

Functional consequences of TGFB1 codon 10 polymorphisms in regulatory T cells and haematopoietic stem cell transplantation

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INTRODUCTION

We have previously shown that the presence of a polymorphism at codon 10, nucleotide 29 T>C, in the TGFB1 gene in patients has a significant impact on the outcome of unrelated donor allogeneic stem cell transplantation by increasing non relapse mortality and overall mortality in individuals with a CC genotype. The biological significance of this polymorphism is not well understood, however the interaction between TGF- β 1 and regulatory CD4+CD25+FOXP3+ T cells (Tregs) is well known.

MATERIALS AND METHODS

To investigate this further we analysed the activation phenotype of Tregs in terms of LAP-bound TGF- β 1 membrane expression, active TGF- β 1 secretion and the suppressive capability of these cells in correlation with TGFB1 codon 10 T/C genotype. We isolated Tregs and effector CD4 T cells by MiniMACS[®] technology (Miltenyi Biotec, Germany) from healthy donors bearing the 3 genotypes (2 C/C, 3 T/T and 3 T/C). Membrane LAP expression after polyclonal activation was measured at different time points by flow cytometry analysis. Similarly, we measured TGF- β 1 in the supernatant of the activated Tregs by Luminex[®] technology (Luminex Corp., USA). Simultaneously, in a subset of samples, we studied Treg autologous suppression activity by radioactive thymidine incorporation.

