

Th17 Cells

Bridging Innate and Adaptive Immunity

Featuring

Antibodies

- AHR (h, m) *New*
- IκBζ (h, m) *New*
- c-Maf (h, m) *New*
- BATF (h) *New*
- IL-17A, AF and F (h, m)

ELISA Kits

- IL-17A Platinum ELISA (h, m)
- IL-17AF Platinum ELISA (h, m)
- IL-22 Single-Use ELISA RSG Standard (h) *New*

ProcartaPlex™ Multiplex Immunoassays

- IL-17A Simplex (h, m) *New*
- IL-21 Simplex (h) *New*
- IL-22 Simplex (h, m) *New*
- Th1/Th2/Th9/Th17/Th22/Treg Cytokine Panel (h, m) *New*

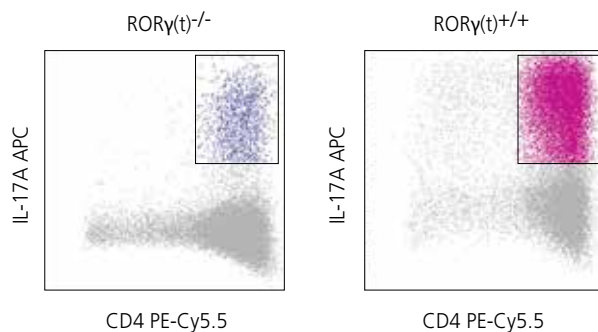
h=human, m=mouse

Th17 cells play a role in host defense against extracellular pathogens as well as a role in the pathogenesis of multiple inflammatory and autoimmune disorders. Th17 cells were an early addition to the Th1/Th2 paradigm of helper cell differentiation when it was shown that IL-17 (also known as IL-17A) was produced and secreted as a homodimer or heterodimer (with IL-17F) by a unique subset of T helper cells. Subsequently, RORγ(t) was identified as the transcription factor responsible for specifying the lineage differentiation of Th17 cells. Th17 cell development is also driven and regulated by the expression of transcription factors STAT3, AHR, BATF, IRF4, c-Maf, IκBζ and RORα.

ROR γ (t) and the Transcriptional Control of Th17 Differentiation

Similar to the role of T-bet and Gata-3 in Th1 and Th2 cells respectively, the transcription factor ROR γ (t) has been identified as the “master regulator” of Th17 cell differentiation. ROR γ (t), which directs a specific and heritable gene expression profile, is induced by the expression of TGF β or IL-6 via direct regulation by STAT3. Deficiency in ROR γ (t) results in diminished Th17 activity and severely reduced expression of IL-17. While deficiency in

ROR α has little effect on Th17 differentiation, ROR γ (t)/ROR α double-deficient mice demonstrate a complete block in Th17 formation. Similarly, the aryl hydrocarbon receptor (AHR) was found to be preferentially expressed by Th17 cells and can promote the expression of IL-17 and IL-22. Additionally, the transcription factors BATF, Runx1 and IRF4 have all been shown to be important for IL-17 expression and may work in conjunction with ROR γ (t) to control cytokine expression.



Identification of Th17 cells by flow cytometric detection of ROR γ (t)

CD4⁺ T cells were sorted from ROR γ (t)-deficient (left) or wildtype (right) mouse spleen and lymph node, cultured in Th17-polarizing conditions for 3 days and stained with Anti-Mouse CD4 PE-Cy5.5 (cat. no. 35-0042), Anti-Mouse IL-17A APC (cat. no. 17-7177) and Anti-Mouse/human ROR γ (t) PE (AFKJS-9) (cat. no. 12-6988). The histogram shows staining of ROR γ (t) in CD4⁺IL-17A⁺-gated events from ROR γ (t)-deficient mice (blue population) and wildtype mice (pink population). Cells in the lymphocyte gate were used for analysis.

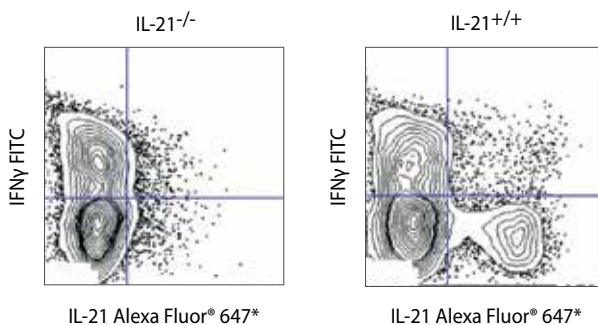
Data provided courtesy of DR Littman, New York University.

