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JNMU
Journal of Nanjing Mecical University, 2007, 21(1): 47-50
www.elsevier.com/locate/jnmu
Research Paper

Response of the xenograft endothelium in the concordant xenotransplantation

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Received 28 April 2006

Abstract

Objective: To investigate the response of the xenograft endothelium in the concordant hamster to rat cardiac xenotransplantation and the mechanism of acute vascular rejection. **Methods**: The animals were divided into 5 groups randomly: control group, CsA group, splenectomy group, D0 splenectomy+CsA group and D3 splenectomy+CsA group. Hamster heart was heterotopicaly transplanted to rat abdominal cavity. The graft survival was monitored by palpation of the rat abdominal wall. The histological and ultrastructural changes of the xenogafts were investigated. NF-κB and P-selectin expression in the xenograft were detected. Heme Oxigenase-1 and Bcl-2 expression were also detected in the xenografts of different groups. **Results**: The mean survival time of the xenografts in control group, CsA group, splenectomy group, D0 splenectomy+CsA group and D3 splenectomy+CsA group was 3.4 ± 0.55, 3.8 ± 0.45, 6.4 ± 1.52, 30 and 7.4 ± 1.14 days. The rejected graft showed typical acute vascular rejection in control group, CsA group, splenectomy group and D3 splenectomy+CsA group. Endothelial cells of the rejected xenograft showed dramatic assembly of ribosomes and expansion of the rough endoplasmic reticulum. However, the endothelium of the long-term survived grafts in D0 splenectomy+CsA group showed normal architecture. NF-κB and P-selectin expression were detected in the rejected xenografts. HO-1 expression was observed in the long-term survived xenografts in D0 splenectomy+CsA group. **Conclusion**: The endothelial cells of the xenograft might be activated during the acute vascular rejection. Expression of HO-1 might inhibit the upregulation of NF-κB and adhesion molecular which decreases the activation of the endothelium of the graft.

Keywords: endothelium; xenotransplantation; cyclosporine

INTRODUCTION

Organ transplantation has become an effective therapy method for advanced organ diseases after nearly 50 years of development. However, the demand for organs far outstrips the supply. Although as an experimental procedure, cross-species transplantation, or xenotransplantation, offers a promising alternative to the use of human organs[1-2]. But the immunological barrier in xenotransplantation is greater than that in allotransplantation. The xenograft can induce severe immune response after it is implanted in the recipient. Xenoreactive natural antibodies (XNA) and complement (C) are thought to be the two major humoral factors that result in hyperacute rejection (HAR) of an discordant xenograft [3-4]. If recipients are modified by various experimental modalities such as removal and/or suppression of XNA- and C-mediated responses, thus abrogating HAR, a process of acute vascular rejection (AVR) with a significant vascular destruction still occurs within days to weeks [5]. The final result is the loss of xenografts, which currently limits the application of xenotransplantation. In this study, the changes of the xenograft endothelium in the concordant hamster to rat xenotransplantation model were investigated and the mechanism of AVR was discussed.

MATERIALS AND METHODS

Cardiac xenotransplantation

Hamsters weighing 80~100 g were used as donors and SD rats weighing 180~200 g as recipients. The

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animals were obtained from the Experimental Animal Center of Xi'an JiaoTong University. Animals were free to food and water. Hamster to SD rat concordant cardiac xenotransplantation models were constructed using 'cuff and sleeve' method which was described previously[6].

Experimental groups

The animals were divided into 5 groups randomly: control group (n=10), receiving hamster to SD rat heart transplantation without immunosuppressant; CsA group(n=10), CsA [10 mg/(Kg·d)] intraperitoneally from the day of transplantation; splenectomy group (n=10), receiving splenectomy without immunosuppressant; D0 splenectomy+CsA group(n=15), receiving splenectomy (performed on the day of the transplantation) and immunosuppressant; D3 splenectomy+CsA group(n=10), receiving splenectomy(performed on the third day after the transplantation) and immunosuppressant. The graft survival in each group was monitored through palpation of the abdominal wall of the recipient. The mean survival time(MST) of the xenograft was defined as the period from the reperfusion of the graft to the time when the heart stopped beating [7]. The rat was sacrificed when the xenograft stopped beating.

Electron microscopy

Samples obtained from control group, D0 splenectomy+CsA group and D3 splenectomy+CsA group were fixed in 1% glutaraldehyde and 4% formaldehyde in 0.1 mol/L phosphate buffer for electron microscopy.

