# PhD THESIS

# The role of mitochondrial receptor translocation and thymic epithelial cells in glucocorticoid induced thymocyte apoptosis


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## INTRODUCTION

Glucocorticoid hormones (GC-s) are secreted by the adrenal gland and have plethora of physiological functions. One of these is to influence the functions of the immune cells. Synthetic GC-analogues (Dexamethasone, Methylprednisolone) are frequently given in the clinical practice to treat autoimmune and other disorders. GC-s act via the intracellular glucocorticoid receptor (GR). Upon ligand binding, the GR initiates different signaling pathways, which can explain both the slowly developing classical genomic effects, and the rapid, alternative, non-genomic effects. GR is a member of a protein complex in the cytoplasm (important members of this complex are heat-shock- and immunophillin proteins). Upon ligand binding, the GR dissociates from this complex and translocates to the nucleus where it binds to well defined DNA-sequences, so called glucocorticoid response elements (GRE-s). GR acts here as a regulator of gene transcription (genomic effects) and these effects are usually detectable after several hours.

Studies from the past decade, however, revealed that besides classical effects GC-s can have non-genomic effects, too, which can be detected within minutes. **1.)** GC-s influence membrane-transport events and membrane permeability **2.)** A certain fraction of the GR is found in the cell membrane in GC-resistant cells, like B-cells and monocytes **3.)** GR associates with other cytoplasmic proteins (eg. in Jurkat T cells, GR is coupled with ZAP-70 kinase, a key molecule of T cell receptor-signalling).

According to the latest reports, in GC-sensitive cells, but not in GC-resistant cells, GR can translocate to the mitochondria and initiate the apoptotic cascade with an unknown mechanism. The mitochondrial GR might exert its effects via the following mechanisms: **1.)** regulation of the mitochondrial membrane potential **2.)** association with pro- and antiapoptotic proteins **3.**) binding to mitochondrial DNA and regulation of the mitochondrial gene transcription **4.)** besides nuclear receptors, transcription factors have been identified in the mitochondria, like NF-κB, AP-1, NFAT, but their exact role needs to be clarified.

T cells, together with B cells, are the main components of the adaptive immune system. Their antigen receptor, the T cell receptor (TcR), is a heterodimeric molecule composed of either α- and β- or γ- and δ-chains. T cells recognise the antigen in processed and presented form, the $CD4^+$ T cells via MHC II, while the $CD8^+$ T cells via the MHC I. T cell maturation begins in the bone marrow and is finished in the thymus. The mature T

cells relocate in the peripheral lymphoid organs to differentiate into effector cells and fulfil their function.

The thymus develops from the 3$^{rd}$ and 4$^{th}$ pharyngeal pouch on day 10 of the embryonic development. This developmental process is strictly regulated by the „forkhead box" transcription factor Foxn1. The expression of this molecule is induced by Wnt-4 glycyoprotein via unknown signalling steps. It is not yet known whether Foxn1 can play a role in the postnatal development of the thymus. By the end of day 14, the cortex-medulla compartmentalization in the thymus is completed, providing the necessary molecular and stromal environment for developing T cells. Further differentiation of the thymus epithelium and segregation of the thymic epithelial cell subsets is dependent on the expression changes of keratin (K) and any other genes. The EpCAM-molecule is expressed on the surface of all thymic epithelial cells (TEC), however, medullary epithelial cells (mTEC) express higher level of EpCAM, than cortical epithelial cells. cTECs coexpress the Ly51 molecule ($EpCAM^{+}Ly51^{+}$), whereas mTECs do not express this marker ($EpCAM^{++}Ly51^{-}$). mTECs can also be characterised as $K5^{+}K8^{-}$ and cTEC as $K5^{-}K8^{+}$ cells.

