A method for enrichment and detection of viable human and mouse IL-17-secreting T cells

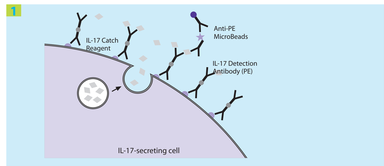
Mario Assenmacher, Michaela Niemöller, Anna Foerster, Claudia Zyntek, Monika Winkels, Jürgen Schmitz, Anne Richter | Miltenyi Biotec GmbH, Research & Development, Bergisch Gladbach, Germany

Introduction

Recent data illustrate the importance of IL-17 in the defense against extracellular bacterial and fungal infections, but also in chronic inflammatory disorders, e.g. autoimmune diseases. We have developed the IL-17 secretion assays that are tools for the enrichment and detection of viable IL-17-producing cells either from human or from mouse tissue.

Results

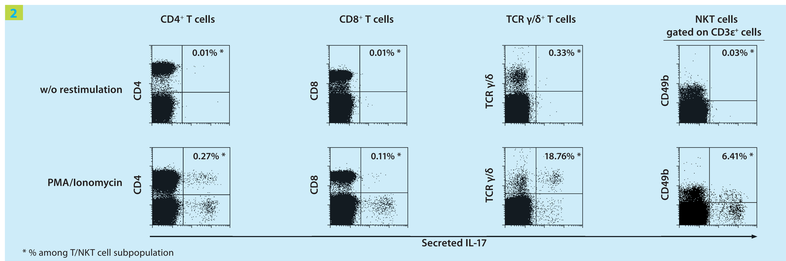
1 The principle of the IL-17 Secretion Assay



After T cell activation, the cells were labeled with a bispecific CD45/IL-17 antibody affinity matrix. Cells were again cultured for 45 minutes to allow IL-17 secretion. Secreted IL-17 was specifically caught onto the affinity matrix of IL-17-producing cells. Subsequently, IL-17-secreting cells were fluorescently stained with a second IL-17 antibody or additionally labeled with paramagnetic MicroBeads.

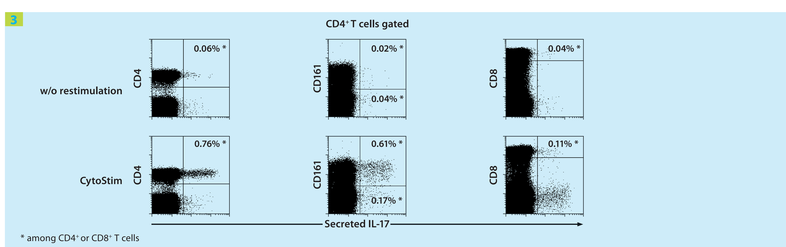
2 Mouse IL-17 is produced by CD4⁺ T cells, CD8⁺ T cells, TCRγδ⁺ cells, and NKT cells

Mouse spleen cells were stimulated with PMA/Ionomycin for 3 hours, and secreted IL-17 was detected as described in figure 1. Figure 2 shows the flow cytometric analysis of IL-17-secreting cells. First, a lymphocyte gate based on forward and side scatter properties was activated. With a second gate, dead cells and B cells were excluded according to PI- and CD45R/B220-PerCP staining in FL-2/FL-3 dot plot. T cells were counterstained using CD4-APC, CD8-APC, and Anti-TCRγδ-PE, and NKT cells were identified using CD3ε-FITC and CD49b-APC. We observed IL-17 production by CD4⁺ T cells, but also by CD8⁺ T cells, TCRγδ⁺ cells, and NKT cells.

