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Reduced Immunogenicity of Rat Renal Allografts After Photochemical Donor Pretreatment and Passive Transfer of Graft Protection

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Summary. Photochemical pretreatment of the kidney donor (Sprague-Dawley rats/SD) with 8-methoxypsoralen (8-MOP) and ex vivo longwave ultraviolet (UVA) irradiation of the kidney graft (PUVA therapy) significantly prolonged survival in allogeneic recipients (BD IX rats). After more than 100 days 7 long-term surviving PUVA-pretreated SD kidneys were retransplanted into BD IX rats. Seven out of 7 secondary recipients survived for more than 100 days. Twenty BD IX recipients of normal SD kidneys were treated at the time of transplantation with serum (1 ml i.v.) and/or spleen lymphocytes (1×10^7 i.v.) obtained from the PUVA-treated long-term survivors. A prolonged graft survival was achieved in 7 our of 20 rats, among them 4 out of 8 recipients of the serum-treated group. In conclusion, the long-term survival of PUVA-treated rat renal allografts is associated with a strong reduction of graft immunogenicity and the development of graft protecting humoral as well as cellular effectors.

Key words: Renal transplantation, PUVA, Donor pretreatment, Ultraviolet irradiation, Immunogenicity.

Introduction

Ultraviolet light of the UVB region (290–320 nm) or the photosensitizer 8-MOP plus UVA light (320–400 nm), which is the PUVA therapy used in the treatment of psoriasis, can inhibit antigen presentation [12, 21]. In murine skin [11], rat kidney [31] and rat pancreatic islet allograft models [25] a beneficial effect of UVB and PUVA on allograft survival has been demonstrated. The results showed that photochemical treatment of grafts can alter their immunogenicity.

The most important mechanism responsible for the long-term survival of rat renal allografts is the reduction of immunogenicity or graft adaptation by loss or depletion of passenger leukocytes [16, 26, 28] including interstitial dendritic cells (DCs, [17, 29, 34]). The purpose of this study was to show that long-term survival of PUVA-treated SD renal allografts in BD IX recipients is mediated by both a reduced immunogenicity and graft protecting effectors.

Material and Methods

Animals. Inbred male rats (310–440 g) were used (Academy of Science of the GDR, Central Institute for Cancer Research, Berlin). SD rats (RT-1^u) served as kidney donors; BD IX rats (RT-1^d) acted as recipients. These strains are different from each other at their major histocompatibility complex (MHC).

Kidney Transplantation. The microsurgical technique used has been published in this journal [30]. Left orthotopic grafts with end-to-end anastomoses of the renal vessels and the ureter were done. Right recipient nephrectomy was performed at the time of transplantation. Ischemic time ranged from 13 to 21 (mean 18) min. Graft function was followed by serial blood urea nitrogen (BUN) estimations (day 2, 7, 10, 14, thereafter weekly). Autopsies were performed on most rats in the manner reported previously [19].

Lymphocytotoxic Antibodies. The determination of complement-dependent lymphocytotoxic antibodies was done by means of the ^{51}Cr-release test. Pooled rabbit serum (dilution 1:10) served as a source of complement. Splenic lymphocytes isolated by centrifugation on a Ficoll-Visotrast-gradient (density: 1.078 g/ml) were used as target cells [10].

Generation of Long-Term Surviving Renal Allografts. An indefinite graft survival was achieved by donor pretreatment with 8-MOP and direct UVA irradiation of the graft as described previously [31].

Experimental Groups. The experimental design is shown in Table 1. In a pilot control study of 3 allogeneic transplants (groups 1a) the effect of pooled serum from BD IX rats injected at the time of grafting into the recipient on the survival time of normal SD kidneys was tested. At the time of retransplantation of PUVA-treated SD kidneys into BD IX recipients (group 2) these long-term surviving rats ($n = 7$) were exsanguinated in parallel and their spleens removed. Thereafter 1 ml serum of the long-term survivors (group 3), 1×10^7 spleen lymphocytes separated by density gradient centrifugation (group 4) or 1 ml serum plus 1×10^7 spleen cells (group 5) were injected into fresh BD IX recipients of normal SD kidneys at the time

Table 1. Experimental group design and results

Experimental groups/treatment		*n*	BUN at day 7 ($\bar{x}$, mmol/l)	graft survival (days)
(1)	Untreated control	10	76	8, 8, 8, 9, 9, 9, 9, 10, 11, 11
(1a)	Non-immune serum	3	74	9, 11, 11
(2)	Retransplantation	7	17	> 100 (7x)
(3)	1 ml serum	8	54	14, 15, 15, 25, > 100 (4x)
(4)	1 x 10^7 spleen cells	6	71	10, 11, 11, 13, 16, > 100 (1x)
(5)	cells + serum	6	58	11, 14, 14, 16, > 100 (2x)

of transplantation. Recipients with elevated BUN levels on the 2nd day after operation as a results of technical complications (ureteral stenosis or fistula, thrombosis) were excluded from the study.

Results

The results in the experimental groups are summarized in Table 1. In the untreated control group 1 and the pilot study group 1a the kidneys were rejected rapidly which was verified histologically. However, 7 out of 7 secondary recipients (group 2) survived for more than 100 days. They produced elevated levels of lymphocytotoxic antibodies with a post transplant peak on day 7 (nearly 60% specific ^{51}Cr release in only 2 determinations) and a slight increases of BUN level on day 7. The survival times after treatment of BD IX recipients of normal SD kidneys with serum or spleen lymphocytes or both (group 3–5) were prolonged. Four out of 8 rats treated only with serum (group 3), 2 out of 6 rats treated with serum + cells (group 5) and 1 out of 6 rats treated only with cells (group 4) survived permanently, but with signs of an acute immune response in the second post-transplant week.

