Review

Advances in CTLA-4-Ig-mediated modulation of inflammatory cell and immune response activation in rheumatoid arthritis

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Abstract

Rheumatoid arthritis (RA) is a multifactorial and polygenic immune-mediated disease, the pathogenesis of which involves different cell types. T and B lymphocytes, macrophages, endothelial cells, fibroblasts and osteoclasts have all been implicated in mediating the production of autoantibodies, proinflammatory cytokines and ultimately bone erosions. Cytotoxic T lymphocyte-associated antigen 4 immunoglobulin fusion protein (CTLA-4-Ig, abatacept) is a unique biologic agent targeting the co-stimulatory molecules CD80/CD86, and is indicated for the treatment of moderate-to-severe RA in patients who have had an inadequate response to one or more disease-modifying anti-rheumatic drugs, including methotrexate or anti-tumor necrosis factor agents. There is a growing body of evidence that, through selective modulation of the CD80/CD86 co-stimulatory molecules expressed by a variety of activated cell types, CTLA-4-Ig may inhibit the pathogenic RA process at several levels, both directly and indirectly. Here, we provide an overview of recent mechanistic studies of the action of CTLA-4-Ig on different cell types involved in mediating inflammation and joint damage in RA.

Keywords: Rheumatoid arthritis - CTLA-4-Ig - Macrophages - Endothelial cells - Osteoclasts - Co-stimulatory molecules

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1. Introduction

Rheumatoid arthritis (RA) is a polygenic, multifactorial and chronic, immune-mediated disease. It is characterized by systemic, chronic joint inflammation that leads to structural damage, as well as by extra-articular involvement. The immunopathology of RA is complex, and involves different cell types. However, despite much research and many advances in recent years, the complete mechanism of disease remains to be fully elucidated [1]. Some possible arthritogenic antigens (including citrullinated antigens) thought to trigger the T cell-initiated inflammatory response characteristic of the RA disease process are the subject of continued research [2,3]. T-cell interaction with B cells leads to increased production of autoantibodies – rheumatoid factor (RF)
and anti-cyclic citrullinated protein (anti-CCP) – that are implicated in the early progression of the disease. A number of polymorphisms associated with RA are involved in T-cell activation, providing additional support for the role of T cells in this disease [4]. The T cell-initiated immune cascade leads to increased migration and proliferation of inflammatory cells, such as macrophages, monocytes, leukocytes and fibroblast-like synoviocytes, which infiltrate the synovial tissue [1]. Activation of these cell types leads to increased production of proinflammatory cytokines (such as anti-tumor necrosis factor [TNF]-α, interleukin [IL]-1, IL-6 and IL-17), and local growth factors, which contribute to joint swelling and pain [1]. The presence of proinflammatory cytokines and local vascular growth factors in the synovial tissue leads to activation of endothelial cells, which subsequently express adhesion molecules that further promote the recruitment of inflammatory cells into the joint as well as induce angiogenesis [5]. In addition to increased production of proinflammatory cytokines, activated macrophages and osteoclasts secrete metalloproteinases, which are responsible for bone and cartilage degradation [1]. Inflammatory cytokines and the receptor activator of nuclear factor-κB ligand (RANKL) stimulate the development of osteoclasts, which also produce metalloproteinases that subsequently exert an effect on structural damage [1]. Interestingly, in pre-RA, anti-citrullinated protein antibody-positive individuals show signs of periarticular bone damage even before the clinical phase of RA has started, demonstrating that autoimmunity is complicated by bone loss [6].

