

# Development and function of human Th17 cells

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## INTRODUCTION/BACKGROUND

CD4<sup>+</sup> T helper (Th) cells are essential regulators of adaptive immune responses. After antigenic stimulation, naïve CD4<sup>+</sup> cells differentiate into specialized effector cell subsets, which are characterized by the secretion of distinct cytokines. Traditionally, effector T helper cells have been classified into two cell lineages: The Th1 cells, which regulate cellular immunity, and the Th2 cells, which mediate humoral immunity and allergic responses. Beside these effector cell subsets, a regulatory T cell subset, called T regulator cells (Tregs), exists. To control and suppress excessive function of effector cells is a key role of these cells.

Recently, the Th17 cells have been proposed to make up a new effector cell subset. These cells are named after their hallmark cytokines IL-17A and IL-17F. Beside these cytokines, they also produce IL-1beta, IL-6, IL-22 and tumor necrosis factor alpha (TNF-alpha). As all of these cytokines have pro-inflammatory properties, it is not surprising that Th17 cells are implicated in a wide range of inflammatory disorders such as rheumatoid arthritis (RA), multiple sclerosis (MS) and inflammatory bowel disease (IBD). Furthermore, they are thought to be involved also in allergy and asthma. On the other hand, Th17 cells have a protective role against some extracellular bacteria and fungi.

Whether naïve T cells develop into Th1, Th2 or Th17 cells depends on the cytokine milieu of their micro-environment (Fig.1). While many reports showed that a combination of transforming growth factor beta (TGF-beta) and IL-6 is crucial for the induction of murine Th17 cells, the situation in man is less clear.

In this project, we investigate the factors that induce development of human Th17 cells and analyze the interaction of Th17 cells with epithelial cells

## MATERIAL & METHODS

### *Isolation of T cells*

Peripheral blood mononuclear cells (PBMC) are isolated from buffy coats of healthy donors using Ficoll (Biochrom KG) density gradient centrifugation. CD4<sup>+</sup> T cells are purified with anti-CD4-Dynal magnetic beads and Detach-a-Bead antibodies (Dynal). CD45RA<sup>+</sup> (naïve) T cells are isolated using MACS magnetic beads.

### *Co-culture with Normal human bronchial epithelial (NHBE) cells*

NHBE cells (LONZA) are maintained in BEBM (LONZA) and are plated in a 24-well dish to be 90% confluent the day of the experiment. T cells are differentiated for 6 days. Then, the T cells are washed and restimulated for 12 h with anti-CD3 (4 ug/ml) and anti-CD28 (4 ug/ml). T cells are added to NHBE cells at a number 5x10<sup>5</sup> cells per well, or cultured alone. Supernatants are collected after 48 h for protein analysis by ELISA. For Immunofluorescence, NHBE cells grown on coverslips are cocultured with T cells for 24 h. Brefeldin A is added

during the last 3 h to block cytokine secretion.

#### *Isolation of RNA and synthesis of cDNA*

Total RNA is isolated using the RNeasy mini kit (Qiagen) according to the manufacturer's protocol. Reverse transcription is performed with TaqMan reverse transcription reagents (Fermentas) using random hexamer primers according to the manufacturer's protocol.

#### *Quantitative real-time PCR*

The PCR primers and probes are designed based on the sequences reported in GenBank with the Primer Express software version 1.2 (Applied Biosystems). The prepared cDNAs are amplified using SYBR-PCR Master mix (Applied Biosystems) according to the manufacturer's recommendations in an ABI PRISM 7900 Sequence Detection System (Applied Biosystems).

#### *Immunofluorescence*

The cells are fixed for 10 min with 1% Paraformaldehyd (PFA, Fluka) and permeabilized with detergent (0.1% Triton (Fluka), 0.02% SDS (Roth) in PBS (Gibco)) for 5 min. Next, the cells are blocked in 2% BSA (Fluka) in PBS for 30 min and probed for 1 h with an anti-IL-6 antibody (FITC, Biolegend) and again fixed in 1% PFA. After mounting using VectaShield mounting medium containing DAPI (Vector Laboratories, Burlingame, CA), the cells are analyzed with a Leica TCS SPE confocal microscope (Leica Microsystems AG, Glattbrugg, Switzerland).

## RESULTS

To determine the mediators required for the differentiation of human Th17 cells, naïve T cells were stimulated in the presence or absence of TGF-beta, IL-6, IL-1beta, IL-23 or TNF-alpha. After 7 days, the cells were analyzed for expression of Th17-specific genes, namely IL-17A, IL-17F and RORC2, the Th17-specific transcription factor. We observe induction of these genes on mRNA level, as well as on protein level when using different combination of TGF-beta, IL-6, IL-1beta, and IL-23. Addition of TNF-alpha alone or in combination with other cytokines does not increase mRNA levels of any of the studied genes.

One major role of Th17 cells is the induction of pro-inflammatory cytokines in several epithelial cells. To test the activity of the *in vitro* differentiated Th17 cells, we investigated their influence on IL-6 production by Normal Human Bronchial Epithelial (NHBE) cells. Naïve T cells were cultured for seven days under either non-differentiating, Th1, Th2, Treg and Th17 conditions. These differentiated cells were added to NHBE cells and expression of IL-6 was assessed by ELISA and Immunofluorescence staining followed by confocal microscopy. Untreated NHBE cells secrete low levels of IL-6. NHBE cells cocultured with Th17 cells show a strongly increased IL-6 production, which is not observed to that extent when the cells were cocultured with other T cell subtypes.

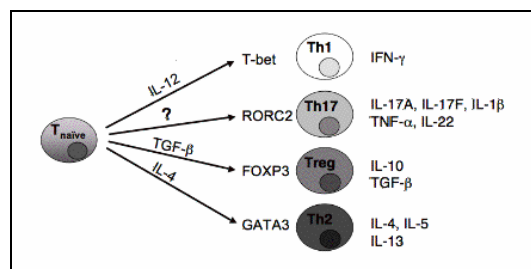


Fig 1: Schematic depiction of T cell differentiation. While development of Th1, Th2 and Treg cells is well established, the factors that are crucial for development of human Th17 cells are still unclear.

## CONCLUSIONS & OUTLOOK

The current study investigates differentiation of naïve human T cells to Th17 cells, discovering similarities as well as differences to Th17 development in mice. Ongoing experiments analyze the particular role of these cytokines in development of human Th17 cells and the underlying molecular mechanisms.

The fact that *in vitro* differentiated Th17 cells can stimulate IL-6 production in epithelial cells underlines the functional relevance of human Th17 cells and is consistent with their role in the attraction of neutrophils during chronic phase of asthma. Future experiments will concentrate on the discovery of new genes in epithelial cells, specifically induced by Th17 cells. This will be a further step to elucidate the role of Th17 cells in asthma.

## REFERENCES

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