Th17 cells and RA

Implications of novel insights of pathogenecity and plasticity of the different T helper subsets in RA

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Abstract

Rheumatoid arthritis (RA) is an autoimmune disorder characterized by chronic inflammation of multiple joints and organs. For many years RA has been recognized as a Th1 mediated disease, but recently Th17 cells appear to play a pivotal role in RA pathogenesis. Studies have revealed that IL-17 producing T cells are important cell types contributing to arthritis. Accordingly, plasticity between different Th1, Th17 and Treg cells has been described, indicating that differentiation of Th subsets is not entirely restricted to separate lineages. Therefore, this report will address these novel insights of pathogenecity and plasticity of different Th subsets in RA.

Abbreviations

RA	Rheumatoid Arthritis
CIA	Collagen induced arthritis
Th cells	T helper cells
Treg cells	Regulatory cells
ROR	Retinoic acid-related orphan receptor
TGF-β	Transforming growth factor- beta
TNF-α	Tumor necrosis factor- alpha
IFN-γ	Interferon-gamma
CXCR	Chemokine receptor family
CCR	Chemokine receptor
RANKL	Receptor activator of NF-κB ligand

Introduction

Rheumatoid arthritis (RA) is a chronic disease characterized by autoimmunity and inflammation of multiple joints and organs. The disease is associated with remissions and relapses. From 60% of patients with arthritis the disease is self-limiting, while 40% develop a chronic form of arthritis from which 62% develops RA [1]. This indicates that RA is typically a progressive disorder which has the potential to destruct joints resulting in functional disability accompanied by symptoms such as pain, swelling and fatigue. RA affects 1-2% of the people worldwide with a man to woman ratio of 1:3, especially middle aged women [2]. The etiology of the disease is unknown, however pro inflammatory cytokines play crucial roles in RA. Moreover, synovial fibroblasts, osteoclasts and infiltrating inflammatory cells have been shown to be important in the disease. Osteoclasts are involved in bone resorption by producing various proteases and acids. In normal bone a balance between bone formation and bone resorption exists, however if osteoclasts over react bone destruction may occur. In addition, inflammatory cytokines such as TNF-α, IL-1 and IL-6 are increased in synovial fluids of RA patients. These cytokines have been shown to induce differentiation and activation of osteoclasts resulting in bone resorption [3]. Furthermore, synovial fibroblasts produce particularly IL-6 and MMPs, which are involved in degradation of intact cartilage matrix [4]. Next to the inflammatory cells mentioned before T cells represent a considered amount of invading cells into the synovial tissue. These activated T cells producing contribute to the progressive inflammation in RA patients by producing pro inflammatory cytokines. As RA is an autoimmune disorder the activation of T cells recognizing auto-antigens and/or the cytokine driven T cell stimulation is implicated in its pathogenesis [5].

T helper subsets

Upon stimulation naïve CD4+ T cells can differentiate into specific T helper (Th) subsets depending on the cytokine milieu (Figure 1). During the process of Th cell differentiation specific transcription factors are required. Differentiation of Th1 cells depends on specific transcription factors such as T-bet and STAT4, whereas the Th2- lineage related transcription factors are GATA3 and STAT6 [6]. Th1 cell differentiation occurs in the presence of IL-12 via STAT4 and T-bet, resulting in up regulation of IFN-γ, which in turn acts as a positive feedback mechanism to enhance T-bet expression leading to a stabilization of Th1

differentiation [7]. Moreover, it has been shown that Th1 differentiation results in down regulation of IL-4 and IL-5 expressions. Considerable evidence has demonstrated that Th1 cells are involved in host defense against intracellular infections by secreting IFN-γ, which can activate macrophages, natural killer cells and CD8^{+} cells [8].

Naïve CD4+ T cells can also differentiate into Th2 cells in the presence of IL-4. Moreover, these cells secrete IL-4, IL-5 and IL-13, which in turn play a key role in humoral immunity and in the control of parasite invasions. IL-4 has been shown to be crucial in Th2 differentiation via activation of STAT6, resulting in the expression of GATA3 [7]. This transcription factor activates its own expression via a positive feedback mechanism and plays a role in the expression of Th2 related cytokines such as IL-4, IL-5 and IL-13. During Th2 differentiation IFN-γ expression is inhibited, while the expression of IL-4 and IL-5 is promoted. Experiments have confirmed that transcription factors of Th1 and Th2 lineage, T-bet and GATA3, counteract Th2 and Th1 development respectively. For example T-bet represses Th2 development by interfering with GATA3 binding to DNA and GATA3 suppresses Th1 development [9, 10]. These findings indicate that Th cell differentiation depends on lineage specific transcription factors which induce a lineage specific gene expression profile in combination with an inhibition of the development of other Th cell subsets.

