SIGNAL TRANSDUCTION PATHWAYS

MEDIATING THE EFFECTS

OF

PROGESTERONE-INDUCED BLOCKING FACTOR

Ph. D. Thesis

Noémi Kozma M.D.

Program leader & Tutor: Júlia Szekeres-Barthó M.D., Ph.D., D.Sc.

Pécs University

Medical School

Department of Medical Microbiology and Immunology

Pécs

2005

SIGNAL TRANSDUCTION PATHWAYS

MEDIATING THE EFFECTS

OF

PROGESTERONE-INDUCED BLOCKING FACTOR

Ph. D. Thesis

Noémi Kozma M.D.

Program leader & Tutor: Júlia Szekeres-Barthó M.D., Ph.D., D.Sc.

Pécs University

Medical School

Department of Medical Microbiology and Immunology

Pécs

2005

INTRODUCTION

Successful pregnancy is a natural model of graft-host relationship, regulated by a bidirectional interaction between the immune and the endocrine systems. Progesterone is essential for the maintenance of pregnancy in most mammalian species. This hormone affects different stages of the immune response and prolongs the survival of xenogenic and allogenic grafts. Peripheral lymphocytes of pregnant women express specific progesterone receptors (PRs). This allows – upon progesterone binding – the synthesis of a mediator, named Progesterone-Induced Blocking Factor (PIBF). By acting on cytokine production this protein exerts an anti-abortive effect in mice. Normal pregnancy is associated with a relative Th2 dominance. PIBF induces a significant increase in IL-10, IL-3 and IL-4 production by activated murine spleen cells and inhibits the IL-12 synthesis of peripheral mononuclear cells of healthy pregnant women.

There are many signalling mechanisms by which cells respond in a specific way to a wide variety of extracellular regulatory substances. The Signal Transducer and Activator of Transcription (STAT) proteins were identified in the last decade as transcription factors critical in mediating virtually all cytokine driven signalling. Latent STATs in the cytoplasm are promptly activated by tyrosine phosphorylation of cytokine receptor associated Janus kinases (Jaks). Phosphorylated STATs from homo- or heterodimers and enter the nucleus, where they bind to specific DNA elements and initiate transcription.

STAT4 plays an important role in Th1 cell function and development. Cellular proliferation and NK cytotoxicity are all abrogated in the absence of STAT4. Although IL-12 appears to be the predominant activator of STAT4, in human cells STAT4 can also be phosphorylated in response to INFγ.

STAT6-deficient T helper cells are unable to differentiate into Th2 cells either *in vitro* or *in vivo*. The primary activators of STAT6 are IL-4 and IL-13. STAT6 is activated by ligation of the IL-4 receptor (IL-4R), which is a heterodimer consisting of the signalling high-affinity α chain and a trans-activating low-affinity chain that can be either the common γ chain or the IL-13 receptor α1 (IL-13Rα1) chain. IL-4Rα and γ chain associate with the Janus kinases, Jak1 and Jak3 respectively. Phosphorylated tyrosine residues of IL-4Rα chain serve as anchor for the SH2 domain of STAT6.

IL-12/STAT4 and IL-4/STAT6 pathways are under negative feedback regulation by Suppressor of Cytokine Signalling (SOCS) proteins. SOCS1 and SOCS3 genes are differentially expressed in Th1 and Th2 cells. IL-12-induced STAT4 phosphorylation is inhibited in Th2 cells, which contain 23-fold higher levels of SOCS3 than Th1 cells. SOCS3 is induced by IL-4 signal, and inhibits IL-12 induced STAT4 activation by binding to the IL-12R.

When IL-12 and IL-4 are present, murine and human T cell differentiation is regulated by the balance of PKC and Ca++ signalling. High levels of PKC activity combined with low Ca++ signals favour Th2 development, whereas high Ca++ signalling together with low PKC activity favours Th1 development. In this respect little is known about the function of individual PKC isotypes. At least 11 isoenzymes have been described and classified into 3 subfamilies: Ca++ dependent or conventional protein kinase C (cPKC) isoforms; α, β, γ, Ca++ independent, novel protein kinase C (nPKC) isoforms; δ, ε, η, θ, μ or atypical phospholipase- and Ca++ independent protein kinase C (aPKC) isoforms ζ, ι, λ.