T cell development in the thymus is regulated by **1.)** direct cell-cell contacts between thymocytes and stromal cells (Notch molecule on thymocytes and its pair Jagged on TEC) and **2.)** soluble factors secreted by stromal cells – cytokines (IL-7), chemokines (CCL19, CCL25), members of the Wnt-glycoprotein family (especially Wnt-4) and different hormones (e. g. GC-s). The developmental steps can be classified to an **early** (proliferation of the double negative (DN) $CD4^{-}CD8^{-}$cells) and a **late** (selection of the double positive (DP) $CD4^{+}CD8^{+}$ cells and the egress of the mature cells to the periphery) stage.

DP cells in the cortex interact with the cTECs during <u>positive selection</u>. cTECs present a variety of peptides for DP cells bound to MHC. If DP cells fail to recognize TEC MHC molecules, they undergo apoptosis due to the lack of survival signal („death by neglect"). Cells recognizing self-MHC molecules survive (positive selection) and proceed to <u>negative selection</u> where autoreactive cells are eliminated by activation induced cell death (AICD). In the end, the phenotype of the thymus derived T cells is $CD4^{+}$ or $CD8^{+}$ single positive (SP). During negative selection mTECs present various not thymic tissue related antigens (TRA) to the thymocytes in certain combinations („promiscuous gene expression") to distinguish self and non-self in the periphery. This process is regulated by the transcription factor AIRE (autoimmune regulator) and plays an important role in shaping the central tolerance in the thymus.

It has been known for a long time, that GC-s induce thymus involution and apoptosis of peripheral T cells and thymocytes. According to recent reports, immunsuppressive agents (Cyclophosphamide, Cyclosporine A) and the GC-analogue Dexamethasone (DX) can block the function of the thymic epithelial cells, too. Thus, thymocyte apoptosis and deteriorated function of thymus epithelium can play an important role of GC-induced thymus involution. A number of studies is available about GC-induced thymocyte apoptosis, but the effect of GC-s on TEC remains to be elucidated.

Interestingly, among thymocyte subpopulations, DP-cells are the most sensitive to GC-induced apoptosis, while expressing the lowest level of GR protein and mRNA. oreover, GR downregulation is also defective in these cells. Thus, the background of this enhanced GC-sensitivity is still contraversial. According to our earlier work in a T cell receptor transgenic mice the GC-induced apoptosis of the DP-thymocytes could be detected within 4 hours (rapid GC effect). Importantly, in the surviving DP-cells, elevated level of the antiapoptotic Bcl-2 protein was found. However, the intracellular distribution of the GR and its trafficking during these processes was still unknown.

Plenty of biochemical events were identified in association with the GC-induced apoptosis in different cell types (also in thymocytes): such as $Ca^{++}$ mobilization, activation of the Src-and Cdk2-kinases, and production of ceramide following the activation of phophatidylinositol-specific phospholipase C and acidic sphyngomyelinase. Studies from our and other laboratories raised the possibility that alternative (rapid) GR-pathways and the Bcl-2 family are involved in this process, and the mitochondria might be key players in GC-induced apoptosis of the DP-thymocytes.

## AIMS

Based on the issues detailed in the Introduction our aims of were the following:
GR can mediate both genomic and non-genomic effects, therefore we wanted to study

**1.)** the morphology and intracellular distribution of the GR in thymocyte subpopulations

The results obtained by microscopy raised the possibility of the **mitochondrial** localization of the **GR** in thymocytes and its role in regulating apoptotic processes. Therefore, we wanted to study

**2.)** the ligand-induced mitochondrial translocation of the GR paying special attention to the GC-sensitive DP-cells.

**3.)** the effect of *in vitro* high dose GC treatment on mitochondrial function.

In the second part of our work, the main objective was to investigate the molecular mechanisms behind the **GC-induced loss-of-function of thymic epithelial cells**

We wanted to characterize

**4.)** the effect of *in vivo* DX-treatment on the organisation of thymic epithelial cells.

**5.)** the genes determining the thymic epithelial cell subtypes and identity, as well as genes influencing T cell development following DX treatment.