Next to the classical Th1 and Th2 subsets a new subset has been described during the last decade, named Th17 cells due to the secretion of IL-17A [11-13]. More importantly, differentiation of Th17 cells depends on RORγt and RORα [14-18]. Yang et al. have demonstrated that RORγt and RORα, two orphan nuclear receptors, have synergistic effects on differentiation of Th17 cells, although RORα has less effect on promoting Th17 differentiation compared to RORγt [17]. Furthermore, STAT3 has been shown to be involved in the expression of the critical transcription factor RORγt [19]. Moreover, Th17 differentiation is dependent on STAT3, which can directly bind to the IL-17 promoter and thereby inducing IL-17A production [20]. In the presence of IL-6/ TGF-β/ IL-23/IL-1β Th17 cells differentiate and preferentially secrete IL-17A, IL-17F, IL-21 and IL-22. These cytokines target for example epithelial cells and fibroblasts. By this means anti-microbial peptides such as Defensin and Lipocalin are produced and therefore Th17 cells have been described to have a protective role in tissue immunity [8]. On the other hand, numerous

experimental studies on autoimmune diseases could not be explained by the Th1/Th2 paradigm, but rather by Th17 cells and therefore these cells have been shown to play a role in autoimmunity [21, 22]. So, next to dealing with infectious agents, Th17 cells have been implicated in various autoimmune disorders and therefore the focus is now set on this relatively new Th subset.
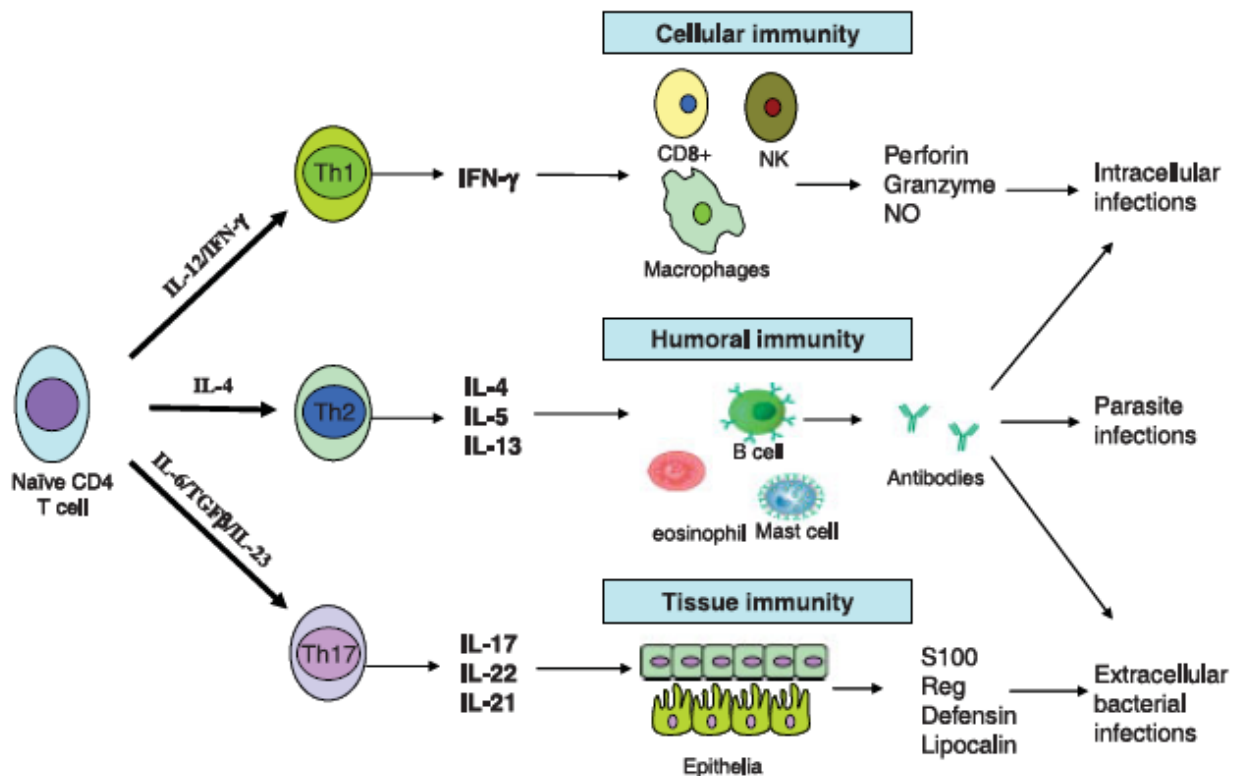
Figure 1 General scheme of T helper differentiation. *Upon stimulation naïve T cells differentiate into Th1, Th2 and Th17 cells, which promote specific immune responses against both bacterial and parasite infections* [8].

