Published in final edited form as:

J Heart Lung Transplant. 2014 September ; 33(9): 950–956. doi:10.1016/j.healun.2014.04.020.

Efficacy of extracorporeal photopheresis in clearance of antibodies to donor and lung specific antigens in lung transplant recipients

Gautam Baskaran, MD^a, Venkataswarup Tiriveedhi, MD, PhD^b, Sabarinathan Ramachandran, PhD^b, Aviva Aloush, RN^a, Brenda Grossman, MD^c, Ramsey Hachem, MD^a, and T Mohanakumar, PhD^{b,c}

^aDepartment of Medicine, Washington University School of Medicine, St. Louis, MO, USA

^bDepartment of Surgery, Washington University School of Medicine, St. Louis, MO, USA

^cDepartment of Pathology & Immunology, Washington University School of Medicine, St. Louis, MO, USA

Abstract

Background—Extracorporeal photopheresis (ECP) has been used to treat chronic rejection after lung transplantation (LTx). Here, we investigate the effect of ECP on several immune parameters which have been associated with poor lung function, including donor specific antibodies (DSA) to HLA, antibodies (Abs) against the lung associated self-antigens (SAg), K α 1Tubulin (K α 1T), Collagen (Col) I and V and circulating levels of pro- and anti-inflammatory cytokines.

Methods—Sera were collected from post-LTx patients diagnosed with bronchiolitis obliterans both before and 6 months after initiation of ECP. DSA and cytokine levels were measured by Luminex. Changes in lung function over the 6 months preceding and subsequent to the initiation of ECP were measured via retrospective analysis of spirometry performed at routine clinic visits.

Results—ECP was associated with a significant decline in DSA levels as well as Abs to lung associated SAg. ECP also reduced circulating levels of pro-inflammatory cytokines and increased levels of anti-inflammatory cytokines. These immunological changes were associated with a significant reduction (63%) in the rate of decline in FeV1 over a one year period. Though statistically insignificant, a higher rate of clearance of Abs to lung associated SAg was strongly associated with better response to ECP.

© 2014 International Society for Heart and Lung Transplantation. Published by Elsevier Inc. All rights reserved.

Address all correspondence to: T. Mohanakumar, PhD, Washington University School of Medicine, Department of Surgery, Box 8109, 3328 CSRB, 660 S. Euclid Ave, St. Louis, MO 63110, Telephone: (314) 362-8463; Fax: (314) 747-1560, kumart@wustl.edu.

Disclosures

None of the authors of this manuscript had any relevant financial relationships, arrangements with any commercial entity with interest in the subjects discussed or other conflicts of interest to report.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conclusion—ECP is associated with a reduction in the levels of circulating DSA, Abs to lung associated SAg (K α 1T, Col-I and V) and circulating levels of several pro-inflammatory cytokines. We propose that these changes contribute to the beneficial effect of ECP in reducing decline in lung function.

Introduction

While the median survival time for patients who are recipients of lung transplants (LTx) has risen over the last three decades, much of the improvement has been due to reduced mortality in the first year post-transplant¹. Despite advances in the management of acute rejection, chronic allograft rejection in the form of the bronchiolitis obliterans syndrome (BOS), continues to be a major barrier to improving mean patient survival, which is currently around 5.5 years¹. Current treatment strategies for BOS are varied and include: recurrent episodes of acute rejection^{2,3}, development of primary graft dysfunction⁴, infections with viruses such as human cytomegalovirus (CMV)⁵, gastroesophageal reflux disease⁶ and damage mediated via antibodies (Abs) to mismatched donor and Abs to various lung associated self-antigens(SAg)^{7,8,9,10}.

Work done by us, and others, have shown that development of Abs to donor human leukocyte antigens (DSA) can lead to the activation of pro-inflammatory cytokines and to an increased risk for development of BOS^{8,9}. Recent data from our laboratory has shown that Abs to the lung tissue-restricted SAg, Collagen V and K-alpha 1, are associated with the development of DSA and can lead to increased levels of pro-inflammatory cytokines and the development of BOS¹⁰. Given this, we hypothesize that therapies designed to decrease their levels will reduce the rate of development of BOS.

