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IMPROVED SURGICAL TECHNIQUE FOR THE ESTABLISHMENT OF A MURINE MODEL OF AORTIC TRANSPLANTATION

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Aortic allotransplantation is a reliable procedure to study the evolvement of chronic rejection in mice. The progressive nature of this process in mice is characterized by diffuse and concentric myointimal proliferation which is inevitably associated with variable degrees of luminal constriction. These vascular changes are comparable to those that are witnessed in organ allografts undergoing chronic rejection in humans, underscoring its utility as a model of choice for the study of the development of this lesion. Whilst improved surgical technique has resulted in markedly enhanced graft survival, the results are far from being acceptable. Realizing this limitation, we embarked on developing a modified technique for aortic transplantation which would allow for improved graft survival in mice. A bypass conduit was created by end-to-side anastomosis of a segment of the donor's thoracic aorta into the infrarenal portion of the recipient's abdominal aorta. Using this technique, the graft survival was >98% with evidence in allotransplanted aorta of morphological changes pathognomonic of chronic rejection. On the contrary, no histopathological anomalies were discerned in aortic grafts transplanted across syngeneic animals. This modified surgical approach ameliorates the unacceptably high graft loss associated with earlier techniques, further extending the utility of this model as a tool to study the molecular and cellular mechanisms rudiment to the evolvement of chronic rejection.

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The advent of increasingly more potent immunosuppressive agents has resulted in marked reduction in the impending peril of organ allograft failure as a consequence of acute cellular rejection. However, despite this remarkable accomplishment, delayed graft loss due to chronic rejection still poses a formidable barrier to prolonged organ survival.[1,2] Although amelioration of acute rejection has been shown to partially obviate the development of chronic rejection,[3] it is still indeterminate as to why the prolonged use of immunosuppressive therapy fails to avert the subsequent evolvement of this disease.

Despite these foregoing arguments, it is evident that the arteriosclerotic changes pathognomonic of chronic rejection have an immune etiopathology.[4] The absence of obliterative bronchiolitis in lung recipients who had evidence of donor cell chimerism with concomitant immune modulation[5] and the retention of normal vascular morphology in heterotopically transplanted hearts following induction of donor-specific tolerance[6] provide unequivocal support to this tenet.

Aortic allograft transplantation as a model to study the evolvement of chronic rejection has been used in mice.[7] However, despite its obvious advantage, the widespread utility of this model has been curtailed by the unacceptably high incidence of graft loss associated with this procedure. In this study, which was initiated to investigate the consequence of liver-induced tolerance on chronic rejection, we illustrate the development of a modified surgical technique which markedly improves graft survival with resultant morphological changes considered to be a hallmark of chronic rejection.

MATERIALS AND METHODS

Animals

Adult male mice weighing 29–32 g were purchased from Jackson Laboratories (Bar Harbor, ME). C57B1/10J (H-2^b) were used as donors and C3H (H-2^k) as recipients of aortic allografts (n = 230); these animals possess disparity

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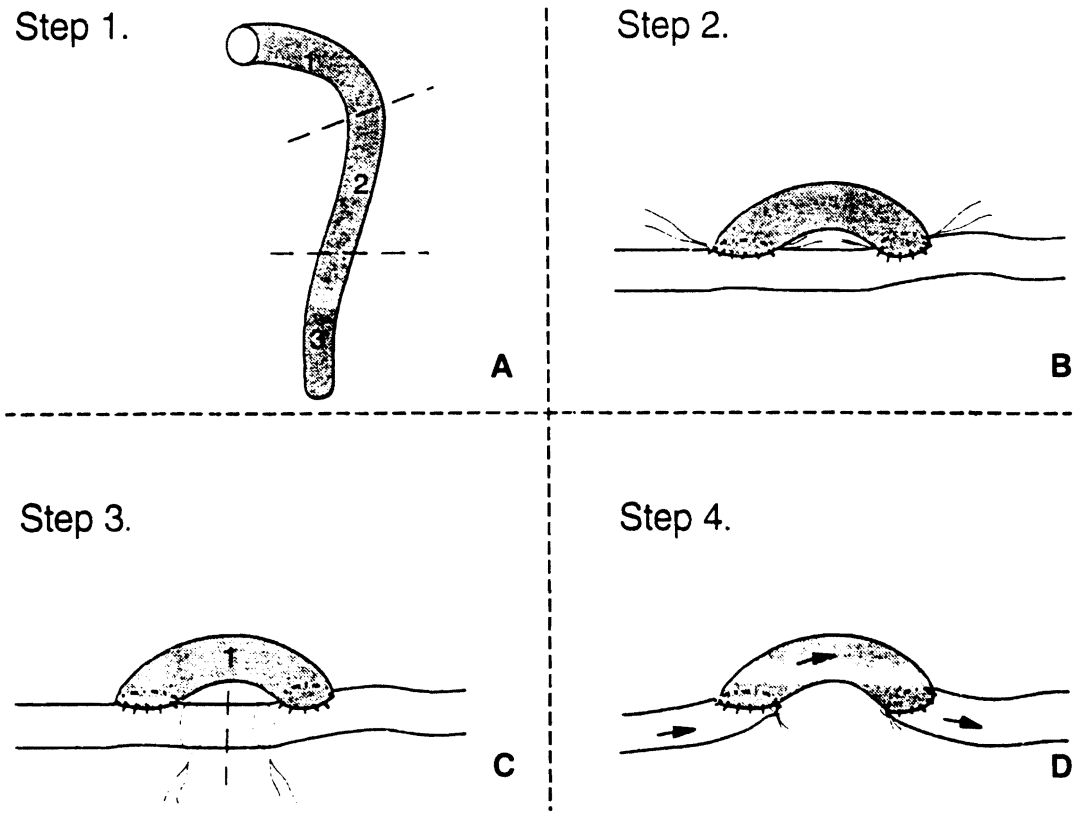

