Ph.D thesis

Regulation of lymphocyte effector functions: The role of steroid membrane lipids and hormones

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Introduction

Due to the progress in biological and medical science, the functional and molecular details of human organ systems became increasingly better known. We can say: "*Diabolus in singulis est*" – i.e. the "devil is in the details". Sometimes, however, we should look back to a higher level, to understand global mechanisms. For example, due to the crosstalk between the immune- endocrine- and central nervous systems, the regulation of the immune response seems much more complex than we have ever thought.

It is widely accepted now that several autoimmune diseases are more prevalent in women than men. This focused the attention on the immuno-regulatory role of estrogen (β-estradiol, E2). There is a growing evidence for immunomodulatory effects of E2, but the mechanisms still remained highly unclear. Exploring the functional details, however, is essential for the safe use of oral contraceptives and hormone replacement therapies, considering the immunolgical effects of E2.

Aims of the study

1) Our group previously demonstrated that E2-BSA (bovine serum albumin covalently conjugated with E2; membrane-impermeable E2 ligand) binds to the membrane of lymphocytes and induces rapid, non-genomal signals in them. Thus, our primary goal was characterization of the membrane associated estrogen receptor(s) (mER), and the relation of classical ER forms to the plasma membrane.

2) Another aim was to search protein data bases for proteins with sex steroid binding motif. In addition we planned to include a hydropathy profile analysis to determine if the known classical ERs are capable to localize in the plasma membrane as a transmembrane protein, upon specialized folding.

3) To better understand the mER function, we examined the receptor dynamics (internalization/recycling). In estrus synchronized female we planned to investigate the relatuinship between serum E2 level and the expression profile of various E2 receptors.

4) Based on the literature, plasma membrane cholesterol may limit the passive E2 uptake of erythrocytes. Thus we also examined how the mER(s) relate to the cholesterol-rich lipid raft microdomains.

5) The different estrogen receptors probably mediate different signals to the cells, so the relative expression ratio of them may determine the outcome of immunoregulation on lymphocyte effector function. Therefore, we explored the expression pattern of the cytoplasmic- and membrane E2 receptors in lymphocyte subpopulations and to would like to see if E2 level influences their subcellular localization.

6) The effect of membrane permeable (E2) and membrane-impermeant (E2-BSA) on lymphocyte proliferation was also planned to be investigated. This could help to distinguish between the mER- and the cytoplasmic ER-mediated signals.

7) As a further detail, we also planned to investigate the role of E2 and E2-BSA on production of various types of Ig antibodies in an i*n vitro* system.

8) Finally, we investigated whether SHBG as a hormone transport protein may play any role in the effect of E2 on B or T lymphocytes.

Methods

- Isolation of thymocytes and splenocytes from mice, and seperation of their subpopulations by MACS and FACS
- Estrus synchronization with PMSG and HCG injections
- E2 and P4 analysis from murine faecal samples with RIA
- Flow- cytometry (FCM)
- Confocal laser-scanning microscopy (CLSM)
- Western blot
- ELISA
- T cell proliferation assay by detecting incorporation of radioactive [H^3] thymidine
- Photobleaching-based analysis of fluorescence resonance energy transfer (pbFRET)

Results

- We described the expressional pattern of ERα, ERβ, GPR30 and mER on T and B lymphocyte subpopulations.

- We have demonstrated plasma membrane associated forms of ERα és ERβ, supposedly localized together with mER in same membrane raft microdomains.

- We have shown that the mER is internalized relatively rapidly after ligand binding and targeted through the endolysomal pathway to the lysosomes. This process was shown to strongly depend on actin polymerization.

- Based on the measurements in estrus synchronized female mice, the expression and localization of the known estrogen receptors are strongly E2 dependent.

- The proliferation of lymphocytes was found to be differentially regulated by E2: by the mER (E2-BSA) positively and by the E2 negatively.

- The E2 receptors also mediated differential outcomes in the production of T-dependent IgG isotype antibodies. Our results suggested that the mER conveys in general positive signals to the cells.

- Finally, we could detect the expression of **megalin** and the presence of **SHBG** (sex hormone binding globulin) on both T and B cell lines and primary, isolated lymphoid cells. Considering also the pbFRET results, it is conceivable, that the SHBG may function as an **alternative mER**.

Discussion

In conclusion, we show here that estrogen has a complex, dynamic receptor network in lymphocytes consisting of several cytoplasmic or plasma membrane-associated (modified) forms of three receptor gene families, ERα, ERβ and GPER. These receptors can either translocate to the nucleus and act as transcription regulators or translocate to plasma membrane compartments, preferentially to lipid rafts, in lymphocytes and mediate rapid E2-signals. Our results suggest that the subcellular localization of ERs and GPER (GPR30) strongly depends on E2 signals mediated by mER, providing an evidence for the linkage between the genomial and the non-genomial E2-signal pathways. The estrogen-

driven regulation of lymphocyte functions is much more complex than we have thought up to now. Presumably the above mentioned receptor forms mediate different signals to the cells depending on their actual, cell- and estrogen level-dependent expression and subcellular localization. Exploring the functional details of this complex E2-receptor network, complemented with a new potential inward E2-shuttling pathway mediated by SHBG and its receptor, may help us to deeper understanding the mechanisms making estrogen a risk factor in various autoimmune diseases and can also help to work out safe strategies for using oral contraceptives and hormonal replacement therapies without 'malfunctions' in the immune system.

