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Human Th17 Cells Comprise Heterogeneous Subsets Including IFN-γ–Producing Cells with Distinct Properties from the Th1 Lineage

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Th17 cells have been named after their signature cytokine IL-17 and accumulating evidence indicates their involvement in the induction and progression of inflammatory diseases. In addition to IL-17 single-producing T cells, IL-17/IFN-γ double-positive T cells are found in significantly elevated numbers in inflamed tissues or blood from patients with chronic inflammatory disorders. Because IFN-γ is the classical Th1-associated cytokine, the origin and roles of these subsets remain elusive. In this paper, we show that not only IL-17+/IFN-γ+ but also IFN-γ+ (IL-17−) cells arise under Th17-inducing condition and have distinct properties from the Th1 lineage. In fact, these populations displayed characteristics reminiscent to IL-17 single-producing cells, including production of IL-22, CCL20, and induction of antimicrobial gene expression from epithelial cells. Live sorted IL-17+ and Th17–IFN-γ+ cells retained expression of IL-17 or IFN-γ after culture, respectively, whereas the IL-17+/IFN-γ− population was less stable and could also become IL-17+ or IFN-γ+ single-producing cells. Interestingly, these Th17 subsets became “Th1-like” cells in the presence of IL-12. These results provide novel insights into the relationship and functionality of the Th17 and Th1 subsets and have direct implications for the analysis and relevance of IL-17 and/or IFN-γ–producing T cells present in patients’ peripheral blood and inflamed tissues. The Journal of Immunology, 2010, 185: 679–687.

Interleukin-17 (also known as IL-17A) has been known to be produced by T cells for the past 15 y (1–3) and initial findings showed that microbial stimuli induced T cell production of IL-17 independently of Th1 or Th2 cytokine secretion (1). However, the notion that IL-17−–producing T cells represent a distinct effector T cell subset only emerged a few years ago when the roles of IL-23 and IL-17 were investigated in mouse both in vitro and in vivo during autoimmunity (4–9). Although IL-17 is the hallmark of Th17 cells, this Th cell subset express a heterogeneous cytokine profile also consisting of IL-17F, IL-22, IL-26, CCL20, and IFN-γ (10). Th17 cells express the chemokine receptor CCR6, the IL-23R, and the C-type lectin CD161 (11–15). For years, Th1 cells producing IFN-γ were thought to be the main disease-inducing cells in inflammatory disorders, such as psoriasis, Crohn’s disease (CD), or multiple sclerosis (MS), but accumulating evidence is now indicating that Th17 cells play a central role during development of these diseases (5–7, 12, 15–18). Nevertheless, a role for IFN-γ in autoimmunity cannot be neglected and blockade of IFN-γ in patients with CD is currently being tested for efficacy in phase 2 clinical trials (19). Furthermore, the importance of IFN-γ during inflammation has been highlighted in animal models: intestinal inflammation induced by Helicobacter hepaticus depended on both IFN-γ and IL-17 functions (20); both myelin-specific IL-12– and IL-23–polarized T cells are able to induce experimental autoimmune encephalomyelitis (21, 22); either Th1 or Th17 cells can be pathogenic during ocular inflammation (23, 24); and severe arthritis in the proteoglycan-induced arthritis model depends on IFN-γ and not IL-17 (25, 26).

In addition to IL-17 and IFN-γ single-producing T cells, IL-17/IFN-γ double-positive T cells are found in elevated numbers in both human- and mouse-inflamed tissues (12, 27–29), and similar populations are observed in human Th17 cells differentiated in vitro from naive CD4+ T cells (15, 30, 31). These populations have been described as Th17, Th1, and Th17/Th1 cells, respectively, terminology based only on their capacity to secrete IL-17 and/or IFN-γ without taking into account their ability to produce other factors. In addition, the existence of IL-17+/IFN-γ+ double-producing cells suggest some relationship between the Th17 and the Th1 differentiation programs. Yet, little is known about the relative profile and function of these cytokine-producing subsets and assessing the heterogeneity within each population is important for deciphering their origin and respective roles during inflammation and host defense.

