Transpl Int (1997) 10: 141–144

ORIGINAL ARTICLE

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Treatment with monoclonal antibodies to ICAM-1 and LFA-1 in rat heart allograft rejection

Received: 1 July 1996
Received after revision: 25 September 1996
Accepted: 28 October 1996

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Abstract During allograft rejection, leukocyte infiltration in the graft is regulated by various adhesion molecules. Treatment with monoclonal antibodies to ICAM-1 and LFA-1 (CD11 a) induces specific tolerance after murine heart transplantation. In this study, we investigated the possibility of tolerance induction using these antibodies in a fully incompatible rat heart transplant model. Heterotopic, intra-abdominal heart transplantation was performed using Dark Agouti (DA) rats as donors and Lewis (LEW) rats as recipients. Group A ($n = 6$) received no immunosuppression and served as controls. In group B ($n = 6$) 500 μg/kg per day 1A29 (anti-ICAM-1) was administered intravenously (i. v.) for 5 days; group C rats received the same dosage of WT.1 (anti-CD11 a) i. v. for 5 days. In group D ($n = 6$), rats received combined i. v. administration of anti-CD11 a and anti-ICAM-1 (500 μg/kg per day of each, for 5 days. The antibodies used were monoclonal mouse anti-rat antibodies produced from hybridomas. Allograft survival was monitored by daily palpation of the graft. There was no statistically significant difference in allograft survival between the groups (A: 5.7 ± 0.5 days; B: 5.7 ± 0.5 days; C: 5.7 ± 0.5 days; D: 6.2 ± 0.4 days). Treatment with monoclonal antibodies to ICAM-1 and LFA-1 alone or in combination had no effect on allograft survival after heart transplantation between fully incompatible rat strains. We conclude that induction of tolerance using these antibodies seems inconceivable in human heart transplantation.

Key words Monoclonal antibodies, heart transplantation, rat · Heart transplantation, rat, monoclonal antibodies · ICAM-1, rat, heart transplantation · LFA-1, rat, heart transplantation

Introduction

In the last 20 years, heart transplantation has emerged an accepted therapy for end-stage heart failure [22]. Acute and chronic rejection represent one of the most significant risk factors leading to death in the postoperative course [5]. Adhesion molecules participate in the recipient's immune response to the allograft and regulate the infiltration of leukocytes [20]. Monoclonal antibodies to adhesion molecules are potential agents to prevent graft rejection. A combination of monoclonal antibodies to ICAM-1 and LFA-1 has been shown to induce tolerance in a mouse heart transplantation model [10]. In this study, the effect of this monoclonal antibody combination after rat heart transplantation was investigated.

Materials and methods

Animals

Two fully incompatible rat strains were used. Three- to four-month-old inbred male Dark-Agouti (DA; RT1a) rats with a body weight of 160–240 g served as donors, and Lewis (LEW; RT1l) rats weighing 239–322 g served as recipients. The rats were bred and kept at the Institute of Immunology, Christian-Albrechts-University, Kiel, Germany. All animals received humane care in compliance with the "Principles of Laboratory Animal Care", published by the National Institute of Health (NIH Publication No. 86–23, revised 1985), and the "German Law on the Protection of Animals".

Heart transplantation

Donor cardiac grafts were transplanted heterotopically into the abdomen of LEW recipient rats according to the method published by Ono and Lindsey [15]. In short, donor rats were anesthetized with ether. Heparin, 100 IU, (Liquemin N, Hoffmann-La Roche, Grenzbach-Wyhlen, Germany) was administered intravenously (i.v.) via the inferior vena cava, the venae cavae and pulmonary veins were ligated with 6–0 silk, and the pulmonary artery and the aorta were cut 2–3 mm above their origin in the heart. Recipients were anesthetized with 3 mg xylazin (Rompun, Bayer, Leverkusen, Germany) and 2.5 mg ketamine (Ketanest, Parke-Davis, Berlin, Germany). A midline abdominal incision was made, the inferior vena cava and abdominal aorta were prepared free from the surroundings, and the graft was implanted with end-to-side anastomoses of the ascending aorta of the graft to the recipient's abdominal aorta, and the pulmonary artery to the inferior vena cava in a running fashion with 9–0 polypropylene (Prolene, Ethicon, Norderstedt, Germany). Total ischemic time was 30–45 min. The recipient was kept in a separate cage to allow for recovery from the operation.

