Regulatory T-cells (Tregs) are the guardians of peripheral tolerance acting to prevent autoimmune diseases such as systemic lupus erythomatosus (SLE) and rheumatoid arthritis (RA). Defects in Tregs have been reported in these two diseases despite significant differences in their clinical phenotype and pathogenesis. In both diseases the potency of Treg fails to keep pace with the activation of effector cells and are unable to resist the ensuing inflammation. This review will discuss the phenotypic, numeric, and functional abnormalities in Tregs and their role in patients and murine models of SLE and RA.

1. Introduction

Autoimmune diseases such as systemic lupus erythomatosus (SLE) and rheumatoid arthritis (RA) arise due to a failure of immunological self-tolerance. Despite central mechanisms of tolerance, some T-cells recognizing self-antigens are released into the periphery. One of the mechanisms employed to eliminate or control these potentially damaging cells is regulatory T-cells (Tregs). The importance of Tregs is underscored by the overwhelming inflammation and autoimmune that results in their absence. This review will highlight the role of Tregs in two autoimmune rheumatic diseases, SLE and RA.

A number of immune regulatory cells have been described but this review will focus on CD4+ Tregs, which can be divided into naturally occurring and adaptive [1]. In mouse, CD4 Tregs constitute around 5% of the peripheral CD4+ lymphocyte population [2] whereas in humans only 1–2% [3]. Human Tregs were originally characterized by Sakaguchi et al. as CD4+ T-cells expressing the highest levels of CD25 (CD25high) have in vitro suppressing activity. Although CD25 was the first Treg marker to be identified, it is also expressed on activated CD4+ T-cells. CD127 (the b chain of the IL-7 receptor) has more recently been shown to be important in the development and function of Tregs and is vital to their phenotypic identification [7–9]. In mice, mutation [10] or depletion [11] of the Foxp3 gene resulted in fatal autoimmune lymphoproliferative disease whereas in human, several mutations of Foxp3 gene have been linked to a disease called immune dysregulation, polyendocrinopathy, enteropathy X-linked syndrome (IPEX) [12,13]. Although Foxp3 is essential for Treg function, as with CD25, CD4+ T-cells can upregulate the expression of Foxp3 upon activation [14,15]. These activated Foxp3+ T-cells are capable of producing IL-2 in vitro indicating a lack of Treg function. This finding could be relevant to diseases such as RA and SLE, which are characterized by marked T cell activation.

Apart from the naturally occurring thymically derived Tregs, stimulation of peripheral CD4+ T-cells with anti-CD3 and anti-CD28 in the presence of IL-2 and TGF-b can induce Foxp3 expression [15,16]. These induced or adaptive Tregs are CD4+CD25+Foxp3+. Thus the peripheral CD4+CD25+Foxp3+ Treg population is a mixture of both natural and induced Tregs. Recently, Helios has been identified as a transcription factor that is expressed by natural, but not induced, Tregs. Thornton et al. showed that all CD4+Foxp3+ thymocytes were Helios+ whereas Helios expression in peripheral Tregs was restricted to approximately 70% of CD4+Foxp3+ in both human and mice. Induced Tregs, either in vitro with anti-CD3 and anti-CD28 antibody in the presence of IL-2 and TGF-b, or in vivo by exposure to antigen orally, do not express Helios suggesting that Helios is a thymic-derived Treg maker [17]. Apart from distinguishing different populations of Tregs, Helios is also important in their function. Helios binds the Foxp3 promoter and partially regulates its expression. Indeed in vitro inhibition of Helios by siRNA oligonucleotides results in down-regulation of Foxp3 [16]. Additionally, natural Tregs...
have a stable Foxp3 gene expression, which is controlled by epigenetic mechanisms. Human CD4+CD25\textsuperscript{high} Tregs display a demethylated FOXP3 promoter in contrast to CD4+CD25\textsuperscript{low} T cells, where FOXP3 is partially methylated. Furthermore, stimulated CD4+CD25\textsuperscript{low} T cells transiently express FOXP3 but remained partially methylated, suggesting promoter methylation as a mechanism for regulation of stable FOXP3 expression and Treg commitment [18]. Based on Foxp3 expression, Tregs can also be divided in different populations. Miyara et al. defined these populations as naive or resting Tregs (CD25\textsuperscript{high}CD45RA\textsuperscript{low}Foxp3\textsuperscript{high}) and activated Tregs (CD25\textsuperscript{high}CD45RA\textsuperscript{low}Foxp3\textsuperscript{high}) both of which are suppressive in vitro. There is also a non-regulatory population that is CD25\textsuperscript{high}CD45RA\textsuperscript{high}Foxp3\textsuperscript{high} [19]. Several additional markers of Tregs have been identified including cytokotic T lymphocyte-associated protein 4 (CTLA-4), glucocorticoid-induced TNF-receptor (GTR), lymphocyte activated gene-3 (LAG-3), neuropilin-1 (Nrp1), CD62L\textsuperscript{high}CD39 and CD72 but once again they are not exclusive to Tregs and integrating these molecules into a clear picture of Treg function remains elusive.

