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Genes associated with T helper 17 cell differentiation and function

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TABLE OF CONTENTS

1. ABSTRACT

Interleukin-17 (IL-17)-producing T helper cells (Th17 cells) constitute a lineage of CD4 effector T helper cells that is distinct from the Th1 and Th2 CD4 phenotypes. In humans, Th17 differentiation is induced in the presence of the cytokines IL-1 beta, IL-6 and TGF beta, whereas IL-23 maintains Th17 survival. Effector human Th17 cells express several cytokines and cell surface markers, including IL-17A, IL-17F, IL-22, IL-26, CCR6 and TNFα. Studies on human cells have revealed that the RORC2 transcription factor plays an effective role in Th17 differentiation. Th17 cells contribute to the host immune response by involving various pathologies, including rheumatoid arthritis, multiple sclerosis and Crohn's disease. However, the full extent of their contribution to diseases is being investigated. The differentiation of Th17 cells is controlled by many transcription factors, including ROR gammat, IRF4, RUNX1, BATF, and STAT3. This review covers the general principles of CD4 T helper differentiation and the known transcription factors that play a role in the recently discovered Th17 cells.

2. INTRODUCTION

CD4 T cells are accepted as important actors in immune responses due to their capacity to regulate and coordinate other cells of the immune system. Because they are major players in the immune response, the characterization of these cells is crucial. Upon antigenic stimulation, naive CD4 T cells activate, proliferate and differentiate into different effector helper T cell subsets referred to as T helper (Th) 1 and Th2 (1,2). These T cell subsets are characterized by their distinct cytokine production profiles and effector functions and carry their own specific, distinctive and heritable markers.

Interferon-gamma is the signature cytokine produced by Th1 cells and is responsible for immunity against intracellular pathogens. In addition, Th1 cells express genes associated with cytotoxicity, such as the Fas ligand and granzymes. Uncontrolled Th1 responses against self-antigens can lead to the development of autoimmunity. Th1 cells are generated from naïve T helper cells by T cell receptor (TCR) engagement and Signal Transducer and Activator of Transcription 1 (STAT1) signaling, which are induced by the IFN-induced activation of the IFNγR. Phosphorylated STAT1 induces the expression of transcription factor T-bet, which drives Th1 differentiation by trans-activating the Th1 signature cytokine IFN-gamma and the specific subunit of the receptor for interleukin (IL)-12, IL-12Rβ2. Thus, the cell becomes responsive to IL-12, which is produced by activated antigen-presenting cells (APCs), and subsequent IL-12 signaling through Signal Transducer and Activator of Transcription 4 (STAT4) further stabilizes the Th1 phenotype. IL-4, IL-5 and IL-13 are secreted by Th2 cells, which play important roles in clearing extracellular pathogens and mediating allergic responses (3-7).

A distinct subset of CD4 T cell effectors was recently identified and named T helper 17 cells (Th17) because these cells produce the cytokine IL-17 (Harrington *et al.*, 2005, Park *et al.*, 2005). Th17 cells

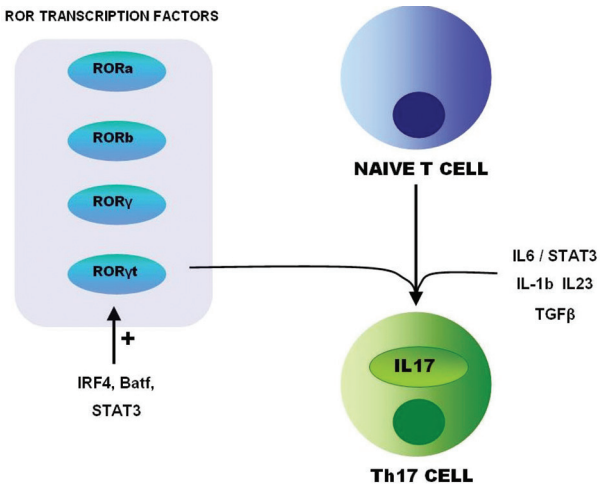

Figure 1. Th17 differentiation. Different members of the ROR family play differing roles in various cellular niches. RORC2 is one of the cytokines required for Th17 differentiation from naive T cells. RORC2 partners with various proteins to affect Th17 differentiation and is positively regulated by a number of factors. IRF4, BATF and STAT3 positively regulate RORC2, which partners with IL-6, STAT3, IL-1b, IL-23 and TGFb to form the interleukin 17 producing Th17 cells.