Monoclonal antibodies

1A29 and WT.1 were used as monoclonal antibodies. 1A29 is a mouse IgG_1 antibody that is reactive against rat ICAM-1. WT.1 is a mouse IgG_2 antibody that is reactive against the α-chain (CD11a) of rat LFA-1. Both antibodies were produced from hybridomas (kindly provided by M. Miyasaka, Osaka, Japan) growing in a microfiber system (Cellmax MPS artificial capillary module, Cellco, Germantown, MD, USA). The concentration in supernatant was adjusted to 4 mg/ml; purification was not necessary. Both antibodies were shown to completely suppress the stimulation index of mixed lymphocyte culture of the rat [21].

Experimental groups

Allogeneic heart transplantation was performed to evaluate monoclonal antibody therapy for acute rejection. In group A ($n = 6$), recipients received no immunosuppressive therapy and served as controls. In group B ($n = 6$), recipients received 500 μg/kg 1A29 (anti-ICAM-1) i.v. on days 0–4. In group C ($n = 6$), recipients received 500 μg/kg WT.1 (anti-CD11a) i.v. on days 0–4. In group D ($n = 6$), a combination of 500 μg/kg 1A29 and 500 μg/kg WT.1 were given i.v. on days 0–4.

The concentration of free antibody in the serum was measured from day 1 to day 7 in a sandwich ELISA for mouse IgG. Goat anti-mouse IgG-Fc serum absorbed against rat serum proteins (Dianova, Hamburg, Germany) was used as a capture antibody and, labeled with peroxidase, as a detection antibody as well. In order to obtain a standard curve, purified monoclonal mouse antibodies were diluted in rat serum. Free antibody concentration in antibody-treated animals was between 1.5 μg/ml (group B, day 1) and 18 μg/ml (group D, day 5). In earlier experiments we found that i.v. but not i.p. administration of 500 μg/kg 1A29 yielded a corresponding concentration of free serum antibody 24 h after injection (data not shown).

Survival of cardiac allografts was assessed by daily palpation, and the cessation of graft beat was interpreted as the completion of rejection. At that point, the animals were sacrificed, and recipient and donor hearts were explanted for histological analysis. Both hearts were fixed in formalin and paraffin.

Microscopic evaluation

Grading of heart allograft rejection was performed on conventional histological paraffin sections stained with hematoxylin and eosin (H&E). Rejection was graded from 0–4, according to the classification of the International Society for Heart and Lung Transplantation [2]: 0, no rejection; 1, focal, or diffuse but sparse infiltration without necrosis; 2, one focus with aggressive infiltration and/or focal myocyte damage; 3, multifocal aggressive infiltrates and/or myocyte damage, or diffuse inflammatory process with necrosis; 4, diffuse aggressive polymorphous infiltration with or without edema, hemorrhage, or vasculitis, with severe necrosis.

Statistical analysis

All values are expressed as mean ± standard deviation. An ANOVA was used to calculate statistical significance. A P value less than 0.05 was considered significant.

Results

The mean graft survival of untreated recipients (group A) was 5.7 ± 0.5 days. Monotherapy with anti-CD11a (group C) or with anti-ICAM-1 (group B) did not show any prolongation of graft survival (B: 5.7 ± 0.5 days, C: 5.7 ± 0.5 days, P = NS). The combination of anti-ICAM-1 and anti-CD11a (group D) showed a slightly, but not significantly prolonged survival (6.2 ± 0.4 days). The survival curves are shown in Fig. 1.

Histologically, explanted grafts from all groups showed severe acute rejection, grade 4. Evaluation of expression of adhesion molecules was not possible due to the necrosis of the allograft.

Discussion

After heart transplantation, acute and chronic rejection, as well as complications of nonspecific immunosuppressive therapy, are limiting factors in graft and patient survival [5, 22]. Therefore, more specific immunosuppressive drugs and/or induction of tolerance would contrib-

