Network: **Combating Cancer through Integrated Functional Genomic Research**

Project: Identification and Characterization of T cell Subsets with Regulatory Function in Cancer

Joachim L. Schultze - University of Cologne, University Hospital - joachim.schultze@medizin.uni-koeln.de

Introduction

In tumor patients an imbalance between inhibitory and stimulatory factors exists that favors the formation of malignant cells. Soluble factors and inhibitory cytokines, e.g. TGF-β and IL-10, have been linked to the altered immune response in tumor patients, while their cellular counterparts have not yet been described. Regulatory T cells (T_{reg} cells) are a newly emerging T cell population that is crucial in the modulation of the immune response. T_{reg} cells have been shown to be important for the balance of tolerance and auto-immunity. In autoimmunity a decrease of T_{reg} cells correlates with the severity of the disease while an increase is associated with milder symptoms. Similarly, the occurrence of chronic Graft-versus-Host disease after allogeneic stem-cell transplantation is closely related with the frequency of T_{reg} cells.

In cancer patients there is growing evidence that the number and function of these cells is altered and thereby negatively regulates the immune system in its effort to attack the tumor. For solid tumors an increase of T_{reg} cells has been described and could be correlated to a decrease in survival. However, in hematological malignancies the frequency and function of T_{reg} cells has not been fully characterized.

Although the importance of regulatory T cells is well established their phenotypical definition is difficult. The $CD4^+$ $CD25^{high}$ T cell subset harbors a significant group of T_{reg} cells but CD25 is also present on activated T cells and not a truly unique marker for this T-cell subpopulation. We have therefore initiated a program to molecularly define T_{reg} cells in cancer patients to facilitate the characterization of T_{reg} cells in future studies.

Recently, the induction of T_{reg} cells has been linked to tolerogenic dendritic cells (DC). So far, DC have been mainly described as the most potent human antigen-presenting cells trafficking through the periphery and responsible for the induction of immunity. However, the induction of indoleamine 2,3-dioxygenase (IDO) in DC leads to decreased tryptophan in their proximity and modulates their activation status. Under these terms DC induce T cell inhibition rather than activation. Prostaglandine E_2 (PGE_2), secreted by a large number of tumors, has been known as a suppressor of T cell activation. New reports show a direct association with the induction of T_{reg} cells as well the modulation of immune responses.

Results

Regulatory T cells

Assessing B cell chronic lymphocytic leukaemia (CLL) as a model for hematological malignancies we demonstrated significantly increased frequencies of $CTLA4^+$ $FOXP3^+$ $GITR^+$ $CD62L^+$ $TGF\text{-}\beta1^+$ $IL\text{-}10^+$ T_{reg} cells in patients with CLL correlating with the stage of the disease. Normal regulatory function of T_{reg} cells in CLL patients never treated with fludarabine was observed while in the majority of CLL patients treated with fludarabine the inhibitory function of T_{reg} cells was decreased or even abrogated. In addition, frequencies of T_{reg} cells were significantly decreased in these patients. First *in vitro* experiments demonstrated a preferential induction of apoptosis in $CD4^+$ $CD25^+$ T cells after incubation with fludarabine. We have extended these findings to patients with multiple myeloma (MM) and were able to show an increased number of fully functional T_{reg} cells in these patients and are currently initiating studies in patients with solid tumors.

Interestingly, we could describe for the first time an increased frequency of human naïve T_{reg} cells in adult patients with MM as well as CLL, further underlining the phenotypical and functional differences between healthy individuals and tumor patients.

Since a truly unique marker for T_{reg} cells is still elusive, we chose genome wide expression analysis of $CD4^+$ $CD25^{high}$ T_{reg} cells and $CD4^+$ $CD25^-$ T cells to identify novel T_{reg} cell associated genes. $CD4^+$ $CD25^{high}$ T cells and $CD4^+$ $CD25^-$ T cells from several CLL patients and healthy individuals were isolated by FACS sorting and used to perform genome wide expression analysis using the whole genome Illumina Sentrix® Human-6 Expression BeadChip.

