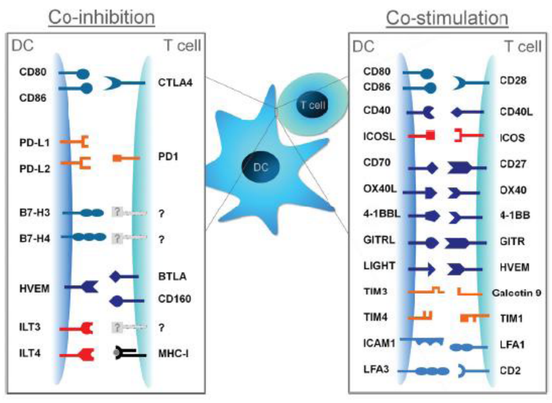
SOLUBLE CO-SIGNALING MOLECULES PREDICT LONG-TERM GRAFT OUTCOME IN KIDNEY-TRANSPLANTED PATIENTS

Beatriz Suárez-Álvarez [1], Susana G. Melendreras [2], Menéndez A[3], Pablo M. Camblor [4], Carvajal Palao R[3], Carmen Díaz-Corte [5,6], Marta Ruiz-Ortega [1], Carlos López-Larrea [3,6].

[1] Cellular Biology of Renal Diseases Laboratory, Instituto de Investigación Sanitaria Fundación Jiménez Díaz, Universidad Autónoma Madrid, Madrid, Spain. [2] Department of Nephrology, Hospital Son Llátzer, Palma de Mallorca, Spain. [3] Department of Immunology, Hospital Universitario Central de Asturias, Oviedo, Spain. [4] Unidad de Apoyo a la Investigación, CAIBER, OIB, Oviedo, Spain. [5] Department of Nephrology, Hospital Universitario Central de Asturias, Oviedo, Spain. [6] Fundación Renal Iñigo Álvarez de Toledo, Madrid, Spain.

BACKGROUND

Activation and differentiation of naive CD4 and CD8 T cells are key processes in the development of the immune response to an allograft. After engagement of the T-cell receptor (TCR) with its antigen on antigen-presenting cells (APCs), a second signal is necessary for full T-cell activation. This signal is sent by the co-stimulatory molecules, which are responsible for full T-cell activation. Following initial activation, co-inhibitory molecules are induced to counteract and avoid an exacerbated activation state. In this way, the set of co-stimulatory and co-inhibitory signals and their expression in time and space (cell type) determine the strength, nature and duration of the immune response during transplantation. But an aberrant expression can lead to the release of a soluble form which could modulate the immune response post-transplant. In fact, an increased presence of soluble co-signaling molecules is associated with persistent activation of T cells in several autoimmune diseases. In kidney transplanted patients, high levels of pre- and post- transplant sCD30 levels had been associated with a higher risk of acute rejection and graft loss.



Bakdash G et al. Frontiers in Immunology; 2013,4:53.

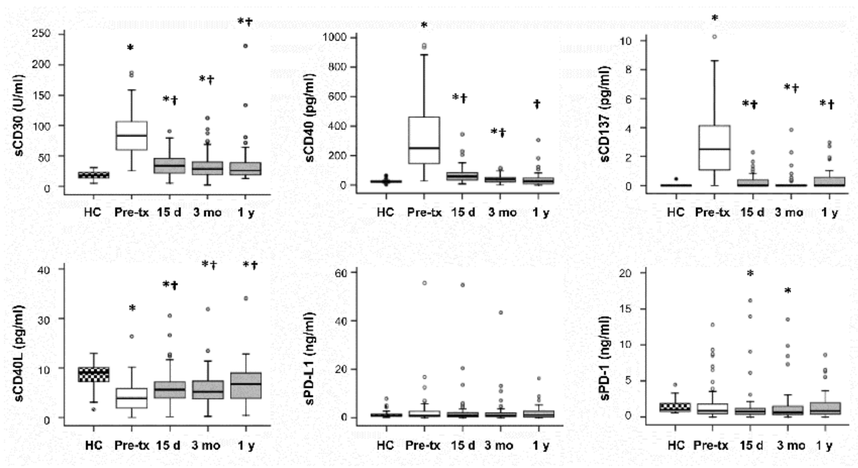
OBJECTIVES AND METHODS

We hypothesized that the presence of soluble co-signaling molecules in serum of kidney-transplanted patients might reveal the activation status of the immune system and be useful for predicting the risk of graft loss.

For this, we analyzed the presence of several soluble co-signaling molecules (**CD30, CD40, CD137, CD40L, PD-1, and PD-L1**) in serum from 25 healthy controls and 59 kidney transplanted patients obtained at different times (before transplant, 15 days, 3 months and 1 year post-transplant) by ELISA. Graft function for every patient was documented at each sampling time and annually therefore until the sixth year post-transplant. The contribution of these soluble molecules to the long-term graft outcome was evaluated using a multivariate principal component analysis.

