The role of the Progesterone-induced blocking factor in normal murine pregnancy.

PhD theses

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Introduction

The role of the immune system is to protect the organism from foreign materials. Although 50% of foetal antigens are of paternal origin, and there is ample evidence that these antigens are recognized, the immune system of the mother tolerates the semi-allogeneic foetus (1). The suitable immunological environment for the proper development of the foetus is established via the dialogue of maternal and fetal cytokines. Progesterone, and the progesterone-induced blocking factor (PIBF) - mediating the immunological effects of progesterone - plays an important role in this process.

PIBF

Following recognition of foetal antigens, activated maternal lymphocytes express progesterone receptors, and the binding of progesterone to its receptors results in the production of PIBF (2).

The phylogenetically conserved PIBF1 gene that codes for the PIBF protein is located on chromosome 13 in humans, and on chromosome 14 in mice. The PIBF1 gene is transcribed to 16 different mRNAs, the longest of which is 3677 base pairs long, and contains 18 exons (3). This codes for the full-length PIBF protein that consists of 756 amino acids and has a molecular weight of 89,6 kilodaltons (kDa). The full length PIBF is a member of the CEP family (4), a component of the pericentriolar satellite, and as such, plays a role in cell cycle regulation and the control of invasion. Smaller isoforms are localized in the cytoplasm. When secreted, they bind to the PIBF receptor and activate the JAK1/STAT6 and PKC/Ca^{2+} pathways that leads to a Th2 type cytokine production (5).

According to the above, PIBF has multiple roles in pregnancy. Besides the inhibition of arachidonic acid release, it exerts the immunomodulatory effects of progesterone, which are the following:

- formation of Th2 cytokine dominance;
- icreasing the production of asymmetric antibodies;
- inhibition of NK cell degranulation.

Th2 cytokine dominance during pregnancy

According to their effect on the immune response, cytokines are categorized as pro-inflammatory (Th1), or anti-inflammatory (Th2).

Pregnancy is characterized by a Th2 dominant cytokine pattern, while implantation requires mild inflammatory conditions, which is provided by the temporary over-expression of interferon gamma (IFNγ) and leukemia inhibitory factor (LIF) at the implantation sites.

The cytokine pattern in peripheral blood is related to the outcome of pregnancy. Th1 cytokine levels are significantly increased in peripheral blood of women with preterm delivery and miscarriage.

Treatment of pregnant mice with interleukin-2 (IL-2), tumor necrosis factor α (TNFα) or IFNγ results in abortion, whereas treatment with granulocyte-macrophage colony stimulating factor (GM-CSF), IL-3 or anti-TNFα prevents foetal loss in abortion-prone mating combinations (6).

Thus Th2 dominance is crucial for normal pregnancy, and one of the major effects of PIBF is the increase of the Th2 cytokine production (7).

The effect of PIBF on degranulation of decidual NK (dNK) cells

NK cells comprise almost 60% of the lymphocytes in the first trimester human or mouse decidua. Decidual NK cells are both phenotypically and functionally different from circulating NK cells. The majority of dNK cells are $CD16^{-}CD56^{bright}$ and though they contain perforin in cytotoxic granules, their main role is establishing proper conditions for placentation, implantation and embryonic development via extensive cytokine production. Nevertheless, they can also fight intrauterine infections if needed.

Miscarriages are characterized by increased NK cell activity (8), while during normal pregnancy, despite the presence of perforin containing granules, these lymphocytes do not degranulate (9).

PIBF is thought to inhibit the degranulation of dNK cells. Earlier data show that PIBF blocks NK activity. Inactivating NK cells corrects increased resorption rates in PIBF-deficient mice, suggesting that PIBF protects pregnancy by controlling NK activity (10).

Both progesterone and PIBF inhibit the release of perforin from the cytotoxic granules *in vitro*, which in part explains the effect of PIBF on the NK activity.

Aims

To further characterize the effect of PIBF on pregnancy and NK activity, we aimed:

- to determine the exon and protein expression pattern of PIBF in murine pregnancy associated tissues, in order to investigate the connection between the presence of different PIBF isoforms and the outcome of pregnancy
- to investigate the role of PIBF in decreasing the cytotoxic activity of dNK cells.

