The effects of chronic glucorticoid stimulation on erythropoiesis in Cushing syndrome

John Strouboulis and Sara El Hoss
Red Cell Haematology, Comprehensive Cancer Centre, School of Cancer and Pharmaceutical Sciences, King's College London, London, UK

Correspondence: J. Strouboulis
john.strouboulis@kcl.ac.uk

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In this issue of *Haematologica*, Varricchio *et al.* report on their use of Cushing syndrome to study stress erythropoiesis under conditions of chronic exposure to glucocorticoids.[1] By studying patients with active Cushing syndrome and in remission, they describe a distinct population of glucocorticoid-responsive hematopoietic progenitors and provide intriguing new insights into the molecular basis of the loss of responsiveness to glucocorticoids in the treatment of anemias.

Glucocorticoids and stem cell factor (SCF) play key roles in stress erythropoiesis.[2] Glucocorticoids 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 glucocorticoid/GRα pathway regulates stress erythropoiesis. This is of clinical significance as glucocorticoids are used to treat hyperproliferative anemias such as Diamond-Blackfan anemia.[3] Glucocorticoid therapy increases red cell mass, thereby alleviating anemia; however, patients become refractory for reasons that are not fully understood. Current models for studying glucocorticoids in erythropoiesis include animal models and *ex vivo* erythroid differentiation of human CD34$^+$ hematopoietic stem and progenitor cells (HSPC), although these have limitations.[4] These include species-specific differences in how murine and human proerythroblasts respond to glucocorticoids[5] and the near-ubiquitous use of dexamethasone and SCF in expanding proerythroblasts *in vitro*, giving rise to confounding effects.[4] Endocrine disorders offer opportunities to study the effects of glucocorticoids on *in vivo* erythropoiesis under conditions of constitutively active (Cushing syndrome) or altogether absent (Addison disease) glucocorticoid/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 patient with an adrenocorticotropic hormone-secreting pituitary adenoma.[7] This is redressed in the study by Varricchio *et al.*, who recruited a relatively large (n=13) cohort of Cushing syndrome patients with active hypercortisolemia (active-phase patients) and an equal number of eucortisolemic patients in remission following surgical removal of the pituitary adenoma (remission-phase patients). Characterization of erythropoiesis showed that active-phase patients had erythrocytosis with normal HbF levels, the latter suggesting that chronic stress conditions do not induce fetal globin expression. Interestingly, CD14$^+$ monocytes in active-phase 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 remission-phase patients, suggesting a cellular memory of monocyte glucocorticoid activation after remission.

Varricchio *et al.* next assessed the immunophenotypic profile of circulating CD34$^+$ cells in active- and remission-phase patients (and healthy controls) using a panel of antibodies that: (i) define a stress-progenitor cell population (CD110$^+$ and CD36$^+$); (ii) detect proteins that regulate (CALR) or respond to (CXCR4) GR activation; and (iii) monitor the response to SCF and interleukin-3 (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 that CD34$^+$ HSPC from patients with active Cushing syndrome had a unique profile characterized by a higher proportion of cells expressing CD36, CD110, CXCR4 and CD133 and a lower proportion expressing CD117 and CD123, compared to cells from healthy controls. These observations are consistent with a stress-like phenotype, activated GR signaling and a greater responsiveness to SCF and IL-3, the receptors of which are down-modulated in response to stimulation.[9] By contrast, CD34$^+$ HSPC in remission-phase 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 Cushing disease patients who achieve this state. As expected, expansion of immature erythroid cells from the HSPC of active-phase patients was similar independently of the absence or

presence of dexamethasone in culture. Interestingly, a similar effect was also seen with HSPC from remission-phase patients. Thus, the immunophenotypic profiles and erythroid expansion characteristics of CD34$^+$ HSPC from remission-phase patients are consistent with GR signaling 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 active- and remission-phase patients and healthy controls, differentiated *ex vivo* with and without dexamethasone. GILZ was analyzed as a gene target activated by GRα. Using antibodies that detect total GRα protein, or the differentially phosphorylated GRα fractions that were marked either for cytoplasmic retention or for translocation to the nucleus, it was shown that, whereas total GRα levels were equivalent in cells from active-phase and remission-phase patients and healthy controls stimulated with dexamethasone, cytoplasmic GRα was lower in cells from patients with active-phase Cushing disease. In addition, GILZ levels were higher regardless of the presence of dexamethasone, indicating constitutive GRα activation in active-phase cells that does not respond further to glucocorticoid stimulation. Interestingly, cytoplasmic GR levels were higher in remission-phase patients than in active-phase patients, yet patients in remission are also insensitive to *de-novo* glucocorticoid stimulation. Taken together, these observations suggest that: (i) the lack of response to glucocorticoid treatment as a result of chronic glucocorticoid 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 dexamethasone, the higher levels of GRα retained in the cytoplasm and the increased fraction of CD163$^+$ monocytes in the circulation may indicate a cellular memory mechanism that reflects the previous hypercorticosolemic state.

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

Disclosures

No conflicts of interest to disclose.

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