To directly assess the gene expression profiles of Th1 and Th17 cell subsets based on their production of IL-17 and/or IFN-γ, we developed a protocol allowing gene expression analysis from cell populations sorted by intracellular cytokine production. We show that human T cells cultured under Th17-inducing conditions and secreting both IL-17 and IFN-γ, or only IFN-γ display characteristics of IL-17 single-producing cells but not of Th1–IFN-γ+ cells. Furthermore, IL-12 induces these populations to become “Th1-like” cells. These findings suggest that the hallmark Th1 cytokine IFN-γ could also be defined as a Th17 cytokine depending on the milieu and other cytokines produced by T cell subsets. Taken together, our study,
in which we profile the phenotype and plasticity of human Th17 cells, provides novel insights into the relationship and functionality of the Th17 and Th1 subsets.

Materials and Methods

**T cell cultures**

Buffy coats were obtained from normal healthy human volunteers participating in the Stanford Medical School Blood Center blood donation program. Permission to perform this investigation was granted by the ethical committee of our institution and written informed consent in accordance with the Declaration of Helsinki from all blood donors. CD4+ T cells were isolated through magnetic bead separation (CD4+ T cell Isolation Kit II; Miltenyi Biotec, Auburn, CA) and isolated from the purified CD4+ T cells by two rounds of depletion with anti-CD45RO and anti-CD25 magnetic beads (Miltenyi Biotec), according to the manufacturer’s instructions. T cells were activated in the presence of beads coated with anti-CD3/CD28/CD2 Abs (1 bead:10 cells). Th17 cells were generated in vitro using anti-CD4 Ab and the IL-17 and IFN-γ coexpression assay kits. As shown in Fig. 1B, differences in gene expression levels of IL17 and INFγ correlated with protein production. Six distinct gene expression clusters were observed when assessing IL17 and INFγ message in all the donors, showing that these cell subsets could be isolated and classified in a reproducible manner based on their cytokine expression. The sorted Th17–IL17−negative cohort, whether INFγ−expressing or not, was found to have higher levels of IL17 gene expression than Th1 cultures, albeit lower levels compared with IL17-positive cells (Fig. 1B). Similar observations were made at the protein levels when assessing the mean fluorescence intensity of the IL17-negative populations (data not shown), suggesting that even IL17-negative populations from the Th1 pool express low levels of IL17, as opposed to Th1 cells.

We subsequently examined the expression of the Th17- and Th1-associated cytokines in the different cell populations. Similar to IL17 single-producing cells, Th17–INFγ+ and IL17−INFγ− cells had much higher expression of IL17F, IL22, CCL20, IL26, lymphotoxin α, and IL1α transcripts than Th1–INFγ− cells (Fig. 1C, 1D). In contrast, Th1–INFγ−populations expressed greater message levels of genes associated with classical Th1 phenotype, such as XCL1, lymphotoxin γ, and IFN γF (Fig. 1E). These results suggest that Th17–INFγ− and IL17–INFγ−producing cells express a cytokine profile more related to the Th17 rather than the Th1 lineage. Interestingly, the expression of IL17F, IL22, and CCL20 genes was significantly increased in IL17 and IL17–INFγ− double-producing cells, whereas IL26 gene expression was slightly higher in Th17–INFγ−cells than other Th17 populations (Fig. 1C). Higher expression of IL-22 protein within IL17+ and IL17−INFγ−cells was also confirmed following intracellular staining, and IL-17F production was mostly confined to cells expressing IL-17 (Fig. 1F). In contrast, Th1–INFγ−cells produced very low levels of IL-22 (<1%), and virtually no IL-17F.
cells were detected. Thus, Th17 but not Th1 cell subsets form heterogeneous populations with respect to IL-22 and IL-17F production.

These results collectively provide evidence that IFN-γ–expressing cells arising from Th17– or Th1-inducing conditions represent distinct Th cell subsets, highlighting the importance of analyzing the expression of additional cytokines besides IFN-γ to distinguish Th1–IFN-γ+ from Th17–IFN-γ+ populations. In addition, our findings suggest that IL-17+, IL-17+/IFN-γ+, and Th17–IFN-γ+ all belong to the Th17 lineage.