Cytokine Production

Th17 cells were originally named based on their unique ability to secrete the cytokine IL-17. The two isoforms IL-17A and IL-17F both bind to IL-17RA and IL-17RC to induce signaling, dependent on the Act1 adaptor protein, resulting in the induction of pro-inflammatory cytokines in many different cell types.

Since their initial discovery it has become clear that in addition to IL-17 family members, Th17 cells also express many other effector cytokines including IL-21 and IL-22.

Expression of IL-21 is dependent on STAT3-mediated IL-6 signaling in Th17 cells and is thought to act in synergy with TGF β to promote Th17 differentiation through autocrine signaling via IL-21R. In contrast to the autocrine function of IL-21, Th17-secreted IL-22 binds IL-22R on target cells (largely epithelial 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.



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 IFN γ FITC (cat. no. 11-7311) and Anti-Mouse IL-21 Alexa Fluor[®] 647 (FFA21) (cat. no. 51-7211).*

Data provided courtesy of Amanda Poholek and Joseph Craft, Yale University.

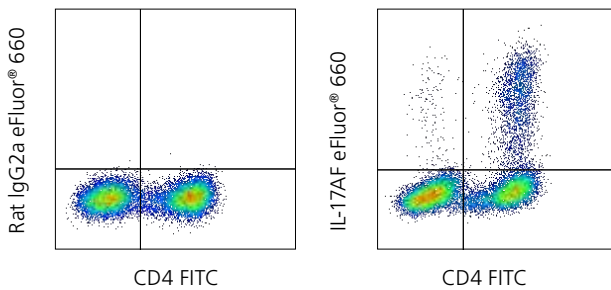
*Alexa Fluor[®] 647 has been replaced with eFluor[®] 660

Defining Th17 T cells											
Surface Expression			Cytokine Expression			Transcription Factor			STAT Regulator	Polarizing Cytokine	
CD4	IL-1R1	IL-12RB1	IL-17A	IL-17F	IL-17AF	ROR γ (t)	ROR α	IRF4	STAT3	IL-6	IL-1 β
IL-23R	IL-21R	CD196 (CCR6)	IL-21	IL-22	IL-26	c-Maf	AHR	Hif1 α		IL-23	TGF β
CD194 (CCR4)	CD161	IL-13Ra1	TNF α	GM-CSF		Runx1				IL-21	

Commitment and Identification of Th17 Cells

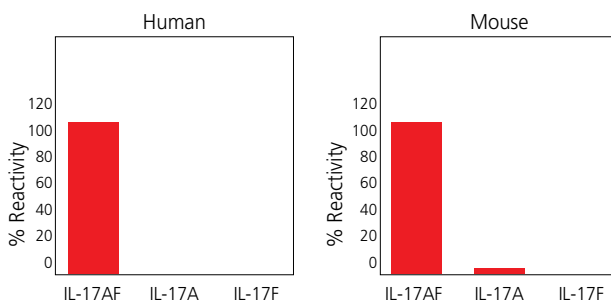
It has been shown that TGF β , IL-6, and IL-1 β can be potent drivers of the differentiation of Th17 cells. While much attention has focused on the cytokine expression patterns of Th17 cells, these cells may also be identified by the surface expression of a number of chemokine and cytokine receptors driven by polarizing cytokines. Th17 cells tend to express CCR6 and CCR4 but lack CCR10 or IL-33R (ST-2).

CCR6 and CCR4 are constitutively expressed on both human and mouse Th17 cells, whereas the marker CD161 (the human homolog of NK1.1) is found only on human cells. After induction toward Th17 commitment by TGF β and IL-6, IL-23R is also selectively up-regulated by Th17 cells, as IL-23 signaling is important for their expansion and survival.