**6.)** the capability of DX-treated TEC to support T cell development in an *in vitro* T cell development model sytem.

## MATERIALS AND METHODS

### Mice

3-4 weeks old BALB/c mice were used for the experiments. The animal experiments were carried out in accordance with the regulations set out by the University's Committee on Animal Experimentations.

### Cell lines

Sp-2 mouse myeloma and RBL2H3 rat basophilic leukaemia cell lines were used in this study. The cells were cultured in DMEM supplemented with 10% FCS, in presence of penicilline (10 U/ml) and streptomycin (10 U/ml).

### Preparation of thymocytes and thymic epithelial cells

After the sacrifice of the animals, the thymi were removed, mechanically disrupted and the suspension was filtered through a nylon mesh. Cell viability was determined by trypan blue exclusion test.

DP-cells were selected by positive selection using Easysep anti-PE (StemCell Technologies) selection cocktail, after a dual labelling with a-CD4-FITC and a-CD8-PE antibodies.

Thymic epithelial cells ($EpCAM^+$-cells) were isolated by magnetic cell separation for RNA preparation after digestion of the thymi with collagenase. Following $CD45^+$ depletion of the resulted cell suspension, cells were selected with positive selection after an anti-FITC labelling (we labelled the cells with a-EpCAM-FITC).

### *In vivo* and *in vitro* Dexamethasone treatment

*In vivo,* mice were injected 20 mg/kg GC-analogue Dexamethasone (DX) (Organon, Oradexon) (dissolved in PBS) intraperitoneally, control animals received PBS. Animals were sacrified after 24 hours, 72 hours and one week following the single injection.

*In vitro*, the thymocytes and the cell lines were treated with a final concentration of 1 μM DX for 30 minutes in serum-free media, the reaction was stopped by ice-cold PBS-azide. Controll cells were treated with DMSO solvent (DX was dissolved in DMSO).

**Loading of the cells with CMX-Ros, cell surface and intracellular labellings**

For labelling mitochondria and for measuring the mitochondrial function CMX-Ros (chloromethyl-X-rosamine) was used with a concentration of 1 μg/ml. It is detectable in the red channel (with flow cytometry in FL2, ex. 579 nm, em: 599 nm). The loading was done parallel with the DX-treatment on 37ºC, for 30 minutes, in serum-free RPMI medium. The cell surface a-CD4 and a-CD8 labelling (conjugated with Pacific blue and Alexa fluor 647 fluorochromes for confocal microscopy or with FITC and CyC fluorochromes for flow cytometry) was performed on ice for 30 minutes, using labelling buffer (0.1 % BSA, 0.1 % $NaN_3$ in PBS). The cells were then washed with labelling buffer, and fixed with 4% PFA in PBS for 20 minutes. Following fixation, cells were incubated with a monoclonal anti-GR antibody (clone 5E4-B1, developed in our laboratory) in the same labelling buffer containing 0.1% saponine for intracellular staining. The cells were finally cytocentrifuged on slides or kept in FACSFix buffer (0,5% PFA in PBS) and analysed by flow cytometry. Flow cytometric analysis was performed using CellQuest software, 10.000 events were recorded, cellular debris and dead cells were excluded from the analysis based on their morphology. Thymocyte subpopulations were distinguished according to their CD4 and CD8 expression. The CMX-Ros fluorescence intensity (MFI) of the thymocyte subpopulations were depicted on histograms.

**Confocal microscopy**

Olympus Fluoview 300 confocal microscopy and Olympus Fluoview FV1000S-IX81 system was used for the experiments. Signals were collected in four different channels: DIC (differential interference contrast), CD4 in UV, GR in FITC (green), a CMX-Ros in red, CD8 in far red. Sequential scanning and Kalman setting was used to eliminate signal crosstalk between the channels. Signals were collected in 3-3 frames and morphological association (colocalisation) between GR and CMX-Ros was analysed.

**Image analysis, quantitative microscopy**

GR and CMX-Ros co-localisation was analysed in 100 control and 100 DX-treated DP-cells. For the analysis, ImageJ software (http://rsb.info.nih.gov/ij) was used with colocalisation plugin. The software displayed the GR – CMX-Ros co-localised pixels as white pixels on a separate image, which pixels were the counted.