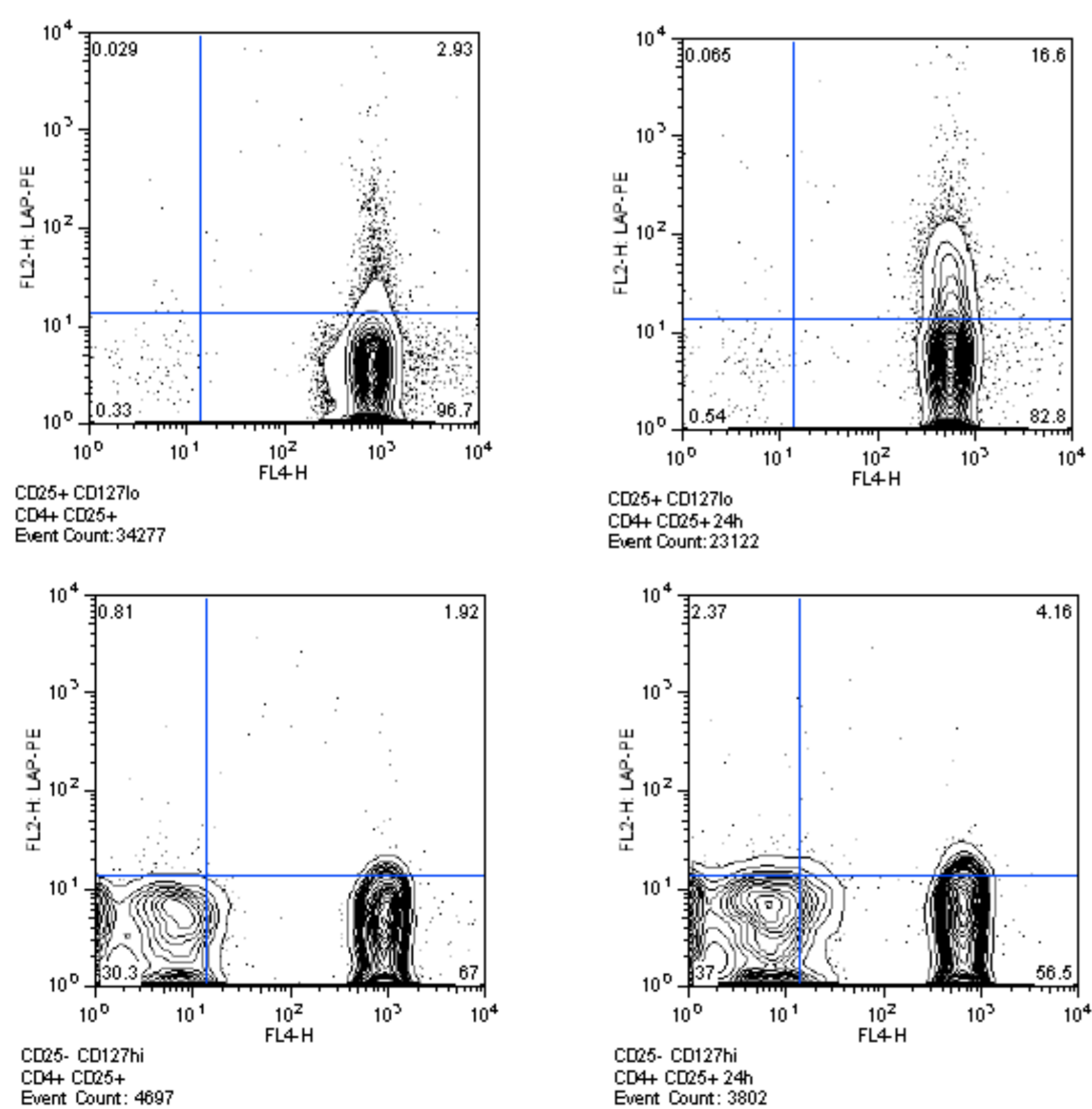


FIGURE 1. Induced LAP expression in polyclonally stimulated Tregs. (FL4-4: α CD4)

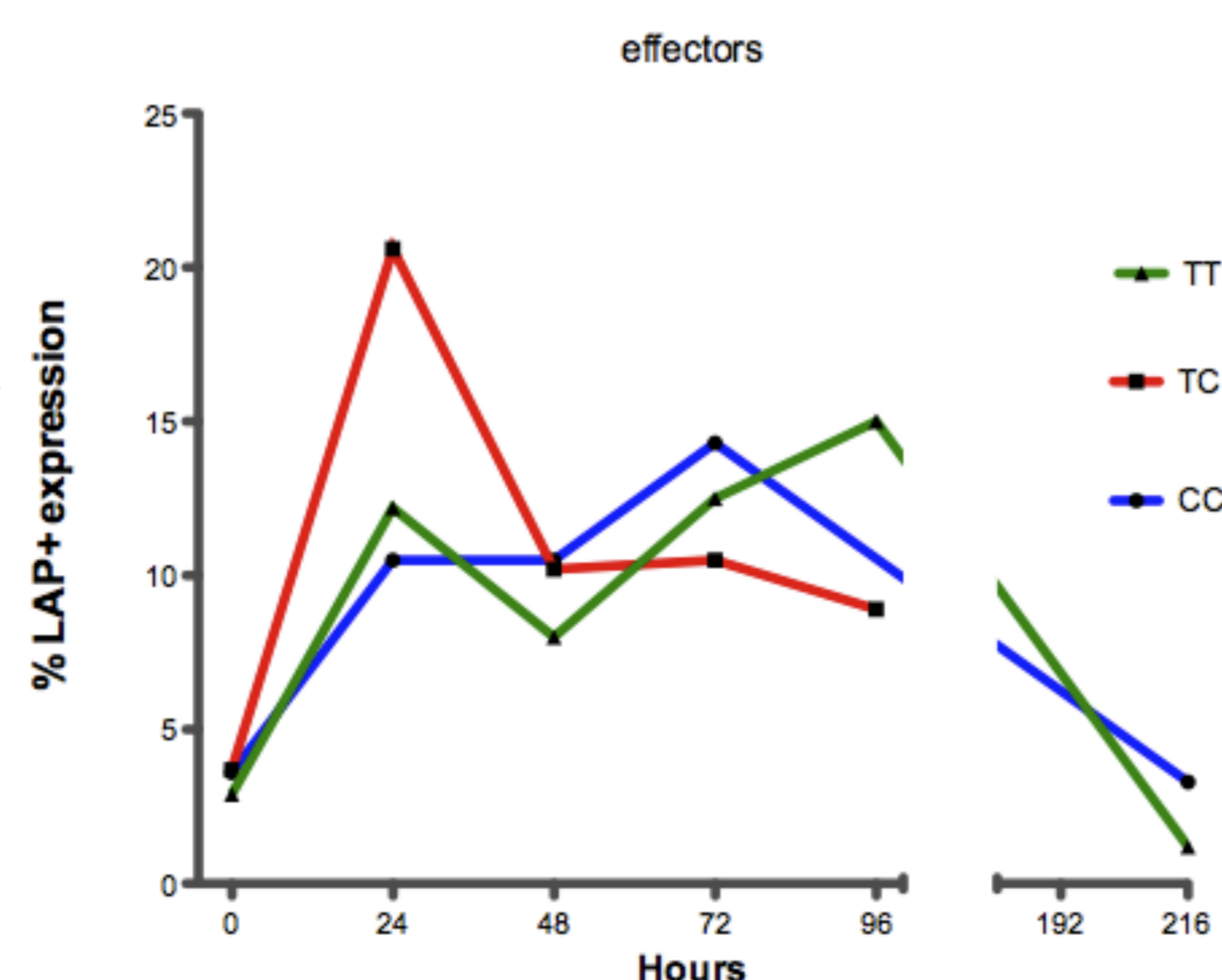


FIGURE 2. Proportion of LAP+ cells in polyclonally stimulated Tregs of different TGFB1 codon 10 genotypes.

