# T cells out of control—impaired immune regulation in the inflamed joint

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**Abstract** | Since the discovery of FOXP3$^+$ regulatory T ($T_{REG}$) cells over 15 years ago, intensive research has focused on their presence, phenotype and function in autoimmune disease. Whether deficiencies in $T_{REG}$ cells underlie autoimmune pathology and whether, or how, therapeutic approaches based on these cells might be successful is still the subject of debate. The potential role of $T_{REG}$-cell extrinsic factors, such as proinflammatory cytokines and resistance of effector T cells to suppression, as the cause of regulatory defects in chronic autoimmune inflammation is an intensive area of research. It is now clear that, at the site of inflammation, antigen presenting cells (APCs) and proinflammatory cytokines drive effector T cell skewing and plasticity, and that these T cells can become unresponsive to regulation. In addition, expansion and function of $T_{REG}$ cells is affected by the inflammatory environment; indeed, new data suggest that, in certain conditions, $T_{REG}$ cells promote inflammation. This Review summarizes the latest findings on changes in effector T cell homeostasis in autoimmune disease and focuses on how mechanisms that normally regulate these cells are affected in the inflamed joints of patients with arthritis. These findings have important clinical implications and will affect the development of new therapeutic strategies for autoimmune arthritis.

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## Introduction

Our understanding of autoimmune diseases, including rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA), has been broadened by the identification of new key players in autoimmune inflammation. The discovery of CD25$^+$ FOXP3$^+$ regulatory T ($T_{REG}$) cells,[1] capable of suppressing T-cell activation, proliferation and effector function, opened an exiting new area of immunological research. FOXP3$^+$ $T_{REG}$ cells originate from the thymus and are therefore commonly referred to as natural $T_{REG}$ cells; they can, however, also be induced in the periphery from naive T cells in the presence of transforming growth factor β (TGF-β) and are then termed induced $T_{REG}$ cells (Figure 1, part 1).[2] Because of their suppressive function, $T_{REG}$ cells are crucial in maintaining self tolerance and preventing autoimmune responses.[2] As a result, their discovery stimulated extensive research into whether deficiencies in the number or function of $T_{REG}$ cells underlie human autoimmune disease, such as RA.[3,4] Accumulating data are now showing, however, that it is not just a question of insufficient or nonfunctional $T_{REG}$ cells in the perpetuation of autoimmune arthritis. The proinflammatory environment in affected joints can also interfere with T-cell regulation.[5] In particular, findings in 2011 indicated that resistance of effector T cells to suppression has an important role in synovial inflammation.[6,7] In addition, insights into T-cell plasticity, especially in inflamed joints,[8,9] have extended our understanding of autoimmune pathology. Here we summarize our current knowledge on how T cells lose control in autoimmune inflammation. We discuss how proinflammatory mediators can induce T-cell plasticity and interfere with T-cell regulation, especially in the inflamed joints of patients with arthritis. We also focus on how highly activated T cells influence antigen presenting cells (APCs) by modifying their infiltration and differentiation. Finally, we consider how these ineffective regulatory mechanisms might contribute to the chronicity of inflammation, which should be kept in mind when developing new therapies for autoimmune arthritis.

## T-cell subsets in autoimmune arthritis

In RA, the synovium becomes infiltrated by multiple types of immune cell, including granulocytes, monocytes and/or macrophages, B cells and high levels of CD4$^+$ and CD8$^+$ T cells, which are mostly activated memory cells, leading to the production of high levels of proinflammatory cytokines.[10,11] Genetic association studies have generated evidence for an important role of T cells, particularly CD4$^+$ T cells, in RA pathogenesis. For example, HLA-DR1 and HLA-DR4, which are involved in antigen presentation and T cell selection, have been associated with the development of RA.[10] In addition, in two spontaneous mouse models of arthritis, adoptive transfer of T cells from diseased mice induced disease in recipient mice.[11,12] Classically, type 1 T-helper ($T_H1$) cells, which produce IFN-γ, were thought to drive RA pathology. Since the discovery of type 17 T-helper ($T_H17$) cells (characterized by the production of IL-17), however, this concept of disease has been revised as these cells seem

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**Key points**

- The study of immune regulation at the site of inflammation is required to improve our understanding of autoimmune pathology
- At the site of autoimmune inflammation, proinflammatory mediators interfere with T-cell regulation and may induce T-cell plasticity
- Regulatory T ($T_{REG}$) cells are less functional, or might even become pathogenic, in an autoimmune inflammatory environment, which should be kept in mind when developing $T_{REG}$-cell-based therapies
- Resistance of effector T cells to suppression markedly contributes to the disturbed immune balance in the inflamed joints of patients with arthritis
- In autoimmune inflammation, a perpetuating loop exists in which antigen presenting cells (APCs) instruct T-cell differentiation and function, and effector T cells promote and shape the infiltration and differentiation of APCs

