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Plastizität von in-vitro induzierten menschlichen immunregulatorischen T-Zell-Phänotypen
(Plasticity of in-vitro induced human immunoregulatory T cell phenotypes)

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Human immunoregulatory T cells, which are reported to have immunosuppressive function in-vivo and in-vitro, are a heterogeneous T cell subset. They are indispensable for the maintenance of dominant self tolerance and immune homeostasis as well as the down-regulation of immune responses, both in organ transplantation and autoimmune disease. Treg are classified into 2 subsets: natural regulatory T cells (nTreg) and induced regulatory T cells (iTreg). Treg are also classified as $CD4^+$ Treg and $CD8^+$ Treg. $CD8^+$ Treg are often referred to as $CD8^+$ Ts. Both T cell subsets appear to be involved in the induction of allograft tolerance. It is supposed that human nTreg primarily emerge from the thymus whereas iTreg are generated in the peripheral blood from naïve T cells via stimulation by allo- or auto-antigen. Although each of the main Treg subsets has been shown to exert immunosuppressive activity, iTreg are believed to be more important in transplant recipients than nTreg.
Hitherto, there is no consensus about the phenotype and induction of human induced Treg in-vitro. We studied plasticity and overlap of human in-vitro induced immunoregulatory cell phenotypes investigating the most accepted biomarkers of human $CD4^+$ iTreg, which are $CD25^{high,}$ $FoxP3^+$ and $CD127^-$. Our data suggest that $CD4^+CD25^{high}FoxP3^+$ and $CD4^+CD25^{high}CD127^-$ phenotypes should not be used exchangeably. Both phenotypes define different, in part overlapping iTreg subsets.
Furthermore, we observed the expression of the co-stimulatory factors CD28/CTLA-4 during polyclonal and allogeneic stimulation. $CD28^-$ and $CD152^+$ provide negative signals for T cells. We found both co-expression and lack of expression of these markers on iTreg, confirming the plasticity of iTreg phenotypes.

$CD28^-$ is also regarded as the main biomarker (and mechanism) on human $CD3^+CD8^+$ Ts. We compared the phenotype of human induced $CD3^+CD4^+CD25^+FoxP3^+$ Treg and $CD3^+CD8^+CD28^-$ Ts, including co-expression of IFN-γ, IL-2, IL-10, and TGF-β, which are believed to be associated with the immunosuppressive function of Treg and Ts. Cells were either stimulated with PMA/Ionomycin or PHA. The data indicate that PMA/Ionomycin and PHA use different stimulation pathways and that both reagents induce different Treg and Ts subsets.

In addition to co-stimulatory factors and cytokines, other molecules are also reported to be related with $CD4^+$ iTreg suppressive mechanisms. Our results suggest that expression of IFN-γ^+, IL-2^-, IL-10^+, TGF-β^+, HLA-DR^+, $CD28^-$, CTLA-4^+, $CD178^+$, $CD279^+$, Granzyme A^+, Granzyme B^+, or Perforin$^+$ are up-regulated on/in both $CD4^+CD25^+FoxP3^+$ and $CD4^+CD25^+CD127^-$ iTreg during polyclonal or allogeneic stimulation. However, there are also $CD4^+CD25^+FoxP3^+$ and $CD4^+CD25^+CD127^-$ iTreg formed during polyclonal stimulation that lack these markers.

Since IFN-γ-producing iTreg have been proposed to play an important role in inducing allograft acceptance, the function of IFN-γ as well as TGF-β, IL-2, and IL-10 in polyclonal and allogeneic stimulation attracted our attention. In MLC experiments, T cell proliferation was reduced in the presence of anti-IFN-γ or anti-TGF-β monoclonal antibody whereas recombinant IFN-γ and TGF-β did not affect responder cell proliferation. In polyclonally stimulated cell cultures, induction of $CD4^+CD25^+FoxP3^+IFN\text{-}\gamma^+$ iTreg was blocked by anti-IFN-γ monoclonal antibody and induced by recombinant IFN-γ but not by anti-TGF-β monoclonal antibody or recombinant TGF-β, IL-2, or IL-10. The data indicate that induction of IFN-γ^+ $CD4^+CD25^+FoxP3^+$ iTreg is IFN-γ-dependent. We believe that $CD4^+CD25^+FoxP3^+IFN\text{-}\gamma^+$ iTreg represent the first line of induced Treg during a beginning immune response. In further MLC experiments, separated IFN-γ^+ iTreg suppressed the proliferation of responder cells. However, the immunosuppressive function of IFN-γ^+ iTreg was not alloantigen specific.

Our findings might have clinical implications. Further analysis of the stimuli and pathways might identify an appropriate agent that might open up new perspectives for human induced immunoregulatory T cells, especially the induction/propagation of IFN-γ-secreting $CD4^+$ iTreg subsets (a) preoperatively in-vivo before organ transplantation, (b) postoperatively after organ transplantation, or (c) in-vitro for subsequent clinical treatment protocols.