Surprise, surprise: STAT5 is not enough to stop the steroids

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Received: May 30, 2022.
Accepted: June 13, 2022.
Early view: June 23, 2022.

https://doi.org/10.3324/haematol.2022.281369

In this issue of *Haematologica*, van der Zwet *et al.*[1] provide evidence that STAT5 is not sufficient to drive resistance to glucocorticoid treatment in T-cell acute lymphoblastic leukemia (T-ALL) cells, although STAT5 promotes significant upregulation of the anti-apoptotic genes *BCL2* and *BCL2L1* (encoding BCL-XL) specifically when in the presence of glucocorticoids.

To understand why these results are intriguing and relevant from biological and clinical standpoints, we should start by recalling that administration of glucocorticoids (steroid hormones such as prednisolone, used by van der Zwet and colleagues) is a pivotal component of frontline pediatric ALL treatment. Refractory/resistant disease constitutes the main clinical challenge in this aggressive but otherwise curable malignancy. Thus, understanding the mechanisms driving resistance to glucocorticoids is of major biological and clinical relevance. Previous studies identified a number of potential mechanisms, including PI3K-AKT pathway blocking translocation of the glucocorticoid receptor (NR3C1) to the nucleus[2] and upregulating BCL-XL and MCL1,[3] or different modes of inactivating *BIM*, a pro-apoptotic gene and major transcriptional target of NR3C1.[4] Additionally, interleukin-7 receptor (IL-7R)-mediated signaling, because of IL-7 in the tumor microenvironment or gain-of-function mutations in IL-7Rα (encoded by *IL7R*) or downstream effectors, can drive T-ALL and promote resistance to steroids.[3,5-8]

IL-7/IL-7R-mediated signaling involves three main pathways: STAT5, MEK-ERK and PI3K-AKT, all of which rely on upstream activation of JAK1 and JAK3.[5] Using patients' data, van der Zwet *et al.* showed that activating mutations in IL-7Rα, JAK1, JAK3 or STAT5B associated, as expected, with STAT5 transcriptional activity. However, sensitivity to prednisolone and event-free survival were comparable in patients with high *versus* low STAT5 transcriptional activity. This constituted a first indication that STAT5 alone cannot be a major reason for steroid resistance in T-ALL patients and that other pathways (for instance PI3K-AKT and/or MEK-ERK) must be taken into account.

Through overexpression of constitutively active forms of IL-7Rα in T-ALL cell lines, combined with pharmacological inhibition of IL-7R-mediated signaling pathways, van der Zwet and colleagues then showed that whereas mutant *IL7R* promoted steroid resistance, this effect did not correlate with upregulation of *BCL2* and *BCL2L1*. As expected, JAK inhibition with ruxolitinib reversed *BCL2* and *BCL2L1* upregulation and steroid resistance in prednisolone-treated *IL7R* mutant cells. However, AKT inhibition (alone or in combination with MEK inhibition) also reversed steroid resistance although *BCL2* and *BCL2L1* transcript levels were not downregulated (if anything they actually increased). Moreover, although a constitutively active form of STAT5B upregulated both anti-apoptotic genes in steroid-treated T-ALL cell lines, this did not result in increased resistance to steroids. These findings are in apparent disagreement with previous studies by Meyer *et al.*, which showed that STAT5 and BCL2 are required for IL-7-mediated resistance to steroids.[8] However, *STAT5B* and *BCL2* were silenced in those studies, whereas van der Zwet *et al.* overexpressed activated STAT5B (with consequent *BCL2* upregulation). Thus, the two observations may be reconciled by concluding that STAT5 is necessary but not sufficient for resistance to glucocorticoids. Obviously, a question ensues: why is STAT5 not sufficient? In line with what was suggested by previous work in B- and T-ALL,[7,9] the authors propose that this may be because activation of the glucocorticoid receptor (NR3C1) leads to BIM upregulation, which counterbalances the effects of increased BCL2 and BCL-XL by binding directly to them. This is based on co-immunoprecipitation studies, which go beyond previous studies, although unfortunately no functional studies were performed addressing the actual importance of the balance between BIM and BCL2 anti-apoptotic family members on sensitivity to glucocorticoids. Nonetheless, the tantalizing corollary, proposed by the authors, is that STAT5 activation only effectively promotes glucocorticoid resistance if cellular defects exist that ultimately disable or decrease steroid-induced pro-apoptotic BIM induction. These defects may be genetic, epigenetic and/or involve the modulation of signaling pathways known to regulate BIM activation, such as MEK-ERK and PI3K-AKT, which can be regulated cell-autonomously and by microenvironmental factors, including IL-7 (Figure 1A). Obviously, this also implies that heterogeneity