Cytokines and regulation

Th cells are important mediators in immune responses due to their ability to secrete cytokines in order to cope with other cellular processes of the immune system. Next to the classical Th subsets, the Th17 subset has been extensively studied. It has been demonstrated that IFN-γ and IL-4 negatively regulate the development of Th17 cells [23]. In addition, it has been demonstrated that enforced GATA3 expression inhibits both Th17 polarization in CD2-GATA3-transgenic mice and RORγt expression *in vitro* and *in vivo* [24]. Several experiments have also shown that Th17 differentiation is induced by the synergistic activation of IL-6 and TGF-β both *in vitro* and *in vivo* [25-27]. In contrast, several studies have reported differences

in human versus mice Th17 cells e.g. human Th17 differentiation was reported to be independent of TGF-β. Instead, IL-1 together with IL-6 was shown to be important for mediating Th17 differentiation [28, 29]. As human and mice Th17 cells differ in various aspects the role of TGF-β in Th17 differentiation is still intensely debated [30, 31] (Figure 2). Beside these cytokines IL-23 has been shown to be critical for Th17 maintenance and pathogenecity [32, 33] e.g. IL-23 induces the proliferation of Th17 cells in vivo. Importantly, initiation of Th17 differentiation does not require IL-23, but this cytokine is required for proliferation and stabilization of Th17 cells in autoimmunity [34]. In addition, IL-23 promotes proliferation of memory CD4+ T cells but not of naïve T cells, as naïve T cells do not express IL-23R [35]. Moreover, IL-23 was shown to be involved in autoimmunity rather than IL-12, although these cytokines share the common subunit p40 [36]. Furthermore, IL-23 has been shown to promote IFN-γ by Th1 cells, probably by sharing overlapping STAT signaling pathways with IL-12. However, this function is not fully studied and requires further investigation [37].

Markers associated with Th17 cells

Th17 cells have been described as a separate lineage of Th cells and to make the difference more apparent studies have demonstrated the presence of chemokine receptors such as CCR6 and CCR4 on human Th17 cells producing IL-17A, but not IFN-γ, whereas cells expressing CCR6 and CXCR3 secrete both IL-17 and IFN-γ (Th17/ Th1) or only IFN-γ (Th1) [38]. This shows that human T cells expressing CCR6 are able to produce IL-17 [15]. Moreover, an additional surface marker has been identified to be associated with human Th17 cells, CD161 [39]. This marker is exclusively expressed by Th17 cells, not by Th1 or Th2 cells. Furthermore, Th17 cells have been shown to arise from CD161+CD4+ precursor cells.
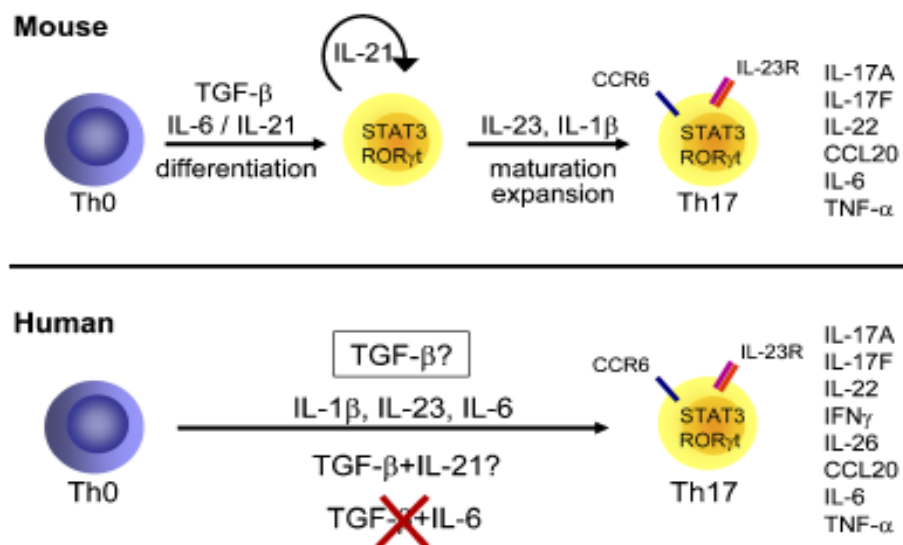
Figure 2 Th17 differentiation in mouse and human. *Th17 differentiation in mice occurs in the presence of TGF-β and IL-6/ IL-21 and is maintained by IL-23 and IL-1β. In contrast, in human TGF-β and IL-6 alone are not sufficient to drive Th17 differentiation. However, a cocktail of cytokines including IL-1β, IL-23, IL-6 and TGF-β induces a Th17 associated gene and cytokine expression profile [31] .*

Th17 related cytokines

Upon activation Th17 cells secrete cytokines such as IL-17A, IL-17F, IL-21, IL-22 and in humans IL-26 [40] . Furthermore, Th17 cells also secrete the chemokine CCL20 that binds to CCR6, which controls the migration of inflammatory cells such as Th17 cells [41].