**Subcellular fractionation**

Mitochondrial fractions were isolated using Mitochondria Isolation Kit (Pierce) following the manufacturer's instructions with minor modifications. Solvent- or DX-treated thymocytes were washed in ice-cold PBS, then the cells were lysed. After centrifugation with 800 g, the resulted nuclear fractions were washed with buffer containing 0.5% NP-40 in TBS, the pellets were resuspended in SDS-sample buffer, (125 mM Tris, 4% SDS, 10% glycerol, 0,006% bromo-phenol-blue) and boiled. The postnuclear supernatants were centrifuged once with 3000g for 15 minutes, and then with 12000g for 5 minutes. We used the supernatant after the 3000g centrifugation as a cytoplasmic fraction.

**Western-blot and densitometry**

The cell fractions were boiled and subjected to 7, 10 or 15% poliacrylamide gel electrophoresis. Gels were blotted overnight onto nitrocellulose membrane using a Bio-Rad Trans-Blot system. After blotting, the membranes were soaked with blocking buffer containing 2% BSA or 1% non-fat dry milk (10 mM Tris, 100 mM NaCl és 0.1% Tween 20, pH 7.4). The blots were probed with a-GR, anti-β-actin, anti-cytochrome c mouse monoclonal or a-histone-H1 rabbit policlonal antibodies, and incubated with the proper a-mouse HRPO or a-rabbit-HRPO secondary antibodies. The blots were analysed by densitometry, the GR relative densities were normalised to the actin, cytochrome C and histone H1 bands in the corresponding fractions. Densitometry was carried out using the BioRad Quantityone software package.

**Immunfluorescence on thymus sections**

Frozen tissue sections (5-10 μm thick) were prepared from the removed thymi, then fixed with acetone and blocked in PBS containing 5% BSA. Then sections were stained with the following antibodies: GR-FITC (5E4-B1) and EpCAM/a-rat-Cy3; EpCAM-FITC and Ly51-PE; EpCAM-FITC and MHC II/a-rat-PE, EpCAM-FITC coupled with policlonal goat Wnt-4/a-goat-PE combinations. Sections were analysed using an Olympus fluorescent microscope

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**Reaggregated thymic organ culture (RTOC)**

TECs were selected upon their lack of CD45 expression and EpCAM-positivity. DN, immature thymocytes ($CD3^-CD4^-CD8^-$) were isolated from untreated, young mice with a magnetic depletion using the antibody cocktail (CD3/CD4/CD8). TECs were mixed

with thymocytes in a 4:1 ratio and centrifuged down. After 1 week, CD4/CD8 expression profile of the thymocytes was analysed. To distinguish the thymocyte subpopulations in the RTOCs, a thymocyte suspension of young mice was also analysed for CD4/CD8 expression.

### Real-time PCR

RNA extraction was performed from the magnetically separated TECs using RNeasy mini kit (Qiagen), then DNase digestion was performed and cDNA was synthetised using „High Capacity RNA to cDNA Kit" (Applied Biosystems). Real-time PCR reaction was carried out using „Sybrgreen master mix" or „Taqman master mix". The primers are found in the table below.