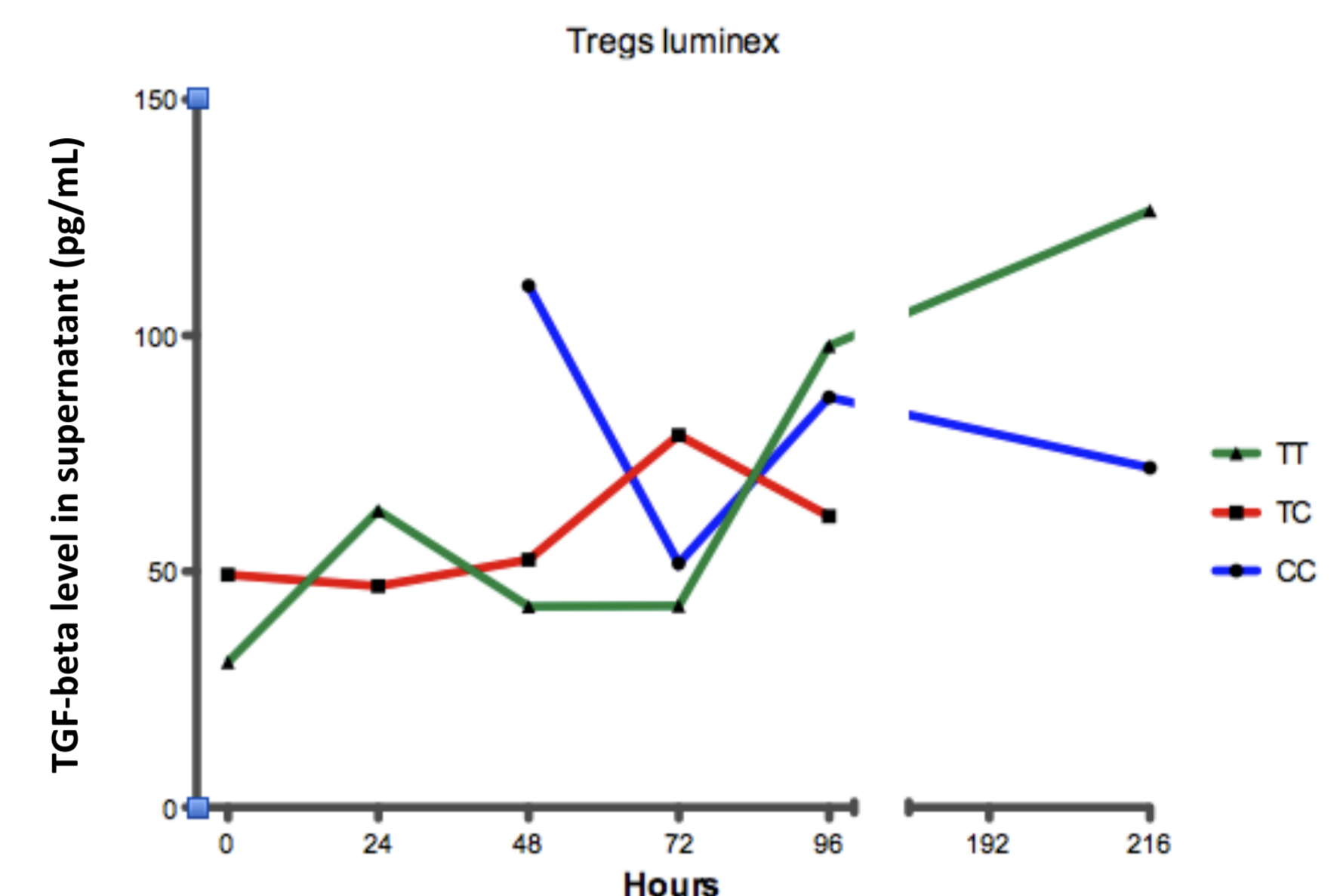


FIGURE 3. TGF-beta levels in supernatants of polyclonally stimulated Tregs of different TGFB1 codon 10 genotypes.