IL-17A and IL-17F

IL-17A is not only secreted by Th17 cells, but also by γδ T cells, NK cells and CD8+ cells [18, 42]. Studies have shown that IL-17A is involved in the pathogenesis of collagen- induced arthritis (CIA) and experimental autoimmune encephalomyelitis (EAE), animal models of RA, and Multiple Sclerosis respectively [2, 43]. This shows that IL-17A is involved in the autoimmunity. IL-17A binds IL17RA, the IL-17 receptor, with high affinity. Studies have shown that IL-17A is a homodimeric cytokine that shares similar biological functions with IL-17F [44]. IL-17F, another signature cytokine secreted by Th17 cells, is a homodimeric cytokine which plays a role in inflammatory bowel disease (IBD) and infection [17, 45]. IL-17A and IL-17F mediate their biological functions via IL-17RA and IL-17RC, components of the IL-17 receptor, which are expressed on various tissue and cell types [46]. Albeit few studies have demonstrated that the biological functions of IL-17A and IL-17F differ [47]. Ishigame et al. have demonstrated that only IL-17F contributes to innate immune responses in

epithelial cells, whereas IL-17A is involved in both allergic and host defense responses [45]. Currently, it is unknown whether Th17 related cytokines have redundant functions in certain diseases. Although, studies have demonstrated that IL-17A deficient mice are resistant to CIA, which suggests a redundant role of IL-17F for IL-17A in the pathogenesis of CIA [48].

Recently, it was shown that activated human CD4+ T cells express heterodimeric IL-17A/IL-17F cytokines. Moreover, IL-17A/IL-17F was also expressed by murine differentiated Th17 cells [49]. These cytokines are able to secrete chemokines in epithelial cells by signaling through the IL-17RA/IL-17RC receptor complex in humans, the same receptor complex as IL-17A and IL-17F.

IL-21

IL-21 is essential for Th17 differentiation and functions as an autocrine growth factor for Th17 cells [50]. Next to that, IL-21 has pleiotropic effects on CD8+ T cell, B cells, NK cells and dendritic cells. Studies have shown that IL-21 in combination with TGF-β results in differentiation of Th17 cells, although IL-6 appears to be a more potent inducer than IL-21 [51]. As IL-17 induces a positive feedback loop for IL-6 expression, it has also been suggested that IL-17 can amplify IL-21 production when present in the proper cytokine milieu [52]. In addition, IL-21 was shown to induce IL-23R expression on Th17 cells, indicating an indirect role of this cytokine in expansion of Th17 cells by IL-23 [46]. Conversely, a study described that Th17 independent IL-21 production can also occur by demonstrating that mice deficient in RORγt produced normal IL-21 levels [53]. Moreover, it was shown that IL-21 producing Th17 cells do not always secrete IL-17A or IL-17F. These findings indicate that next to Th17 cells another cell type might produce IL-21 as well [53]. Young et al. have shown that IL-21 has a pathogenic role in animal models for RA by expanding pathogenic T cell proliferation [54]. In addition, IL-21 has been demonstrated to inhibit Th1 differentiation and down regulate IL-6 production in CIA, indicating that IL-21 contributes to IL-6 production in CIA.

IL-22

IL-22, preferentially secreted by Th17 cells, belongs to the IL-10 family of cytokines. This cytokine is also produced by CD8+ T cells and dendritic cells [55]. Receptors for IL-22 are expressed on epithelial cells and fibroblasts from where IL-22 can mediate its biological

effects [8]. Interestingly, it has been shown that IL-22 can also be produced by Th1 cells [56]. In addition, IL-22 is involved in mucosal immunity by producing anti-microbial peptides and therefore this cytokine has a protective effect against extracellular bacteria [8, 57]. A mechanism by which IL-22 exerts protective responses is through the induction of Reg family of microbial proteins in epithelial cells, which can directly kill bacteria [57]. However, IL-22 has been linked to inflammatory functions in diseases such as dermal inflammation and IBD [16, 56, 58]. Moreover, IL-23 has been shown to directly induce IL-22 production from both human and murine naïve T cells [56]. Recently, it has been described that T cells from both psoriasis and atopic dermatitis (AD) patients could independently produce IL-22 although low levels of IL-17 were co-produced. IL-22 can be produced without co-expression of IL-17 or IFN-γ and might possibly be characterized as a different Th subset, Th22. Furthermore, the new Th subset has been shown to be responsible for the increased levels of IL-22 in AD patients [58]. In addition, Geboes et al. have shown that IL-22 plays a role in CIA by promoting osteoclastogenesis and antibody production, although the mechanism by which IL-22 promotes antibody production needs to be elucidated [59]. Furthermore, IL-22 levels were shown to be increased in RA patients, which was associated with disease severity. Another study revealed that IL-22 was present during proliferation of synovial fibroblast derived from RA patients [60]. These findings indicate an apparent role of IL-22 in relation to RA pathogenecity, albeit further investigation is required to confirm this notion.