| Gene | Forward primer | Reverse primer |
|---|---|---|
| **CD45** | 5'-CCG GAA TTC CGG ATG GGT TTG TGG CT-3' | 5'-CCG CTC GAG CGG CTA ATC ACT GGG TG-3' |
| **GR** | 5'-TGG TGT GCT CCG ATG A-3' | 5'-AGG GTA GGG GTA AGC-3' |
| **FoxN1** | Applied Biosystems TaqMan probe PN4351272 (Mm00477457_m1) | |
| **Keratin 5 (K5)** | 5'-CCCTCT GAA CCT GCA AAT CG-3' | 5'-TGA TCT GCT CCC TCT CCT CAG T-3' |
| **Keratin 8 (K8)** | 5'-TCA GCT ACG GAA TGA GCT CCT T-3' | 5'-GTG GTG CGG CTG AAA GTG T-3' |
| **Keratin 14 (K14)** | 5'-CCT CTG GCT CTC AGT CAT CCA-3' | 5'-TGC ACA TCC ATG ACC TTG GT -3' |
| **Keratin 18 (K18)** | 5'-CGC TTG GTG GAG GAT GGA-3' | 5'-TGC ACA GTT TGC ATG GAG TTG-3 |
| **CIITA** | 5'-CTA GCC AAG TCC CTC CTA AGG-3' | 5'-ATC TCA GAC TGA TCC TGG CAT-3' |
| **AIRE** | 5'-ACC TAA ACC AGT CCC GGA AAG-3' | 5'-CGA GGC TCC AGT GCT T-3' |
| **MHC I (H2D1)** | 5'-ACCT GCA GTT CGC CTA TGA AG-3' | 5'-CCG CCG TCC ACG TTT TC-3' |
| **IL-7** | 5'-ACT ACA CCC ACC TCC CGC A-3' | 5'-TCT CAG TAG TCT CTT TAG G-3' |
| **Wnt4** | 5'-CTC AAA GGC CTG ATC CAG AG-3' | 5'-TCA CAG CCA CAC TTC TCC AG-3' |
| **18S rRNA** | 5'-GGG TCG GGA GTG GGT AAT TT–3' | 5'-AGA AAC GGC TAC CAC ATC CAA–3' |
| **18S rRNA Taqman** | Applied Biosystems TaqMan probe 4352656-0701012 | |

### Statistical analysis

Data are presented as mean ± SD. The effect of various treatments between groups was tested for statistical significance using Student's t-test. $P < 0.05$ denoted statistical significance.

## RESULTS AND DISCUSSION

### Mitochondrial GR in DP-thymocytes

In our earlier work, we demonstrated (together with other groups) with flow cytometry and quantitative PCR measurements that DP-cells express the lowest level of the GR. However, the subcellular distribution of the receptor was still unknown in these cells, therefore, in this work we wished to clarify this first. Similar to our previous flow cytometric and real-time PCR results, with morphology, DP-cells expressed the lowest level of GR compared to immature DN and mature CD4 and CD8 SP cells, respecitvely. Besides the expression differences, we also found unique staining patterns. In DP-cells, the distribution of the GR showed a granular pattern, whereas in CD4 SP cells the pattern was rather homogenous. The granular pattern of the DP-cells raised the possibility of mitochondrial GR localization. When CMX-Ros (mitochondrial) – GR morphological association was analyzed in all four thymocyte subgroups, various levels of colocalisation could be verified in all cell types.

In DP-cells, the morphological assocation between CMX-Ros and GR was further analyzed and interestingly we found that in DP-cells, upon *in vitro* DX-treatment, GR mainly translocated to mitochondria but not to the nucleus. To confirm that GR in DP-cells did not translocate to the nucleus morphological (and methodological) control stainings were performed on Sp-2 myeloma-cells and RBL2H3-cells. In the Sp-2 cell line GR translocated to the mitochondria after 30 minutes high-dose DX-exposition. On the contrary, in the RBL2H3-cell line the GR translocated already after 10 minutes to the nucleus, which was completed after 30 minutes. Thus, our method was sufficient to study the GR subcellular distribution and trafficking.

To follow the mitochondrial GR translocation quantitatively, 100 control and 100 DX-treated DP-cells were randomly selected in different frames and the CMX-Ros – GR colocalised pixels were determined using the ImageJ colocalisation plugin. Interestingly, colocalisation was found also in control cells at a low level, which can be explained by a GC-rich microenvironment of the thymocytes in the thymus, which may result in mitochondrial preference of the GR *in vivo*. Another possible explanation is, that a certain fraction of the GR can be found in the mitochondria as previously demonstrated in some cell lines.