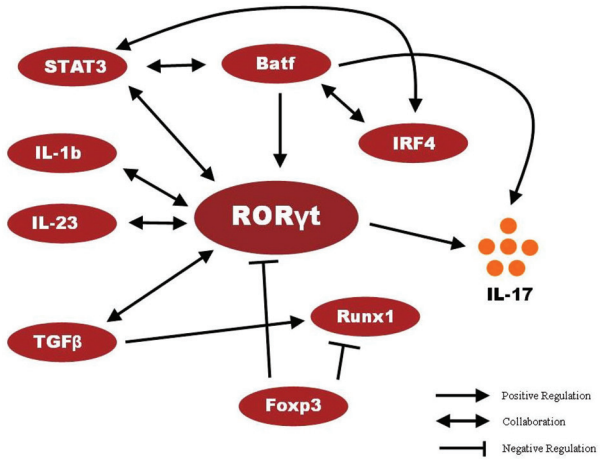

Figure 2. Networking of transcription factors involved in the Th17 differentiation. Most factors regulate differentiation through collaboration (indicated by double-headed arrow), while some regulate the expression or activity of others either positively (unilateral arrow) or negatively (blunt-headed line). Production of IL-17 is the main trait of Th17 cells, which is mainly upregulated by RORC2 and BATF. FOXP3 is known to inhibit RORC2 and RUNX1, while the remaining proteins either collaborate with each other or positively regulate others to drive IL-17 production in Th17 cells.

also produce the cytokines IL-17A, IL- 17F and IL-22 (Annunziato *et al.*, 2007, Wilson *et al.*, 2007) and the chemokine receptor CCR6 (Acosta-Rodriguez *et al.*, 2007b, Annunziato *et al.*, 2007, Hirota *et al.*, 2007, Lim *et al.*, 2008, Singh *et al.*, 2008). Th17 differentiation is directed by lineage-specific transcription factors, including RORγt (or RORC) and RORα, and is controlled by the coordinated activity of a series of positive and negative regulators (8-10).

3. LINEAGE SPECIFICATION OF TH17 CELLS

3.1. The retinoic acid-related orphan receptors (RORs)

ROR is a nuclear receptor containing an N-terminal domain, a DNA-binding domain, a hinge region, a ligand-binding domain and a C-terminal region. In most nuclear receptors, ligand binding to the ligand-binding domain induces a conformational change, recruits transcriptional co-activators and starts the transcription process. The ROR family has three members: RORα, RORβ and RORγ (or RORC). Two different forms of RORγ, RORγ and RORγt (or RORC2), are present in mammalian cells, and the only difference between there isoforms is the N terminus. RORγt is sufficient for IL-17 expression as well as Th17 cell differentiation from human CD4 T cells. RORγt functions with IL6/STAT3, TGFβ, IL-1β and IL-23 to generate Th17 cells. IL-23 and RORgt provide Th17 mediated immunity. Several transcription factors, such as IRF4, BATF, STAT3 and E3 deubiquitinase USP17, control Th17 differentiation by positively regulating RORC expression (11-15).

Foxo1 inhibits RORγt activity through the T cell intrinsic binding of its DNA binding domain, whereas inverse agonists and antagonists (such as TMP778) of RORγt, as well as siRNAs specific to Rorc, have further helped characterize the role of RORγt in Th17 differentiation (16-19).

In addition to the ROR family of receptors, Th17 cells express several other receptors for the differentiation or recognition of other immune cells as well as target tissues. Of these receptors, the following are considered crucial: IL-6R, IL-23R and the TNF receptor CD120a, which are responsible for the initial differentiation mechanisms of Th17 cells, as well as IL-22R, which uses the IL-22 produced by the differentiated cell as its ligand and plays a role in the maintenance of the Th17 phenotype (20-22).

3.2. Interferon-inducible factor-4 (IRF4)

Mammalian interferon regulatory factor (IRF) members play many roles in the immune system, including the regulation of T cell differentiation. IRF4 binds weakly to DNA, and its expression is restricted to immune cells. IRF4 needs cofactors to stably bind DNA. In B cells, the binding of PU.1 to DNA facilitates the recruitment of IRF4. In IL-21 treated B cells, IRF4 interacts with STAT3. $Irf4^{-/-}$ CD4-positive T cells exhibit a failure in STAT3 binding and form defective T follicular helper cells (Tfh). IRF4 can interact with Batf (the ATL-like basic leucine zipper transcription factor)-Jun complex to control transcriptional regulation (23-26).

