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Cytokine-regulated Th17 plasticity in human health and diseases

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Summary

Upon activation, naïve CD4$^+$ T helper (Th) cells differentiate into distinct Th effector cell lineages depending on the local cytokine environment. However, these polarized Th cells can also adapt their function and phenotype depending on the changing cytokine environment, demonstrating functional plasticity. Here, Th17 cells, which play a critical role in host protection from extracellular pathogens and in autoimmune disorders, are of particular interest. While being able to shift phenotype within their lineage, Th17 cells can also acquire characteristics of Th1, Th2, T follicular helper (Tfh) or regulatory T cells. Th17 cell identity is determined by a spectrum of extracellular signals, including cytokines, which are critical orchestrators of cellular immune responses. Cytokine induces changes in epigenetic, transcriptional, translational and metabolomic parameters. How these signals are integrated to determine Th17 plasticity is not well defined, yet this is a crucial point of investigation as it represents a potential target to treat autoimmune and inflammatory diseases. The goal of this review was to discuss how cytokines regulate intracellular networks, focusing on the regulation of lineage-specific transcription factors, chromatin remodelling and metabolism, to control human Th17 cell plasticity. We discuss the importance of Th17 plasticity in autoimmunity and cancer and present current strategies and challenges in targeting pathogenic Th17 cells with cytokine-based approaches, considering human genetic variants associated with altered Th17 differentiation. Finally, we discuss how modulating Th17 plasticity rather than targeting the Th17 lineage as a whole might preserve its essential immune function while purging its adverse effects.

Keywords: Cytokine modulation; Human Th17; inflammation; T-cell plasticity.

Abbreviations: APC, antigen-presenting cell; AS, ankylosing spondylitis; CAPS, cryopyrin-associated periodic syndrome; CMC, chronic mucocutaneous candidiasis; CyTOF, cytometry by time of flight; FFA, free fatty acid; GM-CSF, granulocyte–macrophage colony-stimulating factor; IFN-γ, interferon-γ; IBD, inflammatory bowel disease; IL, interleukin; MALT, mucosa-associated lymphoid tissue; MS, multiple sclerosis; PBMC, peripheral blood mononuclear cell; PsA, psoriatic arthritis; RA, rheumatoid arthritis; RORγt, retinoid-related orphan receptor γt; Sc, single cell; SLE, systemic lupus erythematosus; seq, sequencing; STAT, signal transducer and activator of transcription; SNP, single nucleotide polymorphism; T_{CM}, central memory T cell; TCR, T-cell receptor; TF, transcription factor; TGF-β, transforming growth factor-β, Th, T helper; TNF-α, tumour necrosis factor-α; T_{RM}, tissue-resident memory T cell; Treg, regulatory T cell

INTRODUCTION

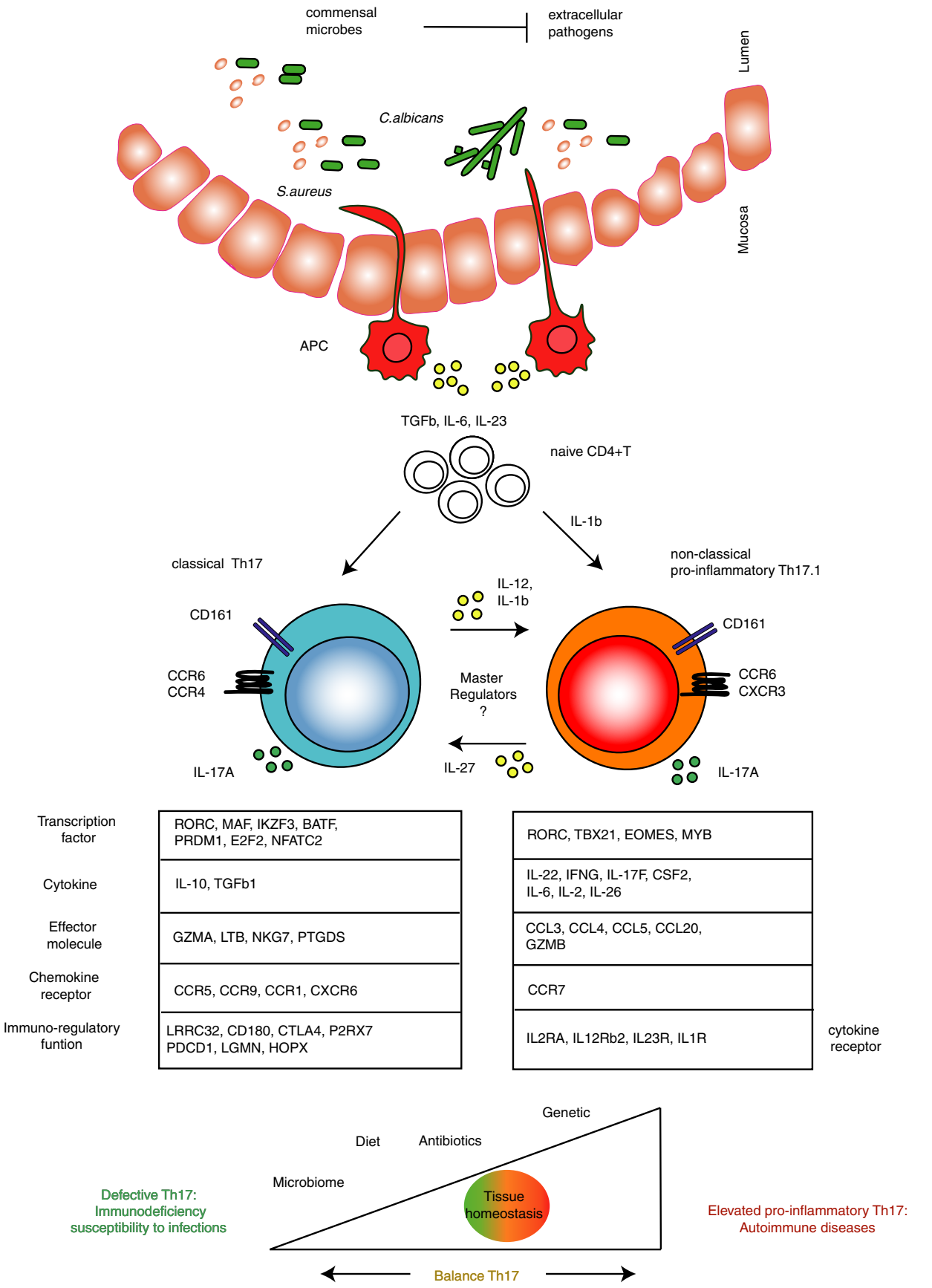
T helper (Th) $CD4^+$ T cells are major players of the adaptive immune system, orchestrating the immunity against pathogens.[1] Following their maturation in the thymus, naïve Th cells circulate through secondary lymphoid organs, including the spleen, lymph nodes and the mucosa-associated lymphoid tissue (MALT) until they recognize antigen presented on the surface of antigen-presenting cells (APCs). Following activation, naïve Th cells differentiate into different effector lineages to carry out specific immunomodulatory roles. Th lineage fate decision is controlled by (a) the identity and activation states of APCs, (b) the magnitude of T-cell receptor (TCR) signalling and 3) environmental signals.[2,3] Here, the local cytokine milieu is critical in determining T-cell differentiation into immunoregulatory cells (T regulatory (Treg)) or pro-inflammatory Th lineages (e.g. Th1, Th2, Th17).[3] These different Th lineages, in turn, produce specific cytokines that define their effector function. TGF-β (transforming growth factor-β) and interleukin-2 (IL-2) drive differentiation of naïve T cells into suppressive Tregs secreting IL-10 to maintain immune homeostasis. IL-12 drives differentiation of (IFN)-γ-producing Th1 that is critical for clearance of intracellular pathogens. IL-4 promotes differentiation into IL-4, IL-5 and IL-13 producing Th2 cells, necessary for targeting parasite infections.[4,5] IL-12, IL-6, IL-23 and IL-21 polarize IL-21-producing Tfh cells that provide B-cell help for optimal humoral immunity.[6] IL-17-producing Th17 cells differentiate from naïve Th cells in the presence of cytokines such as TGF-β and IL-6[7,8] and contribute to host mucosal immunity against bacterial and fungal pathogens.[3] Immunodeficient patients suffering from Job's syndrome, incapable of generating Th17 cells, show recurrent bacterial, fungal and chronic viral infections.[9] However, since their discovery in 2005, Th17 cells have also been implicated in the development of autoimmunity and cancer pathogenesis.[10–12] This apparent functional heterogeneity has sparked interest to better understand Th17 biology in order to manipulate it for therapeutic purposes. A defining feature of Th17 cells is their phenotypic flexibility, here defined as plasticity. While it was initially believed that Th cells remained committed to their acquired lineage,[5,13] differentiated effector Th cells can acquire functional features of other Th lineage characteristics.