According to the latest reports, PKCζ levels are increased during Th2 differentiation of CD4+ T cells. The lack of PKCζ impairs the secretion of Th2 cytokines *in vitro* and *in vivo*, as well as Jak1 phosphorylation and the nuclear translocation of STAT6. PKCθ is

critical for the development of *in vivo* Th2- but not of Th1 cell responses. PKCθ-deficient T cells exhibit impaired IL-4, but normal INFγ production and PKCθ-/- mice fail to develop Th2 cell-dependent allergic airway inflammation.

AIMS OF THE STUDY

The aim of this study was to investigate the mechanisms that play a part in PIBF-induced immunomodulation. The pathways that might be involved in PIBF signalling are shown in Figure 1.

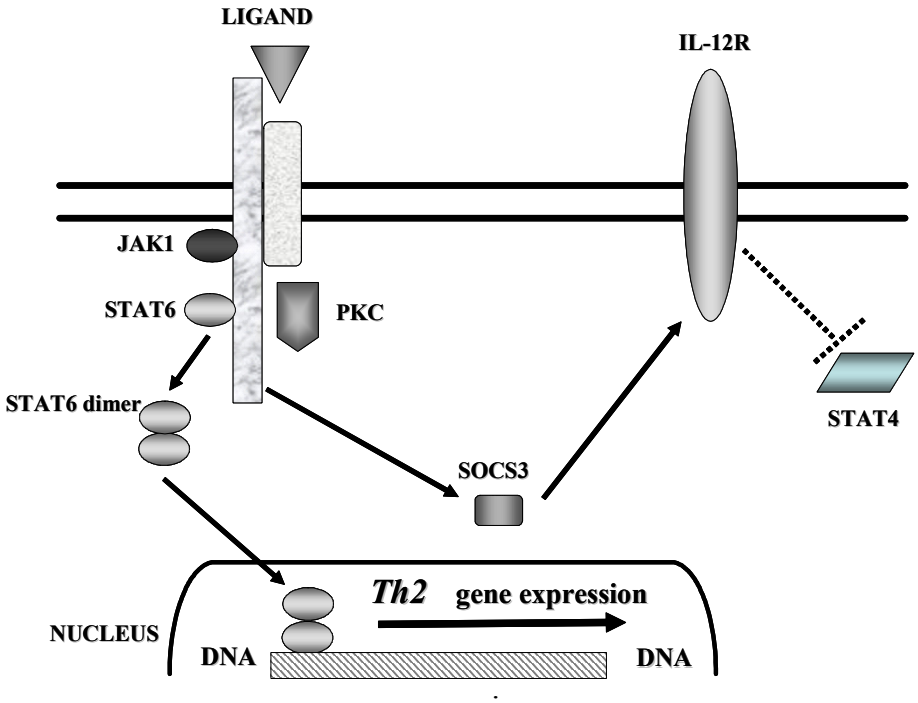

Fig. 1 The Jak/STAT signal transduction pathway

1. Earlier data showed that addition of exogenous arachinodic acid as well as cyclooxygenase inhibitors counteract the immunological effects of PIBF, which suggested that at some point the molecule interferes with arachinodic acid metabolism. Phosholipase A_2 (PLA_2) is needed for the liberation of arachinodic acid. Therefore we investigated the effect of PLA_2 inhibition by quinacrine, on IL-12 production by PIBF-treated lymphocytes.