Histology and Immunohistochemistry assay

The xenografts were harvested 3, 7, 14, 30 days after cardiac transplantation in each group, and were fixed in neutral-buffered formalin, embedded in paraffin, sectioned, and stained for light microscopy. NF-κB and P-selectin expression were detected using monoclonal antibodies to NF-κBp65 and P-selectin. heme oxigenase-1 and Bcl-2 expression were detected using monoclonal antibodies to heme oxigenase-1 and Bcl-2.

Statistical analysis

Results were expressed as mean±SD. The statistical analyses were performed using SPSS10.0 software package. P Value < 0.05 was considered significant.

RESULTS

Survival of the xenografts

55 cases of hamster to SD rats cardiac xenotransplantation were included in the study. The MST of D0splenectomy+CsA group was longer than any other group(P < 0.05). The MST of splenectomy group and D3splenectomy+CsA group was longer than that of CsA and control groups. The MST of the xenografts were shown in ***Table 1***).

Table 1 **Xenograft survival in different groups**

Groups (n = 5)	MST(days)
Control group	3.4 ± 0.55
CsA group	3.8 ± 0.45
Splenectomy group	6.4 ± 1.52*
D0 Splenectomy + CsA group	30*
D3 Splenectomy + CsA group	7.4 ± 1.14*

Compared with control group, *P < 0.05

Histological changes of the xenografts

When rejection occurred in control group, CsA group, splenectomy group and D3splenectomy+CsA group, the xenografts turned pitchy and hardy. The rejected grafts showed diffuse intravascular thrombosis, severe hemorrhage, necrosis of the myocytes and mononuclear cells infiltration (***Fig. 1***). In D0 splenectomy +CsA group, xenografts harvested 7 days after transplantation showed almost normal tissue architecture, and xenografts collected 30 days after transplantation showed excellent preservation of myocardial architecture, with normal vessels and no evidence of chronic injury, although grafts showed mononuclear cells infiltration(***Fig. 2***).

Election microscopy

Ultrastructural analysis of the rejected xenograft in control group showed dramatic assembly of ribosomes and expansion of the rough endoplasmic reticulum in endothelial cells, and an absence of nuclear condensation (***Fig. 3***). In D1 splenectomy +CsA group, xenograft harvested 30 days after transplantation showed normal architecture(***Fig. 4***).

Immunohistochemical evulation

The immunohistological evaluation showed that NF-κB and P-selectin were expressed in endothelium and smooth muscle cells of the rejected xenograft in control group, CsA group, splenectomy group and D3 splenectomy +CsA group (***Fig. 5,6***). In D0 splenectomy+CsA group, HO-1 was expressed in the endothelium of the xenografts 7 days after the transplantation, and the expression was increased 14 days after transplantation(***Fig. 7***); HO-1 expression could also be observed in the endothelium of the xenograftd collected 30 days after transplantation.

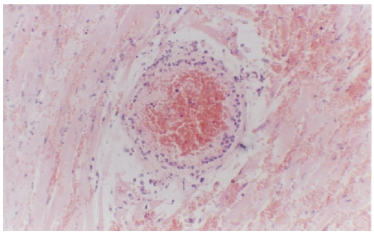

Fig. 1 Xenograft harvested 3 days after the transplantation in Control group. HE ×20

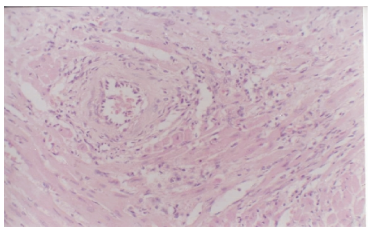

Fig. 2 Xenograft harvested 30 days after the transplantation in D0 Splenectomy+CsA group. HE ×20

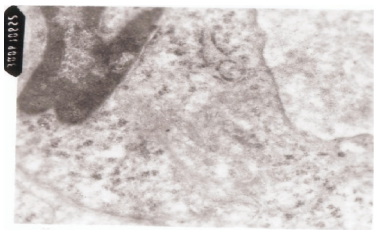

Fig. 3 Ultrastructural of cardiac endothelial cell during AVR in control group electron microscope(×30,000)

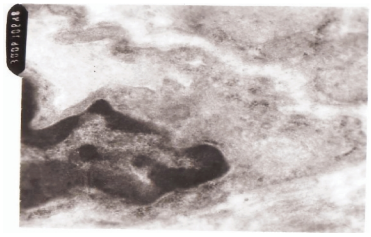

Fig. 4 Ultrastructural of cardiac endothelial cell 30 days after transplantation (Dsplenectomy +CsA group) electron microscope(×30,000)

