Immunotherapy in Rheumatoid Arthritis: Prospects for the Restoration of Tolerance

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Abstract
Rheumatoid arthritis (RA) is an autoimmune disease that can have significant effects on the health and wellbeing of patients and generates a heavy burden for healthcare providers. Severe disability associated with RA has been reduced by the development and use of biological therapies including anti-TNF-α and anti-IL-6R. Here we will review how immune modulating agents currently available for the treatment of RA can effect regulation of inflammation. Furthermore, we discuss how inflammation is regulated by specialized regulatory T cell subsets (Treg) and how defects in regulation have led to the concept that boosting Treg numbers or function in RA may lead to long lasting disease remission. We proceed to describe how immunotherapy may contribute to the modulation of Treg and how cellular therapies such as antigen-specific Treg and tolerogenic dendritic cells may provide a mechanism to re-establish antigen-specific suppression of inflammation in RA.

Keywords: Biologics; Rheumatoid arthritis; Anti-TNF; Regulatory T cells

Introduction
Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease affecting approximately 1% of the population [1]. Nevertheless, in the early 1990s RA was estimated to cost £1.256 billion per year in England, with 52% of this sum as a result of work-related production loss through disability [2]. Initial treatment for RA consists of disease modifying anti-rheumatic drugs (DMARDs) such as methotrexate, sulfasalazine, hydroxychloroquine, prednisolone or leflunomide. Aggressive treatment with a combination of DMARDs soon after diagnosis has been shown to be highly beneficial for the control of inflammation and bone erosion in RA [3-6]. In recent years the treatment of RA has been revolutionized by the advent of biologic therapies that target known inflammatory pathways. In the UK, patients with an inadequate response to DMARDs can be prescribed agents which inhibit TNF-α and IL-6, deplete B cells and block T cell co-stimulation. Not only have these therapies been highly efficacious in the treatment of RA, but each in turn has offered insight into distinct pathways involved in the pathogenesis of disease. Moreover, a number of these therapies have been shown to reset the immune system, permitting the restoration or induction of tolerance by ameliorating regulatory cell number or function. In the future, this capacity to modulate tolerance to self tissue will become more targeted with a focus on cellular therapies and fine tuned modulation of intracellular signaling pathways. This review will discuss the current research on the capacity of biological therapies currently used in RA to modulate tolerance to self-tissue and biological and cellular therapies under consideration for use in the future.

Targeting Inflammation through Induction of Tolerance

T cells are selected on a gradient of reactivity and so some cells that enter the circulation will be more strongly self-reactive than others. Moreover, not all autologous proteins can be expressed in the thymus. Thus, there must be mechanisms in place to suppress immune response to self-antigens in the peripheral tissues. These include anatomical sequestration, the induction of anergy and the activity of regulatory T cells (Treg) [7].

Anatomical sequestration: The circulation of naive T cells, mediated by the chemokine receptor CCR7 and its ligands, ensure that cells that have not encountered antigen never enter the non-lymphoid peripheral tissues. This reduces the possibility of self-antigen being presented to T cells.

Anergy: In contrast to their naive counterparts, T cells that have encountered antigen can migrate into the tissues. However, the transfer of T cells with a TCR specific for the protein ovalbumin (OVA) to mice with expression of OVA on peripheral tissues does not result in autoimmunity. Only upon secondary damage to the tissue could a robust OVA-mediated pathology be triggered [8]. Thus, the presence of auto-reactive T cells in the tissue is not sufficient to drive autoimmunity.

T cell activation in the periphery requires interaction with antigen presenting cells (APC) expressing an antigenic peptide for which the T cell is specific. Additionally, T cells require a co-stimulatory signal to become activated. The best-characterized co-stimulatory interaction is that of CD28 on T cells and B7 family members (in particular B7-1/CD80 and B7-2/CD86) expressed on APC. CD28 lowers the threshold required for activation of T cells via the TCR [9]. Mice which are deficient in CD28 have impaired T cell response to antigen with a reduced activation of helper T cells and reduced antibody class switching [10]. Thus, binding of B7 family members to CD28 in combination with a TCR signal leads to an increase in the transcription and production of interleukin (IL)-2 to promote T cell proliferation [11].

