ZAP-70: A NEWLY IDENTIFIED JUNCTION BETWEEN T-CELL RECEPTOR AND GLUCOCORTICOID SIGNALLING PATHWAYS.

PHD THESIS

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Summary

Glucocorticoids (GC) play essential role in the regulation of the maturation and activation processes in T-cells, regarding especially apoptosis mechanisms. The mutual antagonism between GC signals and T-cell receptor (TcR) derived signals concerned to be crucial in thymocyte selection. The non-genomic actions of GCs are less characterised yet, but based on literature data the existence of cross-talk mechanisms between GC and TcR signal transduction pathways are feasible.
We aimed in our work to reveal rapid, non-genomic GC signal transduction mechanisms in T-cells using *in vitro* cultured Jurkat T-cell leukaemia as a model cell line.
Our results show that glucocorticoid receptor (GR) agonist Dexamethasone (DX) causes changes in tyrosine phosphorylation pattern of resting and activated Jurkat cells. These GC effects occurred rapidly, within 5 minutes.
ZAP-70 kinase has a crucial role in signal transduction originating from the TcR-CD3 complex. ZAP-70 deficient T-cells fail to respond antigenic stimuli *in vitro,* and ZAP-70 deficiency in humans and mice results in Severe Combined Immunodeficiency (SCID syndrome). We demonstrate that DX treatment induces rapid ZAP-70 phosphorylation in both resting and activated Jurkat cells. DX induced ZAP-70 phosphorylation is inhibited by GR antagonist (RU486) pre-treatment suggesting the process is GR dependent. However, DX fails to trigger ZAP-70 phosphorylation in p56-lck deficient JCaM-1 cells proposing the involvement of the upstream src-family kinase p56-lck. We investigated the close physical relation of GR and ZAP-70 by co-immunoprecipitation and confocal microscopy. We show, that the ligand-bound GR associates with ZAP-70 in both Jurkat cells and HeLa/trZAP-70 cells stably expressing transgenic ZAP-70. Examining the role of Hsp-90 we found, that a presumably inactive ZAP-70 fraction is associated with Hsp-90 which is most likely excluded from this signal transduction process.
The association of ZAP-70 with phosphorylated ITAM tyrosines of the CD3 complex is indispensable for the appropriate transmission of signals derived from the TcR. Our results demonstrate that the association of ZAP-70 with the CD3 complex is inhibited in the DX treated samples.
Based on our experimental data we suggest the following signal transduction model: GR, in the presence of its ligand, associates with ZAP-70, triggering tyrosine phosphorylation events. The alteration of tyrosine phosphorylation may influence the kinase activity of ZAP-70 or affect the clustering of other signal transduction molecules with ZAP-70. The GR association with ZAP-70 also inhibits its binding to the ITAMs of the CD3 complex.
Recently GCs are widely used as immunosuppressive drugs although their side-effects are often serious. We revealed here a new non-genomic signal transduction mechanism which may have a crucial role in GC mediated immunosuppression. The characterisation of this new non-genomic pathway may make possible the design and synthesis of new molecules which targets exclusively this immunosuppressive process. In the future this may contribute to the application of new immunosuppressive agents with more favourable side-effect spectra.

Introduction

1.1 Therapeutic application of glucocorticoids

The glucocorticoid hormone (GC) and its derivates are widely used for various therapeutic purposes for a considerably long time. The application spectra of GCs include not only acute and chronic rheumatologic inflammations, asthmatic and allergic conditions, haematological malignancies, but they are also used for immunosuppressive treatment after tissue and organ transplantation. However, in spite of the really wide therapeutic applications of GC hormone derivatives, the exact mechanism of action remained obscure regarding many aspects.

1.2 Structure of the glucocorticoid receptor

GCs exert their effects mainly via the glucocorticoid receptor (GR). GR belongs to the nuclear receptor superfamily. There are multiple steroid hormones acting on receptors of similar structure and other, structurally homologous proteins are known as “orphan receptors” their ligands being unknown.
GR is an evolutionary conserved transcription factor. The alternative splicing of human GR (hGR) mRNA results in the expression of two isoforms: hGRα and hGRβ. The α isoform is the “classical” 777 amino acid protein expressed almost ubiquitously among tissues. The hGRβ isoform is formed via the alternative splicing of exon 9. It differs only in its C-terminal region from hGRβ containing a unique sequence of 15 amino acids. The function of hGRβ is controversial: it does not bind ligand and localised exclusively in the nucleus. Some studies demonstrate the role of hGRβ in pathological GC resistance cases. It may take part in the fine tuning of GR-regulated gene expression.

