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## **Differential responsiveness of human lamina propria and peripheral blood T lymphocytes to CD28 stimulation**

Geboren am 21.8.1977 in Kstovo /Russland  
Examen 22.6.2002 an der Moskow Medical Sechenov's Academy

Promotionsfach: Immunologie  
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This study shows that lamina propria T lymphocytes (LPT) are hyperresponsive to CD28 stimulation in comparison to peripheral blood T lymphocytes (PBT):

1. Compared to PBT, stimulation using a CD28 IgM (clone 428) monoclonal antibody induces vigorous proliferation of LPT. LPT produce high quantities of cytokines (IL-2, TNF- $\alpha$ , TNF- $\beta$ , IFN- $\gamma$ , and GM-CSF) after CD28 triggering, but not PBT. Activation by the superagonistic CD28 IgG mAb (clone 5.11A1) also induced higher cytokine gene expression in LPT than in PBT- although at a low level. The spectrum of secreted cytokines may contribute to the TH1 immune response of LPT and characterizes the development of cell-mediated immunity in the human intestine.
2. Ligation of the CD28 receptor by the CD28 IgM antibody results in activation of the PI3K/AKT pathway. Activated AKT kinase phosphorylates and inhibits GSK-3 $\beta$ . Phosphorylation of AKT and GSK-3 $\beta$  in LPT is significantly higher compared with PBT. LY294002 – a specific inhibitor of PI3- kinase – diminishes the CD28-induced phosphorylation of AKT and GSK-3 $\beta$  in both cellular populations. Besides, the treatment of LPT with LY294002 significantly reduces the CD28-induced proliferative response and cytokine production. Accordingly, the effector functions of LPT depend upon the activation of PI3 kinase by the CD28 triggering.
3. Prolonged stimulation of CD28 promotes expression of CTLA-4 on the surface of LPT compared with PBT, thus revealing a negative feedback mechanism in the regulation of TH1 immune response. Therefore, the mucosal immune system of the human intestine can via this mechanism prevent the inflammation in the gut wall.

In a recent phase I clinical trial application of the superagonistic anti-CD28 mAb TGN1412 induced the rapid onset of a life-threatening “cytokine storm” in all healthy volunteers participating in the study (Suntharalingam et al. 1996). Gastrointestinal symptoms like vomiting, bowel urgency, and diarrhea were among the earliest health problems experienced by the test persons. Our results suggest that given the hyperresponsiveness of LPT to stimulation by CD28 antibodies (CD28 IgM / clone 428 and superagonistic CD28 IgG / clone 5.11A1) activation of these cells may have contributed to the induction of a “cytokine storm” by TGN1412.