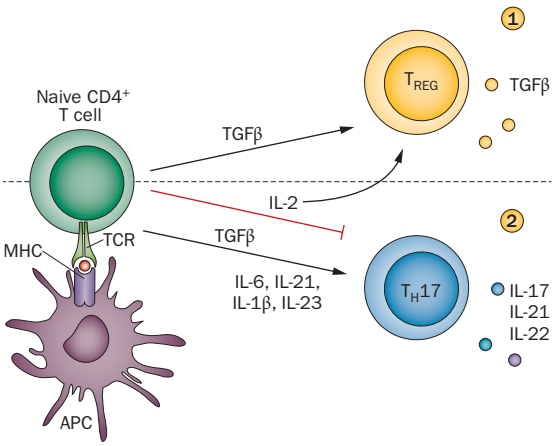


**Figure 1** | Reciprocal development of peripherally induced $T_{REG}$ and $T_H17$ cells. (1) Under specific circumstances, including high levels of TGFβ, $T_{REG}$ cells can be induced in the periphery from naive cells upon interaction with APCs. (2) Development of $T_H17$ cells is also dependent on TGFβ and proinflammatory cytokines, such as IL-6 and IL-21, whereas it is inhibited by IL-2. Therefore, $T_{REG}$ cells can promote the development of $T_H17$ cells in a proinflammatory environment by producing TGFβ and consuming IL-2. Abbreviations: APC, antigen presenting cell; MHC, major histocompatibility complex; TCR, T-cell receptor; TGFβ, transforming growth factor β; $T_H17$, type 17 T-helper cell; $T_{REG}$, regulatory T cell.

to be even more important in promoting autoimmune disease.[11,13] In RA, IL-17 promotes synovial inflammation by enhancing the influx of inflammatory cells, such as neutrophils, and is a major contributing factor to bone and cartilage damage.[10,14]

## Synovial fluid

The relative contribution and commitment of $CD4^+$ T-cell subsets in RA pathology was investigated using epigenetic immune lineage analysis.[15] Epigenetic information is not encoded by changes in DNA sequence but by differential methylation of the DNA and chromatin modification, which results in heritable but plastic modifications. These epigenetic processes have been shown to be key determinants in T-helper ($T_H$)-cell differentiation and stability.[16] By analyzing DNA methylation levels of several key genes in $T_H$ cell differentiation, Janson and colleagues established that demethylation of the *Ifnγ* locus is significantly enhanced in $CD4^+$ T cells isolated from the synovial fluid of patients with RA,[15] which is indicative of enhanced skewing towards the $T_H1$ lineage. In line with this finding, $CD4^+$ T cells in the synovial fluid were found to predominantly produce IFN-γ in response to type II collagen, one of the main constituents of articular cartilage.[17]. Interestingly, both studies also reported $T_H17$ cell responses at the site of inflammation, although this phenotype was less pronounced than $T_H1$ skewing. Moreover, in patients with JIA, higher levels of IFN-γ than IL-17 were observed and $T_H17$ cells were found to co-produce IFN-γ in inflamed joints.[8,9,18] However, as described above, $T_H17$ cells are key players in joint pathology and their lower levels in the inflamed joint compared with $T_H1$ cells might result from limited expansion of these cells at the inflammatory site.[19]

In addition to *Ifnγ* demethylation, Janson *et al.*[15] also observed increased demethylation of the *Foxp3* locus in synovial fluid $CD4^+$ T cells. Thus, in addition to $T_H1$ skewing, an enhanced commitment towards the $T_{REG}$-cell lineage is also observed. This observation is in line with studies showing that $FOXP3^+$ $T_{REG}$ cells are enriched in the synovial fluid of patients with RA and JIA: these studies also confirm the high levels of demethylation of the *Foxp3* $T_{REG}$-cell specific demethylated region (TSDR), suggesting stable FOXP3 expression.[5,6] Enhanced expression of the antigen KI-67 (a marker of cell proliferation) in these cells[5] (and Wehrens *et al.* unpublished observation) further suggest that $T_{REG}$ cells are expanding locally in the inflamed joints. Although little is known about the role of $CD8^+$ T cells in joint pathology, high levels of these cells are present in synovial fluid, mainly with an effector memory phenotype, and produce marked amounts of proinflammatory cytokines, such as tumour necrosis factor (TNF) and IFN-γ.[6,20] Furthermore, in patients with JIA high levels of synovial fluid $CD8^+$ T cells are correlated with a more progressive course of disease than is observed in patients with lower levels of these cells.[21]

## Specific T cell skewing in inflamed joints

Notably, in the peripheral blood of patients with RA, the level of demethylation of *Ifnγ* and *Il-17a* is the same as in healthy controls.[15] Thus, skewed $CD4^+$ T-cell commitment occurs specifically at the site of inflammation, likely as a result of an interaction between $CD4^+$ T cells and local APCs, which are found in increased numbers in inflamed joints.[22–28] Moreover, synovial fluid monocytes[22,24,26] and dendritic cells (DCs)[23,25–28] have a more activated phenotype with enhanced expression of the maturation markers CD40, CD80, CD86 and HLA-DR than those isolated from peripheral blood. Upon interaction with $CD4^+$ T cells these monocytes derived from synovial fluid specifically induce $T_H17$ responses, in contrast to monocytes isolated from peripheral blood.[22] In experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis, DCs promote maintenance of $T_H17$ cells at the site of inflammation via production of IL-23.[29] In addition, the acute phase protein serum amyloid A, which is highly present

during inflammation, induces $T_{REG}$-cellular proliferation without abrogating their suppressive function in a monocyte-dependent manner.[30] Thus, in an inflammatory environment, monocytes may also induce expansion of $T_{REG}$ cells, potentially explaining the high levels of proliferating $T_{REG}$ cells observed in the inflamed joints of patients with arthritis.[5,6] In conclusion, $T_{REG}$, $T_H1$ and $T_H17$ cells are key T-cell subsets in joint inflammation. Although it is unlikely that priming of naive T cells takes place at the site of inflammation, T-cell expansion, differentiation and effector functions are probably further promoted following interactions with local activated APCs (Figure 2).