Most of our knowledge of Th17 biology is based on mouse studies. Deciphering the molecular mechanism and signalling pathways in human Th17 subsets has not been as straightforward. Key similarities and differences in the differentiation programmes of mouse and human Th subsets from naïve Th precursors are discussed elsewhere.[14] In this review, we will explore the contribution of cytokines and their downstream effects (e.g. epigenetic modifications, transcription factor (TF) programmes and metabolic activity) in determining the plasticity and effector function of human Th17 cells. We highlight current therapeutic strategies targeting Th17 cells in the clinic to modulate Th17 plasticity for the treatment of inflammatory diseases and cancer.

HETEROGENEITY AND FUNCTION OF MATURE HUMAN TH17 SUBSETS

While there are few Th17 cells in the circulation, they are abundant in mucosal tissues.[15] Here, they orchestrate the balance between tolerance and inflammation, mucosal repair and healing.[16] Based on the microbiota, pathogens, other environmental factors or genetic predisposition, Th17 cells can acquire phenotypes and effector cytokine profiles that can either be detrimental or re-establish immune homeostasis.[3] Single nucleotide polymorphisms (SNPs) in genes associated with the Th17 pathway have

Figure 1. Heterogeneity, function and plasticity of human Th17 subsets. All ex vivo human Th17 cells express CCR6 and CD161 and produce IL-17A required for pathogen clearance; however, classical Th17 cells have a differential transcriptional profile compared with non-classical Th17.1 cells (genes listed below the two subsets). Classical Th17 cells can be isolated *ex vivo* (through CD161, CCR6 and CCR4) from blood and tissue of healthy individuals or generated in vitro in response to *Staphylococcus aureus* (*S.aureus*). They express the transcription factors (TFs) *MAF*, *AHR* and *IKZF3* implicated in the regulation of immunoregulatory genes (e.g. *CTLA4*, *LRRC32* and others), cytokines (e.g. *IL-10*, *TGF-β1*) and effector molecules (e.g. *GMZA*, *LTB*, *NKG7*, *PTGDS*). Non-classical pro-inflammatory Th17.1 is an heterogenous population characterized by IFN-γ production. Ex vivo Th17.1 cells express CXCR3 and coexpress IL-17 and IFN-γ, and they can be generated *in vitro* from *Candida albicans* (*C. albicans*), although without expressing CXCR3. Pro-inflammatory Th17.1 cells have a transcriptional profile similar to murine pathogenic Th17 cells, coexpressing RORγt and T-bet, IL-23R, IL-1R and IL-12Rβ2. Expression of *EOMES* is a 'marker' of acquired Th1-like phenotype. They express pro-inflammatory cytokine (e.g. *CSF2*, *IL-22*, *IL-6*, *IL-17F*, *IL-2* and *IL-26*) and effector molecules (e.g. *GZMB* and *CCL20*). Th17 cells can be generated directly after pathogen sensing by antigen-presenting cells (APCs) in the mucosa, leading to TGF-β, IL-6, IL-23 and IL-1β production, or acquire a pro-inflammatory phenotype resulting from plastic events (e.g. in the presence of pro-inflammatory cytokines such as IL-12 and IL-1β). *In vitro* human Th17.1 plasticity towards IL-10-secreting phenotype is achieved in the presence of IL-27. So far, specific master regulators directing the Th17 cell phenotypical switch from immunoregulatory to pro-inflammatory and *vice versa* have not yet been identified. Overall, microbiota composition, the nature of invading pathogens, other environmental factors (e.g. diet and antibiotics) or genetic variants shape Th17 cell phenotype and function to maintain immune homeostasis. However, compromised Th17 cell function can lead to immunodeficiency; in contrast, elevated pro-inflammatory Th17 cells can contribute to autoimmune diseases.


commensal microbes
extracellular pathogens
Lumen
C.albicans
S.aureus
Mucosa
APC
TGFb, IL-6, IL-23
naive CD4+T
IL-1b
classical Th17
non-classical pro-inflammatory Th17.1
IL-12, IL-1b
CD161
CCR6 CCR4
IL-17A
Master Regulators ?
IL-27
CD161
CCR6 CXCR3
IL-17A
Transcription factor
RORC, MAF, IKZF3, BATF, PRDM1, E2F2, NFATC2
RORC, TBX21, EOMES, MYB
Cytokine
IL-10, TGFb1
IL-22, IFNG, IL-17F, CSF2, IL-6, IL-2, IL-26
Effector molecule
GZMA, LTB, NKG7, PTGDS
CCL3, CCL4, CCL5, CCL20, GZMB
Chemokine receptor
CCR5, CCR9, CCR1, CXCR6
CCR7
Immuno-regulatory funtion
LRRC32, CD180, CTLA4, P2RX7 PDCD1, LGMN, HOPX
IL2RA, IL12Rb2, IL23R, IL1R
cytokine receptor
Genetic
Diet
Antibiotics
Microbiome
Tissue homeostasis
Defective Th17: Immunodeficiency susceptibility to infections
Elevated pro-inflammatory Th17: Autoimmune diseases
Balance Th17

been linked to autoimmune and inflammatory diseases,[17,18] but also to immunodeficiency.[19] Accordingly, IL-17 levels are increased in inflammatory lesions of patients suffering from multiple sclerosis (MS), psoriasis, rheumatoid arthritis (RA), inflammatory bowel diseases (IBDs) and Sjögren´s syndrome [31–34]; in contrast, impaired Th17 responses are associated with chronic mucocutaneous candidiasis (CMC) and bacterial infections in the lung and skin.[20]

While all human *in vivo* Th17 subsets express CCR6 and the surface marker CD161, they can be distinguished by their differential expression of the chemokine receptors CCR4 and CXCR3: classical Th17 cells ($CCR4^+CXCR3^-$) express high levels of IL-17 and low levels of IFN-γ; non-classical Th17.1 cells ($CCR4^-CXCR3^+$, also called Th17.1 or Th1/Th17) produce low levels of IL-17 and large amounts of IFN-γ; and double-positive ($CCR4^+CXCR3^+$) and double-negative (CCR4-CXCR3-) cells have been identified as Th17 cells in a precursor or transitional state.[21–24] Such heterogeneity can either be generated during initial priming from naïve $CD4^+$ T cells, or it can be the result of plastic events affecting Th effector/memory cells (Figure 1).

Most knowledge about human memory Th17 cells is based on *in vitro* studies using circulating memory T cells ($T_{CM}17$) rather than long-lived tissue-resident memory Th17 ($T_{RM}17$) cells. $T_{RM}17$ cells provide immediate response to bacterial and fungal reinfections at mucosal sites but might equally contribute to tissue damage and amplification of autoimmune diseases.[25–28] Whether different memory Th17 subsets have different potential for plasticity remains to be investigated.