If a T cell recognizes an antigen but does not receive this second co-stimulatory signal it becomes functionally unresponsive, or anergized [12]. In the absence of inflammation APC do not up-
Regulatory T cells are key mediators of peripheral immune tolerance, essential for maintaining immune homeostasis and controlling the magnitude of the effector response. Two main subsets of Treg exist, natural and induced or adaptive. Constituting 5-10% of the CD4+ T cell population in peripheral blood and characterized by their expression of the transcription factor Foxp3 and high levels of CD25, natural Treg are essential for maintaining immune tolerance. Mutations in the Foxp3 gene in both mouse and human results in the development of severe autoimmunity, demonstrating the importance of Foxp3 in Treg function [14,15].

Whilst natural Treg are selected in the thymus, and express genes such as Itkz2 (Helios) and Nrp1 (Neuropilin-1) [16,17], induced Treg, that can develop from CD4+ T cells or CD8+ T cells, develop outside the thymus under a variety of conditions and have been reported to express Dap11 and Igbp4 [18].

As nTreg are thought to be crucial for maintenance of tolerance, why is it that we need iTreg at all? Two recent studies have tried to address the relative importance of both nTreg and iTreg in suppression of chronic inflammation. In the first, a lymphopenic model of inflammatory bowel disease was used to determine the capacity of iTreg to suppress autoimmune inflammation. Transfer of Tconv cells (CD4+ Foxp3 CD45 RB+ into Rag1- mice resulted in the development of colitis but also the induction of Foxp3 expressing CD4+ iTreg. However if Tconv cells were transferred from mice that could not generate iTreg, accelerated disease was observed. Further experiments where nTreg or iTreg or combinations of both were transferred into mice with colitis, revealed that recovery from disease was dependent upon the presence of both nTreg and iTreg [19]. In the second study, it was shown that rescue of Foxp3 deficient mice from autoimmune lymphoproliferative disease was only obtained by the co-transfer of nTreg and Tconv (a proportion of which became iTreg). Transfer of nTreg allowed Foxp3 deficient mice to survive, but this was not sufficient to maintain tolerance as iTreg were required to control pathology at mucosal surfaces [20].

We have also reported that iTreg have functional differences to nTreg. Natural Treg are unable to suppress production of IL-17, yet both CD4+ iTreg and CD8+ iTreg can suppress IL-17 [21,22].

The effects of inflammation on Treg function

A number of cytokines present during inflammation can subvert the function of nTreg. IL-6 was the first cytokine shown to confer resistance upon target cells to the effects of nTreg mediated suppression [23]. Moreover, IL-6 prevented efficient regulation of effector responses in a mouse model of inflammation, despite increased numbers of Treg [24]. In humans however, IL-6 has not been identified as a cytokine that influences nTreg function, although other cytokines such as IL-7 and TNF-α may inhibit the suppressive function of nTreg [25]. IL-6 has also been described to prevent the conversion of conventional CD4+ T cells into iTreg [26], whilst promoting the development of Th17 cells [27]. IL-6 does have opposing effect on CD8+ T cells though, promoting the induction of Foxp3 expression and the development of iTreg [27].

As mentioned earlier, TNF-α is also thought to influence nTreg function. Addition of TNF-α to in vitro suppression assays inhibited nTreg suppressor function by down regulating the expression of Foxp3 [28]. In addition, patients with RA have dysfunctional nTreg with an inability to suppress pro-inflammatory cytokine production, yet in anti-TNF-α treated patients functional CD4+ iTreg that can suppress inflammatory cytokine production have been identified [21,29]. These data suggest that TNF-α may inhibit nTreg function or inhibit iTreg induction in RA during chronic inflammation. However, not all studies agree that TNF-α is detrimental to nTreg, with one report showing that TNF-α actually promotes expansion and function of nTreg due to their high expression of TNFR2 [30]. A further study in diabetic mice confirmed that Tconv cells boost the activation and function of nTreg in vivo via their production of TNF-α [31].

These findings could suggest that there is a balance between the pro-inflammatory actions of cytokines such as IL-6 and TNF-α and their potential anti-inflammatory effects. Under certain conditions during chronic inflammation pro-inflammatory cytokines may enhance nTreg function or induce new functional Treg to limit the collateral damage caused by an excessive immune responses.