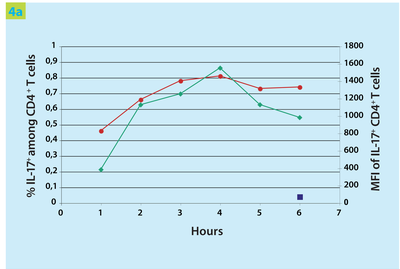


3 Human IL-17 is mainly produced by CD4⁺ CD161⁺ T cells

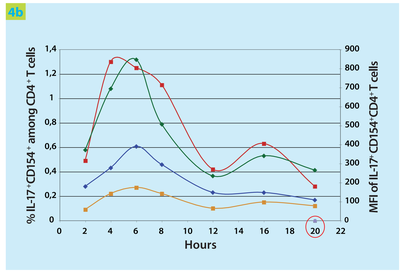
Human PBMC were stimulated with the artificial superantigen CytoStim for 4 hours and secreted IL-17 was detected as described in figure 1. Figure 3 shows the flow cytometric analysis of IL-17-secreting cells. First, a lymphocyte gate based on forward and side scatter properties was activated. With a second gate, dead cells were excluded according to PI-staining in FL-2/FL-3 dot plot. T cells were counterstained using CD4-FITC, CD8-APC, and CD161-APC. Nearly all IL-17-secreting cells were found within the CD4⁺ T cell population with most of them co-expressing the NK cell marker CD161. Only a very low frequency of IL-17-secreting CD8⁺ T cells was observed.



4 Kinetics of IL-17 secretion



Mouse spleen cells were stimulated with PMA/Ionomcyin for 1–6 hours. The frequency of IL-17-secreting CD4⁺ T cells was analyzed as described in figure 2. CD4⁺ T cells showed the maximal frequency of IL-17-secreting cells with the maximal mean fluorescence intensity after 4 hours of restimulation.

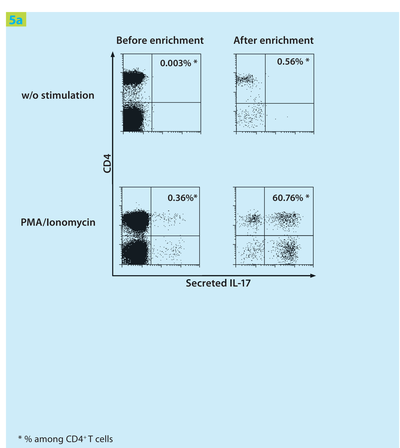


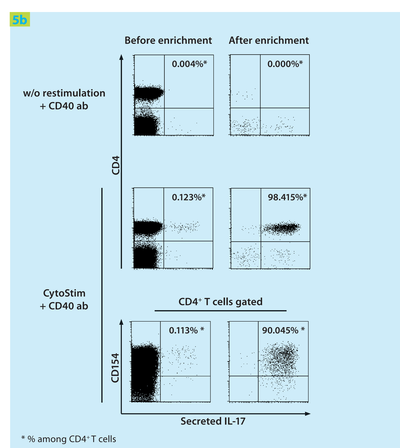
Human PBMC of two donors were stimulated with CytoStim in the presence of CD40 antibody for 2–20 hours and secreted IL-17 was detected as described in figure 3. CD40 antibody was added to maintain the cell surface expression of CD154. The maximal frequency of IL-17-secreting CD4⁺CD154⁺ T cells with the maximal mean fluorescence intensity was determined after 6 hours of activation.

5 Enrichment of viable IL-17-secreting T cells

Mouse spleen cells were stimulated with PMA/Ionomcyin for 3 hours. IL-17-secreting T cells were detected, and additionally labeled with Anti-PE MicroBeads and magnetically enriched using the MACS® Technology. IL-17-producing CD4⁺ T cells from the stimulated sample were enriched from a frequency of 0.36% to >60% with a recovery of 1444 cells out of 10^6 CD4⁺ T cells (= 0.14%). Without restimulation only 0.003% of CD4⁺ IL-17-secreting T cells were found before enrichment, which were enriched to purities of 0.56% and only 6 IL-17-producing cells out of 10^6 CD4⁺ T cells were recovered.

Human PBMC were stimulated with CytoStim in the presence of CD40 antibody for 4 hours. Then secreted IL-17 was detected, additionally labeled with Anti-PE MicroBeads, and magnetically enriched. In the stimulated T cell sample 0.123% of CD4⁺ T cells secreted IL-17. These cells were enriched to a purity of 98% with a recovery of 696 IL-17-producing cells out of 10^6 CD4⁺ T cells (0.07%). In contrast, only 0.004% of CD4⁺ IL-17-secreting T cells were detected without restimulation before enrichment, but less than 1 IL-17-producing cell could be enriched from 10^6 CD4⁺ T cells. 90% of the enriched IL-17⁺CD4⁺ T cells co-expressed the activation marker CD154, confirming the specificity of the detection and enrichment method.



