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Reply to the Editor:

We thank Manji and colleagues for their comments on our article, "Cardiac xenotransplantation technology provides materials for improved bioprosthetic heart valves." There is growing consensus that ectopic calcification, including calcification of bioprosthetic heart valves, may occur in response to local inflammatory reactions.¹ Our research, which builds on earlier fundamental findings that show glutaraldehyde fixed bioprosthetic materials are not totally immunologically inert,^{2,3} sought to identify a potential clinically relevant mechanism for such inflammation and suggests that anti-Gal antibody binding to bioprosthetic materials may be one such signal. As indicated in the article, our work is consistent with the clinical histopathology of explanted porcine valves derived from left ventricular assist devices and extends the earlier observations that antibody from sensitized serum could accelerate calcification.⁴ This work and our earlier study⁵ using Gal-positive and Gal-deficient fixed porcine pericardium show that natural

preformed anti-Gal antibody is sufficient to accelerate the calcification process in the subcutaneous implant model. This suggests, but does not conclusively prove, that a preformed anti-Gal antibody-mediated inflammatory response may occur soon after implantation and that such an antibody-mediated inflammation may initiate an innate immune response that furthers calcification of the device. The α 1,3-galactosyltransferase gene-knockout (GT-KO) pig, which makes anti-Gal antibody, is an appropriate model to test this hypothesis because implantation of Gal-positive bioprosthetic materials would represent an allogeneic implant that differs from the recipient only by the presence of α Gal sugar. Our results support the use of GT-KO tissues for valve development as a means to debulk the major antigen. It may also be of interest to determine if the early inflammatory response is blocked, whether other clinical modalities that block antibody-induced inflammation could further forestall degeneration of bioprosthetic heart valves.

In their letter, Manji and colleagues suggest that additional genetic modification of bioprosthetic materials, including complement regulatory proteins and antithrombotic functions, might be incorporated to further prevent valve degradation. We agree that incorporation of these functions in living tissues may help to prevent rejection of xenogeneic organs or tissues. The high level of glutaraldehyde fixation used in the preparation of bioprosthetic valves, however, would likely inactivate the function of these proteins, thereby eliminating any potential protection to the bioprosthetic valve.

We agree that under the current regulations the cost of bioprosthetic heart valves from genetically modified sources would likely be increased compared with wild-type pigs. It seems likely that the level of biosecurity, which significantly affects the cost of production, needed for fixed valve tissue would be lower than the high

level of biocontainment required by regulatory agencies to produce live tissues and organs for xenotransplantation. If genetically modified valves have greater durability, then the additional cost of the valve will be offset by the benefit to the patient and use in a potentially expanded younger patient population and might be further offset by future regulatory changes governing the disposition of genetically modified animal tissues.

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