FETO-MATERNAL IMMUNE REGULATION BY TIM-3/GAL-9 AND PD-1/PD-L1 PATHWAYS IN HEALTHY, MIFEPRISTONE TREATED AND PACAP-DEFICIENT PREGNANT MICE

Ph.D. thesis

ADRIENN LAJKÓ

University of Pécs Clinical Centre

Department of Medical Microbiology and Immunology

Theoretical Medical Sciences PhD Program

Head of the PhD School: Dóra Reglődi, M.D., Ph.D., D.Sc.

Head of the PhD Program: Júlia Szekeres, M.D., Ph.D., D.Sc.

Tutor: László Szereday, M.D., Ph.D.

PÉCS

2020

I. Introduction

Reproductive immunology refers to a field of medicine that studies interactions (or the absence of them) between the immune system and components related to the reproductive system, such as maternal immune tolerance towards the fetus.

The pregnancy is actually an „immunological paradox", because in a very complex way the mother's immune system helps to maintain the tolerance toward the semi-allogenic fetal antigens. If the immunological tolerance does not work properly, it could lead to severe complications, or even miscarriage.[1]

Understanding the immunological background of pregnancy is very important for clinical medicine and scientific perspectives[2], because the dysregulation of the immune system can even cause recurrent miscarriages.[3] Different immunological failures could be the cause of reduced fertility in women, such as autoimmune diseases, immunodeficiency diseases and asthma.[4] Furthermore, the interaction between the pregnant mother's immune system and infectious agents can also affect fertility.[5]

Immune checkpoint molecules are regulators of our immune system. They regulate the immunological defense against infections, tumors, and autoimmune processes. Therefore, their role in the development and maintenance of the maternal immune tolerance is essential.[6–8] Antigen-presenting cells and dendritic cells express different immune checkpoint ligands. These ligands bind to the receptors on lymphocytes to induce a specific activating or inhibitory pathways. Main activating receptor-ligand pairs: ICOS/ ICOS-L, GITR/GITR-L, CD27/CD70, CD40/CD40-L and CD28/CD80-86. Main inhibitory receptor-ligand pairs: **PD-1/PD-L1**, CTLA-4/CD80-86, **TIM-3/Galectin-9**, MHC-II/LAG-3, and SIRPa /CD47. In the doctoral dissertation, the TIM-3/Gal-9 and PD-1/PD-L1 pathways were examined.

TIM-3 molecule

T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), was first described as a cell surface molecule expressed on IFNγ producing CD4+ Th1 and CD8+ Tc1 cells.[9] Later, the expression was detected in Th17 cells, regulatory T-cells and innate immune cells (dendritic cells, NK cells, monocytes).[10]

The interaction of TIM-3 and Gal-9 molecules inhibits the cytotoxic activity of NK cells and the Th1 and Th17 cells INF-γ secretion. In addition, it regulates the development of T cell tolerance and the activation of Th1 immune cells in mice and humans.[11, 12, 13] It is responsible for the Th1 / Th2 balance.[14] It stimulates the differentiation of naive T cells into Treg cells and promotes the production of Th17 cells by inhibiting naive T cells. Apoptotic activity is prominent in CD4+ Th1, Th17 and CD8+ cytotoxic T cells and CD4 / CD8 double negative or double positive thymocytes.[15]

In the case of mouse pregnancy, the Gal-9 molecule can be detected in the placental and decidual Treg and Th cells. Since the presence of TIM-3 molecule and Gal-9 at the feto-maternal interface has been detected, we can assume that they are also involved in the development of immune tolerance. This hypothesis is supported by the observation that *in vivo* inhibition of the TIM-3 molecule reduces the number of live offspring per pregnancy and increases the chance of the intrauterine absorption of the fetuses.[16,17]

PD-1 molecule

PD-1 molecule (CD279) is an inhibitory immune checkpoint transmembrane receptor, which is member of the B7 / CD28 family. It is expressed by several immune cells such as T cells, B cells, NK cells, and antigen presenting cells.[18] PD-1 has two ligands, PD-L1 and PD-L2, which are members of the B7 family: PD-L1 (B7-H1) and PD-L2 (B7-DC). PD-L1 can inhibit inflammatory T cell responses. It is expressed on T cells, B cells, dendritic cells, and macrophages. PD-L1 mRNA can be detected in the heart, lung, thymus, spleen, and kidney.[19,20] In contrast, PD-L2 expression is restricted only to macrophages and dendritic cells.[21] At the mRNA level it can be detected in the heart, placenta, lung, and liver.[22]

