Th17 Cells

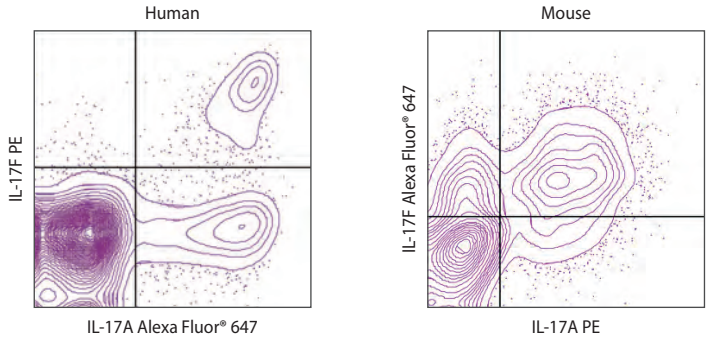
The Complete Solution for Th17 Cell Analysis

2010 | April

A New T Cell Lineage Defined by IL-17A, IL-17F & IL-17AF

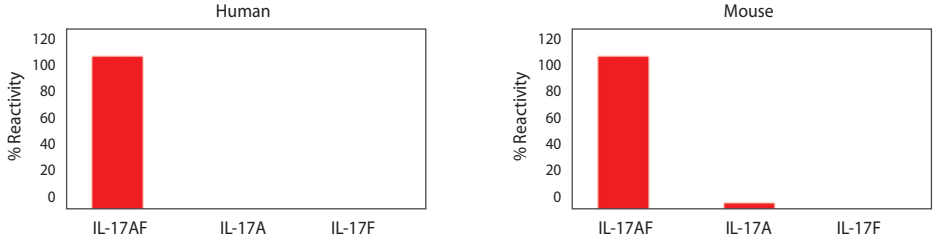
CD4+ T helper cells are critical mediators of the cellular immune response. For many years, due to cytokine expression patterns, it was thought that CD4+ T helper cells existed as a dichotomy of lineages named Th1 and Th2. However, as these subsets were analyzed more closely, it became apparent that the T helper cell population was not limited to these two subsets. Although it has long been appreciated that IL-17 (also known as IL-17A) production by T cells is required for protection against some pathogens, in 2000 it was demonstrated that IL-17A was produced by a unique subset of T helper cells. Subsequently, it was definitively shown that T cells could differentiate into IL-17-producing cells *in vitro* and *in vivo* independently of Th1 or Th2 development thereby establishing Th17 cells as a unique T helper cell lineage. In addition to IL-17A expression, it was recently demonstrated that Th17 cells express another member of the IL-17 cytokine family, IL-17F. Moreover, IL-17A and IL-17F form a heterodimer which is expressed at high levels. IL-17A and IL-17F both bind to IL-17RA and IL-17RC to induce signaling, dependent on the Act1 adaptor protein, which results in the induction of pro-inflammatory cytokines in many different cell types.

IL-17A & IL-17F



Staining of IL-17A and IL-17F in Th17-polarized Human and Mouse T Cells. Human Th17-polarized CD4+ PBMCs were stained with anti-human IL-17A Alexa Fluor® 647 (eBio64DEC17) (cat. no. 51-7179) and anti-human IL-17F PE (SHLR17) (cat. no. 12-7169) (left). Mouse Th17-polarized splenocytes were stained with anti-mouse IL-17A PE (eBio17B7) (cat. no. 12-7177) and anti-mouse IL-17F Alexa Fluor® 647 (eBio18F10) (cat. no. 51-7471) (right). Cells in the lymphocyte gate were used for analysis.

IL-17AF Heterodimer ELISA



Heterodimer-specific IL-17AF Ready-SET-Go!® ELISA. Undetectable cross-reactivity of human IL-17AF ELISA (cat. no. 88-7117) (left) and minimal cross-reactivity of mouse IL-17AF ELISA (cat. no. 88-7272) (right) with recombinant IL-17A or IL-17F alone.

