

Th17 and Non-Classic Th1 Cells in Chronic Inflammatory Disorders: Two Sides of the Same Coin

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Abstract

Th17 lymphocytes, beyond their protective role in the clearance of extracellular pathogens, also play a role in the pathogenesis of several autoimmune and inflammatory diseases, such as multiple sclerosis, rheumatoid arthritis, inflammatory bowel diseases, psoriasis and contact dermatitis. Nevertheless, they are very rare at inflammatory sites in comparison with other T cell subsets. Recently, this rarity has been explained by the finding that Th17 cells rapidly shift into the Th1 phenotype in the presence of IL-12 and/or TNF- α as well as by the fact that they possess self-regulatory mechanisms limiting their own expansion. Th17 lymphocytes that have shifted towards a Th1 phenotype seem to be particularly aggressive and more pathogenic than the Th17 unshifted cells. As a consequence, the Th17-derived Th1 cells, named non-classic Th1 cells, can become a possible target for the therapy of some inflammatory disorders. In particular, convincing evidence has recently been accumulated indicating that this subset can play a role in Crohn's disease and juvenile idiopathic arthritis. More importantly, it has been shown that TNF- α inhibitors, which are used for the treatment of such

diseases, appear to be able to inhibit the transition of Th17 lymphocytes to the non-classic Th1 phenotype, and thus they possibly help to dampen inflammation and arrest disease progression. Based on this context, the definition of the soluble factors involved in the shifting from Th17 towards non-classic Th1 subset as well as the comprehension of their respective pathogenic role in human inflammatory disorders would be of great help for developing novel therapeutic strategies.

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Functional Classification of T Helper Lymphocytes

Human T helper (Th) lymphocytes, on the basis of their immunological functions, can be distinguished into T effector cells, which protect the body from pathogens, and regulatory T cells, which dampen effector responses when they become dangerous for the host. Among effector Th cells, immunologists traditionally distinguished different subsets based on their ability to defend the body from distinct classes of pathogens, which in turn depend upon different profiles of cytokine production as well as transcription factors and homing receptor expression [1]. Over 20 years ago, two main subsets of CD4 Th lymphocytes were identified in both mice and humans, which

were named Th1 and Th2. Th1 cells express the transcription factor T-bet, secrete IFN- γ and protect the host from intracellular infections. Th2 cells express GATA-3, secrete IL-4, IL-5, IL-9 and IL-13 and are involved in the protection from helminthes [2, 3]. Recently, other populations of human Th lymphocytes have been discovered and characterized for both phenotype and function. These include Th17 cells, which express the transcription factor RORC, secrete IL-17 and protect the host mainly from extracellular pathogens [4]; Th22 cells, which play a crucial role in host defense against Gram-negative bacteria [5]; Th9 cells, which are particularly active in promoting inflammation [6], and T follicular helper lymphocytes, which are crucial in helping antibody production [7].

Beyond their protective role, all of these Th cell subsets can play a pathogenic role in a wide spectrum of human diseases [8]. This may happen when the immune response is directed against innocuous environmental antigens (allergens) or self antigens. Indeed, in atopic subjects, allergen-specific Th2 lymphocytes are able to trigger and maintain the inflammation network, whereas in organ-specific autoimmune diseases self-reactive T cells attack different organs and consequently damage the host. Moreover, an inflammation triggered even by unknown pathogens, if it is not adequately dampened by the immune system, can become the main hallmark of the disease [1]. While, before the discovery of Th17 cells, Th1 lymphocytes were considered to be the main actors of chronic inflammatory disorders, such as Crohn's disease (CD), sarcoidosis and atherosclerosis [9], soon after, Th17 cells have been regarded as the true pathogenic cells in many chronic inflammatory disorders, whereas the pathogenic role of Th1 was underestimated or even considered as being protective [10]. More recently, the concept that both Th17 and Th1 cells can play a role in the pathogenesis of autoimmune and inflammatory disorders has been suggested and supported by the finding that these two cell subsets can develop from the same precursors and coexist in the same microenvironment [11]. This hypothesis has been further strengthened by both in vitro and in vivo observations definitively showing the functional plasticity of Th17 cells [12].