Both PD-1 ligands are expressed at the maternal-fetal interface.[23] Expression of PD-L2 can be observed in all layers of the mouse decidua, while expression of PD-L1 is restricted to the basal layer of the decidua.[23]

The *in vivo* inhibition of anti-PD-L1 can cause increased fetal resorption rates, whereas PD-L2 inhibition had no effect on fetal viability.[10,23]

PACAP molecule

Pituitary adenylate cyclase-activating polypeptide is named for its effect on the pituitary gland. It can increase cAMP levels in pituitary cells by stimulating adenylate cyclase. PACAP is a multifunctional neuropeptide, it is the member of the glucagon-secretin vasoactive intestinal polypeptide (VIP) family, with 68% homology to VIP. It can affect fertility, implantation, reproductive behavior, spermatogenesis, and placental functions.[24–26] PACAP molecule can delay puberty and affect follicular maturation in the ovary.[27] It can direct ovarian hormone production,[28] can affects meiotic division and it is important local regulator of the follicular development.[29]

II. Aims

In our experiments, we aimed to investigate the development of feto-maternal immune tolerance, we wanted to study the role and function of TIM-3 and PD-1 immune checkpoint molecules during pregnancy by using different animal models.

We divided our research into three topics:

1. Investigating healthy pregnancy: The role of TIM-3 and PD-1 immune checkpoint molecules

In our experiments, we aimed to analyze phenotypic features and the cytotoxic activity of the immune cells isolated at the peripheral and feto-maternal interfaces. Furthermore, we wanted to investigate the TIM-3/Gal-9 and PD-1 pathways at 14.5 day of mouse pregnancy. We also aimed to determine the expression site of the Gal-9 molecule in the mouse placenta.

2. Investigating the effect of abortion-induced drug treatment: Study the impact of Mifepristone on the TIM-3/Gal-9 pathway

We aimed to investigate the TIM-3/Gal-9 pathway after low dose of Mifepristone treatment. Our test samples were mononuclear cells from the feto-maternal interface of a 14.5 day pregnant mice. We planned to examine by flow cytometer the phenotypic characterization, TIM-3 and Gal-9 expression and the cytotoxic activity of the immune cells. We also aimed to investigate the Gal-9 expression after Mifepristone treatment by immunohistochemistry.

3. Investigating the effect of PACAP molecule on mouse pregnancy: The role of TIM-3 and PD-1 immune checkpoint molecules in PACAP- deficient mice

In the third part of our experiments, we planned to repeat the previously described healthy pregnancy tests with PACAP-deficient mice. Our aims were to analyze phenotypic features of the peripheral and decidual immune cells, as well as to investigate the expression of Gal-9, TIM-3, PD-1 in the mononuclear immune cells and to measure their cytotoxic activities. In addition, we planned to explore the Gal-9 expression of wild-type and PACAP-deficient mice placental samples by immunohistochemistry.

III. Methods

In our experiments the following techniques were used:

- Pairing of BALB-c, CD1 and PACAP- deficient mice
- 24 hours Mifepristone (RU-486) treatment of pregnant BALB-c mice
- Isolation of the decidua and spleen from pregnant BALB-c, CD1 and PACAP-deficient mice
- Isolation of mononuclear cells from decidua and spleen
- Labeling lymphocytes for flow cytometric analyses
- FoxP3 intracellular staining
- CD107a cytotoxicity assay
- Flow cytometry
- Immunohistochemical detection of Gal-9 molecule in mice placenta
- Statistical analyses with SPSS V.20.

IV. Results

<u>Investigation of TIM-3/Gal-9 and PD-1/PD-L1 pathways in healthy pregnant BALB-c mice</u>

Gal-9 was found to be present in the spongiotrophoblast layer of the hemochorial placenta, which separates the labyrinth layer from the decidua.

Within the scope of our research, we discovered significantly enhanced Gal-9 expression by decidual regulatory T cells compared to the periphery. Although there is a decrease in the ratio of CD4 helper T cells in the decidua, the Gal-9 secreting subpopulation is still represented locally without any changes. According to our hypothesis, these dominant presence of the ligands PD-L1, PD-L2 and Gal-9 at the feto-maternal interface suggest subsequent local immunomodulatory potential following maternal immunoactivation.