Receptor-related orphan receptor γt segregates within IL-17–producing cells whereas T-bet is not exclusive to IFN-γ only producing cells

The development of Th17 and Th1 cells requires the expression of a lineage-specific transcription factor (i.e., receptor-related orphan receptor [ROR]γt and T-bet, respectively) (33, 34). RORγt encoding gene RORC was predominantly found within the IL-17+ and IL-17+/IFN-γ+ populations, whereas T-bet encoding gene (TBX21) levels were similar between Th17 and Th1 cell subsets (Fig. 2A, 2B). Interestingly, levels of Eomes transcripts, another T-box family of transcription factor (35), were higher in cells expressing IFN-γ, particularly in Th1–IFN-γ cells (Fig. 2C). Because both the aryl hydrocarbon receptor and runt-related transcription factor 1 (RUNX1) have previously been shown to influence Th17 development (36, 37), we also analyzed whether these genes were differentially regulated among the various T cell populations, but message levels of AHR and RUNX1 were comparable in Th1 and Th17 subsets (Fig. 2D, 2E). However, RUNX2 message was expressed at significantly greater levels in Th17 cells (Fig. 2F). Finally, the gene expression of the AP-1 transcription factor c-MAF was enhanced in Th17 cell subsets compared with Th1 cells. Message levels of c-MAF were, however, reduced in Th17–IFN-γ+ cells compared with cells expressing IL-17 (Fig. 2G), suggesting that c-MAF correlated more to IL-17 production rather than IFN-γ expression within Th17 cell subsets. Taken together, this transcription factor profile further supports the idea that Th17–IFN-γ+ and IL-17+/IFN-γ+ cells are related to the Th17 rather than the Th1 pathway.

Cells coexpressing IL-17 and IFN-γ are less stable than IL-17 or IFN-γ single-producing cells

When considering the pathogenic potential of Th17 cell subsets, a key issue is how stable and plastic these subpopulations are. We therefore investigated whether Th17 populations could retain expression of IL-17 and/or IFN-γ, as well as other proinflammatory cytokines (i.e., IL-17F and IL-22). In vitro polarized human Th1 and CCR6+ Th17 cell subsets were live sorted based on their ability to secrete IL-17 and/or IFN-γ, using a bispecific Ab combining an

**FIGURE 1.** Profiling of human Th17 and Th1 cell subsets based on IL-17 and/or IFN-γ production. Human Th17 and Th1 cells were polarized from naive CD4+ T cells as described in Materials and Methods. Different cell subsets were then sorted based on their IL-17 and/or IFN-γ intracellular production. A, Intracellular IL-17 and IFN-γ staining in CD3+CD8 T cells following stimulation with PMA/ionomycin. Data from one donor are shown and are representative of at least eight independent experiments. B, Real-time PCR analysis of IL17 and IFNG gene expression in sorted populations. Data are plotted as log_{10} (IL17) versus log_{10} (IFNG) gene expression. C–E, Real-time PCR analysis of the indicated gene expression in Th17 and Th1 subsets. F, Intracellular IL-17F and IL-22 staining in CD3+ T cells following stimulation with PMA/ionomycin. Shown populations are gated on IL-17+ and/or IFN-γ–producing cells, as indicated. Data from one donor are shown and are representative of at least six independent experiments. Results from eight donors are shown in B and C; horizontal bars represent median values. Results in D and E are shown as mean + SEM of three independent donors. *p < 0.05; **p < 0.01.
Real-time PCR analysis of the indicated gene expression. Results from eight donors are shown; horizontal bars represent median values. *p < 0.05; **p < 0.01; and ***p < 0.001.

FIGURE 2. Transcription factors associated with human Th17 cell subsets. Human Th17 and Th1 cells were polarized from naive CD4+ T cells as described in Materials and Methods. Different cell subsets were then sorted based on their IL-17 and/or IFN-γ intracellular staining. A–G, Real-time PCR analysis of the indicated gene expression. Results from eight donors are shown; horizontal bars represent median values. *p < 0.05; **p < 0.01; and ***p < 0.001.

FIGURE 3. Gene expression of IL17 and IFNG in populations live sorted using a cytokine secretion assay. Human Th17 cells were polarized from naive CD4+ T cells as described in Materials and Methods. Different T cell populations were then sorted based on their IL-17 and/or IFN-γ secretion using a cytokine secretion assay. IL17 and IFNG gene expression was analyzed by real-time PCR analysis.

anti–IL-17 (or anti–IFN-γ) mAb with an anti-CD45 mAb. Analysis of IL-17 and IFNG gene expression in sorted populations correlated with protein expression (Fig. 3). Each cell subset was then expanded for 1 wk under nonpolarizing conditions, and levels of IL-17, IFN-γ, IL-22, and IL-17F were assessed following intracellular staining.

