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NFκB activation and TGFβ loss signatures are prominent *ex vivo* responses of peripheral blood mononuclear cells

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Background

Homeostasis and activation of immune cells is tidily regulated. A well organized response of different players of the immune system is evoked by disturbance of the steady state homeostasis as seen in e.g. case of inflammation or cancer. One of the major methodologies to study the interactions in the immune system specially in humans is the *ex vivo* analysis of monocytoid cells. This *ex vivo* analysis of human immune cells however might be hampered by the fact that these cells are particularly flexible in reacting to their environment. E.g. specialized cells such as macrophages or DC constantly monitor their environment via so-called pattern recognition receptors. Furthermore several factors present *in vivo* are removed in the *ex vivo* system. For peripheral blood mononuclear cells, some aspects of the response to the *ex vivo* culture conditions have been established. Monocyte adherence was regarded as an activating event resulting in changes of gene expression and protein secretion and mainly increase in IL8 expression was shown to result from adherence of PBMCs to plastic. We and others have recently demonstrated that blood asservation and cell isolation techniques have to be closely controlled when monitoring cellular responses on a global level. Here we asked the question whether there is a global cellular response to the *ex vivo* system itself. Gene expression profiling allows a large scale measurement of changes of gene expression and thereby an unbiased search of cellular changes.

Method

Whole genome expression profiling (HG-U133A, Affymetrix) of 79 samples from PBMCs and $CD4^+$ T-cells maintained for different time courses *in vitro* without further manipulation were performed. Differentially expressed genes where further evaluated using protein based assays.

Results

In vitro culturing did not change the quality of gene expression data. But time dependent changes of gene expression outweighted by far interindividual differences as demonstrated by unsupervised clustering methods. In total 827 genes showed significant changes of expression within the first 24 h. During early time points a strong anti-apoptotic signature was observed. Later the transcription of numerous cytokines and chemokines was significantly upregulated while the expression of the respective receptors was diminished. Analysis of potential upstream elements responsible for the observed changes revealed that a third of the known NFκB target genes showed strong and time dependent regulation by simple *in vitro* culturing. Moreover, a prominent feature observed in *ex vivo* cultured $CD4^+$ T-cells was the early upregulation of several TGFβ antagonists and repression of TGFβ target genes. In summary a strong activation state of cells just maintained in culture was observed.

Conclusion

A rather strong and significant molecular response to the *in vitro* environment itself was detected in PBMCs and CD4+ T cells. Among others activation of NFκB target genes and withdrawal of TGFβ leads to an activation state of cultured cells. These data have significant impact on our interpretation of molecular and functional *in vitro* studies of human immune cells but might also be used as a molecular platform to optimize cellular culture systems for the *ex vivo* production of immunotherapeutically used cells.