

# Th17 Cells

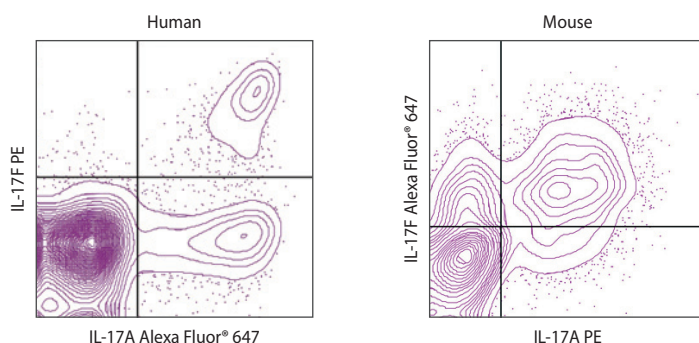
*The Complete Solution for Th17 Cell Analysis*

## 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,

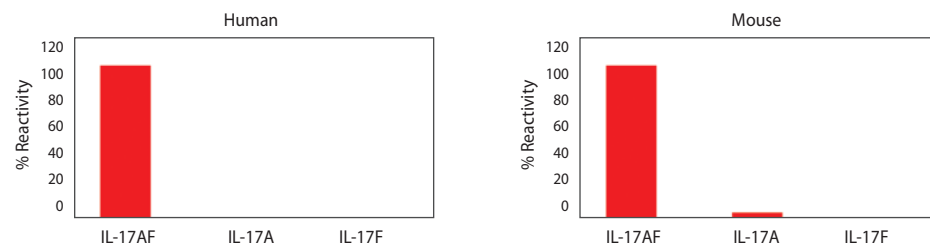
it was definitively shown that T cells could differentiate into IL-17-producing cells *in vitro* and *in vivo* independently of Th1 or Th2 development thereby establishing Th17 cells as a unique T helper cell lineage. In addition to IL-17A expression, it was recently demonstrated that Th17 cells express 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

- CD196 (h) **NEW**
- IL-17A (m) **NEW**
- IL-17F (h) **NEW**
- RORγ(t) (h, m) **NEW**
- Th17 Cytokine Flow Phenotyping Panels (h, m)

### ELISA KITS

- IL-6 Platinum ELISA (h, m, r) **NEW**
- IL-6 High Sensitivity ELISA (h, m) **NEW**
- IL-17A High Sensitivity ELISA (h) **NEW**
- IL-17AF Platinum ELISA (h) **NEW**
- IL-21 Ready-SET-Go!® ELISA (m) **NEW**
- IL-21 Platinum ELISA (m) **NEW**
- IL-23 Platinum ELISA (h) **NEW**
- TGFβ1 Ready-SET-Go!® ELISA (h, m) **NEW**

### FLOWCYTOMIX™ MULTIPLEX

- IL-17A (h, m, r) **NEW**
- TGFβ1 (h, m)

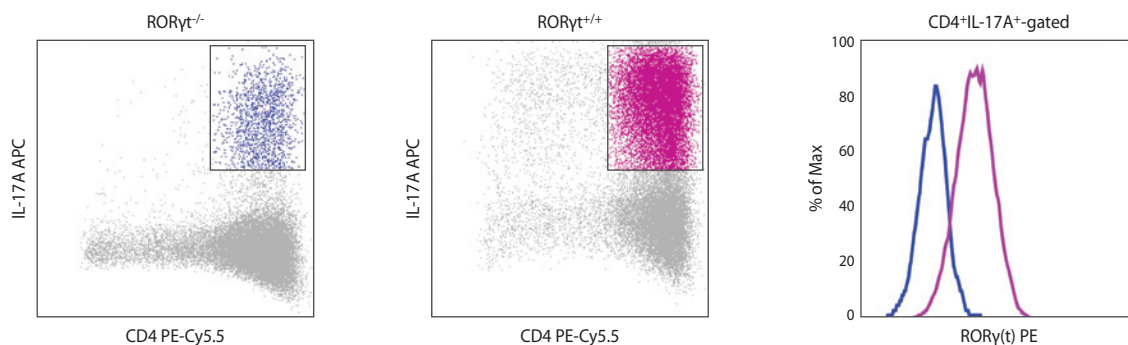
*h=human, m=mouse, r=rat*

## RORyt

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. RORyt, which directs a specific and heritable gene expression profile, is induced by the expression of TGFβ or IL-6. RORyt was

initially identified as a thymus-specific isoform of RORγ, and in 2005, it was discovered that RORyt is also expressed in Th17 cells. Deficiency in RORyt results in diminished Th17 activity and severely reduced expression of IL-17.

