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Humoral immunity in phenotypes of chronic lung allograft dysfunction: A broncho-alveolar lavage fluid analysis☆

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ABSTRACT

Background: Recently, antibody mediated rejection (AMR) has been associated with a higher incidence of chronic lung allograft dysfunction (CLAD) and mortality after lung transplantation (LTx). We investigated markers related to AMR and matrix remodeling in CLAD, with special attention for its two phenotypes being bronchiolitis obliterans syndrome (BOS) and restrictive CLAD (rCLAD).
Methods: Immunoglobulins (IgA, IgE, IgG_1–IgG_4, total IgG and IgM) and complement (C4d and C1q) were quantified in lung lavage samples at the moment of BOS ($n = 15$) or RAS ($n = 16$) diagnosis; and were compared to stable transplant patients who served as control ($n = 14$). Also, airway remodeling and metalloproteinases (MMPs) were investigated via zymography and gelatin degradation. The presence of DSA was additionally assessed in blood.
Results: Total IgG, IgG_1-IgG_4 and IgM were increased in rCLAD versus control ($p < 0.001$) and BOS patients ($p < 0.01$). IgA and IgE were increased in rCLAD compared to control (respectively $p < 0.05$ and $p < 0.01$), but not to BOS. Total IgG and IgE were increased in BOS versus control (respectively $p < 0.01$ and $p < 0.05$). Complement proteins were exclusively present in rCLAD and correlated positively with immunoglobulins. Additionally, in blood, DSA were more present in rCLAD ($p = 0.041$). MMP-9 levels increased in RAS and BOS versus control ($p < 0.001$) and MMP-9 induced gelatin degradation was only increased in BOS compared to control ($p < 0.01$).
Conclusion: We demonstrated increased levels of immunoglobulins and complement proteins dominantly present in rCLAD. This leads to the belief that antibodies and AMR might play a more important role in rCLAD compared to BOS. Therefore, anti B-cell therapy could offer beneficial therapeutic effects in patients diagnosed with rCLAD, which needs further research.

1. Introduction

Despite the improved survival rates over the last decade due to better surgical techniques, perioperative management and more intense immunosuppressive prophylaxis, chronic rejection after lung transplantation (LTx) remains a major obstacle clinically referred to as chronic lung allograft dysfunction (CLAD). CLAD is defined as a chronic decline in FEV_1 (≥20%) compared to the best post-operative values and for which no specific other cause can be identified. Two major phenotypes within CLAD were recently proposed, being restrictive CLAD (rCLAD) or restrictive allograft syndrome (RAS) and the classical obstructive form of CLAD denominated as bronchiolitis obliterans syndrome (BOS) [1].

Chronic rejection has been regarded as a predominantly T-cell mediated process where accordingly standard triple-drug immunosuppressive therapy is given, to target T-cell activation and proliferation [2]. Recent evidence, however, indicated that B-cells might be involved in the pathogenesis of CLAD as well [3]. Activated B-lymphocytes can produce a diverse range of immunoglobulins which all possess specific

Abbreviations: AMR, antibody mediated rejection; BAL, broncho-alveolar lavage; BOS, bronchiolitis obliterans syndrome; CLAD, chronic lung allograft syndrome; CT, computed tomography; DSA, donor specific antibodies; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; HLA, human leukocyte antigen; LTx, lung transplantation; RAS, restrictive allograft syndrome; TLC, total lung capacity.

☆ None of the authors have anything to declare

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biological functions and produce donor-specific antibodies (DSA) directed towards specific human leukocyte antigens (HLA) of the graft [4,5]. Circulating DSAs promote complement activation resulting into lung injury [6].

This phenomenon is, according to the International Society for Heart and Lung Transplantation (ISHLT), denominated as antibody mediated rejection (AMR). AMR is defined by the presence of circulating DSAs, clinical allograft dysfunction and histological evidence for C4d deposition accompanied with representative lung injury [7]. Besides the importance of C4d, in cardiac transplantation, correlations have also been demonstrated between C1q positive antibodies and early AMR [8]. LTx recipients with presence of DSA have an increased risk of CLAD and suffer from a worse survival [9–11]. In a recent study of Roux et al., AMR patients seem to evolve exclusively to RAS [12]. Therefore, at the moment of CLAD diagnosis, we investigated the presence of DSAs in blood combined with complement activation and the presence of immunoglobulins in BAL. Prior work demonstrated lung morphometric differences between BOS and RAS phenotypes, despite the fact they all showed small obliterative airway lesions. In this perspective, we additionally aimed to correlate the investigated humoral markers with airway remodeling.

Therefore, we analyzed broncho alveolar lavage (BAL) fluid and blood of rejecting allografts (BOS and RAS) compared to stable transplant patients.

