Delayed Type Hypersensitivity Responses in Mice Transplanted with Rat Hepatocytes

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ABSTRACT. The delayed type hypersensitivity (DTH) response to xenogeneic hepatocytes (HCs) was investigated in mice received subcutaneous (s.c.), intrasplenic (i.s.), or intravenous (i.v.) transplantation of rat HCs. The DTH response in mice preimmunized i.s. or i.v. with rat HCs (1×10^6 cells) was significantly lower than that in mice preimmunized s.c. with the same doses of rat HCs. Co-transfer of spleen cells from i.s. immunized mice with spleen cells from s.c. immunized mice to naive recipient mice did not suppress the DTH response induced by transfer of spleen cells from s.c. immunized mice. On the other hand, co-transfer of spleen cells from i.v. immunized mice with spleen cells from s.c. immunized mice suppressed the DTH response to rat HCs in recipients. Furthermore, the levels of DTH responses in recipients transferred with spleen cells from mice sensitized i.s. or i.v. with rat HCs, immunized s.c. with rat HCs 6 hr after transfer, and challenged with rat HCs 7 days later was almost similar to those in recipients transferred with spleen cells from s.c. immunized mice. These results suggest that antigen-specific suppression of DTH responses to rat HCs in mice is associated with the presence of suppressive spleen cells induced by i.s. or i.v. immunization with rat HCs.—KEY WORDS: delayed type hypersensitivity, immunosuppression, intrasplenic transplantation, xenogeneic hepatocytes.

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Transplantation of syngeneic or allogeneic hepatocytes (HCs) has been studied in rats with acute fulminant hepatic failure or inherited specific disorders of hepatic metabolism. The transplantation sites of hepatocytes include the spleen [7–9, 14, 15, 21, 22, 26, 30, 32, 33], peritoneal cavity [16, 17, 21, 27], portal vein [13, 16, 17, 27, 34], pulmonary vascular bed [25] and mesenteric fat pad [10]. Most studies have been focused on the maintenance of metabolic functions and histological changes of grafted hepatocytes. With regard to immune responses, allogeneic HCs expressing MHC class I^+ and II^- have been shown to stimulate *in vitro* cytotoxcity of naive responder spleen cells [5]. Macrophages as antigen presenting cells (APCs) also play an important role in the development of allogenic specific cytotoxic T cells in response to purified allogeneic hepatocytes [6]. Immune responses to xenogeneic hepatocytes were associated with an increase in antibody-dependent cellular cytotoxicity after intraperitoneal transplantation [16]. One of cellular immune responses is the delayed-type hypersensitivity (DTH) response. The DTH response to an antigen generally consists of two phases of the induction phase and effector phase. The former is a phase in which antigens internalized, processed, and represented by APCs are presented to helper T cells to proliferation and differentiate precursor T_{DTH} cells into T_{DTH} cells responsible for the induction of the DTH response. The latter is a phase in which antigen-specific T_{DTH} upon resensitization with antigens induce inflammation at the injection site of antigens [20]. However, the cellular events at the DTH response to xenogeneic hepatocytes have not been fully studied. Therefore, in the present study, the DTH response was evaluated in mice transplanted subcutaneously (s.c.), intrasplenically (i.s.), or intravenously (i.v.) with rat HCs.

MATERIALS AND METHODS

Animals: Female inbred F344 rats (9–12 weeks old) were used as donors, and female inbred Balb/c mice (6–7 weeks old at the start of the experiment) as recipients. All of these animals were purchased from Clea Japan Inc., Tokyo, Japan.

Isolation of hepatocytes: Isolation of HCs from rats and mice was performed using a slight modification of Seglen's perfusion technique [24]. Each animal was anesthetized by an intraperitoneal injection of pentobarbital 50 mg per kg of body weight (Somnopentyl®: Pitman-Moore, Inc., IL, U.S.A.), and the peritoneal cavity was opened wide. The inferior vena cava was cannulated. The portal vein was divided, and then the suprahepatic vena cava was ligated. The liver of each rat and mouse was perfused *in situ* at a rate of 10–15 ml/min and 3–5 ml/min, respectively, at 37°C with an ethylene glycol-O,O'-bis-2-aminoethyl-N'-N, N',N'-tetraacetic acid (EGTA)-containing calcium-free salt solution for 3 min, and followed by Hanks' solution supplemented with 0.05% collagenase (Wako Pure Chemical Industries Ltd., Osaka, Japan) for 8 min. The liver was excised and minced gently with two surgical blades on a siliconized glass dish, and then suspended in Hanks' solution (complete medium; CM) containing 1% fetal calf serum (FCS), 0.05 mg/ml gentamicin, 25 mM 2-[4-(2-Hydroxyethyl)-1-piperazinyl] ethanesulfonic acid and 24 mM sodium bicarbonate. The cells were filtered through 60 and 150 steel meshes to remove larger aggregates and debris. The resulting HCs were washed three times with CM, and suspended to the desired concentration in CM. The viability of the resulting cells was more than 90%,