Classical immunoregulatory Th17 cells

Immunoregulatory Th17 are present in blood and tissue of healthy individuals[29] and can be generated *in vitro* from naïve $CD4^+$ T cells in response to *Staphylococcus aureus* (*S.aureus*)-pulsed monocytes[30] (Figure 1). They express IL-17 and immunomodulatory genes normally associated with Tregs (e.g. CTLA-4, LRRC32). Classical Th17 cells are characterized by expression of the transcription factors (TFs) *MAF*, *AHR* and *IKZF3* (encoding for Aiolos), which have been implicated in IL-10 gene regulation in human Th cells.[29,31,32] IL-10-producing Th17 cells contribute to tissue homeostasis as loss-of-function mutations in the IL-10 or IL-10R genes lead to infantile onset of IBD in humans.[33] In MS patients, Th17 cells isolated from blood had reduced expression of IL-10 as compared to healthy controls. Conversely, higher expression of IL-10 in Th17 cells positively correlated with clinically stable disease.[31] It is currently not well understood, in which environmental cues direct the differentiation of immunoregulatory Th17 cells *in vivo*.

Non-classical pro-inflammatory Th17.1

Ex vivo non-classical human Th17.1 cells that express both IL-17 and IFN-γ have been found at sites of inflammation.[34] Given their transcriptional similarity to murine pathogenic Th17 cells,[35] human pro-inflammatory Th17.1 cells are believed to contribute to the pathogenesis of human autoimmune diseases.[31,36] Pro-inflammatory Th17 cells show double-positive Th1/Th17 features as they coexpress the TF retinoid-related orphan receptor γt (RORγt) and T-bet, as well as IL-12Rβ2 and IL-23R.[21,22,37] They are characterized by coexpression of pro-inflammatory cytokines such as granulocyte–macrophage colony-stimulating factor (GM-CSF), IL-26, CCL20 and IL-22.[38] Expression of these cytokines depends on the secretion of IL-6, TGF-β, IL-1β, IL-12 and IL-23 by APCs during differentiation or during reactivation of already-differentiated classical Th17 cells.[3,7,8,30] Cells with similar cytokine production profile can be generated *in vitro* from human naïve $CD4^+$ T cells using *Candida albicans* (*C. albicans*)-pulsed monocytes, however without expressing CXCR3.[30] Alternatively, *in vitro* polarization with *Mycobacterium tuberculosis* induces a population of Th1* (to be read as 'star') which coexpress T-bet and RORγt, CXCR3 and CCR6 and produce IFN-γ (but not IL-17). Furthermore, patients with RORC loss-of-function mutations lack Th1*,[39] suggesting that they could derive from Th17 cells through plastic events in a IL-12-, TNF-α- and/or IL-1β-dependent manner.[40] It is believed that phenotypic instability predisposes Th17 cells to acquire a pro-inflammatory phenotype in chronically inflamed tissues.

In addition to Th17-to-Th1 cell plasticity, Th17 cells can acquire characteristics of other Th or Treg subsets.

Th17 cell acquisition of other Th cell identify

Th17-to-Th2 plasticity

Circulating $CD4^+$ memory T cells producing both IL-4 and IL-17, as well as CRTh2 and CCR6, GATA3 and RORγt, have been identified in patients with allergic asthma.[41,42] However, it is still unclear whether Th17/Th2 cells derive from Th17 or Th2, and whether IL-4 (driving Th17 to Th2 cells) or IL-1β, IL-6, IL-21 (driving Th2 to Th17 cells) or additional cytokines are needed. An in-depth understanding of the origin and the role of these double cytokine producers in the human lung could be vital for targeting respiratory diseases, such as neutrophilic asthma.

Th17-to-Tfh plasticity

A fraction of circulating memory human Tfh cells expresses CXCR5 and CCR6, BCL6 and RORγt, and produces IL-21, IL-22 and IL-17 (hence termed Tfh17).[6,43] It

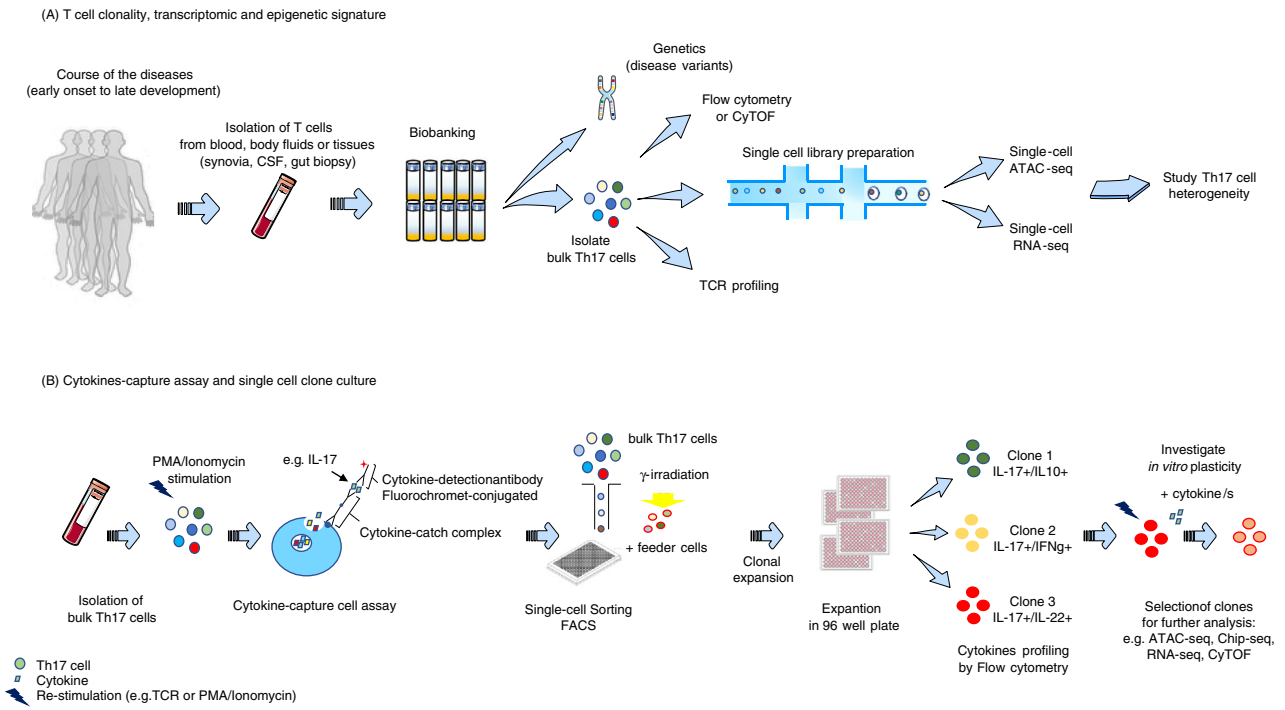


Figure 2. Current methods to study human Th17 cell heterogeneity and plasticity. A) To resolve the heterogeneity and dynamic of specific Th17 populations in a disease setting, it is relevant to isolate T cells from blood, other body fluids or tissues (e.g. synovia, cerebrospinal fluid (CSF), gut biopsy) at several time-points (e.g. before and after treatment, at multiple disease stages). Collected cells can be biobanked for traceability and used at later time-points. Genetic analysis can be performed to identify risk variants associated with the disease and altered Th17 cell phenotype and function, and, in parallel, perform TCRVβ sequencing to investigate clonality. Flow cytometry (FACS) or mass cytometry (CyTOF) allows to immunophenotype bulk Th17 cell populations to dissect their complexity. Th17 cell heterogeneity can be resolved by *ex vivo* epigenetic and transcriptional deep phenotyping at single-cell level combining, for example, ATAC-seq and RNA-seq. Isolation of cytokine-specific Th17 cells by cytokine capture assay allows *in vitro* generation of homogeneous Th17 clones. Isolation of bulk Th17 cells from, for example, blood and their stimulation with PMA/ionomycin allow single-cell sorting, by FACS, of specific cytokine-expressing Th17 cells and culturing with feeder cells. Following clonal expansion, each clone is transferred and expanded in a 96-well plate. Th17 clones can be immunophenotyped before and after restimulation, which allows clone selection for further investigation. Plasticity of a defined Th17 clone can be investigated after reactivation and culturing with cytokines using CyTOF, RNA-seq, ATAC-seq and Chip-seq.

remains to be explored whether Tfh17 cells originate from Tfh or Th17 cells. The Th17-to-Tfh plasticity could have implications for Th17-mediated autoantibody generation.

