A novel isoform of human Glucocorticoid-induced TNFR-related (GITR) protein that modulate activation and proliferation in effectors and regulatory T-cells

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Glucocorticoid-induced TNFR-related (GITR, also know as TNFRSF18) protein is a gene coding for a member of the TNF receptor superfamily. GITR activation influences the activity of effector and regulatory T cells (Treg), thus participating in the modulation of immune response against tumours and infectious agents, as well as in autoimmune and inflammatory diseases[1,2]. In mouse, four GITR splice variants have been identified[3] (GITR, GITR-B, GITR-C and GITR-D), which are characterized by different downstream pathways thus acting in different manner on T-cells differentiation and proliferation. GITR is activated by its ligand (GITR-L)[4] thus resulting in a co-stimulatory signal in effector T-cells[5], both in mouse and humans. Recently *Secreted and transmembrane* (SECTM) 1A protein was identified as a novel GITR ligand in mouse[6]. In humans, the GITR system exerts diverse effect depending on the type of T-cells sub-populations; signaling of human GITR (hGITR) may be different from that of murine GITR (mGITR) due to its structural differences[7,8].The aim of our study was to verify if novel hGITR isoforms exist and modulate the activation and differentiation of human effector T-cells and Treg.We found some hGITR splice variants, one of which, hGITR-4, maintains the fourth intron of GITR gene. This isoform entails a shift in the frame of the cytoplasmic region thus leading to a different translation from the main hGITR splice variant. GITR-4 is the ortholog of murine GITR-C and it appears conserved at protein level. Using qPCR we demonstrated that GITR-4 is expressed at a lower level in effector T-cells than in Tregs of healty donors and after their activation (by aCD3/28 beads or PMA/ionomicine treatment) increases quickly at the mRNA level; moreover, the ratio hGITR/GITR-4 is equal to one in Treg cells isolated from SLE patients and much lower in healthy donors cells.We plan to study the role of GITR-4 in effector T-cells and Treg. To this aim we demonstrated by duolink experiments that SECTM1 is able to bind to the extracellular domain of hGITR; we also demostrated, by qPCR, that SECTM1 is more expressed than GITR-L in T-lymphocytes at basal levels and under activation stimuli. Interestingly the binding of SECTM1 by an anti-SECTM1 antibody inhibits the effector cells and Treg proliferation while the binding of GITR-L by an anti-GITR-L antibody inhibits only effector T-cells proliferation. The possibility that SECTM1 binds hGITR and hGITR-4 at different level is still under investigation.In conclusion we identified a novel hGITR isoform that is involved in T-Cell differentiation and proliferation.

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