Under nonpolarizing conditions, IL-17+ cells remained predominantly IL-17 single-producing cells after culture, with up to 15% converting to IL-17+/IFN-γ- double-producing cells, and only a low percentage changing to IFN-γ+ cells (Fig. 4A). Conversely, most Th1–IFN-γ+ cells maintained IFN-γ expression and a low proportion of the cells coexpressed IL-17, whereas virtually no IL-17+ single-producing cells were detected. IL-22 was produced by both IL-17 and IFN-γ single-producing cells isolated from Th17 cultures, whereas IL-17F expression was predominantly associated with IL-17 secretion (Fig. 4B), in line with our previous observations on bulk CCR6+ Th17 cells (Fig. 1F). Approximately 60% of the Th1–IFN-γ+ cells retained IFN-γ expression after culture (Fig. 4A). Very few Th1–IFN-γ+ cells were able to produce IL-22 (Fig. 3B), showing again that IFN-γ+ cells producing IL-22 belong to the Th17 cell lineage rather than the Th1 subset.

In contrast, only ~40% of the IL-17+/IFN-γ+ cohort maintained expression of both cytokines, and a significant number of cells became single-producing cells for IFN-γ or to a lesser extent for IL-17 (Fig. 4A). IL-17+/IFN-γ+ cells remained heterogeneous for IL-17F+ and/or IL-22 expression, similarly to the IL-17+ cell subset (Fig. 4B). Interestingly, IL-17+/IFN-γ+ cells could become either IL-17+ or IFN-γ-producing cells, suggesting that even cells that were negative for IL-17 and IFN-γ after initial polarization toward a Th17 phenotype can acquire the ability to produce IL-17 or IFN-γ later on, even in the absence of a continued Th17-driving environment. This negative population, to a lesser extent, could also develop into IL-17+/IFN-γ double-positive cells. Together, these results suggest that in vitro differentiated IL-17+ or Th17–IFN-γ+ cells are a stable subset of effector T cells, whereas cells producing both IL-17 and IFN-γ are more plastic and can become IL-17+ and/or IFN-γ-producing cells.

It has been previously reported that expression of CCR6 and CCR4 defines a population of Th17 cells that exclusively produce IL-17 and not IFN-γ (11). CCR6 expression was maintained within the different Th17 cell subsets and was low to absent in Th1 cells (Fig. 4C). Although IL-17 single-producing cells exhibited higher CCR4 on their surface, a significant proportion of IL-17+/IFN-γ double-positive cells also displayed this chemokine receptor (Fig. 4C), suggesting that expression of both CCR6 and CCR4 does not strictly distinguish IL-17 single-expressing cells from IL-17+ cells also producing IFN-γ. Interestingly, CCR4 levels were similar between IL-17–producing cells and Th1–IFN-γ+ cells.

Th17 cell subsets become “Th1-like” IFN-γ+ cells in the presence of IL-12

It is well documented that IL-12 prevents the development of Th17 cells, both in the mouse and human systems (38). Whether IL-12 also regulates cytokine production of differentiated Th17 cells is less clear. We analyzed the plasticity of in vitro-differentiated Th1 and Th17 cells when cultured with lineage-polarizing cytokines. Th1 cells maintained IFN-γ expression when cultured in the presence of a Th17-inducing environment and did not start to produce IL-17, IL-17F, or IL-22 (Fig. 5A and data not shown). In contrast, within bulk Th17 populations, IL-12 reduced the number of IL-17+ cells while increasing the proportion of IFN-γ–producing cells (Fig. 5B). Overall, the proportion of cells expressing both IL-17 and IFN-γ was similar following IL-12 treatment. IL-12 significantly diminished the levels of IL-22 and/or IL-17F–producing cells within the Th17–IFN-γ+ cohort (Fig. 5C), suggesting that these cells become
Th1–IFN-γ like" cells. In addition, the presence of IL-12 led to a downregulation of CCR6 expression by Th17 cells (Fig. 5D). We further analyzed the effects of IL-12 on Th17 cell subsets. First, we failed to observe any significant variation of IL12RB1 or IL12RB2 gene expression among Th17 and Th1 populations (Fig. 5E), suggesting that Th17 populations were equally responsive to IL-12 activities. Cell death was low and comparable among all subsets, whether IL-12 was present or not (Fig. 5F). Furthermore, BrdU incorporation was similar in IL-17F, IL-17/IFN-γ, and Th17–IFN-γ cells in the presence of IL-12 (Fig. 5G), implying that the effects of IL-12 were not due to a reduced proliferation of IL-17+ cells and/or increased proliferation of IFN-γ-producing cells.