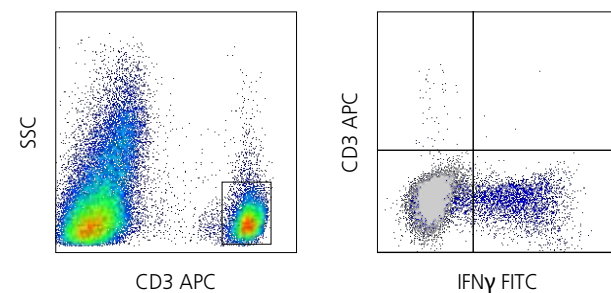
Intracellular staining of IL-17AF heterodimer in Th17-polarized Human T cells

Th17-polarized mouse splenocytes with Anti-Mouse CD4 FITC (cat. no. 11-0042) and Rat IgG2a K Isotype Control eFluor® 660 (cat. no. 50-4321) (left) or Anti-Mouse IL-17AF eFluor® 660 (cat. no. 50-9171)(right). Cells were treated for 5 hours with Cell Stimulation Cocktail plus protein transport inhibitors (cat. no. 00-4975) prior to harvest and staining. Cells in the lymphocyte gate were used for analysis.



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.



Whole Blood Th1/Th17 cytokine analysis

Fresh human whole blood was stimulated at 37°C for 5 hours with Cell Stimulation Cocktail (plus protein transport inhibitors) (cat. no. 00-4975). After stimulation, blood was lysed, fixed and permeabilized with eZKine™ Fix/Lyse and Perm Buffers, then stained with the Th1/Th17 staining cocktail. CD3 positive cells were gated (left) and then analyzed for staining of IFN γ and IL-17A. Isotype cocktail staining for IFN γ and IL-17A is shown in gray (right).

Human Antibodies																
Antigen	Clone	Catalog Number	Purified	Biotin	Functional Grade	Violet Laser	Blue Lasers				Green, Yellow-Green Lasers			Red Lasers		
						eFluor® 450	FTTC	Alexa Fluor® 488	PerCP-Cyanine5.5	PerCP-eFluor® 710	PE	PE-eFluor® 610	PE-Cyanine7	APC	eFluor® 660	Alexa Fluor® 700
Act1	9ACT12	4040	■													
AHR	FF3399	9854	■							■	■				■	
CD161	HP-3G10	1619				■		■	■					■		
CD196 (CCR6)	R6H1	1969	■				■			■	■			■		
c-Maf	SYM0F1	9855								■					■	
IκBζ	HFT2NAP	9853									■					
IL-17A	eBio64DEC17	7179	■	■			■	■	■		■	■	■	■	■	
IL-17AF	20LJS09	9179				■	■				■					
IL-17F	SHLR17	7169			■					■			■		■	
IL-17RA	J10MBS	7517									■					
IL-21	3A3-N2	7219	■								■				■	
IL-22	22URTI	7229				■				■			■		■	
	IL22JOP	7222			■					■				■	■	
IL-23 (p19)	23dcdp	7823									■				■	
IL-23/IL-12 (p40)	eBioHP40	7235								■	■				■	
	C8.6	7129	■			■		■			■				■	
IL-6	MQ2-13A5	7069	■		■	■	■			■	■			■		■
IRF4	3E4	9858	■			■	■			■	■				■	
RORγ(t)	AFKJS-9	6988	■								■			■		
Mouse Antibodies																
AHR	4MEJJ	5925						■			■				■	
BATF	MBM7C7	9860								■	■				■	
c-Maf	SYM0F1	9855								■					■	
IκBζ	LK2NAP	6801	■							■						
IL-17A	eBio17B7	7177		■		■	■	■	■		■			■	■	
IL-17AF	B8KN8R	9171													■	
IL-17F	eBio18F10	7471						■		■	■				■	
IL-17RA	PAJ-17R	7182									■			■		
IL-21	FFA21 (Neutralizing)	7211			■						■			■	■	
	mhalx21	7213									■				■	
IL-22	1H8PWSR	7221								■	■					
	IL22JOP	7222			■					■				■		
IL-23 (p19)	G23-8 (Neutralizing)	7232	■		■											
IL-23/(IL-12 p40)	C17.8 (Neutralizing)	7123		■	■	■		■	■		■				■	
	fc23cpg	7023													■	
IL-6	MP5-20F3	7061	■		■		■				■					
IRF4	3E4	9858	■			■	■			■	■				■	
RORγ(t)	AFKJS-9	6988	■								■			■		
	B2D	6981	■								■			■		