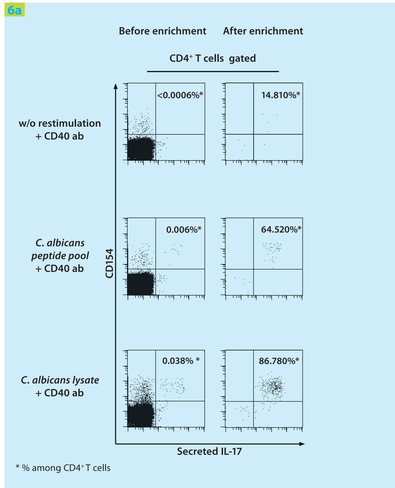


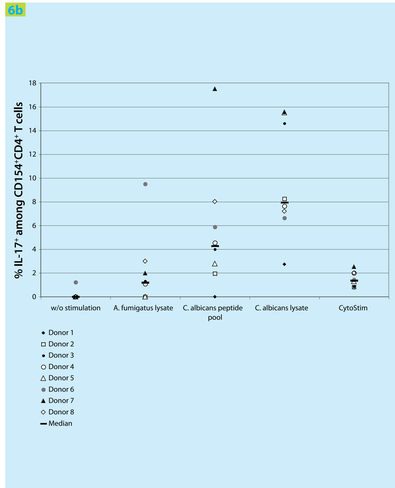
6 Detection and enrichment of antigen-specific IL-17⁺CD154⁺CD4⁺ T cells

Human PBMC were restimulated with an antigen extract of *Candida albicans* in the presence of CD40 antibody for 16 hours or were restimulated with a peptide pool of Mannoprotein 65 of *C. albicans* in the presence of CD40 antibody for 4 hours. Afterwards the IL-17 Secretion Assay was performed, the cells were labeled with Anti-PE MicroBeads, and magnetically enriched.

Without restimulation less than 0.0006% of CD4⁺CD154⁺ IL-17-secreting T cells were found before enrichment, which were enriched to purities of 14.8% and only 2 IL-17-producing cells out of 10^6 CD4⁺ T cells were recovered. After stimulation with the peptide pool only a rare frequency of IL-17–secreting CD154⁺CD4⁺ T cells were detected (0.006%). These cells were enriched to a purity of 65% with a recovery of 18 IL-17⁺CD154⁺ cells out of 10^6 CD4⁺ T cells (0.0018%). And a frequency of 0.038% IL-17⁺CD154⁺CD4⁺ T cells was detected after restimulation with the antigen extract. These cells were enriched to a purity of 87% with a recovery of 188 IL-17⁺CD154⁺ cells out of 10^6 CD4⁺ T cells (0.019%).

Furthermore, we compared the proportion of TH17 cells among all antigen-specific CD154⁺CD4⁺ T cells, activated either with *C. albicans* or *Aspergillus fumigatus* antigens, or with CytoStim.Therefore, PBMC of eight human donors were stimulated with the peptide pool of Mannoprotein 65 of C. albicans, with antigen extracts of *C. albicans* or *A. fumigatus* or with CytoStim for 6 hours and intracellular stained for IL-17. Without restimulation nearly no IL-17⁺CD154⁺CD4⁺ T cells were detected. Cells restimulated with CytoStim showed a median frequency of 1.37% and *A. fumigatus lysate*–stimulated cells a median frequency of 1.2% IL-17⁺ cells among CD154⁺CD4⁺ T cells. In contrast, an increased frequency of *C. albicans*–specific T cells produce IL-17, after stimulation with the peptide pool the median frequency is 4.3% and 7.9% with the antigen extract.





Conclusion

Here, we show the newly developed IL-17 Secretion Assays that allow the detection of IL-17-secreting T cells either from human or from mouse tissue. Additionally, this technique allows the efficient enrichment and therefore the reliable detection of viable IL-17-producing cells, especially of low-frequent T cells specific, for example, to fungal antigens like *A. fumigatus* or *C. albicans*. This enables further to investigate the function of these cells, for example, the role in host defense and disease development.