2. Properties and function of Tregs

Regardless of the origin of Tregs (natural or adoptive Tregs), these cells are considered suppressor cells and able to play a pivotal role in the maintenance of immune tolerance. Their importance in the development of autoimmune diseases was recognised by Sakaguchi and colleagues who were the first to show that transfer of CD4\textsuperscript{+} T-cells depleted of CD25\textsuperscript{+} T-cells, by specific monoclonal antibodies against CD25, into BALB/c athymic nude mice caused spontaneous development of T-cell dependent autoimmune diseases (such as thyroiditis, gastritis, insulitis, sialoadenitis, adenitis, adrenalitis, oophoritis, glomerulonephritis (GN), and polyclonal antibodies against CD25, into BALB/c athymic nude mice caused spontaneous development of T-cell dependent autoimmune diseases (such as thyroiditis, gastritis, insulitis, sialoadenitis, adenitis, adrenalitis, oophoritis, glomerulonephritis (GN), and polyarthritis) [20]. When these mice were reconstituted by CD4\textsuperscript{+}CD25\textsuperscript{+} T-cells within a limited period after CD4\textsuperscript{+}CD25\textsuperscript{+} T-cell transfer, the autoimmune disease development was successfully prevented [4].

Most of our knowledge of the function of human Tregs comes from in vitro experiments. In vitro, Tregs upon antigen stimulation are anergic and characterized by low proliferation rate and low secretion of IL-2. Tregs can be expanded in vitro in the presence of anti-CD3 and IL-2 or when stimulated with anti-CD3/CD28. In vivo, these cells have a high proliferation rate [20]. Tregs after in vitro activation can suppress proliferation and cytokine production of CD4\textsuperscript{+}CD25\textsuperscript{+} T-cells [3,21,22]. Tregs can also suppress monocytes, macrophages [23] B-cells [24] and dendritic cells [25]. Tregs exert their suppressive function through different mechanisms, which includes secretion of inhibitory cytokines and cytotoxic factors or by metabolic disruption, or by modulating antigen presenting cell (APC) maturation and function.

One mechanism by which Tregs exert suppressive function is via the secretion of inhibitory cytokines such as IL-10, TGF-β [26] and IL-35 [27] as well as cytotoxic factors including perforin and granzymes. Deletion of IL-10 specifically in Treg leads to inflammation at mucosal surfaces but does not trigger systemic autoimmunity [28]. IL-10 affects the differentiation of dendritic cells and inhibits the production of IL-12 thus impairing the ability of dendritic cells to promote T-cell activation and Th1 differentiation [29]. Tregs express TGF-β on their surface which is increased upon in vitro stimulation with anti-CD3, in the presence of monocytes its membrane expression is increased. Blockade of Treg TGF-β expression with neutralizing antibodies [26], results in disruption of Treg suppression suggesting that TGF-β is an important mediator of Treg suppression activity. IL-35 is another inhibitory cytokine secreted by Tregs and required for their suppressive activity. It is a heterodimer of Epstein-Barr virus-induced gene 3 (EBi3) and p35 (or IL12x2). Both genes are highly expressed on mouse Foxp3\textsuperscript{+} Tregs [27,30] but not by resting and activated T-effector cells. In contrast the heterodimer cytokine IL-35 is constitutively expressed by Treg but not T-effector cells. Mouse Tregs deficient in EBi3 and p35 had significant reduced suppression activity in vitro, and fail to control homeostatic proliferation and to cure inflammatory bowel disease in vivo. In addition, ectopic expression of IL-35 confers regulatory activity on naive T cells, whereas recombinant IL-35 suppresses T-cell proliferation [27].