FEATURING

ANTIBODIES

- Act1 (h) NEW
- CCR6 (h)
- CD161 (h)
- IL-17A (h, m)
- IL-17F (h, m) NEW
- IL-17RA (h, m) NEW
- IL-21 (h, m) NEW
- IL-22 (h, m) NEW
- RORγ(t) (h, m)
- Th17 Cytokine Flow Phenotyping Panels (h, m) NEW

RECOMBINANT PROTEINS

- IL-17A (h, m, r)
- IL-17F (h, m)
- IL-17AF (h, m)
- IL-21 (h, m)
- IL-22 (h, m)
- IL-23 (h, m)

ELISA KITS

- IL-6 Instant ELISA® (h)
- TGFβ Instant ELISA® (h)
- IL-17A (h, m, r)
- IL-17F (h, m) NEW
- IL-17AF (h, m) NEW
- IL-21 (h, m) NEW
- IL-22 (h, m) NEW

FLOWCYTOMIX™ MULTIPLE ANALYTE DETECTION KITS

- IL-17A (h, m) NEW
- IL-22 (h, m) NEW
- IL-23 (h, m) NEW

h=human, m=mouse, r=rat

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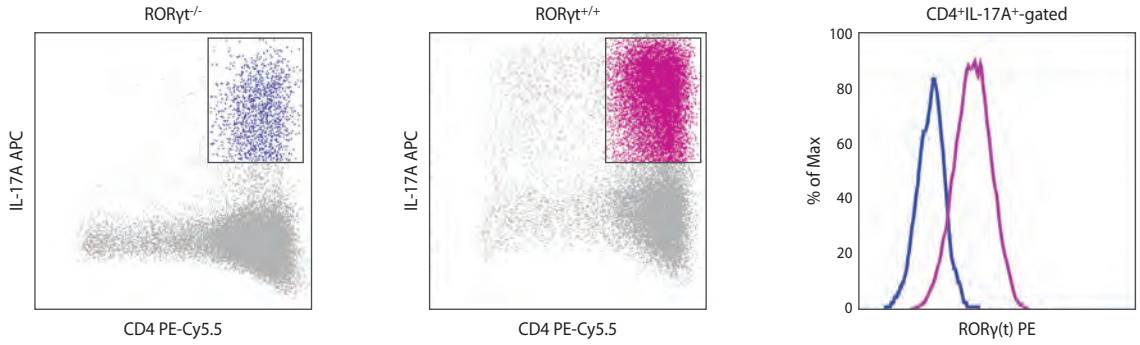
eBioscience®

RORγt

Similar to the role of T-bet and Gata-3 in Th1 and Th2 cells respectively, differentiation of Th17 cells is controlled by a "master-regulatory" transcription factor. RORγt, which directs a specific and heritable gene expression profile, is induced by the expression of TGFβ or IL-6. RORγt was initially identified as a thymus-specific isoform of RORγ, and in 2005, it was discovered that RORγt is also expressed in Th17 cells. Deficiency in RORγt results in diminished Th17 activity and severely reduced expression of IL-17.

Mouse/Human RORγ(t)

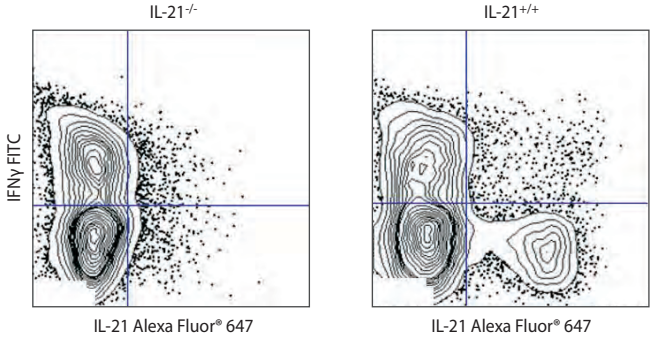


Identification of Th17 cells by flow cytometric detection of RORγ(t). CD4+ T cells were sorted from RORγt-deficient (left dot plot) or wildtype (right dot plot) mouse spleen and lymph node, cultured in Th17-polarizing conditions for 3 days and stained with anti-mouse CD4 PE-Cy5.5, anti-mouse IL-17A APC and anti-mouse/human RORγ(t) PE (AFKJS-9) (cat. no. 12-6988). The histogram shows staining of RORγ(t) in $CD4^+IL\text{-}17A^+$-gated events from RORγt-deficient mice (blue line) and wildtype mice (pink line). Cells in the lymphocyte gate were used for analysis.
Data provided courtesy of DR Littman, New York University.