Regulatory T cells in RA

Regulatory T cells have been shown to be defective at suppressing cytokine production in RA [21,32,33]. The proportion of CD4+ Treg in the peripheral blood of patients with RA appears to be normal, however functionally they are unable to regulate IFN-γ and TNF-α production and display an inability to suppress the highly arthritogenic cytokine, IL-17 [21,32]. It has been shown however that increased numbers of nTreg are present in the synovial fluid from RA patients and that inflammatory cytokines found in the joints of patients with RA may not alter nTreg function per se but influence the ability of activated Tconv to become suppressed by Treg [34]. The restoration of Treg function using biologic therapy or the introduction of new functional Treg in patients is an attractive strategy for treating disease.

The Use of Biologics to Induce Functional Treg

Anti-TNF-α in RA

Inflammation is known to negatively modulate Treg function. Given the abundance of TNF-α in the synovium of patients with RA it was proposed that the response to anti-TNF-α therapy might be mediated, in part, via a reversal of the Treg defect observed in these patients. Indeed, Treg from RA patients treated with infliximab were shown to suppress IFN-γ and TNF-α at comparable levels to healthy controls. Moreover, it was found that these patients had an increase in the number of peripheral Treg [32]. This heavily implicated TNF-α in the modulation of Treg number and function.

The restoration of suppressive function observed in patients responding to infliximab therapy led to an investigation of the phenotypic and functional characteristics of the Treg from these patients. Ex vivo staining revealed that patients treated with infliximab had increased levels of CD62L. Treg compared to healthy controls and patients with active RA [29]. Comparison of the suppressive function...
of CD62L- and CD62L+ Treg showed unsurprisingly, that both were defective in patients with RA. However, whilst CD62L- Treg were the most potent suppressors in healthy controls, in infliximab treated patients there was a distinct shift. The suppressive ability of CD62L- Treg in infliximab treated patients resembled that of patients with active RA but CD62L+ Treg were much more potent suppressors than the same population in healthy controls or patients with active RA. Moreover, in contrast to functional Treg in healthy controls, the neutralisation of TGF-β and IL-10 abrogated the suppressive ability of CD62L- Treg. Furthermore, it was shown that the in vitro addition of infliximab to purified CD25+ cells from patients with RA, but not healthy controls, induced a population of FOXP3+ cells. These cells were shown to be CD62L- and examination of their function in vitro found that they could suppress IFN-γ. Thus, it was concluded that infliximab induced Treg with the capacity to suppress IFN-γ rather than restoring the suppressive ability of existing Treg [29].

A more recent study has demonstrated differences in the capacity of anti-TNF therapeutics to induce Treg. Based on the encouraging data from animal models, clinical trials based on the induction of Treg in rheumatoid arthritis could suppress the severity of disease, the use of cellular therapies in RA has been an attractive option for re-establishing tolerance. Several strategies are under consideration for the development of cellular therapies, each with their own advantages and possible disadvantages.

Anti-CD3 monoclonal antibodies (mAb)

Anti-CD3 mAb was first linked to the induction of tolerance in 1981, when Cosimi et al. demonstrated that it effectively reversed acute organ rejection [35]. Further studies confirmed that anti-CD3 mAb induced permanent tolerance in rats with histocompatibility mismatched heart grafts, yet skin allografts from a third-party rat were rejected [36].

Similar studies using the NOD mouse as a model for type I diabetes, revealed that anti-CD3 mAb could also have beneficial effects in autoimmune diseases. A five day treatment of diabetic mice with anti-CD3 mAb resulted in permanent disease remission, and this was associated with the induction of CD4+ Treg that produced TGF-β [37-39]. Although anti-CD3 mAb treatment has effects on regulatory T cell populations, it is thought that multiple mechanisms contribute to resetting the immune response allowing for the induction of tolerance [40].

