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EDITORIAL

Can We Predict Allograft Tolerance in Experimental Animal Models of Transplantation?

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Allograft tolerance has been successfully induced in many experimental animal models of transplantation.[1] Liver allografts are indeed spontaneously accepted in most mouse and rat strain combinations.[2,3] This contrasts with the difficulties of inducing transplantation tolerance in humans: aggressive conditioning is required to intentionally establish immunosuppression-free long-term graft survival in carefully selected recipients.[4,5] Human liver recipients stand out from the crowd because some patients spontaneously acquire a state of operational tolerance that allows them to safely discontinue all immunosuppressive drugs.[6] This phenomenon can occur in clinical kidney transplantation as well, but it is much more infrequent in kidney transplantation versus liver transplantation.[7] The immune characterization of operationally tolerant liver and kidney recipients has recently received substantial attention from the transplantation community. Operationally tolerant patients might exhibit a signature of tolerance that could potentially be useful for selecting recipients amenable to drug minimization or withdrawal. Furthermore, the elucidation of the molecular pathways associated with the operational tolerance phenotype could provide novel targets for therapy. Recent studies have shown that both liver and kidney recipients who are operationally tolerant exhibit distinct cellular and molecular signatures in their blood.[8-11] Furthermore, in liver transplantation, some of these biomarkers can actually be employed to predict the success of drug withdrawal protocols.[12] In light of the wide clinical implications of these findings, it is somewhat surprising to realize how little effort has been applied to translating them into clinically relevant experimental animal models. As a result, a number of questions that can be safely addressed only in experimental animal models and that would provide very valuable information for clinical transplantation tolerance research remain unanswered. For instance, do tolerant transplant animal recipients exhibit specific biomarkers that are reproducible across different strains and therapeutic strategies? What is the optimal biological specimen for identifying such biomarkers? Do they differ between naive animals and animals with a memory-biased T cell repertoire? Are biomarkers influenced by the quality of the transplanted graft? Finally, is it possible to identify biomarkers capable of predicting the establishment of allograft tolerance?

In this issue of *Liver Transplantation*, Xie et al.[13] report the results of an animal study designed to determine whether a predesigned set of intragraft gene expression markers is capable of discriminating between rejecting grafts and grafts that become tolerant. The investigators employed a mouse model of major histocompatibility complex–mismatched heterotopic heart transplantation in which rejection was averted and tolerance was induced by a short course of rapamycin treatment. An additional set of experiments employing a model of orthotopic liver transplantation in which grafts were spontaneously accepted in the absence of immunosuppressive therapy was also performed. Using a set of 22 genes selected on the basis of their involvement in immunoregulatory pathways, they then analyzed the

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intragraft gene expression differences between rejecting heart allografts, tolerant heart allografts, and tolerant liver allografts. As previously described,[14] before they were ultimately accepted, tolerant liver allografts underwent a phase of inflammation and tissue damage that was spontaneously resolved after several weeks. During this early posttransplant period, tolerant liver allografts up-regulated a set of 9 genes whose expression correlated with the degree of tissue damage, and they were also overexpressed in rejecting heart allografts. In contrast, the expression of 3 additional genes (fibrinogen-like protein 2, killer cell lectin-like receptor G1, and forkhead box P3) was increased only in tolerant liver and heart allografts and not in rejecting heart allografts. These genes could constitute early biomarkers of tolerance. Importantly, similar gene expression analyses were performed on spleen cells. Although the expression of some of the target genes changed in response to allografting, a tolerance-specific gene expression signature could not be identified in spleen cells. The authors concluded that intragraft analysis might be required to identify tolerance-related biomarkers.

This study has some methodological limitations. A group of rejecting liver allografts would have constituted an important group for comparison. In the absence of such animals, whether the identified genes are truly specific for liver tolerance cannot be unambiguously answered. In addition, the study would have greatly benefited from the use of a high-throughput transcriptional platform such as a microarray. This would have allowed the performance of an unbiased functional analysis and might have resulted in conclusions very different from those proposed here. After all, the major findings of recently reported studies on operationally tolerant human recipients would probably have gone unnoticed if such unbiased analytical platforms had not been employed.[8-11] Thus, whether tolerance-related biomarkers in experimental animal models can actually be identified at sites other than the graft itself remains to be more definitely established. Despite these limitations, the authors should be congratulated for conducting one of the few studies attempting to identify biomarkers of allograft tolerance in experimental animal models. Clearly, much more bedside-to-bench research remains to be done. First, animal models that better replicate the clinical situation should be established. For instance, the phenomenon of operational tolerance as described in clinical transplantation (ie, the successful discontinuation of conventional immunosuppressive drugs long after transplantation) has never been modeled in an animal setting. Second, animal models need to be systematically studied in ways that result in clinically relevant information. These kinds of experiments are urgently needed in order to reconcile the apparently discordant observations that have been derived from experimental animal models and studies involving operationally tolerant human recipients. Without these efforts, basic and translational research will fail to effectively address clinical questions, and costly human studies will continue to be performed without adequate background knowledge.[15]

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