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Induction of indirect donor-specific hyporesponsiveness by transportal RT1-peptide pulse in rat skin transplantation

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Abstract In the present study, we examined whether transportal pulse of class I major histocompatibility complex (MHC) allopeptides can induce indirect (non-chimeric) donor-specific hyporesponsiveness, using a high-responder rat skin transplantation model. Two donor-specific 8-amino acid peptides corresponding to residues 58–65 and 70–77 in the α_1 helical region of RT1.A^a were synthesized. In order to test immunogenicity of these peptides, mixed lymphocyte reaction (MLR) was performed. Then, 100-μg portions of peptides were injected into recipient Lewis (LEW, RT1.A^l) rats via the portal vein 14 days before skin transplantation. Skin allografts from August Copenhagen Irish (ACI, RT1^a) or Wistar King A (WKA, RT1^k, third-party) donors were transplanted to LEW (RT1^l) recipients. Transportal pulse of residues 58–65 and 70–77 prolonged graft survival significantly in ACI-to-LEW skin transplantation (17.6 ± 0.40 and 18.0 ± 0.45 days) compared with control (14.2 ± 0.37 days). However, pulse of residues 106–113, a non-donor-specific control, did not prolong graft survival time (14.6 ± 0.40 days) in the same combination. Regarding the third-party donor, residues 58–65 injected into LEW recipients had no effect on survival time of skin grafts (19.0 ± 0.84 days) derived from WKA donors compared with the untreated WKA-to-LEW control (19.4 ± 0.93 days). Transportal pulse of RT1.A^a peptides induced donor-specific hyporesponsiveness even in a high-responder rat skin transplantation model. Our results suggest that graft enhancement by transportal exposure to donor cells may not be induced by a chimeric process but, instead, by an indirect mechanism not involving intervention of viable donor cells.

Keywords Immunohyporesponsiveness · Class I major histocompatibility complex · RT1.A · Skin transplantation

Introduction

The achievement of donor-specific tolerance is the final goal in transplant biology. Administration of donor antigen to transplant recipients, such as blood [12, 18, 26], splenocytes [9, 16, 22, 27], bone marrow cells [11, 15], or extracted histocompatibility antigen [2, 4, 6, 7, 10, 17, 28, 29], has been performed in attempts to induce donor-specific immunohyporesponsiveness. Jin et al. found that portal venous injection of bone marrow cells is more effective in improving skin graft survival time than i.v. injection [11]. More recently, peptides derived from donor major histocompatibility complex (MHC) class I and/or class II were found to prolong graft survival in some rodent models [1, 3, 21, 23, 24, 25, 30]. MHC class I membrane-bound heavy chains (45 kDa) are composed

of membrane-distal polymorphic α_1 (90aa) and α_2 (92aa) domains, each of which includes an α-helix supported by four anti-parallel β-pleated strands. The α_2 domain is attached to a less polymorphic α_3 (92aa) domain, a transmembrane (25aa) domain, and intra-cytoplasmic (30aa) anchor. Our study investigated whether immunological hyporesponsiveness was induced by transportal pulse of synthetic class I MHC RT1.A^a peptides derived from the polymorphic α_1 helical region.

Materials and methods

Animals

Male August Copenhagen Irish (ACI, RT1^a, 8 weeks, 150–200 g) and Lewis (LEW, RT1^l, 8 weeks, 200–250 g) rats were used as donors and recipients, respectively. They were purchased from Charles River Laboratory (Japan). Male Wistar King A rats (WKA, RT1^k, 8 weeks, 190–230 g) were used as third-party donor and obtained from Nippon SLC (Japan). All animals were maintained according to the standard NIH guidelines.

Peptides

ACI-derived 8-mer peptides covering residues 58–65 (RT1.A^a 58–65) or 70–77 (RT1.A^a 70–77) were synthesized by Biologica (Japan). These amino acid sequences correspond to the α_1-helical region of donor RT1.A^a protein [5, 19, 20]. Peptide covering residues 106–113 (RT1.A 106–113), the sequence corresponding to the α_2 domain of RT1.A^a and RT1.A^l in common, was prepared as non-donor-specific control (Fig. 1).

