causes a higher frequency of fetal absorption during first pregnancy. It is known that maternal viral infection, maternal stress, and air pollution are known risk factors for neurodevelopmental disorders [21–24], and that these risk factors could also induce inflammatory responses through TLR signaling [25,26]. Besides relating to IL-6 and the IL-17 based maternal immune activation model, decidual TLR3 signaling might also play a key role in the pathogenesis of the recent increasing worldwide prevalence of neurodevelopmental disorders, and unknown causes of infertility or fetal loss in humans.

Based on our findings, we conclude that the decidua is the initial target of maternal immune activators, and propose that increased maternal decidual pIRF3 expression is likely associated with overall immune activation in the placenta.

Author contributions

T.T, H.Shimada, H.S.-H, H.Shoji, H.I, and T.H conceived and designed the experiments. T.T conducted the experiments. T.T, H.S.-H, and T.H analyzed the data. T.T, H.S.-H, and T.H wrote the paper.

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Transparency document

Transparency document related to this article can be found online at <https://doi.org/10.1016/j.bbrep.2021.100958>.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbrep.2021.100958>.

References

- [1] A.S. Brown, P. Cohen, J. Harkavy-Friedman, V. Babulas, D. Malaspina, J. M. Gorman, E.S. Susser, Prenatal rubella, premorbid abnormalities, and adult schizophrenia, *Biol. Psychiatr.* 49 (2001) 473–486, [https://doi.org/10.1016/S0006-3223\(01\)01068-X](https://doi.org/10.1016/S0006-3223(01)01068-X).
- [2] A.S. Brown, M.D. Begg, S. Gravenstein, C.A. Schaefer, R.J. Wyatt, M. Bresnahan, V. P. Babulas, E.S. Susser, Serologic-evidence of prenatal influenza in the etiology of schizophrenia, *Arch. Gen. Psychiatr.* 61 (2004) 774–780, <https://doi.org/10.1001/archpsyc.61.8.774>.
- [3] A.S. Brown, Prenatal infection as a risk factor for schizophrenia, *Schizophr. Bull.* 32 (2006) 200–202, <https://doi.org/10.1093/schbul/sbj052>, sbj052 [pii].
- [4] H.O. Atladóttir, P. Thorsen, L. Østergaard, D.E. Schendel, S. Lemcke, M. Abdallah, E.T. Parner, Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders, *J. Autism Dev. Disord.* 40 (2010) 1423–1430, <https://doi.org/10.1007/s10803-010-1006-y>.
- [5] L. Shi, N. Tu, P.H. Patterson, Maternal influenza infection is likely to alter fetal brain development indirectly: the virus is not detected in the fetus, *Int. J. Dev. Neurosci.* 23 (2005) 299–305, <https://doi.org/10.1016/j.ijdevneu.2004.05.005>, S0736574804000607 [pii].
- [6] S.E. Smith, J. Li, K. Garbett, K. Mirnic, P.H. Patterson, Maternal immune activation alters fetal brain development through interleukin-6, *J. Neurosci.* 27 (2007) 10695–10702, <https://doi.org/10.1523/jneurosci.2178-07.2007>, 27/40/10695 [pii].
- [7] P.A. Carpentier, A.L. Dingman, T.D. Palmer, Placental TNF- α signaling in illness-induced complications of pregnancy, *Am. J. Pathol.* 178 (2011) 2802–2810, <https://doi.org/10.1016/j.ajpath.2011.02.042>.
- [8] P.A. Carpentier, U. Haditsch, A.E. Braun, A.V. Cantu, H.M. Moon, R.O. Price, M. P. Anderson, V. Saravanapandian, K. Ismail, M. Rivera, J.M. Weimann, T. D. Palmer, Stereotypical alterations in cortical patterning are associated with maternal illness-induced placental dysfunction, *J. Neurosci.* 33 (2013) 16874–16888, <https://doi.org/10.1523/jneurosci.4654-12.2013>.
- [9] T. Tsukada, E. Simamura, H. Shimada, T. Arai, N. Higashi, T. Akai, H. Iizuka, T. Hatta, The suppression of maternal-fetal leukemia inhibitory factor signal relay pathway by maternal immune activation impairs brain development in mice, *PLoS One* 10 (2015), e0129011, <https://doi.org/10.1371/journal.pone.0129011>.
- [10] P.H. Patterson, P.H. Patterson, Activation of the maternal immune system induces endocrine changes in the placenta via IL-6, *Brain Behav. Immun.* 25 (2011) 604–615, <https://doi.org/10.1016/j.bbi.2010.12.017>, S0889-1591(10)00590-8 [pii].
- [11] T. Tsukada, H. Shimada, H. Sakata-Haga, H. Iizuka, T. Hatta, Molecular Mechanisms Underlying the Models of Neurodevelopmental Disorders in Maternal Immune Activation Relevant to the Placenta, *Conogenit Anom (Kyoto)*, 2018, <https://doi.org/10.1111/cga.12323>.
- [12] W.L. Wu, E.Y. Hsiao, Z. Yan, S.K. Mazmanian, P.H. Patterson, The placental interleukin-6 signaling controls fetal brain development and behavior, *Brain Behav. Immun.* 62 (2017) 11–23, <https://doi.org/10.1016/j.bbi.2016.11.007>.
- [13] G.B. Choi, Y.S. Yim, H. Wong, S. Kim, H. Kim, S.V. Kim, C.A. Hoefter, D.R. Littman, J.R. Huh, The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring, *Science* 351 (2016) 933–939, <https://doi.org/10.1126/science.aad0314>.
- [14] K. Koga, I. Cardenas, P. Aldo, V.M. Abrahams, B. Peng, S. Fill, R. Romero, G. Mor, Activation of TLR3 in the trophoblast is associated with preterm delivery, *Am. J. Reprod. Immunol.* 61 (2009) 196–212, <https://doi.org/10.1111/j.1600-0897.2008.00682.x>.
- [15] V.M. Abrahams, T.M. Schaefer, J.V. Fahey, I. Visintin, J.A. Wright, P.B. Aldo, R. Romero, C.R. Wira, G. Mor, Expression and secretion of antiviral factors by trophoblast cells following stimulation by the TLR-3 agonist, Poly(I:C), *Hum. Reprod.* 21 (2006) 2432–2439, <https://doi.org/10.1093/humrep/del178>.
- [16] S.L. Adamson, Y. Lu, K.J. Whiteley, D. Holmyard, M. Hemberger, C. Pfarrer, J. C. Cross, Interactions between trophoblast cells and the maternal and fetal circulation in the mouse placenta, *Dev. Biol.* 250 (2002) 358–373.
- [17] S. Sharma, G. Godbole, D. Modi, Decidual control of trophoblast invasion, *Am. J. Reprod. Immunol.* 75 (2016) 341–350, <https://doi.org/10.1111/aji.12466>.
- [18] A.P. Hess, A.E. Hamilton, S. Talbi, C. Dosiou, M. Nyegaard, N. Nayak, O. Genbecev-Krtolica, P. Mavrogianis, K. Ferrer, J. Krussell, A.T. Fazleabas, S.J. Fisher, L. C. Giudice, Decidual stromal cell response to paracrine signals from the trophoblast: amplification of immune and angiogenic modulators, *Biol. Reprod.* 76 (2007) 102–117, <https://doi.org/10.1095/biolreprod.106.054791>.
- [19] M. Montazeri, J.A. Sanchez-Lopez, I. Caballero, N. Maslehat Lay, S. Elliott, S. López-Martín, M. Yáñez-Mó, A. Fazeli, Activation of Toll-like receptor 3 reduces actin polymerization and adhesion molecule expression in endometrial cells, a

