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T-lymphocyte subsets in lung transplant recipients: association between nadir CD4 T-cell count and viral infections after transplantation

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ABSTRACT

Background: Little is known about the kinetics of T-cell subsets in lung transplant recipients (LTR) and their association with the occurrence of opportunistic infections (OI).
Objectives: To analyze the kinetics of T-lymphocyte subsets in LTR and the association between nadir CD4 T-cell count and viral infections after transplantation.
Study design: Serial measurements of peripheral blood CD4 and CD8 T-cell counts obtained during the first year post-transplantation from 83 consecutive LTR and their correlation with both viral OI and community-acquired infections post-transplantation were retrospectively analyzed.
Results: LTR with a nadir CD4 T-cell count <200 cells/μl had consistently lower CD4 and CD8 T-cell counts than LTR with a nadir CD4 T-cell count >200 cells/μl ($p < 0.001$). In LTR with a nadir CD4 T-cell count <200 cells/μl, the cumulative incidence of viral infections detected in peripheral blood and in bronchoalveolar lavage (BAL) samples was higher than in LTR with a nadir CD4 T-cell count >200 cells/μl ($p = 0.0012$ and $p = 0.0058$, respectively). A nadir CD4 T-cell count <200 cells/μl within the first three months post-transplantation predicted a higher frequency of viral infectious episodes in BAL samples within the subsequent six month period ($p = 0.0066$).
Conclusions: Stratification of patients according to nadir CD4 T-cell count may represent a new and simple approach for early identification of patients at risk for subsequent virus infections.

1. Background

Lung transplantation is the last treatment option for advanced stage lung disease. Despite advances in immunosuppressive regimens and management after transplantation, infections and rejection remain significant complications in lung transplant recipients (LTR). Compared with other solid organ transplant recipients, LTR are more vulnerable to infections. This is related to intensive immunosuppressive treatments as well as to exposure of the allograft to environmental agents [1]. Thus, both opportunistic (OI) and community-acquired (CAI) infections remain a major cause of morbidity and mortality in LTR. In addition, the role of viral infections in favoring the occurrence of chronic lung allograft dysfunction has been suggested [2,3]. Therefore, early detection and appropriate treatment of infections is mandatory to improve the outcome in these patients. While microbial diagnostic techniques have been developed and/or improved over the years, markers of immunological recovery predicting the risk of infection after transplantation are still not routinely applied during the follow-up period.

Abbreviations: LTR, lung transplant recipients; OI, opportunistic infections; CAI, community acquired infections; HCMV, human cytomegalovirus; BAL, bronchoalveolar lavage; ATG, antithymocyte globulin; EBV, Epstein-Barr virus; HSV, herpes simplex virus; VZV, varicella zoster virus; DFA, direct fluorescence antibody; ROC, receiver operating characteristic; SD, standard deviation; IQR, interquartile range; CI, confidence interval.

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Several studies have demonstrated that a low CD4 T-cell count represents a major risk factor for development of opportunistic complications [4,5], malignancies [6] and poor long-term outcome [7,8] in individuals with human immunodeficiency virus type-1 (HIV-1) infection. Additionally, it has been shown that a low nadir CD4 T-cell count predicts limited immune reconstitution [9,10] and poor CD4 T-cell recovery [11] in HIV-1-infected patients who were receiving antiretroviral therapy. Recently, we have shown that patients developing OI after heart transplantation had low nadir CD4 T-cell counts, while low CD8 T-cell counts were associated with the risk of OI following kidney transplantation [12]. Similarly, a more recent study confirmed that low T-cell counts at month 1 post-transplant predicts the subsequent occurrence of OI after kidney transplantation [13]. However, limited data are available in LTR [14,15].

2. Objectives

To analyze the kinetics of CD4 and CD8 T-cell counts in peripheral blood obtained from LTR during the first year after transplantation and evaluated the association between nadir CD4 T-cell count with the development of viral infections post-transplantation.

3. Study design

3.1. Patients and samples

Eighty-three consecutive patients who received a lung transplantation at the Fondazione IRCCS Policlinico San Matteo, Pavia, Italy, between August 2003 and January 2011, with at least one-year follow-up, were retrospectively analyzed. Demographic characteristics, donor and recipient human cytomegalovirus (HCMV) serostatus, transplant indication, type of lung transplant, induction therapy and immunosuppressive treatment, acute rejection episodes and cause of death were obtained from the patient's medical records and are presented in Table 1. Peripheral blood and bronchoalveolar lavage (BAL) samples were obtained as part of routine post-transplant monitoring of infectious complications.