Th17 cells and autoimmunity

Several studies described in literature have linked Th17 related cytokines especially IL-22 and IL-17 to autoimmune diseases such as RA, psoriasis and IBD [16]. In experimental models some pathogens, notably mycobacteria are used to induce autoimmunity and particularly these pathogens are able to produce IL-17 e.g. dendritic cells exposed to zymosan were able to induce Th17 differentiation from CD4+ T cells [61]. Furthermore, SKG mice, a genetic model of RA, spontaneously developed Th17 cells causing arthritis, whereas this phenomenon did not occur in SPF mice, mice free of pathogens [62]. This indicates an apparent role of IL-17 (Th17) and autoimmunity. Interestingly, co-expression of IL-17 and IFN-γ was detected in acute EAE, which was reduced when the disease became chronic [61]. As IFN-γ has an inhibitory effect on Th17 differentiation, it is remarkable that co-production of IL-17 and IFN-γ seems to be associated with pathogenecity. Therefore, there might be some cross-regulation between Th1 and Th17 cells.

Regulatory T cells (Treg) are a subset of cells that differentiate from naïve CD4+ T cells and are involved in controlling immune responses and inflammation triggered by resident microflora. This indicates that Treg cells have a suppressive phenotype and exhibit a protective role against autoimmunity [41, 63]. As TGF-β is involved in Treg cell differentiation, mice lacking TGF-β developed early onset autoimmune disease, indicating an indispensable role for this growth hormone in light of autoimmunity. In contrast, mice with impaired TGF-β signaling in T cells still revealed functional Treg cells [64]. This notion may suggest that other mechanisms are involved in autoimmunity [16].

T regulatory cells versus Th17 cells

Interestingly, Th17 cells have been linked to the generation of induced Treg (iTreg) due to the shared cytokine TGF-β, but distinct transcription factors are required for differentiation of both cell types: RORγt (Th17) and FoxP3 (Treg). TGF-β can induce FoxP3 expression, but in the presence of IL-6 and IL-21 TGF-β can exhibit Th17 differentiation [26], while suppressing Treg differentiation [63]. Consequently, these cells are not only antagonisms of each other, but a reciprocal regulation is induced by Th17 and Treg. Studies in mice have shown that low levels of TGF-β synergize with IL-6 and IL-21 to induce Th17 differentiation via IL-23R expression, whereas high concentrations of TGF-β result in development of FoxP3+ Treg by repressing IL-23R expression [63] . Moreover, it has been shown that Th17 development is inhibited by FoxP3+ via interaction with both RORα and RORγt [65]. In addition, IL-2 has been described as a survival factor for Treg, whereas it inhibits IL-17A expression [66].

RA: Th1 or Th17 mediated?

In RA pro-inflammatory cytokines play an essential role in the pathogenesis of the disease e.g. IL-1, IL-6, TNF-α and IL-17 were elevated in synovial fluids of RA patients [3, 67, 68]. These cytokines can induce osteoclast differentiation resulting in bone resorption. Furthermore, it has been demonstrated that IL-17 acts on different cell types resulting in joint inflammation and bone destruction. For instance, IL-17 producing T cells play a role in TNF-α induced synthesis of IL-1β, IL-8 and IL-6 in synovial fibroblasts [69]. Moreover, synergism between IL-17 and TNF-α have been shown to induce IL-23p19 expression in fibroblast-like synoviocytes [70]. In addition, IL-23 has been shown to be critical for human

osteoclast differentiation via IL-17 [71]. IL-17A is also able to induce chronic destructive arthritis independent of TNF-α and IL-1 as was shown in mice [72]. In the early onset of RA Th17 cells were shown to be up regulated and therefore these cells might also contribute to inflammation [73].

For quite some time RA has been recognized as a Th1 mediated disease [74]. However, recent findings have cast doubt on this proposition e.g. IFN-γ inhibits osteoclast differentiation, indicating that RA is not Th1 mediated [75]. *In vitro* human osteoclastogenesis by Th17 cells can occur via RANKL induction of osteoblasts via IL-17 signaling [76]. In contrast, one study reported that T cells producing IFN-γ were shown to induce osteoclastogenesis via expression of RANKL [77]. But, IL-2 and IFN-γ, Th1 related cytokines, were hardly detectable in joints of RA patients as reported, whereas IL-17 was reported to be elevated in synovial fluids of RA patients [68, 78]. In addition, mice lacking IFN-γ receptor were even more susceptible to CIA [79]. In vivo animal studies have revealed that CIA was partially suppressed in mice lacking IL-17 [48]. Moreover, it has been demonstrated that IL-17 is indispensable in both the early and progressive phase of arthritis [80]. IL-17R deficient mice revealed that synovial expression of IL-1 and matrix metalloproteinases is impaired leading to prevention of cartilage destruction in CIA [81]. These findings suggest that RA is not a Th1 mediated disease, but possibly a Th17 mediated disease.

Treg in RA

As described before Treg and Th17 cells have reciprocal developmental pathways and contradictory functions in the control of inflammation. Accordingly, it would be likely to assume that Treg cells are decreased in RA patients, although this does not account for self-tolerance. In addition, large numbers of Treg cells are present inflamed joints in patients with a mild phenotype compared with patients with a more severe phenotype [82]. As studies have revealed that activated Treg are present in inflamed tissue, these cells however are not capable of suppressing inflammation, especially Th17 mediated immune responses [83]. It could be speculated that Treg cells, a component of pheripheral tolerance, in an early stage of RA are not responsive to Th17 mediated inflammation, which might be explained by escape of T cell selection in the thymus. If a precursor Treg cell escapes appropriate selection in the thymus, this cell possibly becomes non-tolerogenic, which can lead to autoimmunity. Experiments with Treg neither showed suppression of osteoclast differentiation, nor

stimulation of osteoclast differentiation [5]. Therefore the role of Treg in RA remains unresolved.