Cytolysis through secretion of cytotoxic factors like perforin and granzyme B is another mechanism used by Tregs to mediate their suppressive activity. Perforin is a cytotoxic protein that polymerizes in target-cell membranes to form transmembrane pores. Granzymes (A and B) is a serine protease, which activates apoptosis once in the cytoplasm of the target cell. Human adaptive Treg cells preferentially express granzyme B whereas activated human CD4\textsuperscript{+}CD25\textsuperscript{+} natural Treg cells express granzyme A but very little granzyme B. Furthermore, both Treg subtypes display perforin-dependent cytotoxicity against autologous target cells suggesting that the perforin/granzyme pathway is one of the mechanisms that Treg cells harness to control immune responses [31].

Recently, ‘metabolic disruption’ has been described as a further mechanism of T-effector cells suppression by Tregs and is thought to involve cytokine deprivation leading to T-effector cell apoptosis. There is a debate as to whether the high levels of CD25 expression in Tregs results in local consumption of IL-2 causing IL-2 deprivation of T-effector cells and their apoptosis. Pandiyan et al. showed in a mouse model of inflammatory disease that cytokine deprivation-induced apoptosis is a prominent mechanism by which Tregs inhibit T-effector cell responses [32] whereas in humans IL-2 depletion alone was not required by Tregs to suppress T-effector cells [33]. Tregs can also directly suppress T-effectors by transferring the inhibitory second messenger cyclin adenosine monophosphate (cAMP) through gap junctions to T-effector cells [34]. Finally, Tregs express ectoenzymes like CD39 and CD73 that generate pericellular adenosine, which suppressed T-effector cell function through activation of adenosine receptor 2A (A2AR) [35–37]. Adenosine binds to A2AR leading to the induction of Treg cells through inhibition of IL-6 expression while promoting TGF-β secretion [38].

Tregs can also exert their suppressive function by directly affecting dendritic cells (DC). One of the molecules involved in this mechanism is CTLA-4 which is constantly expressed by Tregs but also by CD4\textsuperscript{+} T-cells upon activation. CTLA-4, like CD28, binds to CD80/CD86 co-stimulatory molecules expressed by antigen presenting cells and sends a negative signal that causes inhibition of T-cell activation. Blockade of Treg CTLA-4 results in reduced T-effector cell suppression mediated by DC [39]. Recently, Qureshi et al. revealed the mechanism of CTLA-4 action. CTLA-4 can capture CD80 and CD86 from opposing cells by a process of trans-endocytosis. After removal, these costimulatory ligands are degraded inside CTLA-4 expressing cells, resulting in impaired costimulation via CD80 [40]. Another way that Tregs control APCs is by upregulating expression of indoleamine 2,3-dioxygenase (IDO) which catalyzes tryptophan degradation and kynurenine generation which is associated with T-cell hyporesponsiveness and apoptosis. IDO is up-regulated by proinflammatory cytokines in an attempt to modulate immune responsiveness and therefore represents a negative regulatory pathway. Additionally, CTLA-4 signaling through CD80/CD86 in APC may also up-regulate IDO [41]. IDO expression by DC results in inhibition of T-effector cell function, including proliferation and clonal expansion, and reduced survival. Moreover, IDO-expressing DC may favor the emergence of CD4\textsuperscript{+}CD25\textsuperscript{+}Foxp3\textsuperscript{+} Tregs by the expansion/conversion from naive CD4\textsuperscript{+}CD25\textsuperscript{−}Foxp3\textsuperscript{−} T cells [42,43].
It appears that Treg have multiple mechanisms at their disposal to suppress immune responses which vary according to the environmental and inflammatory context.