Among the lymphocyte subpopulations recruited in the mouse decidua we found predominantly innate immune cells (NK cells, γ/δ T cells and NKT cells) and a reduced number of CD4+ and CD8+ T lymphocytes compared to the periphery.

During the analysis of the PD-1 expression by lymphocytes in the periphery and in the decidua, decidual lymphocytes show a notable increased PD-1 expression in all investigated subpopulations. Lytic activity of PD-1 positive NK, NKT and γ/δ T cells was decreased in the decidua, suggesting the involvement of these lymphocytes in feto-maternal tolerance as a potential result of the PD-1 receptor mediated pathway.

While TIM-3 expression of NK cells and γ/δ T cells is similar both in the periphery and in the decidua, the relative TIM-3 expression is increased locally (higher receptor density on single cell level) indicating decidual TIM-3 expressing cells are more mature and fully functional. However, cytotoxic capacity of decidual TIM-3 expressing NK cells and γ/δ T cells is reduced when compared to the periphery which might be due to their upregulated relative TIM-3 expression on one hand and a much stronger local presence of its ligand Gal-9 on the other hand. Their binding could lead to the subsequent inhibition of effector functions observed here as a reduced cytotoxicity.

Investigating decidual NKT cells, this subpopulation showed a reduced TIM-3 expression with increased relative receptor expression when compared to the periphery.

Comprehensively speaking, our data indicates a very complex, tissue and cell type specific immunoregulatory mechanism by the investigated co-inhibitory receptors at the feto-maternal interface proposing further investigations of their exact role in maternal immune responses.

Investigation of the effect of Mifepristone on the TIM-3/Gal-9 pathway in pregnant BALB-c mice

Here we demonstrated that a relatively high proportion of peripheral NK, NKT and γ/δ T cells also showed Gal-9 positivity. Although Gal-9 expression by peripheral Treg cells was almost negligible, significantly higher percentage of Gal-9 positivity by decidual Treg cells was found in the decidua. Furthermore, we found that the expression of Gal-9 by NK cells was significantly decreased in the normal pregnant decidua compared to the periphery. 0.8mg/kg RU486-treatment resulted in a nearly complete

disappearance of Gal-9 from the junctional zone of the placenta. Furthermore, the treatment significantly decreased the Gal-9 positivity of peripheral NK cells but significantly increased its expression by decidual Treg and CD4+T cells. In addition, the proportion of decidual Gal-9+Th cells with known suppressive capacity was significantly increased after Mifepristone administration.

Our data indicate that even a low dose Mifepristone treatment was effective enough to abrogate Gal-9 production of the placenta. The observed, increased Gal-9 expression by decidual Treg and CD4+Th cells suggest that local immunosuppressive mechanisms are also triggered 24 hours after the treatment, possibly to sustain impaired placental function. These mechanisms might inhibit the pro-inflammatory cytokine production of Th1 and Th17 cell by a Gal-9/TIM-3 dependent fashion[30] and aid the maintenance of the whole embryo placenta unit.

We found that both in control and RU486 treated mice group TIM-3 expression by CD4+T cells was significantly increased in the decidua compared to the periphery. In RU486 treated mice TIM-3 expression by NK cells was significantly increased in the decidua compared to the periphery

In addition, we analyzed the CD107a expression within the TIM-3+ lymphocyte subsets. In untreated pregnant mice our results demonstrated a significantly decreased CD107a expression by decidual TIM-3+ γ/δ T cells together with a significant increase in CD107a expression by NKT cells compared to the periphery. Furthermore, in RU486 treated mice TIM-3+ decidual NKT cells showed significantly higher while NK cells showed significantly lower cytotoxic potential than their peripheral counterparts.

Investigation of TIM-3/Gal-9 and PD-1/PD-L1 pathways in wild-type (CD1) and PACAP-deficient pregnant mice

Both in PACAP KO and wild type mice, a significant increase could be observed in decidual γ/δ T, NK and NKT cell frequency at the feto-maternal interface, while the frequency of decidual CD4+ and CD8+T cells was significantly decreased when compared to the periphery. PACAP KO mice had a significantly elevated Treg frequency in the periphery compared to the decidua. PACAP KO mice had a significantly increased

CD4+ T frequency in the periphery together with a higher number of γ/δ T cells in the decidua compared to the wild type mice.