Figure 1. Schematic representation of the modified method of end-to-side aortic transplantation in mice. ***A:*** *Harvested donor thoracic aorta was divided into three segments of commensurate length.* ***B:*** *Creation of an end-to-side anastomosis in the infrarenal segment of the recipients' abdominal aorta which had been previously clamped both proximally and distally.* ***C:*** *Ligation of the recipients' aorta between the two anastomotic sites and its subsequent severance.* ***D:*** *Release of the clamps and assurance of normal blood flow through the "by-pass" conduit. Shaded: Donor aorta. Unshaded: Recipient's native aorta.*

at MHC class I, II and at the minor histocompatibility loci. Transplantation of aorta across syngeneic (C3H; n = 20) animals served as controls. No exogenous immunosuppressive drug therapy was employed. The animals were maintained in a specific pathogen-free environment at the University of Pittsburgh vivarium and provided with water and chow ad libitum.

Aortic Transplantation

A segment of donor's thoracic aorta was harvested and anastomosed to the infra-renal portion of the recipient's abdominal aorta. Because of the obvious discrepancy in size between the thoracic and the abdominal segments of the aorta, an end-to-side surgical approach was selected in contrast to the end-to-end technique described by Koulack et al.[7] All operative procedures were executed under inhalation anesthesia (Methofane®, Methoxyflurane, Pitman-Moore Inc., Mundelein, IL) using an operating microscope (M-3Z, Leica, Switzerland).

Donor surgery. The entire procedure was performed according to a technique described elsewhere[7] with a single exception; both the ascending and descending parts of the thoracic aorta were harvested and divided into three segments of equivalent length (0.6 cm) for subsequent transplantation (Fig. 1A).

Recipient surgery. Subsequent to the exposure of the abdominal cavity by a transverse incision, the segment of the aorta between the left renal artery (proximally) and its bifurcation (distally) was isolated from the adjacent inferior vena cava. Clamps were applied at both ends of the sequestered vessel and two aortotomies 0.2 cm apart were performed using curve Mini-Vannas style iris spring scissors (Fine Science Tools Inc., Foster City, CA). The magnitude of these incisions was determined by the diameter of the harvested donor aorta. An end-to-side anastomosis was then established between the donor and recipient vessels, respectively, using a running 10-0 polybutester suture (Novafil, Davis+Geck Inc., St. Louis, MO) at ×25 magnification (Fig. 1B). The segment of the native abdominal aorta between the anastomotic sites was ligated at both ends and severed in the middle (Fig. 1C) thus converting an end-to-side to a quasi end-to-end anastomosis (Fig. 1D). At the end of this procedure, the clamps were removed; patency of the transplanted graft was confirmed and blood supply to the pelvis and