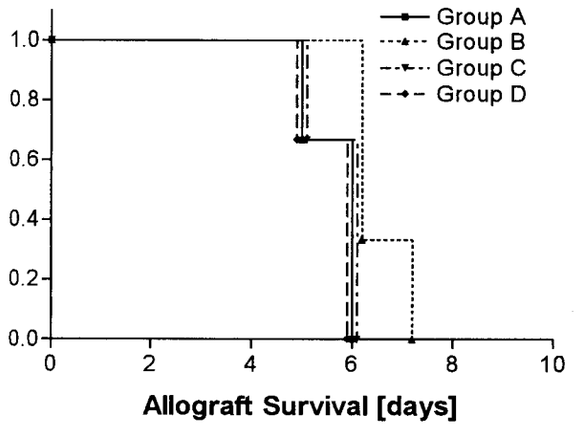

Fig. 1 Survival of heterotopic rat cardiac allografts after treatment with monoclonal antibodies (Kaplan-Meier curves). Group A: no treatment, group B: anti-ICAM-1, group C: anti-CD11a, group D: anti-ICAM-1 and anti-CD11a

ute significantly to progress in clinical organ transplantation.

Adhesion molecules play a central role in regulating the infiltration of leukocytes into the graft during rejection. Lymphocyte function-associated antigen-1 (LFA-1), which is a member of the integrin family, is expressed on T lymphocytes. It consists of two noncovalently linked polypeptide chains, an α_1-chain (CD11a) and a β_2-chain (CD18). Intercellular adhesion molecule-1 (ICAM-1), a surface gylcoprotein of the immunglobulin family, is known as a ligand for LFA-1 and is expressed on the cell membrane of endothelial cells. LFA-1 and ICAM-1 are involved in adhesion of lymphocytes to endothelial cells and play an important role in antigen-specific T-cell recognition, leukocyte migration, and target cell lysis [19].

Under normal conditions, a low level ICAM-1 expression whas been observed on capillary and postcapillary venular endothelia of the heart [14, 20]. In acute rejection, expression of ICAM-1 is strongly upregulated, especially on postcapillary venular endothelia [14, 20]. High numbers of LFA-1-positive graft-infiltrating cells are found during rejection [3].

Blocking adhesion molecule interaction seems to be a promising strategy for the treatment of rejection. The results of various experimental studies show that prolongation of murine cardiac allograft survival is possible with monoclonal antibodies to ICAM-1, LFA-1 (CD11a), CD-18, VCAM-1, and VLA-4 [4, 9, 11, 16]. A combination of two or even three monoclonal antibodies seems to be additionally effective [13, 17]. First clinical trials in human kidney transplantation using monoclonal antibodies against ICAM-1 and LFA-1 are under way [7, 8]. Isobe and coworkers have shown tolerance induction using anti-ICAM-1 and anti-CD11a antibodies in a mouse transplantation model [17].

In this study, the administration of anti-CD11a alone or combined with anti-ICAM-1 had no effect on graft survival or on the histologic grade of rejection in a rat heart transplantation model. Altmann et al. reported synergistic action of ICAM-1/LFA-1 interaction with major histocompatibility complex class II in T-cell activation. ICAM-1/LFA-1 interaction is only important in antigen-presenting cells with a low intensity of HLA-DR, not in cells with high HLA-DR intensity [1]. This may explain why the use of anti-ICAM-1 and anti-LFA-1 antibodies is effective in the weak rat strain combination model of Kameoka et al. but not in the fully incompatible model of this study [12]. Although therapy with anti-VCAM-1 and anti-VLA-4 is effective in a weak rat strain combination, it fails in a strong combination [17]. A combination of monoclonal antibodies with conventional immunosuppressive drugs seems to be more efficient. Harrison and Madwed reported prolonged cardiac allograft survival using the combination of low-dose cyclosporin and anti-ICAM-1 therapy in a rat model [6]. However, tolerance induction by monoclonal anti-adhesion molecule antibodies seems to be easier in a mouse model than in other animal models. The mechanism of action whereby monoclonal antibodies prolong allograft survival is still not precisely understood. The absence of surface expression of ICAM-1 in the donor allograft or in the recipient is insufficient to prolong cardiac allograft survival in a mouse model [18]. Therefore, administration of anti-ICAM-1 antibodies seems to work, not only by inhibiting leukocyte extravasation but also by other mechanisms that remain unclear.

Our results show that the effect of tolerance induction after mouse heart transplantation cannot be transferred across species to rat heart transplantation. Therefore, induction of tolerance using these antibodies seems inconceivable in human heart transplantation.

Acknowledgements We thank Mrs. Katrin Boeke and Mrs. Reina Zühlke for their excellent technical assistance and M. Miyasaka, MD (Osaka, Japan) for providing the hybridomas.

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