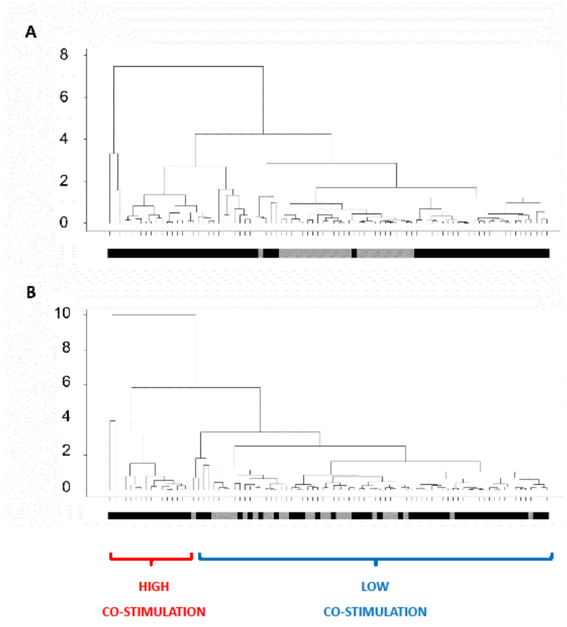
RESULTS

High levels of soluble co-signaling molecules related to cellular activation (sCD30, sCD40 and sCD137) were detected in kidney transplanted patients before transplant. These molecules were early modulated after receiving an allograft but never attained similar levels to those of healthy controls. A group of patients showed aberrant levels of these molecules, which could condition the immune response after transplantation.



Modulation of soluble co-signaling molecules in kidney-transplanted patients over time. The levels of the soluble co-signaling molecules CD30, CD40, CD137, CD40L, PD-1 and PD-L1 were assayed by ELISA in serum samples of healthy controls (n = 25) and kidney-transplanted patients (n = 59) obtained at different times: 15 days, 3 months and 1 year after transplantation. * statistical significant differences healthy controls versus kidney-transplanted patients samples, and † between patients samples obtained at different pre- and post-transplantation times.

Analysis of the combined effect of assayed soluble co-signaling molecules by PCA reveal that at three months post-transplantation, patients interspersed with the healthy control are characterized by low levels of co-stimulatory and co-inhibitory components (LOW CO-STIMULATION group, n=43, 27.1%), whilst patients grouped separately from controls are characterized by high levels of co-stimulatory and co-inhibitory components (HIGH CO-STIMULATION group, n=16, 72.9%).



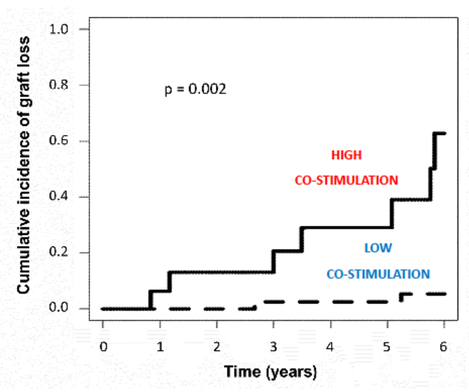
Clustering of patients by levels of co-stimulatory and co-inhibitory molecules. Unsupervised two-dimensional hierarchical clustering was used to group patients and healthy controls based on principal component analysis (PCA) before transplantation (A) and 3 months post-transplantation (B). This analysis reduced the dimensionality of data and identifies underlying variables that most efficiently explain the variation in the data.

We reduced the soluble molecule data to two principal components: the co-stimulatory (sCD30, sCD40, sCD137 and sCD40L) and the co-inhibitory (sPD-1 and sPD-L1). The dendrogram is derived from hierarchical clustering of all patients (n = 59) and healthy controls (n = 25) based on the principal components. Each line represents a single kidney-transplanted patient (black) or healthy control (gray).

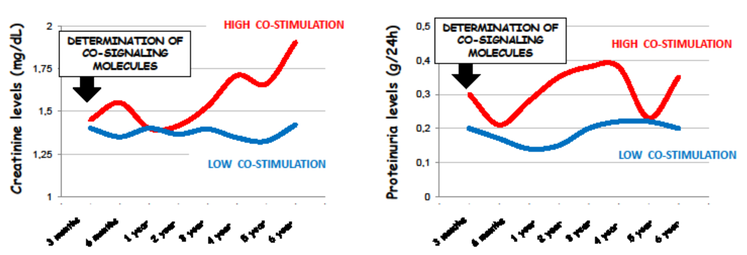
No statistical significant differences were observed in the creatinine (p = 0.58) or proteinuria (p = 0.07) levels between these two groups, suggesting that clustering of patients based on the determination of the soluble molecules at 3 months was independent of kidney function.