3. Tregs in systemic lupus erythematosus

SLE is a systemic autoimmune disease targeting multiple organs including the skin, joints, kidneys, and central nervous system. SLE is characterized by abnormalities in both the B- and T-cell compartments associated with loss of tolerance followed by activation and expansion of autoreactive lymphocytes, the production of inflammatory cytokines and abundant production of a wide array of potentially pathogenic autoantibodies. In SLE, autoantibodies are directed against intranuclear nucleic acids, proteins and nucleoprotein complexes. Thus autoantibodies in conjunction with loss of tolerance play an important role in the pathogenesis and clinical manifestation of disease. In both murine and human SLE, the mechanisms of central tolerance appears to be unaffected implying that there might be a breakdown in the peripheral tolerance. Given that Tregs are involved in controlling peripheral tolerance even subtle defects could contribute to the development of disease. The uncontrolled activation of B- and T-cells and pathogenesis of SLE may be partly attributed to defects in Tregs.

3.1. Tregs in murine models of SLE

The role of Tregs in lupus-prone mice has been extensively studied. The first evidence that Tregs have protective effects came from Treg depletion studies. Hayashi et al. showed that the administration of anti-mouse CD25+ T-cell monoclonal antibody (PC61.5) in autoimmune-prone female NZB x NZW F1 (B/WF1) mice, 3 days after birth, induced the development of nephritis with an increase in IgG2a antinuclear antibody, elevated IL-6 and IFN-γ but decreased TGF-β production, [44]. New Zealand Mixed 2328 (NZM2328) mice spontaneously develop lupus-like disease with high circulating levels of autoantibodies to double stranded DNA and fatal glomerulonephritis. Three-day thymectomy, resulted in an accelerated double stranded DNA autoantibody response and early onset of severe proliferative glomerulonephritis with extensive mesangial immune complexes. Transfer of CD25+ T-cells from 6-week old asymptomatic donors effectively suppressed autoimmune body production and the development of autoimmune diseases, with the exception of proliferative lupus nephritis and sialoadenitis [45]. Scalapino et al. nephritis showed that in (New Zealand Black x New Zealand White) F1 (B/W) lupus-prone mice, adoptive transfer of purified and ex vivo expanded (with IL-2 and TGF-β) CD4+CD25+CD62L+ thymic-derived Tregs reduced the incidence of renal disease, or slowed the progression of renal disease when administered after development of proteinuria [46].

In many of these murine lupus-prone mice strains, such as (New Zealand Black x New Zealand White) F1 (BW/1) and (SWR Xx New Zealand Black) F1 (SNF1) that spontaneously develop lupus-like disease the number CD4+CD25+ Tregs is significantly lower compared to other non-autoimmune mouse strains [46–48]. In addition, NZM2410 mice that are congenic for the Sle1 locus have reduced numbers of Foxp3+ Tregs which correlates with autoantibody production and lupus like features [49]. Some of the data on Treg function in lupus murine models are conflicting. Lupus prone mouse strains have hyperactive B- [50] and T-cells [51] with a lower threshold of activation. Several groups have found that Tregs isolated from lupus-prone mice and cultured in vitro retained their suppressive activities [46,48] whereas other groups have reported the opposite [52,53]. In addition, T cells from MLR/lpr mice appear resistant to Treg suppression [52]. Tregs are therefore not able to control T-cell activation and proinflammatory cytokine production leading to chronic inflammation. MLR/lpr mice also have an altered Treg phenotype (CD62L+CD69*) with a profound reduction in Dicer expression and an altered microRNA profile compared to non-autoimmune strains of mice [53]. Overall, these observations suggest that both the quantity and quality of Tregs are important in the development of lupus-like disease.

These findings also illustrate the important concept that in diseases such as SLE, Treg function needs to keep pace with autoreactive T cells causing inflammation and autoimmunity. Thus if therapies are directed at Treg, restoration of Treg function to healthy levels may not be sufficient. In order to regain control of ongoing inflammation, Treg with increased potency are required. The murine model of chronic graft versus host (cGVHD) disease is characterized by persistent lymphoid hyperplasia and SLE-like disease with splenomegaly, B-cell expansion, autoantibodies and severe immune complex glomerulonephritis. Zhang et al. showed that administration of short-term low-dose anti-CD3 antibody treatment induced significant remission of proteinuria, production of autoantibodies and renal immune complex deposition in lupus nephritic mice. These improvements are associated with up-regulation of renal Foxp3 and IL-10 mRNA expression compared to those treated with control IgG suggesting that anti-CD3 therapy may induce IL-10 secreting Foxp3+ Tregs that eventually suppress T-effector function and ameliorate lupus nephritis [54].