Plasticity

Remarkably, studies have shown that Treg cells seem to be related with Th17 cells mediated by TGF-β and RORγt. It has been demonstrated that Treg cells can be converted to IL-17 producing T cells in both mice and human via up regulation of RORγt and CCR6 [36, 84]. Next to plasticity between Treg and Th17 cells, a relation has been established between Th17 and Th1 cells (Figure 3). In autoimmunity Th1 and Th17 cells develop simultaneously and can be co-localized [85]. It has been shown that IL-17 acts as a positive feedback cytokine that further attracts Th17 cells as well as Th1 cells. This suggests that a possible redundancy between these lineages in pathology of the disease. Studies have shown that fully polarized Th17 cells can be converted into cells producing IFN-γ via IL-12 and IL-23 signaling. Interestingly, Th17 cells maintained by long term TGF-β showed to be persistently responsive to IL-12. This resulted in an induction of a STAT 4 and T-bet dependent conversion marked by loss of RORs, IL-17A and IL-17F. In addition, Th1 related genes were induced upon IL-12 stimulation in Th17 cells of reporter mice [6]. Moreover, in the absence of TGF-β it was shown that Th17 cells do no stably express IL-17 and Th17 cells give rise to Th1 like progeny [86]. These findings imply plasticity between Th17 and Th1 cells. In agreement with this notion, autoimmune myocarditis studied in mice have demonstrated that mice lacking T-bet, the master regulator of Th1, develop more severe disease and increased levels of IL-17 were observed [87]. This suggests that T-bet is a negative regulator of Th17 differentiation. In addition, an epigenetic role for regulation of T cell plasticity has been established for example T-bet is in active epigenetic state in both Treg and Th17 cells, indicating a potential role to up regulate this transcription factor and to differentiate into Th1 cells [88].

As IL-23 is essential for the maintenance of Th17 cells, the role of this cytokine in relation to late Th17 development has been investigated. As a result, IL-23 was not required for maintenance of IL-17 expression [6]. Instead, upon IL-23 stimulation in the absence of TGF-β the frequency of cells producing IFN-γ was increased without co-expression of IL-17A. This indicates an indispensable role of IL-23 in the transition of Th17 cells into Th1 cells together with extinction of IL-17 and increased expression of IFN-γ during late Th17 developmental

plasticity. Therefore, IL-23 could be important for Th17 and Th1 responses. Importantly, fully committed Th1 cells are resistant to the conversion to Th17 cells under the proper conditions, accordingly there is only plasticity between Th17 and Th1 cells in this direction [6, 89].
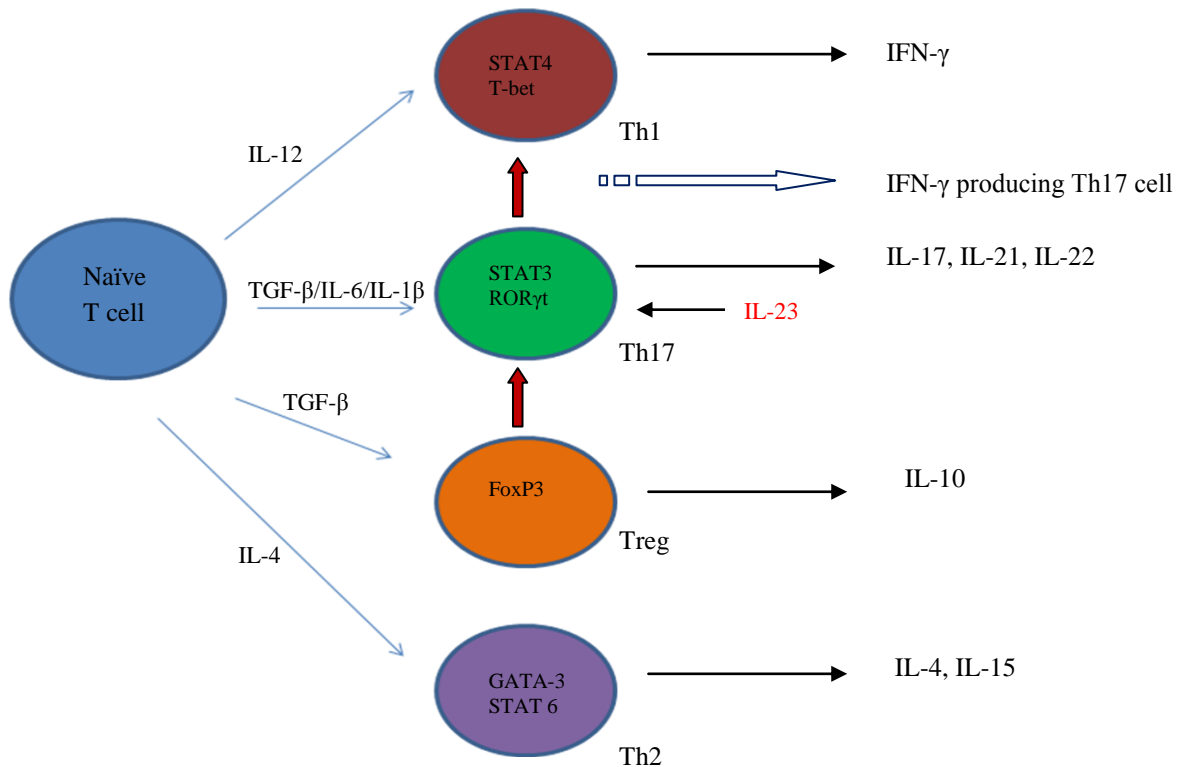
Figure 3 Plasticity between Th1, Th17 and Treg cells. *Treg cells can be converted to IL-17 producing T cells in both mice and human via up regulation of RORγt. In addition, fully polarized Th17 cells can be converted into cells Th1 cells via expression of T-bet and STAT4. However, the opposite does not occur. Next to Th1 and Th17 cells, a population has been described to express both IFN-γ and IL-17.*