Treatment of early RA conventionally starts with non-biologic disease-modifying anti-rheumatic drugs (DMARDs), namely methotrexate (MTX) or leflunomide, which are the gold standard. Patients with established RA who have had an inadequate response to MTX may integrate their therapy with other drugs such as biologic DMARDs [7–9]. Biologics can target a variety of cytokines that are involved in the inflammatory cascade, such as TNF-α and IL-6, whereas some can also target CD20+ B cells, leading to their depletion, or can modulate T-cell co-stimulation and activation (Fig. 1). Newer biologics are being developed that target intracellular JAK/STAT and MAPK signaling pathways involved in the immune cascade (Fig. 1) [10]. Biologics with different mechanisms of action may exert different clinical effects on early and established disease, depending on which cell types they influence; they may also have different clinical effects depending on patient disease characteristics and underlying disease pathology. In fact, two different and prevalent populations of patients

![Fig. 1](image-url). (A) Schematic demonstrating the cell types involved in the immune cascade, in particular at the level of the synovial tissue in rheumatoid arthritis, and the position that different biologics exert their major effects. (B) Impact of CTLA-4-Ig on different cell types following recent advances in basic and clinical research. Anti-CCP, anti-cyclic citrullinated protein; APC, antigen-presenting cell; CTLA-4-Ig, cytotoxic T lymphocyte-associated antigen 4 immunoglobulin fusion protein; IL, interleukin; MMP, matrix metalloproteinases; RF, rheumatoid factor; TNF, tumor necrosis factor.
with RA can be characterized by predominantly T (75%) or B (25%) lymphocyte-driven activation of inflammation [11].

As we understand more about the pathways involved in the inflammatory process, additional therapeutic targets emerge for the immunopathologic responses involved in RA. One such therapeutic target is the CD28:CD80/86 (B7.1/B7.2) co-stimulatory pathway, which has a key role in the full activation of T cells [12] and in determining the fate of immune/inflammatory responses. The co-stimulatory molecules expressed by activated T cells represent a unique target for biologic therapy in RA. As such, the cytotoxic T lymphocyte-associated antigen 4 immunoglobulin fusion protein (CTLA-4-Ig) that selectively modulates the CD28:CD80/86 (B7.1/B7.2) co-stimulation signal (abatacept, Bristol-Myers Squibb, NJ, United States) is one biologic DMARD that is indicated for use in patients with active RA and an inadequate response to other DMARDs (including MTX or anti-TNF). There are various forms of CTLA-4-Ig, including a murine version of CTLA-4-Ig, human CTLA-4-Ig (which does not contain the modified Fc domain described in Section 2) and abatacept. Herein, we refer to these various forms as CTLA-4-Ig; however, all the clinical studies discussed utilized abatacept.

CTLA-4-Ig works early in the inflammatory response – at the point of naïve T-cell activation – and impacts cells involved early in the disease process – both T and B cells. It has proven clinical and radiographic efficacy in the treatment of RA and is well tolerated, with low immunogenicity [13–17], but the mechanisms underlying its efficacy and tolerability remain to be fully elucidated. The objective of this article is to review and provide an expert opinion on recent publications that have contributed to a wider understanding of the consequences of CTLA-4-Ig-mediated co-stimulation modulation, with an emphasis on mechanistic studies of its action on various cell types involved in mediating inflammation and joint damage in RA. Recent publications relevant to the mechanism of action of CTLA-4-Ig in RA, as identified by the authors from their own expertise and knowledge, were included. PubMed searches were conducted using the following search terms to identify additional papers of potential interest that had been published since 2009: “(Abatacept OR CTLA-4-Ig) AND rheumatoid arthritis” in combination with each of the following: (CD80 OR CD86), (co-stimulation modulation OR co-stimulation modulation), T cell, B cell, lymphocyte, macrophage, osteoclast, regulatory T cell (Treg) and endothelial cell.

### 2. Selective modulation of T-cell co-stimulation by CTLA-4-Ig: an overview

The T-cell immune response is triggered by presentation of an antigen by antigen-presenting cells (APC) via a trimolecular complex comprising major histocompatibility complex (MHC) on the APC, the peptide antigen that has triggered the immune response, and the T-cell membrane receptor (TCR) specific for that antigen [18]. However, antigen presentation alone is not sufficient to drive T-cell activation; an additional, regulated...
signal between the APC and naïve T lymphocyte is required. The CD28:CD80/86 co-stimulation signal is one such pathway; CD28 expressed on the T-cell membrane binds to CD80/86 expressed on the APC membrane. Antigen presentation and CD28:CD80/86 co-stimulation activates the full T-cell response of proliferation and cytokine production, enabling a robust antigen–specific immune response [12].