Discussion

Recent evidence has ascribed a key role to MHC class II antigen carrying interstitial DCs in the primary alloimmune response [35]. The investigations of Hart and Fabre [17] have shown that DCs originate from bone marrow and have a high turnover rate in many tissues. It is generally agreed that the primary immunogeneic stimulation provided by transplanted tissue depends directly upon the presence in such tissues of DCs [23, 28]. In the pancreatic islet [8, 24, 25], heart [28] and kidney transplant models [18, 27, 29] intragraft DCs have been shown to constitute the major immunogenic stimulus of allograft rejection in rats.

The theoretical framework of a T helper cell activation by MHC class II antigens and the interaction of activated helper cells plus MHC class I antigen in the differentiation of cytotoxic T cells from their precursors has become more complex by the discoveries of suppressor cells and interleukins both [1, 3, 22]. As MHC class II antigen-responsive cells are generally, but not exclusively, both the helper cells considered and the class I antigen-responsive cells are not exclusively cytotoxic. Therefore, the current concept is to view the T cell lineage in terms of the class of MHC antigens that they use as "guides" for activation [3].

The modulation or depletion of such MHC class II antigen carrying antigen-presenting cells (APCs) like epidermal Langerhans cells or DCs should prevent the helper cell activation and therefore reduce the strength of host-versus-graft reaction.

Pretreatment of rat kidney donor with 300 mg/kg cyclophosphamide on day –5 in addition to 1,000 rad total body irradiation on day –2 which depleted nearly all interstitial DCs, produced a dramatic prolongation in survival of DA recipients of AS kidneys [29]. Similar results were obtained in the rat heart allograft model [28]. Faustman et al. [8] showed that elimination of Ia-positive cells from mouse islets permitted prolonged islet allograft survival in non-immunosuppressed hosts. However, such Ia-positive cells need not be eliminated physically, but may be simply inactivated by ultraviolet irradiation [15]. Furthermore, rejection of murine islet allografts was prevented by pretreatment with anti-dendritic cell antibody [9]. In the same way, the application of donor-specific alloantibodies raised against nylon adherent spleen lymphocytes significantly prolonged the survival time of F_1 (BD IX x SD) kidneys in BD IX recipients [20]. In addition, we have recently published our results on prolongation of rat renal [31] and heart allograft survival time [32] after PUVA donor pretreatment. The main target of this type of therapy also seems to be the intragraft DCs. Gruner et al. [12] have shown a specific inhibition of MHC class II antigen expression by different photochemical treatment protocols. UVB [6] or PUVA [33] treatment of mouse skin leads to a decrease of Ia antigens and the ATPase marker of epidermal Langerhans cells.

The main objective of our experiments was to clarify the mechanisms responsible for long-term renal allograft survival in the SD to BD IX rat model. From the studies of Lechler and Batchelor [27], Chui and Batchelor [5], Batchelor et al. [4], Barber et al. [2], Hart et al. [16] and Kaden et al. [20] it is obvious that the reduced immunogenicity of long-term surviving rat kidneys, obtained by induction of passive or active enhancement or by a short period

of chemical immunosuppression, after retransplantation into naive recipients was attributed to a depletion or loss of DCs and passenger leukocytes. Our results confirm this observation, 7 out of 7 PUVA-pretreated SD kidneys which had been in residence for more than 100 days in BD IX recipients survived indefinitely after retransplantation into naive BD IX rats.

Several possible mechanisms in addition to reduced immunogenicity have been explored to account this phenomenon. If enhancing or blocking antibodies and/or suppressor cells are involved the mechanism is controversial [13]. The presence of blocking factors in the serum was an early explanation – but, with one notable exception [36], it has not proved possible to transfer enhancement successfully with serum from long-term surviving animals. Fabre and Morris [7] were unable to demonstrate graft protecting effectors by passive transfer (serum or spleen cells). Recently Hall et al. [14] have been able to show that splenic T cells from rats with passively enhanced or cyclosporin-induced long-term surviving rat heart grafts can inhibit the capacity of normal lymphocytes to restore heart graft rejection in irradiated hosts, but B cells and sera failed to inhibit the restoration of graft rejection.

In contrast, an adoptive transfer of serum or spleen lymphocytes or both by Kaden et al. [20] was effecitve in preventing deleterious rat renal allograft rejection.

According to the findings of Kaden et al. [20] in passively enhanced rat renal allografts we were successful in demonstrating the presence of both enhancing antibodies and suppressor cells in long-term surviving recipients of PUVA-treated renal allografts. Both adoptively transferred serum and spleen lymphocytes prolonged the survival time of untreated SD kidneys transplanted into naive BD IX recipients. The best results were obtained by application of 1 ml serum at the time of transplantation with long-term survival in 4 out of 8 rats. If would seem, therefore, from our experiments, that maintenance of survival of PUVA-pretreated rat renal allografts was attributed to both the reduced graft immunogenicity or graft adaptation – which probably represents the loss or depletion of highly immunogenic DCs – and the development of humoral as well as cellular graft protecting effectors.

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