2. PIBF acts on the cytokine balance. Cytokine driven signals are mediated by STAT transcription factors. Therefore we aimed at investigating the involvement of the Jak/STAT pathway in the effects of PIBF.
3. Recent data revealed that IL-12/STAT4 and IL-4/STAT6 transcription are under negative control regulation by SOCS1 and SOCS3, which prompted us to examine the effects of PIBF on SOCS induction.
4. Based on the information that STAT6 is activated following ligation of the IL-4R, we tested the involvement of IL-4Rα in PIBF signalling.
5. In order to verify the involvement of the STAT6 pathway in the cytokine effects of PIBF, cytokine production was determined in STAT6 deficient lymphocytes.
6. The development of naive T cells into type 1 or type 2 effector cells is thought to be under the control of PKC/Ca++ pathways. Therefore the role of different PKC isotypes and the level of intracellular Ca++ concentration in PIBF induced signalling were examined.

MATERIALS AND METHODS

1. Cytokine production of the lymphocytes was determined by:

1.1 Immunocytochemistry

Quinacrine treated peripheral mononuclear cells from healthy pregnant women were centrifuged on glass microscope slides. The slides were dried at room temperature, the cells were fixed for 5 min in ice cold acetone and washed in tris-buffered saline (TBS). All incubations were carried out at room temperature in a humid chamber. After blocking endogenous peroxidase activity with 1% H_2O_2 the cells were further incubated in TBS containing 1% bovine serum albumin (BSA) for blocking non-specific protein binding. The cells were incubated for 1 hour with anti-IL-12 monoclonal antibody. HRPO-labeled anti-mouse IgG were applied for 30 minutes. The reaction was developed by diaminobenzidine followed by silver intensification. The nuclei were counter-stained with hematoxylin and the slides were mounted with gelatin-glycerol. The slides were read blind and the percentage of positive cells was determined after counting 500 lymphocytes in the microscope at high power magnification.

1.2 Cytometric Bead Arrays (CBA)

CBA were used to determine the concentrations of IL-10, TNFα and INFγ in the supernatants of lymphocyte cultures. CBA Analysis and Bender MedSystem Softwares were used for analyzing the data.

2. Western blotting

For the detection of different transcription factors, treated or untreated peripheral human lymphocytes were lysed. The cytoplasmic fractions of the cells were separated on SDS-PAGE and transferred to Hybond ECL membranes, which were reacted with anti-phospho-specific antibodies. Controls included the lysate of E. coli that underwent the same purification procedure as the recombinant PIBF, as well as isotype and loading controls for all signalling factors.

3. EMSA Supershift

To test whether PIBF induces nuclear translocation of phosphorylated STAT6 dimers, nuclear extracts were subjected to EMSA. The reaction mixture of samples and double-stranded, labeled oligonucleotides were separated on acrylamide gelelectrophoresis, then exposed to X-ray film. The specificity of the reaction was verified with a supershift assay, which is based on the slower migration of anti-STAT6 IgG - STAT6 complexes, than STAT6 alone.

4. ELISA for detection of receptor binding

Plates were coated with recombinant human IL-4Rα. Logarithmic dilutions (0.01-1 μg/ml) of 48-kDa N-terminal recombinant human PIBF or recombinant human IL-4 were incubated on the plates. For detecting the binding reaction, biotin-conjugated anti-PIBF IgG- streptavidin-biotin-HRPO, and goat anti-IL-4 antibody – anti-goat IgG-HRPO were used respectively.

5. Flow cytometry for determination of PIBF-receptor binding

To test whether blocking of the IL-4R affects PIBF binding, peripheral lymphocytes were incubated with FITC-conjugated PIBF together with increasing concentrations of unlabelled PIBF or monoclonal anti-human IL-4Rα antibody. FITC-PIBF binding was analyzed using a FACSCalibur flow cytometer, equipped with a 488 nm excitation laser with the CellQuest Software program.