Th17-to-Treg plasticity

Evidence from fate mapping experiments in mice identified Th17-to-T regulatory cell transdifferentiation events.[44] While this phenomenon has yet to be reported in humans, studies on skin samples from psoriasis patients show IL-17 production in Treg-like cells.[45] Moreover, the analysis of synovial tissue from active RA patients revealed the presence of IL-17^{+} Foxp3^{+} T cells.[46] For details on the role and origin of IL-17-producing Treg cells in human diseases, see reference.[47] A deeper understanding of mechanisms underlying the Th17-Treg transdifferentiation in human conditions might be critical to restore tolerance to self in autoimmune diseases.

CURRENT METHODS TO STUDY HUMAN TH17 CELL HETEROGENEITY AND PLASTICITY

The study of human Th17 heterogeneity and plasticity requires different sets of tools (Figure 2). While heterogeneity can be resolved by deep phenotyping of Th17 populations in a cross-sectional manner, plasticity requires the longitudinal study of a defined Th17 cell population. Due to the heterogeneity of circulating effector/memory Th17 cells, the study of human Th17 plasticity *ex vivo* is complicated and warrants controlled *in vitro* systems.[3]

Methods to study Th17 heterogeneity

Single-cell RNA-sequencing (scRNA-seq) has proven a powerful technology to study heterogeneity and pathogenicity of mouse Th17 cells,[48] and it holds promise for resolving functional and cellular heterogeneity in human tissues as well.[49] Generating 'transcriptomic' and

'epigenomic' signatures on single-cell (sc) level using, for example, scRNA-seq, scChIP-seq from chromatin modifications or scATAC-seq will allow to identify phenotypically distinct Th17 cell subsets[50,51] at a given location and time-point.

A powerful tool to study human Th17 heterogeneity at the protein level is the cytokine capture assay.[24] Isolation and immunophenotyping of cytokine-specific Th17 cells using flow cytometry or mass cytometry (CyTOF)[52] allows to link function to phenotype in Th17 cells at a single-cell level.[24] Further, single-cell sorting of cytokine-secreting cells can be used to generate single-cell clone cultures of Th17 cells (Figure 2).

Methods to study Th17 plasticity

The generation of human Th17 cell clones provides the opportunity to study the plasticity of a homogenous Th17 cell population.[29] Single-cell clones can be expanded *in vitro* using feeder cells (e.g. immortalized peripheral blood mononuclear cells, PBMCs) and can be immunophenotyped before and after reactivation by, for example, flow cytometry, proteomics, RNA-seq and ATAC-seq. This method was used to address the plasticity of IL-10^+ and IL-10^- Th17 clones when cultured in the presence of IL-1β and IL-27, respectively.[29]

High-throughput sequencing technologies now allow examination of antigen receptor repertoires by resolving the T-cell receptor β variable region (TCRVβ) at single-nucleotide level.[53] This method relies on two basic principles of T-cell biology: (a) every T-cell clone has a unique TCR sequence, and (b) clonal expansion following cognate antigen recognition. In healthy individuals, the TCR repertoire is polyclonal with little overrepresentation of T cells deriving from a single $CD4^+$ T-cell clone.[54] In the context of infections, cancer or immunological disorders,[55] T cells that have been activated and underwent clonal expansion will be overrepresented. Hence, assessing Th17 cell clonality coupled with transcriptional and epigenetic sequencing during the course of a disease or therapy will allow the study of plasticity in T cells *in vivo* (Figure 2).

In the following sections, we will discuss the downstream effects of cytokines involved in human Th17 plasticity, with particular emphasis to Th17-to-Th1 plasticity.

In this review, human memory Th17 cells are defined as $CD4^+$ RA45CD̄ $CCR6^+CCR4^+CXCR3̄$ memory cells unless reported otherwise. Single-cell memory Th17 clones were derived from these cells.

CYTOKINE-MEDIATED SIGNALLING IN TH17 CELLS AND TARGETING FOR AUTOIMMUNITY

Th17 cells and their developmental plasticity are regulated by distinct combinations of cytokines and environmental cues. Here, we discuss the effects of two distinct classes: (1) cytokines that promote heterogeneity and induce plasticity towards pro-inflammatory Th17 cells; and (2) effector cytokines released by Th17 cells in driving autoimmunity and inflammation. For both categories, we reference to completed or ongoing clinical trials that have tried to ameliorate autoimmune and inflammatory disease by cytokine targeting (Table 1).

Cytokines driving Th17 plasticity

Members of the IL-12 family (e.g. IL-12, IL-23 and IL-27) are pleiotropic mediators,[56,57] which are produced by myeloid cells and play a role in shaping human memory Th17 cell plasticity. All IL-12 family cytokines consist of an α- and a β-cytokine subunit, which can be shared among family members. While IL-12 and IL-23 share the p40 β-subunit, they differ in their α-subunit (IL-12p35 and IL-23p19, respectively).[58] Interestingly, IL-12 and IL-23 receptors also share a common β-subunit (IL-12Rβ1), while having a specific α-subunit (IL-12Rβ2 and IL-23R), respectively. Upon receptor binding, both IL-12 and IL-23 activate both STAT3 and STAT4 signalling pathways, with IL-12 predominantly signalling through STAT4 and IL-23 signalling through STAT3.[56] IL-12 induces IFN-γ production in T and NK cells and is increased at sites of chronic inflammation.[59] IL-12 can contribute to the conversion of Th17 to non-canonical Th1 cells as stimulation of both human Th17 and Th17.1 cells with IL-12 downregulates RORγt and IL-17, while upregulating expression of IFN-γ and T-bet.[22,60]

IL-23 is dispensable for initial Th17 differentiation but plays a key role in the stabilization and expansion of pathogenic Th17 cells.[12] IL-23-dependent STAT3 signalling contributes to lineage stabilization, while STAT4 activation promotes IFN-γ expression and plasticity towards a pro-inflammatory phenotype.[61] This mechanism requires upregulation of IL-23R by pre-Th17 cells.[61] In human Th17 cells, IL-23 synergizes with TGF-β and IL-1β to induce upregulation of IL-23R during differentiation.[8] The importance of the IL-23/IL-23R signalling axis is highlighted by the finding that multiple gene polymorphisms in the IL-23R gene correlate with an increase in intestinal Th17.1 cells and an increased risk to develop IBD, psoriasis, RA, psoriatic arthritis (PsA) and ankylosing spondylitis (AS).[17] However, mechanistic studies elucidating how these SNPs contribute to disease pathogenesis are still missing. Clinical trials using monoclonal antibodies either targeting the IL-12p40 subunit (shared by IL-12 and IL-23) or targeting IL-23p19 yielded good results in the treatment of several autoimmune diseases, including Crohn´s disease, psoriasis and psoriatic arthritis (Table 1).