### Mouse/Human RORγ(t)



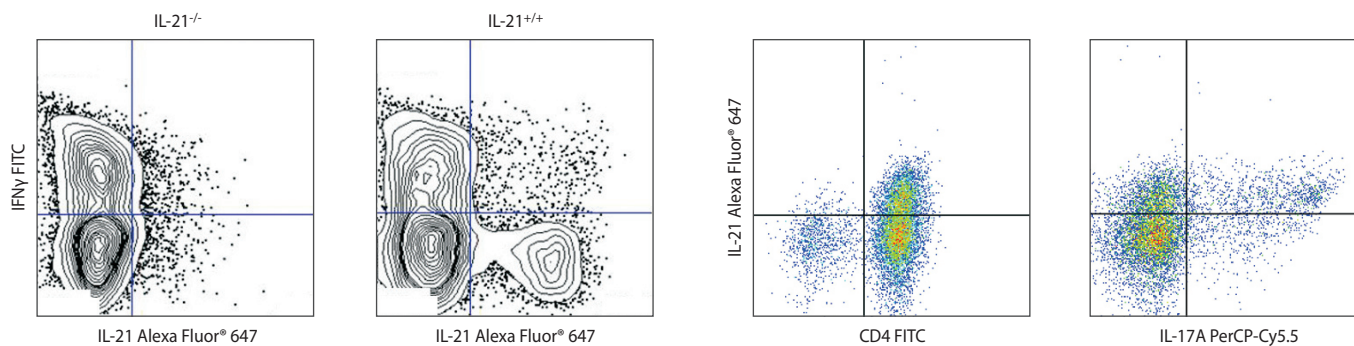
**Identification of Th17 cells by flow cytometric detection of RORγ(t).** CD4<sup>+</sup> T cells were sorted from RORyt-deficient (left dot plot) or wildtype (right dot plot) mouse spleen and lymph node, cultured in Th17-polarizing conditions for 3 days and stained with Anti-Mouse CD4 PE-Cy5.5, Anti-Mouse 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<sup>+</sup>IL-17A<sup>+</sup>-gated events from RORyt-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.

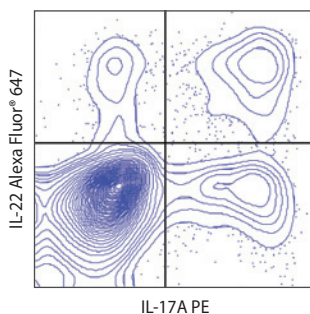
### Mouse IL-21



**Staining of Mouse IL-21 in LCMV-infected CD4<sup>+</sup> T Cells.** CD4<sup>+</sup>CD44<sup>+</sup> 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.

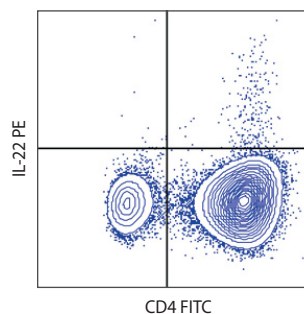
**Staining of IL-21 in Th17-polarized Mouse T Cells.** Mouse Th17-polarized splenocytes were stained with Anti-Mouse IL-21 Alexa Fluor® 647 (FFA21) (cat. no. 51-7211), Anti-Mouse IL-17A PerCP-Cy5.5 (eBio17B7) (cat. no. 45-7177) and Anti-Mouse CD4 FITC. Events displayed in the right plot are CD4<sup>+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

### Human IL-22



**Staining of IL-22 in Human Th17-polarized CD4<sup>+</sup> T Cells.** Human Th17-polarized CD4<sup>+</sup> 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 IFN $\gamma$  and IL-4, cytokines required for Th1 and Th2 maturation, respectively. TGF $\beta$  and IL-6 work in synergy to induce the maturation of Th17 cells, and the addition of TNF $\alpha$  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 TGF $\beta$  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	CD4 CD119 (IFN $\gamma$ R) IL-12RB2 Tim-3	CD4 IL-17RB Tim-1 CD294 (CRTH2)	CD4	CD4 IL-1R1 IL-12RB1 IL-23R	CD196 (CCR6) CD161 IL-13Ra1 IL-21R	CD4 CD140 (PDGFR) CCR10	CD4 Helios CD25 FR4(m) CD39 GITR CD73 GARP CD127 <sup>lo</sup> TIGIT CD152 LAP/TGF $\beta$	CD4 CD84 CD278 (ICOS) CD279 (PD-1) SAP IRF4 TIGIT CD126 (IL-6Ra) IL-21R CD130 (gp130)
Cytokine Expression	IFN $\gamma$ IL-2 TGF $\beta$	IL-4 IL-5 IL-10 IL-13	IL-9	IL-17A IL-17F IL-17AF IL-26	IL-21 IL-22 IL-26	IL-22 TNF $\alpha$	TGF $\beta$ LAP/TGF $\beta$ IL-6 IL-21	
Transcription Factor	T-bet	Gata-3	--	BATF c-maf ROR $\gamma$ t	--	c-maf c-rel Foxp3	BCL6 c-maf IRF4	
STAT Regulators	STAT1, 4	STAT6	--	STAT3	--	STAT5	STAT3	
Polarizing Cytokines	IL-2 IFN $\gamma$ IL-27	IL-4 IL-25 (IL-17E)	IL-4 TGF $\beta$	IL-6 TGF $\beta$ IL-21	TNF $\alpha$ IL-6	TGF $\beta$ LAP/TGF $\beta$	IFN $\gamma$	