In addition to pharmacological treatments, such as IVIG and/or rituximab which have been shown to decrease mortality and the incidence of BOS¹¹, extracorporeal photopheresis (ECP) has also been used as a therapy to ameliorate the progression of BOS. During ECP, the patient's peripheral white blood cells are separated from the general blood and exposed to ultraviolet radiation and 8-methoxypsoralen (a naturally occurring furocoumarin) in a photoactivation chamber. This results in the activation of 8-methoxypsoralen and the formation of covalent bonds with DNA pyrimidine bases which leads to apoptosis. The treated blood is then re-infused into the patient. The end result is the depletion of circulating leukocyte (including helper T-cell) populations and likely changes in dendritic cell populations and cytokine levels¹². ECP is currently used to treat various autoimmune diseases, graft versus host disease, cutaneous T-cell lymphomas and a handful of other esoteric ailments^{12,13}. Within the realm of solid organ transplantation, ECP is an accepted treatment for acute cellular rejection and prophylaxis in heart transplantation¹⁴ and has been theorized to potentially have some benefit in liver transplantation¹⁵. ECP has also been shown to be beneficial in LTx by decreasing the rate of decline in FeV1 at 6 months and 1 year post-initiation of therapy^{16,17,18}. In fact, between 16¹⁷ to 25%¹⁶ of patients not only had stabilization of their lung function but also improvement. Responders to ECP showed greater survival and demonstrated a decreased need for re-transplantation¹⁷.

Despite the demonstrated utility of ECP in reducing the decline in lung function in those with BOS, the mechanism of action continues to remain unclear. Given what is theorized about the role of both DSA and Abs to SAg in the development of BOS, we hypothesized that ECP would be associated with a decrease in levels of these Abs as well as a shift in the cytokine balance towards an anti-inflammatory state.

Methods

Study Design

The study population consisted of 88 patients who were initiated on ECP for the treatment of progressive BOS at Barnes-Jewish Hospital/Washington University between January of 2000 and June of 2011. The protocol for this study was approved by the Washington University School of Medicine Human Research Protection Office for Human Studies. Patient characteristics are described in Table 1.

Our post-transplantation immunosuppression regimen generally consisted of a triple-drug immunosuppressive regimen consisting of systemic steroids, a calcineurin-inhibitor and a cell-cycle inhibitor. Surveillance bronchoscopies with lung biopsies were performed at 1, 3, and 6 months and 1 year post-transplant with additional bronchoscopies and biopsies performed as needed based on acute clinical indications.

Lung function was monitored serially according to established American Thoracic Society (ATS) guidelines¹⁹ and BOS diagnosed according to established International Society for Heart and Lung Transplantation (ISHLT) guidelines²⁰. Initial interventions at the time of diagnosis of BOS consisted of screening for and treatment of GERD (if present) as well as ensuring that the maintenance immunosuppression regimen was as optimized as possible. In patients in whom BOS was diagnosed and who were on optimal immunosuppression therapy, treatment protocol for BOS consisted of three times weekly oral Azithromycin, initiated at the time of diagnosis of BOS, as well as intravenous rabbit or equine anti-thymocyte globulin. ECP was initiated when there was a persistent and progressive decline in lung function despite initiation of the above-mentioned therapies.

Sera was collected from patients prior to the initiation of ECP and again within one month of the completion of the ECP protocol and analyzed for the development of DSA and Abs to SAg as well as serum levels of cytokines IL-1 β , IL-2, IL-4, IL-10, IFN- γ , IL-17 and MCP-1. All blood samples were drawn along with regularly ordered labs on the day of patients' routine transplant clinic visits. To the best of knowledge, no patient had ECP performed on the day of these clinic visits. Spirometry performed before and after initiation of ECP was then analyzed to determine the rate of decline in the forced expiratory volume in one second (Fev1) over the 6 month period before and after initiation of ECP. If the patient was hospitalized at the 6 month mark or being actively treated for a pulmonary infection (this occurred in 11/88 patients), we elected to use the next spirometry measurement, provided it was done within 3 months of the completion of the 6 month ECP protocol (all 11/88 patients had spirometry that met this criteria).

