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Molecular phenotyping of T cell-mediated rejection

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Standfirst

A new study has reported a molecular signature of T cell-mediated rejection in human kidney transplant biopsy samples that is enriched for effector T cells, interferon- γ and macrophages. Inhibitors of T cell activation, such as CTLA4 and PDL1, were also prominent, raising the possibility that these immunological constraints could be harnessed by therapies for treating rejection.

Can molecular phenotyping of graft biopsy samples lead to more accurate diagnosis of underlying causes of renal allograft dysfunction than standard histological approaches? Studies over the past two decades have recognized substantial variance among pathologists in the histological diagnosis of rejection and the need for improved criteria to aid rejection diagnosis. To this end, a new study by Venner *et al.*¹ reports on the use of expression microarrays to identify a molecular signature in biopsy samples with pure T cell-mediated rejection (TCMR); this molecular signature differentiated samples with TCMR from normal biopsy samples and those with other diseases. An important strength of this study was the large number of graft biopsy samples (>700) analyzed for gene expression by microarrays. The study's results confirm that TCMR is associated with a gene signature that is enriched for T cells, activated antigen-presenting cells (APCs) and interferon- γ (IFN- γ). Interestingly a strong upregulation of both co-stimulatory and co-inhibitory molecules was observed. The finding that this molecular signature was highly conserved in TCMR and distinct expression profiles of other diseases represents an impressive step towards successful molecular diagnostics in renal allograft biopsy samples.

A number of reasons exist as to why it is important to correctly diagnose allograft dysfunction. Firstly, antibody-mediated rejection (ABMR) is now recognized as an important cause of graft loss.² Since the Banff Congress on Allograft Pathology in 2003, the criteria adopted for the diagnosis of ABMR has included complement C4d deposition in the microtubular capillaries, microcirculation damage, intra-capillary inflammation and the presence of donor-specific antibody (DSA). In the past few years, however, evidence has

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Competing interests

The authors declare no competing interests.

emerged that DSAs can induce morphologic and molecular features similar to that of ABMR in the absence of C4d deposition.³ In the international prospective INTERCOM study, the incidence of ABMR was reported to have been underestimated by approximately 50%.⁴ Thus, inaccuracies in the diagnosis of TCMR rejection, which may actually include mixed TCMR and ABMR rejection, are likely to have confounded past studies aimed at identifying a robust molecular signature of TCMR. Indeed, differences between TCMR and mixed rejection have been underscored in observations by Liarski *et al.*,⁵ who used computational tools to quantify the frequency of cognate B cell and follicular helper T (T_{FH}) cell interactions in renal biopsy samples. They observed that 80% of $CD4^{+}ICOS^{+}PD1^{+} T_{FH}$ cells conjugated with B cells in mixed rejection, whereas only 15% of T_{FH} cells conjugated with B cells in TCMR. Furthermore, T_{FH} cells in mixed rejection had elevated expression of B-cell lymphoma 6 (BCL6) and interleukin 21 (IL-21) than did T_{FH} cells in TCMR, providing evidence that pure TCMR has a distinct molecular signature from that of mixed rejection. Secondly, early diagnosis of TCMR will enable early therapeutic intervention and improved outcomes. Thirdly, the current association between DSAs and/or ABMR with a higher risk of renal allograft loss suggests a need for more effective therapies. Early and accurate diagnosis of TCMR, with or without ABMR, would aid in the identification of these therapies.

The study by Venner *et al.*¹ follows up on a 2013 study from the same group,⁶ which reported a molecular signature for TCMR that had a low sensitivity (50%) and positive predictive value (62%). The poor performance of this signature was explained by the researchers' findings that the Banff classification for TCMR was error prone, especially when biopsy samples were borderline or unreliable, and that biopsy samples with C4d-negative mixed rejection were often misdiagnosed as TCMR. Using a new classification of ABMR to more accurately identify biopsy samples with pure TCMR, Venner *et al.*¹ have now identified a robust molecular classifier for TCMR. Of 703 biopsy samples, 67 (10%) were classified as having TCMR and were compared to the other biopsy samples, which included 15% with ABMR, 4% with mixed rejection, 55% with other pathologies and 17% with no major abnormalities. The researchers developed a molecular classifier based on 30 transcripts in a discovery set (403 biopsy samples from 315 patients), which they confirmed in a validation set (300 biopsy samples from 264 patients). Of note, 19 of the 30 top transcripts differed between the two datasets; since the classifier transcripts were selected by a rank test of resampled data, which is essentially a nonparametric method, small differences in significance and fold-change between the discovery and validation sets can generate large differences in ranks, leading to the apparent lack of reproducibility. To develop clinically useful classifiers, it will be important to employ more robust approaches to data and pathway analyses.

