Calcification of bioprosthetic heart valves is an unsolved problem ever since. Lim *et al.* [1] recently made another attempt to improve tissue performance by applying the novel fixation agent genipin. Numerous efforts of chemical tissue pretreatment have been tested, but up to now, the longevity of biovalves has not substantially increased, especially not in young recipients. Our group has shown that the implantation of biovalves causes an increase in anti-Gal immunoglobulin M and immunoglobulin G (IgG) titers in patients [2, 3]. Thus, we suggested a mechanism of chronic xenograft rejection and consecutive calcification via that anti-Gal response. The α-Gal barrier is known to prevent xenotransplantation from suitable animals to humans, because humans and old world monkeys are the sole mammals which produce anti-Gal antibodies in high titers and lack the α-Gal epitope [4]. Since recently, biovalve researchers perceive the problem of α-Gal antigenicity in glutaraldehyde-fixed biovalves, and α-Gal knockout pigs are proposed as a possible source for valve tissue [5, 6].

Lim *et al.* [1] delivered a concise report on the use of the cross-linking agent genipin in the pretreatment of bovine pericardium and evaluated calcification in a rabbit intramuscular transplantation model. They proved the superiority of genipin over glutaraldehyde to prevent tissue calcification in a clear sequence of experiments. However, their interpretation of the α-Gal barrier in their setup is disconcerting. They implanted bovine tissue into a rabbit muscle and measured anti-Gal IgG antibodies in plasma before, 12 and 60 days after implantation. Genipin-treated tissue recipients showed significantly lower anti-Gal IgG titers than glutaraldehyde-treated tissue recipients. Furthermore, they showed that decellularization of tissue before implantation abolished this titer increase. But rabbits do not have any anti-α-Gal antibodies, as they express the α-Gal epitope themselves; at least according to the present opinion. How are these results possible then? The authors do not give a clear statement; they indicate an explanation by not correctly citing Macher and Galili [4], saying 'the differences in the fine specificity of natural anti-Gal in various species may cause the multiple B-cell clones to produce anti-Gal antibodies which have specificities that differ slightly from each other, and thus recognize various facets of the a-Gal epitope in its three-dimensional form'. But Macher and Galili never mention 'various species', they merely speak of anti-Gal varieties among individuals. Do the authors suggest differing α-Gal epitopes among different species? This is unlikely, as the sequence homology of α1,3GT is very high among a wide range of species [4]. If uniform Gal epitopes are assumed, one would expect autoimmunologic affection in animals with elevated anti-Gal titers; did the transplanted animals show any signs of systemic inflammation?

Most of the researchers who measure anti-Gal antibodies use self-established ELISAs with internal or no standards. Such ELISAs require extensive titration steps to yield a reliable technique that is suitable for publication. Reevaluation and verification of the results of Lim *et al.* should be considered.

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LETTER TO THE EDITOR RESPONSE

Reply to Mangold and Ankersmit

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We appreciate the comments [1] of Drs Mangold and Ankersmit on our article [2]. Our group proved that genipin fixation is a novel alternative to conventional glutaraldehyde fixation, and the addition of decellularization, organic solvent treatment and detoxification prevent calcification of glutaraldehyde/genipin-fixed bovine pericardium. We also found that the titer of anti-α-Gal(Galα1-3Galβ1-4GlcNAc-R) antibodies (immunoglobulin G) increases after the implantation of bovine pericardium to rabbit, and *in vivo* calcification results are consistent with the increase in anti-α-Gal antibodies titer [2].

Although the cause and significance of the xenoreactive antibody response between the different species in α-Gal concordant animal model have not been exactly known, some possible suggestions may exist: (i) by a species-specific expression of these conjugated compounds including α-Gal epitopes; (ii) a tissue-specific expression of these conjugated compounds including α-Gal epitopes and (iii) the differences in the fine specificity of natural anti-Gal antibodies in various species which recognize various 'facets' of the α-Gal epitope in its three-dimensional form.

A species-specific expression of these conjugated compounds including α-Gal epitopes has been well demonstrated for so long. Thyroglobulin, fibrinogen and immunoglobulins from various species express varying amounts of Galα1-3Galβ1-4GlcNAc residues between 0.01 and 11 residues per molecule [3]. Rabbit red blood cells contain a range of glycolipids of varying lengths that terminate with the Galα1-3Galβ1-4GlcNAc-R structure. These glycolipids vary in size by increments of five monosaccharides, and each increment forms an additional branch. Although rat did not contain Galα1-3Galβ1-4GlcNAc neutral glycosphingolipids in the kidney, pig and sheep contained sialic acid-containing glycolipids (gangliosides) with the Galα1-3Galβ1-4GlcNAc-R structure, most likely the same branched, ganglioside identified in bovine red blood cells [4]. Thymus tissue from sheep, pig and rabbit also contained a range (compounds with 5–11 sugars) of neutral glycolipids terminating with the Galα1-3Galβ1-4GlcNAc-R structure demonstrating a species-specific expression of these compounds. Although the kidney and thymus from sheep, pig and rabbit express Galα1-3Galβ1-4GlcNAc-R glycolipids, these glycolipids were not detected in brain tissue demonstrating a tissue-specific expression of these compounds [5]. The detailed analysis of N-linked glycans from porcine kidney demonstrated that Galα1-3Galβ1-4GlcNAc-R termini were found on a variety of complex bi-, tri- and tetra-antennary N-glycans [6].

As we evaluated recently the immune response in the implantation of bovine pericardium to mice in which the α1,3-galactosyltransferase gene was knocked out ($Gal^{-}/^{-}$ mice), the titer of anti-α-Gal antibodies much increased. But interestingly, we also observed the increase in anti-α-Gal antibodies in wild-type mouse and rat as well as rabbit, although much less than $Gal^{-}/^{-}$ mice. We think that further transplantation or implantation experiments are needed to validate more exact xenoreactive immunologic responses between the different species.

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LETTER TO THE EDITOR

Endoscopic management of post-lung transplantation anastomotic stenosis: metallic, silicone or biodegradable stents

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