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Donor-Specific Transfusion in the Nude Rat Prolongs Survival of Subsequently Transplanted Hamster Cardiac Xenografts

L.A. Valdivia, H. Sun, A.S. Rao, M. Tsugita, C.T. Chen, I.-Y. Park, J.J. Fung, and T.E. Starzl

XENOGRAFT TUMORS have indefinite survival when transplanted into athymic nude rats or mice. However, nude rats reject vascularized hamster cardiac xenografts in tempo (3 to 4 days) similar to that of immunocompetent rats.[1–3] This "slow-motion" hyperacute rejection witnessed in the nude rat is thought to be mediated by a T cell–independent, but antibody- and complement-dependent mechanism(s).[1] In this study, we investigated whether prior exposure of nude rat recipients to hamster xenoantigens in the form of donor-specific transfusion (DST), would modulate the humoral response thus allowing for prolonged survival of subsequently transplanted cardiac xenografts.

MATERIALS AND METHODS

Golden Syrian hamsters were the donors and National Institutes of Health (NIH) athymic nude (rnu/rnu) rats were the recipients of heterotopic cardiac xenografts. Naive Lewis (LEW) rats were used as immunocompetent control recipients. LEW as well as nude rat recipients were pretreated with 1 mL (intravenous [IV]) of heparinized hamster whole blood 7 days before cardiac xenotransplantation. Xenoantibody titers were determined in serum samples of the recipients by a complement-dependent cytotoxicity assay. The quantity of both rat immunoglobulin M (IgM) and IgG was measured using a sandwich enzyme linked immunosorbent assay (ELISA). To ascertain the role of exogenously administered anti-hamster antibodies, 2 mL of decomplemented serum obtained from LEW rats which had on day 3 rejected a hamster heart xenograft were injected (IV) into DST-treated nude rat recipients on day 1 after heart transplantation.

RESULTS AND DISCUSSION

Pretreatment of LEW rats with DST reduced survival of hamster cardiac xenografts from the usual 3 days to less than 10 minutes (n = 5, Fig 1). On the contrary, DST in prospective nude rat recipients did not precipitate hyperacute rejection (HAR), but it instead resulted in marked prolongation of xenograft survival to a median of >100 days (n = 5). Although cellular infiltration was not observed, long-surviving cardiac xenotransplants showed signs of chronic vascular rejection evidenced by marked intimal thickening with varying severity of luminal obliteration. Furthermore, the loss of heart xenografts due to HAR in LEW (but not nude) rats pretreated with DST was associated with the increase of donor-specific IgM in the recipient's serum (232 ± 5 ng versus 64 ± 2 ng) with its marked

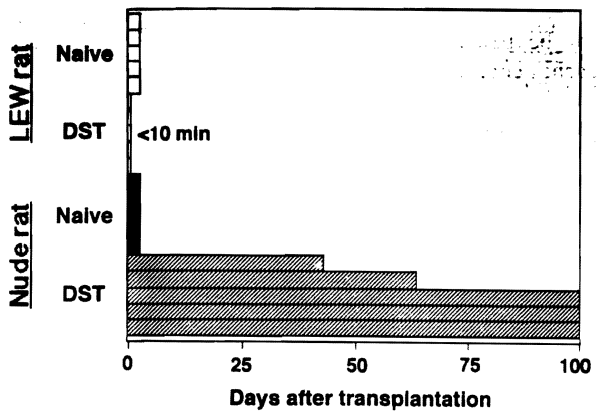

Fig 1. Effect of DST on survival of hamster cardiac xenografts transplanted into immunocompetent LEW or T-cell deficient nude rats.

deposition on the graft endothelium when tested on the day of heart transplantation (day 0). On the contrary, IgG response was minimal or absent throughout the observation period. The presence of cytotoxic antibodies in DST-treated nude rat recipients on day 0 was markedly diminished attaining only the level of preformed xenoreactive natural antibodies present in naive LEW rats (1:32). This observation contrasted with that of rats in whom increased xenoantibody titers (>1:1024) were witnessed. To ascertain that the immunomodulatory effect of DST in the nude rat was due to decreased circulating levels of xenoantibodies, we performed serum transfer experiments on posttransplant day 1. The administration of decomplemented anti-hamster antibody (IgM) into DST-treated nude rat recipients precipitated HAR (<10 minutes; n = 5) of previously transplanted hamster hearts, indicating that, with the ex-

From the Thomas E. Starzl Transplantation Institute and the Departments of Surgery (L.A.V., H.S., A.S.H., M.T., C.T.C., I.-Y.P., J.J.F., T.E.S.) and Pathology (A.S.R.), University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania.

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Address reprint requests to Luis A. Valdivia, MD, PhD, Thomas E. Starzl Transplantation Institute, E1546 Biomedical Science Tower, 200 Lothrop St, Pittsburgh, PA 15213.

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ception of lower antibody titers, the immunological machinery in the nude rat that mediates humoral rejection is uncompromised.

These experiments have emphasized the importance of T cell help for optimal xenoantibody production that ultimately leads to HAR of organ xenografts. Of greater significance is the possibility that T cell–independent IgM responses to xenografts, which are considered to be the main barrier to successful xenotransplantation, could be modulated by DST in immunocompetent hosts with simultaneous administration of T cell–directed immunosuppression. Furthermore, the lack of cellular infiltrate in long-surviving cardiac xenografts in the nude rat indicates that T cells are the main effectors in this concordant xenograft combination. This observation provides little support to recently published reports suggesting a seminal role for natural killer (NK) cells in initiation of xenograft rejection.[4] Despite increased NK cell activity in the nude rat, prolonged survival of cardiac xenografts was witnessed in DST-treated animals, further challenging the role of NK cells in xenograft destruction. Theoretically, the lack of IgG production in nude rat recipients would critically impair antibody-dependent cell-mediated cytotoxicity propitiated by NK cells which are known to bind to the Fc portion of this immunoglobulin for mediation of their actions.[5] There is nevertheless the possibility that NK cells may kill targets directly without the need for IgG antibodies. We are currently investigating the role of NK cells in this model.

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