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Soluble donor MHC class I gene transfer to thymus promotes allograft survival in a high-responder heart transplant model

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Abstract Thymic selection of self and non-self-reactive lymphocytes is a process that may be targeted to induce donor-specific immunologic unresponsiveness in organ transplantation. In the present study, gene transfer was used to preexpose the recipient thymus to soluble donor-specific MHC class I molecules prior to heart transplantation in the high-responder ACI ($RT1^a$) to Lewis ($RT1^l$) rat strain combination. Specifically, cultured Lewis hepatocytes were transfected with DNA encoding a secreted form of the donor allo-MHC class I antigen, $RT1.A^a$. Seven days prior to ACI heart transplantation, genetically altered recipient-strain hepatocytes were injected into the thymus of Lewis recipients which also received a dose of antilymphocyte serum (ALS). Results showed that treatment with both ALS and soluble donor MHC-expressing hepatocytes prolonged transplant survival time by twofold, compared to injection of control hepatocytes and ALS. Therefore, intrathymic gene therapy delivery of soluble donor MHC molecules may be useful for promoting allograft survival in heart transplantation.

Key words Thymus · MHC antigen · Heart transplantation · Immunosuppression · Gene therapy

Introduction

Serious side effects associated with the long-term use of general immunosuppressive drugs in clinical organ transplantation have provided an incentive to seek strategies to induce donor-specific immunologic unresponsiveness. The goal is to make the recipient tolerant to donor antigens that are different from their own, while preserving general immunity to invasive microorganisms and maintaining immunologic defense mechanisms against neoplastic cell development. One possible approach involves the introduction of donor-specific MHC molecules into the thymus before or near the time of organ transplantation. This strategy takes advantage of evidence that tolerance to self-MHC-expressing cells normally develops via presentation of these antigens on thymic epithelium [3]. Therefore, making donor-specific MHC antigens available in the thymic environment has the potential to induce the recipient to be specifically tolerant to organ allografts bearing these antigens. Posselt et al. have applied this strategy in adult animal transplant models by intrathymically injecting murine donor islet [14] or bone marrow cells [15], thereby inducing unresponsiveness to subsequent islet cell transplants. Intrathymic injection of other donor cell types, including splenocytes, has also been shown to be effective at inducing donor-specific unresponsiveness in organ transplantation [11]. However, since donor cells cannot always be readily available prior to organ transplantation, and since some donor-derived molecules may sensitize the recipient, rather than make them tolerant, the focus has recently been on intrathymically injecting genetically synthesized allogeneic peptides. These studies suggest that indirect T cell recognition of allogeneic MHC class I-derived peptides presented by self-antigen-presenting cells

(APC) may be sufficient to induce donor-specific unresponsiveness [2, 16]. Unfortunately, the donor-derived peptides required to induce thymic tolerance must be empirically determined for each donor-recipient situation. The goal of the current study was to avoid the need to predetermine the necessary donor peptides required to induce donor unresponsiveness, and to eliminate the possible negative immunologic effects of multiple donor-derived antigens from viable donor cells or cell extracts. Therefore, we used an ex vivo gene transfer system to deliver complete soluble donor MHC molecules to the thymus prior to allogeneic heart transplantation in a high-responder rat model. Supply of the complete MHC class I molecule can theoretically allow for natural selection of potentially immunosuppressive donor-derived peptides by thymic APC, thus leading to donor-specific prolongation of organ allograft survival.

Materials and methods

Hepatocyte culture and transfection

Viable hepatocytes were obtained from Lewis (RT1.A^l) rat livers by a two-step perfusion system using a collagenase H (Boehringer Mannheim, Mannheim, Germany)-containing solution. Washed hepatocytes were purified using a Percoll gradient as previously described [8]. Purified hepatocytes were cultured for 2 days on collagen-coated (Collagen Corporation, Palo Alto, Calif., USA) Petri dishes. Cells were then transfected with plasmid DNA by lipofection (Lipofectin, Life Technologies, Gaithersburg, Md., USA), as we have described previously [8]. Three plasmid DNA constructs were used: (1) pCMVLux (control plasmid), encoding for firefly luciferase, (2) pcRQ.B3, encoding for a secreted form of the MHC class I molecule, RT1.A^a, and (3) pcRT.45, encoding for membrane-bound RT1.A^a. The DNA constructs and specific expression of secreted or membrane-bound RT1.A^a molecules by transfected hepatocytes have been described in detail elsewhere [4, 5].

