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Profiling of peripheral blood mononuclear cells does not accurately predict the bronchiolitis obliterans syndrome after lung transplantation

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ABSTRACT

After lung transplantation (LTx), circulating mononuclear cell composition and their subsets may be predictive for the bronchiolitis obliterans syndrome (BOS). We investigated the cellular composition in patients developing BOS, or not, by analyzing peripheral blood taken at multiple time points after transplantation. PBMCs of 11 BOS and 39 non-BOS patients were analyzed by FACS for monocytes, dendritic cells, NK-, NKT-, B- and T cells as well as B- and T cell subsets. Analysis of blood samples taken monthly during the first year post-LTx showed that circulating NK, NKT and dendritic cell percentages were not indicative of BOS development, whereas increases in T cells, monocytes and lowered fractions of B cells were related to BOS development. B- and T cell subset analysis at month 5 post-LTx indicated that IgM+IgD− memory B cells and central memory CD8+ T cells were decreased, whereas NKT cells were increased in BOS patients compared to non-BOS patients. Prior to BOS diagnosis, the composition of specific mononuclear cells on a group level differs from patients remaining BOS free. However, given the overlap in percentages of cellular frequencies between the patient groups investigated, this analysis does not allow prediction or risk stratification for development of BOS in individual patients.

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1. Introduction

Lung transplantation (LTx) is the final treatment option for several selected endstage lung diseases [1,2]. The outcome after LTx is severely hampered by the development of chronic lung allograft dysfunction (CLAD), which manifests either as an obstructive CLAD (bronchiolitis obliterans syndrome, BOS) or as a restrictive CLAD (restrictive allograft syndrome, RAS). The two forms of CLAD are diagnosed either by obstructive pulmonary function tests in the case of BOS or restrictive pulmonary function tests in the case of RAS [3].

Approximately 50% of patients survive 5 years after LTx [4,5]. Although the pathogenesis of BOS is mainly unknown, the immune system probably contributes to chronic graft rejection. Prior to BOS development infiltration of immune cells into the allograft has been shown. Accumulation of dendritic cells has been reported as well as submucosal lymphocyte and plasma cell infiltrates [6–8]. These submucosal lymphocyte infiltrates consisted mainly of T cells, both CD4+ and CD8+ T cells, but also NK cells have sometimes been identified [8–11]. In bronchoalveolar lavage fluid (BALF) the alveolar macrophage count is decreased from patients with BOS versus those without [12,13], and differences were reported in numbers of neutrophils, CD4+ and CD8+ T cells between patients with and without BOS [12–15].

Only a few studies have been published on the composition of peripheral blood mononuclear cells (PBMC) after lung transplantation. CD19+ B cells were described to be dramatically decreased and hardly present in the peripheral blood of patients with BOS while CD8+ T cells were increased [16]. NK cells were found to be activated and decreased in the peripheral blood of patients with BOS compared to patients without BOS, although others reported this decrease of NK cells in all LTx patients [11,17]. More detailed research was performed on T regulatory cells showing that levels of CD4+CD25+CD69− and CD4+CD25+ were decreased in patients with BOS [18]. In addition, the frequency and phenotype of peripheral NK cells drastically change after LTx, with immature NK cells being more prominent while mature NK cells are being more activated, but overall less cytotoxic (CD16−CD56dim) [17].

Abbreviations: AR, acute rejection; AUC, area under the curve; BALF, bronchoalveolar lavage fluid; BOS, bronchiolitis obliterans syndrome; CM, central memory T cells; EM, effector memory T cells; FEV_1, forced expiratory volume in 1 s; HLA, human leukocyte antigen; LTx, lung transplantation; NT cells, naive T cells; NK cells, natural killer cells; NKT cells, natural killer T cells; PBMC, peripheral blood mononuclear cells; ROC, receiver operating characteristic; TD, terminally differentiated effector T cells.

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In this study we have focused on the mononuclear cell composition of multiple peripheral blood samples sequentially collected after LTx. Cells characterized include monocytes, dendritic cell, NK cells, NKT cells, and also T/B cells and their subsets. The primary objective was to examine whether this shows differences between patients eventually developing BOS versus patients remaining BOS-free, and if so whether differences found early after transplantation can be used as predictive biomarkers to identify patients at risk for BOS development.

2. Patients and methods

2.1. Patients

Fifty LTx patients transplanted between September 2003 and March 2008 at the Heart Lung Center in Utrecht, The Netherlands, were included in this study, based on patient material availability. BOS was defined as a decline of the FEV_1 from the post-operative baseline at two distinctive time points of more than 20% in the absence of infection or other etiology according to international guidelines [19]. All patients suspected for BOS had a bronchoscopy and CT scanning to exclude large airway complications and infections. BOS grade was assessed at BOS onset. Standard immunosuppressive therapy consisted of tacrolimus, mycophenolate-mofetil and prednisone. Upon suspicion of BOS patients were treated with azithromycin. Directly after lung transplantation induction therapy was given at day 0 and day 4 with basiliximab. No other induction therapy was applied. Clinically there were no signs of severe reflux and all patients were treated with protein pump inhibitors to decrease the effect of acid reflux.

2.2. Blood sampling

Patient follow-up started in September 2003, after approval by the medical–ethical committee and informed consent was obtained from each patient. Blood samples were collected routinely once prior to, once every month during the first year after transplantation, and every quarter thereafter. From 40 ml heparinized whole blood PBMC were isolated by Ficoll Paque Plus (GE Healthcare, Sweden). All samples were frozen in RPMI 20% FCS 10% DMSO and preserved in liquid nitrogen until measurement. In a cross-sectional study blood samples at approximately 5 months after LTx (range 4.2–6.1 months) were used.

