# THROMBOSPONDIN-1 IS ASSOCIATED WITH T AND B LYMPHOCYTE REGULATORY PHENOTYPES IN KIDNEY TRANSPLANTED PATIENTS TREATED BY mTOR-INHIBITORS

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## BACKGROUND AND AIM

Several studies showed that immunological tolerance in kidney transplantation (KT) is associated with increasing levels of circulating T (CD4+CD25+Foxp3+) and B (CD19+Tim-1+) regulatory cells (Tregs/Bregs)[1,2]. Immunosuppressive therapy may strongly influence T and B cell phenotype: in particular, mTOR inhibitors (mTORi) are known to increase Tregs in KT recipients[3]. Thrombospondin-1 (TSP-1) is a 480 KDa extracellular matrix glycoprotein known to exert anti-inflammatory properties[4] and to induce a Treg phenotype after CD47 binding[5]. The aim of this study was to investigate the role of TSP-1 as mediator of mTORi-associated induction of Treg/Breg phenotype in KT recipients.

## METHODS

We enrolled 60 KT patients with stable graft function: 20 in therapy with tacrolimus (TAC), 20 with sirolimus (SRL) and 20 with everolimus (EVE). We studied by FACS the percentage of circulating Tregs, Bregs, T memory (Tmem: CD4+CD45RO+) and B memory (Bmem: CD19+CD27+) cells. Plasma and urine ELISA for TSP-1 was also performed. *In vitro,* TSP-1 mRNA/protein expression was evaluated in T and B cells or in human kidney tubular epithelial cells (TEC) incubated with therapeutic doses of TAC, SRL or EVE. The role of TSP-1 was confirmed by using a blocking monoclonal antibody (mAb) directed to CD47 or by transfection of target cells with TSP-1 small interfering RNA (siRNA).

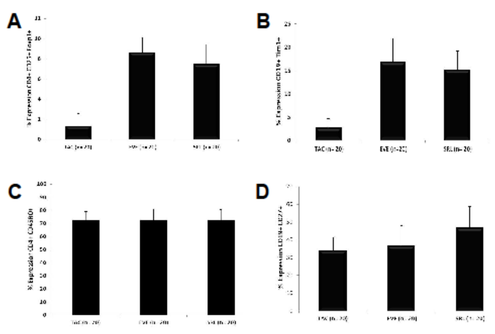


**Figure 1.** FACS Analysis on patients' blood samples: percentage of circulating Treg (A), Breg (B), Tmem (C), Bmem (D) cells.

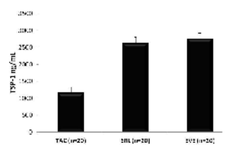

**Figure 2.** TSP-1 level measured by ELISA in patients' blood samples

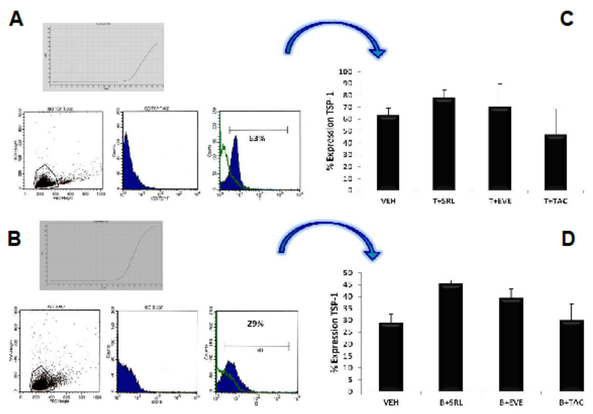


**Figure 3.** RT and FACS analysis of TSP-1 basal production by T cells (A) and B cells (B). FACS analysis of TSP-1 production by T cells (C) and B cells (D), after stimulation with SRL, EVE, TAC.

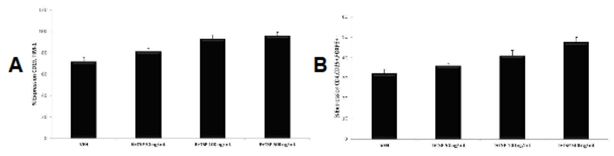


**Figure 4.** FACS analysis: Induction of a regulatory phenotype in T cells (A) and B cells (B) with increasing doses of human recombinant TSP-1.