In our experiments, both PACAP KO and wild type mice on CD-1 background showed the same Gal-9 positivity by the spongiotrophoblast layer of the hemochorial placenta.

Although there is a decrease in the ratio of CD4+ T cells in the decidua both in PACAP KO and in wild type mice, the percentage of the Gal-9 secreting subpopulation (Gal-9+ Th cells) significantly increased locally at the feto-maternal interface compared to the periphery.

The only notable PACAP-specific alteration was related to CD4+ T cells expressing cell surface Galectin-9 (Gal-9+ Th cell). There is a significantly elevated Gal-9+ Th cell frequency at the feto-maternal interface in pregnant PACAP KO mice compared to wild type control mice.

During the analysis of TIM-3 expression by immune cells in the decidua and in the periphery, decidual CD4+ T and Treg cells showed a significantly increased TIM-3 expression in both animal groups, while TIM-3 expression by γ/δ T cells was significantly decreased in the decidua of PACAP KO mice compared to the periphery. Interestingly, TIM-3 expression by γ/δ T cells was significantly increased in the periphery of PACAP KO mice compared to the wild type mice. These results suggest that CD4+ T and Treg cells are presumably under immune-checkpoint control by TIM-3 molecule. Analyzing another immune-checkpoint molecule revealed that PD-1 expression by NK cells is significantly increased in the decidua compared to the periphery in PACAP KO mice.

Analyzing data, the only PACAP specific change is the significantly increased cytotoxicity by γ/δ T cells in the periphery of PACAP KO mice compared to wild type mice. We could not detect any changes in the cytotoxic potential of the investigated cells at the feto-maternal interface between the two groups, suggesting no functional impairment or disturbance locally mediated by either TIM-3/Gal-9 or PD-1/PD-L1 pathways. Furthermore, there is a significantly elevated Gal-9+ Th cell frequency in the decidua at the feto-maternal interface in PACAP KO mice compared to wild type controls, suggesting a possible control of the cytotoxicity of TIM-3 positive cells.

In conclusion, despite the found alterations in the peripheral number and function of immune cells, we could not find any remarkable alteration either in the distribution or in the cytotoxicity of the investigated decidual immune cells which could elucidate any reproductive alterations in pregnant PACAP-deficient mice.

V. Summary

Investigation of TIM-3/Gal-9 and PD-1/PD-L1 pathways in healthy pregnant BALB-c mice

1. Gal-9 is expressed in the spongiotrophoblast layer of the healthy mice's placenta.
2. Decidual Treg cells show higher Gal-9 expression compared to the periphery.
3. Although the proportion of CD4 + Th cells in the decidua is reduced, the Gal-9-producing subpopulation (Gal-9+ Th cells) is present at the same level as the periphery.
4. The decidual PD-1 expression in the NK, NKT, and γ/δ T cells was increased compared to the periphery. The PD-1 positive NK and NKT cells cytotoxic activity was reduced compared to the periphery.
5. While the TIM-3 expression of the NK and γ/δ T cells in the decidua and in the periphery showed similar values, the relative TIM-3 expression was increased in the decidua compared to the periphery. Furthermore, the cytotoxic activity of TIM-3 positive NK and γ/δ T cells in the decidua is lower in contrast the periphery. The NKT cells of the decidua have lower TIM-3 expression than the periphery, but they have higher relative TIM-3 receptor expression. Locally, although their cytotoxicity increased in the decidua, but it was not significant compared to the periphery.
6. The number of PD-1 and TIM-3 double-positive NKT and γ/δ T cells decreased in the decidua compared to the periphery.
7. The PD-1 and TIM-3 positive cells in the decidua are more dominant compared to the periphery. While PD-1+ lymphocytes have decreased cytotoxic activity, the lytic activity of TIM-3 + cells varies depending on the cell type, suggesting that the role of TIM-3 may be various in different lymphocyte subpopulations.