Materials and methods

RNA and protein samples from placenta, uterus, normal and resorbed foetuses of Balb/c mice were analysed by reverse transcription polymerase chain reaction and western blot to determine the exon and protein expression profile of PIBF.

Pregnant CD1 mice were sacrificed at g.d. 7.5; g.d. 10.5; g.d. 12.5; and g.d. 15.5, and dNK cells were characterized by immunohistochemistry and immunoflurescence.

Results

Exon and protein expression profile of PIBF in pregnancy-associated tissues

Earlier data showed that the full-length PIBF plays a role in trophoblast invasion and regulation of the cell cycle, while the smaller splice variants act in a cytokine-like manner, and affect the immunological relationship between the mother and the foetus.

Our aim was to determine the mRNA and protein expression profile of PIBF in pregnancy-associated tissues, from normal and pathological pregnancies. The presence of the individual exons (Ex1-18) was examined by RT-PCR and agarose gel electrophoresis in placenta, uterus, normal and resorbed foetuses from early (g.d. 12-14) and late (g.d. 17-19) pregnancy. The lysates of the same tissue specimens were also analyzed by western blot.

The placenta and uterus showed very similar exon expression pattern, virtually all the exons were present in both stages of pregnancy. At the same time, we observed differences in protein expression. Four PIBF isoforms (90, 66, 55 and 34 kDa) were present in both tissues, but the occurrence of these isoforms differed between the tissues and at the two stages of pregnancy. In the placenta, the expression of all isoforms – especially of the 90 kDa form – decreased towards the end of pregnancy. In late pregnancy uterine samples, we could not detect the full-length PIBF, the expression of the 55 kDa isoform decreased, while the expression of the 66 and 34 kDa variants increased. We compared the expression of PIBF protein isoforms in non-pregnant and pseudo-pregnant uteri. The 90 kDa PIBF was present in the uterus of pseudo-pregnant mice, but missing from the non-pregnant uterus, suggesting that the expression of this isoform is characteristic of the pregnancy-related endometrial transformation, but independent of the presence of the fetus.

All of the exons were present in fetal samples obtained during mid-pregnancy, while the expression of the exons coding for the N-terminal part of the full-length PIBF (exon 1-6, 8-9 and 12) decreased in late pregnancy.

We compared the exon expression profile of normal and resorbed fetuses from late-pregnancy. Exon 3 was missing in resorbed fetuses, and the expression of exons 4-6 and 10 drastically decreased. This was reflected at the protein level. The full-length PIBF was strongly expressed in mid-pregnancy fetuses but not in those from late pregnancy. The protein

expression profiles of the late-pregnancy resorbed fetuses were similar to that of the corresponding normal foetuses, i.e., the 90 kDa protein was missing, and the expression of the 34 kDa isoform decreased. These data suggest that the presence of these isoforms is a key for successful pregnancy.

PIBF positive dNK cells

In the mid-pregnancy decidua, we observed cells with PIBF containing cytoplasmic granules that showed an NK-like morphology. Our aim was to further characterize these cells.

To confirm the lymphocyte origin of these cells, we used decidual sections from alymphoid (Rag2-/-Il2rg-/-) mice, which lacked NK, T and B cells, as well as decidual sections from the same strain, after reconstituting their immune system with bone marrow transplantation.

The day 12.5 of mouse decidua contains a high number of PIBF+ NK cells. The deciduae of alymphoid mice at the same gestational age, were devoid of the PIBF+ granulated cells, however, these cells were present in in the decidua of alymphoid mice reconstituted with bone marrow from male BALB/c mice, in a count similar to that fund in the decidua of normal control mice at the same gestational day. These data suggest that the large, PIBF+ granulated cells belong to the lymphocyte lineage.

