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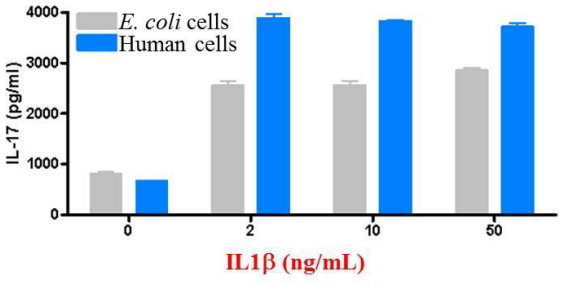
Effective Differentiation of Th17 Cells With HumanKine® Cytokines Expressed in Human Cells

INTRODUCTION

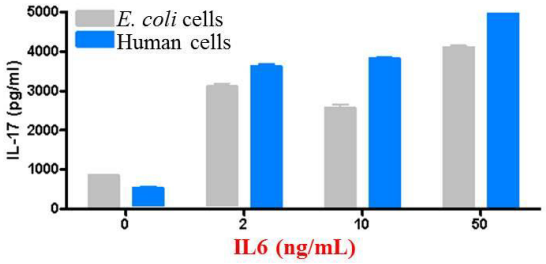
Cytokines are a group of proteins and polypeptides that organisms use as signaling molecules. Most cytokines are glycoproteins less than 30 kDa in size and bind to specific, high-affinity cell surface receptors. Due to their central role in the immune system, cytokines are involved in a variety of immunological, inflammatory and infectious diseases and widely used in research, diagnostics and therapeutics. Cytokines generally alter the gene expression pattern of the target cell which leads to changes in the rate of cell proliferation and/or in the state of cell differentiation. Currently, these proteins are predominantly produced in non-human cells (e.g. E. coli, SF9, CHO) and therefore lack authenticity due to the absence of physiologically relevant glycosylation. In addition, a number of important cytokines are not commercially available due to inadequate proteolytic processing, protein folding or other post-translational modifications that do not occur in the non-human cell expression systems. Proteintech has developed an efficient human-cell based technology, HumaXpress®, for cost-effective and scalable production of human cytokines.

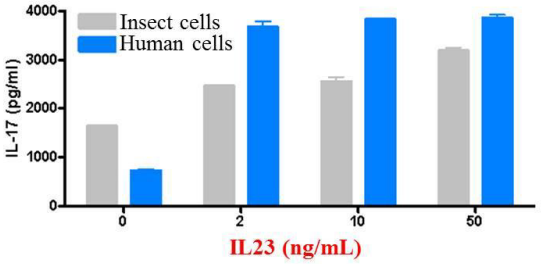
Th17 CELL DIFFERENTIATION

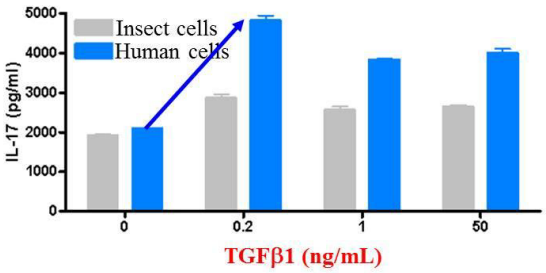
IL-17-producing CD4+ T cells (Th-17 cells) have been identified as a unique subset of Th cells that develop along a pathway that is distinct from the Th1 and Th2-cell differentiation pathways.



This finding has provided exciting new insights into immunoregulation, host defense and the pathogenesis of auto immune diseases. Recently it has been shown that IL1β, IL6 and IL23 are important in driving human Th17 differentiation.







However, TGFβ1, which is important for the differentiation of murine Th17 cells, is reported to be not required, and even inhibits human Th 17 differentiation1,2. In this study, whole CDE4+ cellsisolated 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 Proteintech Inc. (HumanKine® cytokines expressed in a human cell expression system) and from a commercial vendor (cytokines were insect cell expressed). After 5 days

supernatants were harvested for measurement of IL-17 by ELISA. The results show that HumanKine IL1β, HumanKine IL6, and HumanKine IL23 are significantly more effective in inducing IL-17 secretion. More importantly, it demonstrates the HumanKine TGFβ1 is also effective in enhancing the effect. In contrast, this cytokine from insect cells only showed marginal effect. The results indicate that using more authentic cytokines, we can more effectively induce Th17 cell differentiation and lead to more accurate scientific understanding of human biological process.

A rapidly expanding range of HumanKine cytokines are available from Proteintech., manufactured to high quality standards and providing high biological activity, lot-to-lot consistency and low endotoxin levels. The specific products discussed here, HumanKine IL1β, HumanKine IL6, HumanKine IL23, and HumanKine TGFβ1 are available from Proteintech.

References

1. McGeachy, M.J. and Cua, D.J.; *Immunity* vol. 28 April 2008, Elsevier Inc, 445-4532.

2. Chen, Zhi and O'Shea, John J.; *Cytokine* vol. 41 2008 Elsevier Inc., 71-78

PROTOCOL

Purpose: To generate or expand human Th17 cells from naïve, whole or memory CD4 T cells.

Materials:

1. Buffy coat from healthy donor.
2. MACS Buffer – 2% FCS, 2mM EDTA in PBS
3. X-VIVO15 media (Lonza) – 10% Human Serum,Pen/Strep, L-Glut, 2ME
4. Cytokines (all from Proteintech) – TGFβ1, IL1β, IL6, IL23
5. Antibodies – anti CD3 (OKT3 eBioscience) and anti-CD28 (CD29.2, eBioscience)

Methods:

1. Add 500 µl Rosette Sep CD4 T cell enrichmentcoktail (Stem Cell Technologies) to blood, mixthoroughly and incubate for 20 min at RT.
2. Procced to Ficoll separation according to standard protocol.
3. Pipette lymphocyte layer into a new conical tube(being careful not to pipette too much Ficoll), wash with MACS buffer (~ total of 45 ml) and spin at 300xgfor 10 min at 4ºC. NOTE: Keep cells on ice or at 4ºC for remainder of time.
4. Wash with 25 mL MACS buffer and spin at 300 xg for 10 mins at 4ºC.
5. Start magnetic cell sorting for naïve, whole or memory CD4 T cells according to manufacturer's protocol (Miltenyi Biotec). If better purity is desired,FACS sorting is highly recommended.
6. Cell culture:

a) Pre-coat 48 well flat bottom plates with 100 µl per well anti-CD3 (10µg/ml, clone OKT3, eBioscience) in PBS for 2 hr at 37ºC (can also pre-coat overnight at 4ºC). Wash once with PBS andproceed to culture.

b) Culture 1 x 105 CD4 T cells in400 µl X-VIVO15 media (2.5 x 105/ml) containing 5µ/ml soluble anti-CD28 (clone CD28.2 eBioscience),1 ng/ml TGFβ, 10 ng/ml IL1β, 10 ng/ml IL6 and 10ng/ml IL23 at 37ºC.

c) After 5 days, measure supernatant for IL-17 production and perform FACS staining for intracellular IL-17.