6. Confocal microscopy

The relationship of the PIBF and IL-4 receptors was examined by confocal microscopy. Peripheral lymphocytes from healthy volunteers were incubated with 5 μg of FITC-conjugated PIBF for 20 min. at 37°C. The cells were plated on poly-L-lysine coated slides and incubated at 37°C for further 10 min, then washed and fixed with freshly prepared 3% paraformaldehyde in PBS for 10 min. at room temperature. After washing the plates were incubated with 0.5 μg of monoclonal anti-IL-4Rα or 2 μl of PE-labeled mouse anti-CD45RA antibody for 45 min. at room temperature. Cells were washed and incubated with 2 μl PE-labeled rat anti-mouse IgG 2A+B or PE-labeled rat anti-mouse IgG1 for 30 min. at room temperature. Cells were washed and the slides were mounted with DABCO. To control the specificity of the capping formation, all steps were also performed at 4°C. The slides were analyzed with a Biorad confocal microscope with 100x objective, using laser excitation at 473 nm and filters 580+/-16 nm for PE and 522+/-17.5 nm for FITC. Images were analyzed using Adobe Photoshop 7.0 program.

7. RNA interference (RNAi)

Oligonucleotides were hand-designed to interfere exclusively with STAT6 mRNA. As negative control, the same nucleotides were scrambled to form a non-genomic

combination. Lymphocytes culture: intact, control treated with scrambled oligos and after RNAi cultured with PIBF or without PIBF. Lymphocytes were lysed for Western blot analysis.

8. Flow cytometric determination of intracellular Ca++

Intracellular free Ca++ was measured using Fluo-3 AM dye. Activation was carried out with ionomycin as positiv control, and 7-Aminoactinomycin D labelling was used to distinguish dead cells by their higher fluorescence. Increase of fluorescence was measured in a Becton Dickinson FacsCalibur flow cytometer using the CellQuest program. The mean fluorescence intensity of Fluo-3 AM dye was determined at 526 nm. After measuring basal fluorescence the activating agents was added and the measurement continued for further 100 to 400 sec. Gates were created along the time axis of the activation dot plots at definite time points and the mean fluorescence intensity at 526 nm was statistically analyzed from each gate.

RESULTS

1. Neutralization of endogenous PIBF by PIBF specific antibody resulted in increased IL-12 production by peripheral mononuclear cells of pregnant women. This was corrected by inhibiting phospholipase A_2 activity (consequently arachidonic acid release). These data suggest that PIBF acts on IL-12 production by limiting the production of arachidonic acid.
2. Similarly to IL-4, PIBF induced phosphorylation of Jak1 and STAT6. The effect of PIBF on STAT6 induction was concentration and time-dependent: tyrosine phosphorylated STAT6 appeared, after 1 min. incubation with PIBF, which acted in low (200 ng/ml) concentrations. By EMSA Supershift assay, we demonstrated PIBF induced nuclear translocation of phosphorylated STAT6 dimers. At the same time, PIBF inhibited IL-12-induced phosphorylation of STAT4.
3. In order to verify the involvement of the STAT6 pathway in the cytokine effects of PIBF, STAT6 was knocked down in peripheral lymphocytes by siRNA interfering with STAT6 mRNA. In STAT6 deficient cells both the positive (on IL-10 production) and the negative (on TNFα and IFNγ production) effects of PIBF were markedly reduced.
4. Next, we investigated the sensitive feedback regulation by SOCS of PIBF induced STAT activation or inhibition. Similarly to IL-4, PIBF-treatment induced SOCS3 activation, whereas IL-12 induced SOCS1 disappeared after PIBF or IL-4 treatment.
5. Since activation of the STAT6 pathway depends on the ligation of the IL-4R, we tested the involvement of IL-4Rα in PIBF-induced STAT6 activation. We could not demonstrate PIBF binding to IL-4Rα by ELISA, nor did anti-IL-4Rα treatment prevent PIBF binding to its own receptor. On the other hand, treatment of the cells