Because of their morphological resemblance to uterine NK cells, and because murine uterine NK cells are characterized by their PAS and DBA reactivity, sections of g.d. 12.5 mouse uteri were stained with the above markers. Eighty-five per cent of the NK cells in the decidua of g. d.12.5 pregnant mice were both PAS+ and DBA reactive, and only 15% of the cells were PAS+, DBA- . The majority of PAS+DBA- NK cells were located in the spongio-trophoblast, which did not contain PAS+ DBA+ cells.

All of the PIBF positive cells were DBA positive, while neither of the PAS+DBA- cells in the decidua or in the spongiotrophoblast reacted with anti-PIBF antibody. The percentage of PIBF+ cells within the DBA+ population moderately increased as pregnancy progressed.

Deciduae of g.d. 12.5 pregnant mice were reacted with anti-perforin antibody. Labelling sections from the same mice with fluorescent tagged anti- PIBF and anti-perforin antibodies revealed a co-localization of perforin and PIBF in the cytoplasmic granules. The % of perforin+ cells within the PIBF+ population increased throughout pregnancy.

The ratio of both PIBF + cells within the DBA + population and that of PIBF positive cells expressing perforin in the granules increased in control animals, as pregnancy progressed. In contrast to untreated mice, in RU486 treated animals, all the PIBF positive cells expressed perforin (100 vs 54%).

Discussion

By modulating the maternal immune response, PIBF plays a significant role in establishing a favourable environment for the developing foetus. The most important effects of PIBF are the alteration of the cytokine profile and the inhibition of NK activity (7, 11).

The 88% homology between the human and mouse PIBF gene, and the short reproduction period of mice make the murine system an ideal model for studying the effects of PIBF on pregnancy.

In this study we investigated which PIBF isoforms are needed for a normally progressing pregnancy, and how PIBF might influence decidual NK activity.

Our data show that normal murine pregnancy is characterized by gestational age-specific PIBF mRNA exon patterns and PIBF protein isoform profiles, furthermore, that both of the above are altered in failed pregnancies.

PIBF isoforms show typical subcellular localization. Lachmann et al. showed that in PIBF-transfected cell lines the full length PIBF localized exclusively to the nuclear fractions, whereas a 35 kDa splice variant (coded by exon 1-5 +17-18) moved to the cytosolic fractions, suggesting that, the product of the same PIBF gene may potentially act both as a transcription factor and as a cytokine (12).

A Th1 to Th2 switch has been implicated in the maintenance of pregnancy (6).

The effects of PIBF both on cytokine production and NK activity are attributed to the 34-37 kDa secreted isoform, which appears in biological fluids during normal human pregnancy, while pathological pregnancies are characterized by missing or low levels of secreted PIBF (13). Functional analysis of recombinant PIBF constructs (exons 2-9, exons 2-4, exons 5-7, exons 8-9, exons 10-12 and exons 13-16) localized the immunological activity on the N-terminal part of the molecule (3), while the amino acid sequences required for the nuclear/centrosomal localization are located within exons 6–16. Rapidly proliferating cells, e.g., embryonic- or tumor cells express higher amounts of the full length PIBF mRNA than normal cells, and PIBF has been shown to control cell invasion by suppressing pro-invasive genes or inducing genes that positively regulate invasion (14, 15). These results suggest that the transcriptional mechanisms of PIBF1 gene might produce proteins with different functional attributes.

In early pregnancy embryos all exons were present in 100% of the tested samples whereas in late pregnancy the incidence of exons 1-6 and exon 9 was significantly lower. Even more important was the difference between the PIBF mRNA exon-pattern in normal and resorbed fetuses. Compared to fetal samples from identical stages of pregnancy the expression of almost all exons was remarkably reduced in resorbed fetuses. This phenomenon was most prominent for exons encoding the N-terminal region of the full-length protein.

This results in the production of several PIBF isoforms. Whether these are splice variants or post translational modifications- remains to be determined.

Lachmann et al. (12) demonstrated a complex pattern of alternative splicing in tumor samples that generated different PIBF transcripts. In the present study the detected profile of PIBF protein-expression was more complex than the fingerprint of mRNA expression and showed a sort of conserved pattern, which might indicate that either the used primer-pairs were not able to detect all of the possible alternatively spliced PIBF mRNA variants or other mechanisms - such as post-translational modifications, alternative translation initiation - might be also responsible for the appearance of the observed protein-expression profile.