Intrathymic injection and heart transplantation

Transfected hepatocytes were removed from collagen-coated dishes using a trypsin-EDTA solution, and 4×10^6 cells (in a 50 µl volume) were injected into each of the two major thymic lobes. Immediately following intrathymic injection, rats were intraperitoneally injected with 0.6 ml rabbit anti-rat lymphocyte serum (ALS; Accurate Chemical and Scientific, Westbury, N.Y., USA). Seven days after injection, an abdominal ACI heterotopic heart transplant [13] was performed on injected Lewis rats. Heart transplant rejection was defined as the time (in days) at which no heart beat was either palpable or visible at laparotomy. With all animal procedures, the principles of laboratory animal care set forth by the National Institutes of Health were carefully followed (publication number 86–23, revised 1985).

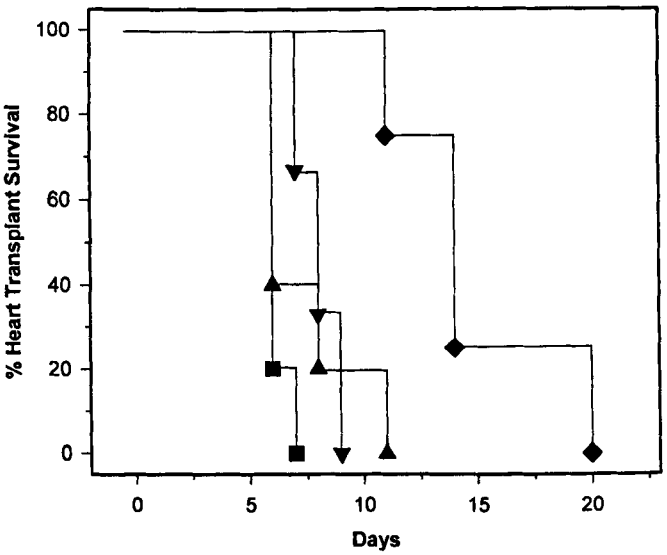

Fig. 1 The effect of intrathymic soluble donor MHC class I gene transfer on heart allograft survival. Results shown represent the percentage of ACI heart transplants that survived in Lewis recipients that were untreated (■), treated with only antilymphocyte serum (ALS; ▼), treated with pCMVLux-transfected control hepatocytes + ALS (▲), or treated with pcRQ.B3-transfected hepatocytes + ALS (◆). Compared to controls receiving pCMVLux-transfected hepatocytes, those recipients treated with pcRQ.B3-transfected hepatocytes demonstrated a significantly prolonged allograft survival time ($P = 0.01$; log-rank test). The number of animals in each group ranged from 3–5

Results

The potential immunosuppressive effect of intrathymic soluble donor MHC class I gene therapy was assessed by determining heart allograft survival times in various experimental groups. More specifically, ACI heart transplants were performed in four groups of Lewis recipients receiving: (1) no treatment, (2) treatment with only ALS, (3) intrathymic injection with pCMVLux-transfected hepatocytes + ALS, and (4) intrathymic injection with pRQ.B3-transfected hepatocytes + ALS (Fig. 1). Untreated recipients in the first group rejected their ACI allografts by 6.4 ± 0.4 days. Rats receiving ALS alone showed a very slight prolongation of heart transplant survival to 8.0 ± 1.0 days. Most importantly, animals receiving both pcRB.B3-transfected hepatocytes and ALS showed a prolongation of ACI heart allograft survival to 14.8 ± 3.8 days, compared to controls receiving pCMVLux-transfected hepatocytes and ALS (7.4 ± 2.2 days). Interestingly, one separate group of recipients was injected intrathymically with Lewis hepatocytes transfected with pcRT.45, encoding membrane-bound RT1.A^a, but ACI heart transplant survival was not consistently prolonged (10.7 ± 4.9 days, $n = 3$). Moreover, heart transplant rejection was accelerated (to 5 days) in 1 of the 3 recipients, compared to controls.