Human Th17 Cells: Markers and Development

In the last years, several phenotypic and functional features of human Th17 lymphocytes have been reported allowing a better ex vivo and in vitro identification of this cell subset. Th17 cells express the transcription factor

RORC [13], the IL-23 receptor, the chemokine receptor CCR6 [12, 14] and the lectin receptor CD161 [15]. The origin of Th17 lymphocytes has been a matter of intense debate in the last years [4]. Some years ago, we found that the simultaneous presence of IL-1 β and IL-23 was required to induce the development of Th17 lymphocytes from a small subset of CD161+CD4+ T cell precursors detectable in both human umbilical cord blood and thymus [15]. Regarding the controversial role of TGF- β signaling for the development of human Th17 lymphocytes, it has recently been reported that it does not have a direct effect, but it can play an indirect permissive role by suppressing Th1 cell differentiation. Indeed, umbilical cord blood naïve CD161+CD4+ T cells, which contain the precursors of human Th17 cells, differentiated into IL-17A-producing cells only in response to IL-1 β plus IL-23, and even in the absence of TGF- β . TGF- β was able to increase the relative proportions of CD161+ T cells differentiating into Th17 cells in response to IL-1 β plus IL-23, whereas under the same conditions it inhibited both T-bet expression and Th1 development [16]. All of these data are in keeping with the observation that patients with mutations in *TGFB1*, *TGFB1* and *TGFB2* (Camurati-Engelmann disease and Marfan-like syndromes) did not exhibit any difference in the numbers of IL-17A-producing T cells compared with healthy controls, whereas patients with STAT3 mutations have impaired Th17 cell development [17]. The dispensability of TGF- β 1 from Th17 cell development has also been confirmed in mice by the observation that ROR γ t-expressing CD4+ lamina propria T cells were found in mice lacking the TGF- β receptor subunit I in their T cells, whereas the numbers of Foxp3+ CD4+ regulatory T cells were dramatically reduced [18].

Protective Role of Th17 Lymphocytes

Th17 lymphocytes play an important role in defending the body from infectious agents, particularly extracellular bacteria and fungi, due to their ability to produce several cytokines, such as IL-21, IL-22, IL-26, TNF- α , GM-CSF and, obviously, IL-17 [8]. The IL-17 molecule is composed of two monomers linked by intramolecular disulfide bonds on cysteine residues to form a homodimer. IL-17 is the founding member of the IL-17 family comprising six members, from IL-17A to IL-17F. The main IL-17 family members are IL-17A and IL-17F, which are predominantly produced by Th17 cells, and both bind and act through the IL-17 receptor (IL-17R), which is composed of chains A (IL-17RA) and C (IL-17RC). IL-

IL-17A and IL-17F monomers can form homodimers and heterodimers, and both are able to bind to IL-17R. This interaction induces the activation of the adaptor protein ACT1, NF- κ B and TNF receptor-associated factor 6, which leads to an increased transcription of the genes encoding IL-6 and IL-8 [19]. Th17 lymphocytes are indeed able to recruit neutrophil granulocytes to the inflammatory sites through the induction of IL-8 and also to activate them mainly through the induction of colony-stimulating factors, which are produced by tissue-resident cells [20]. It has also been reported that activated Th17 cells can directly chemoattract neutrophils via the release of IL-8, thus reinforcing the biological cross-talk between neutrophils and Th17 cells [21]. Moreover, Th17 lymphocytes also stimulate the production of mucins MUC5AC and MUC5 by primary human bronchial epithelial cells in vitro [22], and the expression of human β -defensin-2 [23] and CCL20 by lung epithelial cells [24], thus helping to defend the body from invading pathogens.

The protective role of Th17 lymphocytes is confirmed by the finding that in autosomal dominant hyper-IgE syndrome (Job's syndrome), IL-17 production and Th17 differentiation are impaired due to mutations in the gene encoding STAT3 [25]. This results in recurrent and often severe pulmonary infections, pneumatoceles, eczema, staphylococcal abscesses and mucocutaneous candidiasis. These data are consistent with the crucial role for STAT3 signaling in the generation of Th17 cells and further strengthen the key role of this subset in the clearance of fungal and extracellular bacterial infections [25]. Also, the autosomal recessive hyper-IgE syndrome caused by mutations in DOCK8 is characterized by candidiasis derived by defects in Th17 cell differentiation. Moreover, heterozygous mutations in STAT1 have been found to be involved in chronic mucocutaneous candidiasis. These patients revealed defects in the Candida-stimulated production of IFN- γ , IL-17 and IL-22, probably due to the defective functioning of IL-12 and IL-23 signaling pathways [26].