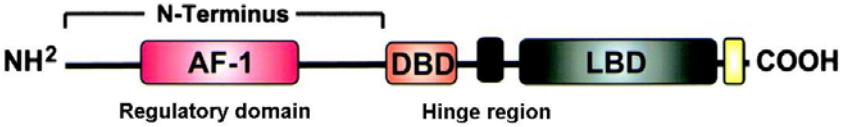

Figure 1.: *The GR structure depicted on ribbon diagram.*

Functionally GR is built up from 4 different domains. The N-terminal domain is the regulatory domain (amino acid residues 1-421). This region is responsible for the constitutive transcription activation function. The DNA binding domain (DBD, amino acid residues 422-486) is the evolutionary most conserved part of the molecule possessing a zinc-finger structure. This part of the molecule binds to the specific palindrome DNA sequences called glucocorticoid response elements (GRE) thus regulating gene transcription. A short hinge region (amino acid residues 487-526) connects the ligand binding domain (LBD, amino acid residues 527-777) which is also responsible for the homodimer formation after ligand binding.

1.3 Genomic GC signal transduction

GCs, being lipophilic molecules, diffuse freely through the cytoplasmic membrane into the cell and bind to inactive GR. The GR, after ligand binding, actively translocates into the nucleus and associates with special regulatory DNA sequences. The ligand-bound GR acts as a transcription factor enhancing or repressing the transcription of certain genes. This is called DNA-dependent regulation. There are also DNA-independent mechanisms of gene expression regulation: in this case GR interacts physically with other transcription factors modifying their activity. In case of GR there are examples of both regulation type.

1.4 Molecular mechanisms of genomic signal transduction mediated by GR

Inactive GR is associated with other proteins (e.g. Hsp-90) in the cytoplasm as a member of a multimeric protein complex. Conformation changes after ligand binding result in dissociation the Hsp-90 complex and migration in the nucleus as a homodimer. Ligand-bound GR in the nucleus acts as a transcription factor: it binds to GRE (glucocorticoid response elements, which are specific, palindrome DNA sequences), enhancing the expression of certain genes. However, GR may exert negative regulation function when binding negative regulatory GREs (nGREs). Hitherto nGREs are less well characterised. Transcription regulation may be achieved also via communication with other transcription factors. In this case GR interacts directly with other transcription factors, modulating their activity. Many transcription factors are known of having physical contact with GR, e.g. NFκB, AP-1, CREB and several STAT family transcription factors. Common characteristic of the previously mentioned genomic signal transduction pathways are that the result is a change of the protein expression pattern in the GC sensitive cells or tissues. Genomic effects always need considerable time (usually a few hours) to manifest.

1.5 Non-genomic effects of GC hormone

Common feature of the non-genomic steroid hormone effects is that no *de novo* protein synthesis is necessary. Non-genomic effects are mainly characterised by post-translational modifications of already existing signal transduction molecules. These reactions may occur considerably fast, as they show within seconds or minutes. The non-genomic GC effects are divided into 3 groups: (1) specific GC effects accomplished through the classical cytoplasmic GR (cGR). These cytoplasmic effects are mainly exerted via GR interactions with other signalling proteins. (2) Specific GC effects involving the putative membrane resident GR (mGR) and (3) non-specific GC effects via physicochemical membrane interactions.

1.6 Importance of communication between GC and TcR signal transduction pathways

GC hormones exert very important regulatory role in T-cells, regarding both thymocyte maturation processes and peripheral T-cell functions. Epithelial cells in the thymus secrete GCs thus being capable of the paracrine regulation of thymocyte maturation. According to the “mutual antagonism theory” GCs together with the signal originating from TcR induce positive selection of thymocytes. GCs presumably play a key role in the apoptosis of the thymocyte clones expressing non-responding TcR. GCs also cause Th2 shift in the cytokine secretion of mature peripheral T-cells and exert profound inhibition effects via multiple mechanisms on the synthesis of various pro-inflammatory cytokines, e.g. IL-1β, IL-2, IL-6 and TNFα. Taken together, the anti-inflammatory effects and the mutual antagonism model strongly suggests the existence of communication mechanisms between the GC and TcR signal transduction pathways.