with blocking concentrations of anti-IL-4Rα abolished the effects of PIBF both on Jak1 and STATs, whereas blocking of IL-13R had no effect. Confocal microscopy analysis of PE–anti-IL-4R and FITC-PIBF labeled cells revealed a co-capping of the two receptors. Based on the above data, we hypothetized that upon ligand binding, the PIBF receptor might form a heterodimer with the alpha chain of the IL-4R, and activates STAT6 pathway. These data however raised the question, why PIBFR needs the IL-4Rα for signalling? Several proteins are anchored to membranes via post-translational lipid modification, the glycosylphosphatidylinositol (GPI) anchor. These molecules have no transmembrane or intracellular domains, thus their signalling capacity is due to association of these molecules with putative transmembrane proteins that can signal. Testing the hypothesis that PIBFR was a GPI anchored protein we digested the putative anchoring region with phosphatidylinositol-specific phospholipase C (PI-PLC). IL-4 was still able to activate STAT6 in PI-PLC digested cells, but PIBF failed to do so, suggesting that the latter was possible a GPI anchored protein.

6. High levels of PKC activity combined with low Ca++ signals favour Th2 development, while predominance of Ca++ signalling with low PKC activity favours Th1 development. Both IL-4 and PIBF treatment induced the phosphorylation of PKCα/β II, PKCθ and PKCζ , without affecting intracellular Ca++ levels.
7. The Ca++ independent, novel PKC isoform PKCθ and the atypical PKC isoform PKCζ are involved in the induction of type 2 development, furthermore PKCζ activity is required for Jak1 phosphorylation during IL-4 signalling. Since PIBF uses the IL-4Rα chain for STAT6 signalling, we tested, whether a functional IL-4Rα was required for PKC activation. The effect of PIBF on the phosphorylation of PKCζ, PKCθ, and PKCα/β was abrogated by the pre-treatment of the cells with blocking concentrations

of anti-IL-4Rα antibody, suggesting that both PIBF and functional IL-4Rα is needed for PIBF-induced PKC-activation. To test if PKC phosphorylation is needed for the effects of PIBF on Jak/STAT activation, Jak1 and STAT6 induction were tested, when PKC activity was blocked. The results suggest that PKCζ and PKCθ phosphorylation, but not that of PKC α/β II. are required for PIBF-induced Jak1 and STAT6 activation.

CONCLUSIONS

PIBF-induced cytokine modulation is manifested via several pathways. By interfering with arachidonic acid metabolism, PIBF reduces IL-12 production, which results in a lowered cytotoxic NK activity. High PKC activity and low intracellular Ca++ levels favour the development of Th2 cytokine sensitive cells, whereas inhibition of STAT4 phosphorylation decreases the sensitivity of the cell to Th1 cytokines. These together might account for the Th2 biased response induced by PIBF.

The concept that both IL-4Rα and PIBFR are required for PIBF signalling is supported by the following:

1. Anti-IL-4Rα does not prevent binding of PIBF to its receptor, suggesting that PIBFR and IL-4Rα are separate entities.

2. Digesting the GPI anchor abolishes PIBF driven signalling, thus a GPI-ancored protein is required for PIBF signalling.

3. Anti-IL-4Rα antibody inhibits PIBF-induced STAT6 phosphorylation in intact cells showing that PIBF can not signal via its own receptor, without the involvement of IL-4Rα.

Taken together, the GPI-ancored PIBFR, is required, but not sufficient for PIBF signalling.

Our data suggest the existence of a novel IL-4R, where upon ligation, the PIBFR combines with IL-4Rα, and induces Jak1 phosphorylation, which in turn activates STAT6. PIBF-induced SOCS3 via binding to the IL-12R inhibits STAT4 phosphorylation and Th1 responses (Fig 2.)

