Immunological Link Between Primary Graft Dysfunction and Chronic Lung Allograft Rejection

Ankit Bharat, MD, Elbert Kuo, MD, Nancy Steward, MS, Aviva Aloush, RN, Ramsey Hachem, MD, Elbert P. Trulock, MD, G. Alexander Patterson, MD, Bryan F. Meyers, MD, and T. Mohanakumar, PhD

Departments of Surgery, Internal Medicine, and Pathology and Immunology, and Division of Cardiothoracic Surgery, Washington University School of Medicine, St. Louis, Missouri

Background. Primary graft dysfunction (PGD) in the immediate post–lung transplant period strongly increases the risk of chronic rejection (bronchiolitis obliterans syndrome). Here, we hypothesized that PGD-induced inflammation augments alloimmunity, thereby predisposing to bronchiolitis obliterans syndrome.

Methods. Primary graft dysfunction and bronchiolitis obliterans syndrome were diagnosed according to the established International Society for Heart and Lung Transplantation criteria. Anti–human leukocyte antigen (HLA) alloantibodies were analyzed using Flow-PRA. Donor HLA class II–specific T cells were analyzed using interferon (IFN)-γ ELISPOT. Serum levels of 25 cytokines and chemokines were measured using LUMINEX.

Results. Of the 127 subjects, 29 (22.8%) had no PGD (grade 0), 42 (33.2%) had PGD-1, 36 (28.3%) had PGD-2, and 20 (15.7%) had PGD-3. Patients with PGD grades 1 to 3 ($PGD_{1\text{-}3}$) had elevated proinflammatory mediators MCP-1, IP-10, interleukin (IL)-1β, IL-2, IFN-γ, and IL-12 in the sera during the early posttransplant period compared with patients with PGD grade 0 (PGD_0). On serial analysis, $PGD_{1\text{-}3}$ patients revealed increased development of de novo anti-HLA-II (5 years: 52.2% versus PGD_0 13.5%, $p = 0.008$). However, no difference was found in anti-HLA-I alloantibody development ($PGD_{1\text{-}3}$ patients 48% versus PGD_0 39.6%, $p = 0.6$). Furthermore, $PGD_{1\text{-}3}$ patients had increased frequency of donor HLA class II–specific $CD4^+$ T cells [$(91.4 \pm 19.37) \times 10^{-6}$ versus $(23.6 \pm 15.93) \times 10^{-6}$, $p = 0.003$].

Conclusions. Primary graft dysfunction induces proinflammatory cytokines that can upregulate donor HLA-II antigens on the allograft. Increased donor HLA-II expression along with PGD-induced allograft inflammation promotes the development of donor specific alloimmunity. This provides an important mechanistic link between early posttransplant lung allograft injury and reported association with bronchiolitis obliterans syndrome.

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Bronchiolitis obliterans syndrome (BOS) represents chronic lung allograft rejection and remains the predominant cause for poor long-term survival after lung transplantation. Bronchiolitis obliterans syndrome develops in about 50% of human lung allograft recipients within 3 years and in more than 90% at 9 years after transplantation [1]. Bronchiolitis obliterans syndrome is postulated to have a multifactorial etiology. Nevertheless, alloimmunity constitutes the predominant form of injury that contributes to the pathogenesis of BOS. Sundaresan and colleagues [2] reported that development of human leukocyte antigen (HLA) class I antibodies was an independent predictor for the development of BOS. These antibodies precede the development of BOS by about 20 months [3]. Jaramillo and coworkers [4] further demonstrated that anti–HLA class I antibodies activate airway epithelial cells (AEC) inducing proliferation and apoptosis. The activated AEC produce growth factors that also lead to smooth muscle and fibroproliferation, characteristic of BOS histopathology [4]. Data from our laboratory [5] and others [6] has further demonstrated that development of HLA class II antibodies is associated with increased risk of BOS. Similarly, both donor HLA class I [7] and class II [5, 8] alloreactive T cells are increased in patients with BOS, suggesting a role for cellular alloimmunity in the pathogenesis of BOS [9].

There is now accumulating evidence that early posttransplant events promote the development of BOS. In a previous study from our laboratory, we found that patients with BOS had elevated proinflammatory mediators including IP-10, MCP-1, interleukin (IL)-1β, IL-2, IL-12, and IL-15 during the early posttransplant interval [5]. The increase in proinflammatory mediators was associated with the development of donor-specific HLA class II alloimmunity. However, the cause of elevated proinflammatory cytokines in patients with BOS remained unclear. Recently, in a retrospective review of 334 adult lung transplant recipients, Hachem and colleagues [10] found that primary lung allograft dysfunction (PGD) was asso-

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Address correspondence to Dr Mohanakumar, Washington University School of Medicine, Department of Surgery, Box 8109-3328 CSRB, 660 S Euclid Ave, St. Louis, MO 63110; e-mail: kumart@wustl.edu.