2.3. Flow cytometry

Cryopreserved PBMC were rapidly thawed in a 37 °C water bath and added to medium containing 10% fetal calf serum (FCS) after which the cells were centrifuged and resuspended in PBS with 0.1% BSA and 0.1% natrium-azide. Two million PBMC were incubated with either relevant antibodies or isotype controls for 30 min on ice in the dark followed by washing and measurement. Data acquisition and analysis were performed on a BD FACS Canto II with 8 color detection (BD Bioscience, CA). Each recipient sample that was measured had approximately 194,000 events (range 69,000–573,000 events) in the lymphocyte gate, which was defined by CD45 + and side-scatter (SSC), and analyzed with FACS DIVA software (BD Bioscience, CA).

2.4. Antibodies

Lymphocyte subsets were distinguished by the use of CD45-PE Cy7 (BioLegend) for lymphocytes, CD3-eFluor 450 (eBioscience) for T cells,

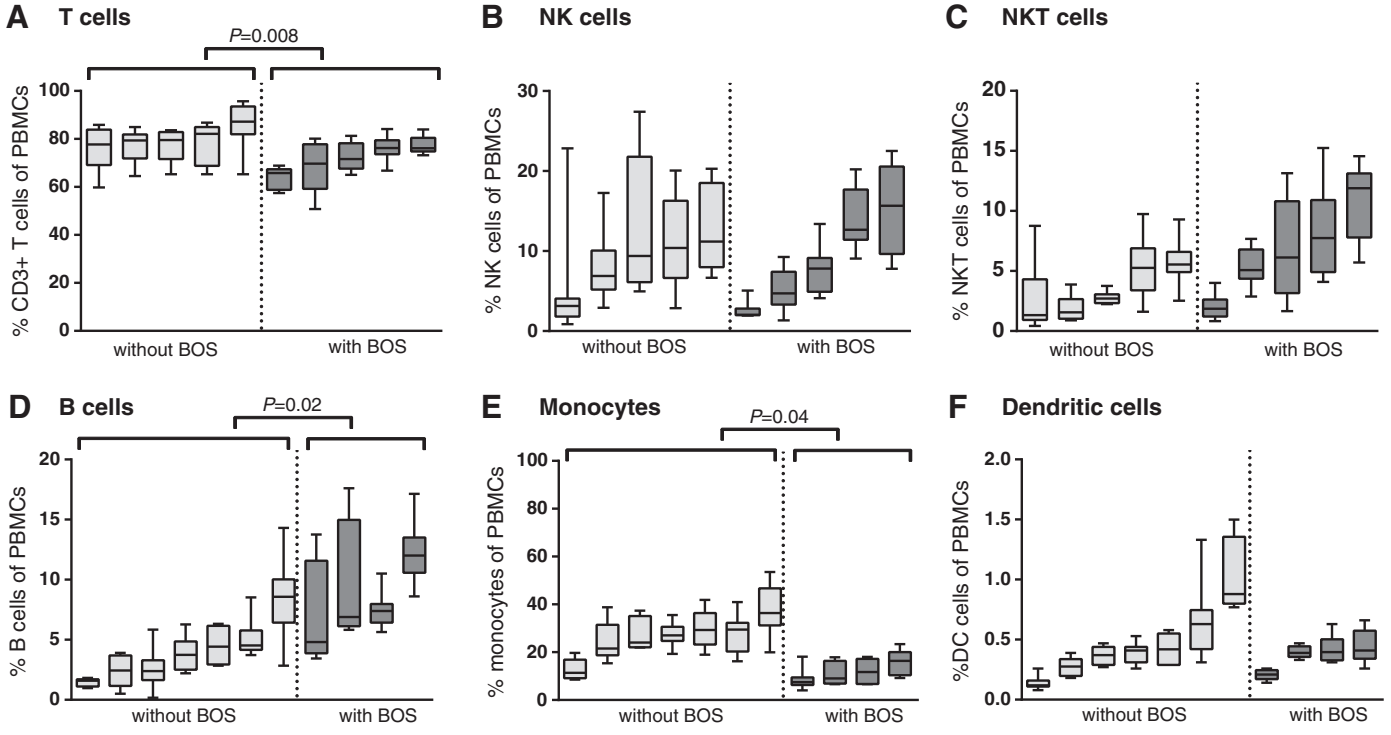

Fig. 1. Composition of mononuclear cells over time after transplantation. Peripheral blood mononuclear cells were obtained monthly after lung transplantation and stored in liquid nitrogen. Since the samples were subjected to Ficoll Paque separation and one freeze–thaw cycle, the CD45 + population only consists of lymphocytes and monocytes, as is also shown in Supplementary Fig. 1. A total of 162 samples with an average of 9 samples per patients (range 5–12) could be analyzed within the first year after transplantation. Results are displayed as box-and-whisker plots with the boxes covering the 75% interval and the median displayed within, and the whiskers displaying the 91st percentile. Each box represents the combined cell numbers of the monthly taken samples during the first year after transplantation for one patient. For T cells, NK cells and NKT cells sufficient material from 5 BOS and 5 non-BOS patients were available, whereas sufficient samples from 4 BOS and 7 non-BOS patients where available for the B cells, monocytes, and DC analyses. Results of patients not developing BOS are displayed on the left side of the figures in boxes filled with light gray whereas those from patients developing BOS are displayed in darker shaded boxes. Significant differences between the total BOS and non-BOS patients cell percentages are indicated in the respective figures. FACS analysis was performed as indicated in Patients and methods in order to determine the percentage of T cells, B cells, monocytes, NK cells, NKT cells, and dendritic cells as indicated in panels A–F.

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