Transpl Int (1994) 7 [Suppl 1]: S602–S605

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Injection of v-mos-transformed and irradiated macrophages leads to longlasting specific acceptance of MHC-allogeneic heart grafts and specific prolongation of skin graft survival in mice

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Abstract In vitro studies have revealed that the v-mos-transformed clone mos2 (mos2) of the murine macrophage cell line P388D1 (D1) (H-2^d) is capable of inducing a state of specific unresponsiveness in MHC-allogeneic unprimed T cells. Here, we present data on the in vivo relevance of these findings. Male C57bl/6 mice (H-2^b) were injected i.p. 6 times with 10^7 of the following irradiated cell types: D1, mos3, mos2, DBA/2-(H-2^d) or C3H-(H-2^k) spleen macrophages. DBA/2 and C3H skin or heart grafts were performed 10 days after the last injection. The normal rejection time for allogeneic skin was 7.5 days and for allogeneic hearts, was 12.8 days. After injection of D1 or mos3, DBA/2 skin grafts were rejected after 4.5 and 6.5 days, respectively, and the hearts, after 15.4 and 18.6 days, respectively. Third-partly C3H grafts were rejected normally (7.0 days). In contrast, injection of mos2 led to prolongation of DBA skin graft survival to 12.3 days. DBA/2 hearts were accepted for more than 160 days as revealed by heart beating. Again, C3H grafts were rejected normally (11.0 days). DBA/2 skin grafts on day 102 after heart grafting survived for 30 days, indicating hyporesponsiveness against these grafts. These results confirmed the in vitro findings. The mos2 cells obviously induced a state of specific unresponsiveness in otherwise unmanipulated recipients. However, the duration of this unresponsiveness induced by the injection of irradiated cells was dependent on the organ type.

Key words P388D1 (D1) transformed with v-mos oncogene Clones mos3 and mos2

Introduction

Specific unresponsiveness is a basic phenomenon in immunology and its induction is the central aim in transplantation. MHC molecules are key structures in this phenomenon. It has been shown that oncogenic transformation influences MHC antigen expression in human and murine cell lines [1, 2, 11]. We used a model of oncogenic transformation of the established murine macrophage cell line P38D1 to induce and investigate non-immunogenicity and tolerogenicity. In previous studies, it has been shown that a v-mos-transformed clone (mos2) of the murine macrophage cell line P388D1 (D1) is incapable of inducing responses of MHC allogeneic naive spleen T cells in primary as well as in secondary proliferation assays despite unaltered MHC class I and class II

expression [13]. This indicates that a tolerogeneic potential of this clone has been detected. Here, we present data on the in vivo relevance of these findings.

Materials and methods

Mouse strains

We used 10- to 12-week-old male C57bl/6/Han (H-2^b) as recipients, and DBA/2/Han (H-2^d) and C3H/He (H-2^k) as donors. Newborn mice (max. 24-h old) served as heart donors.

Cells

The macrophage cell line P388D1 of DBA/2 origin was purchased from ATCC (Rockville, Md., USA) and transformed with the retroviral vector neo^R MPSV as described previously [9]. After selection under G-418 pressure, two stable v-mos-transformed clones, mos2 and mos3, were obtained.

Spleens were removed from DBA/2 and C3H mice under sterile conditions. Purified spleen macrophages were obtained by a controlled adherence procedure [12]. The adherent macrophages served as controls for the cell lines/clones.

Recipient treatment

C57bl/6 mice received a total of six injections of either 10^7 irradiated (60 Gy) D1, mos2 or mos3 cells (H-2^d), or DBA/2 or C3H spleen macrophages (5 Gy) i.p. every second day, followed 10 days later by transplantation of DBA/2 and C3H skin or hearts. Controls received no pretreatment.

Skin grafting

Donor thorax skin was grafted onto the ventral thorax of the recipients. Necrosis was the sign for skin graft rejection.

Heart transplantation

The method established by Judd and Trentin [3] was used. Briefly, the dorsum of the pinna of the recipient ear was cleaned with 70% ethanol. An incision, 3–5 mm in length, was made parallel to the body axis. The incision penetrated the epidermis and dermis, not the cartilage. The total donor heart was excised, with particular attention being paid to leave the sinoauricular node area intact. The excised heart was placed directly into the ear skin pouch. No attempt was made to establish vascular anastomoses between donor and recipient nor was the entrance incision closed by suture or adhesives. Accepted hearts were normally beating at a frequency of 120–180 beats/min. Heart rejection resulted in lack of beating, resorption and necrosis.

Results

Survival of skin grafts

To test the effect of mos-transformed macrophages on skin graft survival, mice were injected with either of the various cell types. After injecting D1 or mos3, DBA/2 skin grafts were rejected after 4.5 and 6.5 days, respectively (normal: 7.5 days), whereas third-party C3H grafts were rejected normally on day 7. In contrast, application of mos2 cells led to significant prolongation of DBA/2 skin graft survival to 12.3 days (Table 1). These data showed that the injected cells had specific immunomodulatory effects.