Th17 Cells in the Pathogenesis of Chronic Inflammatory Diseases

Beyond their protective role in the clearance of extracellular pathogens, Th17 lymphocytes have been described to play a role in the pathogenesis of several autoimmune and inflammatory diseases, such as multiple sclerosis, rheumatoid arthritis and inflammatory bowel

disease, but also in psoriasis and contact dermatitis [27]. Nevertheless, Th17 cells are very difficult to detect in the inflamed tissue of patients suffering from these diseases, and this may be explained at least by two reasons: their limited expansion in response to T cell receptor triggering and their tendency to shift to a Th1 phenotype in the presence of IL-12 [8]. The limited proliferative capacity of Th17 lymphocytes is due to the high expression of the IL-4-induced gene 1, which impairs their expansion in response to T cell receptor triggering [28], and to Tob1, a member of the Tob/BTG anti-proliferative protein family, which prevents cell cycle progression [29].

The second important reason explaining the rarity of Th17 cells at inflammatory sites is their high plasticity, which allows these cells to produce IFN- γ and then rapidly shift to the Th1 phenotype [30]. Some years ago, examination of the cytokine profile of T cells derived from the inflamed mucosa of CD patients disclosed mainly Th1 cells, a few Th17 cells and appreciable levels of cells producing both IL-17 and IFN- γ , which we named Th17/Th1 cells [12]. In the same study, we also showed that culturing Th17 cell clones in vitro in the presence of IL-12 induced them to produce IFN- γ and also to shift towards the Th1 phenotype [12]. Recent evidence indicates that the acquisition of the IFN- γ -producing potential by Th17 lymphocytes as well as the finding of a simultaneous production of IL-17 and IFN- γ is a common occurrence, particularly in inflammatory conditions [1]. How often such a degree of plasticity can be expected in the course of physiologic responses to pathogens and its importance in protective immunity remain to be elucidated. However, it has been reported that human Th17 cells may exhibit a different cytokine profile according to their antigen specificity. *Candida albicans*-specific Th17 cells are able to produce IL-17 and IFN- γ , but not IL-10, whereas *Staphylococcus aureus*-specific Th17 cells produce IL-17 and are able to produce IL-10 upon restimulation. Importantly, IL-1 β was reported to be indispensable for the induction of IL-17/IFN- γ double-producing cells and for the inhibition of the IL-10-producing capacity of differentiating Th17 cells [31].

Th17-derived Th1, named non-classic Th1, can be easily distinguished from classic Th1 cells on the basis of CD161 expression as well as the consistent expression of RORC, IL-17 receptor E, CCR6 and IL-4-induced gene 1 [32], which are all virtually absent in classic Th1 cells. Indeed, it has been found that Th17 cells can induce type 1 insulin-dependent diabetes mellitus efficiently in lymphopenic recipient mice only after their shift to Th1 cells [33]. Moreover, in a model of insulin-dependent diabetes

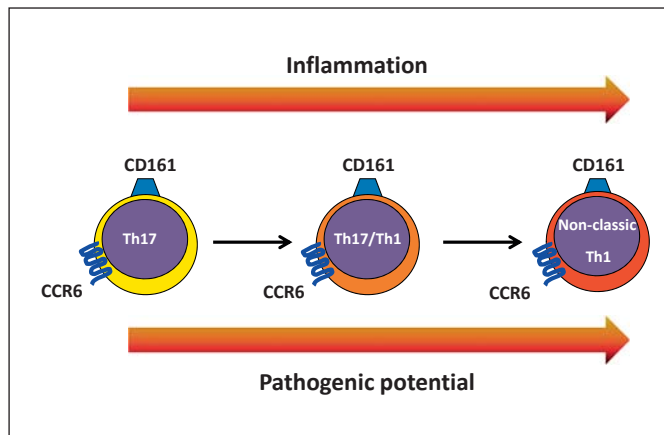


Fig. 1. Th17 lymphocyte transition to the non-classic Th1 phenotype in inflammatory conditions. CD161+ Th17 lymphocytes possess an intrinsic plasticity that allows them to shift towards the Th1 phenotype in the presence of the inflammatory cytokines IL-12 and/or TNF- α . The Th17-derived non-classic Th1 cells maintain the expression of the surface marker CD161 and seem to be particularly aggressive and more pathogenic than the unshifted cells.

mellitus induced by the transfer of highly purified Th17 cells from BDC2.5NOD mice in NOD/SCID recipient mice, the onset of the disease was prevented by the treatment with an anti-IFN- γ neutralizing antibody, but not by the treatment with an anti-IL-17A neutralizing antibody [34]. More importantly, it appears quite clear that both Th17 and Th1 cells can be involved in the pathogenesis of human autoimmune and inflammatory disorders, too. Indeed, treatment with ustekinumab, a monoclonal antibody that binds the shared p40 subunit of human IL-12 and IL-23, thus blocking both the Th1 and Th17 pathway, resulted in an improvement of symptoms in moderate-to-severe psoriasis and in psoriatic arthritis [35, 36]. Moreover, it has recently been suggested that the shifting of Th17 towards the Th1 subset in human chronic inflammatory disease may be associated with disease progression [37–39] (fig. 1).