IL-27 is a heterodimeric cytokine comprised of the p28 and Epstein–Barr virus-induced gene 3 (EBI3) subunits.[62] IL-27 mediates its effects by signalling through IL-27Rα

Table 1. Cytokine-mediated signalling in Th17 cells and targeting for autoimmunity

Targeted cytokine	Potential drug (company)	Indication/status	Identifier
Cytokine driving Th17 plasticity			
IL−12p40 and IL−23p40	Ustekinumab (Janssen)	Psoriasis/ Phase III[A]	NCT01009086
			NCT01077362
		Psoriatic arthritis / Phase III[A]	NCT01009086
			NCT01077362
		Ankylosing spondylitis/ Phase III	NCT02438787
		Ulcerative colitis/ Phase III[A]	NCT02407236
		Crohn´s disease/ Phase III[A]	NCT02407236
		SLE/ Phase III	NCT03517722
		Primary Sjögren's syndrome/Phase I	NCT04093531
IL−23 p19	Tildrakizumab (Merck/Sun Pharma)	Plaque psoriasis/ Phase III[A]	NCT01729754
		Psoriatic arthritis/ Phase III	NCT04314544
		Ankylosing spondylitis/ Phase II-III	NCT03552276
	Guselkumab (Janssen)	Plaque psoriasis/ Phase III[A]	NCT02207244
		Psoriatic arthritis/ Phase III	NCT03158285
		Crohn´s disease/ Phase III	NCT04397263
		Ulcerative colitis/ Phase II-III	NCT04033445
		Lupus nephritis/ Phase II	NCT04376827
	Brazikumab (MEDI2070/ AMG139) (AstraZeneca)	Crohn´s disease/ Phase II	NCT01714726
		Psoriasis/ Phase I	NCT01094093
	Risankizumab (BI655066) (Boehringer Ingelheim, AbbVie)	Plaque psoriasis/ Phase III[A]	NCT02684357
			NCT02672852
		Psoriatic arthritis/ Phase III	NCT03671148
		Ankylosing spondylitis/ Phase II	NCT02047110
		Ulcerative colitis/ Phase II-III	NCT03398148
	Mirikizumab (LY3074828) (Lilly)	Asthma/ Phase II	NCT02443298
		Crohn´s disease/ Phase III	NCT03105128
		Psoriasis/ Phase III	NCT03556202
		Crohn´s disease/ Phase III	NCT03926130
		Ulcerative colitis/ Phase III	NCT03518086
IL−1b	Canakinumab (Novartis)	SJIA / Phase III[A]	NCT00886769
		Gouty arthritis/ Phase III	NCT01362608
		Type 1 diabetes/ Phase II	NCT00947427
IL−1R	Anakinra (Swedish Orphan Biovitrum)	Rheumatoid arthritis/ Phase III[A]	153
		Asthma/ Phase I-II	NCT03513471
		SJIA / Phase I-II	NCT00339157
	Rilonacept	Juvenile idiopathic arthritis/ Phase II	NCT00534495
Th17.1-derived effector cytokines			
IL−17A	Secukinumab (Novartis)	Psoriatic arthritis/ Phase III[A]	NCT02404350
		Ankylosing spondylitis/ Phase III[A]	NCT01358175
		Psoriasis / Phase III[A]	NCT01365455
			NCT01358578
		Rheumatoid arthritis/ Phase III	NCT01350804
	Ixekizumab (Lilly)	Ankylosing spondylitis/ Phase III[A]	NCT02757352
		Psoriasis/ Phase III[A]	NCT01474512,
			NCT01597245
		Psoriatic arthritis /Phase III[A]	NCT01695239
	CNTO6785 (Janssen)	Rheumatoid arthritis/ Phase II	NCT01909427
		COPD/ Phase II	NCT01966549
			NCT01828086
	CJM112 (Novartis)	Psoriasis/ Phase I-II	NCT03299686
		Asthma/ Phase II	

Table 1. *(Continued)*

Targeted cytokine	Potential drug (company)	Indication/status	Identifier
IL−17A and IL−17F	Bimekizumab (UCB)	Rheumatoid arthritis/ Phase II	NCT02430909
		Ankylosing spondylitis/ Phase III	NCT03928743
		Psoriasis/ Phase III	NCT03598790
		Psoriatic arthritis/ Phase III	NCT04009499
IL−17RA	Brodalumab (AstraZeneca)	Plaque psoriasis/ Phase III[A]	NCT04305327
		Psoriatic arthritis/ Phase III	NCT02024646
IFN-g	Fontolizumab (PDL BioPharma)	Crohn´s disease/ Phase II	NCT00072943
		Rheumatoid arthritis/ Phase II[T]	NCT00281294
	AMG811 (Amgen)	Psoriasis/ Phase I	NCT01510951
		SLE/ Phase I	NCT02291588
GM-CSF	Otilimab (GSK3196165) (GlaxoSmithKline)	Rheumatoid arthritis/ Phase III	NCT04333147
	Namilumab (Takeda)	Rheumatoid arthritis/ Phase II	NCT02379091
		Psoriasis/ Phase II	NCT02129777
	Otilimab	Multiple sclerosis/ Phase I-II	NCT01517282
	MOR103 (MorphoSys AG)	Rheumatoid arthritis/ Phase I-II	NCT01023256
		Asthma/ Phase II	NCT01603277
	Lenzilumab (KB003) (Humanigen)	Rheumatoid arthritis/ Phase I	NCT01357759
GM-CSF-R	Mavrilimumab (CAM−3001) (Cambridge Antibody Technology, MedImmune)	Rheumatoid arthritis/ Phase II[T]	NCT01712399
			NCT01050998
			NCT01706926
IL−22	Fezakinumab (Pfizer)	Atopic dermatitis/ Phase II	NCT01941537
		Psoriasis/ Phase I	NCT00563524
TNF-a	Adalimumab (AbbVie) or infliximab (Johnson& Johnson)	Rheumatoid arthritis/ Phase III[A]	NCT00195702
		Juvenile idiopathic arthritis/ Phase III[A]	NCT00048542
		Psoriatic arthritis/ Phase III[A]	NCT00235885
		Ankylosing spondylitis/ Phase III[A]	NCT00195819
		Crohn's disease/ Phase III[A]	NCT00207662
		Ulcerative colitis/ Phase III[A]	NCT00385736
		Plaque psoriasis/ Phase III[A]	NCT01680159
		IBD/ Phase III[A]	NCT00805766
	Certolizumab pegol (UCB)	Psoriatic arthritis/ Phase III[A]	NCT01087788
		Plaque psoriasis/ Phase III[A]	NCT02326298
		Ankylosing spondylitis/ Phase III[A]	NCT02505542
		Rheumatoid arthritis/ Phase III[A]	NCT00160693
		Crohn's disease/ Phase III[A]	NCT00160524
		Axial spondyloarthritis/ Phase III[A]	NCT01087762
	Golimumab (Janssen)	Ulcerative colitis/ Phase II	NCT01090154
		Rheumatoid arthritis/ Phase III[A]	NCT00299546
		Psoriatic arthritis/ Phase III[A]	NCT00265096
		Ankylosing spondylitis/ Phase III[A]	NCT01871649
		Ulcerative colitis/ Phase III[A]	NCT00487539
	Ozoralizumab (Ablynx)	Juvenile arthritis/ Phase III	NCT02277444
		Rheumatoid arthritis/ Phase III	NCT04077567
TNFR	Etanercept (Amgen)	Rheumatoid arthritis/ Phase III[A]	NCT02378506
		Juvenile idiopathic arthritis/ Phase III[A]	NCT03780959
		Psoriatic arthritis/ Phase III[A]	NCT00317499
		Ankylosing spondylitis/ Phase III[A]	NCT01258738
		Plaque psoriasis/ Phase III[A]	NCT01241591

Clinical trial[A], approved by FDA; Clinical trial[T], terminated; IL-12 p40, p40 subunit of interleukin-12; IL-23 p19, p19 subunit of interleukin-23; IL-23 p40, p40 subunit of interleukin-23; IL-17A, interleukin-17A; IL-17F, interleukin-17F; IL-17RA, IL-17 receptor A; IFN-γ, interferon γ; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL-22, interleukin-22; TNF-α, tumour necrosis factor-α; TNFR, tumour necrosis factor receptor; COPD, chronic obstructive pulmonary disease; SLE, systemic lupus erythematosus; SJIA, systemic juvenile idiopathic arthritis.

and the gp130 receptor.[63] IL-27 specifically activates STAT1, STAT3 and to a lesser extent STAT4.[64] While IL-27 seems to have a pro-inflammatory effect by promoting a Th1-like phenotype, it is now also recognized as an anti-inflammatory agent, as it induces IL-10 in human T cells.[65] In addition, IL-27-induced downregulation of IL-22 and upregulation of IL-10 in both human memory $CD4^+$ T cells and memory IL-10^+ Th17 clones *in vitro*[29,66] suggests a role for IL-27 in shaping Th17 plasticity. To date, no clinical trial investigates IL-27 as a target for autoimmune disease.