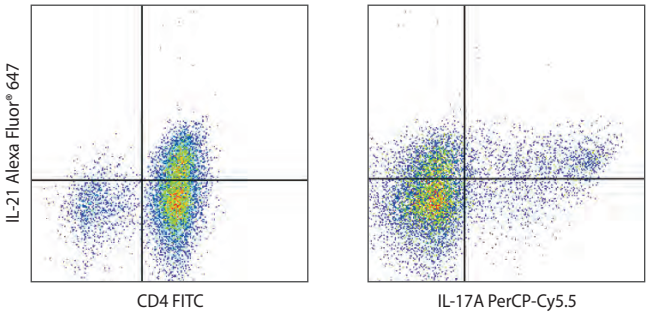
IL-21 & IL-22

Since their initial discovery it has become clear that in addition to IL-17 family members, Th17 cells also express the cytokines IL-21 and IL-22. IL-21 is expressed at high levels by Th17 cells, but it also binds to its receptor, composed of IL-21R and the common γ chain, expressed by Th17 cells to act in an autocrine manner. Expression of IL-21 is dependent on STAT3-mediated IL-6 signaling in Th17 cells and is thought to act in synergy with TGFβ to promote Th17 differentiation. Th17-secreted IL-22 binds to its receptor on target cells to induce the expression of anti-microbial peptides β-defensin-2 and β-defensin-3. Recently, it was demonstrated that IL-22 is able to protect hosts against bacterial infections of the lungs and gut.

Mouse IL-21

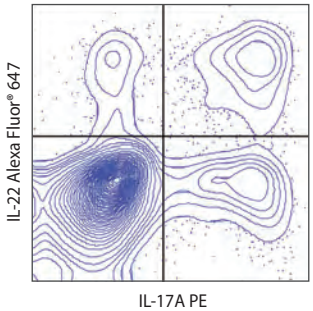


Staining of Mouse IL-21 in LCMV-infected CD4+ T Cells. CD4+CD44+ cells were sorted from IL-21 deficient (left) or wildtype (right) mouse spleen and infected with LCMV. Following restimulation with PMA/Ionomycin, cells were stained with anti-mouse IFNγ FITC and anti-mouse IL-21 Alexa Fluor® 647 (FFA21) (cat. no. 51-7211).
Data provided courtesy of Amanda Poholek and Joseph Craft, Yale University.



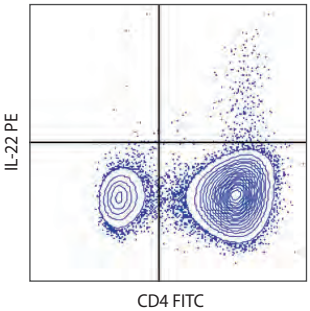
Staining of IL-21 in Th17-polarized Mouse T Cells. Mouse Th17-polarized splenocytes were stained with anti-mouse IL-21 Alexa Fluor® 647 (FFA21) (cat. no. 51-7211), anti-mouse IL-17A PerCP-Cy5.5 (eBio17B7) (cat. no. 45-7177) and anti-mouse CD4 FITC. Events displayed in the right plot are CD4+ gated.

Human IL-22



Staining of IL-22 in Human Th17-polarized CD4+ T Cells. Human Th17-polarized CD4+ PBMCs were restimulated with PMA/Ionomycin and stained with anti-human IL-22 Alexa Fluor® 647 (22URTI) (cat. no. 51-7229) and anti-human IL-17A PE (eBio64DEC17) (cat. no. 12-7179). Cells in the lymphocyte gate were used for analysis.

Mouse IL-22



Staining of IL-22 in Mouse Th17-polarized T Cells. Mouse Th17-polarized splenocytes were restimulated with PMA/Ionomycin and stained with anti-mouse IL-22 PE (1H8PWSR) (cat. no. 12-7221) and anti-mouse CD4 FITC (RM4-5) (cat. no. 11-0042). Cells in the lymphocyte gate were used for analysis.

Defining Th17s

Further proof that Th17 cells are a distinct lineage came from analysis of the cytokines required to promote their differentiation. Th17 development is independent of both IFNγ and IL-4, cytokines required for Th1 and Th2 maturation, respectively. TGFβ and IL-6 work in synergy to induce the maturation of Th17 cells, and the addition of TNF-α and IL-1 further increases this effect. IL-23, which shares its p40 subunit with IL-12, was the first cytokine to be shown to selectively regulate IL-17A expression. It has now been established that while TGFβ and IL-6 direct initial maturation of Th17s, IL-23 regulates their expansion as they acquire expression of the IL-23 receptor.

While much attention has focused on the cytokine expression patterns of Th17 cells, it has recently been demonstrated that Th17 cells may also be identified by the surface expression of CD161 and CCR6. Functionally, Th17 cells play a role in host defense against extracellular pathogens by mediating the recruitment of neutrophils and macrophages to infected tissues. Moreover, it is becoming evident that aberrant regulation of Th17 cells may play a significant role in the pathogenesis of multiple inflammatory and autoimmune disorders.