In the last years, studying juvenile idiopathic arthritis (JIA), the most common form of persistent arthritis in children, we found that CD4+CD161+ T helper lymphocytes are significantly enriched in the synovial fluid (SF) in comparison with peripheral blood (PB) [39]. The proportions of CD4+CD161+ T cells, in particular of the Th17/Th1 subset, in SF correlated positively with parameters of disease activity, such as erythrocyte sedimentation rate and serum levels of C-reactive protein, suggesting that these cells play a major role in the in-

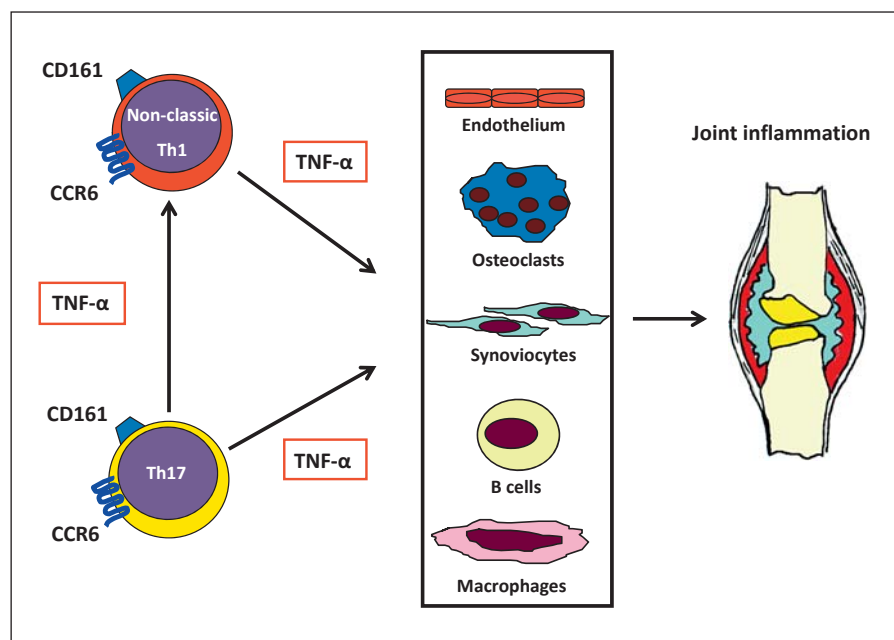
flammatory processes of JIA. Based on these findings, we hypothesized that a Th17-to-Th1 shift occurred *in vivo* in the SF of JIA children. Accordingly, we found that IL-12, which is abundant in the SF of JIA patients, induces a shift from Th17 cells towards the Th1 phenotype, thus also explaining the high frequency of non-classic Th1 cells in the inflammatory environment. The results of this study strongly support the concept that CD161+ lymphocytes possess a functional plasticity that allows them to shift to a Th17/Th1 phenotype, and even to the non-classic Th1, in the presence of IL-12, and probably other still unidentified cytokines present in the inflammatory microenvironment might also be involved [39]. Recently, indeed, we identified TNF- α as another soluble factor, which is particularly abundant in the inflammatory microenvironment, that can drive the shifting from Th17 lymphocytes towards the non-classic Th1 phenotype [40]. Th17 lymphocytes themselves produce TNF- α , which thus drives their transition to the Th1 phenotype by either an autocrine or a paracrine mechanism [40].

Is It Possible to Interfere with the Shifting from Th17 Lymphocytes towards the Th17/Th1 Phenotype? A Lesson from Biologics

T-cell differentiation was once considered linear and irreversible, but there is now increasing evidence to suggest that differentiated CD4+ T cell populations can alter the range of their cytokine production [40]. Under basal conditions, phenotype stability is ensured by the expression of genes turned on by transcription factors, but also by the repression of genes conferring alternative fates. Also, epigenetic modifications and micro-RNA expression contribute to this stability [41]. However, this commitment is not irreversible and, as a consequence, T cells may change their cytokine production profile when exposed to certain microenvironments. Indeed, Th17 lymphocytes represent a paradigmatic example of flexible cytokine production, since it has been clearly shown that they can shift to Th1 in the presence of IL-12 or TNF- α [12, 40], and to Th2 in the presence of IL-4 [42]. Moreover, both Th17/Th1 and Th17/Th2 lymphocytes seem to have a more aggressive and pathogenic phenotype than conventional Th17, Th2 or Th1 phenotypes [39, 43, 44].