CMX-Ros can be used for flow cytometry as well, because the fluorescent intensity is proportional with the mitochondrial membrane potential. Combined with cell surface labelling, we determined the CMX-Ros mean fluorescence intensity in thymocyte subgroups, which was the lowest in DP-cells compared to DN, and CD4 and CD8 SP cells. As DP-cells are the most sensitive to GC-induced apoptosis, we studied the changes in CMX-Ros MFI in DP-cells following DX-treatment. 30 minutes of DX-treatment caused significant CMX-Ros MFI decrease in DP-cells which can be the indicator of the early mitochondrial function loss.

To confirm morphology with Western-blot, cytoplasmic, mitochondrial and nuclear fractions were isolated from bulk thymocytes. Subcellular fractionation was performed on bulk thymocytes (contains every thymocyte subgroups), and later on purified DP-cells. In unseparated thymocytes (Note: in 3-4 weeks old BALB/c mice approx. 80% of the thymocytes are DP), DX-treatment caused a significant decrease of GR-level in the cytoplasm, and a fraction became visible in the mitochondria and in the nucleus. The relatively strong nuclear GR-reactivity can be explained with the higher GR expression levels of the remaining thymocyte subpopulations, which can significantly contribute to nuclear reactivity. When we repeated the subcellular fractionation on magnetically purified DP-cells GR translocated mainly to the mitochondria, and not the nucleus.

It is tempting to speculate, that the GR can initiate the apoptotic cascade by interacting with proapoptotic protein(s) or coupling with different heat-shock proteins after released from the cytoplasmic Hsp-90 and translocating to the mitochondria. To verify this hypothesis, we performed immunoprecipitation with Bax and Bak, as well as Hsp-60 antibodies from the mitochondria of DX-treated purified DP cells. According to our preliminary results, the GR associates with Hsp-60 and Bak in the mitochondria of DP-cells but this needs further confirmation.

**The *in vivo* effects of GC-s on thymus epithelium**

TECs play an important role in T cell development, but little is known about GC-induced thymic epithelial function alterations so far. According to a recent report, high dosage DX-treatment significantly decreases the absolute cell number of both cortical and medullary epithelial cells. Therefore, our aim was to identify the molecules, which govern this process. GC-s exert their effects mostly via the GR, therefore we raised the question, whether the thymic epithelium expressed GR. GR contributes to the homeostasis of the epithelial compartments in many organs, like skin and lungs. The GR expression of the TEC was confirmed by both PCR and immunfluorescence methods.