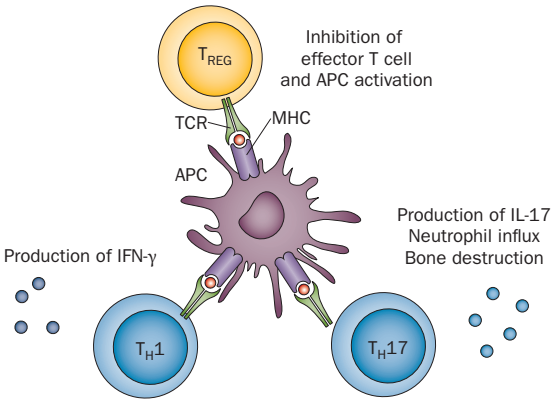


**Figure 2** | The main $CD4^+$ T-cell subsets in synovial inflammation. $T_H1$ cells, which produce IFN-γ, and $T_{REG}$ cells, which can inhibit effector T cells and APC activation, are key T-cell subsets in the inflamed joints of patients with arthritis. $T_H17$ cells, which produce IL-17, contribute to synovial inflammation by enhancing neutrophil infiltration and bone destruction. The differentiation, expansion and effector function of these T cells are modulated by interaction with local APCs. Abbreviations: APC, antigen presenting cell; MHC, major histocompatibility complex; TCR, T-cell receptor; $T_H1$, type 1 T-helper cell; $T_H17$, type 17 T-helper cell; $T_{REG}$, regulatory T cell.

## $T_{REG}$ cells in autoimmune inflammation

Given their central role in maintaining self tolerance and suppressing inflammation, research has focused on the clinical application of $T_{REG}$ cells in patients with autoimmune disease, including RA.[31] However, whether $T_{REG}$ cells are actually deficient in these patients remains controversial.[31,32] Indeed, as described above, high levels of $T_{REG}$ cells are present in the inflamed joints of patients with arthritis and numerous publications have shown that synovial fluid $T_{REG}$ cells are functional *in vitro*.[6,7,33–36] Nevertheless, the proinflammatory environment might interfere with their effectiveness at the site of inflammation. For example, highly activated APCs in inflamed joints might reduce $T_{REG}$-cell function, as strong and prolonged T-cell stimulation through the concomitant stimulation of the T-cell receptor and CD28 signalling pathways can impair $T_{REG}$-cell-mediated suppression.[24,37,38] In addition, in an experimental model of systemic lupus erythematosis (SLE)[39] and in patients with SLE[40], APCs were found to impair $T_{REG}$-cell-mediated inhibition through production of the proinflammatory cytokines IL-6 and IFN-α, respectively.

### TNF

Research over the past 8 years has particularly focused on the effects of TNF on $T_{REG}$-cell function, probably because its receptor (TNF receptor 2 [TNFR2]) is preferentially expressed on the surface of $T_{REG}$ cells in both mice and humans.[41–43] Extensive data on the effect of TNF on $T_{REG}$-cell function have now been retrieved from both human and experimental studies, with partially conflicting results. Indeed, a direct TNFR2-mediated impairment of human $T_{REG}$-cell function has been described *in vitro*[43–45] and, from the reverse perspective, inhibition of TNF improves $T_{REG}$-cell-mediated suppression both *in vitro*[5] and in patients treated with infliximab.[43,45,46] Interestingly, a novel $T_{REG}$-cell population with enhanced suppressive capacity seems to be induced in patients treated with either infliximab[45,47] or adalumimab,[48] although cytotoxic T-lymphocyte protein 4 (CTLA-4) expression by $T_{REG}$ cells remains unaffected.[49] By contrast, data from mouse models revealed that TNF can actually boost $T_{REG}$-cell expansion[50,51] and, as such, protect mice from subsequent induction of autoimmune disease.[51] TNF might, therefore, have different effects on $T_{REG}$ cells—reducing their suppressive function, but, at the same time, promoting their expansion. The latter could be another mechanism behind the high levels of proliferating $T_{REG}$ cells observed in the inflamed joints of patients with arthritis.[5,6] However, the relationship between TNF levels and $T_{REG}$-cell number still remains a conundrum: in humans TNF has a negative effect on $T_{REG}$-cell expansion *in vitro*[52] and, in the inflamed joints of patients with arthritis, a negative correlation between TNF levels and the percentage of $FOXP3^+$ T cells was observed.[5] Furthermore, in mice, TNF was found to increase $T_{REG}$-cell suppressive capacity,[50,51] which contradicts with the data obtained from humans.[5,43,46] Thus, mouse and human $T_{REG}$ cells might respond differently to TNF and so far all human data point towards a negative effect of TNF on $T_{REG}$-cell function. In our opinion, the effect of TNF is species specific: TNF-induced expansion of $T_{REG}$ cells only occurs in mice, whereas, in humans, TNF negatively affects both $T_{REG}$-cell expansion and function. Other cytokines, such as IL-2, IL-7 and IL-15 have also been described to interfere with human $T_{REG}$ cell function.[24,35] Thus, in the inflamed joint of patients with arthritis, proinflammatory cytokines, in particular TNF, and highly activated APCs are thought to interfere with $T_{REG}$-cell function (Figure 3, part 1).

