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Soluble CD30 levels as a diagnostic marker for bronchiolitis obliterans syndrome following human lung transplantation

Anjali .S. Golocheikine¹, Deepti Saini¹, Sabarinathan Ramachandran¹, Elbert P. Trulock³, Alexander Patterson¹, and T. Mohanakumar^{1,2}

¹Department of Surgery

²Department of Pathology & Immunology

³Department of Internal Medicine

Abstract

The long term survival of human lung allograft is hampered by the occurrence of chronic rejection, Bronchiolitis Obliterans Syndrome (BOS). This end-stage disease is normally diagnosed clinically by using the pulmonary function tests. This results in delay of BOS diagnosis and consequently prevents early intervention. It is generally accepted that alloimmunity plays an important role in chronic rejection of the allograft. In this study we analyzed serial serum samples from BOS+ and BOS– patients for sCD30 levels to determine the role of sCD30 to predict the onset of BOS. In contrast to BOS negative patients and normal subjects, 6 out of 9 BOS+ patients ($P < 0.05$) studied had an increase in the sCD30 levels. Significantly, the rise was noted 7.57 ± 2.63 months before the clinical diagnosis was evident. Therefore, we propose that the rise in serum sCD30 levels can be used as a marker for the detection of patients who are at risk of development of BOS.

Keywords

lung transplantation; sCD30; chronic rejection; Bronchiolitis obliterans syndrome (BOS); FEV1

1. Introduction

Lung transplantation is a viable therapeutic intervention for many end-stage pulmonary diseases. The short-term survival post- transplantation has been improved with advanced surgical procedures and immunosuppressive therapy (1). However, chronic rejection in the form of Bronchiolitis Obliterans syndrome (BOS) interrupts the long term survival of the allograft (2,3). BOS is histologically characterized by cellular infiltration, fibrosis, collagen deposition and occlusion of small airways in the allograft (4). The role of T cell immunity in the pathogenesis of chronic lung allograft rejection both in clinical lung transplantation and in animal models of obliterative airway diseases has been well established (5,6). Therefore, it is reasonable to predict that sCD30 which is a marker for T cell activation may increase during rejection of the allograft. Previous studies from our laboratory have already shown that sCD30 levels are increased during chronic rejection post lung transplantation (1).

Corresponding Author: T.Mohanakumar, Ph.D. Washington University School of Medicine Department of Surgery Box 8109–3328 CSRB 660s. Euclid Avenue ST. Louis, MO (314) 362–8463- Voice (314)747–1560-fax kumart@wustl.edu.

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Since increased levels of sCD30 in the sera of lung transplant recipients represents T cell activation and previous data from our laboratory shows increased levels of sCD30 in the sera of BOS + patients with the development of BOS, we hypothesized that the rise in sCD30 levels may precede the clinical evidence of BOS and therefore will assist in their clinical management. Our results strongly suggest that sCD30 levels increases several months prior to clinical evidence of BOS and therefore should assist in identifying lung transplant patients who are at high risk for developing BOS. This test therefore should help in devising strategies which will prevent or treat BOS earlier to clinical evidence of the disease.

2. Materials and Methods

Eighteen Patients who received lung allografts at the Washington University Medical Center/Barnes-Jewish Hospital were studied retrospectively after obtaining informed consent in accordance with a protocol approved by the Institutional Review Board. The mean age of the patient at the time of transplant is 51.72 ± 8.72 years.

Nine BOS+ and 9 BOS- patients were analyzed at an interval of 1–3 months, 1 year before the clinical diagnosis of BOS according to standard ISHLT guidelines (7). Serum samples stored at -70°C were analyzed in this study. 9 normal serum samples from healthy adult volunteer donors were obtained from Washington University Medical Center/Barnes-Jewish Hospital and included in this study.

2.1. sCD30 assay

The samples were analyzed for sCD30 by enzyme-linked immunoassay (ELISA) kit (Bender Med Systems, Vienna, Austria). Briefly, a 96 well plate coated with an anti-human sCD30 monoclonal antibody was used for the analysis. 25 μl of serum sample per well was incubated for 3 hours. All samples were run in duplicates. HRP-conjugated monoclonal anti-human sCD30 antibody was used to bind to the sCD30 that was captured by the 1st antibody. Substrate solution reactive with HRP was then added and reading taken at 450nm. The data was read in Units/ml (U/ mL) using a sCD30 standard.

2.2. Statistical analysis

Students t-test was used to determine the differences in sCD30 levels between normal and BOS + as well as BOS- and BOS+ patients.

3. Results

The levels of sCD30 in the normal human sera were 19.81 ± 3.44 U/ mL. Serial samples from 9 patients each of BOS+ and BOS- were studied for soluble CD30 levels. The clinico-demographic distribution of these patients is shown in Table 1. There was no correlation between HLA mismatch, number of acute rejections, sex or race between the two groups. Further, there was no evidence for infection or rejection at the time of sample collection.

sCD30 levels in BOS+ patients showed significant elevation in comparison to both normal values ($p < 0.047$) and values of BOS- patients ($p < 0.024$) (Fig 1). These results are consistent with the previous study from our center using a larger cohort of patients (1). Goal of our present study is to analyze serial samples and to determine whether sCD30 levels increases prior to clinical evidence of BOS i.e. drop in forced expiratory volume of air in 1 second (FEV1) measured in liters. Six out of the 9 patients with BOS demonstrated significant rise in sCD30 levels (29.5 ± 13.12 U/ml). More significant is our finding that the rise was noted about 7.57 ± 2.63 months before any clinical evidence of BOS (Fig 2a). Representative analysis of sCD30 results and its correlation with FEV1 of a lung transplant recipient with BOS+ is presented in

Fig 2B. sCD30 raised significantly (>120U/ml) 8 months prior to decline in Fev 1 in one patient (Fig 2B). Therefore, the results suggest that serial monitoring of sCD30 is required to correlate increase in sCD30 and clinical evidence of BOS.