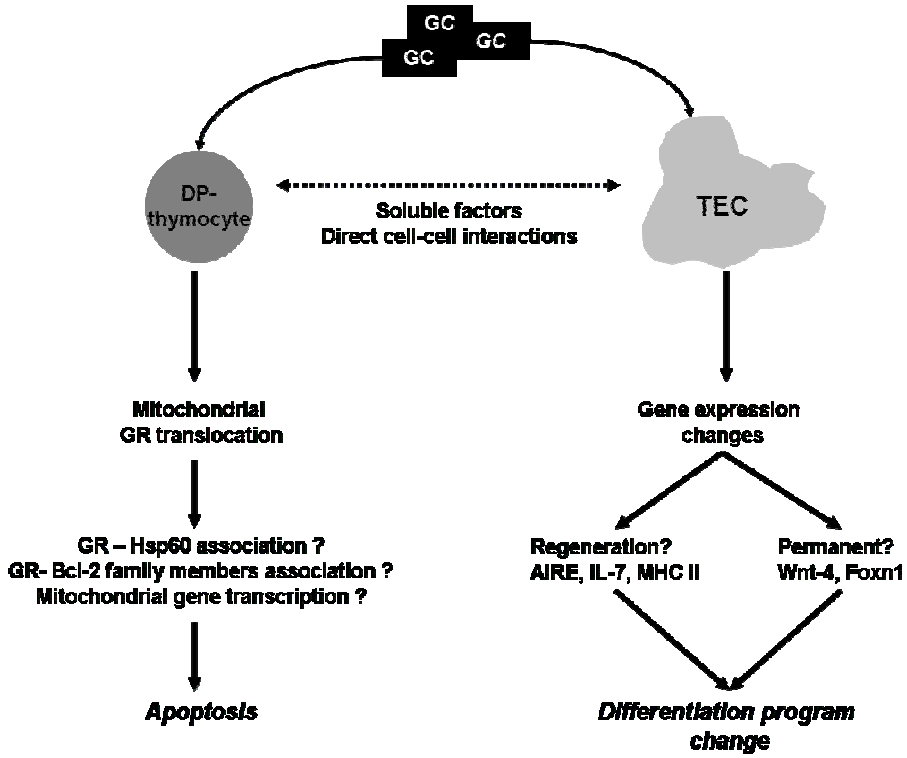
Using immunfluorescence, we showed that TEC lose EpCAM and Ly51 expression after 24 hours of a single DX injection. The expression pattern of these molecules morphologically showed partial regeneration after 72 hours and it was complete after 168 hours of the DX-treatment. Hereafter, we studied the morphology of the most important proteins of TECs, which influence T cell development. The presence of MHC II is important in forming a competent T cell repertoire; therefore we studied its expression changes with immunofluorescence. The loss of MHC II expression was detected after 24 hours of DX-treatment on thymic epithelial cells (EpCAM-co-staining). Among Wnt molecules, Wnt-4 is the most representative in the thymus and it is important for maintaining thymus cellularity, therefore, in our present work we studied its level with immunofluorescence. Similar to MHC II, we found a loss of Wnt-4 expression on TECs in combination with EpCAM.

Together with these morphological differences, real time PCR analysis revealed that in purified TEC, DX induced the loss of Foxn1 expression which did not return to the starting level after 168 hours of DX treatment. Furthermore reduced keratin (K) 5, 8, 14, 18 (main markers of thymic epithelial cells) mRNA levels were measured 24 hours after DX treatment. The decreased mRNA level of these molecules refers to a DX-induced, altered differentiation program and phenotype change of TECs, because the expression of Foxn1 and keratins is the indicator of the TEC identity. Next, we studied the expression levels of important T cell development related molecules in the thymus epithelium. AIRE (autoimmune regulator), MHC I (H2D1), and CIITA (class II transcactivator) molecules have an important role in preventing the export of autoreactive thymocytes from the thymus. The expression level of AIRE, H2D1, CIITA and IL-7 were found to be decreased

24 hours after DX-treatment in TEC, but their mRNA level returned to normal after 1 week. The Foxn1 transcription factor is crucial for the thymus development and is expressed by all subsets of TECs. The mRNA level changes of Wnt-4 strongly correlated to the expression pattern of Foxn1 and it followed closely the immunofluorescence measurements, and did not show recovery after one week.

After having studied the moleculer components of the T cell development, we wished to study whether the function of the TECs became impaired following DX-treatment. Our question was: are DX-treated TEC capable to support T cell development *in vitro*? To test this, we used reaggregate thymic organ cultures (RTOC) and put together 200 thousand TECs and 50 thousand thymocytes and incubated them for one week *in vitro*. After one week, cultures were disrupted, thymocytes were isolated and labelled for CD4 and CD8 to study the composition of the thymocyte subgroups. DX-treated TEC failed to generate SP-cells by the end of the experiments only DN-cells were detectable, whereas in control cultures the thymocyte differentiation was similar to a normal thymus. To evaluate thymocyte subgroups in the RTOCs correctly, we isolated control thymocytes from young mice and performed CD4-CD8 dual labelling.

In sum, we established that high-dose GC can act at multiple levels in the thymus: it induces massive apoptosis in DP-thymoyctes (most likely via non-genomic mitochondrial GR signaling) and inhibits TEC function (probably by inhibiting the Wnt4-Foxn1 axis). The molecular changes in both DP-cells and TECs following high dosage GC-treatment are summarised in the **figure below**. According to this, the network of molecular interactions and fine signalling pathways induced by GC-s is becoming more complex between TECs and thymocytes. However, GC-induced changes in both TECs and T cell precursors have radical temporo-spatial consequences in the thymus.