### Other inhibitory pathways

The proinflammatory synovial environment may also hinder other pathways of T-cell regulation. For instance, the programmed cell death protein 1 (PD-1) is upregulated on synovial fluid T cells, but these cells display impaired responsiveness to PD-1-mediated restriction of proliferation and cytokine production. This phenotype can be mimicked *in vitro* by culturing cells from healthy donors in the presence of synovial fluid,[53] suggesting that soluble proinflammatory mediators have a role in impaired responsiveness to PD-1 signalling. In

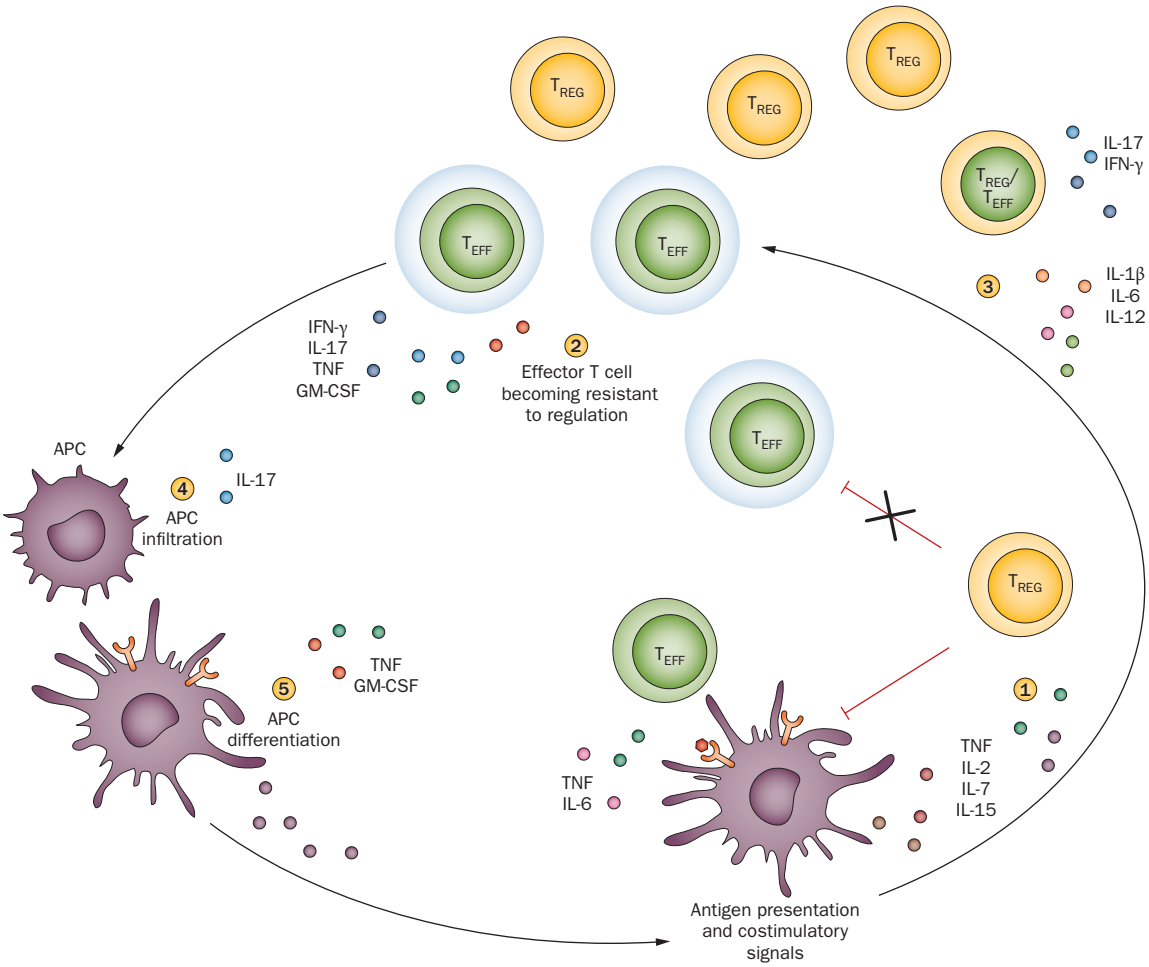


**Figure 3** | Perpetuating loop of uncontrolled synovial inflammation. In the inflamed joints of patients with arthritis, activated APCs and proinflammatory cytokines impair the function of $T_{REG}$ cells (1) and induce resistance of effector T cells (2). In the presence of proinflammatory APCs, a small population of unstable $T_{REG}$ cells might convert into pathogenic effector T cells (3). Cytokines produced by these highly activated uncontrolled effector T cells further drive the inflammatory response by recruiting monocytes to the site of inflammation (4) and promoting their differentiation into dendritic cells (5). Abbreviations: APC, antigen presenting cell; $T_{EFF}$, effector T cell; $T_{REG}$, regulatory T cell.

addition, Hidalgo *et al.*[54] showed that CD130, which is involved in IL-6 signalling, is not downregulated in synovial tissue, presumably owing to the local presence of IL-10. The specific cytokine environment therefore suppresses negative feedback mechanisms in the inflamed synovium, allowing for continuous IL-6 signalling and unresponsiveness to PD-1-mediated inhibition.[54] All together, these data implicate that T-cell regulation, and specifically $T_{REG}$-cell function, are diverted by the proinflammatory environment in the inflamed joint.