The CD28:CD80/86 co-stimulation signal is self-regulating; 24–48 h into T lymphocyte activation, the CD28 signal induces expression of CTLA-4, another T lymphocyte cell surface molecule [19]. CTLA-4 competes with CD28 for CD80/86 binding, but with much higher avidity, thus modulating the signal and regulating naïve T-cell activation and proliferation. Elucidation of these co-stimulatory pathways led to the development of the CD28:CD80/86 signal as a therapeutic target for RA [20,21]. CTLA-4-Ig (abatacept) is a fully humanized molecule consisting of the extracellular domain of human CTLA-4 and a genetically engineered, modified fragment of the Fc region of human immunoglobulin G1 (IgG1), which is devoid of antibody-dependent cell-mediated cytotoxicity and complement fixation activity, and has limited binding to Fc receptors [22]. Generally, and similar to the mechanism of naturally occurring CTLA-4, CTLA-4-Ig competes with CD28 for binding to CD80/CD86 receptors on APCs, thus suppressing the co-stimulatory signal required for full T-cell activation. Consistent with this, quantitative polymerase chain reaction studies and assays of synovial biopsies in clinical trials have identified that treatment of patients with RA using CTLA-4-Ig leads to reduced expression of inflammatory genes and production of proinflammatory cytokines, including the 1h1 cytokine interferon (IFN)–γ, and reduced levels of inflammatory serum biomarkers to within normal levels [23–25]. Interactions between other members of the B7-CD28 family molecules (such as programmed death-1 [PD-L1] and inducible co-stimulator ligand [ICOSL]) may also regulate the T-cell response to an antigen [26,27]. However, the effect of CTLA-4-Ig on these interactions in humans is currently unknown.

3. The wider consequences of CTLA-4-Ig-mediated co-stimulation modulation: furthering our understanding

Research over many years has shown that, in addition to T cells, a number of other cell types play a role in the pathogenesis of RA. B lymphocytes, macrophages, endothelial cells and osteoclasts are implicated in mediating the production of autoantibodies, proinflammatory cytokines and proteinases, and ultimately bone erosion. There is a growing body of evidence that, through selective modulation of the CD28 co-stimulatory pathway or through binding to CD80 and CD86, CTLA-4-Ig may target these additional cell types, providing direct therapeutic effects in addition to T-cell modulation alone [28].

3.1. Lymphocytes

3.1.1. T lymphocytes

It is suggested that CTLA-4-Ig might achieve its clinical effects through modulation of effector (CD28−) T-cell function. CD28− T cells generally display the functional properties of differentiated effector and/or cytotoxic cells, in particular producing large amounts of IFN-γ [29]. Studies have shown that reduction of IFN-γ expression and production in the synovial tissue and serum is a prominent effect of CTLA-4-Ig treatment [23,30]. Downmodulation of CD28 expression is achieved in vitro through engagement with its ligands (CD80/CD86), in a regulatory feedback loop; because this engagement is blocked by CTLA-4-Ig, it has been hypothesized that CTLA-4-Ig may also prevent generation of the CD28− population. Indeed, it has been shown that the number of CD28− T cells is reduced after therapy with CTLA-4-Ig, and that such a decrease in CD28− T cells correlated with reductions in clinical disease activity as evaluated by Disease Activity Score (DAS) 28-defined criteria [31]. Low numbers of CD28− T cells (and consequently high levels of CD28+ T cells) at baseline also correlated with a higher likelihood of achieving disease activity remission (based on DAS28) compared with higher numbers of baseline CD28− T cells (and consequently low levels of CD28+ T cells) [32].

