Identification of Novel Cell Surface Markers on Mouse and Human Th17 Cells

Christine Goetz, Alberto Guerra, and Jody Bonnevier | R&D Systems, Inc., Minneapolis, MN 55413

ABSTRACT

Th17 cells are a subset of CD4+ T helper cells that play a crucial role in protection against extracellular bacteria, but may also be involved in autoimmune disease progression. Consequently, basic and clinical research on Th17 cells has increased tremendously in the last several years, becoming one of the most prominent and active areas of research in immunology. The Th17 subset is defined by the ability to produce cytokines, such as IL-17 and IL-22, and also by the expression of cell surface markers, such as the IL-23 receptor. Much research effort in the area of Th17 cell biology is the search for surface molecules that can be used to consistently characterize Th17 cells in different immune environments, which may ultimately lead to potential therapies for autoimmunity. To date, only a few surface molecules have been found that are exclusively expressed by Th17 cells, making the use of surface markers for this cell type a challenge. Our objective was to identify new surface markers to distinguish Th17 cells from other subsets of CD4+ helper T cells by flow cytometry. In order to accomplish this goal, we used a high-throughput flow cytometer to screen over 700 surface antibodies on mouse and human Th17 cells. We identified 26 novel surface markers on human Th17 cells that also co-express IL-22, IL-17, and RORγt, confirming these markers were on functional Th17 cells. Interestingly, four of these markers (BTLA, CD200, CD99, and IL-18 Rα) overlapped with novel Th17 surface markers identified on mouse Th17 cells. Further study of these markers will enable the development of therapies for autoimmunity and cancer research.

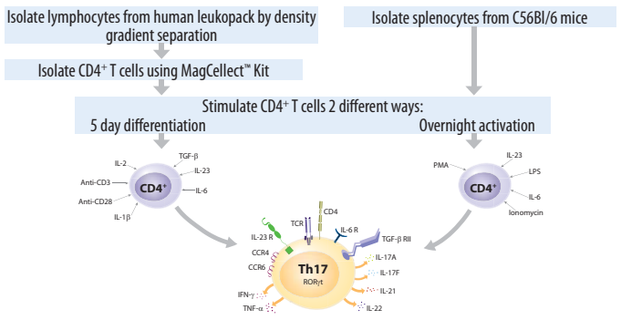
OBJECTIVES

GOAL: To identify novel cell surface markers on mouse and human Th17 cells.

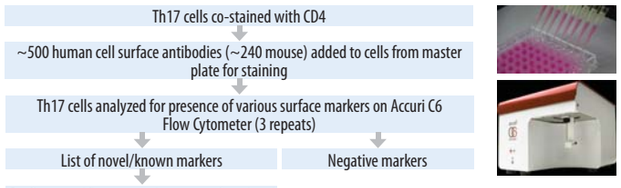
SIGNIFICANCE: Identify unique markers on Th17 cells to enable further research on Th17 cells that can include applications such as autoimmune disease treatment and prevention.

METHODS

Th17 DIFFERENTIATION



SCREENING OF R&D SYSTEMS CELL SURFACE ANTIBODIES



RESULTS

FIGURE 1 Distribution of Markers Identified in our Screen

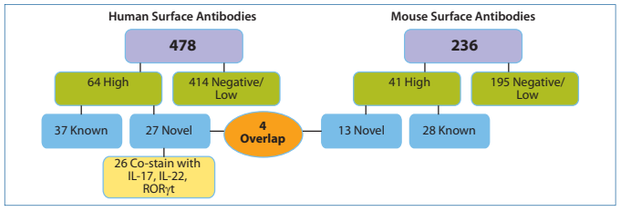
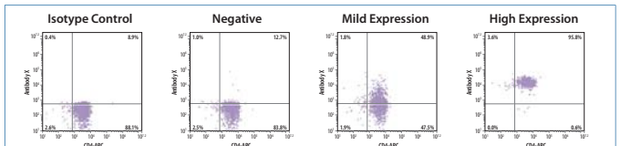
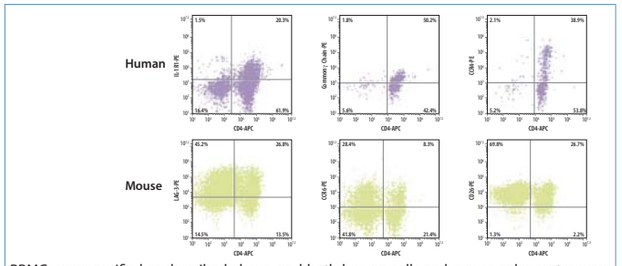


