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The effects on erythropoiesis of chronic glucocorticoid stimulation in Cushing’s syndrome

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In this issue of Haematologica, Varricchio et al. use Cushing’s syndrome to study stress erythropoiesis under conditions of chronic exposure to glucocorticoids (GCs)(1). By studying patients with active syndrome and in remission, they describe a distinct population of GC-responsive hematopoietic progenitors and provide intriguing new insight on the molecular basis of the loss of responsiveness to GCs in the treatment of anemias.

GCs and stem cell factor (SCF) play key roles in stress erythropoiesis(2). GCs bind to the glucocorticoid receptor (GRα in erythroid cells), which translocates to the nucleus to activate stress response genes. However, it is not clear precisely how the GC/GRα pathway regulates stress erythropoiesis. This is of clinical significance as GCs are used to treat hyperproliferative anemias such as Diamond Blackfan Anemia (DBA)(3). GC therapy increases red cell mass alleviating anemia; however, patients become refractory for reasons that are not fully understood. Current models for studying GCs in erythropoiesis include animal models and ex vivo erythroid differentiation of human CD34+ hematopoietic stem and progenitor cells (HSPCs), albeit with limitations(4). These include species-specific differences in how murine and human proerythroblasts respond to GCs(5) and the near-universal use of dexamethasone (Dex) and SCF in expanding proerythroblasts in vitro, giving rise to confounding effects(4). Endocrine disorders offer opportunities to study GC effects on in vivo erythropoiesis under conditions of constitutively active (Cushing’s syndrome) or altogether absent (Addison’s disease) GC/GRα activation(6). Hence it is surprising that only one publication has previously reported erythrocytosis (an increase in red blood cell mass) in one Cushing’s patient with ACTH-secreting pituitary adenoma(7). This is redressed in the present study by Varricchio et al., who recruited a relatively large (n=13) cohort of Cushing’s patients with active hypercortisolemia (A patients) and an equal number of eucortisolemic patients in remission following surgical removal of the pituitary adenoma (R patients). Characterization of erythropoiesis showed A patients as having erythrocytosis with normal HbF levels, the latter suggesting that chronic stress conditions do not induce fetal globin expression. Interestingly, CD14+ monocytes in A patients had a distinct phenotype skewed towards a greater proportion of cells expressing CD163, presumably as a result of
constitutive GR activation(8). This was also maintained in R patients, suggesting a cellular memory of monocyte GC activation after remission.

Varricchio et al. next assessed the immunophenotypic profile of circulating CD34+ cells in A and R patients (and healthy controls) using a panel of antibodies that (i) define a stress-progenitor cell population (CD110 and CD36 positive), (ii) detect proteins that regulate (CALR) or respond to (CXCR4) GR activation and (iii) monitor the response to SCF and IL-3 (CD117 and CD123, respectively) as growth factors used in culture to stimulate erythroid cells. CD133 (prominin), expressed in hematopoietic stem cells, was also included. This analysis showed CD34+ HSPCs from A patients having a unique profile characterised by a higher proportion of cells expressing CD36, CD110, CXCR4 and CD133 and a lower proportion expressing CD117 and CD123, compared to healthy controls. These observations are consistent with a stress-like phenotype, activated GR signalling and a greater responsiveness to SCF and IL-3, the receptors of which are down-modulated in response to stimulation(9). By contrast, CD34+ HSPCs in R patients displayed a greater proportion of CXCR4 expressing cells, but no difference in the fraction of cells expressing stress-like features. These observations suggest that GR activation is sustained even after remission in R patients. As expected, expansion of immature erythroid cells from the HSPCs of A patients was similar regardless of the presence of Dex in culture. Interestingly, a similar effect was also seen with HSPCs from R patients. Thus, the immunophenotypic profiles and erythroid expansion characteristics of CD34+ HSPCs from R patients, are consistent with GR signalling retaining some activity following surgical removal of the pituitary adenoma.

GR activation was also investigated at a molecular level by assessing the profile of GRα protein in erythroid cells from A and R patients and healthy controls, differentiated ex vivo with and without Dex. GILZ was also analysed as a gene target activated by GRα. Using antibodies that detect total GRα protein, or the differentially phosphorylated GRα fractions that are marked is either for cytoplasmic retention or for translocation to the nucleus, it was shown that whereas total GRα levels were equivalent in A, R and control cells stimulated with Dex, cytoplasmic GRα was lower in A cells. In addition, GILZ levels were higher regardless of Dex, indicating constitutive GRα activation in A cells that does respond further to GC stimulation. Interestingly, cytoplasmic GR levels were higher in R patients compared to A patients, yet R patients are also insensitive to de-novo GC stimulation. Taken together, these observations suggest that (i) the lack of response to GC treatment as a result of chronic GC exposure is most likely due to deregulation of the nuclear/cytoplasmic transport of GRα, rather than changes in GRα protein levels and (ii) when in remission, the lack of response to Dex, the higher levels of GRα retained in the cytoplasm and the increased
fraction of CD163+ monocytes in the circulation, suggest a cellular memory mechanism that reflects the previous hypercorticosolemic state in R patients.

Overall, the study by Varricchio et al. adds to our understanding of GC stimulation in erythropoiesis by refining our view on stress-like HSPCs in conditions of chronic GC exposure and in relation to previously characterized Dex-responsive stress progenitors(5). This study also suggests an intriguing explanation for patients becoming refractory to GC therapy, in that prolonged GC exposure leads to GRα retention in the cytoplasm, potentially as a moderating response to constitutive GC exposure. Importantly, as the authors point out, this can be tested using inhibitors of cytoplasmic GRα phosphorylation. Lastly, it is of great interest to investigate in detail the molecular (epi)genetic basis of constitutive GRα activation in stress progenitors and of the potential cellular memory mechanism described here, also in relation to pathways and molecules that have been shown to be deregulated in anemias or following GC treatment(5, 10).

References

1. Varricchio L GE, Martelli F, et al. Hypercortisolemic Cushing’s patients possess a distinct class of hematopoietic progenitor cells leading to erythrocytosis. Haematologica. xxx