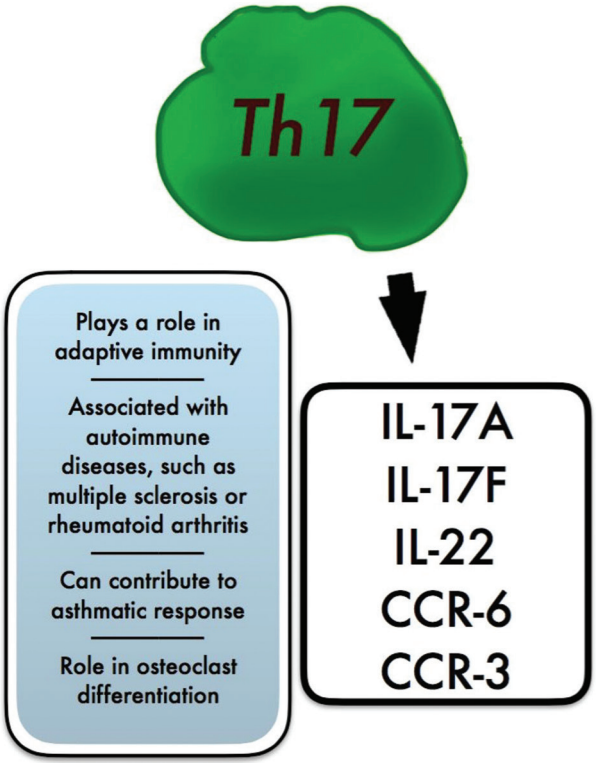

Figure 3. Differentiated interleukin-17 producing T helper (Th17) cells fill important niches in the immune system. The key cytokines produced by Th17 cells are interleukin (IL) 17A, IL-17F, IL-22 and CCR6.

IRF4 aids Th17 cell differentiation and is required for the induction of RORγt and RORα. RhoA-associated kinase (ROCK) 2 is activated under Th17 polarizing conditions and phosphorylates IRF4, which directly binds to IL-17A and IL-21 promoters, inducing their transcriptional activation. The gene expression of IRF4 is induced by IL-1beta, which prompts a 5.4-fold increase in expression, and by IL-6, which results in a 5.5-fold increase in expression. There is a positive relationship between IRF4 and IL-17A gene expression, indicating that IRF4 plays an important role in human Th17 cell differentiation [2-6-7]. IL-6 mainly activates STAT3 through the JAK-STAT pathway. STAT3 binds to the promoters of various genes, such as RORC, IL-17, IL-17F and IL-21, and interacts with the BATF, IRF4 and c-MAF genes. These transcriptional factors are important for Th17 cell differentiation (23-26).

3.3. B-cell activating transcription factor (BATF)

BATF is a basic leucine zipper transcription factor of the activator protein-1 (AP-1) family that helps generate Th17 cells. BATF is required for the generation of T follicular helper (Tfh) cells but not Th1 cells and Treg cells. BATF interacts with IRF4 for the generation of Th2 and Th17 cells and is involved in the induction of the IL-10, IL-17alpha and IL-21 genes in T cells. As a result, IL-6 activates the function of the BATF/IRF4 complex. Previous research has shown that IRF4 is activated upon IL-1 signaling and is critical for early Th17 cell differentiation. IRF4 interacts with NFATp (nuclear factor of activated T-cells) to induce IL-4 expression, suggesting that IRF4 modulates NFATp-dependent IL-2 expression, which is related with IL-17 production (27-30).

During the process of Th17 differentiation, BATF and RORγt directly attach to the promoter of the IL17 gene and induce its transcription in a synergistic manner. However, it has been observed that Th cells lacking BATF fail to differentiate to Th17 cells, despite the presence of RORγt, RORα and IL-6. In addition to these findings, the function of BATF in Th17 differentiation remains unclear (27-30).

3.4. Runt-related transcription factor 1(RUNX1)

Runt-related transcription factor 1 (RUNX1), which is also known as acute myeloid leukemia 1 protein (AML1) and core binding factor subunit alpha-2 (CBFA2), is a protein encoded by the RUNX1 gene in humans. Transforming growth factor-beta (TGFβ) induces the expression of the Runx related transcription factors RUNX1 and RUNX3 in CD4-positive T cells. This induction is required for the binding of RUNX1 and RUNX3 to RUNX binding sites in the FOXP3 promoter. Runx proteins form transcriptional complexes with Foxp3, RORγt and T bet (inhibitor of RORγ gene expression) to carry out Th differentiation and cytokine production. Runx1 induces RORγt expression and is required for the differentiation of Th17 cells and for Foxp3 function. The binding of RORγt and Runx1 together to the IL-17α locus leads to increased expression of IL-17. The Runx1 and Runx3 transcription factors induce the differentiation of both Th1 and Th17 cells depending on the Runx interacting partner (Th1 and Th17 cell transcription factors, respectively). However, Foxp3 prevents both Runx1 and RORgt activity. Runx1 can modify RORγt/Foxp3 complexes and differentially associates with RORγt or Foxp3, thus regulating or repressing transcriptional activity depending on the specific cytokine interactions (31-34).

Both Runx1 and Runx3 maintain high levels of Rorc expression in Th17 cells. The Runx transcription factors are weak transcriptional factors that interact with other transcription factors to activate or repress gene expression. If the concentration of Runx1 is sufficiently high to form both the Runx1-T-bet and Runx1-RORγt transcription complexes, a T helper cell can simultaneously express IFNγ and IL-17A. The overexpression of Runx1 or Runx3 in Th17 cells results in the induction of Stat4 and Tbx21, which are downstream factors of IL12 signaling. Runx1 is a stronger activator of the Tbx21 and Stat4 genes than Runx3, resulting in higher amounts of IFNγ producing cells. Both Runx1 and Runx3 maintain high Rorc expression

