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INVITED COMMENTARY

Predicting tolerance by counting natural regulatory T cells (CD4+25++FoxP+)?

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Immunological tolerance to an allotransplant is the ultimate goal of modern transplant medicine. Using conventional immunosuppressive schemes, organ transplantation was developed to a standard method for replacement of irreversibly injured organs. However, we have to pay a high price for the chronic immunosuppression required – it is not only costly, but also associated with numerous immunological and nonimmunological side effects. As operational tolerance is a rather rare event in patients receiving traditional immunosuppressive protocols, there is a need for both new tolerance supporting protocols and biomarker helping to identify tolerant patients where we can safely wean immunosuppression.

On one hand, the introduction of calcineurin inhibitors (CNI) into transplant medicine dramatically improved the outcome, and on the other hand they exhibit a significant long-term toxicity and seem to interact with tolerance induction. Consequently, many attempts have been done to wean the patients at least from CNI. In fact, kidney transplant patients showed improved creatinine clearance following CNI weaning, but a significant proportion of patients developed graft injury by rejection processes. A complete drug weaning is rarely successful, at least after kidney transplantation. Nevertheless, there exist some reports on operationally tolerant kidney transplant recipients who are drug-free of different reasons (compliance, PTLD, etc.) with stable graft function for years. In about 20–50% of liver transplant patients, however, complete drug weaning seems to be feasible. Nevertheless, a significant proportion of these patients also developed acute rejection episodes after weaning. To guide (partial) weaning strategies in a way which is safe for all patients (also nontolerant ones), we need biomarkers predicting the individual risk.

Regulatory T cells (Treg) control the adaptive immunity and preclinical transplant models suggest their potency in preventing allograft rejection and tolerance induction. During the last decade of the Treg hype, several T-cell subsets with immunoregulatory potency have been described. One important Treg subset is called 'natural Treg' expressing CD4+25++FoxP3+. The absence of this subset (e.g. by defects in the *FoxP3* gene expressing a transcription factor which is essential for Treg development) results in severe autoimmunity in rodents and patients. Therefore, several groups recently looked for the association of this particular Treg subset and allograft outcome.

Braudeau *et al.* studied a quite interesting patient population of rarely detectable operationally tolerant, drug-free kidney transplant patients in comparison with stable patients, patients suffering from chronic rejection, and healthy controls. Recently, the group found decreased levels

of FoxP3 transcripts and of CD4+25++ T cells in chronic rejection while drug-free transplant recipients expressed levels similar to controls [1]. Using the new opportunities of direct intracellular FoxP3 staining, here the authors continued this work and could confirm that, in fact, chronic rejection is associated with a diminished number of natural Treg characterized by CD4+25++FoxP3+. As shown before at transcriptional level, intracellular staining exhibits similar numbers of Treg in healthy individuals and drug-free tolerant patients whereas stable patients on CNI therapy showed intermediate values. Remarkably, the frequency of FoxP3+ expressing CD4+25++ T cells was comparable in all groups suggesting that the difference in cell counts simply reflects a difference in the corresponding CD4+ T-cell numbers between the different groups.

Similar reports came from operational tolerant liver transplant patients [2,3].

There is a clear rational for the detailed analysis of operationally tolerant patients to reveal evidences for the mechanisms of tolerance and biomarkers for detecting candidates. The Nantes group has done a great job to identify operational tolerant drug-free kidney transplant patients as they are quite rarely detectable.

These interesting studies raise two questions:

1. Are natural Treg involved in operational tolerance and would the transfer of (expanded) natural Treg reverse/prevent chronic rejection and support tolerance?

2. Is a normal or even enhanced number of CD4+25++FoxP3+ Treg a robust biomarker for drug weaning attempts?

Unfortunately, this type of studies cannot answer these important questions. If the drop in the number of Treg is mechanistically related to lack of tolerance, it should be down in periphery and graft before rejection occurs. However, if the Treg counts have any predictive value in these studies, it looks rather like a rejection marker then a tolerance marker. Like in chronic rejection, as shown here, acute rejection episodes are also associated with diminished numbers of circulating Treg. However, this does not mean that lack of Treg is causing rejection, as acute/chronic graft rejection is associated with intragraft accumulation of memory T-cell subsets, including Treg expressing several 'memory-type' markers. In fact, the number of graft-infiltrating FoxP3+ cells correlates rather with rejection then tolerance ([4,5], own observations). Inflammatory processes are associated with the release of chemokines attracting memory T cells and the activation of endothelial cells promoting transmigration of the attracted T cells into the inflamed tissue. As result, we see a (transient) drop in T-cell counts, particularly of CD4+ and CD8+ memory T cells in the peripheral blood, including of CD4+25++Foxp3+ cells. The accumulation of memory/effector T cells of irrelevant specificity at the site of inflammation has the biological aim to amplify the adaptive antigen-specific immune response (bystander activation). To control the inflammatory process, on the other hand, it gives sense that Treg also migrate to the site of inflammation. Recently, we could demonstrate that not only natural Treg, but also alloantigen-specific Treg accumulates in the rejecting allografts and can adoptively transfer tolerance to naïve allograft recipients supporting this view of counter-regulation ([6], Tullius St *et al.*, unpublished). Whereas alloantigen-primed Treg (mostly also expressing CD4+25++FoxP3?) are able to adoptively transfer tolerance at relatively low-cell numbers, CD4+25++FoxP3+ natural Treg are less potent and in absence of T-cell depletion mostly insufficient to induce tolerance. Therefore, it is doubtful that adoptive therapy with natural (alloantigen naïve) Treg could reverse chronic rejection and support tolerance, at least in absence of any T-cell depletion.

Is weaning safer in patients with normal or enhanced Treg counts? The data reported here only show that chronic rejection is clearly associated with diminished Treg counts. However, these patients would be never used for (partial) weaning studies. What is about patients with stable graft function, who might be suitable weaning candidates? Braudeau *et al.* suggest intermediate levels of CD4+25++FoxP3+ T cells in this patient subset without any statistical difference to any other subset suggesting a broad distribution from normal (like tolerance) to diminished (like chronic rejection) levels [7]. Unfortunately, the authors do not show the data in detail (e.g. box plot analysis). To use this parameter for guiding drug weaning, clear cut-off levels have to be defined by additional retrospective and prospective studies. This requires a well-validated measurement of this parameter allowing multi-center trials. However, the identification of a single biomarker for identifying tolerance would be a big surprise because of the complexity of graft rejection/tolerance.

In summary, the study reports on a quite interesting observation in an important patient population. To move forward, we need international networks to identify a set of biomarkers to predict tolerance and to allow multicenter trials for their verification.

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