Investigation of the effect of Mifepristone on the TIM-3/Gal-9 pathway in pregnant BALB-c mice

1. After a low-dose (0.8 mg/kg) Mifepristone treatment the Gal-9 expression is almost completely disappeared in the treated mice placenta compared to the control group.
2. After Mifepristone treatment the NK cells Gal-9 expression significantly decreased in the periphery, but the Treg and CD4 + T cells Gal-9 expression significantly increased in the decidua compared to the untreated group.
3. The suppressive Gal-9+ Th cells proportion was significantly increased in the Mifepristone treated mice decidua compared to their periphery and compared to the untreated group's decidua.
4. After Mifepristone treatment the NK and CD4 + T cells in the decidua express significantly more TIM-3 molecules on their surface than the periphery.
5. TIM-3 positive NK cells in the decidua have decreased cytotoxic activity, while the NKT cells in the decidua showed an elevated CD107a expression compared to the periphery.
6. Mifepristone treatment increased the TIM-3 expression in all tested immune cells of the decidua compared to the untreated mice decidua, however, the differences were only significant in the case of CD4 + T cells.

Investigation of TIM-3/Gal-9 and PD-1/PD-L1 pathways in wild-type (CD1) and PACAP-deficient mice

1. In the case of PACAP-deficient and wild-type mice significantly increased the γ/δ T, NK, NKT cell proportion and significantly decreased the CD4+ T and CD8+ T cell proportion in the decidua compared to the periphery.
2. The CD4+ T cells in the periphery were significantly increased in PACAP-deficient mice and the frequency of the γ/δ T cells in the decidua was also increased compared to the control group.
3. There was no significant difference between the PACAP-deficient and wild-type mice Gal-9 expression. In both cases the spongiotrophoblast layers showed the same Gal-9 positivity.

4. The CD4+ T cells decreased in the decidua in both mouse group and significantly increased the proportion of the Gal-9 producing subpopulation (Gal-9 + Th cells) at the feto-maternal interface compared to the periphery.
5. The Gal-9+ Th cells significantly increased in the decidua of the PACAP-deficient mice compared to the wild type's decidua.
6. The CD4+ T and Treg cells TIM-3 expression showed a significant increase in both mouse group compared to the periphery. In contrast, the γ/δ T cells TIM-3 expression was significantly decreased in the PACAP-deficient mice decidua compared to the periphery.
7. The γ/δ T cells TIM-3 expression in periphery was significantly increased in the PACAP-deficient mice compared to wild type periphery.
8. The NK cells PD-1 expression in the decidua was significantly increased in the PACAP-deficient mice compared to their periphery.
9. The γ/δ T cells CD107a expression in the periphery significantly increased in the PACAP-deficient mice compared to the control group.
10. We could not detect any significant differences in cell phenotype and cytotoxic activity in the feto-maternal interface that would clearly explain the low reproductive capacity of the PACAP-deficient mice.

VI. Acknowledgements

Throughout the writing of this dissertation I have received a great deal of support and assistance.

First of all I would like to thank my supervisor, docent László Szereday, whose expertise was invaluable in formulating the research questions and methodology. Your insightful feedback pushed me to sharpen my thinking and brought my work to a higher level. I must also mention here Mátyás Meggyes, an earlier PhD student of my supervisor, who sets a good example before me, and supported my research works from its first steps.

I would like to acknowledge my colleagues from Department of Medical Microbiology and Immunology for their wonderful collaboration. I would particularly like to line out Alíz Barakonyi, Beáta Polgár, Éva Mikó. I want to thank for your courteous help at all times I was given to further my research. Éva Molnár and Réka Bacher-Számel, I would also like to express my gratitude for your help in learning practical techniques.

I would especially like to thank the Department of Pathology of the University of Pécs for their willing and useful cooperation and their permission to use their instruments.

I would like to thank my employer, Soft Flow KFT, for their supportive, flexible attitude and professional competence in all respects in the preparation of my dissertation.

The implementation of my research was also supported by the EFOP-3.6.1-16-2016-00004 project.

Finally, I would like to thank my family and husband for their patience and unbroken support.