Expression of soluble donor MHC class I antigen by pcRQ.B3-transfected Lewis hepatocytes was verified after each group of transfections by testing culture supernatants for RT1.A^a, using an established ELISA method [4]. Consistent with results we have published previously [5], RT1.A^a levels exceeded 1700 ng/ 1.5×10^6 transfected cells by 48–72 h after transfection.

Discussion

Previous studies suggest that systemic delivery of soluble allo-MHC class I antigens can have donor-specific immunosuppressive properties useful in organ transplantation [1, 5, 17]. Recent reports also suggest that donor MHC class I molecules may have an immunosuppressive effect if delivered locally to the thymus. However, most experiments thus far have been performed using donor cells, peptides derived from donor MHC molecules, or a mixture of MHC molecules derived from donor tissue extracts. The use of donor cells or cell extracts has been reportedly effective [11, 12, 14, 15], but the heterogeneity of these types of inoculates makes it difficult to determine exactly which donor antigens are responsible for the immunosuppressive effect. Although synthesized donor-derived peptides have the advantage of being homogenous, actively suppressive peptides must be determined empirically and APC from different recipients may process distinct peptides. To circumvent these issues in the present study we have developed an ex vivo gene transfer system to express complete MHC class I molecules in the thymus. This approach allows expression of only one type of foreign antigen and also permits natural selection of peptides by recipient APC in the thymus. Results from our study indicate that soluble donor MHC class I molecules expressed by this type of gene therapy can prolong heart allograft survival in a high-responder rat strain combination. It is notable that allograft survival was prolonged, but not indefinitely, in this model. One possible explanation for the limited immunosuppressive effect could relate to the intrathymic pretreatment with only one disparate (RT1.A^a) donor MHC class I antigen, in a fully MHC-disparate, high-responder strain combination. Another plausible explanation may be associated with transient high expression levels (approximately 3 days) of the soluble donor MHC with our hepatocyte-gene delivery system, as we have reported recently [5]. In this regard, a previous study indicates that antigen dose may be a critical factor in the thymus to obtain indefinite allograft survival [12]. Efforts are currently being made to address these issues and further improve allograft survival by our gene therapy approach.

Data from the present study suggest that the form of donor MHC class I antigen introduced intrathymically may have an impact on immunity. This was evidenced by the fact that although treatment with membrane-bound donor MHC antigen prolonged allograft survival in most cases, accelerated rejection did occur in one recipient. In contrast, intrathymic exposure to soluble MHC molecules did not show any evidence of recipient sensitization. We have consistently observed and reported a substantial risk of sensitization from hepatocyte-expressed membrane-bound, but not soluble, MHC class I antigens in our experimental systems [5, 6, 9]. It must be considered, however, that we cannot rule out the possibility that a small number of hepatocytes expressing membrane-bound donor antigen leaked out of the thymus after injection. Such a systemic exposure to these cells could explain the immunologic sensitization observed.

In summary, our results suggest that a gene therapy approach can be utilized successfully to intrathymically deliver donor-specific MHC class I molecules to the thymus and promote allograft survival in a heart transplant model. Our results also indicate that soluble donor MHC class I molecules introduced into the thymus are at least as effective as membrane-bound molecules at prolonging allograft survival, and have a lower risk for sensitization. Furthermore, since genetically altered recipient cells, and not donor cells, were introduced to the thymus, donor MHC antigen, and not donor APC, must have been responsible for the immunosuppressive effect. Therefore, although Goss et al. [7] have reported donor MHC class II-expressing APC are required for this type of thymic-mediated unresponsiveness, our present and previous [10] findings contradict this hypothesis. Whether our approach will be effective with other transplanted organs, and among other strain combinations, will be the subject of future studies.

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