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Transfusion of ethylene carbodiimide–fixed donor splenocytes prolongs survival of vascularized skin allografts

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ARTICLE INFO

Article history:
Received 3 March 2017
Received in revised form
24 May 2017
Accepted 6 June 2017
Available online 19 October 2017

Keywords:
Skin transplantation
Mice
Vascularized composite allotransplantation
Tolerance

ABSTRACT

Background: Allograft rejection is a major obstacle to the widespread clinical application of vascularized composite allotransplantation. Recent studies revealed a non-cytoreductive strategy to protect allografts by the transfusion of ethylene carbodiimide–fixed donor splenocytes (ECDI-SPs). To determine whether this approach offers advantages in protecting skin allografts, we examined the immunological protection of infusing ECDI-SPs with a 30-d administration of rapamycin on the skin allografts of mice.

Materials and methods: C57BL/6 recipient mice received BALB/c donor full-thickness skin or vascularized skin transplants at day 0, along with the infusion of donor ECDI-SPs 7 d before and 1 d after allotransplantation and a 30-d course of rapamycin. Recipients received ECDI-untreated splenocytes or C3H allografts as controls. *In vitro* allostimulatory activity of ECDI-SPs and donor-specific *ex vivo* hyporesponsiveness were tested. Production of related cytokines (TGF-β, IL-10, IL-1β, and TNF-α) and expression of $CD4^{+}Foxp3^{+}$ regulatory T cells (Tregs) were also examined.

Results: Transfusion of ECDI-SPs combined with rapamycin significantly prolonged survival of full-thickness skin (median survival time [MST]: 28 d) and full-thickness skin allografts (MST: 71 d) compared with untreated splenocytes (MSTs: 11 d and 30 d) or C3H allografts (MSTs: 11 d and 38 d). This effect was accompanied by increased production of IL-10 and TGF-β, decreased production of IL-1β and TNF-α, and expansion of Tregs *in vitro* and *in vivo*.

Conclusions: ECDI-SP infusion combined with short-term rapamycin administration provides a promising approach to prolong the skin allograft survival.

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0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2017.06.005

Introduction

Vascularized composite allotransplantation (VCA) is a promising clinical approach to restore the forms and functions of devastating facial and limb defects. Since 1998, more than 28 facial and 107 hand allotransplants have been performed worldwide.[1,2] Allograft rejection, triggered mainly by the highly antigenic skin compartment of donor tissues, is one of the major obstacles to the widespread clinical application of VCA. Nearly every facial allotransplant and 85% of hand transplants had at least one acute rejection in the first year after transplantation.[3,4] Although these rejection episodes are readily reversible with pulse doses of immunosuppressants,[4] the associated lifelong complications and toxicities have raised explicit ethical issues. Novel strategies and therapies are needed that can reduce the severity of acute skin rejection and prolong allograft survival.

Most of the current strategies for inducing skin allograft tolerance encompass myeloablation/cytoreduction protocols, which are associated with various toxicities.[5-8] Unlike emergency heart or liver transplantation procedures, skin-containing VCA is not a life-saving surgery. Therefore, the induction and maintenance of tolerance with the assistance of toxic regimens are far from optimal in clinical implementation. Recent studies have shown that intravenous injection of donor splenocytes that have been chemically fixed with 1-ethyl-3-(3′-dimethylaminopropyl)-carbodiimide (ECDI) is an effective method to induce antigen-specific tolerance to allogeneic islet cell and cardiac transplants in mouse models.[9,10] Mechanisms of tolerance involve the induction of T-cell anergy, expansion of regulatory T cells (Tregs), and regulation of the cytokine production profile.[11,12] These immunological changes are also required to establish host immune tolerance against skin allografts.[5,8] Modulation of the host immune response through the infusion of ECDI-fixed splenocytes (ECDI-SPs) avoids cytoreductive conditioning and, therefore, is an appealing strategy for clinical application in skin-containing VCA.

In this study, we tested the hypothesis that infusion of ECDI-SPs combined with a short-term course of rapamycin would prolong the survival of full-thickness skin (FTS) and vascularized skin (VS) allografts in a mouse model. Our results clearly demonstrated that infusions of the ECDI-treated, but not untreated, donor splenocytes in combination with a 30-d administration of rapamycin significantly prolonged donor skin grafts but not third-party grafts. The underlying mechanisms associated with the donor-specific immunological hyporesponsiveness may involve expansion of Tregs and upregulation of tolerance-inducing cytokines.

Materials and methods

Mice

Adult male C57BL/6 (H-2b) and BALB/c (H-2d) mice weighing 20 to 25 g were purchased from the Experimental Animal Center of the Fourth Military Medical University. Adult male C3H (H-2k) mice weighing 20 to 25 g were obtained from Vital River, Inc. (Beijing, China). All animals were housed under specific pathogen-free conditions with accessible food and water. Animal experiments conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 8023, revised 1978) and were approved by the Experimental Animal Committee of the Fourth Military Medical University.

Groups

Fifty-eight recipient mice were randomly divided into 11 groups. Groups 1-5 (Table) received FTS transplantation, and groups 6-11 (Table) received VS transplantation. Group 1 ($n = 6$) and group 6 ($n = 6$) recipients served as controls and only received allogeneic FTS or VS transplantation without other treatments. Group 2 ($n = 5$) and group 7 ($n = 5$) recipients received FTS or VS transplantation without splenocytes injection. Group 3 ($n = 5$) and group 8 ($n = 5$) recipients were treated with ECDI-SP (BALB/c) infusion twice and received FTS or VS from a third-party (C3H) donor. Group 4 ($n = 5$) and group

Table – Treatments assigned for experimental mouse groups.

Group	Number of mice	Label	Immunosuppressants*	Injection of cells	Allograft
1	6	Control	None	None	BALB/c FTS
2	5	Rapa	Yes	None	BALB/c FTS
3	5	C3H+Rapa	Yes	2 × BALB/c ECDI-SPs	C3H FTS
4	5	SP+Rapa	Yes	2 × BALB/c SPs	BALB/c FTS
5	5	2 × ECDI-SP+Rapa	Yes	2 × BALB/c ECDI-SPs	BALB/c FTS
6	6	Control	None	None	BALB/c VS flap
7	5	Rapa	Yes	None	BALB/c VS flap
8	5	C3H+Rapa	Yes	2 × BALB/c ECDI-SPs	C3H VS flap
9	5	SP+Rapa	Yes	2 × BALB/c SPs	BALB/c VS flap
10	5	2 × ECDI-SP+Rapa	Yes	2 × BALB/c ECDI-SPs	BALB/c VS flap
11	6	3 × ECDI-SP+Rapa	Yes	3 × BALB/c ECDI-SPs	BALB/c VS flap

Rapa = rapamycin.
*Immunosuppressant: 30-d course of rapamycin (3 mg/kg/d) administered starting 1 d before skin transplantation.