VII. References

1. Ismétlődő vetélések – miért fontos az immunológiai kivizsgálás? Available at: https://www.immunkozpont.hu/immunologia-hirek/ismetlodo-vetelesek-–-miert-fontos-az-immunologiai-kivizsgalas. (Accessed: 31st March 2019)
2. Carp, H. J. A. & Selmi, C. The autoimmune bases of infertility and pregnancy loss. *J. Autoimmun.* **38**, J266–J274 (2012).
3. Bonney, E. A. & Brown, S. A. To drive or be driven: The path of a mouse model of recurrent pregnancy loss. *Reproduction* **147**, (2014).
4. Pantham, P., Abrahams, V. M. & Chamley, L. W. The role of anti-phospholipid antibodies in autoimmune reproductive failure. *Reproduction* **151**, R79–R90 (2016).
5. Bonney, E. A. Immune Regulation in Pregnancy: A Matter of Perspective? *Obstet. Gynecol. Clin. North Am.* **43**, 679–698 (2016).
6. Sharma, P. & Allison, J. P. The future of immune checkpoint therapy. *Science (80-.).* **348**, 56–61 (2015).
7. Nirschl, C. J. & Drake, C. G. Molecular pathways: coexpression of immune checkpoint molecules: signaling pathways and implications for cancer immunotherapy. *Clin. Cancer Res.* **19**, 4917–24 (2013).
8. Williams, M. A. & Bevan, M. J. Effector and Memory CTL Differentiation. *Annu. Rev. Immunol.* **25**, 171–192 (2007).
9. Monney, L. *et al.* Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. *Nature* **415**, 536–41 (2002).
10. Miko, E., Meggyes, M., Doba, K., Barakonyi, A. & Szereday, L. Immune checkpoint molecules in reproductive immunology. *Frontiers in Immunology* **10**, (2019).
11. Zhu, C. *et al.* The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. *Nat. Immunol.* **6**, 1245–1252 (2005).
12. Sabatos, C. A. *et al.* Interaction of Tim-3 and Tim-3 ligand regulates T helper type 1 responses and induction of peripheral tolerance. *Nat. Immunol.* **4**, 1102–1110 (2003).

13. Seki, M. *et al.* Galectin-9 suppresses the generation of Th17, promotes the induction of regulatory T cells, and regulates experimental autoimmune arthritis. *Clin. Immunol.* **127**, 78–88 (2008).
14. Tang, Z.-H. *et al.* Tim-3/Galectin-9 Regulate the Homeostasis of Hepatic NKT Cells in a Murine Model of Nonalcoholic Fatty Liver Disease. *J. Immunol.* **190**, 1788–1796 (2013).
15. Moritoki, M. *et al.* Galectin-9 Ameliorates Clinical Severity of MRL/lpr Lupus-Prone Mice by Inducing Plasma Cell Apoptosis Independently of Tim-3. *PLoS One* **8**, e60807 (2013).
16. Shi, F. *et al.* PD-1 and PD-L1 upregulation promotes CD8(+) T-cell apoptosis and postoperative recurrence in hepatocellular carcinoma patients. *Int. J. cancer* **128**, 887–96 (2011).
17. Chabtini, L. *et al.* TIM-3 regulates innate immune cells to induce fetomaternal tolerance. *J. Immunol.* **190**, 88–96 (2013).
18. Keir, M. E., Butte, M. J., Freeman, G. J. & Sharpe, A. H. PD-1 and its ligands in tolerance and immunity. *Annu. Rev. Immunol.* **26**, 677–704 (2008).
19. Petroff, M. G. *et al.* B7 family molecules are favorably positioned at the human maternal-fetal interface. *Biol. Reprod.* **68**, 1496–504 (2003).
20. Veras, E., Kurman, R. J., Wang, T.-L. & Shih, I.-M. PD-L1 Expression in Human Placentas and Gestational Trophoblastic Diseases. *Int. J. Gynecol. Pathol.* (2016). doi:10.1097/PGP.0000000000000305
21. Lewkowich, I. P. *et al.* PD-L2 modulates asthma severity by directly decreasing dendritic cell IL-12 production. *Mucosal Immunol.* **6**, 728–39 (2013).
22. Rozali, E. N., Hato, S. V., Robinson, B. W., Lake, R. A. & Lesterhuis, W. J. Programmed death ligand 2 in cancer-induced immune suppression. *Clinical and Developmental Immunology* **2012**, (2012).
23. Guleria, I. *et al.* A critical role for the programmed death ligand 1 in fetomaternal tolerance. *J. Exp. Med.* **202**, 231–7 (2005).
24. Reglodi, D., Tamas, A., Koppan, M., Szogyi, D. & Welke, L. Role of PACAP in Female Fertility and Reproduction at Gonadal Level - Recent Advances. *Front. Endocrinol. (Lausanne).* **3**, 155 (2012).