Preparation of splenocytes

Fresh spleens were harvested from ACI, LEW, or WKA rats under ether anesthesia. We prepared single-cell suspensions by passing the spleen through a 60-gauge stainless steel sieve. Red blood cells were lysed with Tris-ammonium chloride buffer. The splenocyte suspensions were then washed twice with Hank's balanced salt solution and re-suspended in RPMI-1640 medium at a concentration of 3×10^6 cells/ml. The viability of splenocytes was determined by the trypan blue dye exclusion test.

Fig. 1 Published amino acid sequences of RT1.A^a and RT1.A^l (partial). **a** RT1.A^a 58–65 and RT1.A^a 70–77 derived from polymorphic α_1 helical region. **b** RT1.A 106–113 derived from monomorphic α_2 domain

a

	51	58	65	70	77	90
RT1.A^a	W M E R E G P E Y W E Q Q T R I A K E W E Q I Y R V D L R T L R G Y Y N Q S E G					
RT1.A^l	- - - - - - - D - - - R E - Q K - - G N - - N - - - S - - N - - - - - - - - - -					
RT1.A^a 58-65		E Y W E Q Q T R				
RT1.A^a 70-77				W E Q I Y R V D		

b

	91	106	113
RT1.A^a	G S H T I Q E M Y G C D V G S D G S L L R G Y R Q		
RT1.A^l	- - - - - - R - - - - - - - - T - - - - - - - - - -		
RT1.A 106-113		D G S L L R G Y	

Mixed lymphocyte reaction

Stimulator cells, comprising splenocytes obtained from normal ACI rats, were treated with 5% mitomycin C (MMC, Kyowa Hakko, Tokyo, Japan) in RPMI-1640 medium at 37 °C for 40 min. Splenocytes harvested from LEW rats at 3, 7, and 14 days after transportal pulse of RT1.A^a 58–65 were used as responder cells. Similarly, splenocytes from LEW rats at 14 days after transportal pulse of RT1.A^a 70–77 or RT1.A 106–113 were prepared as responder cells. Splenocytes obtained from untreated LEW rats were used as control. In the case of the third-party donor, splenocytes prepared from normal WKA rats were used as stimulator cells, and splenocytes obtained from LEW rats that were untreated or transportally pulsed with RT1.A^a 58–65 at 14 days before mixed lymphocyte reaction (MLR) were used as responder cells. All responder cells were obtained from non-transplanted animals. Each experimental group comprised three animals. The responder cells (3×10^5/100 µl per well) were cultured in 96-well flat-bottomed plates in triplicate, with an equal number of MMC-treated stimulator cells (one-way MLR). The plates were incubated at 37 °C in 5% CO_2 for 4 days, then pulse-labeled for 20–24 h with [^{3}H]-thymidine (1 µCi/well) and harvested. Proliferation was assayed by [^{3}H]-thymidine incorporation measured with a liquid scintillation counter (Aloka, Tokyo, Japan). Values were expressed as counts per minute (cpm) and stimulation index. The stimulation index was calculated from the following formula:

Stimulation index = (cpm of allogeneic MLR)/
(cpm of autologous MLR)

Transportal pulse of peptides and skin transplantation

At 14 days before skin transplantation, donor rats were injected with peptides (100 µg/body) through the superior mesenteric vein with a 27-gauge needle, under ether anesthesia. After injection, hemostasis was secured by gentle pressure with a cotton-wool swab.

Full-thickness skin grafts (1.0×1.0 cm) were harvested from the tail of the donor and sutured to the graft beds on the dorsal wall of the recipients and carefully bandaged. The dressing was removed on day 5, and daily inspections were carried out. Thereafter, graft rejection was noted as the day when the graft was necrotic, non-vascularized, and had dried up or dropped off.