Extracorporeal photopheresis protocol

ECP procedures were performed using the UVAR XTS instrument (Therakos, Exton, PA) via either a peripheral vein or through an indwelling catheter in those patients considered to have difficulty in accessing peripheral veins. All treatments were performed on an outpatient basis per our institution's standard protocol as previously described¹⁶.

Detection of DSA and Abs to SAg and measurement of cytokine levels

All patients in this study had sera obtained before and after initiation of ECP and were screened for Abs to DSA and SAg. DSA determination was performed by the Barnes-Jewish Hospital HLA lab using the LABScreen Single Antigen assay (One Lambda Inc, CA). Results were reported as mean fluorescence intensity values (MFI). In cases where a patient has positive HLA class I and II Abs, the recorded MFI is the sum of both individual MFI measurements.

Abs against SAg (Col-I, Col-V and K α 1T) were measured by an enzyme linked immunosorbent assay (ELISA) and serum levels of cytokines IL-1 β , IL-2, IL-4, IL-10, IFN- γ , IL-17 and MCP-1 were measured using the LUMINEX assay (Invitrogen, CA) as per the manufacturer's protocols and as detailed in our previous publications^{21,22}.

Statistical Analysis

Lung function values were retrospectively obtained by reviewing pulmonary function tests performed one year before initiation of ECP, at the time of initiation of ECP and one year after initiation of ECP. The absolute change in FeV1 over the year preceding initiation of ECP was compared to the absolute change in FeV1 over the year after initiation of ECP. Patients in whom BOS was diagnosed within 6 months of transplantation or who expired within 6 months of initiation of ECP were not included in that portion of the analysis.

All data sets were checked for normality using the Shapiro-Wilks test and in all cases were found to be non-normal. In cases where we examined the effect of ECP on an immunological marker or FeV1 over time in the same patients, we used the Wilcoxon matched pairs signed rank test. Comparison of two different subgroups of patients were performed by the Mann-Whitney test. In cases where we compared more than two groups, we used the Kruskal-Wallis test. In all cases, we considered a p-value of <0.05 as indicative of clinical significance. In the course of our analysis, we noted that our data had a broad range and we consistently noted large standard deviations despite having a relatively low standard error of the mean. Therefore, in cases where our results were statistically significant, we reported the mean and standard deviation as well as the 95% confidence interval. In cases where they were not, we reported the mean and standard error as well as the 95% confidence interval in order to enable clearer and more direct comparison between the reported means in each respective data set. GraphPad Prism 4 (GraphPad, La Jolla, CA) and SPSS 11 (IBM, Armonk NY) were used for statistical analysis.

Results

Significant Reduction in DSA with ECP

Of the 88 patients enrolled in this study, 33 patients developed *de novo* DSA after transplantation and before the completion of ECP. Four of these patients developed DSA following initiation of ECP therapy and were also included in the analysis. Six patients developed DSA post-transplant but cleared them before initiation of ECP and were excluded from the analysis. Thus, 27 patients were included in the final analysis investigating the effectiveness of ECP in clearing DSA. As shown in Figure 1, the majority (19/27 or 70%) had either a decrease or no further increase in DSA levels. In addition, a sizeable number (10/27 or 37%) completely cleared DSA with ECP. Of these 27 patients, all were receiving monthly IVIG infusions at the time of initiation of photopheresis. Furthermore, 18 of these patients (including seven of the eight patients whose DSA levels increased with photopheresis) also received Rituxan in the six-month period prior to the initiation of photopheresis. While literature evaluating the effectiveness of these agents by themselves in clearing DSA is sparse, one study²³ from our institution demonstrated that 61% of patients treated with IVIG and Rituxan eventually cleared DSA by 2 years post treatment. When our own cohort of patients was followed out to two years, 81% of patients who survived (22/27) cleared DSA. It should be noted, however, that the majority of patients in our study also received thymoglobulin which could also account for the increased levels of DSA clearance. The statistical significance and precise cause of this difference is thus unclear.

Reduction in antibodies to lung associated self-antigens following ECP

We analyzed the change in levels of Abs to lung associated SAg (Col-I, Col-V and K α 1T) in all 88 of our patients. As illustrated in Figure 2, ECP was associated with a significant decrease in the mean levels of all three Abs specific to SAg (p value <0.0001). It was interesting to note that all patients had a decline in at least one Ab with 84% (74/88) having a decline in at least 2 Abs and 49% (43/88) having a decline in all three Abs to lung associated SAg.