IL-1β is an inflammasome-related cytokine produced by myeloid cells. IL-1β synergizes with IL-6 and IL-23 to induce differentiation of pro-inflammatory Th17 cells. It activates the p38 mitogen-activated protein kinase (MAPK) and the AKT-mTOR pathways, thus promoting the expansion and effector functions of Th17 cells.[67,68] Moreover, IL-1β-dependent signalling induces IRF4, which in turn promotes pro-inflammatory Th17 differentiation while inhibiting $Foxp3^+$ expression.[69] In fact, IL-1β modulates human memory Th17 plasticity *in vitro* and *in vivo* by downregulating IL-10 production in classical Th17 cells.[70] Targeting IL-1 signalling, both by blocking its receptor (anakinra) and by specifically neutralizing IL-1β, has proven to be effective in several autoimmune diseases (Table 1). Indeed, patients suffering from Schnitzler syndrome or cryopyrin-associated periodic syndrome (CAPS) have decreased numbers of circulating IL-10-producing Th17 cells, likely due to high IL-1β levels.[30,71] The authors showed that defective IL-10 production by the Th17 cells can be restored *in vivo* by IL-1β antibody-blocking therapy.[71] However, whether the effect of IL-1β antibody-blocking therapy on Th17 plasticity drives clinical efficacy is still a matter of debate.

IL-2 is a pleiotropic cytokine that is necessary for T-cell proliferation but influences Th cell differentiation by modulating cytokine receptor expression.[72] While IL-2 supports Th1 differentiation through STAT5-dependent upregulation of IL-12Rβ2 and T-bet, it negatively impacts Th17 cell differentiation by downregulating subunits of the IL-6R, IL-6Rα and gp130. *In vitro*, mouse Th17 cells differentiated in the presence of IL-2 maintain the expression of IL-12β2, possibly allowing the acquisition of IFN-γ production if stimulated by IL-12.[73] In human memory T cells, IL-2 promotes expression of GM-CSF in a STAT5-dependent manner[38] contributing to Th17 plasticity towards pro-inflammatory phenotype. Furthermore, IL-2 may play a role in human memory Th17 cell expansion.[74] It remains to be investigated whether it drives memory Th17 cells towards a pro-inflammatory phenotype in human inflamed tissues. In contrast, immunoregulatory low dose of IL-2, which favours Treg over Th17 differentiation, is under investigation in Crohn´s diseases (NCT02424396), relapsing–remitting MS (NCT02424396), SLE (NCT02424396) and type I diabetes (NCT02265809).

IL-21 is produced by pre-Th17 cells and promotes development and expansion of mature mouse and human Th17 cells in an autocrine manner by inducing IL-23R expression in a STAT3-dependent manner.[75,76] Increased serum levels of IL-21 in several autoinflammatory diseases, including RA and SLE, suggest a pro-inflammatory role of IL-21. In fact, treatment with monoclonal antibodies against IL-21 significantly improved inflammation in patients with active RA (NNC01140006/ Phase II (Novo Nordisk A/S)). However, its role in Th17 plasticity is not clear.

Roles of signature cytokines produced by pro-inflammatory Th17 cells

The IL-17 family comprises six members: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F, which initiate an inflammatory response by inducing the release of mediators from IL-17R-expressing, non-haematopoietic cells (e.g. cytokines, such as IL-6, IL-1, GM-CSF and TNF-α; chemokines such as CXCL1, CXCL2, CXCL5, CCL2, CCL7, CCL20 and IL-8; antimicrobial peptides (AMPs); and matrix metalloproteinases (MMPs)).[77] In the gut, IL-17 contributes to the maintenance of intestinal barrier integrity.[78] Given the range of biological effects of IL-17, which is produced by both pro-inflammatory and immunoregulatory Th17 cells, clinical trials with IL-17- or IL-17R-targeting antibodies both worsened or improved disease symptoms, depending on the type of inflammatory disease. While anti-IL-17 treatment was efficacious in psoriasis, psoriatic arthritis, ankylosing spondylitis and lichen planus,[79–82] it had a moderate or even detrimental effect in patients with rheumatoid arthritis and Crohn´s disease, respectively. This highlights the complexity of targeting the entire Th17 lineage in autoimmune diseases.

TNF-α is a pro-inflammatory cytokine produced by immune and epithelial cells in response to infection and tissue injury,[83] and is one of the major effector cytokines secreted by pathogenic Th17 cells. It initiates production and release of IL-1, IL-6, IL-8 and GM-CSF. expression and is detected at the sites of inflammation in RA, Crohn´s disease and psoriasis.[84–86] Monoclonal antibody-based anti-TNF therapy has emerged as standard of care for many autoimmune diseases. Interestingly, anti-TNF treatment of human $CD4^+$ T cells *in vitro* favours differentiation of IL-17/IL-10 double-positive Th17 cells and prevented accumulation of non-classical Th1 cells.[34]

IL-22 is member of the IL-10 family, binding to the IL-22R1 and IL-10R2 to propagate downstream signals through STAT3 signalling.[87] IL-22 maintains mucosal barrier integrity, cell survival, proliferation and synthesis of antimicrobial peptides by targeting mostly non-haematopoietic cells expressing IL-22R in the skin, intestine, liver, lung and kidney.[88,89] IL-22 is induced by the

IL-23/IL-23R axis, which is hyperactive in autoimmune diseases. Serum levels of IL-22 correlate with disease activity in Crohn´s disease[90]; additionally, patients with Crohn´s disease carrying an IL-23R risk allele have increased levels of serum IL-22.[91] Furthermore, IL-22 serum levels correlate with disease severity in psoriasis, possibly due to the effects of IL-22 on hyperplasia and abnormal differentiation of keratinocytes.[92] Thus, IL-22 acts in concert with IL-17 at the site of inflammation[93] and multiple clinical trials currently investigate the efficacy of targeting IL-22 in autoimmune diseases such as Crohn´s disease, SLE, rheumatoid arthritis and psoriasis, yet critical role of IL-22 in epithelial reaeration and wound healing remains a concern.

GM-CSF was originally identified as a haematopoietic growth factor and immune modulator produced by T and B cells, monocytes, macrophages, endothelial cells and fibroblasts.[94] GM-CSF can polarize inflammatory M1-like macrophages to produce pro-inflammatory cytokines and induce mixed Th1/Th17 responses.[95–97] It has been linked to autoimmune tissue damage and encephalomyelitis in mice and humans.[38,98,99] Mouse pathogenic Th17 cells can produce GM-CSF,[98,99] and human memory IL-10$^-$Th17 clones express significantly more CSF2 (encoding for GM-CSF) compared with IL-10$^+$Th17 cells.[29] However, data from *in vitro* cultures of human naïve T cells suggest that GM-CSF is either expressed alone in a 'GM-CSF-only' (ThGM-CSF) subset or coexpressed with IFN-γ, associating with Th1 rather than Th17 cells. ThGM-CSF can be isolated *ex vivo* as CCR10$^+$CCR4$^+$CXCR3$^-$CCR6$^-$ cells and might derive from plastic Th1 or pro-inflammatory Th17 cells.[38,100] Overall, GM-CSF is an attractive target for treatment of autoimmune diseases.[101–103] However, there are some concerns about possible treatment-related side-effects; thus, better patient stratification is warranted to improve treatment results.

