Introduction

Haematopoietic stem cell transplantation (HSCT) is used to treat malignant and non-malignant diseases. Apart from clinical and Human Leukocyte Antigen (HLA)-related factors, non-HLA immunogenetics is being increasingly recognized to play a role in the outcome of HSCT. A gene that may be relevant for the outcome of HSCT is TGFB1, which encodes Transforming Growth Factor β 1 (TGF-β1), a cytokine that is central in the regulation of numerous immune processes. Several polymorphisms in TGFB1 have been identified and some of them are known to cause alterations in cytokine secretion. We and others have previously shown that polymorphisms within TGFB1 affect the outcome of HSCT. Consequently, the aim of this project is to study the effect of TGFB1 polymorphisms on the function of Treg and their role in HSCT complications such as graft-versus-host disease.

Materials and Methods

Peripheral blood from healthy volunteer donors was obtained by venipuncture. DNA was extracted by an in-house salting-out method. Molecular typing techniques were used to type TGFB1 +29 T>C polymorphism. Volunteer donors were grouped in three genotype categories for further experiments. Peripheral blood mononuclear cells (PBMC) were isolated by ficoll gradient, and Treg (CD4+CD25+CD127low) and effector (CD4+CD25-CD127high) cells were isolated with a microbead-based method (Figure 1).

Isolated Treg and effector cells were stimulated with antiCD3/CD28 antibodies and cultured in vitro in 96-well bottom well plates. Latecy-associated-peptide (LAP, membrane-bound TGF-β1) expression was measured on resting and stimulated cells by flow-cytometric analysis at specific time-points.

Results

Molecular typing techniques for the detection of TGFB1 +29T>C SNP have been employed to type 31 healthy blood donors for functional experiments. Figure 2 shows representative results for the 3 TGFB1 +29 T>C genotypes as identified by sequencing.

Membrane-bound TGF-β1 (LAP positivity) upon TCR stimulation of isolated Treg has been confirmed to be specific to this cell subset (Figure 3). LAP levels have been assessed in 5 donors per TGFB1 +29T>C genotype. The in vitro kinetics of TGF-β1 induction on these cells after TCR stimulation with soluble antibodies against CD3 and CD28 was shown to peak at 24h of incubation and to reach an average of 25.7% of the CD4+CD25+CD127hi cells (range 11.0-51.2%), following by a reduction to low levels by 48h, which remain constant up to 96h (Figure 4). TCR stimulation with plate-bound antibodies produces maximal and sustained LAP expression on Treg (Figure 5). A trend towards a higher percentage of LAP+ Treg generated after soluble activation when the cells bear a +29C allele (Pro10 on its signal peptide) was identified (p=0.066) (Figure 6).

Conclusions

TGFB1 +29 T>C genotype seems to influence the amount of membrane-bound TGFB-β1 expressed exclusively by in vitro TCR-stimulated Treg. The presence of a C allele confers a trend towards higher production of LAP by these cells in a dominant model. Differences in the levels of LAP on Treg may affect the function of these cells in an inflammatory context such as that present in HSCT patients. Consequently, this possibility must be explored and further experiments are being developed in order to address these questions.

Future work

We are currently undertaking RT-Q-PCR experiments in order to characterise the effect of TGFB1 +29 T>C polymorphism on the expression of this cytokine by Treg and effector CD4+ T cells at the RNA level. Additionally, we are developing optimised autologous suppression assays in order to evaluate the functional effect of TGFB1 polymorphisms on immune modulation by Treg.

In the future, we will extend our analysis to other polymorphisms within the regulatory region of TGFB1, and perform haplotype analysis on the joint effect of these polymorphisms on the outcome of HSCT and on the function of Treg.

These data will hopefully help to identify the risk factors associated with TGFB1 expression in patients and donors and to generate clinically useful algorithms of genetic risk assessment in HSCT.

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