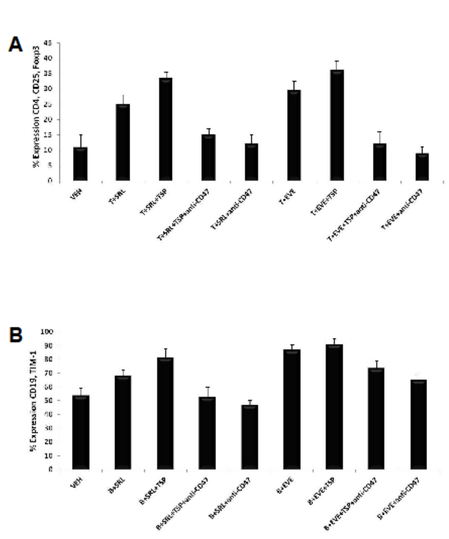


**Figure 5**. FACS analysis of regulatory phenotype in T cells (A) and B cells (B) stimulated by a CD47 blocking mAb.

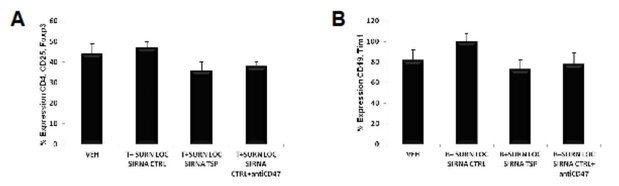


**Figure 6.** FACS analysis of regulatory phenotype in T cells (A) and B cells (B) incubated with supenatants released by TEC transfected by TSP-1 or control siRNA.

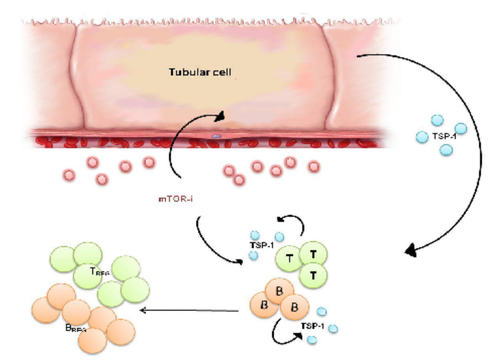


**Figure 7.** Role of TSP-1 as mediator of mTORi-associated Treg/Breg increase in KT patients.

## RESULTS

KT patients treated with both mTORi SRL or EVE showed increased levels of circulating Tregs and Bregs in comparison to TAC-treated patients ($p<0.05$). Of interest, higher levels of Tregs/Bregs were found in EVE patients (Fig.1A-B). By contrast, no differences in percentage of circulating Tmem and Bmem between mTORi and TAC groups were observed (Fig.1C-D). Higher levels of plasma (Fig. 2) and urine (not shown) TSP-1 were found in mTORi-treated patients in respect to TAC. TSP-1 levels correlated with percentage of circulating Tregs. *In vitro,* we found that SRL and EVE but not TAC induced an increased mRNA/protein expression of TSP-1 in T and B cells (Fig.3). In addition, increasing doses of TSP-1 induced a regulatory phenotype in both T and B cells (Fig. 4) without influencing Tmem and Bmem differentiation (not shown). TSP-1-mediated Treg/Breg induction was significantly decreased by using a CD47 blocking mAb (Fig.5). A similar effect was observed incubating T or B cells with supernatants released by TEC cultured with mTORi but not with TAC. Moreover, mTORi-induced Treg/Breg induction was significantly decreased using supernatants of TEC previously engineered to knock-down TSP-1 by siRNA (Fig.6).

## CONCLUSIONS

In conclusion, our results suggest that TSP-1 produced by lymphocytes in an autocrine manner or released by intragraft resident cells such as TEC may induce T and B cells to acquire a regulatory phenotype without affecting the percentage of memory cells (Fig.7). TSP-1 may represent a new biomarker as well as a mediator of mTORi-associated Treg/Breg increase and consequent immunological tolerance in KT.

## References

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