Even when applying very stringent filtering criteria we identified genes that were significantly different between $CD4^+$ $CD25^-$ and $CD4^+$ $CD25^{high}$ T cells. As an important control, we found genes related to T_{reg} cells to be significantly increased in the $CD4^+$ $CD25^{high}$ T cell population. These included FOXP3, which has been described as a lineage marker for T_{reg} cells in mice and in humans, as well as GITR and CTLA4, which have also been closely related to T_{reg} cell function.

Cluster analysis revealed that the $CD4^+$ $CD25^-$ and $CD4^+$ $CD25^{high}$ T cells from healthy individuals cluster separately and that $CD4^+$ $CD25^{high}$ T cells from healthy individuals and CLL patients show differentially expressed genes (Fig 1).

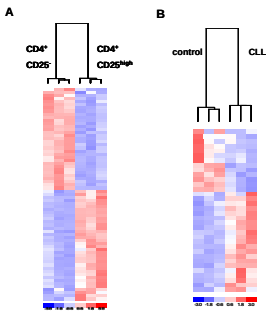

Fig 1: *(A) Hierarchical cluster analysis of (A) $CD4^+$ $CD25^-$ and $CD4^+$ $CD25^{high}$ T cells from healthy individuals (B) $CD4^+$ $CD25^{high}$ T cells from healthy individuals and CLL patients.*

Using pathway analysis groups of genes have been identified that behave similarly to FOXP3, CTLA4, and GITR (Fig 2 and 3).

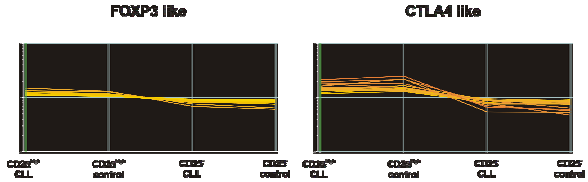

Fig 2: *Expression patterns of FOXP3 and CTLA4 associated genes in $CD4^+$ $CD25^-$ and $CD4^+$ $CD25^{high}$ T cells from healthy individuals and CLL patients.*

NGFN Nationales Genomforschungsnetz

GEFÖRDERT VOM Bundesministerium für Bildung und Forschung

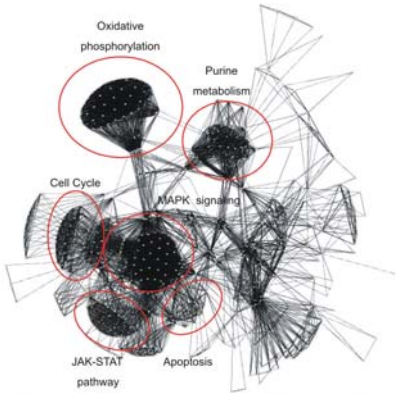

Fig 3: *Pathway analysis with BioRag of differentially expressed genes in $CD4^+$ $CD25^{high}$ T cells.*

These genes are currently validated as potential candidate genes for a specific T_{reg} cell marker.

Tolerogenic DC can induce regulatory T cells

PGE_2 has recently been linked with the induction of regulatory T cells. By using genomic approaches we have been able to uncover some of the mechanisms that are involved in PGE2 mediated induction of regulatory T cells.
By genome wide gene expression profiling we show that PGE_2 mediates induction of indoleamine-2,3 deoxygenase (IDO) in DC and leads to an upregulation and concomitant secretion of CD25. For both factors alone, an inhibition of normal immune responses could be shown and their combination further enhances these effects. In contrast to normally matured DC supernatants from DC matured in the presence of PGE_2 show decreased stimulatory capacity of $CD4^+$ T cells.

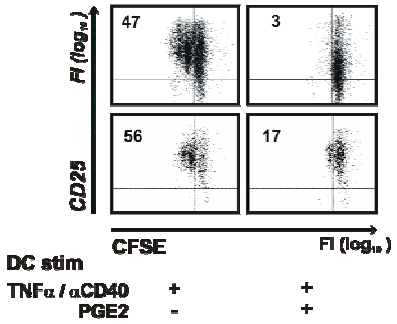

Fig 4: *Inhibition of T cell proliferation through addition of supernatant from PGE_2 treated DC. T cells were stained with a membrane dye and cells dividing are loosing brightness of fluorescence (e.g. upper left)*

This effect is dependent on both factors as blocking of either mechanism only leads to a partial reconstitution of the T cell stimulation.