Based on the encouraging data from animal models, clinical trials were initiated using humanized Fc-engineered monoclonal antibodies teplizumab and otelixizumab [41,42]. Results have been encouraging as patients treated with short courses of otelixizumab have shown preservation of β-cell function and reduced the requirement for insulin for up to 18 months post treatment [42]. One study is of particular interest as it correlated disease remission with the appearance of CD8+CD25+ Treg in the peripheral blood of type 1 diabetes patients treated with teplizumab, suggesting that Treg induction can occur in humans, not just mouse models [43]. More recently, the development of mice expressing human CD3 has allowed for further study of the in vivo effects of teplizumab or otelixizumab in mice with autoimmunity [44,45].

Anti-CD3 mAb in RA

The presence of CD4+ and CD8+ T cells in the synovial fluid of patients with inflammatory arthritis has made them potential targets for therapy. The use of non-mitogenic anti-CD3 monoclonal antibodies have been proposed as biologics with the ability to re-program the immune response and inhibit inflammation [46]. Indeed, a clinical trial involving the use of anti-CD3 mAb in psoriatic arthritis for an 8-10 day period resulted in the reduction of swollen joints in these patients [47].

Collagen-induced arthritis has been used to investigate the potential therapeutic benefits of anti-CD3 mAb in inflammatory arthritis. Our own data has shown that a single dose of anti-CD3 mAb can abrogate CIA in mice. Suppression of disease was accompanied by the induction of Foxp3 expression by CD8+ T cells and an increase in the proportion of CD4+Foxp3+ Treg. The naturally occurring CD4+ Foxp3+ Treg from mice with CIA were unable to suppress Th17 responses in vitro, and this was unaltered in response to anti-CD3 mAb therapy. In contrast however, the induced CD8+Foxp3+ iTreg could suppress both collagen-specific Th1 and Th17 responses in vitro [22].

One of the side effects associated with the use of intra-venous anti-CD3 mAb is the cytokine release observed after the first infusions. Whilst modulation of the Fc portion of anti-CD3 mAb have to some extent reduced the severity of the cytokine release, the use of an alternative route of administration may inhibit cytokine release completely. For example, nasal administration of anti-CD3 mAb has been shown to be sufficient to suppress CIA, with disease remission associated with the induced LAP+ CD4+ Treg and increased IL-10 production [48]. Anti-CD3 mAb appear to be a promising therapy for RA, with the potential to re-program the immune system and re-establish regulatory T cell control. However, the side effects of cytokine release must be carefully controlled, either through an alternative route of administration or via combination with other therapies such as anti-TNF-α [49]. It also remains to be determined whether a short dose of anti-CD3 mAb can induce tolerance via the induction of functional Treg in patients with inflammatory arthritis. It is important to understand whether anti-CD3 mAb will induce a systemic immune suppression or antigen-specific regulation as patients must not be put at risk of developing cancer or being more susceptible to infection.

Cellular therapies

Since the demonstration that the transfer of nTreg into mice with arthritis could suppress the severity of disease, the use of cellular therapies in RA has been an attractive option for re-establishing tolerance. Several strategies are under consideration for the development of cellular therapies, each with their own advantages and possible disadvantages.

In vitro generated iTreg

The ability to generate iTreg in vitro by culturing CD4+Foxp3- T cells in the presence of TGF-β provides a mechanism by which large numbers of functional iTreg could be generated for adoptive transfer into patients with RA [50]. One recent study in mice, comparing the ability of nTreg and TGF-β-induced iTreg to suppress CIA, reported that nTreg were easily converted to Th17 cells upon transfer into arthritic mice, whereas the iTreg sustained Foxp3 expression and remained stable [51]. However, TGF-β induced Treg are thought to be unstable [52,53] and pose a risk of converting into pathogenic T
In humans, there have been studies showing that T cells induced to express Foxp3 by culture with TGF-β lack suppressive capacity and may require additional signals to convert them into functional iTreg. The addition of IL-2 and retinoic acid to cultures of TGF-β stimulated CD4+ T cells can induce stable, suppressive Foxp3+ iTreg [54,55]. Before this method is translated into patients with chronic inflammatory disease it will be important to determine whether these cells remain stable in vivo during inflammation and whether they can regulate in an antigen-specific manner.

