Xenograft rejection—all that glitters is not Gal

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Xenotransplantation is being developed in the hope of resolving the critical shortage of donor organs for transplantation. The Eurotransplant waiting lists [1] for donor organs of various kinds number almost 16,000 patients and the US lists [2] more than 90,000 patients. Renal transplantation, for instance, cost-effectively confers a significant survival advantage [3] and improvement of quality of life [4]. But whereas currently, in Europe, nearly 12,000 end-stage renal disease patients await a suitable donor, only 3383 kidney transplants were performed in 2005, with an average waiting time of 1174 days [1]. Substantial research efforts are being made in the field of xenotransplantation, and the immunological barriers are gradually being elucidated. Pig-to-human xenogeneic organ transplantation is considered the combination of choice for anatomical, physiological and biochemical reasons, and the use of porcine tissues is thought to confer reduced risks for cross species transmission of (viral) agents such as porcine endogenous retroviruses (PERV). Pigs are also easy to breed and their use as organ donors for humans is ethically more accepted than that of non-human primates [5]. However, the pig is immunologically discordant to human and non-human primates and, as a consequence, hyperacute rejection (HAR) and acute humoral xenograft rejection (AHXR) (also called acute vascular rejection) pose major hurdles to xenograft survival in experimental pig-to-baboon models. The HAR occurs within minutes as a result of antibody-mediated complement activation and is histologically characterized by widespread interstitial haemorrhage and thrombosis [6]. The putative antibodies are predominantly directed against Galα1,3Galβ1,4GlcNAc (αGal) carbohydrate residues, present on cell-surface glycoproteins and glycolipids of porcine tissue [7,8]. Primates lack the functional α1,3-galactosyltransferase (α1,3GalT) gene to synthesize αGal, and high titres of ‘natural anti-αGal antibodies’ arise early in life upon encounter of αGal-expressing micro-organisms in the gastrointestinal tract [9]. Strategies have been developed to prevent HAR and include the removal or inactivation of anti-αGal antibodies [10], the depletion or inhibition of complement, or alternatively, the production of donor pigs transgenic for either one or more complement regulatory proteins such as hDAF, MCP and CD59 [11]. However, these approaches have not been able to prevent the return of anti-αGal antibodies, but rather showed that when HAR was avoided, AHXR occurred [12]. This second form of humoral rejection develops within days and is mediated by elicited xenoreactive antibodies, directed to a large extent at αGal but also at non-αGal epitopes, and complement may also be involved [13]. With the successful development of α1,3GT−/− pigs, donor organs became available in which αGal epitope expression was completely eliminated [14,15], and it was hoped that the use of α1,3GT−/− donor organs would not only prevent HAR but also AHXR.

In January 2005, encouraging results were reported by Kuwaki and coworkers [16] and by Yamada and coworkers [17], who achieved prolonged survival of α1,3GT−/− porcine heart, respectively kidney grafts in baboons. Yamada and coworkers [17] performed concomitant recipient thymectomy and splenectomy, with vascularized xenothymus transplantation, T-cell depletion, CD154–blockade and treatment with mycophenolate mofetil, and achieved xenokidney graft survival of up to 80 days. Neither αGal-specific nor non-αGal-specific xenoreactive antibodies were detected, nor did kidney grafts show histological signs of rejection [17]. In the recent 2005 December issue of Nature Medicine, Chen and coworkers [18] equally studied α1,3GT−/− pig xenokidney graft survival in baboons, who were given a preclinical immunosuppressive regimen consisting of a short course of antithymocyte globulin (ATG), tacrolimus, mycophenolate mofetil and steroids, or a single high dose of ATG with subsequent tacrolimus monotherapy. Although HAR was prevented, graft survival was...
limited to 8–16 days, signs of AHXR were found in all rejected grafts, and although anti-αGal antibodies remained absent, an increase was noted in anti-non-αGal antibodies [18].

The confirmation by Chen and coworkers [18] that z1,3GT−/− pig organs are susceptible to AHXR does not come as a surprise. Whereas anti-αGal antibodies are known to monopolize HAR and to play a major role in AHXR, other xenoreactive antibodies, both natural and elicited, are known to contribute to AHXR [13]. The vigour of AHXR, as it occurs in discordant models of xenotransplantation such as hamster-to-mouse or rat, clearly illustrates the significance of elicited xenoreactive antibodies in xenograft rejection [19]. Neither does the report by Chen and coworkers seem to conflict with the two earlier reports. The difference in results probably rather relates to the nature and intensity of the immunosuppressive regimen given. In fact, in the study by Kuwaki et al. [16] HAR was circumvented by combining thymic irradiation, T-cell depletion, cobra venom factor, mycophenolate mofetil, methylprednisolone and anti-CD154 monoclonal antibody therapy, establishing xenogeneic graft survival of up to 179 days. But IgG deposition was noted in some grafts, suggesting a low-level production of xenoreactive antibodies. Moreover, following removal of grafts and tapering of immunosuppression, significant rises in anti-non-αGal antibodies were found, indicating that the level of immunosuppression plays a major role.

It is well-known that successful xenotolerance induction will need to rely on B and T cell tolerance [20,21]. With this respect, it has been shown that anti-αGal antibodies are produced by Mac-1− splenic B1b-like B lymphocytes [22] and depend on T-cell-independent and T-cell-dependent mechanisms. Our studies in models of concordant xenogeneic transplantation in T-cell-deficient nude mice suggest that elicited anti-non-αGal antibodies are produced by marginal zone B-cells, and require ancillary help from NK1.1+ cells through a CD40L-dependent mechanism [23]. These data may explain why splenectomy and/or anti-CD154 monoclonal antibody-treatment can prevent the occurrence of anti-non-αGal antibodies in the studies by Yamada et al. [17] and Kuwaki et al. [16].

In conclusion, although αGal is a dominant determinant of xenograft immunogenicity, it is clearly not the only one. Where the first studies with z1,3GT−/− donor organs in baboons have shown that elimination of αGal represents a major step forward in graft survival, they also show that successful application of xenotransplantation will still require induction of robust T- and B-cell xenotolerance. Studies using heavy immunosuppressive regimens, induction of mixed chimerism, or xenothymus transplantation have delivered proof of concept of xenotolerance induction, but continued research is needed to develop clinically applicable approaches.

Both anatomically and functionally, pig kidneys are similar to human kidneys [24], and the studies discussed above showed kidney xenografts to be life-supporting for up to 80 days [17,18], supporting the previous observation that they can indeed adequately sustain water and electrolyte homeostasis in primates [25]. Physiological incompatibilities, however, do exist. The finding that baboons transplanted with porcine kidneys required blood transfusions suggest that porcine erythropoietin might not be effective in sustaining red blood cell production in primates [25]. Furthermore, incompatibilities in the coagulation pathway [26] and renin-angiotensin-aldosteron pathway [27] have been demonstrated. Tissue injury from rejection, rather than intrinsic, interspecies differences in renal physiology is thought to be the major factor limiting kidney xenograft function [28], but as the immunological barriers are being elucidated, the importance of non-immunological incompatibilities become increasingly apparent, and will need to be addressed in future studies.

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