25. Brubel, R. *et al.* Effects of Pituitary Adenylate Cyclase Activating Polypeptide on Human Sperm Motility. *J. Mol. Neurosci.* **48**, 623–630 (2012).
26. Reglodi, D. *et al.* Disturbed spermatogenic signaling in pituitary adenylate cyclase activating polypeptide-deficient Mice. *Reproduction* **155**, 129–139 (2018).
27. Brubel, R. *et al.* Investigation of pituitary adenylate cyclase activating polypeptide in human gynecological and other biological fluids by using MALDI TOF mass spectrometry. *J. Mass Spectrom.* **46**, 189–194 (2011).
28. Kanasaki, H., Oride, A., Tselmeg, M., Sukhbaatar, U. & Kyo, S. Role of PACAP and Its PACAP Type I Receptor in the Central Control of Reproductive Hormones. in *D. Reglodi, A. Tamas (Eds.), Pituitary Adenylate Cyclase Activating Polypeptide – PACAP, Springer Nature, New York* 375–387 (2016). doi:10.1007/978-3-319-35135-3_22
29. Canipari, R., Di Paolo, V., Barberi, M. & Cecconi, S. PACAP in the Reproductive System. in *D. Reglodi, A. Tamas (Eds.), Pituitary Adenylate Cyclase Activating Polypeptide – PACAP, Springer Nature, New York* 405–420 (2016). doi:10.1007/978-3-319-35135-3_24
30. Hastings, W. D. *et al.* TIM-3 is expressed on activated human CD4+ T cells and regulates Th1 and Th17 cytokines. *Eur. J. Immunol.* **39**, 2492–2501 (2009).

VIII. List of publications

Publications related to the thesis

Lajkó A., Meggyes M., Fülöp BD., Gede N., Reglődi D., Szereday L.: Comparative analysis of decidual and peripheral immune cells and immune-checkpoint molecules during pregnancy in wild-type and PACAP-deficient mice. American Journal of Reproductive Immunology. 2018 Oct;80(4):e13035. doi: 10.1111/aji.13035. **IF: 3,091**

Lajkó A., Meggyes M., Polgár B., Szereday L.: The immunological effect of Galectin-9/TIM-3 pathway after low dose Mifepristone treatment in mice at 14.5 day of pregnancy. PLoS One. 2018 Mar 22;13(3):e0194870. doi: 10.1371/journal.pone.0194870. **IF: 2,776**

Meggyes M., **Lajkó A.**, Palkovics T., Totsimon A., Illés Z., Szereday L, Mikó É.: Feto-maternal immune regulation by TIM-3/Galectin-9 pathway and PD-1 molecule in mice at day 14.5 of pregnancy. Placenta. 2015.07.18.; pii: S0143-4004(15)30018-7. **IF: 2,972**

Other publications with impact factors

Meggyes M., **Lajkó A.**, Fülöp BD., Reglődi D., Szereday L.: Phenotypic characterization of testicular immune cells expressing immune checkpoint molecules in wild-type and pituitary adenylate cyclase-activating polypeptide-deficient mice. American Journal of Reproductive Immunology. 2019 Nov 22:e13212. doi: 10.1111/aji.13212. **IF: 2,739**

Meggyes M., Mikó É, **Lajkó A.**, Csiszár B., Sándor B., Mátrai P., Tamás P., Szereday L.: Involvement of the PD-1/PD-L1 Co-Inhibitory Pathway in the Pathogenesis of the Inflammatory Stage of Early-Onset Preeclampsia. International Journal of Molecular Sciencies. 2019 Jan 29;20(3). pii: E583. doi: 10.3390/ijms20030583. **IF: 4,556**

Meggyes M., Szántó J., **Lajkó A.**, Farkas B., Várnagy Á., Tamás P., Hantosi E., Mikó É, Szereday L.: The possible role of CD8+/Vα7.2+/CD161++ T (MAIT) and CD8+/Vα7.2+/CD161lo T (MAIT-like) cells in the pathogenesis of early-onset pre-eclampsia. American Journal of Reproductive Immunology. 2018 Feb;79(2). doi: 10.1111/aji.12805. **IF: 3,091**