In particular, we have recently suggested that interfering with the acquisition of IFN- γ -producing potential by human Th17 cells in some human inflammatory disorders, such as CD and JIA, may have beneficial effects on

Fig. 2. The role of TNF- α in the orchestration of joint inflammation. TNF- α in JIA activates monocytes, B cells and synovial fibroblasts and stimulates neovascularization and the formation of the pannus, which contains osteoclasts that contribute to cartilage and bone destruction. TNF- α can also act on T lymphocytes by driving the shift from Th17 to non-classic Th1 cells.



their treatment [40, 45]. In patients with fistulizing CD, we found higher frequencies of CD161+ Th lymphocytes in the fistula than in PB. Local administration of adalimumab, a neutralizing anti-TNF- α monoclonal antibody, is associated with fistula resolution in the majority of patients as well as the disappearance of infiltrating T lymphocytes, in particular those expressing CD161, which are able to produce IFN- γ . The finding that the resolution of the fistula is associated with a reduced frequency of non-classic Th1 and Th17/Th1 cells allows us to hypothesize that IFN- γ -producing cells may be pathogenic cells. Moreover, we speculated that the efficacy of TNF- α inhibitors in the treatment of complex fistulizing CD may be due, at least in part, to their ability to interfere with the Th17 polarization towards the Th1 phenotype.

This hypothesis has been indirectly confirmed by our recent finding indicating that etanercept, a soluble dimeric fusion protein that binds soluble TNF- α , inhibits the TNF- α -driven shift from Th17 towards the non-classic Th1 phenotype in JIA [40]. Starting from the observations that CD4+CD161+ T cells play a crucial role in JIA pathogenesis, particularly the Th17/Th1 and non-classic Th1 subsets [39], we assessed the phenotype of CD161+CD4+ T lymphocytes in PB from untreated and etanercept-treated JIA patients and found lower proportions of non-classic Th1 cells and higher proportions of Th17 cells in the latter [40]. The observation that Th17 proportions decrease under anti-TNF- α therapy has also

been reported in rheumatoid arthritis [46]. These data have been further strengthened by in vitro experiments, indicating the ability of etanercept to exert a modulatory activity on the functional phenotype of CD4+ Th subsets obtained from PB of JIA patients. Etanercept was found to act only on CD4+CD161+ T lymphocytes, inhibiting the expansion of Th1 cells, and increasing the proportions of both Th17 and Th17/Th1 cell subsets [40]. Accordingly, we showed that exogenous TNF- α promotes the shifting of highly purified CD161+ IL-17+ IFN- γ -(Th17) cells from PB of healthy donors towards the Th1 phenotype. Interestingly, etanercept was also able to block this shifting towards the non-classic Th1 phenotype not only in the presence of exogenous TNF- α , but also in its absence. This latter observation strongly suggests that Th17 lymphocytes themselves produce TNF- α , which can thus drive their transition to the Th1 phenotype by either an autocrine or a paracrine mechanism [40]. TNF- α -blocking agents induce an amelioration of clinical symptoms, laboratory parameters of inflammation and radiological progression of disease in both rheumatoid arthritis and JIA, but T cells are not generally considered as an important target of such a treatment. The rationale for the usage of TNF- α inhibitors in the treatment of JIA mainly consists in the pleiotropic activity of this cytokine in the inflammatory milieu. Indeed, it has been known for many years that TNF- α in JIA can activate monocytes, macrophages and synovial fibroblasts by

inducing the transcription of genes whose products mediate inflammation and tissue degradation, leading to cartilage and bone destruction. Moreover, TNF- α induces the growth of the synovial membrane with neovascularization and the formation of the pannus, which contains osteoclasts. Also, neutrophils accumulate within the SF and participate in destructive processes, which are mainly mediated by a plethora of metalloproteinases present in the joint and by the activation of osteoclasts [47]. The novel finding that TNF- α can also act on T lymphocytes (by driving the shifting from Th17 to Th1 cells) may represent an additional indication for the use of TNF- α inhibitors in JIA and also in those diseases where such shifting can have a pathogenic role (fig. 2).