IFN-γ is a classical Th1 cytokine[104] and is expressed by pro-inflammatory Th17 cells.[30,59] Clinical trials targeting IFN-γ have demonstrated efficacy, however, to a lesser extent than blocking IL-12p40-dependent upstream signalling, thus terminated (Table 1).

In the following two sections, we will discuss downstream effects of cytokine on changes in Th17 metabolism and epigenetics during T-cell plasticity.

Other microenvironmental factors as a source of Th17 cell plasticity

Recent evidence suggests that microenvironmental factors other than cytokines (e.g. oxygen, vitamins, pollutants, sodium chloride (NaCl), potassium, diet-derived metabolites and commensal microbiota) shape T-cell identity and plasticity.[105] For instance, high NaCl concentrations have an important function for Th17 differentiation and plasticity. NaCl boosts IL-17 production in *in vitro*-differentiated mouse CD4$^+$ T cells and in human memory CD4$^+$ T cells where it also enhances Th2 and suppresses Th1 cell responses.[106,107] In human memory Th17 cells, NaCl, in the presence of TGF-β, promotes their immunoregulatory function through the NFAT5-SGK1-FoxP3 pathway. However, in pro-inflammatory conditions and the absence of TGF-β, NaCl boosts pro-inflammatory cytokine production in both human and mouse Th17 cells and promotes pathogenicity in an EAE model.[108] Also, oxygen concentrations appear to impact on Th17 cell phenotype, as mouse Th17 cells cultured in hypoxic conditions (1% O_2) produced significantly more IL-10 than Th17 cells cultured at 21% O_2.[109] Thus, NaCl and O_2 are two examples of cytokine-independent factors, which modulate Th17 phenotype or plasticity in a context-dependent manner. More examples are discussed elsewhere.[105]

METABOLIC SHIFTS BETWEEN PRO- AND ANTI-INFLAMMATORY TH17 CELLS

A major downstream effect of cytokine signalling is a change in cellular metabolism.[110] However, the specific metabolic needs of different Th subsets are less well understood. PI3 K-AKT-mTOR signalling pathways and the hypoxia-induced factor 1α (Hif1α) are important to determine T-cell differentiation into Th or Treg lineages by shifting cellular metabolism from oxidative phosphorylation to the glycolysis-based Warburg effect.[110] Intriguingly, multiple cytokines, such as IL-2, IL-1β, IL-21 and TGF-β, involved in Th17 differentiation and plasticity promote PI3 K-AKT-mTORC1 activation.[111] In mouse settings, the PI3 K-AKT-mTORC1-S6 K1/2 axis promotes Th17 cell differentiation through the inhibition of *Gfi1* (a negative regulator of Th17 cells) and the nuclear translocation of RORγ.[112] How this pathway alters pro-inflammatory phenotypes of Th17 cells in human autoimmune diseases is yet to be explored.

Several mouse studies have highlighted different metabolic requirements for Th17 cells, other effectors T cells and regulatory T cells.[113,114] In mouse settings, anti-inflammatory Th17 cells have a metabolic profile similar to quiescent or memory cells (mainly using oxidative phosphorylation), while pro-inflammatory Th17 cells are highly glycolytic and glutaminolytic effector cells with increased mTORC1 activation.[115,116,117]. Here, glutaminase, a key enzyme of glutamine metabolism, promotes mouse Th17 cell differentiation, while restraining Th1 cells. These events are accompanied by altered chromatin accessibility and gene expression and suggest possible implications for Th17-Th1 plasticity.[118] In addition, calcium signalling-dependent mitochondrial regulation and

oxidative phosphorylation also play crucial roles in the differentiation of pathogenic Th17 cells in mice.[119]

Tissue-specific metabolic adaptations might also contribute to plasticity of different memory Th17 cell subsets. Circulating mouse T_{CM} relies on oxidative phosphorylation of endogenous free fatty acids (FFA), while skin-resident T_{RM} metabolizes exogenous FFA to support their long-term survival.[120] Whether this has (in)direct implications on the ability of mouse and human memory Th17 cells to acquire new phenotypes remains a matter of investigation.

Knowledge about metabolic requirements of human Th17 cells is more limited, especially in the context of diseases. A recent study showed that activin-A signalling and CD73-dependent molecular pathways restrain pro-inflammatory human Th17 cells in MS patients counteracting HIF1α activity, critical for glycolysis. These events result in increased anti-inflammatory genes through AhR, STAT3 and c-Maf and subsequent IL-10 secretion (in naïve $CD4^+$ T cells polarized towards Th17 cell lineage).[121] In the coming years, it will be vital to discover other metabolic factors that shift the balance between human anti- and pro-inflammatory Th17 subsets, determine plastic events in health and diseases and explore the role of cytokine signalling driving metabolic changes.

EPIGENETIC AND TRANSCRIPTIONAL REGULATION

The transcriptional regulation of Th17 effector functions involves pioneer TFs, which promote chromatin remodelling and thus allow secondary transcription factors to access regulatory genetic elements.

Mechanistic studies suggest that the expression of pro- or anti-inflammatory cytokines in Th17 cells is mutually exclusive. Using reactivated human memory Th17 clones *in vitro*, IL-10 expression correlated with downregulation of IL-17 and upregulation of the TF c-Maf.[29] While overexpressing c-Maf boosted IL-10 production, silencing of c-Maf led to a reduction in IL-10. Conversely, downregulation of c-Maf increased expression of pro-inflammatory cytokines such as IFN-γ, IL-22 and GM-CSF.[29] In another study, IL-10 expression was upregulated after TCR engagement and inversely correlated with IL-17 expression through downmodulation of RORγt, a mechanism dependent on IL-2/STAT5 signalling.[30] In line with this, pharmacological inhibition seems to reduce pro-inflammatory cytokine expression (e.g. IL-22) in Th17 cells (defined as IL-17A-expressing cells generated from mononuclear cells from the synovial fluid (SFMC) of patients with AS and PsA cultured in Th17-promoting conditions 6 days before RORγt inhibitor treatment).[122] These findings suggest a role for RORγt in the transcriptional regulation of pro- and anti-inflammatory cytokines. It remains to be explored whether RORγt directly regulates IL-10 expression in human Th17 cells, as seen in mouse.[123] RORγt inhibitors are currently investigated as therapeutic approach for inflammatory autoimmune disease such as psoriasis.[124]

Human memory Th17 clones cultured *in vitro* with IL-12 produced IFN-γ, an observation that was not accompanied by permissive epigenetic modifications at the IFNG locus.[125] However, converting *in vitro*-generated Th17 cells into these 'non-classical' Th1 cells led to an increase in DNA methylation at the *IL-17A* and *RORC2* gene loci, while both *TBX21* (encoding for T-bet) and *IFNG* genes were demethylated.[126] These non-classical Th1 cells could not be reverted into Th17 as the TF Eomes prevented further chromatin remodelling, a mechanism that required IL-2/IL-12 signalling.[127]

These findings suggest that cytokines can induce epigenetic changes and stabilize the expression profiles depending on the permissiveness of the epigenetic landscape in the target cell or the differentiation state of the cells.[29,127] In fact, a global mapping of H3K4me3 (permissive) and H3K27me3 (repressive) histone marks in different $CD4^+$ T-cell lineages revealed the presence of a mixed, 'poised' state in the promoter of lineage-specific transcription factors, which support the hypothesis of epigenetic plasticity. For instance, in mouse Th17 cells, the *Foxp3* promoter is not epigenetically repressed possibly allowing for Th17-to-Treg cell plasticity.[128]

A recent study showed that systemic inhibition of histone demethylases (corresponding to a genome-wide increase in histone methylation, likely correlated with transcriptional repression) inhibits Th17 differentiation, by downregulation of *RORC*, *IL-17* and *IFNG* expression. Histone demethylase inhibitors had a similar effect *in vitro* on pro-inflammatory cytokine (e.g. IL-17, IL-22 and TNF-α) expression in Th cells enriched from ankylosing spondylitis patients.[129] However, the reduction in pro-inflammatory cytokines was accompanied by a metabolic switch towards an anergic cell state, suggesting that both might impact on effector function in T lymphocytes.[129] Cytokines themselves can induce epigenetic changes, for example, by activating DNA methyltransferases (DNMTs) downstream of the cytokine signalling network.[130] Whether this plays a role in human Th17 plasticity is currently unknown. It is of great interest to understand whether those epigenetic regulators can be specifically targeted to modulate pro-inflammatory Th17 cells. Similarly, it remains to be understood which other key TFs govern the function of pro- versus anti-inflammatory Th17 cells.