when determined by the trypan blue (0.16%) exclusion test. The rate of contamination of nonparenchymal cells in 200 cells in the smear stained with hematoxylin and eosin was less than 1%.

Preparation of sheep red blood cells (SRBC): SRBC in Alsever's solution (Nippon Bio-test Lab. Inc., Co., Tokyo, Japan) were washed three times with Hanks' solution, and suspended to the desired concentration in Hanks' solution.

Immunization: Mice were injected s.c., i.s., or i.v. with rat HCs at various doses (1×10^3 – 1×10^6). At the same time, control mice were also injected s.c., i.s., or i.v. with Hanks' solution. During i.s. injection under pentobarbital anesthesia, the hilar vessels of the spleen were not clamped. For one experiment, each mouse was injected i.s. or i.v. with 10^8 SRBC.

Measurement of DTH: Mice were injected s.c., i.s., or i.v. with rat HCs. Four or 7 days later, for challenge, rat HCs and syngeneic mouse HCs (1×10^5 cells) in 25 μl Hanks' solution were injected into the right hind footpad (RFP) and the left hind footpad (LFP), respectively. The footpad thickness was measured by a dial thickness gauge (Ozaki Engineering, Tokyo, Japan) at 6, 12, 18, 24, 30, 48 and 72 hr after challenge. The results were expressed as the difference of thickness between RFP and LFP, as described previously [29].

Adoptive cell transfer: Spleen cells, obtained from mice at 4 or 7 days after s.c., i.s., or i.v. immunization with 10^6 rat HCs under pentobarbital anesthesia, were supplemented with ammonium chloride-Tris buffer to lyze contaminated erythrocytes, and then suspended to the desired cell concentrations, as described previously [29]. Adoptive cell transfer was performed by i.v. injection of the resulting spleen cells (3×10^7 or 1×10^8) into the tail vein of naive recipients. Six hr after the transfer, rat HCs (1×10^6 cells) were injected into the s.c. tissue of the footpad of the recipients for DTH responses.

Statistical analysis: The results were expressed as the mean ± standard deviation (SD), and differences among the various parameters were analyzed by the repeated measure ANOVA, two factorfactorial ANOVA, and scheffés F. Differences $P<0.05$ were considered significant.

RESULTS

Changes in DTH responses in mice sensitized with rat HCs via different routes: Mice were sensitized s.c., i.s., or i.v. with different doses of rat HCs (1×10^3 – 1×10^6 cells). Four or 7 days later, 10^5 rat HCs were injected into the

