

Efficient Differentiation of TH17 Cells With Authentic HumaXpress™ Cytokines Expressed in Human Cells



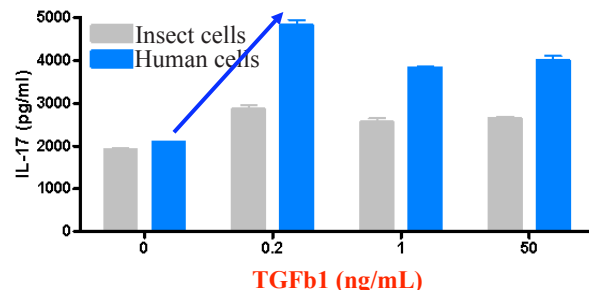
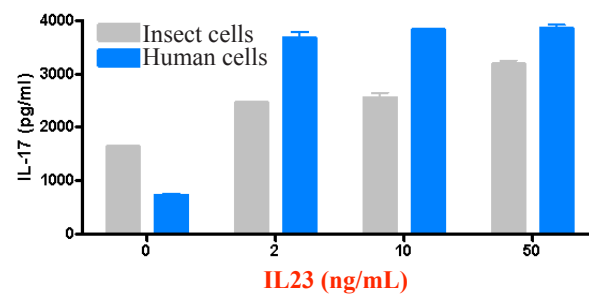
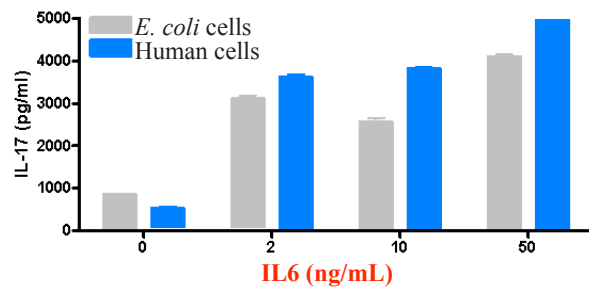
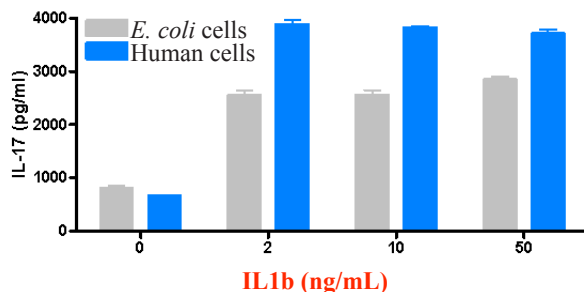
INTRODUCTION

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 can lead 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. HumanZyme has developed an efficient human-cell based technology, HumaXpress™, for 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 autoimmune diseases. Recently

it has been shown that IL1 β , IL6 and IL23 are important in driving human Th17 differentiation. However, TGF β 1, which is important for the differentiation of murine TH17 cells, is reported to be not required, and even inhibits human Th17 differentiation^{1,2}. In this study, whole CDE4+ cells isolated from a healthy donor were stimulated with 10 μ g/mL plate bound anti-CD3 and 10 μ g/ml soluble anti-CD28 in the presence of Th17 polarizing cytokines from HumanZyme Inc. (HumaXpress™ 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 IL1 β ^{HuXp}, IL6^{HuXp} and IL23^{HuXp} are



significantly more effective in inducing IL-17 secretion. More importantly, it demonstrates the TGF- β 1^{HuXp} 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 HumaXpress™ cytokines are available from HumanZyme Inc., manufactured to high quality standards and providing high biological activity, lot-to-lot consistency and low endotoxin levels. The specific products discussed here, IL1 β ^{HuXp}, IL6^{HuXp}, IL23^{HuXp} and TGF- β 1^{HuXp}, are available in trial size and in bulk.

References

1. McGeachy, M.J. and Cua, D. J.; Immunity vol. 28 April 2008 Elsevier Inc, 445-453
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 HumanZyme) - TGF- β 1, IL-1 α , IL-6, IL-23
5. Antibodies - anti-CD3 (OKT3, eBioscience) and anti-CD28 (CD28.2, eBioscience)

Methods:

1. Add 500 ul Rosette Sep CD4 T cell enrichment cocktail (Stem Cell Technologies) to blood, mix thoroughly and incubate for 20 min at RT.
2. Proceed 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 300xg for 10 min at 4°C. NOTE: Keep cells on ice or at 4°C for remainder of time.
4. Wash with 25 mL MACS buffer and spin at 300xg for 10 min at 4°C.
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 ul per well anti-CD3 (10 ug/mL, clone OKT3, eBioscience) in PBS for 2 hr at 37°C (can also pre-coat overnight at 4°C). Wash once with PBS and proceed to culture.
b) Culture 1×10^5 CD4 T cells in 400 ul X-VIVO15 media (2.5×10^5 mL) containing 5 ug/mL soluble anti-CD28 (clone CD28.2 eBioscience), 1 ng/mL TGF- β 1, 10 ng/mL IL-1 α , 10 ng/mL IL-6 and 10 ng/mL IL-23 at 37°C.
c) After 5 days, measure supernatant for IL-17 production and perform FACS staining for intracellular IL-17.