## Resistance of effector T cells

In addition to the impairment of $T_{REG}$-cell function described above, resistance of effector T cells to suppression also markedly contributes to uncontrolled inflammation. In 2003, *in vitro* mouse experiments showed that activated APCs could render effector T cells unresponsive to suppression by a mechanism dependent, in part, on IL-6.[55] The phenomenon of effector T cells becoming refractory to suppression was additionally suggested *in vivo* in EAE.[56,57] $T_{REG}$ cells isolated from the site of inflammation in EAE were functional, as these cells suppressed naive effector T cells *in vitro*. However, suppression was reduced following co-culture with primed effector T cells from the inflamed central nervous system, demonstrating that effector T cells from the site of inflammation are less responsive to suppression.[56,57] Notably, these primed effector T cells produced high levels of TNF and IL-6; adding these cytokines to naive effector T cells reversed their suppression by $T_{REG}$ cells.[56]

Resistance of effector T cells to suppression was also reported for cells from the site of inflammation in patients with JIA. We[6] and others[7] have shown that $T_{REG}$-cell-mediated suppression is impaired when cells are isolated from the inflamed joints of these patients. Interestingly, this finding is not a result of a functional defect in synovial fluid $T_{REG}$ cells, as these cells suppressed effector T cells isolated from the peripheral blood to a similar level as was observed in healthy controls.[6,7] Instead effector T cells from the site of inflammation showed reduced responsiveness to suppression, which, although not associated with a memory phenotype of the cells, did

correlate with their activation status.[6,7] More specifically, we established that protein kinase B (PKB) hyperactivation contributed to resistance of synovial fluid T effector cells to suppression. In our opinion, PKB hyperactivation results from the proinflammatory synovial environment, because TNF and IL-6, both highly present in inflamed joints, induce PKB activation and subsequent resistance to suppression in effector T cells from healthy donors.[6] These findings are in agreement with the before mentioned studies in EAE showing a role for TNF and IL-6 in effector T-cell resistance to suppression.[55,56] In patients with JIA, unresponsiveness to suppression was not only observed in $CD4^+$ T cells, but also highly apparent in synovial fluid $CD8^+$ T cells,[6] in line with their correlation to a more severe outcome of disease.[21] Furthermore, although this resistance to suppression is intrinsic to T cells and maintained *in vitro* even in the absence of synovial fluid APCs,[6] it seems that synovial fluid APCs are responsible for the initial induction of resistance to suppression in JIA (Wehrens *et al.* unpublished observations).

In patients with RA, resistance of effector T cells to suppression—although to a lesser degree than resistance to suppression observed in the synovial fluid of patients with JIA—was also detected in the peripheral blood of patients when compared with healthy controls,[58] perhaps owing to a more systemic pathology in RA than JIA. In other autoimmune diseases, including type I diabetes mellitus (T1DM), SLE and inflammatory bowel disease, resistance of effector T cells to suppression has been described, both in patients[59–63] and in animal models[39,64–67] (Table 1). Effector T-cell resistance to suppression therefore seems to be a general mechanism involved in autoimmune pathology and chronic inflammation. In line with this conclusion, $T_H17$ cells, identified as key players in autoimmune inflammation, are less susceptible to suppression than other $T_H$-cell subsets.[57,68–70] In conclusion, effector T cells in the inflamed joints of patients with arthritis are refractory to suppression, which probably contributes to uncontrolled synovial inflammation (Figure 3, part 2).

## Inflammation-induced T-cell plasticity

### $T_{REG}$ cells

The proinflammatory environment is known to interfere with $T_{REG}$-cell-mediated suppression, but it might also induce plasticity of these cells. Purified human $T_{REG}$ cells can downregulate FOXP3 expression in the presence of proinflammatory cytokines, such as IL-1β and IL-6, *in vitro*.[71,72] This loss of FOXP3 expression is associated with reduced suppressive function[72] and, more importantly, production of IL-17.[71,72] Although it is impossible to exclude outgrowth of contaminating $FOXP3^-$ cells in a human system, data from transgenic mice confirmed that cells expressing FOXP3 tagged with green fluorescent protein could differentiate into effector cells that produce IL-17 and IFN-γ in an autoimmune inflammatory environment.[73,74] Together these data suggest that $T_{REG}$ cells from patients with autoimmune disease exhibit enhanced plasticity because of ongoing inflammation. In patients with multiple sclerosis[75] and T1DM,[76] increased numbers of peripheral blood $FOXP3^+$ T cells co-express IFN-γ and, as a result, display reduced suppressive capacity. These unstable $T_{REG}$ cells are thought to arise as a consequence of their proinflammatory environment, as exposure to IL-12 *in vitro* also induced IFN-γ production in $FOXP3^+$ cells from healthy donors.[75,76]

