Suppression of Delayed-Type Hypersensitivity (DTH) Responses in Xenografts by Pretreatment with Ultraviolet (UV)-Irradiated Hepatocytes

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ABSTRACT. F344 Rat hepatocytes (HCs) that had been exposed to ultraviolet (UV) light were transplanted into the dorsal subcutaneous tissues of Balb/c mice. Four days after the transplantation, the anti-HC delayed-type hypersensitivity (DTH) response was assessed by determining the response to a direct challenge with non-irradiated HCs. The DTH response in mice transplanted with 600 J/m²-UVB-irradiated HCs was suppressed significantly compared with that with non-irradiated HCs. Furthermore, the DTH responses evoked by challenge with non-irradiated HCs were similar to those evoked by UV-irradiated HCs.—KEY WORDS: delayed-type hypersensitivity, hepatocyte, ultraviolet irradiation.


Direct ultraviolet (UV)-irradiation to graft cells is known to attenuate their immunogenicity. It has been reported that the pretransplant exposure of islet cells to UV light causes graft survival to significantly prolong after allograft to rat) [5, 7] and xenotransplantation (rat to mouse) [8]. It was also elucidated that allotransplantation of UV-irradiated hepatocytes (HCs) elongates survival of rats with toxic (D-galactosamine)-induced hepatic failure [4] and increases plasma albumin levels in rats with inherited lack of plasma albumin [5]. There have, however, been no studies for clarifying the immunospecific effect of UV-irradiated hepatocyte and inducing tolerance with UV-irradiated donor xeno-HCs prior to grafting of other organs (for example, kidney), although we have reported that pretransplantation with UV-irradiated peripheral blood lymphocytes and whole blood induced tolerance [10, 11]. In this study the effects of transplanting UV-irradiated xenogeneic HCs from rats into mice on mice were therefore investigated and compared with those of non-irradiated xenogeneic HCs by the DTH reaction.

Female F344 rats (9–12 weeks old) were used as donors and female Balb/c ByJ mice (6–8 weeks old) as recipients. HCs from rats and mice were isolated by the Seglen’s perfusion technique with a slight modification [8]. Each animal was anesthetized with an intraperitoneal injection of pentobarbital and the peritoneal cavity was opened wide. The inferior vena cava was cannulated, the portal vein was divided, and the suprahepatic vena cava was ligated. The liver of each rat and mouse was perfused in situ at 10–15 m/min and 3–5 m/min, respectively at 37°C with an ethylen glycol-6,6'-bis-2-aminoethyl-N,N',N'-tetraacetic acid (EGTA) containing calcium-free salt solution for 3 min followed by perfusing with Hanks’ solution supplemented with 0.05% W/V collagenase (Wako Pure Chemical Industries Ltd., Osaka, Japan) for 12–15 min. The liver was excised and minced gently with two surgical blades on a siliconized glass dish, suspended in Hanks’ solution, and filtered through 60 and 150 meshes to remove large aggregates. The resulting HCs were washed three times with Hanks’ solution and centrifuged at 500×g for 1 min between washing. The viability of this cell preparation, determined by 0.2% W/V trypan blue in PBS, was 80–90%. In order to increase the viability rates, each crude suspension was layered onto a discontinuous Percoll (approximately 1.06 g/ml, Pharmacia, Uppsala, Sweden) in a 15 ml conical tube and centrifuged at 100 × g for 5 min. The supernatant was discarded and the pure HCs in the pellet were washed three times with Hank’s solution and centrifuged at 50 × g. The resulting viability was over 90%. In 200 cells stained with hematoxylin and eosin, the rate of contamination with nonparenchymal cells was less than 1%. Next the HCs were suspended in 10 ml Hank’s solution and an aliquot was placed on a siliconized glass dish (10 cm in diameter). The aliquots were exposed to UV light from a UV lamp (Model UVM-37, 302 nm, UVP Inc., San Gabriel, CA, U.S.A.) 10 cm away. For the first experiment, UV energies of 0, 300, 600, 1,400, 3,500, and 7,000 J/m² were used to irradiate the HCs. The viabilities of all the HCs subjected to UV-irradiation exceeded 90%.

A total of 1 × 10⁶ HCs in 0.1 ml Hank’s solution was transplanted into the subcutaneous dorsal tissues of the mice. Four days later, the mice were tested for DTH by a challenge injection of 1 × 10⁶ rat HCs in 0.025 ml Hank’s solution into the right hind footpad. Twenty-four hours after the challenge, the footpad swelling was determined by measuring the thickness by using a dial thickness gauge (Ozaki Engineering, Tokyo, Japan). The results were expressed as the mean ± S.D. and the differences among the various groups were analyzed by Student’s t test for unpaired data. Differences at P<0.05 were considered significant.

It was found that the footpad thickness due to DTH after inoculation with HCs exposed to UV energies of 0, 300, 600, 1,400, 3,500, and 7,000 J/m² were 54 ± 9, 49 ± 18, 33 ± 5, 54 ± 10, 82 ± 25, and 76 ± 9 (× 10⁻² mm), respectively. The mean DTH response in mice transplanted with 600 J/m²-UV-irradiated HCs was significantly suppressed (P<0.005), by approximately 39%, compared with that of control mice transplanted with non-irradiated HCs, in which the mean footpad thickness was 49 ± 15 × 10⁻² mm, similar to that of the mice injected with 600 J/m²-UV-irradiated HCs (Fig. 1).

The DTH response depends on T lymphocytes and helper T cells (Th-1) [2], which recognize antigen — major histocompatibility class II complexes on antigen-presenting cells (APC). The antigen that comprises this
complex on APC is allogeneic-major histocompatibility complex (MHC) class I or non-MHC type antigen taken up and processed by APC [1, 9, 10]. It has not yet to be elucidated that the mechanism of the DTH response to xenogeneic antigens is similar to that to allogeneic antigens. Direct UV-irradiation of murine cells has been shown to induce an initial loss in H-2K, IA, and IE antigen densities. Moreover, IA antigen synthesis in UV-irradiated cells was inhibited [6]. Murine hepatocytes isolated by the collagenase perfusion expressed an MHC class I, but not class II, antigen [1]. In our study, the DTH response was suppressed significantly by subcutaneous injection of 600 J/m²-UV-irradiated HCs, when compared with that evoked by non-irradiated HCs. This immuno-suppressive effect may be due to a reduction of MHC class I and/or minor MHC antigens on UV-irradiated HCs. The DTH immune response to the challenge with 600 J/m²-UV-irradiated HCs 4 days in mice transplanted with non-irradiated HCs did not differ from that with non-irradiated HCs. This suggests that UV-irradiation may have no effect on the antigenicity of the HCs, but cause the immunogenicity to decrease. It was concluded that 600 J/m²-UV-irradiated HC treatment is an effective method for monitoring immunospecific responses to xenogeneic antigens without initiating host versus-graft rejection. Further investigations are required to determine whether after transplantation of graft (for example, liver or skin) challenge with UV-irradiated HCs induce the reduced graft rejection compared with non-irradiated HCs.

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