1.7 TcR-originating signal transduction pathways

Seconds after the TcR engaged the antigen-primed MHC, tyrosine phosphorylation events occur in T-cells. This activation signal initiates a cascade of biochemical alterations which cause a profound change in the metabolism of T-cells. Within 2 hours, expression of cytokine receptors occur (the most characteristic is the IL-2R), and secretion of various cytokines begins in the first 2-6 hours. DNA replication is initiated within 24 hours followed by cell division within 48 hours. Optimal signal transduction needs the participation of co-receptors, such as CD4 and CD8. These co-receptors are required for the stabilization of the intercellular connection and they also participate in signal transduction. Non-receptor tyrosine kinases of the Src-family are attached to the intracellular part of CD4 and CD8 co-receptors, mediating tyrosine phosphorylation events. After the antigen binding of the TcR and the MHC-binding of the co-receptors mainly CD3 ζ (zeta) chains are phosphorylated on tyrosine, mediated primarily by p56-lck, a src-family kinase. The phosphorylated tyrosine residues of the ζ chains serve as docking sites for ZAP-70 kinase (Zeta-chain associated protein of 70 kilodaltons)

1.8 Role of ZAP-70 kinase in TcR-originating signal transduction

ZAP-70 is a non-receptor tyrosine kinase of the Syk-family. It is expressed only in T- and NK cells, its role is fulfilled in B cells, neutrophils, eosinophils and mast cells by Syk kinase, showing high structural and functional homology to ZAP-70. Regarding its primary structure, ZAP-70 can be divided to a C-terminal kinase domain, two tandem SH2 domains and interdomain A and B regions.

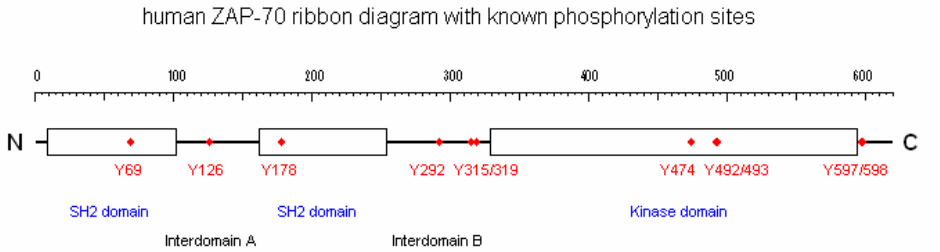

Figure 2. *Primary structure of ZAP-70 showing known tyrosine phosphorylation sites*

Main role of ZAP-70 is the augmentation of TcR-derived signal. ZAP-70 plays an indispensable role in TcR-CD3 signalling. In ZAP-70 deficient cells no Ca^{2+} signal is detected after TcR activation or CD3 cross-linking. Human ZAP-70 deficiency results in SCID syndrome (Severe Combined Immunodeficiency) characterised by the complete lack of cytotoxic T cells and immunological unresponsiveness of peripheral T helper cells.

The activity regulation of non-receptor tyrosine kinases occur mainly via phosphorylation. There are 30 tyrosine residues in ZAP-70 and 11 of them are known phosphorylation sites. The phosphorylation of some tyrosine residues directly influence the kinase activity (either positive or negative) while others provide docking sites for other signal transduction proteins which can also modulate ZAP-70 function.

2 Objectives

1. Studying the rapid non-genomic GC effects on T-cells using an *in vitro* cultured model cell line (Jurkat). Definition of the alterations in the tyrosine phosphorylation pattern in Jurkat cells after high dose Dexamethasone (DX, GR agonist) treatment.

2. Characterisation of the rapid changes in the phosphotyrosine content of ZAP-70 kinase after high dose DX treatment.

3. Determination of the upstream kinase responsible for DX induced phosphorylation of ZAP-70. Examination the role of GR in the process.

4. Studying the kinetics of the DX induced ZAP-70 phosphorylation.

5. Examination the possible physical linkage between GR and ZAP-70 using co-immunoprecipitation and confocal microscopy in the presence and absence of GR agonist in Jurkat cells.

6. Clarification of the relationship of Hsp-90 chaperone towards the GR and ZAP-70.

7. Studying the relation of GR, ZAP-70 and Hsp-90 in a non-T cell environment using HeLa cells stably transfected with ZAP-70.