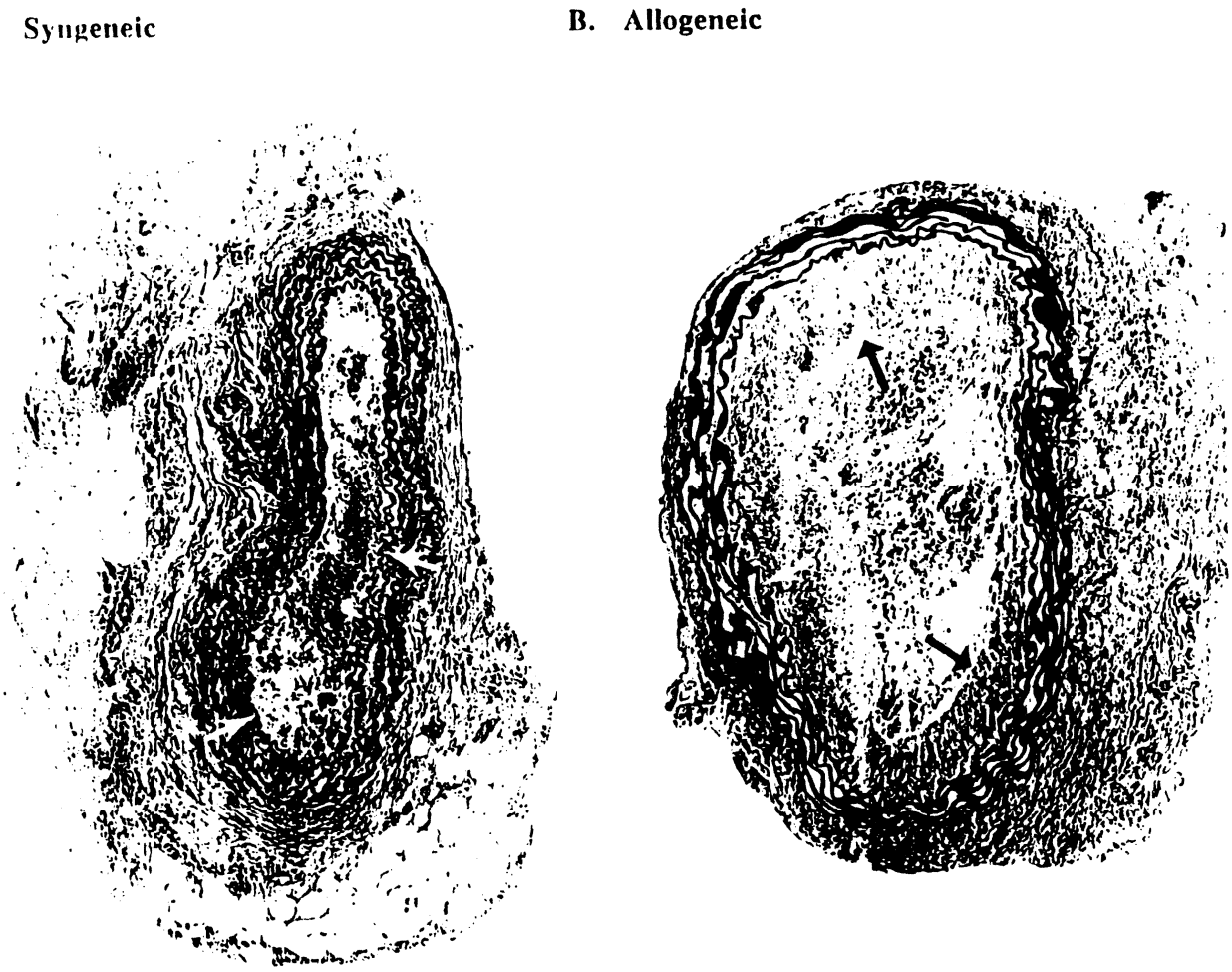

*Figure 2. Staining for elastic fibers in cross-sections of transplanted aorta using Verhoeff-van Gieson's stain. The graft was harvested at day 50 following transplantation across **(A)** syngeneic (C57B1/10 → C57B1/10) and **(B)** allogeneic (C57B1/10 → C3H) mouse strain combinations. Whilst no morphological changes were evident in aorta obtained from syngeneic recipients (A), those harvested from allogeneic hosts (B) had evidence of intimal thickening and luminal narrowing. White arrows: Internal elastic limiting membrane. Black arrows: Thickened intima. Magnification ×100.*

lower extremities was restored. The abdominal incision was closed with two layers of running 3-0 Dexon® sutures (Davis+Geck, Inc., St. Louis, MO).

Histopathological examination. Under anesthesia and at variable times postaortic transplantation, the abdomen was exposed and after visual examination the segment of the grafted aorta between proximal and distal sutures was harvested. The graft was subsequently divided into three segments of commensurate length, one of which was fixed in 10% neutral buffered formalin for 2–3 days and consequently processed and embedded in paraffin. Remnants of the 10-0 suture used for anastomosis were employed to orient the specimen in a vertical position at the time of embedding, thus facilitating cross-sectioning. Sections (4-μ-thick) were obtained using a microtome (American Optical, Buffalo, NY) and subjected to Verhoeff-van Gieson's stain using a method described previously.[8] The ensuring morphological examination was performed using an Olympus Microscope (Model BX40).

