CASE REPORT

IL-6 receptor blockade for allograft dysfunction after lung transplantation in a patient with COPA syndrome

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Abstract

Objective. COPA syndrome is a genetic disorder of retrograde cis-Golgi vesicle transport that leads to upregulation of pro-inflammatory cytokines (mainly IL-1β and IL-6) and the development of interstitial lung disease (ILD). The impact of COPA syndrome on post-lung transplant (LTx) outcome is unknown but potentially detrimental. In this case report, we describe progressive allograft dysfunction following LTx for COPA-ILD. Following the failure of standard immunosuppressive approaches, detailed cytokine analysis was performed with the intention of personalising therapy. Methods. Multiplexed cytokine analysis was performed on serum and bronchoalveolar lavage (BAL) fluid obtained pre- and post-LTx. Peripheral blood mononuclear cells (PMBCs) obtained pre- and post-LTx were stimulated with PMA, LPS and anti-CD3/CD28 antibodies. Post-LTx endobronchial biopsies underwent microarray-based gene expression analysis. Results were compared to non-COPA LTx recipients and non-LTx healthy controls. Results. Multiplexed cytokine analysis showed rising type I/II IFNs, and IL-6 in BAL post-LTx that decreased following treatment of acute rejection but rebounded with further clinical deterioration. In vitro stimulation of PMBCs suggested that myeloid cells were driving deterioration, through IL-6 signalling pathways. Tocilizumab (IL-6 receptor antibody) administration for 3 months (4 mg kg⁻¹, monthly) effectively suppressed IL-6 levels in BAL. Mucosal gene expression profile following tocilizumab suggested greater similarity to normal. Conclusion. Clinical effectiveness of IL-6 receptor blockade was not observed. However, we identified IL-6 upregulation associated with graft injury, effective IL-6 suppression with tocilizumab and evidence of beneficial effect on molecular transcripts. This mechanistic analysis suggests a role for IL-6 blockade in post-LTx care that should be investigated further.

Keywords: COPA syndrome, IL-6, lung transplantation, tocilizumab
INTRODUCTION

COPA syndrome is a monogenic disorder of immune dysregulation associated with mutations in the COPA gene on chromosome 1 encoding the coatamer-associated protein subunit alpha. The syndrome was first described in 2015 based on whole exome sequencing. The pathogenesis is of dysfunctional retrograde Golgi to endoplasmic reticulum (ER) protein transport, leading to the accumulation of unfolded proteins and increased ER stress. This causes an upregulation of pro-inflammatory cytokines (mainly IL-1β and IL-6) and skew the T-helper (Th) response towards a Th17 phenotype, associated with autoimmune disease. Clinical manifestations reported with this syndrome include inflammatory arthropathies, glomerulonephritis, interstitial lung disease (ILD) and pulmonary haemorrhage. High autoantibody titres are also common.

The impact of the innate immune dysfunction associated with COPA syndrome on post-lung transplant (LTx) outcomes is unknown. The potential risk is that impaired regulation of pro-inflammatory pathways will exacerbate immune-mediated allograft injury and impair outcome. The recent identification of COPA syndrome means that experience of performing LTx for this indication is limited. Encouragingly, a recent case report described stable lung function with no episodes of acute cellular rejection (ACR) or antibody-mediated rejection (AMR) after 15 months of follow-up. The immunosuppressive approach was reported to included peri-operative plasmapheresis (PLEX) and rituximab, induction anti-thymocyte globulin (ATG), and long-term maintenance intravenous immunoglobulin (IVIg).

In this case report, we describe our experience with lung transplantation (LTx) for ILD associated with COPA syndrome. We report progressive allograft dysfunction that occurred early post-LTx and provide detailed immunologic analysis that informed therapeutic approach. Although the outcome was not successful, we hope that the description of the challenges faced will provide mechanistic insights into disease pathways that may be investigated in the management of COPA syndrome and post-LTx allograft dysfunction.