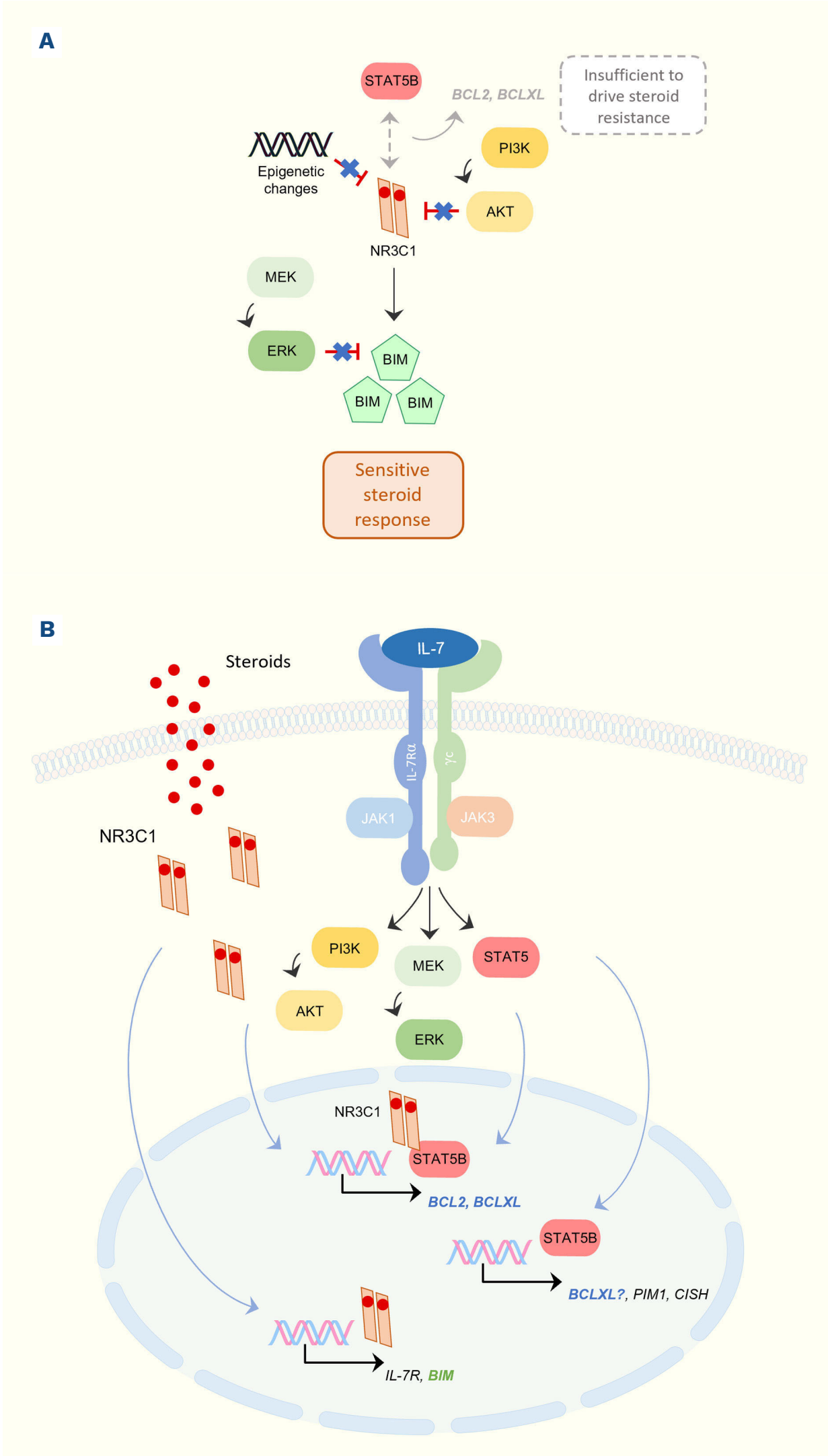

Figure 1. STAT5 and response to steroids in acute lymphoblastic leukemia. (A) Van der Zwet *et al.*[1] propose that, in the absence of the indicated mechanisms that downregulate NR3C1-mediated BIM activation, NR3C1/STAT5 cooperation in regulating *BCL2* and *BCLXL* expression is insufficient to induce steroid resistance. (B) Upon activation, both NR3C1 and STAT5 translocate to the nucleus. Activation of NR3C1 by steroids promotes the upregulation of *BIM* and *IL7R*, whereas STAT5 activation by IL-7R-mediated signaling, enhances the expression of *PIM1*, *CISH* and possibly *BCL2L1/BCLXL*, but not of *BCL2*. The concomitant activation of glucocorticoid- and IL-7R-mediated signaling leads to NR3C1 and STAT5 cooperating in inducing the expression of *BCL2* and *BCL2L1/BCLXL*.

should exist in the molecular landscape of T-ALL cell lines and patients' samples which may justify differences regarding the relevance of STAT5 for resistance to glucocorticoids. Characterizing those differences will likely be essential to identify the best combination therapies required to overcome resistance in particular patients.

There are other notable features in the article, namely regarding the role of IL-7R-mediated STAT5 activation in upregulating BCL2 in T-ALL cells. The link between IL-7, STAT5 and BCL2 upregulation in healthy developing T cells has long been established. Because IL-7 activates STAT5 and upregulates BCL2 it has been assumed that BCL2 is upregulated due to STAT5 transcriptional activity also in T-ALL cells. However logical it may seem, this view was proven wrong. Ribeiro *et al.* showed that STAT5 does not bind to the *BCL2* locus and that STAT5 silencing or pharmacological inhibition does not prevent IL-7-mediated BCL2 upregulation in T-ALL cells.[10] These observations have now been corroborated by van der Zwet and colleagues, who also showed that a constitutively active mutant form of STAT5B was unable to upregulate *BCL2*. While this is another nail in the coffin of a longstanding "dogma", why STAT5 alone does not transcriptionally upregulate *BCL2* in T-ALL cells remains a puzzling question.