Animals were divided into the following six groups: group 1, no transportal pulse (sham operation); group 2, transportal pulse of residues 58–65 (RT1.A^a 58–65); group 3, transportal pulse of residues 70–77 (RT1.A^a 70–77); group 4, transportal pulse of residues 106–113 (RT1.A 106–113, non-donor-specific control); group 5, skin graft obtained from WKA (RT1^k) rats transplanted to untreated LEW rats; group 6, skin graft from WKA rats transplanted to LEW rats transportally pulsed with RT1.A^a 58–65. Each group consisted of five animals.

Statistical analysis

All values were expressed as mean ± SEM. Differences between groups were examined for statistical significance with Student's *t*-test. *P* values of less than 0.05 were considered to indicate statistical significance.

Results

Immunogenicity of synthetic class I MHC peptide in MLR

To assess the immunogenicity of synthetic class I MHC peptide, we performed one-way MLR (Fig. 2). Responder cells from LEW rats at 7 and 14 days after transportal pulse of RT1.A^a 58–65 exhibited significant proliferative response against ACI stimulator cells compared with normal LEW responder cells (10,541 ● 1,912 and 16,481 ± 3,990 vs 3,827 ± 249 cpm). Similarly, responder cells from LEW rats at 14 days after transportal pulse of RT1.A^a 70–77 exhibited significant proliferation to ACI stimulator cells (7,848 ± 2,460 cpm), but responder cells from LEW rats treated with RT1.A 106–113 did not (3,361 ± 84 cpm). Regarding the third-party donor, LEW responder cells treated with RT1.A^a 58–65 exhibited no proliferative response to WKA stimulator cells compared with normal LEW responder cells (3,416 ± 45 vs 4,218 ± 252 cpm). These findings demonstrate that the synthetic class I MHC peptides RT1.A^a 58–65 and RT1.A^a 70–77 exhibited immunogenicity in recipient animals in a donor-specific fashion.

Survival of skin allografts

Mean graft survival in groups 2 and 3 (17.6 ± 0.40 and 18.0 ± 0.45 days) was significantly longer than that in group 1 (14.2 ± 0.37 days). In group 4, the non-donor-specific control, prolongation of graft survival was not observed (14.6 ± 0.40 days). There was no significant difference in graft survival between groups 5 and 6 (19.0 ± 0.84 and 19.4 ± 0.93 days). Indeed, donor-specific RT1.A^a 58–65 and RT1.A^a 70–77 prolonged skin graft survival, but RT1.A 106–113, a sequence common to RT1^a and RT1^l, had no effect on graft survival in ACI-to-LEW rat skin transplantation. Peptide pulse of RT1.A^a 58–65 to LEW recipients did not prolong survival time of skin grafts obtained from WKA donors significantly (Table 1). These findings show that this hyporesponsiveness occurred in a donor-specific manner.

Discussion

Various donor antigens, such as donor blood [12, 18, 26], splenocytes [9, 16, 22, 27], bone marrow cells [11, 15], and extracted histocompatibility antigens [2, 4, 6, 7,