Human Proteins and Immunoassays

Antigen	Recombinant Protein	ELISA Ready-SET-Go! [®] (RSG)	Single-use RSG ELISA Standard	ELISPOT Ready-SET-Go! [®] Kit	Platinum ELISA	High Sensitivity ELISA	Instant ELISA	ProcartaPlex [™] Simplex
GM-CSF	14-8339	88-8337	39-8339		BMS283		BMS283INST	EPX010-10283
IL-17A	14-8179	88-7176	39-8179		BMS2017	BMS2017HS		EPX010-12017
IL-17AF	14-8178	88-7117	39-8178		BMS2082			EPX010-12082
IL-17F	14-8479	88-7478	39-8479		BMS2037/2			EPX010-12160
IL-21	14-8219	88-8218	39-8219		BMS2043			EPX010-12043
IL-22	14-8229	88-7522	39-8229		BMS2047			EPX010-12047
IL-23	14-8239	88-7237	39-8239		BMS2023/3			EPX010-12023
IL-6	14-8069	88-7066	39-8069		BMS213/2	BMS213HS	BMS213INST	EPX010-10213
TGFβ1	14-8348	88-8350 88-50390	39-8348		BMS249/4		BMS249/3INST	EPX010-10249
TNFα	14-8329	88-7346	39-8329		BMS223/4 BMS2034	BMS223HS	BMS223INST	EPX010-10223

Mouse Proteins and Immunoassays

GM-CSF	14-8331	88-7334	39-8331		BMS612			EPX010-20612
IL-17A	14-8171	88-7371	39-8171	88-7370	BMS6001			EPX010-26001
IL-17AF	14-8172	88-8711	39-8172		BMS6026			EPX010-26026
IL-17F	34-8471	88-7472			BMS6020			EPX010-26020
IL-21	14-8211	88-8210	39-8211	88-7210	BMS6021			EPX010-26021
IL-22	14-8221	88-7422	39-8221		BMS6022			EPX010-26022
IL-23	14-8231	88-7230	39-8231		BMS6017			EPX010-26017
IL-6	14-8061	88-7064	39-8061		BMS603/2	BMS603HS		EPX010-20603
TGFβ1	14-8342	88-8350			BMS608/4			EPX010-20608
TNFα	14-8321	88-7324	39-8321	88-7328	BMS607/3	BMS607HS	BMS607/2INST	EPX010-20607

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	eFluor [®] 660
	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	eFluor [®] 660
	IL-22	1H8PWSR	PerCP-eFluor [®] 710
	CD4	RM4-5	eFluor [®] 450