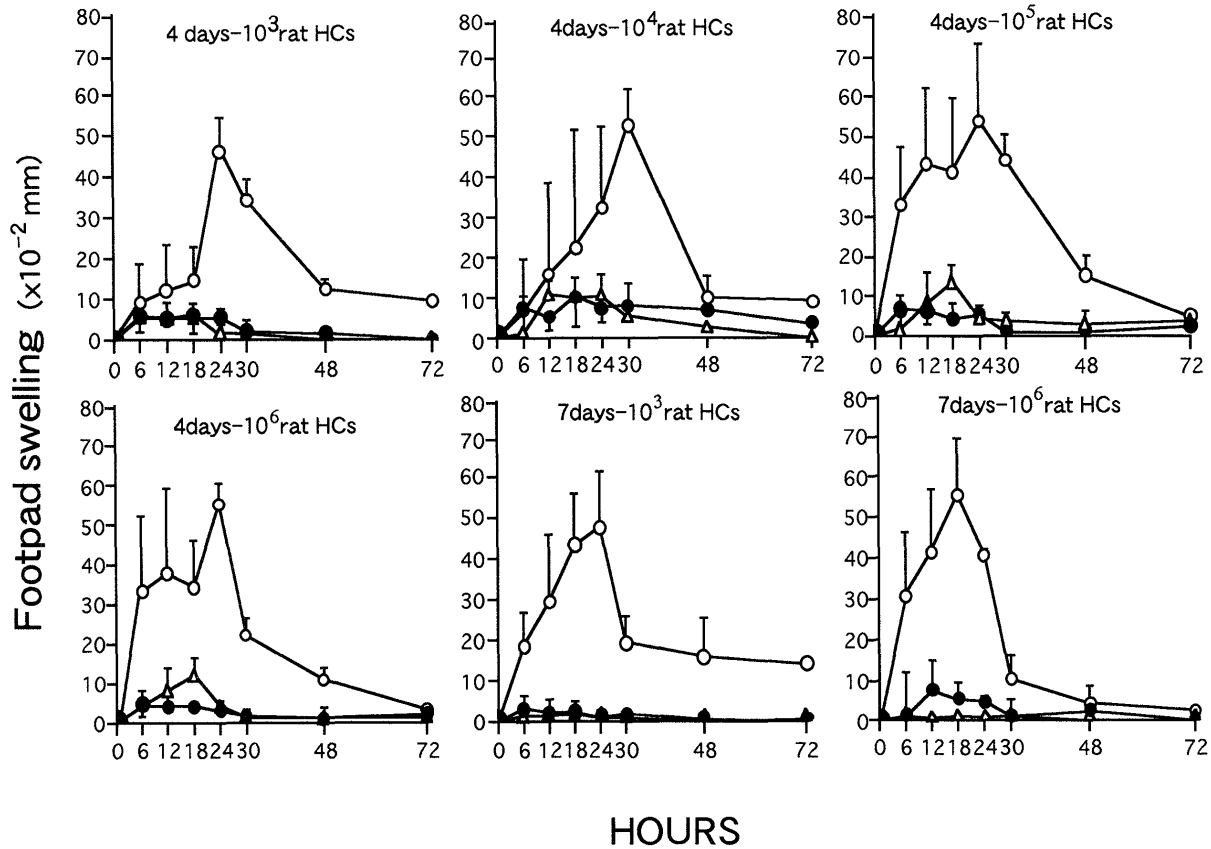

Fig. 1. Changes in DTH responses in mice sensitized s.c., i.s., or i.v. with various doses of rat HCs. Mice were sensitized s.c. (○), i.s. (●), i.v. (△) with 10^3, 10^4, 10^5, or 10^6 rat HCs, respectively. Four or 7 days later, rat HCs (1×10^5 cells) were injected into the s.c. tissue of the footpad. Footpad swelling was measured at 6, 12, 18, 24, 30, 48 and 72 hr after challenge. The means ± SD of 5 to 6 mice are given.

s.c. tissue of the footpad to evaluate DTH responses. Footpad swelling in mice sensitized s.c. with 10^3 – 10^6 rat HCs reached a maximum at 18–24 hr after challenge (Fig. 1). However, DTH responses in mice sensitized i.s. or i.v. with 10^3–10^6 rat HCs were significantly lower than those in mice sensitized s.c. with the same number of rat HCs.

Induction of antigen-specific suppression of DTH responses by i.s. or i.v. preimmunization: To examine antigen-specific suppression of DTH responses in mice sensitized i.s. or i.v. with rat HCs, DTH responses in mice preimmunized i.s. or i.v. with SRBC were evaluated. As shown in Table 1, footpad thickness in mice preimmunized i.s. (Expt. IV in Table 1) or i.v. (Expt. VI) with 10^6 rat HCs 1, 4, 7, or 20 days before s.c. immunization with 10^6 rat HCs, and followed by s.c. challenge with 10^5 rat HCs 7 days later was significantly lower, when compared with that in mice preimmunized s.c. (Expt. III). Footpad swelling in mice preimmunized i.s. (Expt. V) or i.v. (Expt. VII) with 10^8 SRBC 1, 4, 7, or 20 days before s.c. immunization with 10^6 rat HCs, and followed by challenge 7 days later was almost similar to that in mice preimmunized s.c. with 10^6 rat HCs (Expt. III).