### 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 <sup>®</sup> 647
	IL-22	22URTI	PerCP-eFluor <sup>®</sup> 710
CD4	RPA-T4	eFluor <sup>®</sup> 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 <sup>®</sup> 647
	IL-22	1H8PWSR	PerCP-eFluor <sup>®</sup> 710
	CD4	RM4-5	eFluor <sup>®</sup> 450

### Antibodies

Human	Cat. No.	Clone	Purified	FG Purified	Biotin	FITC	Alexa Fluor 488	PE	APC	PE-cy7	PerCP-Cy5.5	Alexa Fluor 647	Alexa Fluor 700	PerCP-eFluor 710
Act1	4040	9ACT12	•											
CD161	1619	HP-3G10					•			•	•			
CD196 (CCR6)	1969	R6H1	•			•	•	•						
IL-6	7069	MQ2-13A5	•	•		•	•						•	
IL-17A	7179	eBio64DEC17	•		•	•	•	•			•	•		
IL-17F	7169	SHLR17		•			•					•		
IL-17RA	7517	J10MBS					•							
IL-21	7219	3A3-N2	•				•					•		
IL-22	7229	22URTI					•					•		•
ROR $\gamma$ (t)	6988	AFKJS-9	•				•	•						
Mouse	Cat. No.	Clone												
IL-6	7061	MPS-20F3	•	•		•	•							
IL-17A	7177	eBio17B7			•	•	•	•	•	•	•	•		
IL-17F	7471	eBio18F10				•	•					•		
IL-17RA	7182	PAJ-17R					•							
IL-21	7211	FFA21		•			•	•				•		
		(Neutralizing)												
	7213	mhalx21					•					•		
IL-22	7221	1H8PWSR					•							•
IL-23/(IL-12 p40)	7123	C17.8 (Neutralizing)		•	•		•				•	•		
IL-23 (p19)	7232	G23-8 (Neutralizing)	•	•										
ROR $\gamma$ (t)	6988	AFKJS-9	•				•	•						
	6981	B2D	•				•							•

## Th17 Cell Proteins &amp; Immunoassays

Mouse	Cat. No.	ELISA Ready-SET-Go! <sup>®</sup>	ELISPOT Ready-SET-Go! Kit	FlowCytomix <sup>™</sup> Multiplex	High Sensitivity ELISA	Platinum ELISA	Single-use ELISA Standards	Recombinant Protein
IL-6	7064	•						
	7864		•					
	8061						•	•
	BMS603				•	•		
	BMS8603FF			•				
IL-17A	7371	•						
	7370		•					
	8171						•	•
	BMS6001					•		
IL-17F	BMS86001FF			•				
	7472	•						•
IL-17AF	8471							•
	BMS86020FF			•				
	7272	•						
IL-21	8172						•	•
	BMS6026					•		
	BMS86026FF			•				
	7210		•					
IL-22	8210	•						
	8211						•	•
	BMS6021					•		
	BMS86021FF			•				
	7422	•						
IL-23	8221							•
	BMS6022					•		
	BMS86022FF			•				
	7230	•						
TGFβ1	8231						•	•
	BMS6017					•		
	BMS86017FF			•				
TGFβ1	8350	•						
	BMS608					•		
	BMS8608FF			•				

Rat	Cat. No.	ELISA Ready-SET-Go! <sup>®</sup>	ELISPOT Ready-SET-Go! Kit	FlowCytomix <sup>™</sup> Multiplex	High Sensitivity ELISA	Platinum ELISA	Single-use ELISA Standards	Recombinant Protein
IL-6	8060							•
	BMS625					•		
IL-17A	7170	•						
	8170						•	•
	BMS635					•		
IL-17F	BMS8635FF			•				
	8116							•
TGFβ1	8117							•
	BMS623					•		

Human	Cat. No.	ELISA Ready-SET-Go! <sup>®</sup>	ELISPOT Ready-SET-Go! Kit	FlowCytomix <sup>™</sup> Multiplex	High Sensitivity ELISA	Instant ELISA	Platinum ELISA	Single-use ELISA Standards	Recombinant Protein
IL-6	7066	•							
	7869		•						
	8069							•	•
	BMS213				•	•	•		
IL-17A	BMS8213FF			•					
	7176	•							
	7876		•						
	8179							•	•
IL-17F	BMS2017				•		•		
	BMS82017FF			•					
	7478	•							
IL-17AF	8479							•	
	BMS2037						•		
	BMS82037FF			•					
IL-21	7117	•							
	8178							•	
	BMS2082						•		
	BMS82082FF			•					
IL-22	7216	•							
	8219							•	•
	BMS2043						•		
IL-23	7522	•							
	8229								•
	BMS2047						•		
IL-23 (p19/p40)	BMS82047FF			•					
	7237	•							
	8239							•	•
	BMS2023						•		
TGFβ1	BMS82023FF			•					
	7239						•		
TGFβ1	BMS2023						•		
	8348							•	•
	8350	•							
	BMS249					•	•		
	BMS8249FF			•					