- potential mechanism for viral-induced implantation failure, *Hum. Reprod.* 30 (2015) 893–905, <https://doi.org/10.1093/humrep/deu359>.
- [20] S.X. Yu, F.H. Zhou, W. Chen, G.M. Jiang, C.T. Du, G.Q. Hu, Z.Z. Liu, S.Q. Yan, J. M. Gu, X.M. Deng, T.J. Lin, E.K. Duan, Y.J. Yang, Decidual stromal cell necroptosis contributes to polyinosinic-polycytidylic acid-triggered abnormal murine pregnancy, *Front. Immunol.* 8 (2017) 916, <https://doi.org/10.3389/fimmu.2017.00916>.
- [21] A.S. Khashan, K.M. Abel, R. McNamee, M.G. Pedersen, R.T. Webb, P.N. Baker, L. C. Kenny, P.B. Mortensen, Higher risk of offspring schizophrenia following antenatal maternal exposure to severe adverse life events, *Arch. Gen. Psychiatr.* 65 (2008) 146–152, <https://doi.org/10.1001/archgenpsychiatry.2007.20>.
- [22] D.K. Kinney, K.M. Munir, D.J. Crowley, A.M. Miller, Prenatal stress and risk for autism, *Neurosci. Biobehav. Rev.* 32 (2008) 1519–1532, <https://doi.org/10.1016/j.neubiorev.2008.06.004>.
- [23] A. Ronald, C.E. Pennell, A.J. Whitehouse, Prenatal maternal stress associated with ADHD and autistic traits in early childhood, *Front. Psychol.* 1 (2010) 223, <https://doi.org/10.3389/fpsyg.2010.00223>.
- [24] L. Pagalan, C. Bickford, W. Weikum, B. Lanphear, M. Brauer, N. Lanphear, G. E. Hanley, T.F. Oberlander, M. Winters, Association of prenatal exposure to air pollution with autism spectrum disorder, *JAMA Pediatr* (2018), <https://doi.org/10.1001/jamapediatrics.2018.3101>.
- [25] J. Cienciewicki, L. Brighton, W.D. Wu, M. Madden, I. Jaspers, Diesel exhaust enhances virus- and poly(I:C)-induced Toll-like receptor 3 expression and signaling in respiratory epithelial cells, *Am. J. Physiol. Lung Cell Mol. Physiol.* 290 (2006) L1154–L1163, <https://doi.org/10.1152/ajplung.00318.2005>.
- [26] A. Friebe, A.J. Douglas, E. Solano, S.M. Blois, E. Hagen, B.F. Klapp, D.A. Clark, P. C. Arck, Neutralization of LPS or blockage of TLR4 signaling prevents stress-triggered fetal loss in murine pregnancy, *J. Mol. Med. (Berl.)* 89 (2011) 689–699, <https://doi.org/10.1007/s00109-011-0743-5>.