Development and Characterization of Monoclonal Antibody Clone 41802 to Human IL-17A

Jody L. Bonnevier, Ricardo deMedeiros, Qi Cheng, Molly Thomas, David Galitz, Kristin Coley, Cyrus Munshi, Julio Herrera, Kurt LaBresh, J.P. Houchins and Jessie H.T. Ni
R&D Systems, Inc. 614 McKinley Place NE, Minneapolis, MN 55413, U.S.A.

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ABSTRACT

Although IL-17A is made by CD4$^+$ Th17 cells and there is an ever-increasing body of literature on these cells, other cells make IL-17A, including CD8$^+$ (Tc17) and γ-δ T cells, NK cells, and neutrophils. There are many factors that contribute to published findings of the percentage of cells producing IL-17A, including the activation or differentiation conditions, time course of activation, and antibody used for detection. It has been published that high levels of IL-17A are secreted by human PBMCs after anti-CD3/CD28-treatment alone for only 24 hrs. We have developed a new monoclonal antibody (Clone 41802) that is specific for human IL-17A. The specificity of this Ab has been rigorously tested. Clone 41802 detects an increase in IL-17A in PMA/ionomycin-treated human PBMCs over resting cells by intracellular flow cytometry. This finding correlated with Western blot data in Th17-differentiated vs resting CD4$^+$ PBMCs. This clone also detects IL-17A in a population of activated CD3$^+$ PBMCs that is also positive for IL-22 and IL-23 R by flow cytometry. Importantly, clone 41802 detects IL-17A in transfectant cells overexpressing human IL-17A, but not in cells overexpressing human IL-17F. In conclusion, clone 41802 is specific for human IL-17A. Although a larger percentage of IL-17A$^+$ cells are detected in activated PBMCs than with other commercially available clones, this indicates an interesting biological finding, not a lack of antibody specificity.

INTRODUCTION

Although IL-17 is made by CD4$^+$ Th17 cells and there is an ever-increasing body of literature on these cells, other cells make IL-17 as well, including CD8$^+$ (Tc17) and γ-δ T cells, NK cells, and neutrophils (Weaver, CT *et al.* (2007) Ann. Rev. of Immunol. **25**:821). Detection of IL-17 in non-CD3$^+$ or non-CD4$^+$ PBMCs does not imply lack of specificity of an antibody. There are many factors that contribute to published findings of the percentage of cells producing IL-17; including the activation or differentiation conditions, time course of activation, and the antibody used for detection. Detection of different numbers of cells with different antibodies does not imply that one antibody must be non-specific. Although many studies have examined IL-17A production in differentiated CD4$^+$ Th17 cells using IL-6, TGF-β, IL-23, etc treatment for several days, it has been published that high levels of IL-17A are secreted by human PBMCs after anti-CD3/CD28-treatment alone for only 24 hrs (Chen, Z. *et al* (2007) Arthritis & Rheumatism **56**:2936).

MATERIALS AND METHODS

CELLS AND ACTIVATION

Human PBMCs were obtained by Ficoll gradient from peripheral blood leukopacs. In some experiments, PBMCs were activated overnight with 50 ng/mL PMA and 200 ng/mL ionomycin in the presence of 3 µM monensin. In other experiments, 500 ng/mL LPS and 10 ng/mL rhIL-23 (R&D Systems Catalog # 1290-IL) were also added (see figure legends for details). Th17 cells were differentiated as previously described (Annunziato, F. *et al* (2007) J. Exp. Med. **204**:1849). Briefly, CD4$^+$/CD45RO- cells were cultured in the presence of anti-CD3/CD28-coated beads (Dynal), 10 ng/mL rhIL-1β (R&D Systems Catalog # 201-LB), 50 ng/mL rhIL-6 (Catalog # 206-IL), 100 ng/mL anti-human IL-4 (Catalog # AB-204-NA) and 100 ng/mL anti-human IFN-γ (Catalog # AB-285-NA) for 3 days. On day 3, 20 ng/mL rhIL-2 (Catalog # 202-IL) and 20 ng/mL rhIL-23 (Catalog # 1290-IL) were added. Cells were harvested on day 12.