In vitro expansion of antigen-specific nTreg

Animal models have provided some promising data for the use of antigen-specific nTreg in suppressing transplant rejection and graft versus host disease (GVHD) [56,57]. In patients, expansion of pre-existing Treg Ex vivo may be helpful in regulating ongoing inflammation, however, there are several problems associated with this method. Firstly, it is important to obtain a relatively pure population of Treg as Treg proliferate poorly in vitro and will soon be outgrown by T cells in prolonged cultures. Live cells cannot be isolated based on Foxp3 expression and therefore high expression of CD25 is generally used as a marker for Treg isolation. This is a problem in autoimmunity where Tconv may transiently upregulate CD25 and Foxp3 upon activation [58]. Currently the best marker available to differentiate between Treg and Tconv with high expression of CD25 is CD127, the IL-7 receptor α-chain, which is expressed at low levels on Treg [59]. Secondly, obtaining large numbers of pure Treg from patients for this approach may also be problematic. Although there are several technical issues that need to be overcome before this method is put into practice, it offers a standardized protocol in which patients could be treated with their own antigen-specific Treg.

Generation of antigen-specific nTreg via TCR gene transfer

Modulation of Treg TCR via gene transfer is an attractive option for boosting the antigen-specificity of Treg in autoimmunity. Genetic manipulation of the TCR expressed by T cells has been explored in cancer in order to boost cytotoxic cell activity and clearance of tumour cells [60]. Lentiviral TCR gene transfer has been used to re-direct human Treg, making them capable of recognizing the melanoma antigen tyrosinase. This resulted in the generation of antigen-specific human Treg that were capable of regulating T cell tumor responses in vivo [61].

In RA, the auto-antigen responsible for chronic stimulation of the immune system is unknown and may be different between individuals, therefore developing an antigen-specific TCR is more challenging. We do know however that Treg, once activated by its cognate antigen can regulate T cell responses within the immediate vicinity, regardless of specificity. This phenomenon has been utilized to develop a system whereby designer Treg specific for a “bystander” antigen can suppress inflammatory arthritis caused by a separate antigen [62]. Mice induced to develop a methylated BSA (mBSA) specific inflammatory arthritis were either injected intra-articularly with mBSA or mBSA and ovalbumin (OVA). CD4+Foxp3+ nTreg or CD4+ T cells transfused with Foxp3 were re-directed via the transduction of an OVA-specific TCR, and when adaptively transferred into arthritic mice, could regulate disease in the knees containing mBSA and OVA but not mBSA alone [62]. This linked suppression was dependent upon the antigen targeted by the designer Treg being present in the inflamed joint. This data offers a tempting glimpse at a future where Treg can be targeted to suppress in an antigen dependent manner without knowing the disease-initiating antigen. Indeed, one target of this therapy upon translation into patients with RA may be citrullinated proteins. These proteins make good candidates not least because approximately 80% of RA patients present with anti-citrullinated protein antibodies (ACPA) [63]. Citrullination is the post-translational conversion of an arginine residue to a citrulline. This post-translational conversion, usually under extreme intracellular conditions makes it unlikely that these proteins will have been expressed in the thymus during T cell development, permitting the ‘escape’ of T cells reactive to citrulline residues into the circulation. Interestingly, citrulline has been shown to fit into a region of major histocompatibility complex (MHC) class II known as the ‘shared epitope’ [64]. The genes which code for this region of the antigen presenting molecule are the greatest genetic risk factor for RA [65,66]. Indeed, MHC class II susceptibility genes have been found to associate with production of ACPA [67]. Citrullinated proteins that have been described in the synovium of RA patients include fibrin, vimentin, type II collagenase and alpha enolase [68-71]. The requirement of citrullination of these proteins was shown in experiments where DR4-IE transgenic mice which express an RA susceptible MHC were injected with citrullinated or non-citrullinated fibrinogen and only animals injected with the citrullinated protein proceeded to develop arthritis [72]. Thus, citrullinated proteins such as fibrinogen or vimentin, that are highly specific to RA, could provide target antigens for designer Treg.

Tolerogenic Dendritic Cells

In addition to targeting Treg to the site of inflammation directly, there is also the possibility of modulating the way in which antigens are presented to T cells to favor the induction of tolerance. Dendritic cells (DCs) are specialized antigen presenting cells and these cells play a fundamental role in tolerance to self-tissue. In the absence of inflammation DCs express low levels of co-stimulatory molecules and other markers of activation – these cells are described as immature DCs. If immature DCs present antigen to a T cell, the cells may undergo proliferation but will fail to become pathogenic [7]. Furthermore, it has been shown that targeting of antigen to immature DCs leads to the inhibition of antigen specific effector T cell function and the appearance of IL-10 producing antigen specific regulatory T cells [73,74]. Thus, immature DCs not only prevent the activation of the immune response but also promote the induction of tolerance through the generation of Treg.