Survival of heart grafts

Because the neonatal heart is considered to be less immunogenic than skin, the effects of the injection protocol were studied on heart grafts. After injection of D1 or mos3 cells, DBA/2 heart grafts were rejected after 15.4 and 18.6 days, respectively, i.e. slightly later compared to the normal rejection time of 12.8 days. Third-party C3H grafts were rejected after 11.0 days. In contrast, after injection of mos2 cells, DBA/2 hearts were accepted for more than 160 days (Table 2). These effects

Table 1 Skin grafting: prolongation of skin graft survival after injection of mos2 in untreated MHC fully allogeneic recipients. Survival time of the transplants in days

Pretreatment of C57 recipients (number per group: 6)	Strain combination		
	C57→C57 (H-2^b)	DBA→C57 (H-2^d)	C3H→C57 (H-2^k)
None	> 100	7.5 (7–8)	7.0
D1 (H-2^d)	n.d.	4.5 (4–5)	7.0
Mos3 (H-2^d)	n.d.	6.5 (6–7)	7.0
Mos2 (H-2^d)	n.d.	12.3 (10–14)	7.2 (7–8)

Table 2 Heart transplantation: longstanding transplantation tolerance in adult mice after injection of mos-transformed macrophages in untreated MHC fully allogeneic recipients. Survival time of the transplants in days

Pretreatment of C57 recipients (number per group: 10)	Strain combination		
	C57→C57 (H-2^b)	DBA→C57 (H-2^d)	C3H→C57 (H-2^k)
None	10/12: > 100	12.8 (10–14)	11.0 (10–14
D1 (H-2^d)	> 100	15.4 (15–17)	10.9 (10–14)
Mos3 (H-2^d)	> 100	18.6 (17–20)	10.9 (10–14)
Mos2 (H-2^d)	> 100	7/10: > 160	11.0 (10–14)

Table 3 Specific prolongation of heart donor skin graft survival 80 days after heart transplantation in mos2-pretreated C57 recipients

Skin donor	Survival time (days)
C57	>60 ($n=7$)
DBA	29.6 (29–30, $n=7$)
C3H	7.0 ($n=7$)

indicated that mos2 cells induced a state of specific unresponsiveness. This unresponsive state depended on the tissue grafted.

Survival of secondary skin grafts after prolonged survival of DBA/2 heart grafts in mos2-pretreated C57bl/6 recipients

To study the state of unresponsiveness observed after injection of mos2 cells and heart transplantation, heart-donor skin was grafted 80 days after heart transplantation. DBA/2 skin grafts were accepted for 30 days (Table 3). These data indicated a state of hyporesponsiveness. The specificity of this state was revealed by normal rejection of third-party skin. The survival of the previous heart grafts was not effected.

Discussion

The data presented here clearly demonstrated that the v-mos-transformed clone mos2 of the murine macrophage cell line P388D1 expressing MHC class I and class II structures is normally capable of inducing long-standing specific unresponsiveness towards MHC fully allogeneic grafts in non-immunosuppressed recipients. The parental cell line led to accelerated skin graft rejection. The slight prolongation of heart graft survival by these cells suggested that the requirements for acceptance or rejection of different organs may vary from organ to organ.

The fact that in the experiments described no immunosuppressive regimen was added to the cell injection, neither by drugs [4] nor by antibodies [5, 6], makes them clearly different from other recent in vivo approaches towards transplantation tolerance. Injection of cells that are MHC-identical to the subsequent grafts may be an equivalent to the donor-specific blood transfusions that have been reported to be of beneficial effect in clinical renal transplantation [7, 10]. However, the experiments described here were different from the usual clinical situation where only partial MHC mismatches are accepted by immunosuppressed recipients, in that the organ grafts are fully MHC-incompatible to the non-immunosuppressed recipient.

The observation that the two clones, mos2 and mos3, showed completely different features in terms of immunogenicity and tolerogenicity suggested that the tolerogenic capacity of mos2 is induced by mos transformation but may not be mos oncogene dependent. This is likely since the insertion of the oncogene in the genome of the cells occurs randomly [8] in as yet unpredictable loci. The ongoing work on identification of the insertion loci in the genome and the analysis of the flanking sequences and their function in the mos2 cells may allow direct in vitro genetic intervention, e. g. by gene targeting, in other, non-transformed cells to induce the features observed in the clone mos2 and to avoid the risk of malignancies in vivo. This may lead to a new approach to induce transplantation tolerance without immunosuppression of the recipient.

Acknowledgement The authors wish to acknowledge the expert technical assistance of Mrs. Gudrun Scherer and Mrs. Friederike Steigleder.

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