ANTIBODIES

R&D Systems anti-human IL-17A (Clone 41802) was used to detect IL-17A protein by flow cytometry and Western blot (Catalog # MAB3171, IC3171P, IC3171C). For comparison, cells were also stained with PE-conjugated anti-human IL-17A from eBioscience (Catalog # 12-7179; Lot # E030194) and Biolegend (Catalog # 512306; Lot # B114014).

INTRACELLULAR FLOW CYTOMETRY

Cells were harvested, washed in PBS, then fixed in 4% paraformaldehyde and permeabilized in PBS containing 0.1% saponin for intracellular staining using R&D Systems Flow Cytometry Fixation and Permeabilization Kit I (Catalog # FC009). Prior to analysis, cells were resuspended in R&D Systems Flow Cytometry Staining Buffer (Catalog # FC001).

DEVELOPMENT & TESTING OF MONOCLONAL ANTI-HUMAN IL-17A ANTIBODY (CLONE 41802)

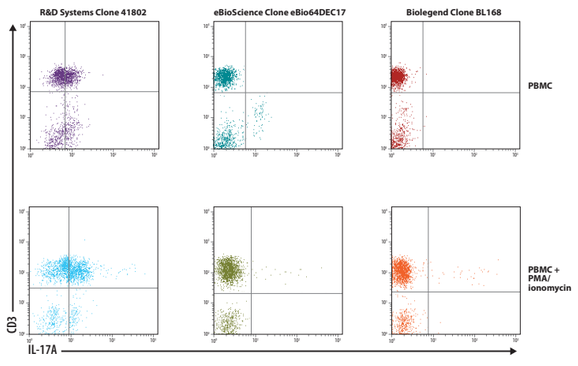
Development

✓ Immunize mouse with rhIL-17A (aa 20 – 155; Accession # Q16552)
✓ Produce hybridoma
✓ Protein G affinity purify antibody from hybridoma tissue culture supernatant

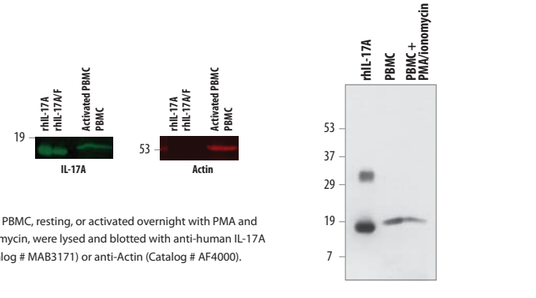
Testing for Antibody Specificity

✓ Western blot with rhIL-17A
✓ Direct ELISA with rhIL-17A (Clone 41802 does not cross-react with rhIL-17B, rhIL-17C, rhIL-17E, rhIL-17F or rmIL-17)
✓ Intracellular flow cytometry of HEK293 cells overexpressing rhIL-17A
✓ Intracellular flow cytometry of resting and activated PBMCs
✓ Western blot of resting and activated PBMCs

R&D SYSTEMS CLONE 41802 DETECTS A LOW LEVEL OF IL-17A IN RESTING PBMC

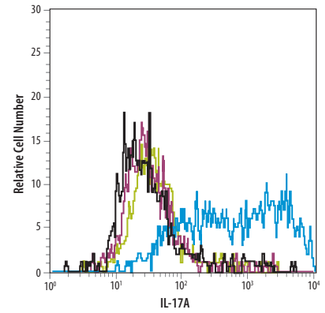


Human PBMCs were stimulated overnight with PMA (50 ng/mL) plus ionomycin (200 ng/mL) and 3 µM monensin. Cells were harvested and stained with PE-conjugated anti-human IL-17A (Clone 41802, R&D Systems Catalog # IC3171P) or PE-conjugated anti-human IL-17A from other commercial sources, and APC-conjugated anti-human CD3 (R&D Systems Catalog # FAB100A). Data shown was gated on lymphocytes based on forward and side scatter parameters. Quadrant markers were set based on staining of isotype control antibodies.



Total PBMC, resting, or activated overnight with PMA and ionomycin, were lysed and blotted with anti-human IL-17A (Catalog # MAB3171) or anti-Actin (Catalog # AF4000).