2. Materials and methods

Patients' characteristics included gender, age, type of LTx (single or bilateral), underlying disease and immunosuppressive therapy at moment of CLAD. CLAD was diagnosed as a persistent decline in FEV_1 of at least 20% compared to the two best post-operative values, without any other identifiable cause. A further subdivision was made between BOS and RAS: if total lung capacity (TLC) was available, RAS diagnosis was made using a decrease in TLC ≥ 10% in combination with a ≥ 20% decrease in FEV_1. If TLC was unavailable, a FEV_1/FVC ratio > 0.70 in combination with persistent infiltrates on CT and a persistent decrease in FEV_1 and/or forced vital capacity (FVC), was diagnosed as rCLAD [1]. Retrospectively, only patients were included with confirmed histopathology and with available BAL sample at the moment of BOS (n = 15) or rCLAD (n = 16) diagnosis. HLA typing was assessed by complement dependent cytotoxicity assay (CDC) or, more recently, via a validated SSO methodology (LIFECODES HLA SSO Typing Kit; Immucor Transplant Diagnostics) which is routinely used by the Laboratory for Histocompatibility Immunogenetics of the Belgian Red Cross. Serum antibodies against HLA antigens, were performed via enzyme immunoassay (EIA) (Quick Screen Lifecodes, Immucor, Norcross, GA) or Luminex (LMX Lifecodes, Immunocor). An anti-HLA screening result was considered negative if median fluorescence intensity (MFI) was <500 and all adjusted values (based on internal control beads) were 0. Patients with MFI ≥ 500were defined as positive. A positive screening was always retested with a single antigen bead (SAB) (Lifecodes, Immucor) assay to identify the HLA specificity. Patients with MFI ≥ 500 were defined as positive. Donors were systematically screened for HLA typing of which we could assess whether these HLA antibodies were directed against the donor, thus donor specific (DSA).

2.1. BAL samples

To assess the role of humoral immunity, broncho-alveolar lavage (BAL) fluid was retrospectively analyzed at diagnosis of BOS (n = 15) or RAS (n = 16). Briefly, BAL was performed with 2 aliquots of sterile saline (50 ml) of which the returned fractions were pooled and processed for cell counting as previously described [13]. Time-matched BAL samples (n = 14), retrieved at routine follow-up, of stable lung transplant

Table 1
Patient characteristics.
Results are shown in numbers (percentage) or as mean ± SEM or as median (IQR). The p-value displayed on the right shows the results of the Kruskall-Wallis ANOVA in case of continuous data. Significances of the Dunn's post-hoc test are presented as: # (BOS versus control); * (RAS versus control); † (BOS versus RAS) (*p < 0.05, **p < 0.01, ***p < 0.001). In case of discrete data, the results of the contingency table are shown. A p-value < 0.05 was considered significant and is indicated in bold.

	Control	BOS	rCLAD	p-Value
Number of patients, n	14	15	16	
Female gender, n (%)	10 (71%)	8 (53%)	6 (38%)	0.18
Age, y	50 ± 4	38 ± 4	36 ± 4	**0.047**
Indication for LTx, n (%)				0.26
Emphysema, α-1ATD	4 (29%)	2 (13%)	6 (38%)	0.31
Pulmonary fibrosis	3 (21%)	1 (7%)	0 (0%)	0.11
CF/Bronchiectasis	5 (36%)	9 (60%)	5 (31%)	0.23
Eisenmenger/PAH	2 (14%)	1 (7%)	3 (19%)	0.61
Others (e.g. sarcoidosis, LAM)	0 (0%)	2 (13%)	2 (12%)	0.37
Type LTx (SS-S), n (%)	14 (100%)	15 (100%)	16 (100%)	1.00
Pulmonary function (absolute value)				
FEV_1 (%Pred)	97 (87–105)	59 (48–72) ###	52 (44–68)***	<**0.0001**
FEV_1 (L)	2.8 (2.2–3.5)	2.0 (1.5–2.5)	1.9 (1.3–2.2)*	**0.014**
FVC (%Pred)	101 (94–115)	79 (70–92) #	64 (48–75)***,†	<**0.0001**
FVC (L)	3.3 (2.2–4.5)	3.1 (2.8–4.0)	2.5 (1.5–3.4)	0.055
FEV_1/FVC (%)	80 (74–87)	64 (49–76) #	80 (63–85)	**0.018**
TLC (%Pred)	89 (78–94)	88 (83–104)	68 (57–86)*, †	**0.016**
TLC (L)	4.6 (4.1–6.2)	5.9 (4.4–6.2)	4.4 (3.5–5.2)	0.13
Pulmonary function (relative to baseline)				
FEV_1 (%Best)	101 (98–108)	68 (52–78) ###	65 (55–70)***	<**0.0001**
FVC (%Best)	99 (95–103)	95 (83–99)	66 (62–79)***, ††	<**0.0001**
TLC (%Best)	98 (96–102)	102 (95–112)	72 (65–98)††	**0.006**
Treatment, n (%)				
AZA-MMF-everolimus-none	6(43%)-6(43%)-0(0%)-2 (14%)	6 (40%)-6(40%)-0(0%)-2(13%)	8(50%)-4(25%)-1(6%)-1(6%)	0.77
Tacrolimus-cyclosporine	14 (100%)-0	14 (93%)-1 (7%)	14 (88%)-2 (12%)	0.39
Steroids (mg)	4.0 (4.0–4.0)	4.0 (4.0–12.0)	4.0 (4.0–10.0)	0.24
Azithromycin	7 (50%)	8 (53%)	11 (69%)	0.53
Time between LTx and BAL, y	2.0 ± 0.0	3.0 ± 0.6	3.2 ± 0.6	0.99
Time between diagnosis and death, y	1.8 ± 0.2	2.2 ± 0.5	1.2 ± 0.4*	0.020

Abbreviations: LTx = lung transplantation; SS = sequential single sided LTx; S = single sided LTx; α-1ATD = alpha-1-antitrypsin deficiency; CF = cystic fibrosis; LAM = Lymphangioleiomyomatosis; PAH = pulmonary arterial hypertension; FEV1 = forced expiratory volume in 1 s; FVC = forced vital capacity; TLC = total lung capacity; MMF = mycophenolate mophetil; AZA = azathioprine; y = years.