Patients with high levels of soluble co-stimulatory molecules (HIGH CO-STIMULATION) determined at 3 months post-transplantation have a greater risk of graft failure than those with low levels (LOW CO-STIMULATION) (HR 11.88, 95% CO 2.458-57.402, p = 0.002).

	HIGH COSTIMULATION n=16	LOW COSTIMULATION n=43	p
Donor age ≥ 60 y	8 (50)	13 (30.23)	0.22
Female Donor	8 (50)	21(48.8)	1
Recipient age ≥ 60 y	9 (56.25)	14 (32.55)	0.13
Female Recipient	3(18.75)	14 (32.55)	0.35
Previous transplant	3 (18.75)	5 (11.6)	0.67
Type of dialysis:			
Hemodialysis (HD)	11 (68.75)	13 (30.2)	0.03
Peritoneal dialysis (PD)	5 (31.25)	27 (62.8)	
Predialysis	0	3 (6.97)	
ESRD etiology:			
Vascular nephropathy	2 (12.5)	5 (11.62)	0.67
Diabetes mellitus	3 (18.75)	7 (16.27)	
Polycystic kidney disease	0	10 (23.25)	
Glomerulonephritis	5 (31.25)	10 (23.25)	
Pyelonephritis	3 (18.75)	5 (11.62)	
Unknown	1 (6.25)	3 (6.97)	
Others	2 (12.5)	3 (6.97)	
Cold ischemia time (h)	14.25 ± 3.52	12.48 ± 4.41	0.12
Delayed graft function	7 (43.75)	12 (37.9)	0.34
HLA mismatches ≥ 5-6	4 (25)	13 (30.23)	0.75
Post-transplantation DSA/no DSA at 3 mo	0/2 (12.5)	0/4 (9.3)	1
Acute rejection episode in first 3 mo	2 (12.5)	4 (9.3)	1
Immunosuppressive therapy			
Induction therapy	12 (75)	25 (58.1)	0.36
CsA + MMF	8 (61.5)	10 (27)	0.042
FK506 + MMF	5 (38.5)	27 (73)	
Serum Cr (mg/dL) at 3 mo	1.54 ± 0.5	1.46 ± 0.43	0.58
Total Pr (g/24 h) at 3 mo	0.44 ± 0.4	0.23 ± 0.17	0.07



Long-term graft survival based on the determination of soluble co-signaling molecules. Nelson-Aalen estimator was used to analyze the incidence of graft loss due to the existence of competing events.



Creatinine and Proteinuria levels in kidney transplanted patients over time. Clustering of patients according to the quantification of soluble costimulatory molecules at 3 months post-transplant allow the early identification of patients at high risk of poor graft outcome before than the kidney function will be detectable.

CONCLUSIONS

- **High levels of soluble co-signaling molecules in the sera of kidney-transplanted patients determined at 3 months post-transplantation were significantly associated with long-term graft outcome.**
- The quantification of these soluble molecules by a non-invasive method enables the immune status of transplanted patients to be characterized and will allow the early identification of patients at high risk of graft loss before the damage becomes irreversible.
- The use of soluble co-signaling molecules as biomarkers is a novel field in the transplant immunology, so far restricted to the CD30 molecule, which will open new routes in the development of transplant biomarkers.

REFERENCES

1. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat Rev Immunol. 2013; 13:227.
2. Kinnear G, Jones ND, Wood KJ. Costimulation blockade: current perspectives and implications for therapy. Transplantation. 2013; 95:527.
3. Wan B, Nie H, Liu A, Feng G, He D, Xu R, et al. Aberrant regulation of synovial T cell activation by soluble costimulatory molecules in rheumatoid arthritis. J Immunol. 2006; 177:8844.
4. Velásquez SY, García LF, Opelz G, Alvarez CM, Süsal C. Release of Soluble CD30 After Allogeneic Stimulation Is Mediated by Memory T Cells and Regulated by IFN-γ and IL-2. Transplantation. 2013; 96:154.
5. Süsal C, Döhler B, Sadeghi M, Salmela KT, Weimer R, Zeier M, et al. Posttransplant sCD30 as a predictor of kidney graft outcome. Transplantation. 2011; 91:1364.

51ERA-EDTA
607--MP
N) Renal Transplantation. N1) Transplantation basic science and immune-tolerance of allogenic and xenogenic transplants.
Beatriz Suarez-Alvarez
DOI: 10.3252/pso.eu.51era.2014
Poster presented at: 51 ERA-EDTA CONGRESS AMSTERDAM 2014
PosterSessionOnline.com