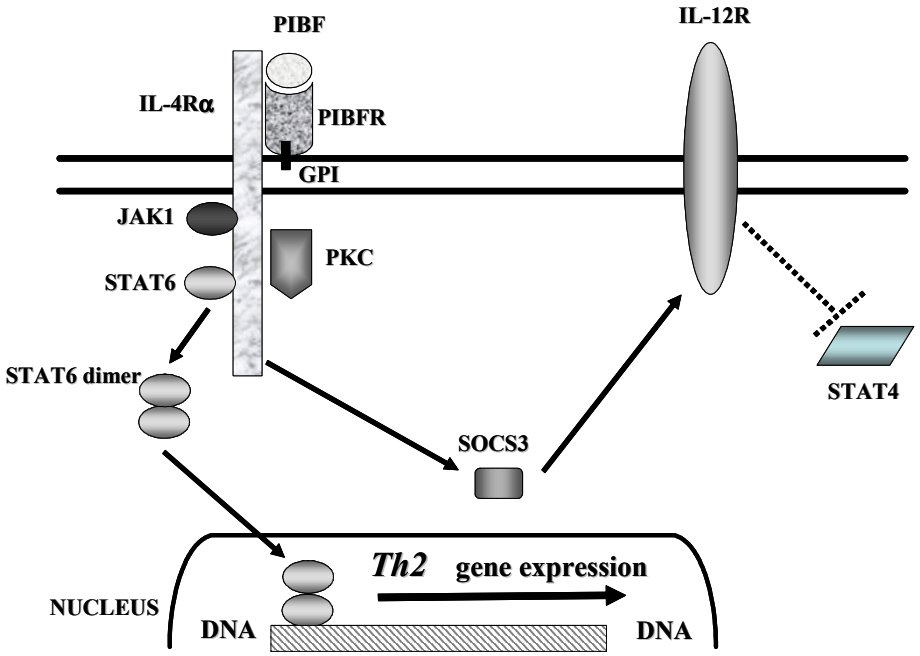

Fig. 2 PIBF acts on the cytokine balance via a novel type of IL-4R

THESES

1. PIBF inhibits arachinodic acid release. The subsequent block of prostaglandin synthesis reduces IL-12 production and results in a lowered cytotoxic NK activity, which may contribute to a normal pregnancy outcome.
2. PIBF induced Th2 shift is explainable by specific signal pathways in the background. Low concentrations of PIBF immediate phosphorylate Jak1, STAT6 and activate SOCS3, but inhibit IL-12 induced STAT4 phosphorylation and SOCS1 induction. Phosphorylated STAT6 dimers translocate into the nucleus.
3. PIBF is not a ligand of IL-4Rα, yet the latter is indispensable for PIBF signalling.
4. The PIBF receptor is a GPI-anchored protein. The STAT6 activating effect of PIBF is abolished after PI-PLC digestion of the cells, whereas that of IL-4 remains intact.
5. PIBF phosphorylates PKC without affecting intracellular Ca++ levels supporting another possible mechanism for explaining the cytokine effects of PIBF.

LIST OF PUBLICATIONS

Progesterone regulates IL-12 expression in pregnancy lymphocytes by inhibiting Phospholipase A2
G. Par, J. Geli, N. Kozma, P. Varga, J. Szekeres-Bartho
American Journal of Reproductive Immunology, 2003;49:p:1-5. IP: 2.088

Progesterone-dependent immunomodulation
J. Szekeres-Bartho, B. Polgar, N. Kozma, E. Miko, G. Par, L. Szereday, A. Barakonyi, T. Palkovics, O. Papp, P. Varga
Chemical Immunology and Allergy, 2005;89:118-125

The Progesterone-Induced Blocking Factor modulates the balance of PKC and intracellular Ca++
N. Kozma, M. Halasz, T. Palkovics, J. Szekeres-Bartho
American Journal of Reproductive Immunology (in press) IP: 2.088

Progesterone-Induced Blocking Factor activates STAT6 via binding to a novel IL-4 receptor
N. Kozma, M. Halasz, B. Polgar, T. G. Poehlmann, U. R. Markert, T. Palkovics, M. Keszei, G. Par, K. Kiss, J. Szeberényi, L. Grama, J. Szekeres-Bartho
The Journal of Immunology (in press) IP: 6.486

