Confounding effect of cyclosporine dosing when comparing horse and rabbit antithymocyte globulin in patients with severe aplastic anemia

We read the interesting paper recently published by Feng *et al*.[1] They found that double negative ($CD3^+CD4^-CD8^-$) regulatory T cells (Tregs) where more frequent in the peripheral blood of rATG than hATG patients, despite no significant effect on the absolute number of these cells between the two patient groups. This was in agreement with findings of the study previously published by some of the authors in which absolute number of ($CD4^+CD25^{high}CD127^{low}$) Tregs was lower in rATG patients.[2] This study reported a randomized trial comparing rATG with hATG in acquired severe aplastic anemia (SAA) showing a better response rate with hATG when combined with cyclosporine with dose adjustments to maintain a blood concentration at 200-400 ng/mL.[2] The patient data of this previous study were used in those of Feng *et al*.[1] Nevertheless, cyclosporine (CsA) presents a concentration-dependent effect on interleukine-2 (Il2) inhibition,[3] and Il-2 production is only partially inhibited by low CsA concentrations. It has been shown that Il-2 plays an important role in promoting the development and proliferation of Tregs. Subsequent studies have established that only ultra-low dose Il2 was necessary for a better expansion of Tregs.[4] This is consistent with previous studies who found that CsA allows the development of Tregs at low concentrations or doses while Tregs are inhibited when high concentrations or doses are used.[5,6]

Considering the interaction between CsA and Tregs, caution should be exercised when interpreting the results of studies comparing the effect of ATG on Tregs expansion in SAA patients receiving CsA concomitantly. In particular, ATG effect on Tregs should be evaluated in each patient according to the measured CsA blood levels. It is well known that lack of Tregs plays an important role in the pathogenesis of autoimmune disorders. It has been shown that Tregs are deficient in patients with SAA at diagnosis and increased in responders.[7] Thus, SAA treatment should benefit from strategies allowing their expansion.

Rabbit but not horse ATG has been shown to be able to expand Tregs *in vitro*.[8] Results *in vivo* are controversial, possibly because Tregs induced by rabbit ATG may not have the same phenotype as native Tregs, and so are not always found.[9] On the other hand, the number of Tregs is certainly not the only parameter to take into account. Indeed, Shi *et al*. demonstrated that Tregs of SAA patients had impaired function and could not suppress T effectors.[10] But rATG-induced Tregs may have enhanced functional activity which could compensate for low numbers.[11] It may not be appropriate to conclude that horse or rabbit ATG may be superior in treatment of SAA because the peripheral number of Tregs is higher with one or with the other. Moreover, the ratio between bone marrow and peripheral blood Tregs seems to be more relevant to characterize Tregs deficiency in SAA patients.[10]

All these observations deserve further investigation, based on the role of CsA on regulatory T cells (Treg) when combined to ATG, and notably on its synergistic effect on Tregs expansion with rATG. In the past, some studies in SAA used 17.5 mg/kg of rATG or 75 mg/kg of hATG combined with 5 mg/kg/d of CsA.[12] Response rates were about 77% in each case, even in refractory or relapsed patients. More recent studies used rather 10 or 15 mg/kg/d of CsA or high CsA target trough blood (TBC) levels (150-250 ng/mL), despite the fact that no evidence of the superiority of high doses of CsA has been shown. In these studies, response rates ranged from 40% to 68%.[2,13] We simulated the values of CsA blood levels obtained with 5 or 10 mg/kg/d by using a physiologically-based pharmacokinetic model.[14] TBC were respectively 92.7±4.9 and 153.5±7.3 ng/mL. We also looked at the 18 SAA patients of our cohort in whom we had carried out strict monitoring of CsA levels post ATG treatment (at least one measure per week); 9 of them were responders. Among them, 8 had a mean CsA TBC between 99 and 129 ng/mL during the first three months. Moreover, there was a trend for a higher probability of hematopoietic stem cell transplantation in patients who had mean CsA TBC over 130 ng/mL (66.7% *vs.* 16.7%; log rank, P=0.08). In contrast, we reported 3 cases of refractory SAA patients treated with hATG and 15 mg/kg of CsA who responded after decreasing CsA doses while targeting trough blood concentrations around 100 ng/mL.[15] These preliminary results led us to explore the relationships between CsA exposure and neutrophil response by using a pharmacokinetic/pharmacodynamic modeling approach. We found that CsA TBC should be maintained between 87 and 120 ng/mL to maximize the probability of response (M. Philippe *et al.,* submitted manuscript, 2015).

All these considerations may lead to the conclusion that rATG should not be definitively disgraced. They also raise some questions: could the results of immunosuppressive therapy of SAA be improved by the use of lower CsA doses and by targeting CsA TBC around 100 ng/mL? Answers can only be provided by a randomized trial comparing rATG with hATG, where results concerning Tregs expansion should be balanced by the CsA blood levels actually observed in patients.

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