Adoptive transfer of the DTH response: To examine spleen cells involving in the suppression of the DTH response, recipient mice were transferred with 10^8 or 3×10^7 spleen cells from donor mice 4 or 7 days after s.c., i.s., or i.v. immunization with 10^6 rat HCs. The results are shown in Table 2. Transfer of 10^8 spleen cells from mice immunized s.c. with rat HCs showed the DTH response with a peak 12–18 hr after challenge with rat HCs (Expt II

Table 1. Antigen-specific suppression of DTH responses induced by i.s. or i.v. preimmunization with rat HCs

Expt.	Preimmunization[a]		s.c. immunization	Footpad swelling ($\times10^{-2}$ mm)[b] at various times between preimmunization and s.c. immunization			
	Route	Antigen		1 day	4 days	7 days	20 days
I	all[c]	Hanks	Hanks	N.D.[d]	N.D.	1.8± 1.9[e]	N.D.
II	all	Hanks	10⁶ HCs	57.0± 8.2	46.2±11.9	61.2±19.9	63.0± 6.4
III	s.c.	10^6 HCs		55.2± 4.4	41.2±10.3	42.0± 9.2	63.2±12.3
IV	i.s.	10^6 HCs		9.8± 4.0*	9.2± 4.8*	16.2± 1.8*	10.8± 5.1*
V	i.s.	10^8 SRBC		51.6±16.0	42.4±10.1	62.4±13.7	55.8± 3.7
VI	i.v.	10^6 HCs		10.8± 1.2*	9.8±11.1*	20.2± 8.5*	32.8±16.2*
VII	i.v.	10^8 SRBC		49.2± 5.4	42.4±10.1	55.0±19.0	53.4±15.6

a) Mice were preimmunized s.c., i.s., or i.v. with rat HCs (1×10^6 cells) or SRBC (1×10^8 cells), and immunized s.c. with 10^6 rat HCs or Hanks' solution 1, 4, 7, or 20 days later.
b) Rat HCs and syngeneic mouse HCs (1×10^5 cells) were injected into the RFP and LFP, respectively 7 days after immunization. Data express footpad swelling at 18 hr after challenge.
c) All represents the routes injected with Hanks' solution for preimmunization.
d) N.D.: Not determined.
e) The means±SD of 5 to mice are given.
* There was a significant difference between Expt. III and Expt. IV or VI ($P<0.05$).

Table 2. Adoptive transfer of spleen cells from mice immunized i.s. or i.v. with rat HCs

Expt.	Immunization[a]		Number of spleen cells transferred	Footpad swelling ($\times10^{-2}$ mm)[b] at various times between immunization and transfer	
	Route	Antigen		4 days	7 days
I	all[c]	Hanks	10^8	5.5±1.7[d]	1.6±1.6
II	s.c.	10^6 HCs	10^8	24.2±6.9	24.6±6.2
III	s.c.	10^6 HCs	3×10^7	8.0±2.3	2.4±2.6
IV	i.s.	10^6 HCs	10^8	8.0±4.1*	3.0±3.7*
V	i.v.	10^6 HCs	10^8	1.0±0.6*	2.0±4.3*

a) Mice were immunized s.c., i.s., or i.v. with rat HCs (1×10^6 cells) or Hanks' solution.
b) Four or 7 days after immunization, donor spleen cells (3×10^7 cells or 1×10^8 cells) were transferred to naive recipient mice. Six hr later, HCs, (1×10^5 cells) were injected into the s.c. tissue of the footpad of recipient mice, and footpad swelling was measured at 18 hr after challenge.
c) All represent the routes injected with Hanks' solution for immunization.
d) The means±SD of 5 to 6 mice are given.
* There was a significant difference between Expt. II and Expt. IV or V ($P<0.01$).

in Table 2). However, transfer of 3×10^7 spleen cells from mice immunized s.c. with 10^6 rat HCs failed to induce significant DTH responses to rat HCs in naive recipient mice (Expt. III). Spleen cells from mice 4 or 7 days after i.s. (Expt. IV) or i.v. (Expt. V) immunization with 10^6 rat HCs were also not effective in the induction of DTH responses to rat HCs in recipients.

Co-transfer of s.c.-immunized spleen cells with i.s. or i.v. immunized spleen cells: In order to examine the effect of transfer of spleen on DTH responses, naive recipient mice were co-transferred i.v. with spleen cells from mice immunized s.c. with 10^6 rat HCs and spleen cells from each group of normal mice, i.s. injected mice and i.v. injected mice. As shown in Table 3, recipient mice transferred with 10^8 spleen cells from mice immunized s.c. with rat HCs together with 3×10^7 or 10^8 spleen cells from i.s. immunized mice showed a considerable level of DTH responses (Expt. II and III). However, transfer of spleen cells from mice immunized s.c. with rat HCs together with spleen cells from mice immunized i.v. with rat HCs failed to induce the DTH response in recipients (Expt. IV).