Feature	Th1	Th2	Th9	Th17	Th22	Treg	Tfh
Surface Expression	IL-12RB2 IFN-γR Tim-3	IL-17RB Tim-1	--	IL-1R1 IL-12RB1 IL-23R CCR6 (h) CD161 (h) IL-13Rα1	PDGFR CCR10	CD25 CD39 CD73 CD101 CD127^{lo} FR4(m) GITR/AITR	CD84 CXCR5 IL-6R IL-21R gp130
Unique Cytokine Expression	IFN-γ	IL-4 IL-5 IL-13	IL-9	IL-17A IL-17F IL-17AF IL-21 IL-22	IL-22 TNF-α	TGF-β	IL-6
"Master Regulator" Transcription Factor	T-bet	Gata-3	--	RORγt	--	Foxp3	BCL6
STAT Regulators	STAT-1, 4	STAT-6	--	STAT-3	--	STAT-5	STAT-3
Polarizing Cytokines	IL-12 IFN-γ IL-27	IL-4 IL-25 (IL-17E)	IL-4 TGF-β	IL-6 TGF-β IL-21	TNF-α IL-6	TGF-β	IL-6 IL-21 CXCL13

NEW Th17 Cytokine Flow Phenotyping Panels

eBioscience has made characterization and identification of Th17 cells easy!

As Th17 cells become increasingly appreciated for their role in pathogenesis, it will be critical to accurately identify and characterize them. eBioscience has developed both human and mouse Th17 cytokine phenotyping panels for the identification of Th17 cells by flow cytometry. These panels include all reagents needed for the simultaneous flow cytometric detection of the major cytokines produced by this important T helper cell lineage; IL-17A, IL-17F, IL-21 and IL-22, plus all necessary intracellular staining buffers.

Th17 Cytokine Flow Phenotyping Panels			
Human Panel	Target	Clone	Format
88-8419 (includes intracellular staining buffers)	IL-17A	eBio64DEC17	FITC
	IL-17F	SHLR17	PE
	IL-21	eBio3A3-N2	Alexa Fluor® 647
	IL-22	22URTI	PerCP-eFluor® 710
	CD4	RPA-T4	eFluor® 450
Mouse Panel	Target	Clone	Format
88-8411 (includes intracellular staining buffers)	IL-17A	eBio17B7	FITC
	IL-17F	eBio18F10	PE
	IL-21	FFA21	Alexa Fluor® 647
	IL-22	1H8PWSR	PerCP-eFluor® 710
	CD4	RM4-5	eFluor® 450

New Th17 Cell Reagents

Mouse	Cat. No.	Clone	Formats
ELISA/ELISPOT			
IL-6	7064	--	ELISA Ready-SET-Go!® Set
	7964	--	ELISA Ready-SET-Go!® Kit (w/ pre-coated plates)
	7864	--	ELISPOT Ready-SET-Go!® Set
IL-17A	7371	--	ELISA Ready-SET-Go!® Set
	7971	--	ELISA Ready-SET-Go!® Kit (w/ pre-coated plates)
	7370	--	ELISPOT Ready-SET-Go!® Set
IL-17F	7472	--	ELISA Ready-SET-Go!® Set
IL-17AF	7272	--	ELISA Ready-SET-Go!® Set
IL-21	8210	--	ELISA Ready-SET-Go!® Set
IL-22	7422	--	ELISA Ready-SET-Go!® Set
IL-23	7234	--	ELISA Ready-SET-Go!® Set
	7231	--	ELISA Ready-SET-Go!® Kit (w/ pre-coated plates)
TGFβ1	7344	--	ELISA Ready-SET-Go!® Set
	7449	--	ELISA Ready-SET-Go!® Kit (w/ pre-coated plates)
Recombinant Proteins			
IL-6	8069	--	Purified, Carrier-free, Single-use ELISA Standards (4 pack)
IL-17A	8171	--	Purified, Carrier-free, Single-use ELISA Standards (4 pack)
IL-17F	8471	--	Carrier-free
IL-17AF	8172	--	Purified, Carrier-free
IL-21	8211	--	Purified, Carrier-free
IL-22	8221	--	Purified, Carrier-free
IL-23	8231	--	Purified, Carrier-free, Single-use ELISA Standards (4 pack)
Antibodies			
IL-6	7061	MP5-20F3	Purified, FG Purified, FITC, PE
IL-17A	7177	eBio17B7	Biotin, FITC, PE, APC, Alexa Fluor® 488, Alexa Fluor® 647, PerCP-Cy5.5
IL-17F	7471	eBio18F10	PE, Alexa Fluor® 488, Alexa Fluor® 647
IL-17RA	7182	PAJ-17R	PE
IL-21	7211	FFA21 (Neutralizing)	Alexa Fluor® 647, PE, Functional Grade, APC
	7213	mhalx21	Alexa Fluor® 647, PE
IL-22	7221	1H8PWSR	PE, PerCP-eFluor® 710
IL-23/IL-12 p40	7123	C17.8 (Neutralizing)	FG Purified, Biotin, PE, Alexa Fluor® 647, PerCP-Cy5.5
IL-23	7232	G23-8 (Neutralizing)	Purified, FG Purified
RORγ(t)	6988	AFKJS-9	Purified, PE, APC
	6981	B2D	Purified
FlowCytomix™			
IL-6	BMS8603FF	-	-
IL-17A	BMS86001FF	-	-
IL-22	BMS86016FF	-	-
IL-23	BMS86017FF	-	-
TGFβ	BMS8608FF	-	-