CASE REPORT

We report the case of a 38-year-old Asian woman who underwent LTx for progressive ILD associated with COPA syndrome. Prior to LTx, COPA syndrome management had included immunosuppression (cyclophosphamide, azathioprine and prednisone) in a similar approach taken by other groups. In addition to ILD, COPA syndrome had manifested as glomerulonephritis and arthropathy. Other relevant medical history included hypertension, osteoporosis and a pulmonary embolus. At the time of referral for LTx, she presented with advanced restrictive lung disease [TLC 3.2 L or 63% predicted; DLCO unrecordable; cellular and fibrotic NSIP pattern with emphysematous/cystic changes (Figure 1)], secondary pulmonary hypertension and hypoxic respiratory failure. Pre-LTx human leucocyte antigen (HLA) testing revealed a high level of sensitisation, with a calculated panel reactive antibody (cPRA) of 28% for class I and 75% for class II HLA.

At the time of LTx, a positive antibody crossmatch – identifying both auto and donor specific-antibodies (DSA) – necessitated antibody desensitisation [intra- and post-operative PLEX, IVIg (1 g kg⁻¹) and ATG (5 mg kg⁻¹)]. Bilateral LTx surgery was performed on central vena-arterial extracorporeal membrane oxygenation support due to high pulmonary pressures and low lung volumes. Surgery was uneventful, post-operative recovery was routine, and grade 1 (mild) primary graft dysfunction was noted at 72 h. She was discharged from hospital on the 22nd post-operative day (POD), mobilising independently on room air. Maintenance oral immunosuppression at discharge composed of cyclosporin, mycophenolate and prednisone.

Despite this good early outcome, acute graft dysfunction (decline in FEV1 of 0.5 L from baseline; ill-defined, inflammatory nodules on CT chest; BAL neutrophilia with Enterococcus cloacae on BAL culture) was observed 2 months post-transplant. This was associated with grade 1 acute cellular rejection (ACR) and probable antibody-mediated rejection (AMR) (de novo HLA DQ DSA but C4d stain negative) on transbronchial biopsies. Intravenous antibiotics were administered for infection, guided by culture result. Despite this antibiotic course, allograft function did not recover. Treatment of ACR and AMR was commenced, including high-dose methylprednisolone, PLEX, IVIg and rituximab.
Cyclosporin was also changed to tacrolimus at this time. Although a small improvement in FEV1 was
seen with this treatment, allograft function did not return to baseline (Figure 2) and an ongoing
requirement for supplementary oxygen was noted. Immunosuppressive strategy was
complicated by leucopenia, low-grade CMV viraemia, Influenza A and the identification of
*Aspergillus fumigatus* in BAL necessitating mycophenolate dose reduction.

**Investigations**

As described above, COPA syndrome results in the upregulation of pro-inflammatory cytokines
leading to interstitial lung disease amongst other disorders. In this case, we hypothesised that
persistent immune dysregulation might underlie the pathogenesis of progressive graft injury, as IL-
6, IL-1β and Th17 upregulation have all been associated with COPA syndrome. If so, these
processes may augment or exacerbate the alloimmune response. Therefore, to evaluate
whether pro-inflammatory cytokines presented potential therapeutic targets in this unique case,
we obtained peripheral blood mononuclear cells (PBMC), serum, bronchoalveolar lavage (BAL) and
lung mucosal tissue for analysis from the patient, non-COPA LTx recipients with or without acute
rejection (*n* = 3 for each), and two healthy volunteers.

Multiplexed cytokine analysis of BAL at 45, 62, 91 and 102 POD showed rising type I and II IFNs,
IL-17, and IL-6 in BAL that decreased after treatment of ACR and AMR, but subsequently rebounded following clinical deterioration (Figure 3a). In our patient, plasma IL-6 and TNFα exhibited a particularly sharp rise post-LTx in comparison with non-COPA LTx recipients with acute rejection (Figure 3b). PBMCs from the patient at 3 months post-LTx exhibited marked LPS-recruitable IL-6 production but minimal responsiveness to anti-CD3, suggesting that T-cell responses were controlled and that cells of the myeloid lineage were driving clinical deterioration through an IL-6 signalling pathway. Interestingly, analysis of cytokine production of PBMCs in response to different stimuli showed that this patient’s post-LTx management effectively controlled type I interferon production in her T cells and myeloid cells (Figure 3f). Microarray-based gene expression profiling of endobronchial biopsies at POD 102 revealed a very unusual pattern of gene expression distinct from other biopsies in the reference set (Figure 3c).