ECP induces changes in the circulating levels of cytokines

Given that likely mechanisms linking ECP and Ab clearance may involve changes in the populations of circulating lymphocytes and cytokines, we measured the pre- and post-ECP levels of various pro- and anti-inflammatory cytokines in all 88 of our patients using a 25-Plex cytokine luminex assay. The results (Figure 3) show that there was a statistically significant decrease in the levels of the pro-inflammatory cytokines IL-1 β , IL-2, IFN- γ , IL-17, IP-1 and MCP-1 and an increase in the levels of cytokines IL-4 and IL-10 (p-value <0.0001 in all cases). Taken as a whole, we propose that this suggests that ECP facilitates a reduction in the level of systemic inflammation in patients with BOS.

Change in FeV1 with ECP

ECP has previously been shown to ameliorate decline in lung function¹⁶. In our cohort of patients who developed BOS and underwent ECP, we measured the absolute change in FeV1 over the 6 month period before and after initiation of ECP. Out of 88 patients, we

excluded 19 as they developed BOS within the first six months of transplant and we were unable to establish a clear baseline rate of decline in lung function prior to the initiation of ECP. In the remaining 69 patients, the average change in FeV1 was -0.76 liters over the 6 months before initiation of ECP compared to -0.28 liters in the 6 months after initiation of ECP; a reduction in the rate of decline of 63% ($p < 0.0001$).

Association between rate of decline in Fev1 and immune parameters

Since results presented earlier demonstrate that ECP resulted in a reduction in DSA, Abs to lung associated SAg and pro-inflammatory cytokine levels, we determined if there was a direct correlation between reduction in these markers and improvement in FeV1. In the 69 patients for whom we had reliable FeV1 data, 27 patients had positive DSA at time of ECP initiation. Of these, those patients who demonstrated stability or a reduction in DSA levels ($n=19$) had a reduction in rate of decline in FeV1 (average reduction of 0.14 liters) compared to those patients ($n=8$) who had rising DSA levels (average reduction of 0.36 liters). Difference did not reach statistical significance with a p-value of 0.77.

Similarly, we were unable to demonstrate a clear relationship between percentage reduction in Ab to SAg or pro-inflammatory cytokine levels and improvement in FeV1. However, the amount of decrease in pro-inflammatory cytokine levels was greater in patients who cleared Abs to all three SAg as opposed to only two (Table 2). Interestingly, when we examined the difference in rate of deterioration of lung function in patients who cleared one Ab (-0.83 ± 0.25 [95%CI: 0.28 : 1.38]) versus two (0.03 ± 0.15 [95%CI: -0.29 : 0.35]) versus three (0.33 ± 0.15 [95%CI: 0.03 : 0.63]), we noted a similar pattern where patients who cleared all three Abs appeared to have less decline in lung function compared to those who cleared fewer.

Continuing our exploration of the association between change in lung function and change in various cytokine and Ab levels, we subdivided our 69 patients into patients who appeared to have no change or improvement in Fev1 ($n=15$, with an average change in FeV1 of 0.26 liters) and patients who did not ($n=54$, with an average change in FeV1 of -0.40 liters, $p < 0.0001$). Interestingly, as summarized in Table 3, in the subpopulation of patients whose FeV1 appeared to be stable/have increased as a result of ECP we observed a higher degree of clearance of Abs to SAg compared to those patients who continued to have a decline in FeV1 levels though this did not approach statistical significance. However, we were unable to demonstrate differences in the reduction of pro and anti-inflammatory cytokine levels (Table 4) between these two groups.

Discussion

Studies from at our institution, and elsewhere^{16,17,18} have demonstrated that ECP ameliorates the progression of BOS though the precise mechanism is unknown. In this study, we have demonstrated that ECP clears DSA and Abs against SAg which we propose leads to decreased circulating levels of pro-inflammatory cytokines resulting in reduced rate of FeV1 decline.

