A knock-out punch?

To the editor:

Earlier this year, we published our initial studies of renal xenotransplantation in baboons, using a line of α1, 3-galactosyltransferase (GalT)-knockout (GalT-KO) pigs as donors, and using a preparative regimen directed toward induction of T-cell tolerance through cotransplantation of vascularized donor thymus and costimulatory blockade with CD154-specific monoclonal antibody[1]. The survival of life-supporting renal xenografts was prolonged from a previous maximum of 30 days using the same regimen with Gal-positive donors[2] to more than 80 days without rejection using the new GalT-KO donors. Four animals survived longer than 50 days, two of which survived longer than 80 days. Even control animals that received the same immunosuppressive preparation but without concomitant thymus survived 20–34 days[1]. In contrast, Chen and colleagues report in this issue the survival of pig-to-baboon GalT-KO renal xenografts for only 8–16 days[3].

What are the reasons for this discrepancy and what can it teach us about the future of xenotransplantation? Chen *et al.* suggest that "further modification of the donors" may be required for successful xenotransplantation. Although further genetic modifications will undoubtedly be advantageous, the comparison of these two sets of results suggests to us instead that the use of a tolerance-induction regimen will be the best, if not the only, way to avoid the problem of humoral rejection.

Both sets of baboons had similarly low starting levels of non-Gal-specific antibodies, and both sources of GalT-KO donors seemed to be equivalent with regard to lack of Gal expression. Therefore, we expect that the antibodies causing rejection in the Chen studies were probably the result of new, T cell–dependent, pig-specific antibody responses. The immunosuppression that was used in these baboons, despite being directed predominantly at T cells, was apparently insufficient to prevent these responses. Our own control animals not receiving thymus cotransplants likewise developed non-Gal-specific antibodies and showed evidence for both cellular and humoral rejection[1]. The fact that our control animals survived for 20–34 days as compared to the 8–16 days achieved by Chen *et al.* suggests that our immunosuppressive regimen may be more effective in delaying T cell–dependent antibody responses, perhaps as a result of the use of costimulatory blockade with CD154-specific antibody. However, only the animals also receiving donor thymus avoided non-Gal-specific antibody responses and humoral rejection completely.

Contrary to the suggestion that our immunosuppression led to a "high complication rate and mortality," the fraction of animals dying from early complications seen in our studies was actually lower than that in Chen *et al.*, with only 3 of 14 recipients of life-supporting kidneys, with or without thymus, dying in less than 18 days[1]. This was also true for the 10 heterotopic GalT-KO heart xenotransplants also reported from our research center[4], for which no deaths attributable to complications of the chronic immunosuppressive regimen were observed, suggesting that the regimen might indeed be clinically applicable[4]. Although CD154-specific antibody is unlikely to be available for clinical use because of its tendency to cause vascular thromboses in large vessels[5], there are several other available reagents for costimulatory blockade that may be just as effective, should it turn out that such blockade is essential to induce tolerance.

In summary, although the Chen data are discouraging with regard to the likelihood of prolonging xenografts indefinitely with immunosuppression alone, they do not diminish our enthusiasm for the potential applicability of an approach combining the use of GalT-KO donors with tolerance induction.

David H Sachs

Department of Surgery, Harvard Medical School, and Transplantation Biology Research Center, Massachusetts General Hospital, Boston, Massachusetts 02129, USA.
e-mail: david.sachs@tbrc.mgh.harvard.edu

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