LIST OF PRESENTATIONS

Poster presentations

Arachidonic acid metabolism is involved in the IL-12 expression by pregnancy lymphocytes
G. Pár, J. Géli, N. Kozma, J. Szekeres- Barthó
Alps Adria Society for Immunology of Reproduction
AASIR Hungarian Immunological Society
Jun. 27-30, 2000. Pécs, Hungary

Arachidonic acid metabolism is involved in the blocking of NK activity induced by immunomodulation of progesterone
N. Kozma, G. Pár, J. Géli, J. Szekeres- Barthó
Jubilee (XXX.) Meeting of the Hungarian Society for Immunology
Oct. 25-27, 2000. Budapest, Hungary

Signal transduction mediating the effects of PIBF
N. Kozma, G. Pár, J. Szekeres-Barthó
Jubilee (XXXI.) Meeting of the Hungarian Society for Immunology
Oct. 17-19, 2001. Eger, Hungary

Signal transduction pathways mediating the effects of Progesterone Induced Blocking Factor (PIBF)
N. Kozma, G. Pár, M. Keszei, Cs. Szalai, A. Falus, K. Kiss, J. Szeberényi, J. Szekeres-Barthó
European Congress of Reproductive Immunology
As the 9th Congress of the Alp-Adriatic Society for Immunology of Reproduction
As the 5th Congress of the European Society of Reproductive and Developmental Immunology
As the 10th meeting of the Czech Reproductive Immunology (Section of Czech Society of Immunology)
Jun. 30 – Jul. 3, 2004. Pilsen, Czech Republic

The Progesterone Induced Blocking Factor (PIBF): Focusing on intracellular signal transduction
N. Kozma, G. Pár, M. Keszei, Cs. Szalai, A. Falus, K. Kiss, J. Szeberényi, J. Szekeres-Barthó
1st International Conference on Basic and Clinical Immunogenomics
Oct. 3-7, 2004. Budapest, Hungary

PIBF effects on the JAK/STAT pathway depend on the IL-4 receptor (IL-4 R)
N. Kozma, G. Par, B. Polgar, T. Palkovics, M. Halasz, M. Keszei, Cs. Szalai, A. Falus, J. Szekeres-Bartho
Embryo implantation: from basics to clinics
The First EMBIC Summer School
Jun. 4-10, 2005. Malinska, Croatia

Investigations of the receptor
T. Palkovics, N. Kozma, J. Szekeres-Bartho
Embryo implantation: from basics to clinics
The First EMBIC Summer School
Jun. 4-10, 2005. Malinska, Croatia

PIBF effects on the Jak/Stat pathway depend on the IL-4 receptor.
N. Kozma, G. Par, B. Polgar, M. Halasz, M. Keszei, Cs. Szalai, A. Falus, J. Szekeres-Bartho
13th EFIS „Signals and Signal Processing in the Immune System"
Sept. 7-11, 2005. Balatonoszod, Hungary

Oral presentations

The role of histamine in reproduction
G. Pár, N. Kozma, É. Buzás, J. Szekeres-Barthó, A. Falus
Jubilee (XXX.) Meeting of the Hungarian Society for Immunology
Oct. 25-27, 2000. Budapest, Hungary

Intracellular mechanisms involved in PIBF action.
J. Szekeres-Barthó, N. Kozma, T. Palkovics, G. Pár, B. Polgár
VIII. International Congress of Reproductive Immunology
Jul. 2-6, 2001. Opatija, Croatia

Signal transduction pathways mediating the effects of Progesterone Induced Blocking Factor (PIBF)
N. Kozma, G. Pár, J. Szekeres-Barthó
11 th EFIS Symposium on Signals and Signal Processing in the Immune System
Sept. 2-6, 2001. Pécs, Hungary

Mechanisms of immunomodulatory action of progesterone
J. Szekeres-Barthó,A. Barakonyi, N. Kozma, T. Palkovics, G. Pár, B. Polgár, L. Szereday
12 th Symposium on Signals and Signal Processing in the Immune System
and EFIS symposium
Sept. 3-7. 2003. Sopron, Hungary