Failure to transfer suppression of DTH response at induction phase: To examine the effect of transfer of spleen cells from mice immunized i.s. or i.v. with rat HCs on the induction phase of the DTH responses, 10^8 spleen cells from mice immunized i.s. or i.v. with rat HCs were injected into the tail vein of naive mice. Six hr later, they were immunized s.c. with rat HCs (1×10^6 cells). Seven days after s.c. immunization, rat HCs, (1×10^5 cells) were injected into the s.c. tissue of the footpad of recipient mice. The DTH responses were evaluated at 24 hr after challenge. As shown in Table 4, the level of DTH responses in recipient mice transferred with spleen cells from i.s. or i.v. immunized mice was extremely similar to that in recipient mice transferred with spleen cells from s.c. immunized mice.

Table 3. Co-transfer of spleen cells from mice immunized i.s. or i.v. with rat HCs and spleen cells from s.c. immunized mice

Expt.	Combination of spleen cells co-transferred[a]	Footpad swelling ($\times10^{-2}$ mm)[b]	
		12 hr	18 hr
I	s.c./10^8+normal/10^8	19.2±5.6[c]	29.4±4.3
II	s.c./10^8+i.s./10^8	29.8±5.3	24.6±11.8
III	s.c./10^8+i.s./$3x10^7$	23.0±3.5	23.6±4.9
IV	s.c./10^8+i.v./10^8	2.2±3.7*	2.0±1.7*

a) Mice were immunized s.c., i.s., or i.v. with 10^6 rat HCs. Seven days later, co-transfer of 10^8 spleen cells from s.c. immunized mice with 10^8 spleen cells from each group of normal mice, i.s. immunized mice and i.v. immunized mice to naive mice was performed.
b) Six hr after transfer, recipient mice were challenged with 10^5 rat HCs into the RFP and with 10^5 syngeneic mouse HCs into the LFP. Data express footpad swelling at 12 and 18 hr after challenge.
c) The means±SD of 5 to 6 mice are given.
* There was a significant difference between expt. I or II and IV ($P<0.05$).

Table 4. Failure to transfer the suppression of the DTH response at the induction phase

Expt.	Immunization with 10^6 rat HCs via various routes[a]	Number of spleen cells transferred at 6 hr before s.c. immunization[b]	Footpad swelling ($\times10^{-2}$mm) at 24 hr after challenge[c]
I	s.c.	10^8	54.4±4.9[d]
II	i.s.	10^8	48.2±12.3
III	i.v.	10^8	53.2±6.9

a) Mice were immunized s.c., i.s., or i.v. with 10^6 rat HCs.
b) Seven days later, transfer of spleen cells (1×10^8 cells) from s.c., i.s., or i.v. immunized mice to naive recipient mice was performed. Six hr after cell transfer, the recipient mice were immunized s.c. with rat HCs (1×10^6 cells).
c) Seven days after s.c. immunization, the recipient mice were challenged. Data express footpad swelling 24 hr after challenge.
d) The means±SD of 5 to 6 mice are given.

DISCUSSION

The results in the present study are summarized as follows; (1) the DTH response in mice sensitized i.s. or i.v. with rat HCs was significantly lower than that in s.c. sensitized mice, (2) preimmunization with rat HCs via i.s. or i.v. routes resulted in antigen-specific suppression of the DTH response induced by s.c. immunization with rat HCs, (3) co-transfer of spleen cells from mice immunized i.v. with rat HCs with spleen cells from s.c. immunized mice induced the suppression of the effector phase of the DTH response. However, transfer of spleen cells from mice immunized i.s. with rat HCs together with spleen cells from s.c. immunized mice failed to suppress the DTH responses at both effector phase and induction phase. These results suggest that the DTH response to rat HCs in mice is dependent on the routes sensitized, and lower DTH responses to rat HCs in i.v. immunized mice are associated with suppressive spleen cells induced after sensitization with rat HCs.