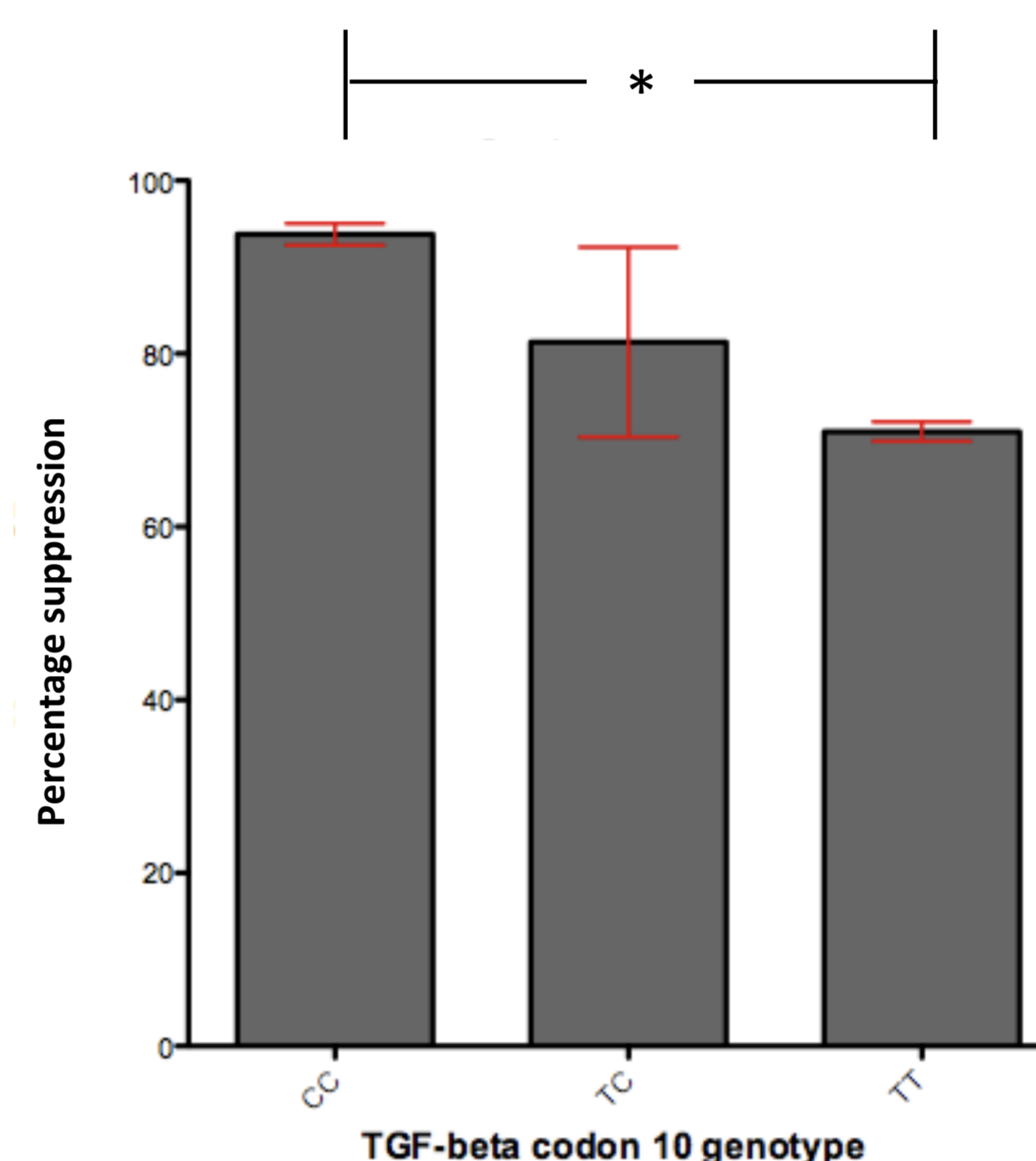


FIGURE 4. Suppressive capability of Tregs of different TGFB1 codon 10 genotypes. (*p=0.013)

RESULTS

We observed that LAP expression in CD4+/CD25+/FOXP3+ cells peaked between 24 and 48 hours (13-20%) and kept high levels up to 96 hours with similar kinetics between different genotypes (Figures 1-2). TGF- β 1 secretion had a later peak between 96 and 216 hours (Figure 3). Compellingly, we found that Tregs from C/C individuals showed significantly higher suppression capability (Figure 4) (95%) compared to T/T (70%) cells (p=0.013).

CONCLUSIONS

In conclusion, we found a higher suppressive capability of Tregs in samples with a TGFB1 codon 10 C/C genotype, which may explain our observations of a worse transplant outcome in these individuals. We hypothesise that higher suppression could impair patients' response to infection. We are currently conducting additional cellular and Q-PCR experiments in order to further clarify the effect of this polymorphism on TGF- β 1. Moreover, we are also analysing the *in vivo* expression of LAP by Tregs in patients after an allogeneic-HSCT.

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