Prevention of lung allograft rejection by combined treatment with adhesion molecule antibodies and cyclosporine

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Received 5 March 2003; received in revised form 5 June 2003; accepted 16 June 2003

Abstract

Infiltration of leukocytes into the lung allograft is regulated by adhesion molecules during acute rejection. The purpose of this study was to assess the effect of monoclonal antibodies against ICAM-1 (1A29) to prevent rejection after lung transplantation. Left lateral orthotopic lung transplantation was performed using Dark Agouti rats as donors and Lewis rats as recipients. Recipients received 1A29 alone (group A), cyclosporine A alone (group B), a combination of both drugs (group C) or no therapy (group D). Animals were killed on day 5 and 10, respectively. Rejection was graded by histology. Myeloperoxidase activity (MPO) was measured in the graft. In allografts treated with cyclosporine and 1A29 histologically a lower grade of rejection was seen and less MPO were detected compared to groups A, B and D. Anti-ICAM-1 monoclonal antibodies alone as well as a subtherapeutic dose of cyclosporine are not effective to prevent acute allograft rejection after lung transplantation. However, the combination of both strategies significantly reduces rejection in this model.

Keywords: Adhesion molecule; Monoclonal antibody; Rat lung transplantation; Cyclosporine; Rejection

1. Introduction

Acute rejection represent one of the most important risk factors of death in the early postoperative period after lung transplantation. Infiltrating leukocytes have been shown to participate in the development of tissue injury during pulmonary allograft rejection. Adhesion molecules participate in the recipient’s immune response to the allograft and regulate the infiltration of leukocytes [1]. Four steps of the infiltration process have been described: (1) rolling of the cells on the activated endothelium; (2) activation of leukocytes; (3) firm adhesion of leukocytes to the endothelium; (4) migration of leukocytes into the surrounding tissue [1]. Adhesion molecules, which are expressed on both endothelial cells and leukocytes, are regulating this process. As such, rolling of leukocytes is mediated by selectins. Firm adhesion and migration of leukocytes are regulated by β2-integrins (CD11a/CD18, CD11b/CD18) interacting with endothelial ICAM-1 [1]. Therefore, monoclonal antibodies to adhesion molecules are potential agents to prevent graft rejection. In this study, the effect of monoclonal antibody therapy against ICAM-1 after rat lung transplantation was investigated.

2. Material and methods

2.1. Animals

Three- to four-month old inbred male Dark-Agouti (DA; RT1n) rats with a body weight of 250–300 g, and Lewis (LEW; RT1l) rats with a weight of 200–250 g were used. The rats were bred and kept at the Institute of Immunology, Christian-Albrechts-University, Kiel, Germany. All animals received human care in compliance with the European Convention on Animal Care and the study was approved by the institutional ethics committee.

2.2. Lung transplantation

Donor lung grafts were transplanted orthotopically into recipient rats according to the methods published by

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1569-9293/03/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S1569-9293(03)00131-2
Marck and Prop [2]. In short, the recipient rat was intubated after sedation with 60 mg/kg phenobarbital i.p., application of atropine 0.05 mg i.m. and mechanically ventilated with 100% oxygen. A left lateral thoracotomy was performed. The donor animal was anesthetized by ether. A one stage laparotomy and median sternotomy was performed followed by intravenous injection of 1300 IE heparin. The main pulmonary artery was intubated with a 19-gauge cannula and cold flush perfusion (10 ml of cold Ringer’s lactate; perfusion pressure 10 cmH2O) was initiated after incision of the left atrial appendix. The left lung was then excised and the vessels anastomosed using 10-0 polypropylene sutures. Lung reperfusion was started. Bronchial anastomosis was then performed using a 8-0 polypropylene suture and thereafter the left lung was ventilated. The chest was closed and the animal extubated when spontaneous breathing was sufficient.

2.3. Monoclonal antibodies

1A29, a mouse IgG1 antibody, which is reactive against rat ICAM-1, was used. The antibody was produced from hybridomas growing in a microfiber system (Cellmax MPS artificial capillary module, Cellco Inc., Germantown, MD, USA). Concentration in supernatant was 4 mg/ml, so that purification was not necessary. The antibody was shown to completely suppress the stimulation index of mixed lymphocyte culture of the rat [3].