**TREATMENT**

In view of the results of the cytokine analysis seen in this case, in addition to progressive allograft
dysfunction, we administered tocilizumab 4 mg kg⁻¹ monthly for three doses. Tocilizumab was well tolerated by the patient and effectively suppressed IL-6 in BAL (Figure 3d). Mucosal gene expression profile following treatment suggested improvement in features of T-cell-mediated rejection and greater similarity to normal biopsies (Figure 3e). Tocilizumab was discontinued after

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**Figure 1.** Pre-transplant CT chest and explanted lung pathology. (a) Thoracic CT scan performed 1 week prior to lung transplant showed extensive cystic lung disease and fibrosis. (b) Explant pathology showed emphysematous/cystic changes, bronchiectasis, and a cellular and fibrotic nonspecific interstitial pneumonia pattern of interstitial lung disease. The upper image shows 20× magnification and the lower image 40× magnification.
three doses, due to a lack of substantial clinical improvement. After tocilizumab discontinuation, an increase in serum IL-6 suggested that ongoing IL-6 suppression may have been efficacious (Figure 3d).

**DIFFERENTIAL DIAGNOSIS**

**Baseline lung allograft dysfunction**

The failure to achieve ‘normal’ pulmonary function following transplant (based on non-LTx population reference ranges) has been termed baseline lung allograft dysfunction (BLAD). The presence, and severity, of FEV1 impairment compared to ‘population norms’ has been associated with increasing mortality risk. Factors associated with BLAD include ILD as a LTx indication and donor smoking history (> 20 packs per years). Conceptually, one might also expect other factors to contribute to a failure to achieve a ‘normal’ FEV1, such as size-mismatching, primary graft dysfunction (PGD), and early or ongoing lung injury (infection, aspiration, rejection). In this case, whilst the diagnosis of ILD may have been a risk factor for BLAD, the donor did not have a smoking history and the donor lung was well size-matched (donor-recipient predicted TLC ratio 1.02). Peri-operative donor and recipient BAL cultures were negative, and mild PGD (grade 1) was experienced at 72 h.

**T- or B- cell-mediated acute rejection**

Bronchoscopy at POD 46 revealed minimal ACR (grade A1Bx) and a positive BAL culture for *E. cloacae*. Despite appropriate intravenous antibiotics, a decline in lung function occurred and treatment of ACR with IV methylprednisolone (1 mg kg\(^{-1}\) for 3 days) was indicated. The diagnosis of ‘probable AMR’ based on graft injury, histology and de novo DSA (DQ4 and DQ6) was managed with PLEX, IVIg and rituximab. No further TBBx were performed due to concerns associated with low lung function. Following AMR treatment, DQ6 DSA resolved but DQ4 DSA persisted. Autopsy histology did not identify evidence of ACR or AMR (Figure 3g), suggesting that the immunosuppressive approach taken in this case was successful in treating acute rejection but that other immune processes contributed to progressive allograft dysfunction.
Infection as a cause of progressive graft dysfunction

During post-LTx follow-up, this patient underwent frequent screening for infection, including regular bronchoscopy (including BAL with bacterial, fungal and mycobacterial cultures), sputum C+S and nasopharyngeal swabs for viral PCR. In view of the augmented immunosuppressive strategy, infection was carefully considered as the aetiology for ongoing graft dysfunction. Notably, bacterial organisms were only identified on two occasions post-LTx and treated appropriately. Influenza A was identified on two separate occasions and treated with antivirals. Aside from pneumonitis, respiratory viral infection has been associated...
with the potentiation of an alloimmune response in LTx recipients. Whilst we are unable to confirm whether influenza contributed to the potentiation of immune-mediated injury in this case, we did not identify an expansion of either class 1 or 2 HLA antibodies following these infections. Finally, the identification of *A. fumigatus* in BAL was managed with preemptive antifungal therapy, in view of the risk of invasive aspergillosis associated with further immunosuppression. With this strategy, we did not identify radiographic evidence of aspergillosis on CT imaging and serum galactomannan remained negative.