The suppression of DTH responses was also induced in mice preimmunized i.v. with non-irradiated or irradiated allogeneic spleen cells [3, 31], and hapten-modified syngeneic or allogeneic spleen cells [18, 19]. This suppression has been shown to be mediated by T cells acting at the induction phase and effector phase of the DTH response [18, 19]. Our results on co-transfer of spleen cells from mice immunized i.v. with rat HCs and spleen cells from mice immunized s.c. with rat HCs (Table 3) suggest that the spleen in mice immunized i.v. with rat HCs has suppressive cells acting at the effector phase of DTH responses. The suppression of the DTH response to allogeneic antigens induced by i.v. preimmunization was induced even in splenectomized mice, suggesting the independence of the presence of the spleen [1, 3]. On the other hand, the spleen was needed to be present for the suppression of the DTH responses at the induction phase to 2, 4-dinitro-1-fluorobenzen in mice preimmunized i.v. with 2,4-dinitrobenzene sulfonic acid sodium salt [28].

With regard to antigens injected, C^{51}-labelled allogeneic spleen cells have been shown to be distributed in the spleen and liver rather than in the lymph node, and the amount of transplanted C^{51}-labelled allogeneic spleen cells in the spleen of recipients is similar to that in the liver [4]. The liver has also been suggested to be essential for the induction of specific unresponsiveness to skin allografts in mice, which is mediated by suppressor T cells [4]. Injection of allogeneic spleen cells into the portal vein resulted in the suppression of the DTH response mediated by suppressive serum factor of IgG class at the induction phase [11, 23]. These results have suggested that the induction of suppressive spleen cells involving in the suppression of the DTH response depends on the condition to be injected with antigens and the routes to be sensitized. In the present study, suppressed DTH responses in mice immunized with rat HCs were shown to be induced by i.v. and i.s. sensitization but not by s.c. sensitization. I.s. immunization of $10^3 - 10^6$ rat HCs used in the present study induced the suppression of DTH responses in mice, whereas i.s. immunization with small doses of antigens, which were aberrantly expressed molecules immunoprecipitated from the lysate of triazene-xenogenized L5178Y/DITC cells, has been shown to induce the DTH response and production of antibody [12]. The difference in these DTH responses may be due to the different doses of antigens rather than antigenicity.

Our results showed that a considerable level of the DTH response to xenogeneic HCs was induced in the footpad of mice immunized s.c. with rat HCs. On the other hand, in our preliminary experiment, the DTH response in Balb/c mice immunized s.c. with 10^6 allogeneic (C57BL/6 mouse) HCs 4 days before challenge with 10^5 allogeneic HCs was significantly weak ($14.7 \pm 6.1 \times 10^{-2}$ mm), when compared with that ($55.2 \pm 10.8 \times 10^{-2}$ mm) in mice immunized s.c. with 10^6 rat HCs. These differences in the magnitude of the DTH responses in mice immunized s.c. with xenogeneic HCs and allogeneic HCs may be due to the difference of these MHC class antigens. Rat HCs have been reported to have MHC class I antigens on their surface but not class II antigens [5]. The cell types involving in the immune response may also be responsible for the difference in the level of the DTH responses to xenogeneic HCs and allogeneic HCs. Cells expressing porcine MHC class I antigens in the skin transgeneic mice have been demonstrated to be rejected by $CD4^+$ cells but not by $CD8^+$ cells [2], suggesting that $CD4^+$ cells play a more major role rather than $CD8^+$ cells. On the contrary, allogeneic hepatocyte MHC class I antigens have been reported to stimulate cytotoxicity of responder cells but not mixed hepatocyte-lymphocyte reaction, suggesting that $CD8^+$ cells play a more major role rather than $CD4^+$ cells [5, 6].

In summary, our results showed that antigen-specific suppression of DTH responses to rat HCs in mice was closely associated with the induction of suppressive spleen cells by i.s. immunization and i.v. immunization with rat HCs.

More detailed studies on immunohistochemistry of the footpad, lymph node and spleen with anti-$CD8^+$ mAb recognizing suppresser T cells and anti-$CD4^+$ mAb recognizing helper T cells are needed to understand the cellular events in the suppression of the DTH response to xenogeneic HCs.