The 90 kDa full-length PIBF shows the highest expression in the 12-14-day (less differentiated) fetuses, while during late pregnancy – in the more differentiated tissues - the 90kDa full-length PIBF becomes the least-translated isoform.

The tissue-specific and time-dependent expression of PIBF isoforms during murine pregnancy suggests specific functional properties in several respects. The full length 90kDa PIBF that is overexpressed in undifferentiated cells (e.g. tumors and embryo) has been identified as a component of the pericentriolar satellite, this way it may play a role in cell cycle regulation. The CEP family of proteins is the active component of centrosome and plays a role in cell cycle progression control. Kim et al demonstrated that CEP90 is a larger isoform of PIBF1 molecule, and plays a crucial role in maintaining the integrity of the mitotic spindle pole (4).

Based on the fact that the full-length PIBF is preferentially expressed in tissues with high rate of proliferation (especially in the mid-pregnancy embryo) and its transcription down-regulates before birth (after organic differentiation) this isoform might control embryonic cell differentiation, development and/or stem cell maintenance in the developing fetus. A point mutation in PIBF1 gene identified by ENU mutagenesis screen caused a mid-gestational (E94-10.5) lethality that was likely owing to vascular defects and lack of nutrient supply (16) and T. O'Brien, personal communications.

The fact, that the 90 kDa isoform was present in the pseudopregnant endometrium suggests that the expression of this isoform is independent of the presence of the fetus. PIBF is constitutively expressed in endometrial mesenchymal stem cells and progesterone treatment induces PIBF secretion from these cells (17).

Taken together, the loss of the N-terminal exons in late and failed pregnancy might have two important functional consequences. The absence of the full length PIBF will interfere with cell cycle regulation, while the lack of the N-terminal exons might prevent the production of the isoforms endowed with immunomodulatory functions. In the latter case, the favorable effects of PIBF on the cytokine pattern and NK activity will not be manifested. Therefore, the lack of the N terminal exons could be detrimental, both because of failed

production of the isoforms involved in cell cycle regulation, and of those with immunoregulatory functions.

During normal conditions decidual NK cells contribute to creating a favourable environment for placentation, implantation and embryo development (18), but at the same time they are fully armed to fight intrauterine infections, if necessary (19). NK cells, - which constitute 60 to 70 % of all decidual lymphocytes in the first trimester of human pregnancy (20) - are both phenotypically and functionally different from peripheral NK cells. Most decidual NK cells are $CD16^{-}CD56^{bright}$, and though they contain cytotoxic granules and selectively overexpress genes of perforin and granzymes A and B (20), show a low cytotoxic activity.

Cytotoxic mechanisms exerted by NK cells can potentially damage the trophoblast and induce ablation of placenta, though there is no direct proof that NK cells attack the trophoblast. Yet, the fact that certain pregnancy pathologies are associated with increased decidual NK activity suggests that such a mechanism might exist. In humans, recurrent miscarriages are associated with an increased number of endometrial NK cells (21). Gulan et al. (22) demonstrated decreased perforin content of decidual lymphocytes from failed pregnancies as compared to those from normal pregnancy deciduae, suggesting that an increased rate of degranulation might have had taken place in the former case. These data suggest that a part of human recurrent miscarriage with unknown aetiology might be explained by deficiency in $CD16^{-}CD56^{bright}$ uNK cells, though the precise mechanism has been unknown.

Decidual NK cells express perforin and granzymes A and B (23). Under certain conditions, e.g., when exposed to hCMV infected autologous decidual cells (24), or during spontaneous abortion in mice (25) they degranulate yet despite the abundant presence of cytotoxic molecules in their cytoplasmic granules, during a normal pregnancy, these cells are not cytotoxic (5).

In this study we showed that in decidual NK cells perforin co-localizes with PIBF in the granules, and that while in RU486-treated mice PIBF+ NK cell counts decrease by approximately 50%, the ratio of perforin+ cells increases within the PIBF+ population.