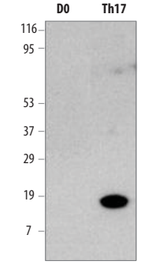
DETECTION OF HUMAN IL-17A IN HEK293-hIL17A TRANSFECTANTS



TRANSFECTANT CELL TYPE	ANTIBODY
EGFP Only	Mouse IgG$_1$ Isotype Control
EGFP Only	R&D Systems Clone 41802
hIL-17F Transfectant	R&D Systems Clone 41802
hIL-17A Transfectant	R&D Systems Clone 41802

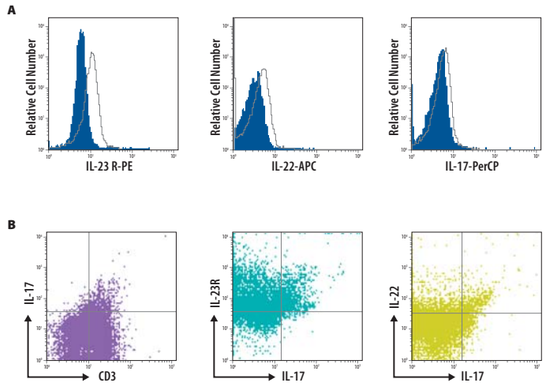
HEK293 cells were transfected with EGFP vector alone, or with human IL-17A or IL-17F. Cells were harvested and stained with anti-human IL-17A (Catalog # MAB3171) or mouse IgG$_1$ isotype control (Catalog # MAB002) followed by PE-conjugated anti-mouse antibody (Catalog # F0101B).

WESTERN BLOT DETECTION OF HUMAN IL-17A IN Th17 CELLS



Primary human CD4$^+$CD45RO$^-$ naive cells were purified from peripheral blood via negative selection (R&D Systems Catalog # HCD41). Th17 cells were differentiated *in vitro* as previously described (J Exp Med 2007 **204**:1849; Semin Immunol 2007 **19**:400). On day 12 of culture, the Th17 cells were re-stimulated with 10 ng/mL PMA + 200 ng/mL ionomycin for 48h. 30 µg total protein of lysate from naive CD4$^+$CD45RO$^-$ cells (D0) or Th17 cells re-stimulated on day 12 with PMA/iono (Th17) was resolved on a 5-20% gel under non-reducing conditions. Proteins were transferred to PVDF membranes, membranes were blocked with 5% milk/TBS, then probed overnight at 4 °C using 2 µg/mL MAB3171 in 5% milk/TBS. Membranes were subsequently developed using standard procedures.

DETECTION OF HUMAN IL-17A, IL-22, AND IL-23 RECEPTOR IN ACTIVATED PBMCS



Human PBMCs were stimulated overnight with PMA (50 ng/mL), ionomycin (200 ng/mL), IL-23 (10 ng/mL, R&D Systems, Catalog # 1290-IL), and LPS (500 ng/mL) followed by 5 hours re-stimulation with PMA/ionomycin and 3 mM monensin. Cells were harvested and stained with human Th17 Cell 4-Color Flow Cytometry Kit (Catalog # FMC007). Histograms (A) show the relative intensities of the indicated antibodies (open histograms) or isotype controls (filled histograms) in CD3$^+$ cells from activated PBMCs. Dot plots (B) show the percentage of IL-17$^+$ cells in relation to CD3$^+$, IL-23 R$^+$, or IL-22$^+$ populations from activated PBMCs. Quadrants were set based on isotype controls.

SUMMARY

1. R&D Systems anti-human IL-17A (clone 41802) detects a low level of IL-17A in resting PBMCs as detected by flow cytometry, Western blot and Real-Time PCR (data not shown). An increased level of IL-17A protein was detected in activated human PBMCs over resting cells by flow cytometry.
2. Clone 41802 does not cross-react with rhIL-17B, rhIL-17C, rhIL-17E, rhIL-17F, or rmIL-17 by direct ELISA (data not shown).
3. Clone 41802 specifically detects IL-17A in cells over-expressing human IL-17A.
4. Clone 41802 detects human IL-17A in the same population of activated CD3$^+$ PBMCs that also express IL-22 and IL-23 receptor.
5. Clone 41802 specifically detects IL-17A in Th17 differentiated cells by Western blot and flow cytometry (data not shown).