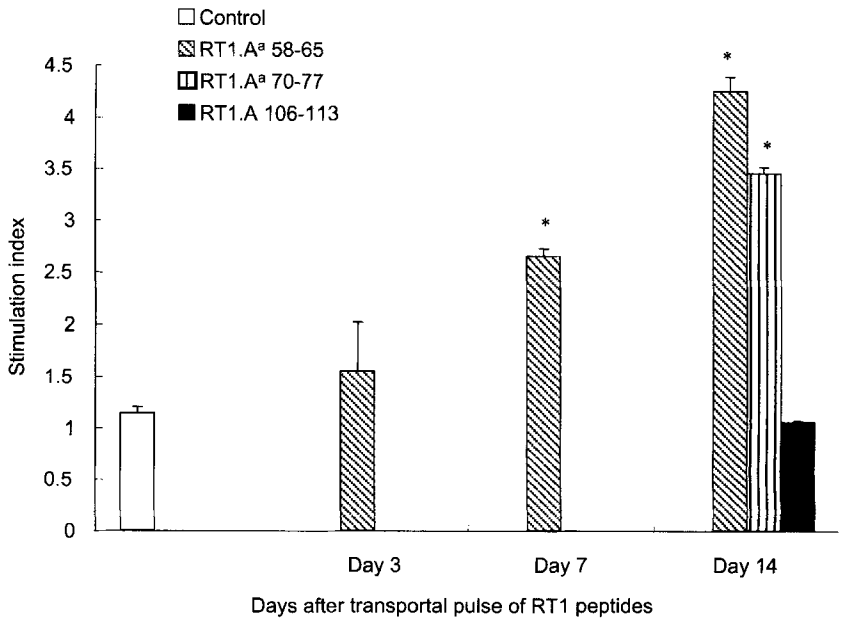

Fig. 2 [^{3}H]-thymidine incorporation by LEW-derived splenocytes was significantly higher at 7 and 14 days after transportal pulse of RT1.A^a 58–65 than by normal LEW responder cells. Similarly, splenocytes derived from LEW rats transportally pulsed with RT1.A^a 70–77 showed significantly increased proliferation compared with control, but splenocytes derived from responder animals transportally pulsed with RT1.A 106–113 (non-donor-specific control) did not exhibit significant proliferation as expected. Stimulation index = experimental cpm/autologous cpm. *$P < 0.05$

Table 1 Effect of RT1 peptides on skin graft survival (*PV* portal venous injection, *NS* not significant)

Donor	Recipient	Peptide	Graft survival (days)	Mean ± SEM (days)	*P* value
ACI	LEW	–	13, 14, 14, 15, 15	14.2 ± 0.37	–
ACI	LEW	RT1.A^a 58–65 (PV)	17, 17, 17, 18, 19	17.6 ± 0.40	$P < 0.01$
ACI	LEW	RT1.A^a 70–77 (PV)	17, 17, 18, 19, 19	18.0 ± 0.45	$P < 0.01$
ACI	LEW	RT1.A 106–113 (PV)	13, 15, 15, 15, 15	14.6 ± 0.40	NS
WKA	LEW	–	16, 19, 19, 20, 21	19.0 ± 0.84	–
WKA	LEW	RT1.A^a 58–65 (PV)	18, 18, 19, 19, 23	19.4 ± 0.93	NS

10, 17, 28, 29], have been used to induce donor-specific tolerance. Recently, several types of MHC-derived proteins have been used to induce tolerance [1, 3, 21, 23, 24, 25, 30]. Intra-thymic inoculation of peptides derived from the polymorphic region of donor MHC class I peptides prolonged graft survival [1]. Similarly, allochimeric donor/recipient class I MHC proteins resulted in long-term acceptance of cardiac allografts, when administered by the intra-portal route or by oral gavage in combination with cyclosporin A [24, 25]. In our study, transportal pulse of RT1.A^a 58–65 or RT1.A^a 70–77 derived from the polymorphic region of donor MHC class I molecules prolonged skin graft survival in a high-responder rat strain combination, but RT1.A 106–113 derived from monomorphic regions of recipient class I MHC molecules had no effect on skin transplantation. Thus, this hyporesponsiveness occurred in a donor-specific fashion.

There are two distinct pathways for the recognition of alloantigens, the direct and the indirect pathway. In the direct pathway of allorecognition, recipient T cells recognize intact allogeneic MHC molecules directly on the surface of donor antigen-presenting cells (APCs). In the indirect pathway, recipient T cells recognize processed donor MHC peptides presented in association with recipient class II MHC on the surface of self-APCs. Our skin transplantation model was free from intervention of viable donor APCs. Therefore, this hyporesponsiveness occurred through the indirect pathway.

The mechanism underlying tolerance induced by administration of alloantigen has been described as being due to clonal deletion [13], clonal anergy [8, 14], or micro-chimerism [12]. In this study, micro-chimerism was ruled out because no donor cells existed in transplant recipients. Our findings demonstrated proliferation of lymphocytes in MLR. Thus, anergy rather than clonal deletion might be responsible for donor-specific immunohyporesponsiveness.

In conclusion, transportal pulse of donor-specific MHC peptides prolonged skin graft survival in our study, but this effect was not persistent. Additional approaches would be required to induce transplantation tolerance.

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