2.4. Experimental groups

In all groups allogeneic lung transplantation was performed. DA donors and LEW recipients were used. Average total ischemic time was 45 min. In group A (n = 10), rats received 500 μg/kg per day 1A29 i.v. on days 0–4. In group B (n = 10), rats received a low-dose of cyclosporine (1.5 mg/kg per day i.p.) (Sandimmun, Novartis Pharma, Nürnberg, Germany). In group C (n = 10), rats received 500 μg/kg per day 1A29 i.v. on days 0–4 and cyclosporine (1.5 mg/kg per day i.p.). Group D rats (n = 10) served as controls without immunosuppression. Animals were killed on days 5 (n = 5 in each group) and 10 (n = 5 in each group), respectively. Animals were randomized to be killed on days 5 or 10.

2.5. Histology and immunohistology

A segment of left and right lung was fixed in formalin and paraffin, and paraffin slides were stained with hematoxylin eosin (HE) for diagnosis of rejection. The remaining lung tissue was snap frozen and stored in liquid nitrogen. For immunohistological staining WT.1 (a mouse IgG3a monoclonal antibody against rat CD11a) (Serotec, Wiesbaden, Germany), ED2 (a mouse IgG1 monoclonal antibody against rat ED2 antigen) (Serotec), and TLD-4C9 (a mouse IgG1 monoclonal antibody against rat ICAM-1(CD54)) (Serotec) were used. Immunohistological staining was performed on cryostat sections of 7 μm and cut at −25 °C (Micron, HM 500 OM, Germany). Sections were fixed in an acetone and methanol (2:1) mixture for 10 min at −4 °C and dried at room temperature. After blocking of the sections with 20% normal rat serum for 10 min, the primary mouse monoclonal antibody was applied for 1 h. A peroxidase-conjugated rabbit anti-mouse monoclonal antibody (Dako, Copenhagen, Denmark) was used as the second antibody. Slides were stained with 3,3’-diaminobenzidine (Sigma, Frankfurt, Germany) and hemalaun counterstaining.

2.6. Microscopic evaluation

It is already known that CD11a is expressed on alveolar macrophages and lymphocytes in normal lungs, and that the pattern of expression remained unchanged during rejection. But the number of CD11a-positive infiltrated leukocytes is markedly increased during rejection and can therefore used as grading of the severity of infiltration. ED2 is expressed on macrophages infiltrating lung tissue and can also use as grading of the severity of infiltration [1]. To evaluate CD11a- and ED2-positive infiltrating leukocytes, the number of cells were counted in five different fields of each slide using a magnification of 1000×, then the mean number of positive cells/field was calculated. To analyze ICAM-1 expression, the number of arterioles with ICAM-1-positive and -negative endothelium in the whole slide was counted using a magnification of 400×, then the percentage of arterioles with ICAM-1 expression on endothelium was calculated.

Grading of lung allograft rejection was performed on conventional histological paraffin sections stained with hematoxylin–eosin (H&E). Rejection was graded from grade 0 to 5 according to the degree of perivasculary and peribronchiolar mononuclear infiltration, alveolar interstitial edema and infiltration, local degeneration and necrosis as described elsewhere [4]: 0: no rejection; 1: minimal; 2: mild; 3: moderate; 4: severe lung rejection; 5: tissue necrosis.

2.7. Myeloperoxidase

Lung tissue was homogenized and sonicated. Myeloperoxidase activity in supernatants was measured by the change in optical density (at 460 nm) resulting from decomposition of H2O2 in the presence of o-dianisidine as described elsewhere [5].

2.8. Statistical analysis

All values were expressed as mean ± standard deviation. Analysis of variance was used to calculate statistical
3. Results

On day 5, in animals of group C and group B perivascular mononuclear infiltrates were seen on H&E-stained slides, according to mild acute rejection (grade 2). In contrast, animals of group A and group D showed diffuse perivascular, interstitial and peribronchial infiltration according to severe rejection (grade 4). This difference in the grade of rejection was significant between the groups ($P < 0.05$) (Fig. 1). On day 10, all grafts in the control group without therapy (group D) and in group A were necrotic, whereas the grafts in group B showed grade 3–4 rejection, and the grafts in group C showed grade 2–3 rejection. This difference was statistically significant ($P < 0.05$) (Fig. 1).

On day 5, fewer CD11a-positive leukocytes, infiltrating the allograft, and less myeloperoxidase activity (Fig. 2) were found in the combined treatment group (group C) compared to the other groups (Table 1). Evaluation of CD11a expression and myeloperoxidase on day 10 after lung transplantation was not performed due to graft necrosis in most animals.

The infiltration of ED2-positive cells (macrophages) was markedly reduced in groups B and C (Table 1).