GC
GC
GC
DP-
thymocyte
Soluble factors
Direct cell-cell interactions
TEC
Mitochondrial
GR translocation
GR – Hsp60 association ?
GR- Bcl-2 family members association ?
Mitochondrial gene transcription ?
Apoptosis
Gene expression
changes
Regeneration?
AIRE, IL-7, MHC II
Permanent?
Wnt-4, Foxn1
Differentiation program
change

**The summary of new results:**

1. We characterised the intracellular distribution of the GR in thymocyte subpopulations and confirmed our flow cytometric and qPCR data, furthermore, we gained new information about the intracellular localization of the GR.
2. We proved that upon ligand-binding GR translocates to the mitochondria and not to the nucleus in DP-thymocytes, this phenomenon was demonstrated both by confocal microscopy and subcellular fractionation coupled with Western blot.
3. We could observe a certain GR fraction in the mitochondria of DP-cells.
4. We found that DP-cells possess the lowest level of basal mitochondrial membrane potential among the thymocyte subpopulations.
5. According to our results, high dose, short DX-treatment leads to decrease of the mitochondrial function.
6. We showed that thymic epithelial cells express GR at both mRNA and protein level.
7. High dose GC-treatment *in vivo* negatively regulates the function of TEC and induces the degeneration of cortex-medulla organisation.
8. High dose DX-treatment in vivo induced the decrease of Foxn1 and keratin mRNA levels in TEC, which refers to an altered differentiation program following pharmacological dosage of GC-s.
9. Furthermore, upon DX-treatment, TECs show decreased mRNA expression of molecules strongly related to T cell development (AIRE, CIITA, MHC I, IL-7).
10. Using an *in vitro* T cell development model (RTOC), we showed TEC isolated from DX-treated mice fail to support T cell differentiation.

**PUBLICATIONS:**

**Publications related to the thesis (cumulative impact factor: 10,453):**

1. **Gergely Talabér,** Ferenc Boldizsár, Domokos Bartis, László Pálinkás, Mariann Szabó, Gergely Berta, György Sétáló jr., Péter Németh, Timea Berki: *Mitochondrial translocation of the glucocorticoid receptor in double-positive thymocytes correlates with their sensitivity to glucocorticoid-induced apoptosis.* Int Immunol. 2009 Nov 21 (11): 1269-1276. [IF: 3,403]

2. Ferenc Boldizsár, **Gergely Talabér**, Mariann Szabó, Domokos Bartis, László Pálinkás, Péter Németh, Timea Berki: *Emerging pathways of non-genomic glucocorticoid (GC) signalling in T cells.* Immunobiology 2010 Jul; 215(7):521-6. [IF: 3,586*]

3. László Pálinkás, **Gergely Talabér**, Ferenc Boldizsár, Domokos Bartis, Péter Németh, Timea Berki: *Developmental shift in TcR-mediated rescue of thymocytes from glucocorticoid-induced apoptosis.* Immunobiology 2008; 213 (1): 39-50. [IF: 3,464]

4. **Gergely Talabér**, Krisztián Kvell, Zoltán Varecza, Eric J. Jenkinson, Graham Anderson, Ferenc Boldizsár, Péter Németh, Timea Berki, Judit E. Pongrácz: *Wnt-4 down-regulation is a characteristic feature of Dexamethasone (DX) induced reversible thymic epithelial depletion*. (submitted for publication, Mol. Immunol.)

*According to the latest (2009) impact factors.

**Citable abstracts (first authored): 4 (1)**
**Lectures on international conferences (first authored): 3 (2)**
**Lectures on hungarian conferences (first authored): 3 (1)**
**Posters on international conferences (first authored): 9 (7)**
**Posters on hungarian conferences (first authored): 10 (5)**