Although decidual NK cells do not express nuclear progesterone receptor, they appear to be affected by PIBF. The increased resorption rates of pregnant BALB/c mice induced by anti-PIBF antibody were corrected by treating the mice with anti NK-1.1 antibody (10). PIBF blocked up regulation of perforin expression in decidual lymphocytes, cultured with decidual adherent cells and anti-PIBF antibodies reversed the progesterone mediated reduction in cytolytic activity of decidual lymphocytes (11). Faust et al. (26) showed that PIBF inhibits cytotoxicity of peripheral NK cells via a block of degranulation without

interfering with target conjugation. Based on these data, it cannot be ruled out that PIBF present in the cytoplasmic granules, contributes to low decidual NK activity.

Summary

We showed that murine resorptions are characterized by the lack of the N-terminal PIBF exons and a consequently altered protein expression pattern. These findings suggest, that both the full length PIBF and the smaller isoforms are required for the maintenance of normal pregnancy.

The presence of PIBF in the cytoplasmic granules of the potentially cytotoxic decidual NK cells contributes to their low cytotoxic activity, consequently; to normally progressing pregnancy.

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References

1 Medawar BP: Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. Symp. Soc. Exp. Biol. 1953; 7: 320.

2 Szekeres-Bartho J, Szekeres G, Debre P, Autran B, Chaouat G: Reactivity of lymphocytes to a progesterone receptor-specific monoclonal antibody. Cell Immunol. 1990; 125(2):273-283.

3 Polgar B, Kispal G, Lachmann M, Paar C, Nagy E, Csere P, Miko E, Szereday L, Varga P, Szekeres-Bartho J: Molecular cloning and immunologic characterization of a novel cDNA coding for progesterone-induced blocking factor. J Immunol 2003; 171(11): 5956–5963.

4 Kim K, Lee K, Rhee K: CEP90 is required for the assembly and centrosomal accumulation of centriolar satellites, which is essential for primary cilia formation. PLoS One 2012; 7(10): e48196.

5 Kozma N, Halasz M, Polgar B, Poehlmann TG, Markert UR, Palkovics T, Keszei M, Par G, Kiss K, Szeberenyi J, Grama L, Szekeres-Bartho J: Progesterone-induced blocking factor activates STAT6 via binding to a novel IL-4 receptor. J Immunol 2006; 176(2):819-826.

6 Wegmann TG, Lin H, Guilbert L, Mosmann TR: Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th2 phenomenon? Immunol Today 1993; 14(7): 353-356.

7 Szekeres-Bartho J, Wegmann TG: A progesterone-dependent immunomodulatory protein alters the Th1/Th2 balance. J Reprod Immunol 1996; 31(1-2): 81-95.

8 Quenby S, Nik H, Innes B, Lash G, Turner M, Drury J, Bulmer J: Uterine natural killer cells and angiogenesis in recurrent reproductive failure. Hum Reprod 2009; 24:45–54.

9 Rukavina D, Rubesa G, Gudelj L, Haller H, Podack ER: Characteristics of perforin expressing lymphocytes within the first trimester of human pregnancy. Am J Reprod Immunol 1995; 33:394–404.

10 Szekeres-Bartho J, Par G, Dombay G, Smart YC, Volgyi Z: The antiabortive effect of progesterone-induced blocking factor in mice is manifested by modulating NK activity. Cellular Immunology 1997; 177:194–199.

11 Laskarin G, Strbo N, Sotosek V, Rukavina D, Faust Z, Szekeres-Bartho J, Podack ER: Progesterone directly and indirectly affects perforin expression in cytolytic cells. Am J Reprod Immunol 1999; 42(5):312-320.

12 Lachmann M, Gelbmann D, Kálmán E, Polgár B, Buschle M, Von Gabain A, Szekeres-Barthó J, Nagy E: PIBF (progesterone induced blocking factor) is overexpressed in highly proliferating cells and associated with the centrosome. Int J Cancer 2004; 112(1):51-60.

13 Polgár B, Nagy E, Mikó E, Varga P, Szekeres-Barthó J: Urinary progesterone-induced blocking factor concentration is related to pregnancy outcome. Biol Reprod 2004 71(5):1699-705.