PGE_2 mediated direct effects on T cells

By genome-wide transcription profiling we also attempted to elucidate direct inhibitory mechanisms of PGE_2 on T cells. T cel cells were stimulated by signaling through the T cell receptor (TCR) and CD28.
Incubation with PGE_2 completely abolished T cell proliferation. This inhibition was induced by an interference of PGE_2 with an early step of TCR signaling as demonstrated by an inactivation of important molecules for TCR mediated proliferation, e.g. lck and ZAP70 (Fig 5).

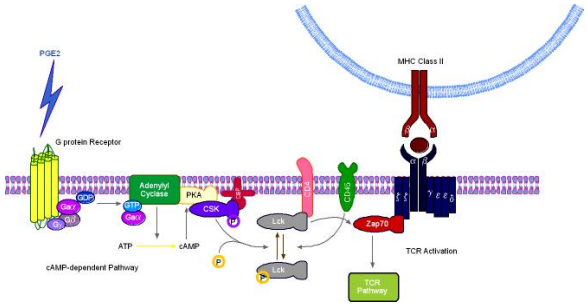

Fig 5: *Model proposed for integration of PGE_2 derived signals into the TCR signalling cascade.*

Among the genes, which escaped PGE_2 induced inhibition, we identified a number of anti-apoptotic genes. Interestingly, upregulation of these genes was associated with a partial protection of PGE_2-stimulated T cells from apoptosis.
Hodgkin's lymphoma is characterized by an enormous accumulation of immune cells at tumor sites. However, these cells lack the capacity to mount an efficient anti-tumor immune response. PGE_2 is one of the main factors released by the infiltrating macrophages.
Gene expression profiling of tumor-infiltrating T cells from patients with Hodgkin's lymphoma revealed a similar expression pattern to PGE_2 treated T cells. Taken together these two mechanisms might explain the absence of an anti-tumor response in these patients while an accumulation of senescent inefficient T cells in the tumor microenvironment occurs.

Outlook

As human tumors seam to be associated with an overall increase of T_{reg} cells, the exact characterization of the underlying mechanism as well as the exact cause of this increase are warranted. Identifying new molecular targets on T_{reg} cells will not only help us to further portray these cells and understand their importance in human malignancies but also guide us to new targets for therapeutic interventions by either reducing T_{reg} cell numbers or their functions.
PGE_2 skews DC to a more tolerogenic phenotype, leads to the induction of impaired T cells and has inhibiting effects on T cell activation and proliferation. Furthermore, it can lead to the induction of T cells with a regulatory phenotype either via tolerogenic DC or by converting conventional T cells to T_{reg} cells. We will need to study the effects of PGE_2 treated DC on T cells to determine in which state of differentiation the T cells are stopped. Further on, it will be important to determine, how we can overcome this immune inhibition, as it might place a central role within tumor-host interactions. We strongly believe that it will be critical to understand and overcome these barriers to develop clinically efficient cancer immunotherapies such as cancer vaccines.

Lit.: ***1.*** *Beyer M et al. Reduced frequencies and suppressive function of $CD4^+$ $CD25^{high}$ regulatory T cells in patients with chronic lymphocytic leukemia after therapy with fludarabine. Blood. 2005 Sep 15;106(6).* ***2.*** *von Bergwelt-Baildon MS et al. Concomitant induction of indoleamine 2,3-dioxygenase and soluble CD25 by prostaglandin E2 in human dendritic cells inhibits T cell responses. Submitted.* ***3.*** *Chemnitz JM et al. Prostaglandin E_2 impairs $CD4^+$ T cell activation by inhibition of lck – implications in Hodgkin´s lymphoma. Submitted.*