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Transitional B cell subsets – a convincing predictive biomarker for allograft loss?

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Abstract:

In this issue, Cherukuri and colleagues describe a convincing association between the proportion of transitional B lymphocyte subsets in kidney transplant recipients and long-term outcomes, and present a biologically plausible mechanism, based on differential ability of T1 and T2 cells to regulate in vitro T cell responses to explain the link. Further work is clearly needed to validate their claim that measurement of T1/T2 ratios may represent a reliable and reproducible predictive biomarker of transplant outcomes.
The majority of kidney transplants fail before the end of the natural lifespan of the recipient; most because of immune mediated injury [1] and one of the major challenges facing clinicians involved in renal transplantation is how to improve long-term allograft survival. However, an incomplete understanding of the complex mechanisms driving immune-mediated damage and the difficulties of identifying patients at higher risk of graft loss hamper progress in this area. Coupled with the potential benefits of identifying those at low risk of graft failure, in whom the detrimental effects of excess immunosuppression might be avoided, progress in identifying new predictive biomarkers for allograft outcome is desirable.

Over recent years there has been an explosion of interest in the role and importance of B lymphocytes in transplantation, beyond their role in antibody production. It is now well recognised that they play a complex role in renal transplant recipients, capable of both promoting and regulating anti-donor T cell responses [2]. The precise phenotype of human regulatory B cells is still incompletely defined, but ‘transitional’ cells, which regulate via production of IL-10, are perhaps the most studied population [3]. Transitional B cells (immature B cells that have recently migrated from the bone marrow into the peripheral blood) are characterised by expression of high levels of IgM, IgD, CD24, CD38 and CD10. In this issue, Cherukuri and colleagues build on previous work and report that the relative proportions of T1 and T2 subsets, differentiated on the basis of the level of CD24 and CD38 expression, predict regulatory B cell activity and is significantly predictive of subsequent graft dysfunction [4].

This group has already contributed significantly to our understanding of transitional B cell biology after kidney transplantation. In previous work, they showed that multiple B cell subpopulations secreted IL-10 and were capable of suppression in vitro [5], but that non-transitional populations also typically secreted TNFα, which inhibited regulatory activity. The regulatory phenotype of healthy transitional B cells after polyclonal stimulation was therefore due to the uniquely polarised production of IL-10. However, transitional B cells from patients suffering graft rejection had a diminished ability to regulate in vitro T cell responses due to co-secretion of TNFα with IL-10 (rather
than a reduced ability to secrete IL-10). This ‘defect’ associated with poorer graft outcomes over 3 years.

In this new work, they study the relative proportions of transitional cell subsets in two independent populations of kidney transplant recipients; the first a group undergoing late ‘for-cause’ biopsy, and the second, patients with stable function two years post-enrolment to an induction trial comparing alemtuzumab with basiliximab.

In the first group of 45 patients, those with subsequent functional deterioration (defined as halving of eGFR or graft loss) were found to have a significantly lower frequency and absolute number of transitional B cells than those with stable function; more specifically there was a very significant decrease in the numbers of T1 cells, such that the T1/T2 ratio exhibited by those patients with deteriorating function was significantly lower than those with stable function. ROC curve analysis demonstrated that the T1/T2 ratio in peripheral blood collected at the time of for-cause biopsy strongly predicted the development of late allograft dysfunction and death-censored graft loss. Of particular interest is the detailed analysis of the 25 patients with biopsy-proven rejection, in whom the T1/T2 ratio strongly classified graft dysfunction compared to stable function, whilst the histological parameters based on BANFF scores lacked predictive capacity.

These findings were replicated in the second group of 97, in which patients were allocated to either a “stable” or “deteriorating” group according to their clinical progress over the subsequent five years. Again, patients with deteriorating function were found to have a significantly lower frequency and absolute number of T1 cells, with a lower T1/T2 ratio than patients with stable function. ROC curve analysis again confirmed the T1/T2 ratio to be a strong predictor of allograft function.