Table 1. Genes and interactions in Th17 function and differentiation

Gene name	Role in Th17	Role in immune system	Associations with other proteins	References
BATF			Induces IL-17 transcription	27,30
CCR6	Surface expression factor	Associated with colorectal cancer and Crohn's disease	Chemokine receptor	8,9,10
Foxo1			Inhibits the RORC program	16
Foxp3			Antagonizes ROR production by Th17	39
IL-17A	Defining gene of Th17	Recruits monocytes and neutrophils to inflammation site	Induced by IL-22	8,9,10
IL-17F		Pro-inflammatory role in asthma	IL-17RA and IL-17RC as receptors	8,9,10
IL-1beta			Induces expression of IRF4	24
IL-22		Adaptive innate immune response	IL-22R1 and IL-10R2 complex as receptor	9
IL-23		Cytokine expression		14
IL-26			Induces rapid phosphorylation of STAT3	39
IL-6		Cytokine expression	Activates STAT3	14
TGFbeta			Reciprocal relationship between TGFbeta and Foxp3	5,6,7,8,39
IRF4			Induces RORgammat, RORalpha, IL-17A, IL-22	23,24
RORγt	Master regulator transcription factor			11,13
RUNX1			Binds to RORgammat, Foxp3 and T-bet	31,32,33
STAT3	STAT regulator of Th17 cells		Controls transcription of Th17 differentiation	44,45,46,47
TNFα	Th17 cytokine	Involved in systemic inflammation		46
USP17			Positively regulates RORC expression	15

despite increased expression of Tbx21 and Stat4 (inhibitors of the Rorc gene) (31-34).

3.5. FOXP3 and T-BET

Th17 and Treg cells exhibit a close, reciprocal relationship through the molecules TGFβ and FOXP3 (forkhead box P3). TGFβ induces Treg differentiation and collaborates with or regulates proteins in the Th17 differentiation pathway, whereas the Foxp3 produced by Treg cells antagonizes the RORγt and RORα produced by Th17 cells. In addition, retinoic acid causes the preferential differentiation of Treg over Th17 cells because it enhances TGFβ and inhibits IL-6 receptor expression. Furthermore, the transcription factor hypoxia inducible factor 1-alpha (HIF1alpha) enhances RORγt expression and inhibits Foxp3 expression, which leads to the preferential differentiation of Th17 cells compared with Treg cells. These findings show that the immune system has mechanisms that can induce suppression or inflammation as desired, but the implications of this conclusion are not known yet (35-39).

Th1 specific T box transcription factor (T-bet) exerts an indirect antagonistic effect on Th17 differentiation by blocking Runx1 from binding and activating the RORC gene, which encodes the RORγt protein required to activate IL-17. Runx1 and T-bet interact during the early Th17 cell differentiation process. However, this interaction mutually prevents both proteins from activating their target genes, resulting in an equal amount of each protein completely inhibiting the other's function in the differentiation process. A higher amount of Treg favors Th1 differentiation, whereas a lower amount favors Th17 differentiation. T-bet can also bind to a protein binding element in the RORC promoter, preventing Runx1 from binding to its own adjacent binding site. It is unknown whether similar adjacent binding sites exist in other Runx1-activated genes, indicating that further investigation is required to understand the details and specificity of the repression mechanism (40-42).

3.6. Signal transducer and activator of transcriptions (STATs)

Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that is activated by the cytokines IL-17, IL-23, IL-6 and IL-21. The importance of STAT3 has been shown by the finding that the

deletion of STAT3 in T cells leads to the prevention of Th17 differentiation. STAT3 directly binds the IL-17 promoter, as was shown by a ChIP assay. STAT3 binds to enhancers and promoters of genes that are responsible for Th17 specification. STAT3 binds not only to the IL-17 promoter but also to the IL-21, IL-21r and IL-23r genes. The important role of STAT3 is the control of transcription factors involved in Th17 differentiation, such as IRF4, RORγt and Batf. STAT3 also plays an interesting role involving Th17 proliferation and survival. It was recently shown that the phosphorylation status of Smad2/Smad3 allows them to act as opposite cofactors of STAT3, which regulates Th17 differentiation through RORγt (43-47).

STAT proteins affect Th17 differentiation in a positive manner but can also affect this process in a negative manner. STAT1, which is activated by IL-23, plays an important role in Th17 differentiation. Th17 differentiation is inhibited in the absence of STAT1 (48). Another negative regulator of Th17 differentiation is STAT4, which is activated by IL-12 and acts as a positive regulator of Th1 differentiation and INFγ production. The production of IL-17 is suppressed by IL-2 and IFNγ (49). IL 2 activated STAT5 is involved in Treg and Th17 differentiation (50).

4. SUMMARY AND PERSPECTIVE

A distinct subset of CD4 T cell effectors denoted T helper 17 cells was recently identified. Th17 cells contribute to the host immune response by involving various pathologies, including multiple sclerosis, Crohn's disease and rheumatoid arthritis. The differentiation factors of Th17 cells in humans remain under investigation. An increasing body of evidence in the literature emphasizes that human CD4 T cells have a huge capacity to display plasticity during the differentiation of the T helper phenotype. Th17 cells can differentiate into Th1 and Th2 phenotypes, whereas FoxP3-positive Tregs can differentiate into Th1, Th2 and Th17 cells. Therefore, it is important to fully understand the differentiation mechanisms of T helper cells.

