Lymphocytosis disorder in Glucocorticoid-induced Leucine Zipper (GILZ) KO mice is intrinsic to B cell lineage

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Glucocorticoids (GC) are strong immunosuppressive drugs and they are used in the treatment of some lymphomas and leukemia. Molecular mechanisms of GC action include induction of GC target genes, among which we found Glucocorticoid-induced leucine zipper (GILZ). This gene mediates a number of GC effects, such as control of cell proliferation, differentiation and apoptosis. GILZ belongs to TSC22d family, sharing conserved TSCbox domain and is an important regulator of Ras/MAPK/Erk pathway; recently it has been shown that TSC22d mutated forms are implicated in diffuse large B cell lymphoma patients, suggesting that also TSC22d family members may play a role in lymphoma development. GILZ KO mice develop a B lymphocytosis overtime. Here we investigated whether the defect observed in GILZ KO mice is intrinsic to B cell lineage, so we have generated mice lacking GILZ only on B cell cKO (obtained by crossing FLOX GILZ mice with transgenic CD19 Cre mice).

Results obtained confirms that the defect is related to the absence of the GILZ gene in B cells, in fact, cKO mice show a phenotype similar to the total body KO mice. The defect appears in the bone marrow of young mice where there is an increase percentage and number of all B220+ cell subsets starting from the pre-B stage of differentiation. After 6-8 months the B220+ increase is detectable also in peripheral blood of cKO mice.

With the aim to elucidate the mechanisms responsible pof this B-cell expansion, we analyzed the apoptosis and proliferation of B cells by caspase, annexin V and with Ki67 staining analyzed by flow cytometry. Results showed a decreased apoptosis in cKO B cells compared to WT, while no differences in cell proliferation were detected. These data were confirmed also by western blot analysis on caspase-3, and -8 where a decreased expression in B cell of KO mice was found. The decrease in apoptosis in B cell lacking GILZ was confirmed by increased levels of the anti-apoptotic gene *Bcl-2*.

Finally, since lymphocytosis in cKO mice could result in increased immunoglobulins (Ig) levels we performed ELISA to measure levels of IgG, IgM and ANA sera concentration in WT and cKO mice. Results didn't show any difference between WT and cKO mice.

Altogether our data show that lack of GILZ results in specific defect in B cell development, with a decrease of apoptosis in GILZ deficient, leading to the expansion of $B220^{+}$ cells compartment, starting in the bone marrow in the young mice, causing an accumulation of B cells overtime. These results indicate that GILZ could be a pharmacological target in the pathologies of B cell lineage.