PARADOXICAL ROLE OF TH17 SUBSETS IN TUMOUR IMMUNITY

In addition to their well-appreciated role in autoimmunity, the pluripotent nature of Th17 cells has motivated extensive investigation into their biological roles and

Table 2. Overview of pleiotropic function of Th17 cells in different tumours

Type of cancer	Outcomes	Th17-derived cytokine profiles	Effect of Th17 cells on tumour	References
Ovarian	Protective	IL−17, IFN-γ, GM-CSF, IL−2	Increased intratumoral Th17 cell numbers were associated with improved survival rates.	138,154,155
Colorectal	Protective	TNF-α, IL−21, IL−22, GM-CSF, IFN-γ and IL−8	Positive contribution of tumour-infiltrating Th17 cells in mediating tumour immunity through the recruitment of cytotoxic $CD8^+$ T cells.	132,140,156,157
Gastric	Adverse	IL−17, IL−21	Th17 was shown to promote tumour growth via secretion through upregulation of VEGF prostaglandins.	158–160
Non-Hodgkin lymphoma	Protective	IL−17, IFN-γ (?)	Lymphoma limits Th17 generation and maintenance to mediate tolerance.	146,161
Hodgkin lymphoma (HL)	N/A	IL−17, IL−1, GITR	Compared with Epstein–Barr virus (EBV) + HL patients, EBV- HL patients upregulated IL−23 that displayed a pro-inflammatory Th17 phenotype.	162
Multiple myeloma	Adverse	IL−17, IFN-γ	Higher numbers of Th17 cells were associated with tumour growth and impeded host tumour immunity	144,163
Acute myeloid leukaemia	Adverse	IL−17, IL−10	Increased frequencies of Th17 cells were associated with poor prognosis. Th17 promotes tumour growth via secretion of IL−17, while Th7-producing IL−10 suppresses immune activity.	143,164

N/A, not applicable; IL-17, interleukin-17; IFN-γ, interferon γ; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL-2, interleukin-2; IL-22, interleukin-22; IL-23, interleukin-23; TNF-α, tumour necrosis factor-α; IL-10, interleukin-10.

functions in cancer. Th17 cells are prevalent in many different types of cancer, including melanoma, pancreatic cancer, prostate cancer, and colon and gastric cancer.[131] This suggests that the microenvironment within tumours promotes the recruitment and/or expansion of Th17 cells. Recent studies suggest that tumours secrete Th17-attracting chemokines, including CCL17, CCL20, MCP-1 and RANTES,[132] and support Th17 development through secretion of cytokines, including IL-1β, TGF-β and IL-6.[131] Other cytokine-independent factors such as hypoxic tumour microenvironment and tumour-derived glutamine-dependent metabolites also promote development of Th17 cells.[118,133] Tumour-infiltrating Th17 cells can exert both pro- or antitumour effects depending on the types of cancer,[131] suggesting functional heterogeneity within tumour-associated Th17 cells. The dual nature of Th17 cells in both promoting tumour growth and mounting an antitumour response has been discussed extensively elsewhere.[134,135] Here, we summarize Th17 phenotypes that are associated with different cancer types (Table 2). On the one hand, Th17 cells were found to directly promote tumour growth in cancers such as hepatocellular carcinoma, and colorectal and pancreatic cancers by inducing vascularization and immunosuppressive activities through secretion of effector cytokines, including IL-17A and IL-10.[132,134,136,137] On the other hand, in some tumour types such as ovarian, prostrate and colorectal cancers, Th17 cells can induce antitumor immune responses through recruitment of cytotoxic effector T cells and production of effector cytokines, including IFN-γ.[138,139] These functions are associated with increased chances of survival of cancer patients. Notably, a recent study highlighted that the frequency of Th17 in colorectal tumour cells themselves was not predictive of the number or proportion of Th17 cells in the tumour tissue. The authors demonstrated that although the increased frequency of intra-epithelial Th17 cells is associated with a pronounced cytotoxic T-cell infiltration and prolonged survival in colorectal cancer, the stromal Th17 infiltrates were not.[140,141] Hence, the functional plasticity of Th17 cells within the same tumour types depending on their location and the cytokine milieu at those sites may allow a pro- or antitumorigenic effect.

Th17 cells and their associated cytokines have also been implicated in the pathogenesis of haematological cancers, including acute myeloid leukaemia (AML), lymphoma and multiple myeloma (MM). Here, an increased frequency of Th17 cells in AML patients is associated with tumour burden.[142,143] Furthermore, Th17 cell frequency was reduced in patients who achieved complete remission following chemotherapy. Th17 cell numbers and associated cytokine levels (e.g. IL-17, IL-21, IL-22 and IL-23) are also increased in the blood and bone marrow of myeloma patients.[144] Elevated inflammatory cytokine levels within bone marrow niches of MM patients support development of Th17 cells, which in turn sustains myeloma growth via secretion of cytokines, possibly by secretion of IL-17 and IL-21.[145] In contrast to AML and MM, lymphoma B cells were shown to inhibit differentiation of Th17 cells in favour of promoting immunosuppressive Treg cells to promote disease

progression.[146] Whether Th17 cells transdifferentiate into Treg cells is not clear. These studies overwhelmingly suggest that Th17 plasticity and functional heterogeneity are regulated by tumour-specific microenvironment.

Efforts to develop therapies that modulate the tumour microenvironment in order to promote antitumour T-cell functions, while inhibiting their tumour-promoting potential, are undergoing. Current checkpoint blockade therapies have been shown to boost Th17-mediated antitumour responses by enhancing their production of IFN-γ, IL-17 and TNF-α.[147,148] Furthermore, in mouse models of adoptive T-cell therapy, Th17 cells grown *in vitro* under pro-inflammatory conditions exhibited potent antitumour activity upon adoptive transfer *in vivo*.[149,150] The systemic administration of the Th-supporting cytokine IL-23 or autocrine secretion of IL-23 by CAR-T cells mediates antitumour immunity in solid tumour models.[151,152] Future work characterizing functional heterogeneity Th17 cells in different cancer settings will be required to define the therapeutic potential of targeting Th17 plasticity to mount effective anticancer response.

CONCLUDING REMARKS

Understanding mechanisms behind the context-dependent generation of anti-inflammatory and pro-inflammatory Th17 cells is at the core of future treatments for autoimmunity. In this review, we highlighted the interconnected relationship between cytokines, intracellular signalling and metabolic pathways, epigenetic regulators and transcription factors, which together orchestrate the ever-changing human Th17 cell identity. A thorough characterization of the epigenetic status of Th17 cells from autoimmune patients and healthy controls, from both inflamed and non-inflamed tissue, would provide a better understanding of how cytokine-mediated changes are linked to disease pathogenesis, which is critical for designing immunomodulatory therapies for chronic inflammatory diseases.

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CONFLICT OF INTEREST

S.C. is a fellow of the AstraZeneca R&D postdoc programme. S.C., S.P. and U.G. are employees of AstraZeneca and may own stock or stock options.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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