In our cohort, the majority (70%) of patients had a reduction in DSA levels following ECP (Figure 1). This was associated with a statistically significant decline in levels of Abs to SAg and the pro-inflammatory cytokines IL-1 β , IL-2, IFN- γ , IL-17 and MCP-1 (Figures 2 and 3) as well as an increase in the levels of the anti-inflammatory cytokines IL-4 and IL-10 ($p < 0.0001$ in all cases). When analyzing change in lung function over a period of 6 months prior to and after the initiation of ECP, we demonstrated that there was a 63% reduction in the rate of decline ($p < 0.0001$). In 23% of our patients there was not only a reduction in deterioration of lung function but rather complete stability and even an increase in FeV1 at the end of 6 months of ECP. This is consistent with previously reported values (16¹⁷–25%¹⁶) for the number of patients who had improvement in lung function after ECP.

Results presented in Table 2 and 3 suggest that increased clearance of Abs to SAg may lead to greater reduction of circulating pro-inflammatory cytokines and a greater amelioration of decline in lung function. Even when patients cleared a single Ab to a SAg, they showed significant improvement in FeV1 and a greater reduction in circulating pro-inflammatory cytokine levels. Reduction of Abs by ECP is also accompanied by a reduction of pro-inflammatory and increase in anti-inflammatory cytokines suggesting that ECP may downregulate T helper cells and therefore lead to decreased activation of antibody producing B cells. We postulate that this may partially account for the mechanism by which ECP stabilizes or reduces the rate of decline in FeV1 in patients with BOS following LTx.

Overall, our study had several limitations, the most significant of which were the small sample size, lack of a true control group (i.e. a group that did not receive ECP) and lack of randomization. The ECP protocol that our patients were treated with, while in accordance with the practice at our institution, is also by no means universal. Other groups¹⁷ reporting improvement in lung function in LTx recipients have employed different regimens. The effects of variations in the number or frequency of treatment cycles is thus unknown. It should also be noted that in light of studies indicating the ECP may modulate immune function by increasing the amount of circulating regulatory T-cells, an assessment of the change in the amount of and activation of circulating T-cells may provide some mechanistic insights that could be exploited to treat or delay BOS development.

In conclusion, ECP has been shown to be associated with a statistically significant decreases in circulating levels of autoantibodies to lung associated SAg, Col-I, Col-V and K α 1T, as well as the pro-inflammatory cytokines IL-1 β , IL-2, IFN- γ , IL-17 and MCP-1. We have also shown that ECP appears to be associated with increased levels of the anti-inflammatory cytokines IL-4 and IL-10 and confirmed the previously reported phenomenon of a reduction in the rate of decline in FeV1. Further investigation with a larger cohort of patients and with characterization of regulatory T-cell function and activation may shed further light on the topic as well as potentially link our observations with previously reported mechanisms by which ECP is thought to function.

Acknowledgments

This work was supported by NIH HL HL056643 and the BJC Foundation (TM). The authors thank Ms. Billie Glasscock for her help in preparing this manuscript.