Polymorphisms

Many studies have suggested that RA is partially a genetic disorder which can be explained by the presence or absence of a shared epitope (SE) in various HLA-DR molecules [90]. Previous studies have shown an association between the presence of SE and RA onset at an earlier age [91, 92]. Next to correlations with HLA other genes are also involved in determining a risk for RA as genome wide association studies have provided evidence for various susceptibility loci on chromosomes [93]. For example associations have been established between RANK, RANKL, osteoprotegerin (OPG) and IL-17 genotypes and early

RA in Japanese patients [90]. These results are in agreement with previous studies in light of results wherein RANKL and IL-17 contribute to bone resorption in RA patients [76, 77]. This indicates that SNPs in genes may be predictive for disease progression in RA patients with an early disease phenotype.

Therapies

Upon the discovery of new molecules and pathways new treatments are created in order to treat diseases such as RA. With other words targeting a range of molecules may have therapeutic potential for treatment of autoimmune diseases. Over the past years different therapeutic treatments have been considered and tested in RA patients and therefore the array of potential therapeutic targets is impressive. Many of these targets are at various stages of clinical development and these potential drugs can be categorized in different groups in light of cytokines and receptors, cell recruitment and intracellular pathways [94]. To treat RA common treatments such as anti-inflammatory drugs have been used, however disease-modifying anti-rheumatic drug (DMARD) therapy is often used to prevent long-term damage [95]. Additionally, agents have been produced to block TNF-α (Etanercept), IL-1β (Canakinumab), IL-1 receptor antagonist (Anakinra) and IL-6 receptor (Tocilizumab). In addition, rituximab targets B cells (CD20) and CD28, a costimulatory molecule on T cells, is blocked by abatacept in RA patients [95]. Some of the experiments, such as Tocilizumab, are ongoing to test their efficacy in relation to the disease. Despite pivotal successes the therapies that are currently available are not effective in many RA patients and can have severe side effects. These notions emphasize the need for safer and more effective drugs.

The largest focus now, regarding RA has been laid on anti-cytokine therapy, especially IL-17 therapy. It has already been demonstrated that IL-17 blockade is very effective in CIA [80]. As IL-17 is found in the synovial fluids and in synovial tissue of RA patients and enhances the production of cytokines by fibroblast-like synoviocytes, it is tempting to speculate that blockade of this cytokine in humans might be useful [68, 73]. IL-17 blockade can be established via monoclonal anti-IL-17A antibodies, which have been developed now and via soluble antagonists which can bind members of IL-17 [94]. Currently, AIN457, a biological against IL-17 (Novartis AG), is being tested in psoriatic arthritis patients to test its efficacy (ClinicalTrials.gov identifiers: NCT00809614). As the subunit p40 is shared with IL-23 and

IL-12 [32] currently, a trial is ongoing to analyze whether IL-23p40 inhibition is associated with reduction of inflammation in RA patients (ClinicalTrials.gov identifiers: NCT00642629). Although many targets are not listed in table1, it gives an overview of few therapies for RA. More target for RA are listed in the review by Waldburger et al. [94]. These new drugs, which are being tested in clinical trials, seem promising, but time will tell whether these targeted therapies are indeed useful. Although, targeting IL-23p40 would be a good target for RA, as IL-23p40 is involved in maintenance of Th17 cells and has been shown to promote Th1 cell differentiation. This speculation includes T cell plasticity, which makes it more likely to target IL-23p40 in RA patients.