To further define the potential of IL-12 to directly regulate each Th17 subset, we sorted live IL-17+, IL-17+/IFN-γ, and Th17–IFN-γ+ populations and expanded these cells in the presence or absence of IL-12. The addition of IL-12 to IL-17+ cells resulted in a reduction of IL-17 single-producing cells and a strong upregulation of cells coexpressing IL-17 and IFN-γ or IFN-γ single-producing cells (Fig. 5H). Within the IL-17+/IFN-γ+ population, IL-12 inhibited IL-17 single-producing cells as well as IL-17/IFN-γ double-producing cells while enhancing IFN-γ cells. Similarly, the number of IL-17+/IFN-γ+ cells that develop in the Th17–IFN-γ subset was reduced by IL-12 (Fig. 5H). These results imply IL-12 can directly induce an IL-17+ cell to change its cytokine secretion profile and become a Th1–IFN-γ-producing cell.

Similar to IL-17 single-producing cells, IL-17+/IFN-γ+ and Th17–IFN-γ+ subsets induce antimicrobial and inflammation-associated gene expression on keratinocytes

With the cytokine profile and stability of IL-17, IL-17/IFN-γ, Th1–IFN-γ, and Th1–IFN-γ-producing cells defined, we compared in vitro the functional potential of these populations on NHEKs. Supernatants from activated Th17 and Th1 cell subsets were harvested and compared for their effects on NHEK antimicrobial
and inflammation-associated gene expression. Supernatants derived from IL-17+ or IL-17+/IFN-γ populations highly increased expression of β-defensin 2, S100A7, S100A8, and S100A9 transcripts (Fig. 6), in line with the important role of IL-17, IL-17F, and IL-22 in the induction of these genes (15, 32, 39, 40). Supernatants from Th1–IFN-γ+ cells also induced significant upregulation of these genes, albeit lower levels than cells producing IL-17 cells, whereas supernatants from Th1–IFN-γ− cells had little effect (Fig. 6), which could be partly due to the higher IL-22 production by Th17–IFN-γ+ cells. These results further highlight that IL-17+/IFN-γ− and Th17–IFN-γ− populations belong to the Th17 and not the Th1 lineage, despite their ability to produce IFN-γ.

Discussion
Whether Th1 and Th17 cells are mutually antagonistic remains incompletely elucidated, and the presence of T cells producing IL-17 and IFN-γ in both human and mouse inflammatory tissues complicates this relationship. By profiling Th17 and Th1 cell
population or a mix of Th1– and Th17–IFN-γ subset, and further studies should assess whether IFN-γ-expressing IFN-γ-producing IL-17 and IFN-γ (or T-bet) is not sufficient to distinguish a Th17–IFN-γ Th1 cells, our data suggest that the sole analysis of IFN-γ expression, IL-17, and such “Th22” cells may represent a distinct helper T cell. With respect to previous studies identifying When looking at transcription factor expression, RORγt was tightly linked to IL-17 production, as expected. However, T-bet appears more promiscuous, being present in all subsets. T-bet expression in IL-17–producing cells is in accordance with previous studies showing that T-bet was required for mice in optimal IL-17 production in response to IL-23 and that T-bet was critical during development of EAE and encephalitogenicity of Th1 and Th17 cells (44–49). In contrast to previously held assumptions that IFN-γ+ (IL-17+) cells are always Th1 cells, our data suggest that the sole analysis of IFN-γ expression (or T-bet) is not sufficient to distinguish a Th17–IFN-γ from a Th1–IFN-γ–producing cell. With respect to previous studies identifying Th17–IFN-γ+ and Th17–IFN-γ–producing cells that all belong to the Th17 subset, and further studies should assess whether IFN-γ–producing cells identified ex vivo in inflamed tissues represent a homogeneous population or a mix of Th1– and Th17–IFN-γ–expressing cells.