**Allograft injury related to a dysregulation of the immune response associated with COPA syndrome**

Throughout the post-LTx course, this patient underwent a CT thorax approximately monthly. At 3 months post-LTx, there was radiological evidence of bronchiolitis obliterans and this progressed on interval scans. The development of BOS at this early time-point is uncommon and likely driven by allo-immune responses to noxious stimuli. The lack of clinical benefit to ACR/AMR treatment, in addition to the pro-inflammatory cytokine profile seen, raises the question as to whether the progressive graft injury in this case was a result of, or exacerbated by, the immunological dysfunction intrinsic to COPA syndrome.

**DISCUSSION**

The likely pathophysiology underpinning this case is of a dysfunctional immune response causing early and ongoing graft injury leading to progressive chronic lung allograft dysfunction (CLAD). Whether the trigger for this injurious immune response was infection, alloimmunity and/or COPA-associated immune dysregulation is unclear. However, the pro-inflammatory cytokine response described with an over-expression of IL-6 presented a potential therapeutic target following the failure of standard immunosuppression.

Tocilizumab – a monoclonal anti-IL-6 receptor antibody – is FDA approved for use in rheumatoid arthritis, juvenile idiopathic arthritis and giant cell arteritis. IL-6 is thought to play an important role in the progression of autoimmune disease and has been implicated in the expansion and activation of both B and T cells, as well as in the initiation of the acute phase inflammatory response. In organ transplant animal models, improved survival has been reported in IL-6-deficient heart and kidney allografts. In renal transplant medicine, tocilizumab has been investigated in pre-transplant HLA desensitisation and in the treatment of chronic AMR. In lung transplantation, raised IL-6 levels in BAL have been reported to be associated with primary graft dysfunction, ACR and CLAD.

Unfortunately, the administration of tocilizumab did not provide a clinically meaningful benefit in this case. This patient experienced progressive allograft dysfunction and death at 9 months post-LTx. However, we hypothesise that if IL-6 has a role in ongoing graft injury, the commencement of tocilizumab at an earlier phase of alloimmune injury may be more beneficial than that seen in this case when radiographic evidence of BOS was present (Figure 3h).

**Lessons to be learnt**

We identify IL-6 as a potentially important cytokine in the development of graft dysfunction and report effective suppression of IL-6 in BAL with tocilizumab. In addition, we report the improvement of injury as measured by mucosal gene transcription, suggesting reduced cellular injury and rejection with this treatment. This insight has value to the LTx community in general, as there is a growing evidence for IL-6 receptor blockade in promoting immune tolerance. We further support this theory by identifying elevated BAL IL-6 concentrations in non-COPA LTx patients experiencing acute rejection (Figure 3a). Further research is required to determine whether IL-6 suppression leads to clinically meaningful endpoints.

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**CONFLICT OF INTEREST**

Peter Riddell, Sajad Moshkelgosha, Liran Levy, Prodipto Pal, Kieran Halloran, Lianne Singer and Shaf Keshavjee declare
no conflict of interest. Phil Halloran is Owner/founder of Transcriptome Sciences Inc. and received honoraria for lectures from Thermo Fisher and Astellas. Michael Parkes is Employee of Transcriptome Sciences Inc. Tereza Martinu and Stephen Juvet received research grant support from Sanofi.

AUTHOR CONTRIBUTIONS

Peter Riddell: Conceptualization; Formal analysis; Visualization; Writing–original draft; Writing–review & editing. Sajad Moskheldoosh: Data curation; Investigation; Methodology; Visualization; Writing–review & editing. Liran Levy: Data curation; Formal analysis; Writing–review & editing. Nina Chang: Data curation; Visualization; Writing–review & editing. Prodipto Pal: Data curation; Visualization; Writing–review & editing. Michael Parkes: Data curation; Formal analysis; Visualization; Writing–review & editing. Kieran Halloran: Data curation; Formal analysis; Visualization; Writing–review & editing. Shaf Keshavjee: Conceptualization; Methodology; Writing–review & editing. Tereza Martinu: Conceptualization; Data curation; Formal analysis; Methodology; Visualization; Writing–review & editing. Stephen Juvet: Conceptualization; Data curation; Formal analysis; Methodology; Project administration; Supervision; Writing–review & editing.

ETHICS BOARD APPROVAL

Studies on samples from the healthy volunteers and LTx recipients were approved by the institutional review board at the University Health Network, Toronto.

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