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REFERENCES

1. Asheerson, G. L., Zembala, M., Mayhew, B., and Goldstein, A. 1976. Adult thymectomy prevention of the appearance of suppressor T cells which depress contact sensitivity to picryl chloride and reversal of adult thymectomy effect by thymus extract. *Eur. J. Immunol.* 6: 699–703.
2. Auchincloss, H. Jr., Moses, R., Conti, D, Sundt, T., Smith, C., Sachs, D. H., and Winn, H. J. 1990. Xenograft rejection of class I-expressing transgeneic skin is CD4-dependent and CD8-independent. *Transplant. Proc.* 22: 2335–2336.
3. Bianchi, A. T. J., Hussaarts-Odijk, L. M., van der Kwast, T. H., Bril, H., and Benner, R. 1984. Suppression of antigraft immunity by preimmunization. II. Characterization of the suppressor cells. *Transplantation* 37: 490–499.
4. Brent, L. and Opara, S. C. 1979. Specific unresponsiveness to skin allografts in mice. V. Synergy between donor tissue extract, procarbazine hydrochloride, and antilymphocyte serum in creating a long-lasting unresponsiveness mediated by suppressor T cells. *Transplantation* 27: 120–126.
5. Bumgardner, G. L., Chen, S., Hoffman, R., Cahill, D. C., So, S. K., Platt, J., Bach, F. H., and Ascher, N. 1989. Afferent and efferent pathways in T cell responses to MHC class I^+, II^--hepatocytes. *Transplantation* 47: 163–170.
6. Bumgardner, G. L., Chen, S., Almond, S. P., Ascher, N. L., Payne, W. D., and Matas, A. J. 1990. Role of macrophages in the immune response to hepatocytes. *J. Surg. Res.* 48: 568–572.
7. Cobourn, C. S., Makowka, L., Falk, J. A., and Falk, R. E. 1987. Allogeneic intrasplenic hepatocyte transplantation in the Gunn rat using cyclosporine a immunosuppression. *Transplant. Proc.* 19: 1002–1003.
8. Cuervas-Mons, V., Cienfuegos, J. A., Maganto, P., Golitsin, A., Eroles, G., Gastillo-Olivares, J., and Segovia de Arana, J. M. 1984. Time-related efficacy of liver cell isografts in fulminant hepatic failure. *Transplantation* 38: 23–25.
9. Ebata, H., Okikawa, I., Sawa, M., Kasai, S., and Mito, M. 1987. Survival of adult hepatocytes and fetal hepatic tissue transplanted into the spleens of allogeneic rats. *Transplant. Proc.* 19: 998–1001.
10. Fujii, H. 1993. The regeneration of transplanted hepatocytes within the mesenteric fat pad of mice. *Transplantation* 55: 452–455.
11. Fujiwara, H., Qian, J-H., Sato, S., Kokudo, S., Ikegami, R., and Hamaoka, T. 1986. Studies on the induction of tolerance to alloantigens. II. The generation of serum factor(s) able to transfer alloantigen-specific tolerance for delayed-type hypersensitivity by poral venous inoculation