Target	Representative molecules	Representative clinical trials
TNF-α	Etanercept	Phase IV
IL-1β	Canakinumab	Phase II
IL-1RA	Anakinra	Phase III
IL-6R	Tocilizumab	Phase III
B cells (CD20/CD28)	Rituximab	Phase II
IL-17	AIN457 mAb	Phase II
IL-23p40	STA-5326 Mesylate	Phase II

Table 1 Examples of targeted therapies for RA *(ClinicalTrials.gov)*

Next to mono-therapies, combination therapies for RA patients are also considered e.g. combination therapy of a targeted immune modulator and methotrexate has conferred the best results [96]. In contrast, much more side effects can occur in patients using the drugs as multiple targets are 'attacked'. Therefore novel studies are needed to address these issues.

Conclusion

Rheumatoid arthritis (RA) is an autoimmune disorder with an unknown etiology, characterized by chronic inflammation of multiple joints and organs [1]. For many years RA has been recognized as a Th1 mediated disease, but recently Th17 cells, a newly described Th subset, appear to play a pivotal role in RA pathogenesis as elevated IL-17 levels have been detected in the synovial fluids of RA patients [68, 73]. In the presence of IL-6/ TGF-β/ IL-23/IL-1β Th17 cells differentiate and secrete cytokines such as IL-17A, IL-17F, IL-22, IL-21 and IL-26 in humans [11-13, 40]. These novel T cells induce autoimmunity far more efficiently than Th1 cells in mice [2]. As IFN-γ has an inhibitory effect on Th17 differentiation, it is remarkable that co-production of IL-17 and IFN-γ seems to be associated with pathogenecity. Therefore, there might be some cross-regulation between Th1 and Th17 cells. Moreover, IL-23 could be important for Th17 and Th1 responses as this cytokine is important for maintaining Th17 cells and promoting IFN-γ by Th1 cells [35, 37]. Notably, studies have demonstrated plasticity between Th1, Th17 and Treg cells [6, 36, 84].

Treg and Th17 cells have reciprocal developmental pathways and contradictory functions in controlling inflammation. Currently, experiments are conducted to investigate whether Treg cell administration could be useful to treat RA patients as Treg cells have suppressive functions. Notably, it should be considered that Treg cells can convert to Th17 cells and thereby side effects and worsening of the disease may occur. Therefore, the role of Treg in RA remains unresolved and further research is required.

Currently, new and promising therapies are being tested in clinical trials such as anti-IL-17 therapy [94]. However, one should take into account T- cell plasticity. Due to T-cell plasticity the effect on therapies for RA may need to be reconsidered as targets can be converted to other cell types i.e. Th17 cells can be transformed to Th1 cells. In addition, the efficacy of new drugs being developed should also be considered due to T-cell plasticity. Moreover, both side effects and safer therapeutic strategies have to be taken into consideration in order to treat RA patients. The Th17 pathway has been described thoroughly, however the effects on RA patients are still not clear, as clinical trials are being conducted. It could be speculated that the effects of targeting Th17 cells will be promising as depletion of Th17 cells would probably result in less IL-17 production and the ratio of Th17 cells converting to Th1 cells would possibly be lower, due to few Th17 cells available. The effects of the other Th subsets will

theoretically result in more Th1 and Th2 cells compared to the numbers of Th17 cells. As IFN-γ (Th1) is able to repress Th17 differentiation, over expression of this cytokine would keep Th17 cells suppressed. If RA is indeed a Th17 mediated disease, targeting Th17 cells will be promising.

Aware of these notions, the question which therapy is appropriate to treat RA patients is addressed: a therapy based on T cell targeting or a therapy based on targeting cytokines produced by Th17 cells. As Th17 cells are not the only source of Th17 related cytokines such as IL-17A, IL-21 and IL-22, it would probably be wise to target these cytokines. In addition, targeting IL-6 might be sensible as IL-6 is a potent inducer of Th17 differentiation [33, 51]. Moreover, targeting IL-23, specifically IL-23p40, might be reasonable as the p40 subunit is shared with IL-12. This indicates that both IL-12 and IL-23 are targeted and thereby the Th1 and Th17 functions are diminished or completely abolished in the case of Th17 cells, because IL-23 is no longer available to maintain these cells. Only time will tell whether blocking of the cytokines mentioned afore will be effective in RA patients in conjunction with determination of the side effects.

From 60% of patients with arthritis the disease is self-limiting, while 40% develop a chronic form of arthritis from which 62% develops RA [1]. This could possibly be explained by polymorphisms of genes such as HLA, as studies have provided evidence that certain genes are involved in determining a risk for RA. These results were obtained from studies that provided evidence for various susceptibility loci on chromosomes [93]. It is more likely that Th17 cells are involved in RA, due to IL-17 genotype associations and early RA in Japanese patients [90]. This indicates that SNPs in genes may be predictive for disease progression in RA patients with an early disease phenotype.

Albeit, predicting the development of RA using T cell profiles might not give a complete prediction of the disease, it might partially envisage the development of RA in patients. Overall, RA may be both a Th1 and Th17 mediated disease due to T- cell plasticity and remains unresolved. However, further investigation is required to confirm this hypothesis.

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