T helper 17 cells can express more than one master transcription factor. The interactions of transcription factors with other transcription factors and/or regulatory factors need to be further studied in detail. For example, BATF transcription factor expression is not limited to Th17 cells; in fact, this transcription factor can be expressed by all T helper cells, even though its role in other Th cells remains unclear. In addition to RORs, IRF4 and RUNX1, peroxisome proliferator activated receptor gamma (PPARγ) (51) and epidermal fatty acid binding protein (E-FABP) (52) have been shown to play a role in IL17 gene expression. However, the molecular mechanism underlying these regulations is not fully understood. Furthermore, it is also important to obtain a more in-depth understanding of the epigenetic regulation of T helper cells in order to comprehend the plasticity and stability of T helper cells.

Although the transcription factor RORC2 has been shown to be expressed in human Th17 cells, the relationships of RORC with other transcription factors remain unclear. It has been proposed that microRNAs in addition to cytokines and transcription factors can also play a role in T helper differentiation. Therefore, the roles of microRNAs in Th17 differentiation need to be clearly elucidated. MicroRNAs (miRNAs) are 17- to 23-nt RNA molecules that regulate the expression of protein-coding genes. It is very likely that induced or suppressed miRNAs regulate key transcription factors and other genes involved in T helper cell differentiation. Understanding the molecular mechanisms underlying Th17 cell differentiation and the regulation of this process will help scientists develop better therapeutic approaches for the treatment of Th17 cell-driven pathologies.

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6. REFERENCES

1. TR Mosmann, H Cherwinski, MW Bond, MA Giedlin, RL Coffman: Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 136(7), 2348-2357 (1986) PMid:2419430
2. AK Abbas, KM Murphy, A Sher: Functional diversity of helper T lymphocytes. *Nature* 383, 787-793 (1996) DOI: 10.1038/383787a0 PMid:8893001
3. S Constant, K Bottomly: Induction of TH1 and Th2 CD4 T cell responses: the alternative approaches. *Annu Rev Immunol* 15, 297-322 (1997) DOI: 10.1146/annurev.immunol.15.1.297 PMid:9143690
4. IC Ho, D Lo, LH Glimcher: c-maf promotes T helper cell type 2 (Th2) and attenuates Th1 differentiation by both interleukin 4- dependent and -independent mechanisms. *J Exp Med*

188, 1859-1866 (1998)
DOI: 10.1084/jem.188.10.1859
PMid:9815263, PMCid: PMC2212398

5. L Gorelik, PE Fields, RA Flavell: Cutting edge: TGF-beta inhibits Th type 2 development through inhibition of GATA-3 expression. *J Immunol* 165, 4773-4777 (2000)
DOI: 10.4049/jimmunol.165.9.4773
PMid:11045997

6. L Gorelik, S Constant, RA Flavell. Mechanism of transforming growth factor beta-induced inhibition of T helper type 1 differentiation. *J Exp Med* 195, 1499-1505 (2002)
DOI: 10.1084/jem.20012076 PMid:12045248
PMCid: PMC2193549

7. W Chen, W Jin, N Hardegen, KJ Lei, L Li, N Marinos, G McGrady, SM Wahl: Conversion of peripheral CD4+CD25- naive T cells to CD4+ CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J Exp Med* 198, 1875-1886 (2003)
DOI: 10.1084/jem.20030152
PMid:14676299, PMCid: PMC2194145

8. E Betteli, Y Carrier, W Gao, T Korn, TB Strom, M Oukka, HL Weiner, VK Kuchroo: Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 441, 235-238 (2006)
DOI: 10.1038/nature04753
PMid:16648838

9. EV Acosta-Rodriguez, G Napolitani, A Lanzavecchia, F Sallusto: Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat Immunol* 8, 942-949 (2007)
DOI: 10.1038/ni1496
PMid:17676045

10. E Betteli, T Korn, VK Kuchroo: Th17: the third member of the effector T cell trilogy. *Curr Opin Immunol* 19, 652-657 (2007)
DOI: 10.1016/j.coi.2007.07.020
PMid:1776609, PMCid: PMC2288775

11. II Ivanov, BS McKenie, L Zhou, CE Tadokoro, A Lepelley, JJ Lafaille, DJ Cua, DR Littman: The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* 126, 1121-1133 (2006).
DOI: 10.1016/j.cell.2006.07.035
PMid:16990136

12. AN Mathur, HC Chang, DG Zisoulis, GL Stritesky, Q Yu, JT O'Malley, R Kapur, DE Levy, GS Kansas, MH Kaplan: Stat3 and Stat4 direct development of IL-17- secreting Th cells. *J Immunol* 178, 4901-4907 (2007).
DOI: 10.4049/jimmunol.178.8.4901
PMid:17404271