FIGURE 2 Representative Flow Data in Human Th17 Cells



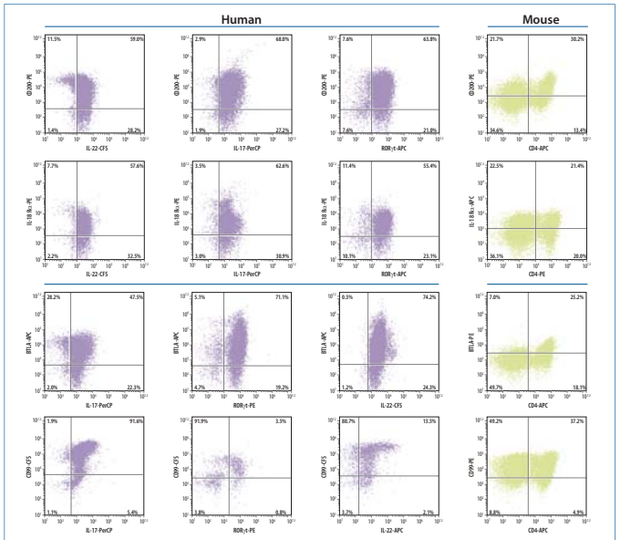
PBMCs were isolated, CD4-purified using the MagCellect™ Human Naïve CD4+ T Cell Isolation Kit (Catalog # MAGH115), and stimulated for 5 days to induce Th17 differentiation. The resulting Th17 cells were then stained with APC-conjugated Mouse Anti-Human CD4 Monoclonal Antibody (Catalog # FAB3791A) and various human surface markers. These data show the various levels of staining with the surface markers ranging from negative to high expression. These results are representative of three experiments.

FIGURE 3 Examples of Known Human and Mouse Th17 Markers Identified in our Screen



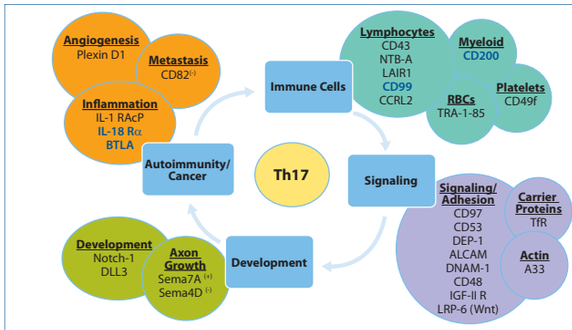
PBMCs were purified as described above, and both human cells and mouse splenocytes were stimulated for 5 days to induce Th17 differentiation. The resulting Th17 cells were stained with an APC-conjugated Mouse Anti-Human CD4 Monoclonal Antibody (Catalog # FAB3791A) or an APC-conjugated Rat Anti-Mouse CD4 Monoclonal Antibody (Catalog # FAB554A) and the surface markers shown. These data validate that known surface markers associated with Th17 cells were identified in our screen. These results are representative of three experiments.