Conclusion

Several features of human Th17 cells have emerged in the last years, particularly those which concern their involvement in human diseases. Human Th17 cells are unable to strongly proliferate after T cell receptor triggering and this can account for their rare occurrence at inflammatory sites. This probably represents an important mechanism for the self-regulation of Th17 cell expansion, which is certainly devoted to minimize their dangerous proinflammatory activity. Th17 lymphocytes possess an intrinsic functional plasticity that allow them to shift to Th1-like but also Th2-like cells, at the level of inflamed sites in response to peculiar environmental signals. In inflammatory conditions, Th17 lymphocytes that have shifted towards a Th1 or Th2 phenotype, thus acquiring the ability to produce IFN- γ or IL-4, seem to be particularly aggressive and more pathogenic than the unshifted cells. For this reason, the possibility to interfere with this

shifting can be considered a potential target for developing novel therapeutic strategies in chronic inflammatory diseases. In this context, recent findings suggest that TNF- α inhibitors, which are currently used in the management of CD and JIA, can help to dampen inflammation also by inhibiting the TNF- α -induced transition of CD161+ Th17 lymphocytes to the Th17/Th1 and the Th1 phenotype. Moreover, other possible biologics may be evaluated in the next future for their capacity to interfere with the shifting from Th17 towards the Th1 phenotype, for example IL-1 inhibitors, since this cytokine has been reported to be crucial in the development of the Th17/Th1 subset [18].

The comprehension of the mechanisms by which TNF- α inhibitors and also other biologics act on different T cell subsets in human inflammatory disorders could represent an important issue to establish better therapeutic strategies in such diseases. Moreover, it could be useful to follow up patients treated with biologics and to determine response to therapy.

- Th17 lymphocytes are low proliferating cells that can shift towards a non-classic Th1 phenotype in the presence of IL-12 and/or TNF- α .
- Non-classic Th1 lymphocytes are more pathogenic than Th17, and the inhibition of this shifting can be considered a therapeutic tool in inflammation.
- IL-12 and TNF- α inhibitors can block disease progression by interfering with the shifting of Th17 cells towards the non-classic Th1 phenotype.
- The identification of other inflammatory mediators potentially involved in the shifting of Th17 to the non-classic Th1 phenotype may represent a new tool in the development of biologics for the treatment of Th17-Th1-mediated diseases.

References

- 1 Cosmi L, Maggi L, Santarlasci V, Liotta F, Annunziato F: T helper cells plasticity in inflammation. *Cytometry A* 2014;85:36–42.
- 2 Mosmann TR, Coffman RL: TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989;7:145–173.
- 3 Romagnani S: Lymphokine production by human T cells in disease states. *Annu Rev Immunol* 1994;12:227–257.
- 4 Annunziato F, Cosmi L, Liotta F, Maggi E, Romagnani S: Defining the human T helper 17 cell phenotype. *Trends Immunol* 2012;33:505–512.
- 5 Honda K: IL-22 from T cells: better late than never. *Immunity* 2012;37:952–954.
- 6 Jabeen R, Kaplan MH: The symphony of the ninth: the development and function of Th9 cells. *Curr Opin Immunol* 2012;24:303–307.
- 7 King C, Tangye SG, Mackay CR: T follicular helper (TFH) cells in normal and dysregulated immune responses. *Annu Rev Immunol* 2008;26:741–766.
- 8 Annunziato F, Cosmi L, Liotta F, Maggi E, Romagnani S: Main features of human T helper 17 cells. *Ann NY Acad Sci* 2013;1284:66–70.
- 9 Gately MK, Renzetti LM, Magram J, et al: The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. *Annu Rev Immunol* 1998;16:495–521.
- 10 Chen Z, O'Shea JJ: Th17 cells: a new fate for differentiating helper T cells. *Immunol Res* 2008;41:87–102.
- 11 Annunziato F, Cosmi L, Romagnani S: Human and murine Th17. *Curr Opin HIV AIDS* 2010;5:114–119.
- 12 Annunziato F, Cosmi L, Santarlasci V, Maggi L, Liotta F, Mazzinghi B, Parente E, Fili L, Ferri S, Frosali F, Giudici F, Romagnani P, Parronchi P, Tonelli F, Maggi E, Romagnani S: Phenotypic and functional features of human Th17 cells. *J Exp Med* 2007;204:1849–1861.