Subsequent multivariate Cox analysis showed the T1/T2 ratio was independently associated with declining allograft function in both cohorts of patients, whereas other parameters examined, including the presence of DSA and eGFR, were not able to predict late allograft decline.

To link these new data to their earlier work they show that the most immature T1 subset of transitional cells, characterised by the highest expression of CD24 and CD38, had the greatest
polarisation of cytokine production towards IL-10 after polyclonal stimulation, such that the IL-10/TNFα ratio was significantly higher in T1 compared to T2 cells. The impact of a low ratio of T1/T2 was therefore to reduce the regulatory ability of the whole transitional B cell population and this explains the ‘defect’ in B cell regulatory ability they previously associated with poor graft outcomes. Measuring the T1/T2 ratio of peripheral blood B cells by flow cytometry is clearly a more easily measurable predictive biomarker than measuring the cytokine secretion profiles of polyclonally activated B cells.

There are several potentially problematic issues with this work, which the authors discuss. First is the issue about distinguishing between T1 and T2 cells; the threshold of CD24 and CD38 expression used to define the two subgroups is somewhat arbitrary. However, the authors have attempted to establish a sound footing for their methodology, basing their flow cytometric gating strategy on earlier reports of B cell repopulation in patients treated with rituximab, in whom a discrete population of T1 cells repopulates peripheral blood from the bone marrow, followed by the appearance of T2 cells with distinctly lower expression of both markers. In healthy volunteers, these gates gave a T1/T2 ratio of approximately 1:3 and the same gates were applied to patient samples in a non-biased manner.

The second issue is that meaningful interpretation of the results also relies on the allocation of patients to either the “stable” or “deteriorating” groups, which rests on how deteriorating function is defined. Here the authors choose halving of eGFR as an established surrogate for the progression of kidney disease, or alternatively the harder endpoint of graft loss. They sought to address the recognised association between deteriorating renal function and overall numbers of B cells in the peripheral blood by examining the relationship between transitional cell subsets and eGFR; although a weak positive correlation was observed in the larger patient group, none was seen in patients undergoing for-cause biopsy. Furthermore the multivariate Cox proportionate hazard analysis, which
included eGFR as a covariate, demonstrated the T1/T2 ratio was independently associated with graft deterioration.

Finally, immunological biomarker discovery in renal transplant recipients is made more difficult by the potential confounding impact of immunosuppression on different cell populations [6]. These authors have previously reported higher numbers of B cells, including transitional subsets, in patients receiving induction alemtuzumab compared to basiliximab [7] and in this new data, twice as many patients with the highest T1/T2 ratios had received alemtuzumab. The authors were careful to show that, irrespective of their induction regimen, patients with a low T1/T2 ratio had a comparable degree of graft deterioration and change in eGFR. An additional consideration was that significantly more patients found to have a low T1/T2 ratio were taking maintenance prednisolone, but once again the authors do a careful subgroup analysis and show that the association of outcome with low T1/T2 ratio was independent of steroid use.

In summary, Cherukuri and colleagues propose that the relative proportions of transitional B cell T1 and T2 subsets in peripheral blood can serve as a biomarker for late graft deterioration in renal transplant recipients, with a plausible biological explanation for the association with graft outcomes, based on the functional differences between T1 and T2 cells at regulating T cell responses (Fig 1). Their findings are complementary to and build on their previous work, and are consistent with other recent work examining transitional B cells in the early post-transplant period [8, 9]. Of particular interest for future work will be understanding the dynamic changes in transitional cell subsets in individual patients and relating this to changes in eGFR, as well as understanding whether specific treatments influence the relationship between T1 and T2 subsets. Further study of transitional B cells to determine the generalizability of these observations, and to understand the importance of regulation of the anti-donor alloresponse by B cells is clearly required.
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References