Publications related to the Ph.D thesis:

Mónika Ádori*, Endre Kiss*, Zsuzsanna Barad, Klaudia Barabás, Edda Kiszely, Andrea Schneider, Erna Sziksz, Dorottya Kövesdi, István M. Ábrahám, János Matkó, Gabriella Sármay. **Estrogen augments the T-cell-dependent but not the T-independent immune response *in vivo* and induces rapid non-classical effects on B- and T-Cells**. Cell Mol Life Sci, (2010) 67:1661–1674 (IF: 7.047)

Andrea E. Schneider, Éva Kárpáti, Kitti Schuszter, Eszter A. Tóth, Endre Kiss, Margit Kulcsár, Glória László, Janos Matko. **A dynamic network of estrogen receptors in murine**

lymphocytes: fine-tuning the immune response. Journal of Leukocyte Biology (2014) (in press) (IF: 4.568)

Other publications:

Máté Maus, David Medgyesi, Endre Kiss, Andrea E. Schneider, Ágnes Enyedi, Nóra Szilágyi, János Matkó, Gabriella Sárma. **B cell receptor-induced Ca2+-mobilization mediates F-actin rearrangements and is indispensable for adhesion and spreading of B lymphocytes** Journal of Leukocyte Biology (2013) Apr. 93(4):537-47. (IF: 4.568)

Conference abstracts and presentations:

E. Kiss, M. Ádori, Zs. Barad, K. Barabás, E. Kiszely, A. Schneider, D. Kövesdi, I. Ábrahám[2,4], G. Sármay[1,3], J. Matkó: „Estrogen regulates T-dependent humoral immune response and exerts rapid, non-genomic effects on B- and T lymphocytes." ***15th Symposium on Signals and Signal Processing in the Immune System (Balatonöszöd, 2009)***

Schneider Andrea, Kiss Endre, Ádori Mónika, Ábrahám István, Matkó János **Funkcionális membrán ösztrogén receptor Limfocitákon,** ***Magyar Immunológiai Társaság 39. Vándorgyűlése, 2010. november 3-5., Szeged***

Schneider Andrea, Kiss Endre, Ádori Mónika, Ábrahám István, Matkó János **Funkcionális membrán ösztrogén receptor Limfocitákon,** ***„Universitates Nostrea- Scientia Nostra", az ELTE fenállásának 375. évfordulója alkalmából az Eötvös Loránd Tudományegyetem és a Semmelweis Egyetem közös ünnepi ülése, 2010 november 18., Budapest***

Sármay Gabriella, Ádori Mónika, Kiss Endre, Barad Zsuzsanna, Barabás Klaudia, Kiszely Edda, Schneider Andrea, Sziksz Erna, Kövesdi Dorotty, Ábrahám István, Matkó János **Az ösztrogén**

fokozza a T-sejt függő, de nem befolyásolja a T-independens immunválaszt, és gyors nem-genomiális változásokat vált ki T- és Bsejtekben, ***Magyar Farmakológiai Anatómus Mikrocirkulációs Élettani Társaságok Közös Tudományos Konferenciája, 2011. június 8-11., Pécs***

Andrea Schneider, Kitti Schuszter, Endre Kiss, Glória László, János Matkó. **Membrane estrogen receptor(s) on lymphocytes: Who are they and can they regulate the immune responses?** ***Immun-related Phatologies: Understanding Leukocyte Signaling and Emerging therapies (1st IMPULSE), 2011. szeptember 3-6., Visegrád***

Schneider Andrea, Schuszter Kitti, László Glória, Matkó János, **Az ösztrogén szex hormon, mint immunmoduláns: funkcionális membrán receptor limfocitákon** ***Centrumban a tudás, TÁMOP konferencia az Eötvös Loránd Tudományegyetemen, 2011. november 24., Budapest***

E. Schneider Andrea, Schuszter Kitti, László Glória, Matkó János, **Limfociták membrán ösztrogén receptora: kapcsolat a genomiális és nem-genomiális útvonalak között,** ***41. Magyar Immunológiai Társaság vándorgyűlése, 2012. október 17-19., Debrecen***

Andrea E. Schneider, Éva Kárpáti, Kitti Schuszter, Glória László, János Matkó, **Membrane estrogen receptor(s) on lymphocytes: The linkage between the genomial and non genomial pathway,** ***Immun-related Phatologies: Understanding Leukocyte Signaling and Emerging therapies (2nd IMPULSE), 2013. augusztrus 31-szeptember 3, Mátraháza***