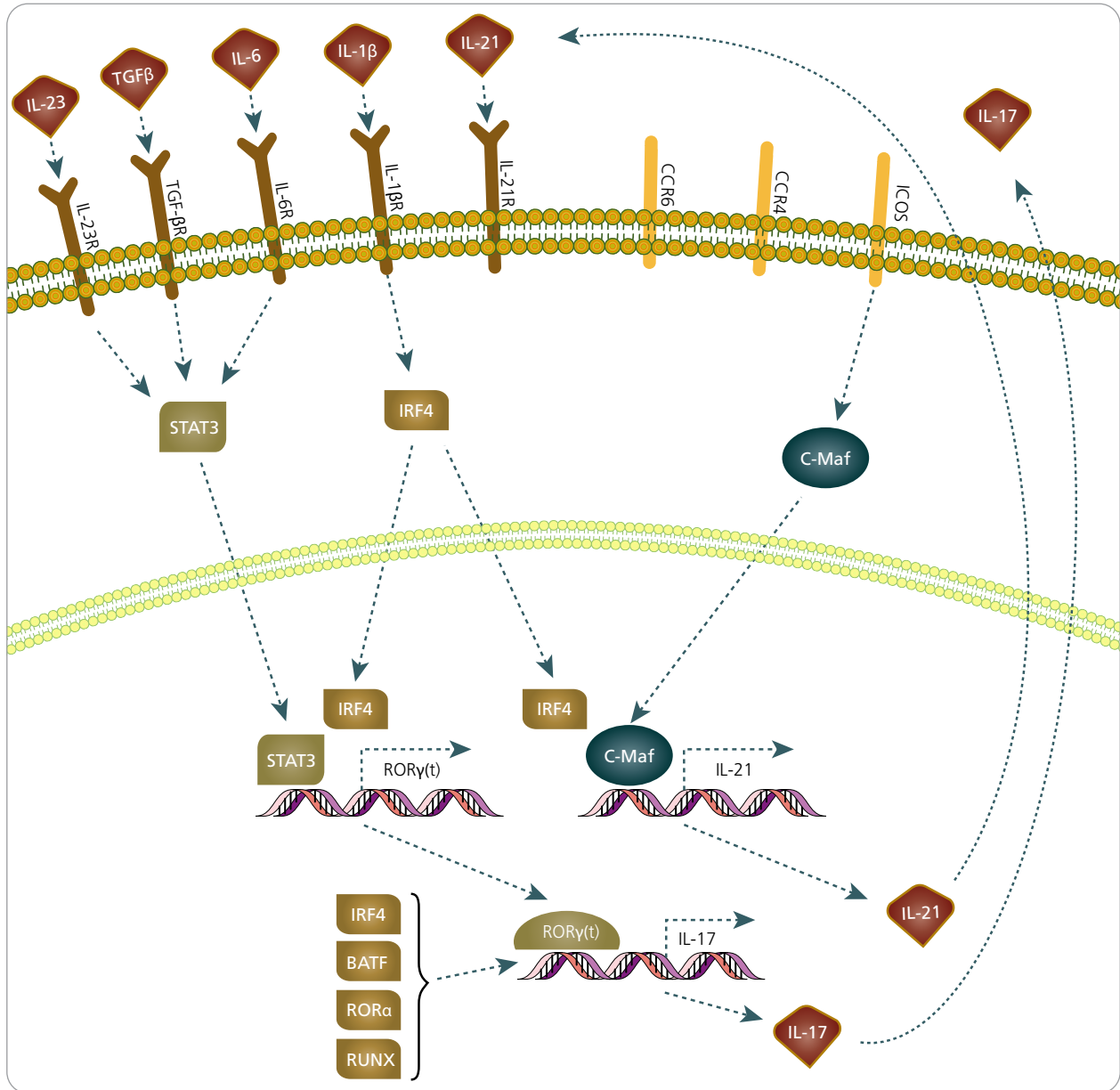
eZKine[™] Whole Blood Cytokine Staining Kits

Cat. No.	eZKine [™] Kits	APC	PerCP-eFluor [®] 710	FITC	PE
8822-6850	eZKine [™] Th1/Th17	CD3	CD4	IFNγ	IL-17A
8822-6851	eZKine [™] Th1 Activation 1	CD3	CD4	IFNγ	CD69
8822-6852	eZKine [™] Th1 Activation 2	CD3	CD4	IFNγ	TNFα
8822-6853	eZKine [™] Th17/Th22	CD3	CD4	IL-17A	IL-22
8822-6854	eZKine [™] CD8 Activation 1	CD3	CD8	IFNγ	CD69
8822-6855	eZKine [™] CD8 Activation 2	CD3	CD8	IFNγ	TNFα
8822-6856	eZKine [™] Compensation Kit				

Related Dyes and Buffers

Reagent	Laser Excitation	Format	Cat. No.
Fixable Viability Dye	UV (355 nm)	eFluor [®] 455 (UV)	65-0868
	Violet (405 nm)	eFluor [®] 405	65-0863
	Violet (405 nm)	eFluor [®] 506	65-0866
	Violet (405 nm)	eFluor [®] 520	65-0867
	Red (633 nm)	eFluor [®] 660	65-0864
	Red (633 nm)	eFluor [®] 780	65-0865
Cell Stimulation Cocktail			00-4970
Cell Stimulation Cocktail (with transport inhibitors)			00-4975
Foxp3 / Transcription Factor Staining Buffer Set			00-5523

Signals Influencing Th17 Differentiation and Effector Function



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