RESULTS

Graft Outcome

Over 250 aortic transplants (allogeneic n = 230; syngeneic n = 20) have been performed using the end-to-side technique. Precluding the initial technical difficulties, the overall graft survival rate has been >98%. This is unlike what has been reported previously, where even after the acquisition of experience with 150 aortic transplants the success rate was only 74%.[7] The only complication that contributed to the infrequent graft attrition in our studies was thrombosis. Interestingly, this was also the major cause for transplant failure in end-to-end anastomosis reported by Koulack et al.[7]

Histopathology

Aortic grafts implanted across syngeneic barriers do not develop any morphological aberrations (Fig. 2A) as compared to that to normal aorta when examined at variable times during the course of follow-up (for up to 120 days post-transplantation). Contrarily, allotransplantation of aortic grafts precipitated a self-limiting cellular infiltration which resolved at day 7 postimplantation with little or no evidence of residual damage. However, all allografts obtained at day 50 post-transplantation exhibited morphological changes pathognomonic of chronic rejection (Fig. 2B). There was evidence for progressive luminal narrowing, myointimal thickening and patchy destruction of internal elastic limiting membrane which resulted in complete occlusion of the vessel by day 120 post-transplantation. The observed arteriosclerotic changes involved the entire length and the circumference of the aorta, a finding consonant with that witnessed in the vasculature of human organ allografts undergoing chronic rejection.

DISCUSSION

Our inability to attenuate or abrogate chronic rejection with currently available therapeutic modalities has mandated an urgent evolvement of a strategy which would allow for reprieve from the debilitating outcome of this process. Given its immune etiopathology,[4] we have argued that perhaps prior induction of tolerance may assist in alleviating this disease. Having previously documented the establishment of donor-specific tolerance by orthotopic liver allotransplantation,[9] we proceeded to ascertain its role in mitigating the process of chronic rejection in mice. Towards the realization of this goal, we have developed a modified technique for aortic transplantation in mice which results in >98% graft survival. The progressively worsening histopathological changes in aortic allografts are harmonious with those that are witnessed in organs undergoing chronic rejection in humans. Contrarily, no morphological anomalies were discerned in grafts transplanted across syngeneic barriers. These observations underscore the utility of this model towards the elucidation of mechanisms involved in the development of chronic rejection. The rationale for improved graft survival using this technique is not entirely distinct. However, the obvious disparity in size between the thoracic and abdominal segments of the aorta may predispose to the increased incidence of thrombosis observed in aortic grafts transplanted using an end-to-end technique, an incongruity obviated by the use of an end-to-side anastomosis. It is our contention that with the establishment of this model and the ever increasing availability of immunological and molecular tools for studies in mice, a more comprehensive understanding of this disease process should now be possible.

CONCLUSION

The considerably improved graft survival obtained by the utilization of this modified technique for aortic transplantation in mice has extended the usefulness of this model to study the process of chronic rejection. The observed histopathological changes are in concert with those witnessed in humans, providing a unique opportunity to initiate intervention studies with novel drugs and other reagents for the prevention and/or reversal of this disease.

REFERENCES

1. Azuma H, Tilney NL: Chronic graft rejection. *Curr Op Immunol* 6: 770–776, 1994.
2. Billingham ME: Pathology and etiology of chronic rejection of the heart. *Clin Transplantation* 8:289–292, 1994.
3. Geerling RA, de Bruin RW, Scheringa M, et al.: Suppression of acute rejection prevents graft arteriosclerosis after allogeneic aorta transplantation in the rat. *Transplantation* 58:1258–1263, 1994.
4. Paul LC: Immunobiology of chronic renal transplant rejection. *Blood Purification* 13:206–218, 1995.
5. Keenan R, Zeevi A, Banas E: Microchimerism is associated with a lower incidence of chronic rejection after lung transplantation. *J Heart Lung Transplant* 13:S32, 1994.
6. Murase N, Starzl TE, Tanabe M, et al.: Variable chimerism, graft-versus-host disease, and tolerance after different kinds of cell and whole organ transplantation from Lewis to brown Norway rats. *Transplantation* 60:158–171, 1995.
7. Koulack J, McAlister VC, Giacomantonio CA, Bitter-Suermann H, MacDonald AS, Lee TD: Development of a mouse aortic transplant model of chronic rejection. *Microsurgery* 16:110–113, 1995.
8. Bradbury P, Gordon KC: Connective tissue and stain, in Bancroft JD, Stevens A (eds): *Theory and Practice of Histological Techniques*. New York, Churchill Livingston Press, 1990, p 119.
9. Qian S, Demetris AJ, Murase N, Rao AS, Fung JJ, Starzl TE: Murine liver allograft transplantation: Tolerance and donor cell chimerism. *Hepatology* 19:916–924, 1994.