The in vitro generation of TolDCs has been successful via a variety of methods, including genetic and pharmacological manipulation, and transfer of these cells into animal models of autoimmunity has led to suppression of disease [75-78]. The generation of antigen-loaded TolDCs for therapy in RA offers a promising way to modulate the entire adaptive immune response. However, similar to the Ex vivo manipulation of Treg, there are safety issues associated with the generation of TolDCs from monocytes isolated from RA patients. Monocyte-derived DCs from RA patients have been described as having a more pro-inflammatory phenotype compared to those from healthy individuals [79,80], which may make them more difficult to convert to TolDCs. Identifying markers for TolDCs, other than those similar to immature DCs, will be helpful for studying their stability in vivo in the presence of inflammation. However, clinical grade human monocyte-derived TolDCs have already been generated from patients with RA. These cells are phenotypically comparable to TolDCs generated from healthy individuals and suppressed T cell proliferation and pro-inflammatory cytokine production. Moreover, these cells were stable when immunosuppressive drugs were withdrawn and when challenged with pro-inflammatory mediators [81]. The generation of antigen-
specific tolerance in RA using TolDCs also has similar challenges as the use of designer Treg. Not knowing the auto-antigen to target has led to several approaches being considered, including the loading of TolDCs with autologous synovial fluid that contains a variety of auto-antigens or the loading with citrullinated peptides derived from RA candidate auto-antigens [82]. The generation of an antigen-specific TolDC, either by generation in vitro or manipulation of DC in vivo could present an exciting cellular therapy that acts to reprogram the immune system, switching off pathogenic T cell responses whilst inducing antigen-specific Treg.

Future Therapeutics

Recent research has focused on immunotherapies that target intracellular signaling pathways. Two recent papers have described the successful use of JAK pathway inhibitors in patients with RA [83,84]. It remains to be seen if this represents a novel immunomodulatory therapeutic with the capacity to reset peripheral tolerance in RA. However, the prevalence of JAK-STAT signaling in cells of the immune system including Treg and DCs may suggest that this therapy could ameliorate disease through restoring or enhancing tolerogenic mechanisms.

Summary

There is a broad spectrum of treatments available for use in RA that range from disease modifying drugs such as methotrexate to biological therapies including anti-TNF-α. Current therapies target the inflammatory response; either the cells involved in disease progression or the inflammatory cytokines they produce. This improves disease through a reduction in inflammation, pain and joint destruction (Figure 1). Although successful, patients are required to continue therapy for the rest of their lives leading to significant costs to the health service, increased risk of side-effects and increased incidence of relapse. The ability to take a short course of drugs to induce tolerance through the induction of Treg or the capacity to transfuse patients with autologous antigen-specific Treg or TolDCs to re-program the immune system and suppress disease long term is an attractive concept. Improved understanding of the mechanisms of tolerance induction and the function of immunoregulatory cells will aid in the translation of these cellular therapies into patients with RA providing short term, long-lasting disease specific therapy.

Figure 1: Therapeutic strategies for the treatment of RA

Immunotherapies for the treatment of RA have directly targeted pro-inflammatory cytokines such as TNFα and IL-17, and others, such as IL-6, through the blockade of the IL-6 receptor (tocilizumab). Agents that deplete subsets of cells such as T cell depleting anti-CD3 antibodies may also be helpful in re-setting the immune response via the induction of Treg. In future it is hoped that cellular therapies will add to the cannon of therapeutic agents to treat RA either through the in vitro expansion of naturally occurring Treg from patients or the in vitro induction of antigen specific Treg. Alternatively, the generation of TolDCs in vitro and transfer back to the patient could result in altering the balance of inflammation towards tolerance via the reduction in T cell activation and cytokine production, the increase in production of anti-inflammatory cytokines and the induction of functional Treg.

References


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