A Promoter Polymorphism in the CD59 Complement Regulatory Protein Gene in Donor Lungs Correlates With a Higher Risk for Chronic Rejection After Lung Transplantation


Soluble CD59 is a Novel Biomarker for the Prediction of Obstructive Chronic Lung Allograft Dysfunction After Lung Transplantation

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Despite innate immune proteins contributing to graft dysfunction post-lung transplantation, data on the role of the complement system is scarce, primarily focusing on endothelial C4d deposition during graft injury [1-4]. One reason for the lack of a mechanistic understanding of how complement proteins modulate graft dysfunction is that the system is so diverse, comprising of over 60 proteins [5]. Additionally, many of the proteins and their cleavage products are labile and hard to measure [6]. Among these, some proteins promote inflammation, while others regulate it [7]. Regulators acting through different mechanisms can mitigate inflammation either in the fluid-phase or on the cell-surface [5,7]. One of these cell-surface, membrane-bound regulators, CD59, inhibits the formation of the membrane-attack complex (MAC) by blocking the binding of C9 to the C5b-9 complexes. As suggested by preclinical models of renal transplantation, endothelial cells (EC) not only express CD59, but also have the ability to become resistant to complement-mediated cytotoxicity by increasing the expression of CD59 in the setting of stress, a process termed as accommodation [8,9].

Budding et al recently evaluated a donor single nucleotide polymorphism occurring in the promoter region of CD59 that was associated with significantly lower freedom from bronchiolitis-obliterans syndrome (BOS, p=0.046) [10]. Among 137 lung transplant (LTx) recipients between 2004 and 2012, the authors sequenced the CD59 promoter and adjacent regions. After carefully screening 11 SNPs, they found that those patients who received a graft from a donor with an insertion of adenine at SNP rs147788946 (A/x genotype, at position -314 in the promoter region of CD59) had a trend towards lower 5-year survival (p=0.094), in addition to it being an independent predictor of BOS on multivariate analysis (hazard ratio 3.1, 95% confidence interval: 1.0-9.9, p=0.058). Peripheral blood-derived mononuclear cells (PBMCs) of donors with the A/x genotype had lower CD59 expression and a higher proportion of them lysed when sensitized with W6/32 (anti-HLA A/B/C antibody) or anti-thymocyte globulin (ATG) and incubated with increasing doses of human serum, as compared to those with the -/− genotype. Additionally, SNP-genotyped A/x vascular ECs had significantly higher secretion of IL-6 upon sublytic MAC activation with ATG and human serum compared to -/- ECs, though they were resistant to ATG-mediated cytotoxicity. Thus, the authors proposed that this novel SNP, associated with increased risk of BOS in a limited sample, functionally increased complement-mediated cytotoxicity in PBMCs and increased sublytic MAC-induced cytokine secretion from ECs, promoting local inflammation and graft dysfunction.

The authors found that ECs were resistant to substantial lysis by complement, which was partly explained by the high CD59 expression on these cells. Incubating the cells with phospholipase-C [which cleaves off GPI (glycosylphosphatidylinositol)-anchored proteins
including CD59] increased complement-mediated lysis. Hence, in a follow-up manuscript, they evaluated **soluble CD59 (sCD59) levels as a prognostic biomarker for BOS** [11]. They measured sCD59 levels using an ELISA in 90 LTx recipients, 20 of whom had BOS. In the pilot analysis, they found that 8 of 10 recipients with BOS had elevated sCD59 levels compared to the median of matched recipients without BOS (n=10). In the same group, mean sCD59 levels were comparable at 1 months and 3 months post-LTx in those with and without BOS. However, **at 6 months post-LTx, sCD59 levels were significantly higher in those who went on to develop BOS** (517.0 ± 72.22 pg/ml vs. 271.8 ± 35.22 pg/ml, p=0.0069). The authors validated these findings in an independent cohort of 10 LTx recipients who developed BOS versus 59 recipients who did not, and performed an ROC analysis, which showed that using a cutoff of 400 pg/mL to predict BOS 6 months post-LTx resulted in an area under the curve of 0.72 (95% confidence interval 0.58-0.80), sensitivity of 60% and specificity of 84%. The predictive ability of sCD59 held true in a multivariate Cox proportional hazards model where **sCD59 > 400 pg/mL** was an independent predictor of BOS at 6 months post-LTx [n=89, hazard ratio 6.2 (2.4-15.8), p <0.0001]. Of note, BOS was diagnosed on an average of 35 months post-LTx in this cohort, well after the discriminant sCD59 levels would be detected.

Through these two manuscripts, the authors have discovered a novel polymorphism in a membrane regulator of the complement system in donors, showed how it protects against complement-mediated cell damage and identified a novel biomarker in the blood of recipients to predict the development of BOS. A few caveats must be considered. **First, these manuscripts were from single center cohorts** and both the rs147788946 polymorphism as well as the sCD59 biomarker needs to be independently validated in other multi-centric LTx cohorts. **Secondly, sCD59 needs to be better understood as a biomarker.** How gender, ethnicity, immunosuppression and other forms of lung injury (such as acute rejection or infection) affect levels needs to be better understood before it can be entertained as a predictive marker for BOS. **Third, there was no correlation between sCD59 levels and markers of alveolar injury** (such as CC16, Clara Cell secretory protein), or markers of systemic inflammation (such as C-reactive protein or absolute neutrophil counts). This goes against the hypothesis that sCD59 is released from cell damage, but raises a possibility that enzymes such as phospholipase-C may be locally active in recipients who have ongoing graft injury resulting in BOS. This would be especially important in designing therapies to mitigate the development of BOS [12-14]. Currently, ocular gene therapy with an adeno-associated virus (AAV2) vector expressing human CD59 with a deletion in the GPI anchor of CD59 (AAVCAGsCD59) is being tested in a Phase I clinical trial for advanced dry age-related macular degeneration (NCT03144999) [15]. Once the kinetics and cell-specific nature of CD59 in graft dysfunction are better understood, this may be an attractive target for novel therapies to mitigate the risk of BOS post-LTx.


Review provided by:

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