8. Investigation of the crosstalk between the TcR-CD3 complex and GR-related signal transduction pathways.

3 Experimental procedures

3.1 Cell lines

For studying rapid GC effects in vitro, we used Jurkat cells (human acute T-cell leukaemia cell line) and its p56-lck deficient subclone (JCaM1.6). For some experiments, we transfected HeLa cells with a lentiviral vector carrying full-length ZAP-70.

3.2 Lentivirus production and transduction

To examine the ZAP-70 association with GR in non-T-cells, we transfected HeLa human epithelial carcinoma cells using a lentiviral vector containing human full-length ZAP-70 cDNA. The transfer plasmid (pWPTS with EF1-ZAP-70), packaging plasmid and envelope construction were transiently co-transfected by calcium-phosphate method into 293T cells. The supernatant of the virus producing cells was used to transfect HeLa cells by spinoculation. The expression level of transgenic ZAP-70 was controlled by flow cytometry and western blotting.

3.3 Geldanamycin, GR agonist/antagonist treatment

Cells were plated and incubated for 4 hours at 37^{o}C in the presence or absence of RU486 (Mifepristone) or 1,78µM Geldanamycin. After a washing step, Dexamethasone (DX) or solvent (DMSO) were added at a concentration of 10µM for 5 minutes at 37^{o}C. The treatment was stopped by placing the tubes into liquid nitrogen. In case of confocal microscopy samples DX treatment was stopped by addition of 9 volumes of ice-cold PBS supplemented with 0.1% sodium-azide.

3.4 Cell activation and lysis

OKT-3 anti-CD3 monoclonal antibody was added to the DX treated or untreated samples. Following incubation for the indicated time at 37^{o}C the activation was stopped by quickly freezing the cells in liquid nitrogen. The samples were lysed for 30 minutes in 500 µl ice-cold TX-100 or TEM lysis buffer freshly supplemented with protease inhibitor coctail and sodium orthovanadate. When TEM buffer was used for co-immunoprecipitation experiments, an additional sonication step was included.

3.5 Immunoprecipitation and Western blot

Equal amounts of cell lysates were incubated on a rotator platform at 4^{o}C with Protein G coupled Sepharose beads for 30 minutes. After the pre-clearing step, 10 µl precipitating antibody was added to the pre-cleared lysates for 2 hours. Immunocomplexes were fixed on Protein-G Sepharose beads. After extensive washing, beads were resuspended in 100 µl of SDS sample buffer and the immunoprecipitates were boiled for 10 minutes. The supernatants were collected and loaded on SDS-polyacrylamide gels. SDS-PAGE was performed according to Laemmli. Western blotting was carried out using standard protocols. For Western blot visualization chemiluminescent substrate was used, and the signal was recorded using X-ray film.

3.6 Confocal microscopy

Dexamethasone treated or untreated Jurkat cells were fixed in 4% PFA, then permeabilized using saponine buffer. The fluorochrome labelled monoclonal antibodies or the appropriate negative control antibodies were added at a concentration of 1 µg/ml. After one hour incubation on ice the cells were washed and carefully layered onto slides. After the cells settled the remaining fluid was carefully aspirated and the slides were covered using 50 % glycerol-PBS. In case of HeLa cells the labeling procedure was carried out on monolayers grown on coverslips. An Olympus Fluoview 300 confocal microscope or later a Olympus Fluoview FV1000S-IX81 system was used for the examination of the samples.

4 Results

4.1 Dexamethasone alters the tyrosine phosphorylation pattern of Jurkat cells

5 minutes of 10μM DX treatment alone caused increased tyrosine - phosphorylation of numerous proteins in whole Jurkat cell lysates, compared to the solvent-treated control. Activation with monoclonal anti-CD3 antibody markedly increased the tyrosine-phosphorylation of several proteins. Compared to activated cell lysates, 5 minute DX pre-treatment at 10 μM concentration inhibited the anti-CD3 induced tyrosine-phosphorylation of severeal phosphoproteins.