- 13 Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelletier A, Lafaille JJ, Cua DJ, Littman DR: The orphan nuclear receptor ROR γ directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* 2006;126:1121–1131.
- 14 Acosta-Rodriguez EV, Rivino L, Geginat J, Jarrossay D, Gattorno M, Lanzavecchia A, Sallusto F, Napolitani G: Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. *Nat Immunol* 2007;8:639–646.
- 15 Cosmi L, De Palma R, Santarlasci V, Maggi L, Capone M, Frosali F, Rodolico G, Querci V, Abbate G, Angeli R, et al: Human interleukin-17-producing cells originate from a CD161+ CD4+ T cell precursor. *J Exp Med* 2008;205:1903–1916.
- 16 Santarlasci V, Maggi L, Capone M, Frosali F, Querci V, De Palma R, Liotta F, Cosmi L, Maggi E, Romagnani S, et al: TGF- β indirectly favors the development of human Th17 cells by inhibiting Th1 cells. *Eur J Immunol* 2009;39:207–215.
- 17 de Beaucoudrey L, Puel A, Filipe-Santos O, Cobat A, Ghandil P, Chrabieh M, Feinberg J, von Bernuth H, Samarina A, Janni  re L, et al: Mutations in STAT3 and IL12RB1 impair the development of human IL-17-producing T cells. *J Exp Med* 2008;205:1543–1550.
- 18 Ghoreschi K, Laurence A, Yang XP, Tato CM, McGeachy MJ, Konkel JE, Ramos HL, Wei L, Davidson TS, Bouladoux N, et al: Generation of pathogenic T(H)17 cells in the absence of TGF- β signaling. *Nature* 2010;467:967–971.
- 19 Miossec P, Kolls JK: Targeting IL-17 and Th17 cells in chronic inflammation. *Nat Rev Drug Discov* 2012;11:763–776.
- 20 Ouyang W, Koli JK, Zheng Y: The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity* 2008;28:454–467.
- 21 Pelletier M, Maggi L, Micheletti A, Lazzeri E, Tamassia N, Costantini C, Cosmi L, Lunardi C, Annunziato F, Romagnani S, et al: Evidence for a cross-talk between human neutrophils and Th17 cells. *Blood* 2010;115:335–343.
- 22 Chen Y, Thai P, Zhao YH, Ho YS, DeSouza MM, Wu R: Stimulation of airway mucin gene expression by interleukin (IL)-17 through IL-6 paracrine/autocrine loop. *J Biol Chem* 2003;278:17036–17043.
- 23 Kao CY, Chen Y, Thai P, Wachi S, Huang F, Kim C, Harper RW, Wu R: IL-17 markedly up-regulates beta-defensin-2 expression in human airway epithelium via JAK and NF- κ B signalling pathways. *J Immunol* 2004;173:3482–3491.
- 24 Kao CY, Huang F, Chen Y, Thai P, Wachi S, Kim C, et al: Up-regulation of CC chemokine ligand 20 expression in human airway epithelium by IL-17 through a JAK-independent but MEK/NF- κ B-dependent signalling pathway. *J Immunol* 2005;175:6676–6685.
- 25 Milner JD, Brenckley JM, Laurence A, Freeman AF, Hill BJ, Elias KM, Kanno Y, Spalding C, Elloumi HZ, Paulson ML, Davis J, Hsu A, Asher AI, O'Shea J, Holland SM, Paul WE, Douek DC: Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. *Nature* 2008;452:773–776.
- 26 McDonald DR: Th17 deficiency in human disease. *J Allergy Clin Immunol* 2012;129:1429–1435.
- 27 Fouser LA, Wright JF, Dunussi-Joannopoulos K, Collins M: Th17 cytokines and their emerging roles in inflammation and autoimmunity. *Immunol Rev* 2008;226:87–102.
- 28 Santarlasci V, Maggi L, Capone M, Querci V, Beltrame L, Cavalieri D, D'Aiuto E, Cimaz R, Nebbioso A, Liotta F, De Palma R, Maggi E, Cosmi L, Romagnani S, Annunziato F: Rarity of human T helper 17 cells is due to retinoic acid orphan receptor-dependent mechanisms that limit their expansion. *Immunity* 2012;36:201–214.
- 29 Santarlasci V, Maggi L, Mazzoni A, Capone M, Querci V, Rossi MC, Beltrame L, Cavalieri D, De Palma R, Liotta F, Cosmi L, Maggi E, Romagnani S, Annunziato F: IL-4-induced gene 1 maintains high Tob1 expression which contributes to TCR unresponsiveness in human T helper 17 cells. *Eur J Immunol* 2014;44:654–661.
- 30 Annunziato F, Santarlasci V, Maggi L, Cosmi L, Liotta F, Romagnani S: Reasons for rarity of Th17 cells in inflammatory sites of human disorders. *Semin Immunol* 2013;25:299–304.