with allogeneic cells. *J. Immunol.* 136: 2763–2768.
12. Grohmann, U., Romani, L., Binaglia, L., Fioretti, M. C., and Puccetti, P. 1992. Intrasplenic immunization for the induction of humoral and cell-mediated immunity to nitrocellulose-bound antigen. *J. Immunol. Methods* 137: 9–16.
13. Groth, C. G., Arborgh, B., Bjorken, C., Sundberg, B., and Lundgren, G. 1977. Correction of hyperbilirubinemia in the glucuronyltransferase-deficient rat by intraportal hepatocyte transplantation. *Transplant. Proc.* 9: 313–316.
14. Kasal, S., Sawa, M., Kondoh, K., Ebata, H., and Mito, M. 1987. Intrasplenic hepatocyte transplantation in mammals. *Transplant. Proc.* 19: 992–994.
15. Kusano, M. and Mito, M. 1982. Observation on the fine structure of long-survived isolated hepatocytes inoculated into rat spleen. *Gastroenterology* 82: 616–628.
16. Makowka, L., Rotsein, L. E., Falk, R. E., Falk, J. A., Langer, B., Nossal, N. A., Blendis, L. M., and Philips, M. M. 1980. Reversal of toxic and anoxic induced hepatic failure by syngeneic, allogeneic, and xenogeneic hepatocyte transplantation. *Surgery* 88: 244–253.
17. Matas, A. J., Sutherland, D. E. R., Steffes, M. W., Mauer, S. M., Lowe, A., Simmons, R. L., and Najarian, J. S. 1976. Hepatocellular transplantation for metabolic deficiencies: decrease of plasma bilirubin in Gunn rats. *Science* 192: 892–894.
18. Miller, S. D., Sy, M. S., and Claman, H. N. 1977. The induction of hapten-specific T cell tolerance using hapten-modified lymphoid cells. II. Relative roles of suppressor T cells and clone inhibition in the tolerant state. *Eur. J. Immunol.* 7: 165.
19. Miller, S. D., Sy, M. S., and Claman, N. H. 1978. Suppressor T cell mechanisms in contact sensitivity. II. Afferent blockade by alloinduced suppressor T cells. *J. Immunol.* 121: 274–280.
20. Miller, S. D. and Jenkins, M. K. 1986. Detection of suppressor cells and suppressor factors for delayed-type hypersensitivity responses. pp. 77.1–77.13. *In*: Cellular Immunology, 4th ed. (Weir, D. M. ed.), Blackwel Scientific Publication, Oxford.
21. Minoto, M., Houssin, D., Demma, I., Morin, J., Szekely, A. M., and Bismush, H. 1984. Transplantation of hepatocytes for treatment of surgically induced acute hepatic failure in the rat. *Eur. Sur. Res.* 16: 162–168.
22. Mito, M., Ebata, H., Kusano, M., Onishi, T., Saito, T., and Sakamoto, S. 1979. Morphology and function of isolated hepatocytes transplanted into rat spleen. *Transplantation* 28: 499–505.
23. Qian, J-H., Hashimoto, T., Fujiwara, H., and Hamaoka, T. 1985. Studies on the induction of tolerance to alloantigens I. The abrogation of potentials for delayed-type hypersensitivity responses to alloantigen by portal venous inoculation with allogeneic cells. *J. Immunol.* 1374: 3656–3661.
24. Seglen, P. O. 1976. Preparation of isolated rat liver cells. *Methods Cell. Biol.* 13: 29–83.
25. Selden, C., Gupta, S., Johnstone, R., and Hodgson, H. J. F. 1984. The pulmonary vascular bed as a site for implantation of isolated liver cells inbred rats. *Transplantation* 38: 81–83.
26. Sommer, B. G., Sutherland, D. E. R., Mats, A. J., Simmons, R. L., and Najarian, J. S. 1979. Hepatocellular transplantation for treatment of D-galactosamine-induced acute liver failure in rats. *Transplant. Proc.* 11: 578–584.
27. Sutherland, D. E. R., Matas, A. J., Steffes, M. W., Simmons, R. L., and Najarian, J. S. 1977. Transplantation of liver cells in an animal model of congenital enzyme deficiency disease: The Gunn rats. *Transplant. Proc.* 9: 317–319.
28. Sy, M. S., Miller, S. D., Kowach, H. B., and Claman, H. N. 1977. A splenic requirement for the generation of suppressor T cells. *J. Immunol.* 119: 2095–2099.
29. Tanabe, S., Taura, Y., Tanaka, M., Nakaichi, M., and Nakama, S. 1993. Suppression of delayed-type hypersensitivity (DTH) responses in xenografts by pretreatment with ultraviolet (UV)-irradiated hepatocytes. *J. Vet. Med. Sci.* 55: 853–854.
30. Toledo-Pereya, L. H., Gordon, D. A., and Mackenzie, G. H. 1982. Immunologic response to liver cell allografts. *Am. Surg.* 48: 28–31.
31. Van der Kwast, T. H., Bianchi, A. T. J., Bril, H., and Benner, B. 1981. Suppression of antigraft immunity by preimmunization. I. Kinetic aspects and specificity. *Transplantation* 31: 79–85.
32. Vromen, J. P. A. M., Blanckaert, N., Buurman, W. A., Heirwegh, K. P. M, and Kootstra, G. 1985. Treatment of enzyme deficiency by hepatocytes transplantation in rats. *J. Surg. Res.* 39: 267–275.
33. Vromen, J. P. A. M., Buurmann, W. A., Heirwegh, K. P. M., van der Linden, C. J., and Kootstra, G. 1988. Hepatocyte transplantation for enzyme deficiency disease in congeneic rats. *Transplantation* 42: 130–135.
34. Wiederkehr, J. C., Kondos, G. T., and Pollak, R. 1990. Hepatocyte transplantation for the low-density lipoprotein receptor-deficient state. *Transplantation* 50: 466–476.