4. Discussion

sCD30 molecule was originally characterized as a marker for Reed- Sternberg cells in Hodgkin's disease (8). CD30 is a membrane bound glycoprotein and is a member of TNF-superfamily (9). CD30 protein has been shown to be expressed on activated T-cells (10) and correspond to an increase in sCD30 found in the serum. Soluble form of the CD30 antigen has a smaller molecular weight compared to membrane bound form and is produced by proteolytic cleavage of the membrane bound CD30 (11). There are several recent reports suggesting that measurement of sCD30 may be of clinical use including acute rejection (12) and rheumatoid arthritis (13). In normal human subjects, sCD30 levels are low (14), but the levels are increased in conditions with T-cell activation (15).

Our previous study demonstrated low levels of sCD30 in the sera of normal subjects (1). In addition, similar values were noted in pre transplant sera from human lung transplant recipients. However, sCD30 levels were significantly high in lung transplant recipients who have clinical evidence of BOS (1). In this report, we performed serial analysis of sCD30 levels in a cohort of lung transplant recipients with and without BOS. Our results confirmed our earlier finding of significant ($p<0.05$) increase in the sCD30 levels in the serial serum samples of BOS + cases whereas the sCD30 values remained within the normal range in patients with normal lung function (BOS-ve patients). Further, we demonstrate in this report that sCD30 levels were elevated in the serum 7.57 ± 2.63 months prior to the clinical diagnosis of BOS. However, the levels did not remain persistently elevated in the serum samples analyzed. There was no correlation in increased sCD30 levels and infection or acute rejection episodes.

There was a slight increase in sCD30 levels in 2 out of 9 BOS – patients. These patients did not have any evidence of acute rejection or infection and had stable lung function during the time of sample collection. We are currently following these patients to analyze whether they are at higher risk for developing BOS.

Although the exact role of CD30 in the pathogenesis of BOS is unknown, alloimmune response is considered as a major contributor in the immuno- pathogenesis of BOS (5,16,17). We have shown that BOS+ patients develop early post- transplant inflammation which may activate allo- as well as auto-immunity (18). Further, loss of regulatory T cells which down regulate both auto as well as alloimmunity, may lead to increased immune response leading to the immunopathogenesis of BOS (19). Our finding that sCD30 levels are elevated several months prior to clinical onset of BOS strongly suggests activation of the immune response leading to an increase in the serum sCD30 levels.

In summary, our results show that raise in sCD30 levels may correlate with T cell activation in the lung transplant recipients who develop BOS. More significant is our finding that a raise in serum sCD30 levels takes place several months (mean 7.57 months) before the clinical diagnosis of BOS. Therefore, sCD30 analysis may provide a useful non-invasive marker for identifying lung transplant recipients who are at high risk for development of BOS so that new therapeutic strategies can be instituted to prevent or control BOS, a major risk factor for the continued function of the transplanted organ.

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Figure 1. sCD30 levels in serial plasma levels of normal, BOS – and BOS+ patients post transplantation

The plasma levels of sCD30 was analysed by ELISA in normal healthy individuals (n=9), BOS – patients (n=9) and BOS+ patients (n=9). The plasma sCD30 levels are significantly high ($p<0.05$) in 6 BOS+ Patients when compared with BOS– and normal.

Figure 2A

Figure 2B

Figure 2. Levels of soluble CD30 in serum increases prior to clinical diagnosis of BOS

Levels of soluble CD30 in serial serum samples from lung transplant recipients were measured by sCD30 ELISA before and at clinical diagnosis of BOS. A) The bars represent the mean $\pm$ SD concentrations of soluble CD30 in serum at the given time point. The horizontal line represents the normal $\pm$ SD concentrations observed in normal human subjects. The levels of the soluble CD30 were elevated above the mean values 5 to 8 months prior to clinical diagnosis of BOS. 2B) Serial CD 30 measurements in serum from a single lung transplant recipient along with the FEV1 values are plotted. A significant increase in the soluble CD30 levels in the serum was observed 8 months prior to clinical diagnosis of BOS (FEV1 decline).

Table 1
Clinical and demographic characteristics of LT patients

	BOS+ n =9	BOS- n =9
Age	57.88 ± 8.86 Yrs	45.55 ± 10.10 Yrs
Sex		
Female	6	5
Male	3	4
Race		
Caucasian	9	9
African American	0	0
Original Disease		
COPD	6	4
PPH	1	1
IPF	1	1
A1E	1	1
CF	0	2
HLA mismatch		
Class I	1	1
Class II	1	1
Both class I & II	0	4
Type of Transplant		
Bilateral	6	8
Single	3	1
patient survival	4	7

COPD= Chronic Obstructive Pulmonary Disease

PPH= Primary Pulmonary Hypertension

IPF= Interstitial Pulmonary Fibrosis

A1E= Alpha-1 Antitrypsin deficiency emphysema

CF= Cystic Fibrosis