GILZ mediates the inhibition of migration of neutrophil granulocytes induced by glucocorticoids by interaction with PU.1 and consequent up-regulation of Annexin I in a mouse model of peritonitis

E. Ricci[1], M. Gentili[1], E. Pericolini[2], E. Gabrielli[2], A. Vecchiarelli[2], L. Cari[1], G. Nocentini[1], S. Ronchetti[1], C. Riccardi[1]
[1]Section of Pharmacology, Department of Medicine, University of Perugia, Piazzale Lucio Severi 1, S. Andrea delle Fratte, 06132 Perugia
[2] Section of Microbiology, Department of Experimental Medicine, University of Perugia, Piazzale Lucio Severi 1, S. Andrea delle Fratte, 06132 Perugia

The glucocorticoid-induced leucine zipper protein, GILZ, mediates the glucocorticoid anti-inflammatory effects in a variety of experimental diseases, particularly in T lymphocytes, macrophages and dendritic cells. Here we show that GILZ is necessary for the function and the migration process of neutrophils into the inflamed tissue. In a mouse model of thyoglicollate-induced peritonitis, GILZ knock-out peritoneal neutrophils show higher phagocytocis and killing activities, while producing less NETosis than WT. Interestingly GILZ knock-out neutrophils can migrate into the peritoneum four hours after peritonitis induction, regardless of dexamethasone (DEX) pre-treatment, whereas wild type cells cannot. GILZ is indeed upregulated during peritonitis in peritoneal neutrophils and by *in vivo* DEX treatment in peripheral neutrophils. Therefore, GILZ up-regulation results in inhibition of their migration. While no differences in adhesion molecule expression (CD11a, CD11b, TLR2, CXCR2) nor in peritoneal cytokines (IL-12p40, MIP-2, IL-6, IL-8, TNF-alpha) can be observed, a missed upregulation of Annexin a1 in DEX-treated GILZ-KO cells can be revealed both in peritoneal and in peripheral neutrophils, in DEX treated mice. Annexin a1 allows neutrophil detachment from endothelial wall, thus preventing neutrophil migration into inflamed tissues. Although lacking GRE regulatory elements, Annexin a1 is an important glucocorticoid-induced gene. Since GILZ is unable to directly bind DNA, it needs a co-factor to upregulate Annexin a1 gene expression. Luciferase assays of annexin A1 promoter in the presence of GILZ revealed a GILZ-dependent activation of the promoter in a region comprising a large putative binding site for PU.1 transcription factor, known to be a negative regulator of Annexin a1 expression. Protein co-immunoprecipitation and *in situ* proximity ligation assays demonstrate a direct interaction between GILZ and PU.1. GILZ-dependent activation of Annexin A1 promoter through PU.1 was confirmed by chromatin immunoprecipitation assay (CHIP).
Altogether our results show for the first time that Annexin a1 upregulation by glucocorticoid treatment is GILZ-mediated, thus providing a new tool to either prevent or treat inflammation through GILZ.