14 Miko E, Halasz M, Jericevic-Mulac B, Wicherek L, Arck P, Arato G, Skret Magierlo J, Rukavina D, Szekeres-Bartho J: Progesterone-induced blocking factor (PIBF) and trophoblast invasiveness. J Reprod Immunol 2011; 90:50-7.

15 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(23):4617-30.

16 Hagarman JA, O'Brien TP: An essential gene mutagenesis screen across the highly conserved piebald deletion region of mouse chromosome 14. Genesis 2009; 47:392-403.

17 Kyurkchiev DS, Ivanova-Todorova E, Kyurkchiev SD: Effect of progesterone on human mesenchymal stem cells. Vitam Horm 2011; 87:217-37.

18 Redhead ML, Portilho NA, Felker AM, Mohammad S, Mara DL, Croy BA: The transcription factor NFIL3 is essential for normal placental and embryonic development but not for uterine natural killer (uNK) cell differentiation in mice. Biol Reprod 2016; 94(5):101.

19 Quillay H, El Costa H, Duriez M, Marlin R, Cannou C, Madec Y, de Truchis C, Rahmati M, Barré-Sinoussi F, Nugeyre MT, Menu E: NK cells control HIV-1 infection of macrophages through soluble factors and cellular contacts in the human decidua. Retrovirology 2016; 13(1):39.

20 Koopman LA, Kopcow HD, Rybalov HD, Boyson JE, Orange JS, Schatz F, Masch R, Lockwood CJ, Schachter AD, Park PJ, Strominger JL: Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. J Exp Med 2003; 198:1201-12.

21 Quenby S, Farquharson R: Uterine natural killer cells, implantation failure and recurrent miscarriage. Reprod Biomed Online 2006; 13:24-8.

22 Gulan G, Podack ER, Rukavina D, Gudelj L, Rubesa G, Petrovic O: Perforin-expressing lymphocytes in peripheral blood and decidua of human first-trimester pathological pregnancies. Am J Reprod Immunol 1997; 38:9-18.

23 Bogovic Crncic T, Laskarin G, Juretic K, Strbo N, Dupro J, Srsen S, Randic L, Le Boutellier P, Tabiasco J, Rukavina D: Perforin and Fas/FasL cytolytic pathways at the maternal-fetal interface. Am J Reprod Immunol 2005; 54:241-8.

24 Siewiera J, El Costa H, Tabiasco J, Berrebi A, Cartron G, Le Boutellier P, Jabrane-Ferrat N: Human cytomegalovirus infection elicits new decidual natural killer cell effector functions. PloS Pathog 2013; 9(4):e1003257.

25 Lima PD, Tu MM, Rahim MM, Peng AR, Croy BA, Makrigiannis AP: Ly49 receptors activate angiogenic mouse DBA+ uterine natural killer cells. Cell Mol Imunol 2014; 11(5):467-76.

26 Faust Zs, Laskarin G, Rukavina D, Szekeres-Bartho J: Progesterone induced blocking factor inhibits degranulation of NK cells. Am J Reprod Immunol 1999; 42:71-75.

A, The theses was based on the following publications

Bogdan A, Polgar B, Szekeres-Bartho J: Progesterone induced blocking factor isoforms in normal and failed murine pregnancies. Am J Reprod Immunol 2014; 71(2): 131-136. **IF: 2,438**

Bogdan A, Berta G, Szekeres-Bartho J: PIBF positive uterine NK cells in the mouse decidua. J Reprod Immunol 2017; 119: 38-43. **IF: 2,322**

Other publications related to the theses;

Segerer SE, Martignoni F, **Bogdan A**, Muller N, Kapp M, Dietl J, Rieger L, Kammerer U: Thrombopoietin modulates the proliferation, migration and cytokine profile of decidual cell subsets during early gestation. Mol Hum Reprod 2013; 19(6): 361-368. **IF: 3,483**

Mori M, **Bogdan A**, Balassa T, Csabai T, Szekeres-Bartho J: The decidua - the maternal bed embracing the embryo - maintains the pregnancy. Semin Immunpathol 2016; 38(6): 635-649. **IF: 5,296**