4.2 Dexamethasone rapidly induces ZAP-70 phosphorylation

We investigated the tyrosine-phosphorylation of ZAP-70 after DX and/or anti-CD3 treatments. 5 minutes exposure to 10 μM DX caused an average of 4 fold increase in tyrosine-phosphorylation of the ZAP-70 kinase in the anti-ZAP-70 precipitated samples. Anti-CD3 treatment caused similar increase. The tyrosine-phosphorylation after combined DX + anti-CD3 treatment was higher than in case of anti-CD3 or DX treatment alone. Phosphorylation increase after single DX or anti-CD3 or combined treatments proved significant compared to the solvent treated control sample (Student's t-test, $P < 0.05$). We observed higher phosphorylation after combined DX + anti-CD3 treatment than single DX or anti-CD3 treatment in all of our independent experiments, but this further rise was statistically not significant by Student's t-test.

4.3 Dexamethasone induced ZAP-70 phosphorylation is p56-lck and GR dependent

In addition to acting as a tyrosine kinase, ZAP-70 is a substrate of p56-lck, a lymphocyte specific src-family tyrosine kinase. When JCaM1.6, a p56-lck deficient subclone of Jurkat cells was subjected to high dose DX treatment, we observed no rise in the degree of tyrosine-phosphorylation of ZAP-70 kinase.
We also investigated, whether DX induced tyrosine-phosphorylation alterations were GR dependent in Jurkat T-cells using RU486 (Mifepristone), a glucocorticoid receptor antagonist. Pre-treatment of Jurkat cells for 4 hours with equimolar RU486 prevented rapid phosphorylation of ZAP-70 induced by subsequent DX exposure. RU 486 treatment alone caused no remarkable tyrosine-phosphorylation change in ZAP-70.

4.4 Time kinetics of Dexamethasone induced ZAP-70 phosphorylation

Since the kinetics of tyrosine-phosphorylation is a key feature to understand cellular signalling events, we further investigated the time course of DX induced ZAP-70 tyrosine-phosphorylation. The tyrosine-phosphorylation of ZAP-70 occurs rapidly after the addition of high dose DX. We measured a rise in tyrosine-phosphorylation after 1 and 2 minutes after DX administration, respectively. Dephosphorylation of ZAP-70 occurred after 5 minutes.

4.5 Upon ligand binding GR associates with ZAP-70 kinase

Since previously we have found that sort term high dose DX treatment increased the phosphotyrosine content of the ZAP-70 molecule, we sought a possible association between the ZAP-70 and the GR molecules in Jurkat cells. We performed immunoprecipitation on DX or vehicle treated Jurkat cell lysates, both with anti-ZAP-70 and anti-GR antibodies, to investigate whether there is a physical link between GR and ZAP-70 in the cytoplasm. The results of both experiments were similar: DX induced the association of GR and ZAP-70 compared to the vehicle–treated control samples. To further characterise the localization and relation of the ZAP-70 and GR molecules in the cytoplasm, we visualised the two molecules parallel by confocal microscopy. In vehicle treated, resting Jurkat cells both ZAP-70 and GR showed even, mostly cytoplasmic distribution, with almost no co-localization. Upon 5 min high dose DX treatment we found near-membrane co-localisation of GR and ZAP-70 molecules in Jurkat cells.

4.6 A fraction of ZAP-70 is associated with Hsp-90 in the cytoplasm

Since the unliganded GR complexes Hsp-90 in the cytoplasm and the upstream kinase p56-lck is also an Hsp-90 client protein, we aimed to check the relation of ZAP-70 to Hsp-90. We found that Hsp-90 co-precipitated with ZAP-70 from Jurkat cell lysates. Hsp-90 and ZAP-70 co-precipitation was not influenced by the presence or absence of DX. To further elucidate the relations of ZAP-70, GR and Hsp-90 in Jurkat cells we used geldanamycin (GA) to specifically inhibit Hsp-90. We performed immunoprecipitation with anti-ZAP-70 antibody. Our results showed, that Hsp-90 failed to co-precipitate with ZAP-70 in the GA treated samples. Although GA inhibited the co-precipitation of ZAP-70 with Hsp-90, it could not abrogate the DX induced GR-ZAP-70 association. Therefore we conclude that the ZAP-70-GR association is Hsp-90 independent.

