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Heart allograft survival in rats following immunization with soluble peptide MHC class I donor antigens: evidence for the role of indirect recognition in rejection

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Abstract The current series of experiments addressed the question of whether indirect priming with donor MHC antigens affects heart allograft survival. LEW (RT-1^1) rats were immunized with a mixture of two peptides corresponding to the variable region of MHC class I locus A^a antigen ($\alpha 1$ and $\alpha 2$ domain). The recipients were transplanted with a DA (RT1-1^a) heart 1 month after immunization, and graft survival was closely monitored by ECG. All peptide-treated recipients presented with anti-peptide antibodies at the time of transplantation and developed a strongly accelerated graft rejection. These findings indicated that indirect recognition of MHC I donor antigens promotes heart allograft rejection.

Key words Allorecognition · MHC Graft rejection · Peptide

Introduction

To be recognized by T cells, foreign antigens must be taken up by antigen-presenting cells (APC), digested, and reexpressed on the cell membrane in association with self MHC molecules [1]. Among the few known exceptions to this indirect recognition is the recognition of foreign MHC molecules. MHC antigens presented on donor cells can be directly recognized by recipient T cells [2]. In addition, MHC antigens can also be indirectly recognized [3]. Whereas the role of direct recognition in graft rejection has been extensively analyzed, little is known about the influence of indirect recognition.

In rat skin transplant experiments, Fangmann et al. [4] first showed that priming with indirect allorecognition with peptides derived from donor MHC causes second set rejection. Whereas untreated rats rejected donor skin in 9.3 days, primed animals rejected in 7.5 days. We studied the effect of indirect priming with donor MHC antigens on heart allograft survival in a rat model.

Materials and methods

Two peptides corresponding to the variable region of DA MHC class I (RT1-A^{av1}) were synthesized. They corresponded to the α helical region of the $\alpha 1$ and $\alpha 2$ domains, with the sequence for $\alpha 1$ as follows: HN-Pro-Glu-Tyr-Trp-Glu-Gln-Gln-Thr-Arg-Ile-Ala-Lys-Glu-Trp-Glu-Gln-Ile-Tyr-Arg-Val-Asp-Leu-Arg-Thr-OH and for $\alpha 2$ as follows: H2N-Thr-Arg-Asn-Lys-Trp-Glu-Arg-Ala-Arg-Tyr-Ala-Glu-Arg-Leu-Arg-Ala-Tyr-Leu-Glu-Gly-Thr-Cys-OH. Nine LEW rats were immunized s.c. with a mixture of $\alpha 1 + \alpha 2$ peptide (combined 100 µg + 100 µg) in Freund's adjuvant (FA) 1 and 2 months before transplantation. Eleven controls received FA only. Subsequently, the recipients were transplanted heterotopically with DA hearts and graft survival was monitored by ECG. Rejection was defined as the time at which the transplanted heart stopped beating.

Anti-peptide antibodies were measured in sera of immunized animals using a stepwise incubation procedure in microtiter plates coated with 0.5 µg/well of $\alpha 1$- or $\alpha 2$-peptide, with test serum, mouse F(ab')2 anti-rat Ig (Jackson Immunoresearch Lab, West Grove, Pa), and PNP substrate. Optical density was determined at 405 nm.

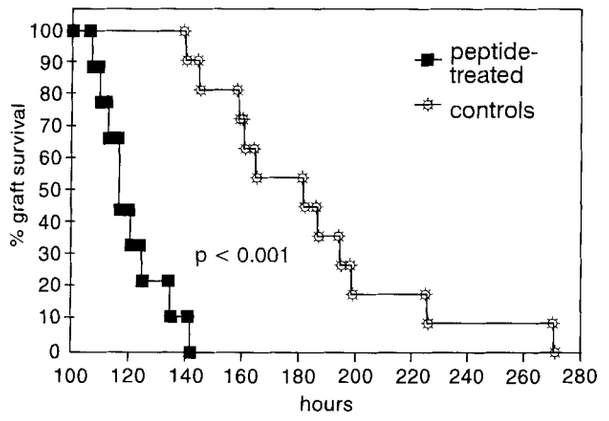

Fig. 1 Heart (DA) allograft survival in LEW recipients preimmunized with donor MHC I peptides

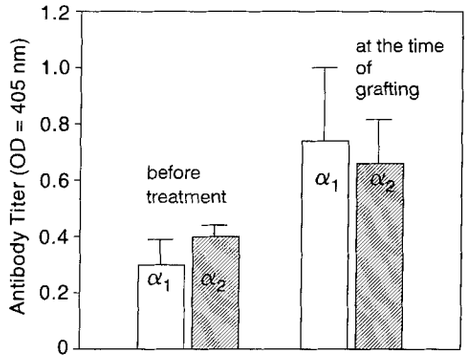

Fig. 2 Antibody response against α1 and α2 peptides in sera of rats before immunization and at the time of transplantation

Results and discussion

Kaplan-Meier survival curves of heart allografts in peptide-sensitized and -unsensitized recipients are shown in Fig. 1. The survival time was significantly shortened in sensitized (indirectly primed) recipients.

After immunization the recipients developed antibodies against both α1- and α2-donor type peptides (Fig. 2). This demonstrated that the peptides were immunogenic, i.e., able to induce an immune response. Formation of antibodies to MHC peptides is T cell dependent. Therefore, the presence of antibodies in our experiments indicated that the peptides were recognized by T cells. T cell activation by MHC peptides can be induced only by a costimulatory signal provided by APC [3]. The involvement of APC demonstrated the indirect recognition of donor antigen.

Depending on the species, accelerated graft rejection may be the consequence of preformed antibodies or primed T cells. Previous experiments have shown that, in rats, antidonor antibodies have a graft-protective rather than a deleterious effect [5–7]. Moreover, it has been shown [4] that antibodies to α1 and α2 peptides do not react with intact MHC molecules expressed on cells and, therefore, are not able to promote graft rejection. The accelerated graft rejection in rats following peptide immunization described herein can be explained by the presence of indirectly primed donor-reactive T cells.

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