13. XO Yang, BP Pappu, R Nurieva, A Akimzhanov, HS Kang, Y Chung, L Ma, B Shah, AD Panopoulos, KS Schluns, SS Watowich, Q Tian, AM Jetten, C Dong: T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. *Immunity* 28, 29-39 (2008)
DOI: 10.1016/j.immuni.2007.11.016
PMid:18164222, PMCid: PMC2587175

14. L Zhou, II Ivanov, R Spolski, R Min, K Shenderov, T Egawa, DE Levy, WJ Leonard, DR Littman: IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol* 8, 967-974 (2007)
DOI: 10.1038/ni1488
PMid:17581537

15. L Han, J Yang, X Wang, Q Wu, S Yin, Z Li, J Zhang, Y Xing, Z Chen, A Tsun, D Li, M Piccioni, Y Zhang, Q Guo, L Jiang, L Bao, L Lv, B Li: The E3 deubiquitinase USP17 is a positive regulator of retinoic acid-related orphan nuclear receptor γt (RORγt) in Th17 cells. *J Biol Chem* 289(37), 25546-25555 (2014)
DOI: 10.1074/jbc.M114.565291
PMid:25070893, PMCid: PMC4162160

16. A Lainé, B Martin, M Luka, L Mir, C Auffray, B Lucas, G Bismuth, C Charvet: Foxo1 Is a T Cell-Intrinsic Inhibitor of the RORγt-Th17 Program. *J Immunol* 195(4), 1791-1803 (2015)
DOI: 10.4049/jimmunol.1500849
PMid:26170390

17. J Skepner, M Trocha, R Ramesh, XA Qu, D Schmidt, E Baloglu, M Lobera, S Davis, MA Nolan, TJ Carlson, J Hill, S Ghosh, MS Sundrud, J Yang: In vivo regulation of gene expression and T helper type 17 differentiation by RORγt inverse agonists. *Immunology* 145(3), 347-256 (2015)
DOI: 10.1111/imm.12444
PMid:25604624

18. S Xiao, N Yosef, J Yang, Y Wang, L Zhou, C Zhu, C Wu, E Baloglu, D Schmidt, R Ramesh, M Lobera, MS Sundrud, PY Tsai, Z Xiang, J

Wang, Y Xu, X Lin, K Kretschmer, PB Rahl, RA Young, Z Zhong, DA Hafler, A Regev, S Ghosh, A Marson, VK Kuchroo: Small-molecule RORγt antagonists inhibit T helper 17 cell transcriptional network by divergent mechanisms. *Immunity* 40(4), 477-489 (2014) DOI: 10.1016/j.immuni.2014.04.004 PMid:24745332, PMCid: PMC4066874