References

1. Stehlik J, Edwards LB, Kycheryavva AY, Benden C, Christie JD, Dipchand A, Dobbels F, Kirk R, Rahmel AO, Hertz M. The Registry of the International Society for Heart and Lung Transplantation: 29th official Adult Heart Transplant Report—2012. *J Heart Lung Transplant*. 2012; 31(10):1052–1064. [PubMed: 22975095]
2. Boehler A, Kesten S, Weder W, Speich R. Bronchiolitis obliterans after lung transplantation: a review. *Chest*. 1998; 114(5):1411–1426. [PubMed: 9824023]
3. Burton CM, Iversen M, Carlsen J, Mortensen J, Andersen CB, Steinbrüchel D, Scheike T. Acute cellular rejection is a risk factor for bronchiolitis obliterans syndrome independent of post-transplant baseline FEV1. *J Heart Lung Transplant*. 2009; 28(9):888–893. [PubMed: 19716040]
4. Whitson BA, Prekker ME, Herrington CS, Whelan TP, Radosevich DM, Hertz MI, Dahlberg PS. Primary graft dysfunction and long-term pulmonary function after lung transplantation. *J Heart Lung Transplant*. 2007; 26(10):1004–1011. [PubMed: 17919620]
5. Snyder LD, Finlen-Copeland CA, Turbyfill WJ, Howell D, Willner DA, Palmer SM. Cytomegalovirus pneumonitis is a risk for bronchiolitis obliterans syndrome in lung transplantation. *Am J Respir Crit Care Med*. 2010; 181(12):1391–1396. [PubMed: 20167845]
6. King BJ, Iyer H, Leidi AA, Carby MR. Gastroesophageal reflux in bronchiolitis obliterans syndrome: a new perspective. *J Heart Lung Transplant*. 2009; 28(9):870–875. [PubMed: 19716037]
7. Palmer SM, Davis RD, Hadjiliadis D, et al. Development of an antibody specific to major histocompatibility antigens detectable by flow cytometry after lung transplant is associated with bronchiolitis obliterans syndrome. *Transplantation*. 2002; 74:799–804. [PubMed: 12364858]
8. Hachem RR, Tiriveedhi V, Patterson GA, Aloush A, Trulock EP, Mohanakumar T. Antibodies to K-alpha 1 Tubulin and Collagen V Are Associated With Chronic Rejection After Lung Transplantation. *Am J Transplant*. 2012; 12:2164–71. [PubMed: 22568593]
9. Tiriveedhi V, Sarma N, Mohanakumar T. An important role for autoimmunity in the immunopathogenesis of chronic allograft rejection. *Int J Immunogenet*. 2012; 39:373–80. [PubMed: 22486939]
10. Saini D, Weber J, Ramachandran S, et al. Alloimmunity-induced autoimmunity as a potential mechanism in the pathogenesis of chronic rejection of human lung allografts. *J Heart Lung Transplant*. 2011; 30:624–31. [PubMed: 21414808]
11. Hachem RR, Yusen RD, Meyers BF, et al. Anti-human leukocyte antigen antibodies and preemptive antibody-directed therapy after lung transplantation. *J Heart Lung Transplant*. 2010; 29:973–80. [PubMed: 20558084]
12. Foss FM, Gorgun G, Miller KB. Extracorporeal photopheresis in chronic graft-versus-host disease. *Bone Marrow Transplantation*. 2002; 29:719–725. [PubMed: 12040467]
13. Sanford KW, Balogun RA. Extracorporeal photopheresis: clinical use so far. *J Clin Apher*. 2012; 27(3):126–131. [PubMed: 22467383]
14. Szczepiorkowski ZM, Winters JL, Bandarenko N, Kim HC, Linenberger ML, Marques MB, Sarode R, Schwartz J, Weinstein R, Shaz BH. Guidelines on the use of therapeutic apheresis in clinical practice--evidence based approach from the Apheresis Applications Committee of the American Society for Apheresis. *J Clin Apher*. 2010; 25(3):83–177. [PubMed: 20568098]
15. Urbani L, Mazzoni A, Colonbatto P, Biancofiore G, Bindi L, Tascini C, Menichetti F, Brunetto M, Scatena F, Filipponi F. Potential applications of extracorporeal photopheresis in liver transplantation. *Transplant Proc*. 2008; 40(4):1175–1178. [PubMed: 18555142]
16. Morrell MR, Despotis GJ, Lublin DM, Patterson GA, Trulock EP, Hachem RR. The efficacy of photopheresis for bronchiolitis obliterans syndrome after lung transplantation. *J Heart Lung Transplant*. 2010; 29(4):424–31. [PubMed: 19853479]
17. Jaksch P, Schneid A, Kepliner M, Ernst MB, Dani T, Just U, Nahavandi H, Klepetko W, Knobler R. A prospective interventional study on the use of extracorporeal photopheresis in patients with bronchiolitis obliterans syndrome after lung transplantation. *J Heart Lung Transplant*. 2012; 21(9): 950–957. [PubMed: 22884382]