How these findings translate to $T_{REG}$ cells present in the inflamed joints of patients with arthritis is still unclear and might be obscured by the transient upregulation of FOXP3 expression in human activated effector T cells.[77,78] Although IFN-γ levels are very low, low levels of IL-17 expression can be found in $FOXP3^+$ T cells isolated from synovial fluid of patients with arthritis, but it is not clear how these levels compare to $T_{REG}$ cells from peripheral blood.[5] In addition, we did not observe any IFN-γ and IL-17 production in synovial fluid $FOXP3^+$ T cells in our cohort of patients with JIA (Wehrens *et al.* unpublished observation). Furthermore, $FOXP3^+$ cells from synovial fluid of patients with JIA have high TSDR demethylation levels, similar to $FOXP3^+$ cells from peripheral blood,[5,6] suggesting that these cells have a relatively stable phenotype despite their proinflammatory environment. Data in mice have now shown that only a minor subpopulation of $FOXP3^+$ T cells with a fully methylated TSDR region become unstable,[79,80] whereas the majority of $FOXP3^+$ T cells are resistant to conversion into effector T cells and have a fully demethylated TSDR region.[80] Notably, these unstable $FOXP3^+$ cells are mainly present within peripherally induced and not natural $T_{REG}$ cells and preferentially expand under inflammatory conditions.[80] Thus, unstable peripherally induced $T_{REG}$ cells might predominantly contribute to $T_{REG}$-cell plasticity observed in the presence of proinflammatory cytokines.[71,72,74] It is not possible to distinguish between natural and induced $T_{REG}$ cells in humans, however, but in support of the data from mice studies, only a subpopulation of human $FOXP3^+$ cells was found to be unstable and displayed impaired suppressive capacity.[72,81] Natural $FOXP3^+$ $T_{REG}$ cells might, therefore, be more stable than initially recognized. Although no data so far indicate that synovial fluid $T_{REG}$ cells are unstable, it is still possible that in the inflamed synovium some $T_{REG}$ cells display enhanced plasticity due to the highly proinflammatory environment (Figure 3, part 3).

### $T_H17$ cells

Although it is still unclear whether $T_{REG}$-cell plasticity takes place in the inflamed joint, plasticity of $T_H17$ cells in the synovium of patients with JIA has been reported. Compared with peripheral blood, a high proportion of $T_H17$ cells in synovial fluid co-express IFN-γ[8,9,18] and display both $T_H17$ (RORC)[8,9] and $T_H1$ (T-bet) transcription factors.[9] Instability of these synovial fluid $T_H17$ cells is confirmed *ex vivo* as, following culture, a substantial proportion start to produce IFN-γ, whereas $T_H17$ cells from peripheral blood remain IL-17 single positive.[8] However, $T_H17$ cells derived from peripheral blood can convert into $T_H1$ cells when cultured in the presence of synovial fluid in a mechanism dependent on high levels of IL-12 in the synovial fluid.[8,9] Thus, $T_H17$ cells in the synovium of patients with JIA probably become

**Table 1** | Resistance of effector cells to suppression in human and experimental autoimmune disease

| Disease | Subject | Suppression assay | Type of effector cell analyzed | Resistant compared to | Suggested mechanism | Study |
|---|---|---|---|---|---|---|
| JIA | Human | *In vitro* allogeneic | Synovial fluid $CD4^+CD25^-$ effector T cells | Effector T cells from healthy controls | Enhanced activation | Haufe *et al.* 2011[7] |
| | | *In vitro* autologous | Synovial fluid $CD4^+$ and $CD8^+$ effector T cells | Peripheral blood effector T cells from the same patient | PKB hyperactivation (in response to TNF and IL-6) | Wehrens *et al.* 2011[6] |
| RA | Human | *In vitro* allogeneic | Peripheral blood $CD4^+CD25^-$ effector T cells | Effector T cells from healthy controls | Increased TRAIL expression (inducing $T_{REG}$ cell apoptosis) | Xiao *et al.* 2011[58] |
| T1D | NOD mice | *In vitro* syngeneic | Splenic $CD4^+CD25^-$ effector T cells | Effector T cells from pre-diabetic mice | ND | You *et al.* 2005[67] |
| | DO11.10 RIP-mOVA mice | *In vivo* syngeneic | Lymph node $CD4^+CD25^-$ effector T cells | Effector T cells from pre-diabetic mice | High IL-21 production | Clough *et al.* 2008[64] |
| | NOD mice | *In vitro* allogeneic | Splenic $CD4^+CD25^-$ effector T cells | Effector T from B6 mice | ND | D'Alise *et al.* 2008[65] |
| | Human | *In vitro* allogeneic | Peripheral blood $CD4^+CD25^-$ effector T cells | Effector T from healthy controls | ND | Schneider *et al.* 2008[61] |
| | Human | *In vitro* allogeneic | Peripheral blood $CD4^+CD25^-$ effector T cells | Effector T cells from healthy controls | ND | Lawson *et al.* 2008[60] |
| SLE | MRL/lpr and NZB/WF1 mice | *In vitro* allogeneic | Splenic and lymph node $CD4^+CD25^-$ effector T cells | Effector T cells from CBA/Ca mice | ND | Monk *et al.* 2005[66] |
| | MRL/lpr mice | *In vitro* allogeneic | Lymph node $CD4^+CD25^-$ effector T cells | Effector T cells from CBA/J mice | ND | Parietti *et al.* 2008[39] |
| | Human | *In vitro* allogeneic | Peripheral blood $CD4^+CD25^-$ effector T cells | Effector T cells from healthy controls | ND | Venigalla *et al.* 2008[63] |
| | Human | *In vitro* allogeneic | Peripheral blood $CD4^+CD25^-$ effector T cells | Effector T cells from healthy controls | ND | Vargas-Rojas *et al.* 2008[62] |
| IBD | Human | *In vitro* allogeneic | Lamina propria $CD4^+$ effector T cells | Compared to lamina propria and peripheral blood effector T cells from healthy controls | High expression of Smad7 (interfering with TGFβ signalling) | Fantini *et al.* 2008[59] |
| EAE | *Foxp3gfp*.KI mice | *In vitro* autologous and allogeneic | CNS $CD4^+FOXP3/GFP^-$ effector T cells | Splenic effector T cells from the same mice and effector T cells from 2D2 mice | High TNF and IL-6 production | Korn *et al.* 2007[56] |
| | C57BL/6 mice | *In vitro* allogeneic | CNS $CD4^+CD25^-$ effector T cells | Effector T cells from 2D2 mice | ND | O'Connor *et al.* 2007[57] |