Cumulative impact factor: 19,225

Presentations

Lajkó A., Meggyes M., Mikó É., Szereday L.: Alternatív állatkísérleti módszerek a Pécsi Tudományegyetemen. Interdiszciplináris Doktorandusz Konferencia. 2017.05.19-21., Pécs

Lajkó A., Meggyes M., Mikó É., Szereday L.: A „3R szabály" alkalmazása a Pécsi Tudományegyetem Orvosi Mikrobiológia és Immunitástani Intézetében. Tavaszi szél konferencia. 2017.01., Miskolc

Lajkó A., Meggyes M., Szántó J., Mikó É., Szereday L.: Feto-maternal immune regulation by PD-1 molecule in pregnant mice. 13th Congress of the International Society for Immunology of Reproduction. 2016.06.22-26., Erfurt

Lajkó A., Meggyes M., Szántó J., Mikó É., Szereday L.: Immune regulation by PD-1 molecule in mice at day 14.5 of pregnancy. Interdiszciplináris Doktorandusz Konferencia. 2016.05.27-29., Pécs

Szántó J., **Lajkó A.**, Szereday L., Meggyes M.: Immunreguláció pathológiás terhesség alatt: PD-1/PD-L1 expresszió összehasonlítása egészséges terhes és early-onset preeclampsiás nőknél. Interdiszciplináris Doktorandusz Konferencia. 2016.05.27-29., Pécs

Lajkó A.: Immunhisztokémia optimalizálása és Galektin-9 molekula vizsgálata egészséges és kóros placenta mintákon. Tavaszi szél konferencia. 2016.04.15-17., Budapest

Lajkó A., Meggyes M., Tótsimon A., Szántó J., Mikó É., Szereday L.: Galektin-9 molekula vizsgálata perifériás és deciduális mononukleáris sejteken terhes egérmodellben. Doctoral Workshop. 2015.10.10., Pécs

Lajkó A., Meggyes M., Tótsimon A., Szántó J., Mikó É., Szereday L.: Investigating Galectin-9 molecule expression by peripheral and decidual mononuclear cells in pregnant mice. Tavaszi szél konferencia. 2015.04.10-12., Eger

Meggyes M., Mikó É., Polgár B., **Lajkó A.**, Szekeres-Barthó J., Szereday L.: TIM3/Galectin-9 in normal pregnancy and in early-onset preeclampsia. Magyar Immunológiai Társaság XLIII. Vándorgyűlése, 2014.10.15-17. Velence, Immunológiai Szemle, 2014, 3:36

Poster presentations

Lajkó A., Meggyes M, Szántó J, Polgár B, Szereday L: The rol of Galectin-9/TIM-3 pathway in Mifepristone induced medical aborted mice. 46th Annual Meeting of the German Society for Immunology, 2016.09.27-30., Hamburg

Lajkó A., Meggyes M., Szántó J., Mikó É., Szereday L**.:** PD-1 molekula immunregulációja terhes egérben. Magyar Farmakológiai, Anatómus, Mikrocirkulációs és Élettani Társaságok Közös Tudományos Konferencia (FAMÉ 2016), 2016.06.1-4., Pécs

Lajkó A., Meggyes M., Tótsimon A., Szántó J., Mikó É., Szereday L.: The possible role of Galectin-9/TIM-3 pathway in Mifepristone induced medical abortion in mice. Magyar Immunológiai Társaság 44. Vándorgyűlése. 2015.10.14-16. Velence

Meggyes M., Szántó J, Mikó É, **Lajkó A**, Szereday L.: Investigating PD-1 and PD-L1 expression in normal pregnancy andin early onset preeclamsia. Magyar Immunológiai Társaság 44. Vándorgyűlése. 2015.10.14-16. Velence

Meggyes M., **Lajkó A.,** Mikó É., Illés Z., Szekeres-Barthó J., Szereday L.: The significance of Galectin-9/TIM3 pathway in mifepristone induced madical abortion in BALB/c mice. 12th Conference of the European Society for Reproductive Immunology. 2015.09.21-24. Oxford

Lajkó A., Meggyes M., Tótsimon A., Szántó J., Mikó É., Szereday L**.:** The possible role of Galectin-9/TIM3 pathway in fetomaternal tolerance in pregnant mice. 4th European Congress of Immunology, 2015.09.6-9., Bécs