Table 1
Characteristics of the study population ($n = 83$).

Characteristic	Median [interquartile range] or n (%)
Age (years) at transplantation	51.6 [37.2–58.8]
Gender	
Male	63 (75.9)
Female	20 (24.1)
Donor (D)/Recipient (R) human cytomegalovirus serostatus	
D+/R+	66 (79.5)
D+/R−	8 (9.6)
D−/R+	7 (8.4)
D−/R−	2 (2.4)
Transplant indication	
Idiopathic pulmonary fibrosis	34 (41.0)
Cystic fibrosis	14 (16.9)
Pulmonary emphysema	13 (15.7)
Pulmonary hypertension	9 (10.8)
Bronchiectasis	5 (6.0)
Eisenmenger syndrome	2 (2.4)
Histiocytosis X	1 (1.2)
Bullous dystrophy	1 (1.2)
Nonspecific interstitial pneumonia	1 (1.2)
Ebstein's anomaly	1 (1.2)
Acute respiratory distress syndrome	1 (1.2)
Lymphangioleiomyomatosis	1 (1.2)
Type of lung transplant	
Single	28 (33.7)
Double	48 (57.8)
Heart-lung	7 (8.4)
Induction therapy	
Antithymocyte globulin	24 (28.9)
No	59 (71.1)
Immunosuppressive regimen	
CsA or tacrolimus/AZA/steroids	40 (48.2)
CsA or tacrolimus/MMF/steroids	43 (51.8)
Acute rejection (AR) ≥A2	44 (53)
AR episodes per patient	2 [1–3]
Deceased patients	14 (16.9)
Time (months) to death after transplant	16.8 [14-21.6]
Infections	7 (50.0)
Bronchiolitis obliterans syndrome	3 (21.4)
Neoplasia	3 (21.4)
Hepatic failure	1 (7.1)

CsA, cyclosporine A; AZA, azathioprine; MMF, mycophenolate mofetil.

3.2. Immunosuppressive therapy

Induction therapy with antithymocyte globulin (ATG) was used in 28.9% of LTR. All LTR were receiving a standard triple immunosuppressive regimen including calcineurin inhibitors (cyclosporine A or tacrolimus), azathioprine or mycophenolate mofetil, and steroids and were treated with steroid pulses when an episode of acute rejection was diagnosed; grade ≥ A2 rejection were treated with a steroid bolus. No patient received antiviral drugs (ganciclovir or valganciclovir) for HCMV prophylaxis, while pre-emptive antiviral therapy was initiated when the HCMV DNA level exceeded 3×10^5 copies/ml blood or 1×10^5 copies/ml BAL [16,17].

3.3. Viral infections

In the OI group were considered those infections related to cellular and humoral immunosuppression, which included in our study HCMV, Epstein-Barr virus (EBV), herpes simplex virus (HSV), varicella zoster virus (VZV), human herpesvirus 6 (HHV-6) and polyomavirus [12,13,18]. Infections were defined as the presence of viral DNA in blood or BAL samples, determined using quantitative real-time PCR [16,19,20], in the absence of symptoms. Viral syndrome and disease were defined according to established criteria, which included quantification of viral DNA in blood as well as body fluids or biopsies in the presence of symptoms [21]. In the CAI group were included infections that could be acquired in the community or during hospitalization (nosocomial infection) independently from the patient's immune status, which include in our study parvovirus B19, rhinovirus, coronavirus, parainfluenza virus, adenovirus, influenza A and B. Parvovirus B19, rhinovirus and coronavirus were quantified by real-time PCR as described [20,22,23]. Samples were tested for parainfluenza virus, adenovirus, influenza A and B by direct fluorescence antibody (DFA) staining as described [24]. Samples positive by DFA were quantified by real-time PCR for adenovirus [25] and influenza A and B [26,27].

3.4. T-cell subsets

Peripheral whole blood was stained with anti-CD3 (FITC-conjugated), anti-CD45 (APC-Alexa Fluor 750-conjugated), anti-CD4 (APC-conjugated) and anti-CD8 (PE-conjugated) monoclonal antibodies (Beckman Coulter, Milan, Italy). After lysis of red cells, the percentage of CD4 and CD8 T lymphocytes was determined using a Navios™ Flow Cytometer System (Beckman Coulter). Absolute CD4 and CD8 T-cell counts (cells/μl) were calculated taking into account the total (WBC) and differential lymphocyte counts estimated by an automated hematology analyzer used in our institution's clinical laboratory. Measurements were performed in a