Rat	Cat. No.	Clone	Formats
ELISA/ELISPOT			
IL-17A	7170	--	ELISA Ready-SET-Go!® Set
Recombinant Proteins			
IL-6	8060	--	Purified
IL-17A	8170	--	Purified

Human	Cat. No.	Clone	Formats
ELISA/ELISPOT			
IL-6	7066	--	ELISA Ready-SET-Go!® Set
	7966	--	ELISA Ready-SET-Go!® Kit (w/ pre-coated plates)
	7869	--	ELISPOT Ready-SET-Go!® Set
	BMS213INST	--	Instant ELISA®
IL-17A	7176	--	ELISA Ready-SET-Go!® Set
	7976	--	ELISA Ready-SET-Go!® Kit (w/ pre-coated plates)
	7876	--	ELISPOT Ready-SET-Go!® Set
IL-17AF	7117	--	ELISPOT Ready-SET-Go!® Set
IL-21	7216	--	ELISPOT Ready-SET-Go!® Set
IL-22	7522	--	ELISPOT Ready-SET-Go!® Set
IL-23	7237	--	ELISA Ready-SET-Go!® Set
	7239	--	ELISA Ready-SET-Go!® Kit (w/ pre-coated plates)
TGFβ1	7344	--	ELISA Ready-SET-Go!® Set
	7449	--	ELISA Ready-SET-Go!® Kit (w/ pre-coated plates)
	BMS249/2INST	--	Instant ELISA®
Recombinant Proteins			
IL-6	8069	--	Purified, Carrier-free, Single-use ELISA Standards (4 pack)
IL-17A	8179	--	Purified, Carrier-free, Single-use ELISA Standards (4 pack)
IL-17F	8479	--	Purified, Carrier-free, Single-use ELISA Standards (4 pack)
IL-17AF	8178	--	Purified, Carrier-free
IL-21	8219	--	Purified, Carrier-free, Single-use ELISA Standards (4 pack)
IL-22	8229	--	Purified, Carrier-free
IL-23	8239	--	Purified, Carrier-free, Single-use ELISA Standards (4 pack)
TGFβ1	8348	--	Purified, Carrier-free, Single-use ELISA Standards (4 pack)
Antibodies			
Act1	4040	9ACT12	Alexa Fluor® 647, Purified
CD161	1619	HP-3G10	PE, PerCP-Cy5.5
CD196 (CCR6)	1969	R6H1	FITC, PE, PE-Cy7, Purified
IL-6	7069	MQ2-13A5	Purified, FG Purified, FITC, PE, Alexa Fluor® 700
IL-17A	7179	eBio64DEC17	Biotin, FITC, PE, Alexa Fluor® 488, Alexa Fluor® 647, PerCP-Cy5.5, Purified, APC
IL-17F	7169	SHLR17	PE, Alexa Fluor® 647
IL-17RA	7517	J10MBS	PE
IL-21	7219	3A3-N2	PE, Alexa Fluor® 647, Purified
IL-22	7229	22URTI	PE, Alexa Fluor® 647, PerCP-eFluor® 710
RORγ(t)	6988	AFKJS-9	Purified, PE, APC
FlowCytomix™			
IL-6	BMS8213FF	-	-
IL-17A	BMS82017FF	-	-
IL-22	BMS82047FF	-	-
IL-23	BMS82023FF	-	-
TGFβ	BMS8249FF	-	-

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