Consistent with our understanding of TCMR, transcripts associated with T cells and T cell signalling, co-stimulation and co-inhibition, APC activation, and IFN- γ expression and effects, were most prominently upregulated. These TCMR-associated transcripts curated most strongly into three pathways: CD28-Cytotoxic T-lymphocyte protein 4 (CTLA-4) costimulation-coinhibitor pathways, T-cell receptor (TCR) signalling pathways and T-helper cell differentiation pathways. Weaker associations for inflammasome activation, innate

immunity pathways, cytotoxic molecules and parenchymal injury were also identified. Taken together, these data support a model of T cell infiltration and interaction with APCs within the graft, IFN- γ production by T cells and down-stream effects of IFN- γ modulation of macrophages, monocytes and dendritic cells in TCMR (Figure 1). The observation of a strong IFN- γ signal is particularly satisfying for basic transplant immunologists who have modelled acute rejection as a type 1 T helper (T_H1) cell process.⁷ As mentioned above, a notable finding is the prominence of T cell coinhibitory-related transcripts such as CTLA4, BTLA, PD-L1, LAG3,⁸ and other inhibitory molecules such as SLAM family member 8 (SLAMF8)⁹ and CD96.¹⁰ The conspicuous presence of these transcripts reinforce the concept that regulatory responses are indelibly linked to effector responses, but that these processes may nevertheless be insufficient to curtail the T cell-mediated responses released within the allograft during TCMR.

Although Venner et al.¹ linked the top 50 transcripts associated with TCMR to CD8⁺ or CD4⁺ effector cells, IFN- γ -treated macrophages, natural killer cells and B cells, and performed pathway analyses, in reality these data cannot differentiate whether the changes in transcript levels reflect changes in individual cells or an accumulation of specific T cell subsets. For instance, ICOS, CTLA4 or LAG3 may be upregulated in activated T cells, or they may reflect an accumulation of T_{FH} or regulatory T cells. Furthermore, the TCMR biopsy samples were compared with a pool of biopsy samples with multiple diagnoses, so additional studies are necessary to ascertain whether this molecular approach can indeed distinguish between the diagnoses of TCMR, ABMR and events mediated by non-allospecific processes. It is also important to note that the analysis of 'pure' TCMR versus 'pure' B cell-mediated rejection represent extremes in a continuum of rejections with different degrees of T or B cell contribution; so molecular diagnoses may in fact be more complex. Finally, the intragraft processes reflect, at least in part, events occurring in the peripheral immune system that are more readily accessible in animal models. Nevertheless, the strong conservation of the molecular associations with TCMR illustrates the potential utility of this approach, sets the stage for refining these signatures into a clinically applicable diagnostic test, and points to future studies aimed at more fully understanding the cellular and mechanistic bases for these signatures of TCMR in human renal recipients maintained on pharmacological immunosuppression.

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Biographies

Anita S Chong received her Ph.D. from the Australian National University, Canberra, (Australia), and is currently a Professor of Surgery at The University of Chicago (USA). Anita Chong's research focuses on the mechanisms of transplantation tolerance and how infections affects the acquisition and maintenance of tolerance. Her group is also investigating T and B cell-mediated immune responses in sensitized transplant recipients, as well as the utility of self-assembling nanoparticles as vaccine platforms.

Dr. David L Perkins received his M.D. from the Albert Einstein College of Medicine, and his PhD from Boston University. After a nephrology fellowship at Boston University and a post-doctoral fellowship at Massachusetts Institute of Technology, Dr. Perkins was a clinical and research faculty at Harvard Medical School and subsequently at the University of California at San Diego. He is currently a Professor of Surgery at the University of Illinois at Chicago, and his research focusses on the genomics of transplantation rejection, alloimmunity and inflammation.

Figure 1.

Model of TCMR Gene expression analysis suggests a model of TCMR in which effector CD4⁺ T cells engage in cognate interactions with DCs and possibly B cells, facilitating their differentiation into TH1 cells, which secrete IFN-γ to promote DC maturation, macrophage recruitment and CD8⁺ T effector cell differentiation. Some of the effector T cells may upregulate coinhibitory molecules; alternatively, TREG cells expressing these inhibitory molecules might accumulate in the rejecting allograft. Abbreviations: DC, dendritic cell; IFN-γ, interferon-γ; TCMR, T cell-mediated rejection; TCR, T cell receptor; TH1, type 1 T helper; TREG, T regulatory.