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Abbreviations and Acronyms

APC = antigen presenting cells
BOS = broncholitis obliterans syndrome
HLA = human leukocyte antigen
HLA-DR = human leukocyte antigen–donor
ISHLT = International Society for Heart and Lung Transplantation
IL = interleukin
PBMC = peripheral blood mononuclear cell
PGD = primary graft dysfunction

ciated with increased risk of BOS (relative risk, 1.73 to 2.53), independent of acute rejection, lymphocytic bronchitis, and community-acquired respiratory viral infections. Primary graft dysfunction has been proposed to produce inflammation and amplify the immunogenicity of the allograft. Here, we hypothesized that PGD-induced inflammation upregulated major histocompatibility complex expression on the allograft, leading to increased alloantigen presentation and production of antidonor antibodies that are known to contribute to the immunopathogenesis of BOS.

Material and Methods

Study Subjects

Adult patients undergoing lung transplantation at Washington University Medical Center/Barnes-Jewish Hospital were prospectively enrolled in the study between May 1990 and January 2005 after obtaining informed consent, in accordance with a protocol approved by the Institutional Review Board. Only those patients who consented to donate blood at posttransplant follow-up visits were included. The peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood by Ficoll-Hypaque density gradient centrifugation (Pharmacia, Uppsala, Sweden), and stored in the laboratory sample bank at −135°C until further use. The plasma separated from peripheral blood was stored at −70°C. Patients were excluded if they had HLA antibodies before transplant, had hyperacute rejection, got retransplanted, or died within 6 months after transplantation. All patients were free of acute rejection or respiratory infection for at least 1 month before the time of analysis. The standard immunotherapy protocol consisted of cyclosporine, azathioprine, and prednisone. After BOS was diagnosed, the immunotherapy protocol was modified to FK506 (Tacrolimus), mycophenolate mofetil, and prednisone.

Definitions

Broncholitis obliterans syndrome was diagnosed according to the International Society for Heart and Lung Transplantation (ISHLT) criteria [11] based on the percentage decline in forced expiratory volume in 1 second (FEV_1) compared with baseline and graded as follows: 1 = 80% to 66% of baseline value, 2 = 65% to 51% of baseline value, and 3 = 50% or less of baseline value. Other causes of decreased lung function such as infection and bronchial anastomotic stricture were ruled out.

Primary graft dysfunction was diagnosed immediately after transplant on arrival of the patient to the intensive care unit according to the established definition of the ISHLT [12]. Grade 0 is characterized by a partial pressure of arterial oxygen to the fraction of inspired oxygen (PaO_2/FiO_2) ratio greater than 300 mm Hg and a clear chest radiograph; grade 1 by a PaO_2/FiO_2 ratio greater than 300 mm Hg and radiographic infiltrates consistent with pulmonary edema; grade 2 by a PaO_2/FiO_2 ratio = 200 to 300 mm Hg and pulmonary infiltrates; and grade 3 by a PaO_2/FiO_2 ratio less than 200 mm Hg with pulmonary infiltrates. The absence of other potential causes of lung allograft dysfunction such as hyperacute rejection, venous anastomotic complications, cardiogenic pulmonary edema, and pneumonia are implicit in this definition.

Assays and Reagents

Flow-panel reactive antibodies (PRA) analysis for the detection of HLA antibodies was done using flow-cytometry as per the manufacturer's protocol (One Lambda, Canoga Park, California). The percent PRA is determined by the percent of microparticles that are bound by the antibodies in the serum. A PRA of 2.9% or greater for HLA class I and 2.4% for HLA class II was considered positive. Serum levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, Eotaxin, IP-10, MIG, MCP-1, MIP-1α, MIP-1β, RANTES, tumor necrosis factor (TNF)-α, IFN-α, IFN-γ, GM-CSF, IL-1Rα, and IL-2R were analyzed using the solid phase sandwich multiplex bead LUMINEX immunoassays (Biosource International, Carlsbad, California) according to the manufacturer's protocols. To detect donor specific anti-HLA class II $CD4^+$ T-cell alloreactivity, we used peptides corresponding to the to the β-chain hypervariable region of the mismatched HLA-DR*0101, HLA-DR*0301, HLA-DR*0701, and HLA-DR*1501 (Research Genetics, Huntsville, Alabama) [8]. These techniques have been described in detail in our previous publication [5].

ELISPOT Assay

The ELISPOT assay for IFN-γ was performed as previously described [13]. ELISPOT is a recent technique to analyze the frequency of antigen-specific T cells in a given sample. Briefly, multiscreen 96-well filtration plates (Millipore, Billerica, Massachusetts) were coated with 5.0 μg/mL capture human cytokine-specific mAb (BD Biosciences) at 4°C overnight. The plates were then blocked with 1% BSA for 2 hours and washed with phosphate-buffered saline. Subsequently, 3×10^5 PBMCs were cultured in triplicate in the presence of donor HLA-DR peptides (10 μg/mL) and irradiated feeder autologous PBMCs (antigen presenting cells [APC]; 1:1 ratio). After 48 hours, the plates were washed, and then 2.0 μg/mL biotinylated human cytokine-specific mAb (BD Biosciences, San Jose, Califor-