18. Benden C, Speich R, Hofbauer GF, Irani S, Eich-Wanger C, Russi EW, Weder W, Beohler A. Extracorporeal photopheresis after lung transplantation: a 10 year single-center experience. *Transplantation*. 2008; 86:1625–1627. [PubMed: 19077900]
19. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, can der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J. Standardisation of spirometry. *Eur Respir J*. 2005; 26:319–398. [PubMed: 16055882]
20. Estenne M, Maurer JR, Boehler A, Egan JJ, Frost A, Hertz M, Mallory GB, Snell GI, Yousem S. Bronchiolitis obliterans syndrome 2001: an update of the diagnostic criteria. *J Heart Lung Transplant*. 2002; 21(3):297–310. [PubMed: 11897517]
21. Goers TA, Ramachandran S, Aloush A, Trulock E, Patterson GA, Mohanakumar T. De novo production of K-alpha1 tubulin-specific antibodies: role in chronic lung allograft rejection. *J Immunol*. 2008; 180:4487–94. [PubMed: 18354170]
22. Fukami N, Ramachandran S, Saini D, et al. Antibodies to MHC class I induce autoimmunity: role in the pathogenesis of chronic rejection. *J Immunol*. 2009; 182:309–18. [PubMed: 19109162]
23. Hachem RR, Tusen RD, Meyers BF, Aloush AA, Mohanakumar T, Patterson GA, Trulock EP. Anti-human leukocyte antigen antibodies and preemptive antibody-directed therapy after lung transplantation. *J Heart Lung Transplant*. 2010; 29(9):973–980. [PubMed: 20558084]
24. Lamioni A, Parisi F, Isacchi G, Giorda E, Di Cesare S, Landolfo A, Cenci F, Bottazzo GF, Carsetti R. The immunological effects of extracorporeal photopheresis unraveled: induction of tolerogenic dendritic cells in vitro and regulatory T cells in vivo. *Transplantation*. 2005; 79(7):846–850. [PubMed: 15818329]
25. George JF, Gooden CW, Guo L, Kirklin JK. Role for CD4(+)CD25(+) T cells in inhibition of graft rejection by extracorporeal photopheresis. *J Heart Lung Transplant*. 2008; 27(6):616–622. [PubMed: 18503960]
26. Meloni F, Cascina A, Miserere S, Perotti C, Vitulo P, Fietta AM. Peripheral CD4(+)CD25(+) TREG cell counts and the response to extracorporeal photopheresis in lung transplant recipients. *Transplant Proc*. 2007; 29(1):213–217. [PubMed: 17275508]
27. Burlingham WJ, Love RB, Jankowska-Gan E, et al. IL-17-dependent cellular immunity to collagen type V predisposes to obliterative bronchiolitis in human lung transplants. *J Clin Invest*. 2007; 117:3498–506. [PubMed: 17965778]

Figure 1. Percentage change in DSA levels in patients who underwent ECP. Change in DSA levels, expressed as percentage increase or decrease from pre-ECP treatment values, in patients (1–23) who underwent ECP.

Figure 2. Changes in autoimmune antibody (SAg) levels with ECP

Levels of antibodies to Col 1, Col 5 and K α 1T pre and post photophoresis (pp). Symbols represent means and error bars represent the 95% confidence interval.

Figure 3. Mean cytokine levels pre and post initiation of ECP

Cytokine levels pre and post photopheresis (pp). Symbols represent means and error bars represent the 95% confidence interval.

Table 1

Patient characteristics at ECP initiation

Characteristic	Number (percentage of total) (total patients: 88)
Age (in years)	Avg: 53 (range: 20–71)
Gender	
Male:	46 (52%)
Female:	42 (48%)
Pre Transplant Diagnosis	
COPD:	27 (31%)
IPF:	21 (24%)
Cystic Fibrosis:	17 (19%)
α_1 -Antitrypsin deficiency:	9 (10%)
Primary Pulmonary Hypertension:	6 (7%)
Bronchiectasis:	4 (5%)
Idiopathic non-specific interstitial pneumonitis:	2 (2%)
Sarcoidosis:	2 (2%)
Type of Transplant	
Single Lung:	84 (95%)
Double Lung:	4 (5%)
Time to development of BOS (days since transplant):	Avg: 805 (range: 121–4207)
Concurrent 3-drug immunosuppression (prednisone plus two of the following: tacrolimus, cyclosporine, sirolimus or mycophenolate):	86 (98%)
Concurrent Azithromycin:	80 (91%)
Prior Thymoglobulin:	83 (94%)
Prior IVIG:	28 (32%)

Amount of decrease in cytokine levels as a result of ECP in all patients with decline in one autoimmune antibody versus two autoimmune antibodies versus all three autoimmune antibodies.