3.2. Tregs in human SLE

Considerable efforts have been made to delineate the role of Tregs in the development of SLE in patients. Most of the initial studies have focused on phenotypic characterization of circulating Tregs in SLE patients and comparing them with healthy individuals as well as other patients with autoimmune diseases. Unfortunately, due to limitation in Treg specific markers, this type of investigation has been challenging to interpret and the results obtained by different investigators are contradictory. Early reports revealed that in patients with SLE the number of circulating CD4+CD25+ Tregs were decreased [55–58]. These studies were open to doubt since identification of Tregs relied on CD25 which can be expressed by activated CD4+ T-cells. In more recent studies, investigators analyzed Tregs as CD4+CD25high and still found a decrease percentage in SLE patients [59–62]. A number of groups have described that CD4+CD25+ inversely correlate with disease activity. Active SLE patients also appear to have decreased levels of CD4+CD25high Tregs compared to inactive SLE patients and healthy individuals [60]. In addition, different types of treatments used on SLE patients such as corticosteroid therapy [63], therapeutic plasmapheresis [64] and B-cell depletion with Rituximab [65,66] increase the circulating percentage of CD4+CD25high Tregs. In contrast to most studies, three different groups reported similar levels of circulating CD4+CD25high Tregs in SLE patients and healthy individuals [67–69].

The phenotypic characterization of circulating Tregs in SLE patients has also been analyzed based on expression of intracellular Foxp3 transcription factor. The results from these studies did little to resolve the contradictory results from studies based on CD25 expression. Three reports have shown a decrease of CD4+Foxp3+ Tregs in SLE patients compared to healthy individuals [61,70,71] and another three revealed normal levels of CD4+CD25Foxp3+ Tregs [67,68,72]. Studies that showed a decrease in CD4+Foxp3+ Tregs in SLE patients found an inverse correlation with disease activity [61,70] or showed no correlation [71]. There are also reports showing an increase of circulating CD4+CD25Foxp3+ Tregs that correlates with disease activity.
[59,73–76]. Recently, Bonelli et al. showed that SLE patients have increased numbers of CD4+CD25+Foxp3+ Tregs rather than activated T-cells. In vitro functional analysis on these cells revealed that they are able to suppress T-cell proliferation but not IFN-γ production [77]. Zhao et al. reported that the proportion of blood CD4+CD25+CD127dim T-cells of the SLE patients was not significantly different from that of the healthy individuals. However, the proportions of CD4+CD25+FOX3+ T-cells and CD4+CD25high T-cells of SLE patients were significantly lower than those of the healthy individuals [72].

Trying to quantify circulating Tregs in patients with SLE may be influenced by the deposition of these cells in lymph nodes and inflamed tissues. Unfortunately, there is limited information about Tregs infiltration and deposition in lymph nodes and inflamed organs such as the skin and kidney in SLE patients. Miyara et al. reported a decreased number of Foxp3+ Tregs in lymph nodes from patients with SLE and reduced levels of Foxp3 miRNA isolated from SLE patients with active nephritis [60]. Another study done in patients with cutaneous SLE, also found a decreased number of Foxp3+ Tregs in skin specimens obtained despite the fact that these patients had a normal number of circulating Foxp3+ Tregs [69].

Apart from the quantitative analysis of Tregs in SLE patients, several studies were also focused on the qualitative and functional analysis of Tregs in SLE patients. These studies also show diverse results. Several groups have reported abnormalities in the suppressive function of CD4+CD25high Tregs [59,61,62,67,70,71,74,78], whereas other groups did not confirm such alteration [57,75,79–81]. In most studies, the suppressive defects of SLE patients were attributed to Tregs, whereas others have indicated an increased resistance to suppression by T-responder cells. Venigalla et al. showed that CD4+CD25– T-responder cells isolated from patients with active SLE were significantly less sensitive than those from patients with inactive SLE to the suppressive function of autologous or normal donor CD4+CD25+CD127dim T-cells. Furthermore, a significant inverse correlation was observed between regulatory T cell suppressor function and the level of lupus disease activity [67]. Consistent with this finding, other groups have reported impaired Treg function when cultured with autologous T-effector cells but not when T-effector cells come from healthy individuals suggesting a possible resistance of lupus T effector cells to Treg suppression [62,67,78].