Abbreviations: CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; IBD, inflammatory bowel disease; JIA, juvenile idiopathic arthritis; ND, not determined; PKB, protein kinase B; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; T1D, type 1 diabetes; TGFβ, transforming growth factor β; TNF, tumour necrosis factor; TRAIL, TNF-related apoptosis inducing ligand; $T_{REG}$, regulatory T cells.

unstable owing to their proinflammatory environment containing high levels of IL-12. This inflammatory mediated conversion of $T_H17$ has also been observed *in vivo* in mouse models of autoimmune disease.[82–84] CD161 expression by a proportion of $T_H1$ cells in the synovium[9] and clonal overlap of these cells with $T_H17$ cells and $T_H17$ cells co-expressing IFN-γ[8,9] suggest that unstable $T_H17$ cells give rise to a population of $T_H1$ cells in the inflamed joints, potentially explaining the more pronounced $T_H1$ responses in the inflamed synovium described earlier.[8,16,17]

## T cells modulate APC: closing the loop

Highly activated APCs at the site of autoimmune inflammation influence T-cell homeostasis, which is reflected by the preferential induction of $T_H17$ cells by monocytes from the inflamed joints of patients with RA.[22] Studies in mice and humans over the past 3 years have demonstrated that T cells also influence the infiltration and differentiation of APCs. Human monocytes express high levels of IL-17 receptor and IL-17 serves a chemoattractant for these cells. More specifically, synovial fluid from patients with RA, which contains high levels of IL-17, induces monocyte migration, which is abrogated by antibodies against IL-17 or its receptors.[85] Thus, in inflamed joints, monocytes induce $T_H17$ cell differentiation whereas IL-17 produced by these cells promotes the infiltration of new monocytes into the synovium (Figure 3, part 4). In addition to enhancing monocyte infiltration, the differentiation of these cells into DCs is also promoted by cytokines produced by effector T cells (Figure 3, part 5). Human $CD4^+$ T cells induce differentiation of monocyte-derived DCs *in vitro*, which is triggered by production of granulocyte-macrophage colony stimulating factor (GM-CSF) and TNF.[86] Furthermore, during inflammation *in vivo* the generation of monocyte-derived DCs seems to be dependent on $CD4^+$ T cells producing GM-CSF.[87] When acute inflammatory arthritis is induced in mice that lack GM-CSF producing $CD4^+$ T cells, the numbers of monocyte-derived DCs in draining lymph nodes are significantly reduced. As a result, histological arthritis scores in these mice are moderated, indicating that T-cell dependent differentiation of monocyte-derived DCs contribute to the severity of joint inflammation.[87]

In addition to promoting DC differentiation in general, one study now indicates that human $CD4^+$ T cells can actually modulate the type of DC that is generated, depending on the subset of $T_H$ cell and the cytokines it