4.7 Studying the relation of ZAP-70, GR and Hsp-90 in transgenic HeLa cells stably expressing ZAP-70

We aimed to clarify the molecular relations between ZAP-70, GR and Hsp-90 in non-T-cells. ZAP-70 is T- and NK-cell specific molecule, while GR and Hsp-90 is ubiquitously expressed. We prepared for these experiments transgenic HeLa cells stably expressing ZAP-70 (HeLa-trZAP-70) using a lentiviral vector. We performed co-immunoprecipitation and confocal microscopy similarly to Jurkat cells to examine the molecular relations in transgenic cells. We found that DX treatment induces the association of GR and ZAP-70 in HeLa-trZAP-70 cells which is similar that of we found in Jurkat cells. Interestingly, the co-localization of GR and ZAP-70 in transgenic HeLa cells showed perinuclear pattern upon DX treatment, while in Jurkat cells the two molecules clustered underneath the cell membrane. We found that a fraction of ZAP-70 associates in HeLa-trZAP-70 cells with Hsp-90 independent of the presence of DX.

4.8 High dose DX treatment rapidly abrogates ZAP-70 association with the CD3 complex in Jurkat T-cells.

ZAP-70 plays a key role in the signal transduction pathways originating from the TcR-CD3 complex. T-cell activation caused by either TcR engaged by peptide primed MHC or cross-linking with anti-CD3 antibody triggers tyrosine phosphorylation events. After ZAP-70 molecules becomes phosphorylated (autophosphorylation and src-family kinase mediated phosphorylation) associate with the ITAM-tyrosines by their tandem SH2 domains. This step is crucial considering further activation events. Our results demonstrate that ZAP-70 association with the CD3 complex in anti-CD3 activated Jurkat cells is abrogated in the presence of high dose DX, as it failed to co-precipitate in the DX treated samples.

5 Discussion

Considering our results summarized above and the referring literature data we suggest that the p56-lck - ZAP-70 hierarchy might represent a new, hitherto unknown regulatory mechanism of GC and TcR signal transduction pathways. We published first that GC treatment causes rapid tyrosine phosphorylation changes of several molecules in T-cells. Antigen binding induced TcR signalling has different influence on T-cells during their different developmental stages. GCs are thought to play primary role in the elimination of thymocytes bearing non-responsive TcR, while clones with high-affinity TcR - being potentially autoreactive - are deleted via activation-induced apoptosis. According to the “mutual antagonism theory”, thymocytes receiving simultaneous signals from both TcR and GCs survive, because the two apoptotic signals are mutually antagonistic. Regarding mature, peripheral T-cells, TcR-activation together with the proper co-stimulatory signals causes T-cell activation, resulting in IL-2 secretion and clonal proliferation. GC hormones antagonize this effect, making them useful drugs in immunosuppressive and anti-inflammatory therapy.

Hitherto multiple communication points are known on GC and TcR signal transduction pathways, but all of them concerned the genomic signal transduction pathway. These genomic effects need considerable time (typically a few hours) to manifest and require *de novo* protein synthesis. In contrast to that, non-genomic effects in our studies occur within minutes via post-translational modifications (namely tyrosine-phosphorylation) of already existing signal transduction molecules. Our result, that GC treatment induces rapid ZAP-70 tyrosine phosphorylation suggests that GCs modulate TcR-derived signal at this level, too. Tyrosine phosphorylation is an important issue regarding the regulation of kinase activity. Currently 11 tyrosine phosphorylation sites are known on ZAP-70 making possible the fine-tuning of kinase activity and other signal transduction processes.

Since our experimental results showed that DX induced ZAP-70 phosphorylation was GR dependent we examined the possibility of physical linkage between the two molecules. The results of our co-immunoprecipitation experiments and confocal microscopic images strongly suggest, that ligand binding (GR agonist) promotes the direct association of GR and ZAP-70. These results confirm our assumption of ZAP-70 being a communication point between GC and TcR signal transduction pathways.

It is known that both inactive GR and p56-lck are Hsp-90 client proteins. Therefore we investigated ZAP-70 relation to Hsp-90. Interestingly we found that a fraction of ZAP-70 co-precipitates with Hsp-90, examining both Jurkat and HeLa-trZAP-70 cells. However, the association of the two molecules is independent of the presence of GR ligand. Geldanamycin, a specific inhibitor of Hsp-90 prevented ZAP-70 association with Hsp-90 but showed no effect on ZAP-70-GR co-precipitation. These results strongly suggest that the association of GR and ZAP-70 is independent of Hsp-90.