FIGURE 4 CD200, IL-18Rα, BTLA, and CD99 are Novel Surface Markers on Mouse and Human Th17 Cells



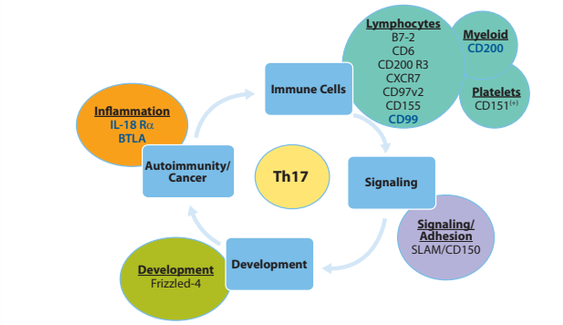
PBMCs were purified as described above, and both human cells and mouse splenocytes were stimulated for 5 days to induce Th17 differentiation. The resulting Th17 cells were stained with an APC- or PE-conjugated Mouse Anti-Human CD4 Monoclonal Antibody (Catalog # FAB3791A or FAB3791P, respectively) or an APC- or PE-conjugated Rat Anti-Mouse CD4 Monoclonal Antibody (Catalog # FAB554A or # FAB554P, respectively) and the surface markers shown. In order to identify if these markers were expressed on functional human Th17 cells, a separate experiment was carried out to examine CD200, IL-18 Rα, BTLA, and CD99 levels in conjunction with IL-22, IL-17, and RORγt. These data are representative of three and two experiments, respectively.

FIGURE 5 Novel Surface Markers on Human Th17 Cells



PBMCs were purified as described above and stimulated for 5 days to induce Th17 differentiation. The resulting Th17 cells were stained with APC-, Fluorescein-, or PE-conjugated Mouse Anti-Human CD4 Monoclonal Antibody (Catalog # FAB3791A, # FAB3791F, or # FAB3791P, respectively) and the surface markers shown. Cells expressing 26/27 of these novel markers also expressed IL-22, IL-17, and RORγt. The four novel markers that overlap with mouse Th17 cells are shown in blue. These results are representative of three and two experiments, respectively.

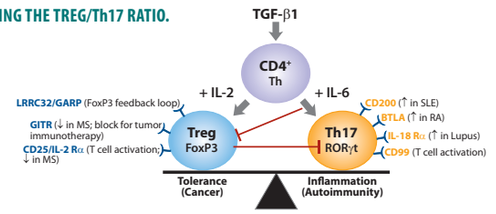
FIGURE 6 Novel Surface Markers on Mouse Th17 Cells



Mouse splenocytes were stimulated for 5 days to induce Th17 differentiation. The resulting Th17 cells were co-stained with APC-, Fluorescein-, or PE-conjugated Rat Anti-Mouse CD4 Monoclonal Antibody (Catalog # FAB554A, # FAB554F, or # FAB554P, respectively) and the surface markers shown. The four novel markers that overlap with human Th17 cells are shown in blue. These results are representative of three experiments.

SUMMARY

BALANCING THE TREG/Th17 RATIO.



The intricate balance of immune cells prevents the development of autoimmunity. T regulatory cells (Tregs) and Th17 cells derive from CD4+ precursors in the presence of TGF-β1. The key cytokine that skews these cells toward the Th17 lineage is IL-6, while IL-2 promotes Treg differentiation. IL-6 signaling induces STAT3, which upregulates RORγt, the master regulator of Th17 cells. IL-2 signaling induces STAT5, which thereby induces FoxP3, leading to Treg differentiation. FoxP3 has been shown to be antagonistic towards Th17 cells, whereas IL-6 blocks Treg differentiation. When there is a skewing of the ratio towards the Th17 lineage, inflammatory cytokines are produced, which sets the stage for autoimmune disease progression. The immunosuppressive function of Tregs helps prevent the development of autoimmunity, but this state of tolerance can hinder the immune system from destroying cancer cells. Representative surface markers for Tregs and Th17 cells illustrate these points. Thus, understanding the correct balance of Tregs and Th17 cells in autoimmunity and cancer is critical for developing immunotherapies for these patients in the clinic.

RnDSystems.com

P5_12.12_Th17_MidwinterInfmm.