Profiling of Th17 and Th1 subsets identified Runx 2 and c-MAF as Th17 rather than Th1 related transcription factors. Interestingly, Zhang et al. (37) showed that the differential interaction of Runx 1 with RORγt or FOXP3 could regulate mouse Th17 differentiation. We nevertheless observed a similar expression of RUNX 1 gene among human Th17 and Th1 cell subsets at the time point analyzed. Both Runx 1 and Runx 2 can upregulate RORγt-induced IL-17 expression, and conversely, silencing of Runx 1 or Runx 2 led to downregulated IL-17 production, although Runx 1 activities were more potent (37). Despite the recent association of c-MAF with IL-10 production by Th17 cells in the mouse system (50), we did not observe any correlation between c-MAF and IL10 gene expression (data not shown). This suggests that either cMAF is not able to induce IL-10 expression in our system, which could be due to the inhibitory effect of PGE2 on IL-10 production (51), and/or that c-MAF, besides regulating Th17 production of IL-10, could also be implicated in other processes in the Th17 program. In this regard, the B cell-activating transcription factor, another member of the AP-1 family of transcription factors, was recently shown to be required for development of Th17 cells (52).

The emergence of new Th cell subsets, including Th17 cells, or induced regulatory T cells, has led to a rethinking of the concept that effector T cells are not plastic and represent terminal endpoints in the CD4 T cell developmental program. Studies performed in mice suggest that Th17 cells derived from naive T cells in vitro fail to retain IL-17 and IL-17F expression without continuous exposure to TGF-β and IL-6 (53, 54). In contrast, we show in this study that in vitro-derived human IL-17+ or Th17–IFN-γ+ cells could maintain the expression of IL-17 or IFN-γ, respectively, without additional exposure to Th17-inducing conditions. Even though cells expressing both IL-17 and IFN-γ presented characteristics of both IL-17+ and Th17–IFN-γ+ single-producing cells, these cells were, however, less stable and could maintain their propensity to produce both IL-17 and IFN-γ but could also become IL-17 or IFN-γ single-producing cells, suggesting that this subset could possibly be more pathogenic. In this regard, Kebir et al. (27) recently reported that IL-17 and IFN-γ double-producing cells preferentially cross the human blood-brain barrier, and these cells are present in brain lesions of MS patients.

Th1 cells were not as flexible and production of IL-17 could not be induced in the presence of a Th17 inducing milieu. In contrast, Th17 cells are sensitive to IL-12, in line with observations previously made both in the human and mouse systems (12, 53, 55, 56). Such plasticity of Th17 but not Th1 cells could be explained by recent epigenetic studies examining histone modifications and DNA methylation that regulate gene expression in effector T cell subsets (57, 58). The plasticity of Th17 cells was also recently highlighted in vivo in mouse models of ocular inflammation, colitis, or diabetes showing that after adoptive transfer of IL-17+ cells, these cells converted rapidly into IFN-γ–producing cells that were critical for disease (53, 56, 59). In addition, Th17 cells only induced diabetes efficiently after conversion into IFN-γ–producing cells in lymphopenic hosts (60). Further in vivo studies should address whether such IFN-γ–producing cells belong purely to the Th1 or have more the characteristics of the Th17–IFN-γ+ subset.

Th17–IFN-γ+ population became “Th1-like” cells in the presence of IL-12, as measured by downregulation of CCR6 expression as well as IL-17F and IL-22 production. Such effects of IL-12 were not due to a differential expression of the IL-12R or to a differential proliferation of Th17 subsets. Thus, there appear to be three potential mechanisms whereby IL-12 directly regulates IL-17 and IFN-γ production: first, by inducing IL-17+ cells to coexpress IFN-γ; second, by inhibition of IL-17 in a cell producing both IL-17 and IFN-γ; and finally, by converting an IL-17+ cell into an IFN-γ+ cell. These observations are in line with work done on human or mouse T cell clones showing the ability of IL-12 to induce IL-17 clones to produce IFN-γ, together with an increase of T-bet expression and a downregulation of RORγt and IL-17 (12, 46, 56).
In conclusion, this work provides new insights into the heterogeneity, functionality, and relationship between human Th17 and Th1 cell subsets. In addition, our results suggest that the hallmark Th1 cytokine IFN-γ could also be defined as a Th1 cytokine depending on the milieu and other cytokines produced by T cell subsets.

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