19. M Ganjalikhani Hakemi, K Ghaedi, V Homayouni, A Andalib, M Hosseini, A Rezaei: Positive and Negative Regulation of Th17 Cell Differentiation: Evaluating The Impact of RORC2. *Cell J* 16(3), 343-352 (2014) PMid:24611151, PMCid: PMC4204187
20. S Nakae, Y Iwakura, H Suto, SJ Galli: Phenotypic differences between Th1 and Th17 cells and negative regulation of Th1 cell differentiation by IL-17. *J Leuk Bio* 81, 1258-1268 (2007) DOI: 10.1189/jlb.1006610 PMid:17307864
21. G Huang, Y Wang, H Chi: Regulation of Th17 cell differentiation by innate immune signals. *Cell Mol Immunol* 9(4), 287–295 (2012) DOI: 10.1038/cmi.2012.10 PMid:22504954, PMCid: PMC3423893
22. T Korn, E Bettelli, M Oukka, VK Kuchroo: IL-17 and Th17 Cells. *Anna Rev Immunol* 27, 485-517 (2009) DOI: 10.1146/annurev.immunol.021908.132710 PMid:19132915
23. A Brüstle, S Heink, M Huber, C Rosenplänter, C Stadelmann, P Yu, E Arpaia, TW Mak, T Kamradt, M Loboff: The development of inflammatory T(H)-17 cells requires interferon-regulatory factor 4. *Nat Immunol* 8, 958-966 (2007) DOI: 10.1038/ni1500 PMid:17676043
24. Q Chen, W Yang, S Gupta, P Biswas, P Smith, G Bhagat, AB Pernis: IRF-4-binding protein inhibits interleukin-17 and interleukin-21 production by controlling the activity of IRF-4 transcription factor. *Immunity* 29:(6), 899-911 (2008) DOI: 10.1016/j.immuni.2008.10.011 PMid:19062315 PMCid: PMC2633410
25. M Huber, A Brüstle, K Reinhard, A Guralnik, G Walter, A Mahiny, E von Löw, M Lohoff: IRF4 is essential for IL-21-mediated induction, amplification, and stabilization of the Th17 phenotype. *Proc Natl Acad Sci USA* 105, 20846-20851 (2008) DOI: 10.1073/pnas.0809077106 PMid:19088203, PMCid: PMC2634912
26. Y Chung, SH Chang, GJ Martinez, XO Yang, R Nurieva, HS Kang, L Ma, SS Watowich, AM Jetten, Q Tian, C Dong: Critical regulation of early Th17 cell differentiation by interleukin-1 signaling. *Immunity* 30, 576-587 (2009) DOI: 10.1016/j.immuni.2009.02.007 PMid:19362022, PMCid: PMC2705871
27. DR Echlin, HJ Tae, N Mitin, EJ Taparowsky: B-ATF functions as a negative regulator of AP-1 mediated transcription and blocks cellular transformation by Ras and Fos. *Oncogene* 19, 1752- 1763 (2000) DOI: 10.1038/sj.onc.1203491 PMid:10777209
28. GJ Martinez, RI Nurieva, XO Yang, C Dong: Regulation and function of proinflammatory TH17 cells. *Ann N Y Acad Sci* 1143, 188-211 (2008) DOI: 10.1196/annals.1443.021 PMid:19076351
29. GJ Martinez, C Dong: BATF: bringing (in) another Th17-regulating factor. *J Mol Cell Biol* 1, 66-68 (2009) DOI: 10.1093/jmcb/mjp016 PMid:19726487
30. BU Schraml, K Hildner, W Ise, WL Lee, WA Smith, B Solomon, G Sahota, J Sim, R Mukasa, S Cemerski, RD Hatton, GD Stormo, CT Weaver, JH Russell, TL Murphy, KM Murphy: The AP-1 transcription factor Batf controls T(H)17 differentiation. *Nature* 460, 405-409 (2009) PMid:19578362 PMCid: PMC2716014
31. IM Djuretic, D Levanon, V Negreanu, Y Groner, A Rao, KM Ansel: Transcription factors T-bet and Runx3 cooperate to activate Ifng and silence Il4 in T helper type 1 cells. *Nat immunol* 8, 145-153 (2007) DOI: 10.1038/ni1424 PMid:17195845
32. T Egawa, RE Tillman, Y Naoe, I Taniuchi, DR Littman: The role of the Runx transcription factors in thymocyte differentiation and in homeostasis of naive T cells. *J Exp Med* 204, 1945-1957 (2007) DOI: 10.1084/jem.20070133

PMid:17646406 PMCid: PMC2118679

33. A Collins, DR Littman, I Taniuchi: RUNX proteins in transcription factor networks that regulate T-cell lineage choice. *Nat Rev Immunol* 9, 106-115 (2009)
DOI: 10.1038/nri2489
PMid:19165227 PMCid: PMC4231139

34. Y Wang, J Godec, K Ben-Aissa, K Cui, K Zhao, AB Pucsek, YK Lee, CT Weaver, R Yagi, V Lazarevic: The transcription factors T-bet and Runx are required for the ontogeny of pathogenic interferon-γ-producing T helper 17 cells. *Immunity* 40(3), 355-366 (2014)
DOI: 10.1016/j.immuni.2014.01.002
PMid:24530058 PMCid: PMC3965587

35. D Mucida, Y Park, G Kim, O Turovskaya, I Scott, M Kronenberg, H Cheroutre: Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* 317, 256-260 (2007)
DOI: 10.1126/science.1145697
PMid:17569825

36. M Veldhoen, K Hirota, AM Westendorf, J Buer, L Dumoutier, JC Renauld, B Stockinger: The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature* 453, 106-109 (2008)
DOI: 10.1038/nature06881
PMid:18362914

37. FJ Quintana, AS Basso, AH Iglesias, T Korn, MF Farez, E Bettelli, M Caccamo, M Oukka, HL Weiner: Control of Treg and TH17 cell differentiation by the aryl hydrocarbon receptor. *Nature* 453, 65-71 (2008)
DOI: 10.1038/nature06880
PMid:18362915

38. EV Dang, J Barbi, HY Yang, D Jinasena, H Yu, Y Zheng, Z Bordman, J Fu, Y Kim, HR Yen, W Luo, K Zeller, L Shimoda, SL Topalian, GL Semenza, CV Dang, DM Pardoll, F Pan: Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. *Cell* 146, 772-784 (2011)
DOI: 10.1016/j.cell.2011.07.033
PMid:21871655 PMCid: PMC3387678

39. L Zhou, JE Lopes, MMW Chong, II Ivanov, R Min, GD Victora, Y Shen, J Du, YP Rubtsov, AY Rudensky, SF Ziegler, DR Littman: TGF-b-induced Foxp3 inhibits Th17 cell differentiation by antagonizing RORct function. *Nature* 453, 236-241 (2008)
DOI: 10.1038/nature06878
PMid:18368049 PMCid: PMC2597437

40. SJ Szabo, ST Kim, GL Costa, X Zhang, CG Fathman, LH Glimcher: A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 100l, 655-669 (2000)
DOI: 10.1016/S0092-8674(00) 80702-3