Table 2

	One autoimmune Ab (pg/mL) (n=14) Mean decrease ± Stand. Error [95% CI]	Two autoimmune Abs (pg/mL) (n = 31) Mean decrease ± Stand. Error [95% CI]	Three autoimmune Abs. (pg/mL) (n = 43) Mean decrease ± Stand. Error [95% CI]	p Value
IL-1β	617 ± 255 [95% CI: 67 : 1167]	435 ± 142 [95% CI: -145 : - 726]	634 ± 101 [95% CI: -427 : - 840]	0.35
IL-2	567 ± 104 [95% CI: 344 : 791]	231 ± 61 [95% CI: -106 : - 357]	306 ± 51 [95% CI: -202 : - 409]	0.22
IL-4	-392 ± 51 [95% CI: -501 : - 282]	-346 ± 38 [95% CI: -423 : - 268]	-347 ± 30 [95% CI: -408 : - 286]	0.67
IL-10	-656 ± 143 [95% CI: -966: - 347]	-585 ± 113 [95% CI: -816 : - 354]	-567 ± 102 [95% CI: -773 : - 361]	0.24
IL-17	643 ± 46 [95% CI: -543 : - 742]	466 ± 38 [95% CI: -388 : - 544]	586 ± 27 [95% CI: -532 : - 640]	0.39
IFN-γ	544 ± 129 [95% CI: -265 : - 823]	424 ± 82 [95% CI: -437 : - 731]	584 ± 72 [95% CI: -437 : - 731]	0.23
IP-10	246 ± 48 [95% CI: -142 : - 350]	235 ± 33 [95% CI: -167 : - 303]	262 ± 24 [95% CI: -214 : - 310]	0.36
MCP-1	339 ± 49 [95% CI: -232 : - 446]	347 ± 54 [95% CI: -236 : - 458]	321 ± 44 [95% CI: -232 : - 410]	0.38

Table 3

Percent decrease in autoimmune antibody levels as a result of ECP in patients with stable/increased FeV1 versus patients with decline in FeV1.

	Average decrease in patients with stable/increased Fev1 (n= 15). Mean $\pm$ Stand. Error [95% CI]	Average decrease in patients with decline in Fev1 (n=54) Mean $\pm$ Stand. Error [95% CI]	p Value
Col I	48% $\pm$ 9 [95%CI: 28 : 68]	14.8% $\pm$ 16 [95%CI: 17 : 47]	0.23
Col V	33% $\pm$ 17 [95%CI: 19 : 70]	14% $\pm$ 15 [95%CI: -18 : 45]	0.74
KaT	40% $\pm$ 14 [95%CI: 11 : 70]	12% $\pm$ 14 [95%CI: -16 : 40]	0.17

Table 4

Percent decrease in cytokine levels as a result of ECP in patients with stable/increased FeV1 versus patients with decline in FeV1.

	Avg. change in cytokine levels in patients with stable Fev1 (n = 15) Mean decrease $\pm$ Stand. Error [95% CI]	Avg. change in cytokine levels in patients with decline in Fev1 (n = 54) Mean decrease $\pm$ Stand. Error [95% CI]	p Value
IL-1 β	24% $\pm$ 16 [95% CI: -11: 60]	11% $\pm$ 13 [95% CI: -13: 42]	0.67
IL-2	38% $\pm$ 5 [95% CI: 27: 49]	35% $\pm$ 8 [95% CI: 18: 52]	0.82
IL-4	-315% $\pm$ 45 [95% CI: -414: -218]	-260% $\pm$ 20 [95% CI: -302: -220]	0.45
IL-10	-536% $\pm$ 115 [95% CI: -780: -60]	-361% $\pm$ 196 [95% CI: -769: -303]	0.16
IL-17	56% $\pm$ 4 [95% CI: 47: 64]	50% $\pm$ 3 [95% CI: 46: 58]	0.7
IFN- γ	36% $\pm$ 3 [95% CI: -28: 44]	30% $\pm$ 5 [95% CI: 19: 44]	0.6
IP-10	64% $\pm$ 3 [95% CI: -49: -72]	60% $\pm$ 5 [95% CI: 57: 70]	0.56
MCP-1	55% $\pm$ 26 [95% CI: -47: -64]	45% $\pm$ 30 [95% CI: 28: 62]	0.19