Intracellular signal mechanisms involved by PIBF
N. Kozma, G. Pár, K. Kiss, J. Szeberényi, J. Szekeres-Barthó
Jubilee (XXXIII.) Meeting of the Hungarian Society for Immunology
Oct. 15-17, 2003. Győr, Hungary

Genomic bases of progesterone-dependent immunomodulation
J. Szekeres-Bartho, B. Polgar, E. Nagy, E. Miko, N. Kozma, T. Palkovics, O. Papp and M. Halasz
Dept. of Medical Microbiology and Immunology, Pecs University, Pecs, Hungary
1st International Conference on Basic and Clinical Immunogenomics
Oct. 3-7, 2004. Budapest, Hungary

The Progesterone Induced Blocking Factor (PIBF): Intracellular signal transduction
N. Kozma, G. Pár, K. Kiss, J. Szeberényi, M. Keszei, Cs. Szalai, A. Falus, J. Szekeres-Barthó
Jubilee (XXXIV.) Meeting of the Hungarian Society for Immunology
Oct. 27-29, 2004. Szeged, Hungary

Role of progesterone in the immuno-endocrine control of successful pregnancy
J. Szekeres-Barthó, M. Halasz, N. Kozma, E. Miko, T. Palkovics, B. Polgar, L. Szereday, P. Varga
Embryo implantation: from basics to clinics
The First EMBIC Summer School
Jun. 4-10, 2005. Malinska, Croatia

PIBF is a ligand of a novel type of IL-4 receptors
M. Halasz, N. Kozma, B. Polgar, T. Palkovics, N. Halidi, L. Grama, M. Nyitrai, B. Somogyi, J. Szekeres-Bartho
Embryo implantation: from basics to clinics
The First EMBIC Summer School
Jun. 4-10, 2005. Malinska, Croatia

PIBF is the ligand of a novel type of IL-4-receptors
M. Halasz, N. Kozma, B. Polgar, N. Halidi, L. Grama, M. Nyitrai, B. Somogyi, J. Szekeres-Bartho
13th EFIS „Signals and Signal Processing in the Immune System"
Sept. 7-11, 2005. Balatonoszod, Hungary

Jak/STAT and PKC/Ca++ pathway mediating the effects of PIBF
N. Kozma, M. Halasz, B. Polgar, T. Palkovics, G. Par, M. Keszei, J. Szekeres-Bartho
Jubilee (XXXV.) Meeting of the Hungarian Society for Immunology
Oct. 19-22, 2005. Sopron, Hungary

PIBF is the ligand of a novel type of IL-4R?
M. Halasz, N. Kozma, B. Polgár, N. Halidi, L. Grama, J. Szekeres-Bartho
Jubilee (XXXV.) Meeting of the Hungarian Society for Immunology
Oct. 19-22, 2005. Sopron, Hungary

Published abstracts

Intracellular mechanisms involved in PIBF action
J. Szekeres-Bartho, N. Kozma, T. Palkovics, G. Par, B. Polgar
American Journal of Reproductive Immunology, 2001;46:p:62-63

Signal transduction pathways mediating the effects of Progesterone Induced Blocking Factor (PIBF)
N. Kozma, G. Pár, M. Keszei, Cs. Szalai, A. Falus, K. Kiss, J. Szeberényi, J. Szekeres-Barthó
American Journal of Reproductive Immunology, 2004;51:p:476-477

The Progesterone Induced Blocking Factor (PIBF): Intracellular signal transduction
N. Kozma, G. Pár, K. Kiss, J. Szeberényi, M. Keszei, Cs. Szalai, A. Falus, J. Szekeres-Barthó
Tissue Antigens, 2004;64:p:421-422

Genomic bases of progesterone-dependent immunomodulation
J. Szekeres-Bartho, B. Polgar, E. Nagy, E. Miko, N. Kozma, T. Palkovics, O. Papp and M. Halasz
Tissue Antigens, 2004;64:p:356-357