Meggyes M, Szereday L, Jakso P, Bogar B, **Bogdan A**, Nörenberg J, Miko E, Barakonyi A: Expansion of CD4 phenotype among CD160 receptor-expressing lymphocytes in murine pregnancy. Am J Reprod Immunol 2017; 78(6). doi: 10.1111/aji.12745. Epub 2017 Sep 16. **IF: 2,745**

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(1): 4662. **IF: 4,122 (2017)**

cumulative IF of A (of publications on which the theses are based): 20,406

Other publications related to the theses in Hungarian;

Bogdán Ágnes, Polgár Beáta, Szekeres-Barthó Júlia: A progeszteron-indukálta blokkoló faktor szerepe a terhesség fenntartásában. EGÉSZSÉG-AKADÉMIA 2014; 5(4): 254-258.

Book chapter related to the theses

Julia Szekeres-Bartho, Melinda Halasz, Beata Polgar, **Agnes Bogdan**: Immuno Endocrine Interactions: An Example; Progestagen Treatment for Threatened and Recurrent Abortion and Molecular Mechanisms. In: Gérard Chaouat, Olivier Sandra, Nathalie Lédéé (Eds) Immunology of Pregnancy 2013. Online kiadás: Bentham Science Publishers Ltd., 2013. pp. 614-624.

B, Other publications

Bányai K, Forgách P, Erdélyi K, Martella V, **Bogdán A**, Hocsák E, Havasi V, Melegh B, Szucs G: Identification of the novel lapine rotavirus genotype P[22] from an outbreak of enteritis in a Hungarian rabbitry. Virus Res 2005; 113(2): 73-80. **IF: 2,562**

Bányai K, Jiang B, **Bogdán A**, Horváth B, Jakab F, Meleg E, Martella V, Magyari L, Melegh B, Szucs G: Prevalence and molecular characterization of human group C rotaviruses in Hungary. J Clin Virol 2006; 37(4): 317-322. **IF: 2,630**

Bányai K, **Bogdán A**, Kisfali P, Molnár P, Mihály I, Melegh B, Martella V, Gentsch JR, Szücs G: Emergence of serotype G12 rotaviruses, Hungary. Emerg Infect Dis 2007; 13(6): 916-919. **IF: 5,775**

Bányai K, Martella V**, Bogdán A**, Forgách P, Jakab F, Meleg E, Bíró H, Melegh B, Szucs G: Genogroup I picobirnaviruses in pigs: evidence for genetic diversity and relatedness to human strains. J Gen Virol 2008; 89(2): 534-539. **IF: 3,092**

Bányai K, **Bogdán A**, Domonkos G, Kisfali P, Molnár P, Tóth A, Melegh B, Martella V, Gentsch JR, Szucs G: Genetic diversity and zoonotic potential of human rotavirus strains, 2003-2006, Hungary. J Med Virol 2009; 81(2): 362-370. **IF: 2,470**

Bányai K, **Bogdán A**, Szücs G, Arista S, De Grazia S, Kang G, Banerjee I, Iturriza-Gómara M, Buonavoglia C, Martella V: Assignment of the group A rotavirus NSP4 gene into genotypes using a hemi-nested multiplex PCR assay: a rapid and reproducible assay for strain surveillance studies. J Med Microbiol 2009; 58(3): 303-311. **IF: 2,272**

Bányai K, Kisfali P, **Bogdán A**, Martella V, Melegh B, Erdman D, Szucs G: Adenovirus gastroenteritis in Hungary, 2003-2006. Eur J Clin Microbiol Infect Dis 2009; 28(8): 997-999. **IF: 2,605**

Bányai K, Gentsch JR, Martella V, **Bogdán A**, Havasi V, Kisfali P, Szabó A, Mihály I, Molnár P, Melegh B, Szücs G: Trends in the epidemiology of human G1P[8] rotaviruses: a hungarian study. J Infect Dis 2009; 200 Suppl 1: S222-227. **IF: 5,865**

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