The fact that DX treatment inhibited the ZAP-70 kinase association with the CD3 complex in activated cells, raises the possibility that this non-genomic GC effect described here takes part in the well-known GC effect of suppressing T-cell activation and function. According to our recent knowledge in cellular signalling, the exact temporospatial organization of the participating molecules is of key importance in the proper signalling function. We found that DX treatment disrupts the CD3-associated signalling complex preventing ZAP-70 associating with the TcR-CD3 complex. If the

docking of ZAP-70 on the CD3 chains is inhibited, the T-cell activation is at least defective or can lead to anergy.

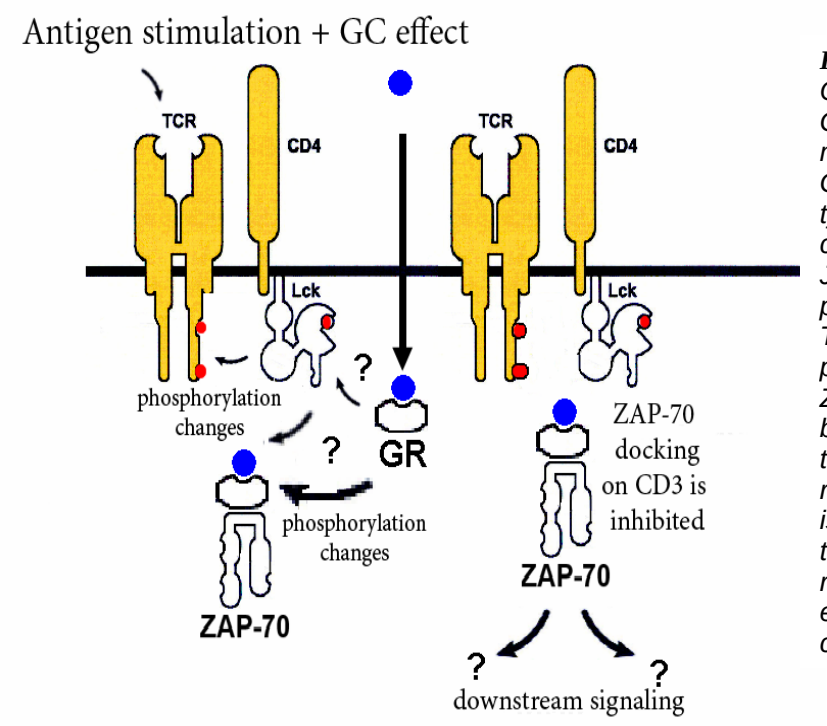

Figure 3
Our concept on TcR-CD3- and GR signal transduction crosstalk mechanisms. We suggest that GC hormone causes multiple tyrosine phosphorylation changes as we demonstrated on Jurkat cell lysates and precipitated ZAP-70 samples. The GC hormone caused rapid phosphorylation changes of the ZAP-70 kinase. After ligand binding, the GR associates with the ZAP-70 kinase and ZAP-70 recruitment to the CD3 complex is inhibited. We assume that these processes might be partly responsible for the inhibitory effect of the GC hormone on T-cell activation.

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Publications related to this thesis:

Domokos Bartis, Ferenc Boldizsár, Krisztián Kvell, Mariann Szabó, László Pálinkás, Péter Németh, Éva Monostori and Tímea Berki: *Intermolecular relations between the glucocorticoid receptor, ZAP-70 and Hsp-90* (Biochem Biophys Res Commun 354 (2007) 253-258) *IF: 3,000*

Domokos Bartis, Ferenc Boldizsár, Mariann Szabó, László Pálinkás, Péter Németh and Tímea Berki: *Dexamethasone induces rapid tyrosine-phosphorylation of ZAP-70 in Jurkat cells* (J Steroid Biochem Mol Biol 98. (2006) 147-154) *IF: 2,715*

Other publications:

Ferenc Boldizsár, László Pálinkás, Tamás Czömpöly, **Domokos Bartis**, Peter Németh, Tímea Berki: *Low glucocorticoid receptor (GR), high Dig2 and low Bcl-2 expression in double positive thymocytes of Balb/c mice indicates their endogenous glucocorticoid hormone exposure* (Immunobiology, 211 (2006), 785-796) *IF: 1,812*

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Boldizsár, F, Berki, T., Pálinkás, L., **Bartis, D.**, Németh, P: *Antigen and glucocorticoid hormone (GCs) induce positive selection of DP thymocytes in a TCR transgenic mouse model.* Immunol Lett. Special Issue: Abstracts of the 15th European Immunology Congress, EFIS 2003, June 8-12, 2003, Vol. 87.(1-3) W11.09

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