Despite the plethora of studies focused on Tregs and SLE, their role in disease initiation and development remains elusive. A number of reasons are likely to account for this lack of clarity. The markers that define Treg such as CD25 and Foxp3 can also be influenced at least transiently upon T-cell activation [1,14,82,83]. One of the basic features of SLE is persistent T cell activation. For this reason, studies based on phenotypic characterization of circulating Tregs must be regarded with caution. In addition, SLE is a disease with a very heterogeneous patient population and their pathogenesis is unlikely to be uniform. Disease activity and different therapies could also alter Treg numbers or function. For example active disease was positively correlated with decreased numbers and suppressive function of Tregs whereas patients with inactive disease showed no difference to healthy individuals [60]. Additionally, patients treated with corticosteroid therapy [63], therapeutic plasmapheresis [64] and B-cell depletion with Rituximab [65,66] have increased numbers of Tregs in their circulation. Discrepancies in findings related to Treg function could be due to different methods used to isolate Tregs from blood (e.g. Treg isolation with magnetic beads versus FACS sorting), the different cell markers used to isolate Tregs, the different in vitro stimuli used to activate T-effector cells as well as the presence or absence of accessory cells (e.g. monocytes) in the in vitro assays.

### 4. Tregs in rheumatoid arthritis

Rheumatoid arthritis (RA) is characterized by swelling of the synovium and damage of the cartilage around the joints leading finally to the joint destruction. Several factors play a role in disease development. The role of TNF-α and IL-6 as key mediators of RA has been proven and blockade of these cytokines has been successfully introduced as therapies in RA patients. The role of Tregs has been demonstrated in both murine models of autoimmune arthritis and patients with RA. On the whole, Treg function has been reported to be impaired whereas the number of circulating Tregs varies depending on the study. Recently investigations into Tregs focus on the balance between Tregs and other proinflammatory T-cell populations such as Th17 cells [85].

#### 4.1. Tregs in murine models of rheumatoid arthritis

Collagen-induced arthritis (CIA) is the most common murine model used to investigate the role of Tregs in disease and is induced after immunization of mice with bovine type II collagen emulsified in complete Freund’s adjuvant [86,87]. Morgan et al. targeted CD4+CD25+ Tregs in these arthritic mice by administration of a monoclonal antibody specific for CD25. As a result, these anti-CD25 treated mice had worse disease than controls. Disease exacerbation was accompanied by higher antibody titers against collagen, and in vitro tests showed increased proliferation of collagen-specific T cells. When CD4+CD25+ Tregs were administered back into CD25+ depleted mice, at the time point of immunization, disease severity was reduced [88]. Tregs slowed disease progression although there was no difference in T- and B-cell responses. Adoptively transferred CD4+CD25+ Tregs were located in the synovial tissue of affected joints soon after transfer indicating that regulation may occur locally in the joint [89]. Transfer of Foxp3-transduced CD4+ T-cells also ameliorated CIA although the time point of transfer is critical for modulating disease severity. CD4+Foxp3+ Tregs were able to reduce the severity of CIA when transferred prior to collagen immunization. When transferred 20 days after collagen immunization, a higher number of CD4+Foxp3+ Tregs was needed suggesting increased resistance to suppression whereas if transferred even later after the booster immunization they had no effect on disease modulation. Apart from the time point of Treg transfer, the route of administration is also important. Tregs administered systemically rather than directly into the inflamed joint were more effective in modifying disease [90].