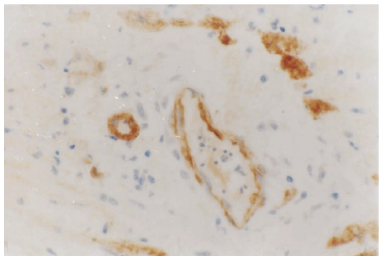

Fig. 5 HO-1 expression in the endothelial cell 14 days after the transplantation in D0 Splenectomy+CsA group (×40)

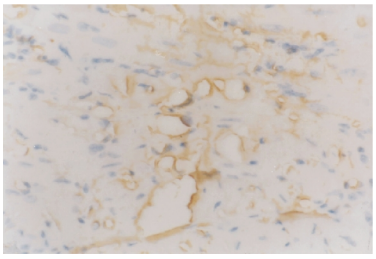

Fig. 6 P-selectin expression in the endothelial cells 3 days after the transplantation in control group (×40)

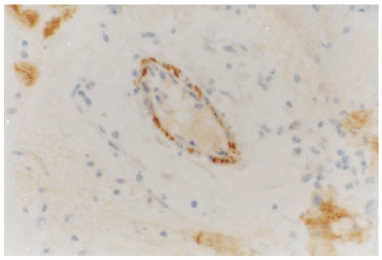

Fig. 7 NF-κB expression in the endothelial cell 3 days after the transplantation in Control group (×40)

However, the expression of the Bcl-2, NF-κB and P-selectin were not detected in those grafts.

DISSCUSSION

Severe immune response is still the main obstacle of xenotransplantation in the clinical application. After years of study, the hyperacute rejection can be prevented through suppression of XNA and/or the complement activation in the disconcordant xenotransplantation. However, this procedure could only delay the rejection for about several days to one week. Another vigorous immune reactions, AVR will lead to graft loss eventually [8-9]. Although the mechanism of AVR is still not clear, the pathogenesis of AVR has been thought to be initiated by the activation of the endothelium of the grafts [10]. The binding of the xenoreactive antibody in xenograft activates the endothelium, causing upregulation of the adhesion molecular and inflammatory cytokines that eventuate in thrombosis and loss of the graft[11].

The binding of xenoreactive antibody in endothelium is the first step that induces AVR. It was reported that the relatively lower titers of xenoreactive antibody might induce accommodation of the xenograft [12-13]. In the previous study, it was observed that secretion of xenoreactive antibody in the recipients could be partially inhibited by splenectomy. Long term survival of xenograft was successfully induced through D0 splenectomy (splenectomy performed on the day of the transplantation) combined with CsA. In this study, the expression of NF-

κB and P-selectin were significant in endothelial cells and smooth muscle cells in rejected xenografts. The ultrastructure analysis of the endothelial cells showed increased number of ribosomes and expansion of the rough endoplasmic reticulum. However, no evidence of nuclear condensation was observed, which indicated typical endothelial cell activation. Therefore, the binding of xenoreactive antibody might activate the xenograft endothelium, and the activated cell express NF-κB and P-selectin could aggravate the immunologic injury (mononuclear cell infiltrate, platelet aggregation). The ultrastructure of the endothelial cells of the long term survived graft in D0 splenectomy+CsA group showed normal architecture, which implied that the activation of the graft endothelium was inhibited. In this study, HO-1 expression was observed in the grafts harvested 7 days after the transplantation and increased on the 14th day. However, no evidence of NF-κB and P-selectin expression was observed in the xenografts harvested at the same time. It was reported that HO-1 played a key role in maintaining antioxidant and oxidant homeostasis during cellular injury[14]. The HO-1 system is thought to exert four major functions: (1) antioxidant function; (2) the maintenance of microcirculation; (3) modulatory function upon the cell cycle; and (4) anti-inflammatory function[15]. Vachharajani et al [16] observed that HO-1 and biliverdin modulated leukocyte infiltration by altering the expression of P-selection and E-selectin in lung, kidney, liver, and intestine in an endotoxin model. This study postulated that HO-1 expression might inhibit the expression of NF-κB and P-selectin, and inhibit the activation of endothelium of the graft, which was thought to be the key step to initiate the AVR. In this study, Bcl-2 expression was not observed in the xenograft as previously reported[17]. The role of Bcl-2 in the xenotransplantation needs further investigation.

In conclusion, the activation of endothelium of xenograft in concordant xenotransplantation model might be the key step to cause AVR. HO-1 expression might inhibit the upregulation of NF-κB and adhesion molecules, which protected the xenograft.

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