MECHANISM OF REJECTION IN ORTHOPAEDIC XENOGRAFTS

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INTRODUCTION: Although several xenograft bioscaffolds are commercially available for musculoskeletal reconstruction, few studies have addressed the host antibody reaction to a xenogeneic cell and matrix surface carbohydrate antigen called the ∝-galactosyl epitope (α-Gal). Humans and Old World primates lack the α-Gal epitope, but all other mammals produce and incorporate α-Gal epitopes into cellular and extracellular structures using the ∝1,3-galactosyl transferase enzyme [1]. Humans and Old World primates continuously produce anti-Gal antibodies constituting about 1% of circulating immunoglobulins, and are therefore not immunotolerant towards grafts presenting with α-Gal epitopes. Therefore, previous studies where grafts were tested in lower order species did not show the rejection response [2]. To date, no decellularization, washing or sterilization processing technique has been shown to remove the Gal epitope leaving xenografts with short residence times when transplanted to humans. Our previously reported studies have characterized the immunological response elicited by porcine tissues and examined ∝-Gal epitope enzymatic cleavage methods as applied to porcine cartilage grafts [3]. The current study was performed to prove efficacy of ∝-Gal epitope depletion to avoid immunological rejection in a rhesus ACL reconstruction model, as a precursor to a human xenograft ligament reconstruction study [4].

MATERIALS AND METHODS: A unilateral rhesus ACL reconstruction model was implemented using 20 rhesus monkeys with two, six and twelve-month sacrifice time points with clinical, serological, histological and biomechanical evaluations. This report will review immunological findings. Three animals were reconstructed with treated grafts for the 2-month cohort , with three and five at 6 and 12 months respectively. Controls consisted of one untreated and one rhesus allograft at two-month and five rhesus allograft s at 12-months.

Patellar tendons were harvested from fresh frozen porcine stifles, thawed and pulse lavaged to remove cellular components. Removal of the ∝-Gal epitope by enzymatic treatment using recombinant ∝-galactosidase was verified by enzyme-linked immunosorbent assay (ELISA) with an ∝-Gal specific antibody and homogenized treated porcine tendon as solid phase antigen as previously reported.[3] Cross-linking of the devices was accomplished with 0.10 % glutaraldehyde for 12 hours followed by a glycine endcapping to block un-reacted glutaraldehyde molecules. The final packaged devices were terminally sterilized by electron beam irradiation at 17.8 kGy (validated sterility assurance of 10^{-6}) and stored frozen at -70°C until use. The test and control devices were pre-fabricated into 30mm long by 4mm wide tendon grafts with proximal 5mm diameter by 7mm in length bone plug ends. Final test articles were rinsed and wrapped in sterile gauze with 0.1% Bacitracin until implantation.

Blood samples were taken pre-surgically and on days 10, 14, 21, 28, 42, 56, and at 3, 6, 9 and 12 months for analysis of anti-Gal and anti-non-Gal antibodies (i.e. antibodies to pig tendon proteins).

Serum anti-Gal IgG and IgM activity was determined by ELISA, using standard methods. ELISA wells were coated with synthetic ∝-Gal epitopes coupled to bovine serum albumin (∝-Gal-BSA) as the solid phase antigen and blocked with 1% BSA solution in carbonate buffer. Primate sera at serial two-fold dilutions in PBS with 1% BSA was then incubated for 2-hours at room temperature. Peroxidase coupled rabbit anti-human IgG or IgM were used as secondary antibodies incubated in wells for 1-hour. The titer increase was determined by matching the initial pre-implant absorbance value to a post-implantation absorbance dilution value. The effective increase is expressed as a multiple or "x" fold increase over pre-implantation titer and additionally in reciprocal titer at half-maximal binding.

Serum anti-non-Gal activity was determined using homogenized treated porcine patellar tendon as the solid phase antigen. This assay system monitors the production of antibodies to pig tendon antigens other than the ∝-Gal epitope. Porcine tendon homogenates were brought to 200 ug/mL in carbonate buffer, pH 9.5, and plated in 50ul aliquots in ELISA wells. After drying for 24 hours the implant fragments adhere strongly to the wells. The dried ELISA plates were blocked with 1% BSA in carbonate buffer. The sera were depleted of any anti-Gal activity by adsorption to 30% rabbit red cells (RRBC) (vol/vol) for 1 hr at 4°C. Subsequently, serum samples in two-fold serial dilutions were measured for IgG or IgM binding to the treated tendon. As with the anti-Gal assay, the labeled secondary antibody was a horseradish peroxidase-conjugated rabbit anti-human IgG. Antibody binding to tendon homogenate was determined by evaluation of titers similarly to anti-Gal response.

RESULTS: Post-process analysis of treated grafts showed an effective removal of cellular debris, as assessed by embedding, sectioning and photo-microscopy and a two-log efficacy in epitope cleavage as assessed by immunoassay.

Post-implantation anti-Gal titers were attenuated by greater than 95% as compared to untreated porcine grafts (Fig.1). This result confirms previously published studies, and supports the efficacy of ∝-galactosidase in epitope cleavage and attenuation of immunological recognition. Anti-Gal titers resolve to pre-implantation range by 8 to 12 weeks post-implantation. The robust anti-Gal response to untreated porcine tissue, indicating acute rejection, resolves only in coordination with a rapid destruction and resorbtion of the graft. Anti-non-Gal titers were comparatively minimal and yielded no adverse hematological or systemic changes. Additionally, anti-Gal and anti-non-Gal IgM titers were monitored, with only nominal changes observed (data not shown).

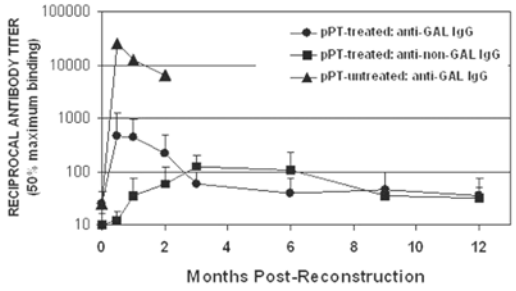

Figure 1. Anti-Gal and anti-non-Gal IgG titers for 13 rhesus implanted with untreated and treated porcine grafts for ACL reconstruction. Error bars represent standard deviation of reciprocal titers (N=13).

DISCUSSION: Two-month host cellular response to the enzymatically treated graft were comparable to the rhesus allograft exhibiting no signs of immunological rejection The two-month time point in this study was chosen to attain short-term comparative local and systemic host response to untreated and treated porcine tissues as compared to rhesus allograft. Histological and serological results support an acute humoral and local immunologically mediated rejection of the untreated porcine graft.

Functional patency of the ∝-Gal depleted and mildly cross-linked porcine grafts continued through the 6 and 12-month time points and was histologically and biomechanically similar to rhesus allograft. These results parallel 5-year findings from current studies in human ACL reconstruction with the immunochemically modified device.

Although a discrete rejection threshold level of galactosyl epitopes has not been derived for graft materials, a clear relationship exists between the presentation of galactosyl epitopes, resulting immunological rejection and limited functional integrity of xenograft materials. These findings may explain the the short term response and variable long term viability of current xenogeneic materials for orthopaedic applications. The use of enzymatic depletion of the gal epitopes provides a methodology for eliminating the rejection response.

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