Antigen-induced arthritis (AIA) is another model of murine rheumatoid arthritis and is induced by pre-immunization of mice with methylated bovine serum albumin in complete Freund’s adjuvant followed 21 days later by an intra-articular injection of the inducing antigen. Transfer of CD4+CD25+ cells reduced the presence of CD4+Foxp3+ Tregs in these arthritic mice by administration of a monoclonal antibody specific for CD25. As a result, these anti-CD25 treated mice had worse disease than controls. Disease exacerbation was accompanied by higher antibody titers against collagen, and in vitro tests showed increased proliferation of collagen-specific T cells. When CD4+CD25+ Tregs were administered back into CD25+ depleted mice, at the time point of immunization, disease severity was reduced [88]. Tregs slowed disease progression although there was no difference in T- and B-cell responses. Adoptively transferred CD4+CD25+ Tregs were located in the synovial tissue of affected joints soon after transfer indicating that regulation may occur locally in the joint [89]. Transfer of Foxp3-transduced CD4+ T-cells also ameliorated CIA although the time point of transfer is critical for modulating disease severity. CD4+Foxp3+ Tregs were able to reduce the severity of CIA when transferred prior to collagen immunization. When transferred 20 days after collagen immunization, a higher number of CD4+Foxp3+ Tregs was needed suggesting increased resistance to suppression whereas if transferred even later after the booster immunization they had no effect on disease modulation. Apart from the time point of Treg transfer, the route of administration is also important. Tregs administered systemically rather than directly into the inflamed joint were more effective in modifying disease [90].
Manipulation of Tregs either directly or indirectly has also been proven successful in treating arthritis in murine models of disease. Wright et al. showed that adoptive transfer of antigen specific Tregs (generated by retroviral T-cell receptor gene transfer into purified CD4+CD25+ Tregs) in mice with AIA resulted in reduction of Th17 cells and a significant decrease in arthritic bone destruction [93]. Recently, an indirect way of targeting Tregs in the AIA model of arthritis by using tolerogenic DC loaded with antigen has been described. Administration of DC transfected with an ERK activator and OVA in mice with AIA, maintains DC in an immature state. As a result, DC present antigen at suboptimal levels, leading to inhibition of CD8 T-cell expansion and secretion of TGF-β that directs antigen specific Treg differentiation and proliferation rather than expansion of CD4+ T-effector cells. As a result arthritis is significantly inhibited through antigen specific Tregs [94]. Finally, administration of anti-CD3 monoclonal antibody in the CIA murine model of arthritis has proven to be effective in treating disease severity through expansion of naturally occurring CD4+CD25+Foxp3+ Tregs and the generation of CD8+CD25+Foxp3+ Tregs [95].

4.2. Tregs in human rheumatoid arthritis

As with SLE, many human RA studies focused on quantifying Treg numbers in the peripheral blood, but also at the site of inflammation. In RA patients the number of Tregs present in synovial fluid is higher than that in the peripheral blood. Tregs accumulated in inflamed joints express high levels of surface and intracellular CTLA-4, GTR, OX-40, and Foxp3 [96]. Data regarding the number of Tregs in the circulation of RA patients compared to healthy individuals are inconclusive and contradictory. Some studies showed a decrease of circulating Treg in RA patients [97–99] whereas others showed no difference in circulating Treg numbers compared to healthy individuals [96,100–102] and some indicated an increase in circulating Treg numbers [103,104].

Studying Treg function in patients with RA has been informative. Although Tregs present in the synovial fluid of patients with RA have an enhanced capacity to suppress both T-cell proliferation and cytokine production (TNF-α and IFN-γ) disease is still able to progress. T-responder cells present in the synovial fluid were less susceptible to suppression compared with circulating T-responder cells [103]. This data is consistent with the observation that strongly activated CD4+ T-cells are resistant to Treg suppression [105]. Our group has reported that CD4+CD25+ circulating Tregs are able to suppress proliferation of T-effectors but were unable to suppress proinflammatory cytokine secretion from activated T-cells and monocytes [102]. This deficiency of RA Tregs is associated with defective expression and function of CTLA-4, a key molecule linked to their suppressive function. Expression of CTLA-4 is reduced in RA Tregs compared to healthy Tregs which correlates with reduced function. Artificial induction of CTLA-4 expression on RA Treg in vitro restored their suppressive capacity. Furthermore, CTLA-4 blockade impaired healthy Treg suppression of T-cell IFN-γ production, but not T-cell proliferation. These data suggest that Tregs control T-cell proliferation and cytokine production through different mechanisms [106]. Furthermore, CTLA-4 gene polymorphisms have also been correlated with autoimmune diseases like RA [107] and SLE [108].