On the other hand, Meyer *et al.* demonstrated that IL-7-mediated activation of STAT5 could upregulate BCL2 in T-ALL cells, with a major "nuance": the leukemia cells were treated with glucocorticoids.[8] The work from Meijerink's laboratory is a leap forward in the molecular understanding of how this happens and in integrating the two previous studies (Figure 1B). In the absence of steroids, STAT5 is incompetent to bind the locus or upregulate *BCL2*, even if activated mutationally or by IL-7 stimulation. However, in the presence of steroids, STAT5 is required for NR3C1 to bind to the *BCL2* locus at two sites, including one with a STAT5 binding motif. Because STAT5 and NR3C1 physically interact, it is possible that, as speculated by the authors, STAT5 is required for heterodimeric or multimeric STAT5-NR3C1 complexes to activate *BCL2* and other antiapoptotic genes (Figure 1B). Intriguingly, STAT5 and NR3C1 appear to interact in a 'constitutive' fashion, independently of STAT5 or NR3C1 activation status, which raises a number of questions. Where in the cell does the interaction occur (cytoplasm, nucleus, both compartments)? Is the interaction required for nuclear translocation and DNA binding? Does it potentially allow for non-phosphorylated, inactive STAT5 to translocate to the nucleus in the presence of steroids? These and other outstanding questions merit investigation, because they may be of great use for the understanding of the crosstalk between glucocorticoid therapeutic signaling and IL-7R-mediated resistance signaling.

Overall, the findings of van der Zwet and colleagues are provocative and may have a considerable impact, not only by augmenting the understanding of the underlying biology of resistance to treatment in T-ALL (extending some of the current views and challenging others) but also, in doing so, by providing hints for devising effective combination strategies to overcome resistance and avoid relapse.

Disclosures

No conflicts of interest to disclose.

Contributions

MF and JTB wrote the manuscript.

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