41. V Lazarevic, X Chen, JH Shim, ES Hwang, EJang, AN Bolm, M Oukka, VK Kuchroo, LH Glimcher: T-bet represses T(H)17 differentiation by preventing Runx1-mediated activation of the gene encoding RORgammat. *Nat Immuno*l 12l, 96-104 (2011)
DOI: 10.1038/ni.1969
PMid:21151104 PMCid: PMC3077962

42. R Nurieva, XO Yang, G Martinez, Y Zhang, AD Panopoulos, L Ma, K Schluns, Q Tian, SS Watowich, AM Jetten, C Dong: Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature* 448, 480-483 (2007)
DOI: 10.1038/nature05969
PMid:17581589

43. XO Yang, AD Panopoulos, R Nurieva, SH Chang, D Wang, SS Watowich, C Dong: STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. *J Biol Chem* 282, 9358-9363 (2007)
DOI: 10.1074/jbc.C600321200
PMid:17277312

44. TJ Harris, JF Grosso, HR Yen, H Xin, M Kortylewski, E Albesiano, EL Hipkiss, D Getnet, MV Goldberg, CH Maris, F Housseau, H Yu, DM Pardoll, CG Drake: Cutting edge: An in vivo requirement for STAT3 signaling in TH17 development and TH17-dependent autoimmunity. *J Immunol* 179(7), 4313-4317 (2007)
DOI: 10.4049/jimmunol.179.7.4313
PMid:17878325

45. L Durant, WT Watford, HL Ramos, A Laurence, G Vahedi, L Wei, H Takahashi, HW Sun, Y Kanno, F Powrie, JJ O'Shea: Diverse targets of the transcription factor STAT3 contribute to T cell pathogenicity and homeostasis. *Immunity* 32(5), 605-615 (2010)
DOI: 10.1016/j.immuni.2010.05.00
PMid:20493732 PMCid: PMC3148263

46. F Chalmin, G Mignot, M Bruchard, A Chevriaux, F Vegran, A Hichami, S Ladoire, V Derangere, J Vincent, D Masson, SC Robson, G Eberl, JR´ Pallandre, C Borg, B

Ryffel, L Apetoh, C Rebe, F Ghiringhelli: Stat3 and Gfi-1 Transcription Factors Control Th17 Cell Immunosuppressive Activity via the Regulation of Ectonucleotidase Expression. *Immunity* 36, 362-373 (2012)
DOI: 10.1016/j.immuni.2011.12.019
PMid:22406269

47. JH Yoon, K Sudo, M Kuroda, M Kato, IK Lee, JS Han, S Nakae, T Imamura, J Kim, JH Ju, DK Kim, K Matsuzaki, M Weinstein, I Matsumoto, T Sumida, M Mamura: Phosphorylation status determines the opposing functions of Smad2/Smad3 as STAT3 cofactors in TH17 differentiation. *Nat Commun* 6, 7600 (2015)
DOI: 10.1038/ncomms8600
PMid:26194464 PMCid: PMC4518312

48. R Duhen, S Glatigny, CA Arbelaez, TC Blair, M Oukka, E Bettelli: Cutting edge: the pathogenicity of IFN-gamma-producing Th17 cells is independent of T-bet. *J Immunol* 190(9), 4478-4482 (2013)
DOI: 10.4049/jimmunol.1203172
PMid:23543757 PMCid: PMC3633668

49. WT Watford, BD Hissong, JH Bream, Y Kanno, L Muul, JJ O'Shea: Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. *Immunol Rev* 202, 139-156 (2004)
DOI: 10.1111/j.0105-2896.2004.00211.x
PMid:15546391

50. Z Yao, Y Kanno, M Kerenyi, G Stephens, L Durant, WT Watford, A Laurence, GW Robinson, EM Shevach, E Moriggl, L Hennighausen, C Wu, JJ O'Shea: Nonredundant roles for Stat5a/b in directly regulating Foxp3. *Blood* 109(10), 4368-4375 (2007)
DOI: 10.1182/blood-2006-11-055756
PMid:17227828 PMCid: PMC1885496

51. L Klotz, S Burgdorf, I Dani, K Saijo, J Flossdorf, S Hucke, J Alferink, N Nowak, M Beyer, G Mayer, B Langhans, T Klockgether, A Waisman, G Eberl, J Schultze, M Famulok, W Kolanus, C Glass, C Kurts, PA Knolle: The nuclear receptor PPAR gamma selectively inhibits Th17 differentiation in a T cell-intrinsic fashion and suppresses CNS autoimmunity. *J Exp Med* 206, 2079-2089 (2009)
DOI: 10.1084/jem.20082771
DOI: 10.1084/jem.20082771120409c
PMid:19737866

52. B Li, JM Reynolds, RD Stout, DA Bernlohr, J Suttles: Regulation of Th17 differentiation by epidermal fatty acid-binding protein. *J Immuno*l 182, 7625-7633 (2009)
DOI: 10.4049/jimmunol.0804192
PMid:19494286 PMCid: PMC2707838

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