In RA, Tregs especially those present in the synovial fluid are also influenced by the cytokine profile. TNF-α, IL-6, IL-15, and IL-1 present in the inflamed joint act to increase the number of infiltrating Tregs in the inflamed joint but at the same time impair their function. For example IL-6 secreted by DC after TLR stimulation induces T-responder cell resistance to Treg mediated suppression [109]. TNF-α and IL-7 secreted by activated monocytes in the inflamed joint have a direct effect on CD4+CD25+ Tregs by abrogating their suppressive activity [110]. In vitro addition of TNF-α at high concentrations in Treg suppression assays inhibit the suppressor function of Tregs by down regulating Foxp3 expression [111].

Different types of RA treatment can also affect the function of Tregs. Our group has demonstrated that after the resolution of inflammation by administration of anti-TNF therapy (anti-TNF antibody infliximab) the Treg function that was originally impaired appeared to be restored [102]. Patients treated with infliximab had two different populations of Tregs. Natural Tregs characterized by CD62L Ligand expression (CD62L+) and induced Tregs that were CD62L+. In patients who responded to anti-TNF therapy, the CD62L+ Tregs were still unable to suppress TNF-α and IFN-γ production by T-responder cells whereas induced CD62L+ Tregs from the same patients suppressed TNF-α and IFN-γ production by T-responder cells through the production of IL-10 and TGF-β rather than CTLA-4 [112]. Although anti-TNF treatment works for most patients, after withdrawal of treatment relapse usually ensues raising the possibility that to induce long lasting remission natural Tregs need to be functionally restored. Some of these findings have recently been confirmed using a mouse model of arthritis. Regulatory T cells with a reduced expression of CD62L are increased in a TNF driven arthritic mouse model treated with anti-TNF [113].

Treg stability in inflammatory conditions like RA and their relationship with inflammatory Th17 cells has been studied in the last few years. In an inflammatory condition like RA, it is quite possible that Tregs in the presence of the different proinflammatory cytokines will become unstable and convert to pathogenic T-cells. Treg secreting Th17 have been described though their function is not necessarily impaired. Ayyoub et al. showed that circulating memory Tregs secrete IL-17 ex vivo [114]. In addition, memory Tregs when cultured in vitro with IL-10 and IL-6 secrete IL-17 whereas IL-17 secretion was prevented in the presence of TGF-β suggesting that inflammatory conditions impair Tregs cell function and promote IL-17 production [115]. The mechanisms affecting Treg stability under inflammatory conditions are linked to regulation and epigenetic modification of the Foxp3 gene. Complete demethylation of CpG residues in the proximal promoter of Foxp3 gene is required for stable Foxp3 expression. IL-6 and possibly other proinflammatory cytokines like TNF-α and IL-1β promote demethylation of CpG residues in the proximal promoter of Foxp3 gene resulting in downregulation of Foxp3 expression by Tregs [116,117].

5. Conclusion

Tregs play a pivotal role in controlling autoimmune responses and inflammation. Subtle changes in Treg number, function or phenotype could lead to the development of autoimmune diseases such as SLE and RA, but also may be a consequence of the inflammatory environment (Fig. 1). In comparison to data from murine models of disease, clear conclusions as to the nature of Treg defects in patients with SLE and RA have yet to be realized due to the many contradictory reports. The discovery of new specific Treg markers will help to resolve these issues but disease heterogeneity and the number of therapies available also contributes to some of the conflicting findings. Accurate patient classification and analysis of subgroups of patients will be important in future studies. In addition, the importance of Treg at different phases of disease may vary. Evidence from both diseases suggest that the potency of T-effectors and the suppressive effects of Treg need to be carefully balanced. Further research is needed in human diseases to determine how to increase the suppressive power of Tregs, perhaps only for a short period, in order to restore immunological tolerance.
References


Beriou, G. et al. (2009) IL-17-producing human peripheral regulatory T cells retain suppressive function. Blood 113 (18), 4240–4249.