No significant difference regarding the expression of ICAM-1 on arterioles in the allograft could be detected (Table 1).

4. Discussion

After lung transplantation acute allograft rejection as well as complications of non-specific immunosuppressive therapy represent major problems in the early postoperative period. Therefore, more specific immunosuppressive drugs and/or induction of tolerance would represent significant progress in clinical organ transplantation.

During rejection leukocytes infiltrate the allograft. Precipitated by an adhesion receptor-dependent endothelial binding a transmigration process through the vessel wall occurs [1,6]. As lung injury after transplantation is dependent on leukocyte–endothelial interaction, immunosuppressive strategies are targeted to the binding of activated leukocytes. New insights on the sequence of molecular events in the adhesion of leukocytes to the endothelium raise the hope that receptor-directed antagonism of selected steps of cell adhesion may be a promising therapeutic strategy after 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 $\alpha$- (CD11a) and a $\beta$-chain (CD18). Intercellular adhesion molecule-1 (ICAM-1), a surface glycoprotein of the immunoglobulin 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 [1]. Under normal conditions ICAM-1 expression was observed on almost all
endothelial cells with less staining on larger vessels. In acute rejection expression of ICAM-1 is strongly upregulated, especially on larger arteries and veins [6].

Blocking of adhesion molecule interaction seems to be a promising strategy for rejection treatment. 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, and VCAM-1 [7–9]. Combination of two or more monoclonal antibodies seems to be additionally effective [7]. The first clinical trials in human kidney transplantation using monoclonal antibodies against ICAM-1 are underway [10]. Isobe and coworkers have shown tolerance induction using anti-ICAM-1 and anti-CD11a antibodies in a mouse heart transplantation model [7].

In our rat lung transplantation model, unmodified lung allograft rejection resulted in rejection on day 10. We demonstrated that neither anti-ICAM-1 monoclonal antibodies nor low-dose cyclosporine therapy alone were able to reduce the rejection process significantly at this time. However, a combination of low-dose cyclosporine and anti-ICAM-1 monoclonal antibodies prolonged allograft survival. Anti-rejection prophylaxis by antibodies and/or cyclosporine was discontinued on day 4 and unmodified rejection followed thereafter, leading to necrosis in nearly all animals without or with monotherapy within 5 days. Thus, the effect of anti-ICAM-1 monoclonal antibodies on allograft rejection appears to be limited to the time of treatment, but does not lead to tolerance induction or prolonged abrogation of the immune response. In this study, administration of anti-ICAM-1 alone shows no effect on histologic grade of rejection in a rat lung transplantation model. This corresponds well to the missing effect of anti-ICAM-1 antibodies in rat heart transplantation, as reported previously by our group [11].

Combination of monoclonal antibodies with conventional immunosuppressive drugs seems to be more efficient. In vitro data suggested that combination of suboptimal concentrations of a single immunosuppressant, e.g. cyclosporine, with a suboptimal concentration of an ICAM-1 monoclonal antibody results in an additive suppressive effect on the mixed lymphocyte reaction [12]. Harrison and Madwed reported prolonged cardiac allograft survival using the combination of low-dose cyclosporine and anti-ICAM-1 therapy in a rat model [13]. The mechanism of action whereby monoclonal antibodies prolong allograft survival still remains 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 [14]. Therefore, administration of anti-ICAM-1 antibodies seems to work not only by inhibiting leukocyte extravasation but also by other mechanisms which are still unclear. There are several reports of rejection treatment by using monoclonal antibodies against ICAM-1, LFA-1 and several other adhesion molecules in heart, liver and kidney transplantation [10, 11,15]. To our knowledge, however, no data showing effects of treatment with monoclonal antibodies against adhesion molecules on lung allograft rejection have been published as yet.

To summarize, although intravenous administration monoclonal antibodies against ICAM-1 alone could not prevent acute lung allograft rejection, combination with cyclosporine significantly reduces infiltration of leukocytes into the allograft during acute allograft rejection. This combination show major effects on leukocyte infiltration and subsequent tissue injury. Furthermore, the use of monoclonal antibodies against adhesion molecules may allow for a reduction in the dose of cyclosporine for immunosuppression. Such a reduction could lead to lowering of side effects of long-term treatment with cyclosporine like nephrotoxicity. In further studies, these potential benefits should be evaluated by long-term therapy models in comparison to other clinically used immunosuppressive protocols.

Acknowledgements

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

References


