

Effective Polarization of Human Th17 Cells with Biologically Relevant HumanKine[®] TGFβ1, TGFβ2, and TGFβ3 Expressed in Human Cells

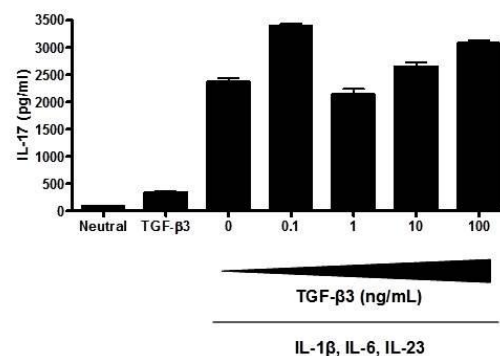
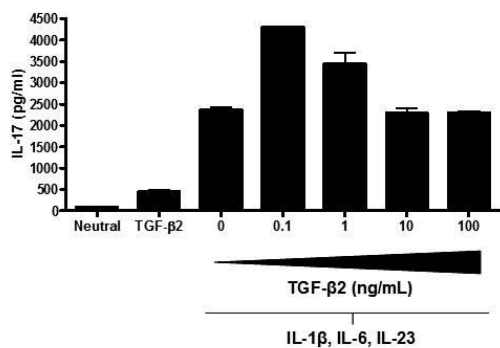
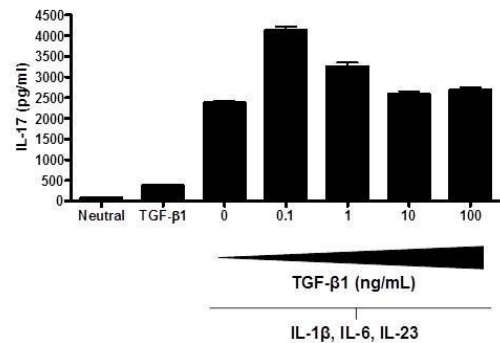
INTRODUCTION

Transforming growth factors beta (TGFβ) are highly pleiotropic cytokines that act as cellular switches and regulate immune function, proliferation and epithelial-mesenchymal transition. These proteins are produced as precursors then a furin-like convertase processes the proprotein to generate an N-terminal latency-associated peptide (LAP) and a C-terminal mature TGFβ. Disulfide-linked homodimers of LAP and TGFβ remain non-covalently associated after secretion, forming the small latent TGFβ complex. Covalent linkage of LAP to latent TGFβ binding proteins creates a large latent complex that may interact with the extracellular matrix. Commercially available TGFβ proteins are produced as a recombinant protein expressed in CHO cells or as purified native protein from human platelets. Due to complex post-proteolytic modifications, TGFβ yield is low and the products are not available in economic bulk quantity. HumanZyme has developed an efficient human-cell based technology, HumaXpress[®], for scalable production of human cytokines and produces TGFβ1, β2, and β3 from engineered human 293 cells. The proteins are highly purified disulfide-linked dimers of 25kD that can be cost-effectively produced in large scale.

Th17 POLARIZATION

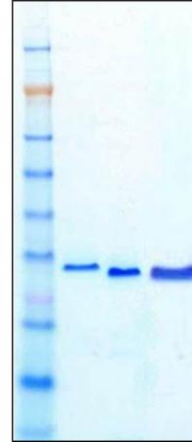
TGFβs, which are important for the polarization of murine Th17 cells, are reported not required, and are even inhibitory, for human Th17 polarization. In this study, whole CD4⁺ cells isolated from a healthy donor were stimulated with 10 μg/ml plate bound anti-CD3 and 10 μg/ml soluble anti-CD28 in the presence of Th17 polarizing cytokines from HumanZyme and another commercial vendor. After 5 days, supernatants were harvested for measurement of IL-17 by ELISA. The results show that all the HumanKine TGFβs are effective at inducing IL-17 secretion with an optimal concentration of 0.1 ng/ml TGFβ.

In contrast, TGFβ1 from insect cells showed only marginal or even inhibitory effects. The results indicate that using biologically relevant cytokines can more effectively induce Th17 cell polarization and lead to a more accurate scientific understanding of the human biological process.



A rapidly expanding range of HumanKine cytokines are available from HumanZyme Inc. The proteins are manufactured to high quality standards and provide high biological activity, lot-to-lot consistency and low endotoxin levels. The specific products discussed here, HumanKine TGFβ1, HumanKine TGFβ2, and HumanKine TGFβ3 are available from Proteintech at www.ptglab.com

Mature TGF-β1, -β2, -β3



Purified HumanKine TGFβ1, HumanKine TGFβ2, and HumanKine TGFβ3 were resolved on an SDS-PAGE with Coomassie Blue staining.