Lack of glucocorticoid-induced leucine zipper (GILZ) deregulates B-cell survival and leads in B-cell lymphocytosis in mice.

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Glucocorticoids (GC) are widely used as immunosuppressive drugs and antitumor agents in some acute leukemias and multiple myeloma. Therapeutic doses of GC induce growth-suppressive and cytotoxic effects on various leukocytes including B cells. Molecular mechanisms of GCaction include induction of GC target genes. Glucocorticoid-induced leucine zipper (GILZ), a member of Tsc22d family, is a rapidly, potently and invariably GC-induced gene. One of the TSC22d members were recently found mutated in diffuse large B cell lymphoma patients, suggesting that also TSC22d family members may play a role in lymphoma development. Our studies are needed to characterize the role of GILZ in normal and pathological hematopoiesis using of mouse models of knock-out mice (KO) for GILZ.

GILZ expression and function have been characterized in many hematopoietic lineages, including T cells, macrophages, and dendritic cells, but its role in B lymphocytes has not been addressed. To determine whether GILZ is expressed in B lymphocytes, BM cells from WT mice were analyzed by qPCR for the expression of GILZ at various stages of B-cell development. We have found that GILZ is expressed in all B-cell developmental stages at levels comparable with those of $CD4^+CD25^-$ T cells. Young gilz KO mice showed normal body and lymphoid tissue weight and absolute counts of white blood cells, as well as B and T lymphocytes. However, the total number of BM cells was significantly higher in gilz KO mice compared with controls. Flow cytometry analysis of BM revealed an increate frequency and number of $B220^+$ cells in gilz KO mice compared with WT controls, whereas development of other hematologic lineages appeared normal. Flow cytometry analysis showed in KO mice a mild but significant increase in the frequency and absolute number starting from the PreB stage. Then we studied older mice to see if they develop leukemia/lymphoma overtime. We observed increased cellularity of BM, spleen and pLN in gilz KO mice compared with WT littermates. The increased lymphocyte number was the result of a specific accumulation of $B220^+$ B cells in mice lacking GILZ. Conversely, the number of $CD4^+$, $CD8^+$, and Mac-1^+ cells did not differ in respective organs of WT and gilz KO mice. To investigate whether the B-lymphocyte accumulation is a result of a decreased cell death, we tested the ability of WT and gilz KO B cells to survive in vitro. Purified $CD19^+$ cells lacking GILZ showed increaste survival in vitro compared with WT B cells, as measured by cell counting after 48 hours in culture. Moreover we compared apoptosis in WT and GILZ-deficient B cells by AnnexinV staining. Results demonstrate that cells lacking GILZ have a decreased frequency of apoptotic cells in BM. Furthermore we evaluated cell proliferation and death in different B-cell subpopulations ex vivo by flow cytometry. Expression of ki67 was comparable in WT and gilz KO mice in all of the B-cell subsets, instead, B cells isolated from gilz KO mice revealed decreased caspase activation in all B-cell subsets starting from the PreB stage of B development. Then we monitored the expression levels of different Bcl-2 family members in cultured BM cells isolated from WT and gilz KO mice. the results gave the same directions. Furthermore here we found that GILZ interacts with the NF-kB p65 subunit in purified $CD19^+$ B cells as revealed by proximity ligation assay, suggesting that GILZ regulates NF-kB also in B cells. Consistently, we observed an increased nuclear translocation of the NF-kB p65 subunit in gilz-deficient B cells. Then we performed ChIP assay in $CD19^+$ B cells purified from WT or gilz KO animals. Results indicate that p65/NF-kB binds more to the Bcl-2 promoter in purified B cells lacking GILZ.

These results establish GILZ as an important regulator of B-cell survival and suggest that the deregulation of GILZ expression could be implicated in the pathogenesis of B-cell disorders.