- 31 Zielinski CE, Mele F, Aschenbrenner D, Jarrossay D, Ronchi F, Gattorno M, Monticelli S, Lanzavecchia A, Sallusto F: Pathogen-induced human T(H)17 cells produce IFN- γ or IL-10 and are regulated by IL-1 β . *Nature* 2012;484:514–518.
- 32 Maggi L, Santarlasci V, Capone M, Rossi MC, Querci V, Mazzoni A, et al: Distinctive features of classic and non-classic (Th17 derived) human Th1 cells. *Eur J Immunol* 2012;42:3180–3188.
- 33 Martin-Orozco N, Chung Y, Chang SH, Wang YH, Dong C: Th17 cells promote pancreatic inflammation but only induce diabetes efficiently in lymphopenic hosts after conversion into Th1 cells. *Eur J Immunol* 2009;39:216–224.
- 34 Bending D, De la Pena H, Veldhoen M, Phillips JM, Uyttenhove C, Stockinger B, Cooke A: Highly purified Th17 cells from BDC2.5NOD mice convert into Th1-like cells in NOD/SCID recipient mice. *J Clin Invest* 2009;119:565–572.
- 35 Papp KA, Langley RG, Lebwohl M, Krueger GG, Szapary P, Yeilding N, Guzzo C, Hsu MC, Wang Y, Li S, et al: Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 52-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 2). *Lancet* 2008;371:1675–1684.
- 36 Goldminz AM, Gottlieb AB: Ustekinumab for psoriasis and psoriatic arthritis. *J Rheumatol* 2012;89:86–89.
- 37 Luger D, Silver PB, Tang J, Cua D, Chen Z, Iwakura Y, Bowman EP, Sgambellone NM, Chan CC, Caspi RR: Either a Th17 or a Th1 effector response can drive autoimmunity: conditions of disease induction affect dominant effector category. *J Exp Med* 2008;205:799–810.
- 38 Nistala K, Adams S, Cambrook H, Ursu S, Olivito B, de Jager W, Evans JG, Cimaz R, Bajaj-Elliott M, Wedderburn LR: Th17 plasticity in human autoimmune arthritis is driven by the inflammatory environment. *Proc Natl Acad Sci USA* 2010;107:14751–14756.
- 39 Cosmi L, Cimaz R, Maggi L, Santarlasci V, Capone M, Borriello F, Frosali F, Querci V, Simonini G, Barra G, et al: Evidence of the transient nature of the Th17 phenotype of CD4+CD161+ T cells in the synovial fluid of patients with juvenile idiopathic arthritis. *Arthritis Rheum* 2011;63:2504–2515.
- 40 Maggi L, Cimaz R, Capone M, Santarlasci V, Querci V, Simonini G, Nencini F, Liotta F, Romagnani S, Maggi E, et al: Brief report: etanercept inhibits the tumor necrosis factor α -driven shift of Th17 lymphocytes toward a nonclassic Th1 phenotype in juvenile idiopathic arthritis. *Arthritis Rheumatol* 2014;66:1372–1377.
- 41 O'Shea JJ, Paul WE: Mechanisms underlying lineage commitment and plasticity of helper CD4 T cells. *Science* 2010;327:1098–1102.
- 42 Cosmi L, Maggi L, Santarlasci V, Capone M, Cardilicchia E, Frosali F, Querci V, Angeli R, Matucci A, Fambrini M, Liotta F, Parronchi P, Maggi E, Romagnani S, Annunziato F: Identification of a novel subset of human circulating memory CD4(+) T cells that produce both IL-17A and IL-4. *J Allergy Clin Immunol* 2010;125:222–230.
- 43 Wang YH, Voo KS, Liu B, Chen CY, Uygungil B, Spoede W, Bernstein JA, Huston DP, Liu YJ: A novel subset of CD4+T(H)2 memory/effector cells that produce inflammatory IL-17 cytokine and promote the exacerbation of chronic allergic asthma. *J Exp Med* 2010;207:2479–2491.
- 44 Cosmi L, Liotta F, Maggi E, Romagnani S, Annunziato F: Th17 cells: new players in asthma pathogenesis. *Allergy* 2011;66:989–998.
- 45 Maggi L, Capone M, Giudici F, Santarlasci V, Querci V, Liotta F, Ficari F, Maggi E, Tonelli F, Annunziato F, Cosmi L: CD4+CD161+ T lymphocytes infiltrate Crohn's disease-associated perianal fistulas and are reduced by anti-TNF- α local therapy. *Int Arch Allergy Immunol* 2012;161:81–86.
- 46 Aerts NE, De Knop KJ, Leysen J, Ebo DG, Bridts CH, Weyler JJ, Stevens WJ, De Clerck LS: Increased IL-17 production by peripheral T helper cells after tumour necrosis factor blockade in rheumatoid arthritis is accompanied by inhibition of migration-associated chemokine receptor expression. *Rheumatology (Oxford)* 2010;49:2264–2272.
- 47 Smolen JS, Steiner G: Therapeutic strategies for rheumatoid arthritis. *Nat Rev Drug Discov* 2003;2:473–488.