produces.[86] DCs differentiated in the presence of $T_H1$, $T_H2$ or $T_H17$ cells differ in phenotype and cytokine production. As a result, following co-culture with naive T cells they preferentially induce the same type of $T_H$ cell that they were generated with. Some evidence now indicates that this T-cell instructed DC differentiation also occurs during inflammation *in vivo*, as DCs isolated from psoriatic skin lesions in which $T_H1$ and $T_H17$ cells predominate resemble those that are generated in the presence of $T_H1$ and $T_H17$ cells *in vitro*.[86] Together, these data suggest that at the site of autoimmune inflammation, a loop exists in which T cells act back on APCs, not only by enhancing their recruitment (Figure 3, part 4), but also by promoting and probably modulating their differentiation into DCs (Figure 3, part 5). As a result, the proinflammatory type of immune response is further reinforced. Reduced killing of activated monocytes might also contribute to this ongoing loop of inflammation. Under homeostatic conditions effector cells can induce monocyte apoptosis, but this response is impaired in patients with RA, especially in cells from synovial fluid,[88] which might result from resistance of synovial fluid monocytes to FAS-induced apoptosis.[89] Finally, although $T_{REG}$ cells normally inhibit APC function, APCs become less responsive to suppression following activation.[90] Therefore, it will be very interesting to investigate whether $T_{REG}$ cells are capable of suppressing the highly activated APCs found in the inflamed joints of patients with arthritis. In conclusion, insufficiently controlled effector T cells in the inflamed synovium (Figure 3, part 2) can further enhance the ongoing inflammation by promoting monocyte infiltration (Figure 3, part 4) and differentiation into DCs (Figure 3, part 5).

### $T_{REG}$ cells and chronic inflammation

So far we have described several pathways involved in T-cell regulation that are upregulated at the site of autoimmune inflammation. However, their regulatory outcome is clearly restricted by the proinflammatory environment. In particular, $FOXP3^+$ $T_{REG}$ cells are present at high levels in the inflamed joints of patients with arthritis,[5,6] but ineffective in controlling effector T cells, at least partly owing to the resistance of effector T cells to suppression.[6,7] Besides being ineffective, could these $T_{REG}$ cells actually contribute to ongoing inflammation? For example, as induction of pathogenic $T_H17$ cells is dependent on TGFβ[84,91–93] but inhibited by IL-2,[84,94] $T_{REG}$ cells could promote $T_H17$ differentiation by producing TGFβ and consuming IL-2[84,95] (Figure 1). Indeed, in mice, highly purified $FOXP3^+$ $T_{REG}$ cells induce $T_H17$ differentiation, in a manner dependent on production of TGFβ[73,96] or consumption of IL-2, or both.[97,98] Moreover, this $T_H17$ induction specifically occurs under inflammatory conditions both *in vitro*[73,96] and *in vivo*.[97,99] In addition, in humans, $T_{REG}$ cells not only fail to suppress IL-17 levels in culture, but actually increase the percentage of IL-17 producing cells.[100] Of note, $T_{REG}$ cells enhance IL-17 production by effector memory T cells in these assays, suggesting that in inflamed joints of patients with arthritis, in which memory T cells predominate,[6,7] $T_{REG}$ cells could promote $T_H17$ cellular responses. In addition, if $T_{REG}$ cells become unstable and subsequently convert into pathogenic effector T cells producing proinflammatory cytokines, there could be a highly detrimental effect on autoimmune inflammation. Indeed, mouse $T_{REG}$ cells become pathogenic following downregulation of FOXP3 and induce diabetes in $RAG2^{-/-}$ mice.[101] Finally, although $T_{REG}$ cells inhibit proinflammatory cytokine production by monocytes,[88,102] they do not induce apoptosis of these cells, as shown for effector T cells.[88] Therefore, reduced killing of activated monocytes might also be a negative consequence of high levels of $T_{REG}$ cells present at the site of inflammation. Thus, besides being ineffective in controlling the inflammatory response, through a variety of mechanisms, $T_{REG}$ cells might actually contribute to the ongoing inflammation.

## Conclusions

In the past decade, biologic agents that specifically target essential mediators of autoimmune inflammation have been introduced in the clinic to treat autoimmune disease. Of these, monoclonal antibodies against TNF (infliximab and adalimumab), or the soluble TNF receptor (etanercept), have been very promising in the treatment of autoimmune arthritis. Anti-TNF therapy is still not curative and only partially effective in the majority of patients.[103] The search for new treatment options therefore continues. Over the past years the therapeutic application of $T_{REG}$ cells has been thoroughly investigated because of their profound anti-inflammatory properties.[31] However, the local proinflammatory environment can interfere with $T_{REG}$-cell function in patients with autoimmune inflammation and resistance of effector T cells to suppression could restrict the effectiveness of $T_{REG}$-cell targeted approaches.[6,7,58,59,61,63] Looking to the future, more information on how this resistance is induced and maintained will be essential in the development of more effective therapeutic strategies. Data on whether and how current therapies target resistance of effector T cells to suppression will also help to further understand its importance in autoimmune pathology. Indeed, alternative targets might be more efficient in the treatment of autoimmune arthritis or could enhance effectiveness of $T_{REG}$ based therapies. Moreover, $T_{REG}$ cells could promote the development of pathogenic effector T cells in a proinflammatory environment.[71–73,75] Overall, $T_{REG}$-cell-based therapies should be approached with great caution in patients with ongoing inflammation—a combination with a profound anti-inflammatory strategy might be indispensable in preventing adverse events.

### Review criteria

PubMed was searched for full-text, English-language original and review articles published between 2005 and 2012. The search terms used were: "rheumatoid arthritis", "juvenile idiopathic arthritis", "synovial/synovium T cells", "FOXP3 regulatory T cells", "Th17", "T cell plasticity", "inflammation", "synovial fluid" and "resistance to suppression", either alone or in combination. The reference lists of identified articles were searched for further relevant papers. The reference list was last updated May 15, 2012.

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