Murine models have provided a mechanistic insight into the effect of CTLA-4-Ig on other aspects of T-cell biology. Administration of CTLA-4-Ig was associated with failure of antigen-specific T cells in lymph nodes to acquire a phenotype (CXCR5+ICOS+) associated with migration to B-cell follicles, which in turn led to reduced specific antibody responses, despite normal B-cell clonal expansion [33]. These data suggest a direct effect of CTLA-4-Ig in lymph nodes, leading to an effect on a T cell-dependent process in vivo. The pathologic significance to RA of T cell-dependent B-cell response suppression by CTLA-4-Ig was confirmed by the finding that treatment with CTLA-4-Ig prevented the breach of B-cell tolerance and the emergence of self-reactivity [33]. A recent study using a severe combined immunodeficiency (SCID) mouse model suggests that CTLA-4-Ig does not act directly on synovial T cells, but more likely prevents T-cell activation at a systemic level of the immune system [34], consistent with the data described above. A novel role for CD28 co-stimulation in regulating the expansion and resultant homing capacities of pathogenic ovalbumin and influenza peptide-specific effector memory CD4+ T cells was suggested following observations in CTLA-4-Ig-treated mice [35,36]. Immunopathology in RA is thought to be perpetuated, at least in part, by memory CD4+ T cells [1], so if CTLA-4-Ig is able to influence their activation and homing to joint tissue, it could add to our understanding of its actions in RA.

3.1.2. B lymphocytes

The co-stimulatory molecules CD80 and CD86 are also expressed on B lymphocytes and have roles in modulating B-cell activity. Triggering of CD86 signaling in B cells has been observed to enhance antibody production induced by IL-4 [37,38]. More recently, a direct role for CD80/CD86 expressed by B cells on the regulation of the IgG secretion by previously activated and class-switched B cells has been demonstrated in a study of virus-specific humoral responses in mice [39]. According to the investigators, such a finding may be of interest in the context of the interpretation of data from clinical studies on the use of CTLA-4-Ig therapy in systemic lupus erythematosus, an autoimmune disease that is linked to altered B-cell activation [40]. The function of CD86 as a B-cell regulatory molecule is consistent with the finding that multiple signaling pathways are activated in the regulation of B-cell activity by CD86 [41].

B cells have been used to demonstrate the potential to induce peripheral T-cell tolerance by tolerizing APCs [42]. Gene transfer of CTLA-4 using lentiviral vectors was able to reduce the expression of CD86 in human and mouse B-cell lines [42]; such B cells were found to have reduced co-stimulatory capacity as evidenced by reduced antigen-specific T-cell proliferation in vitro.

Immunohistological analysis of synovial tissue from patients with RA and an inadequate response to anti-TNF agents following treatment with CTLA-4-Ig plus MTX (compared with MTX alone as control) has found that inhibition of not only T cells, but also B cells in the synovium, probably plays a role in the efficacy of CTLA-4-Ig [43]. Reduced cell proliferation and downregulation of the expression of B-cell markers, including CD20, CD80 and CD86, were apparent in the histological samples taken from patients who received CTLA-4-Ig and MTX compared with samples taken from control patients (MTX alone) [43]. Clinical trial data from the first mechanistic study of CTLA-4-Ig in patients with an inadequate response to anti-TNF treated with CTLA-4-Ig [23] also provide evidence for modulation of B cells, with a modest but significant reduction observed in CD20+ cells in synovial biopsies [23]. Further research is needed to determine whether these T- and B-cell effects occur in lymph nodes as well as the articular joints.

3.2. Regulatory T cells

Treg cells are of interest in RA, given their constitutive expression of CTLA-4 and ability to suppress autoimmunity. They protect against local and systemic TNF-mediated bone loss and preserve bone mass
during physiologic and pathologic bone remodeling through inhibition of osteoclast differentiation [44,45]. Expansion of the numbers or activity of Treg cells could, therefore, be beneficial in reducing inflammation-induced bone destruction in patients with RA. Although early mouse studies with CTLA-4-lg suggested a potential effect on Treg numbers (decrease) and function [46], these dramatic effects in the mouse do not appear to translate into human RA studies. A role for the Treg population in suppressing the development of collagen-induced arthritis in a mouse model has been explored more recently, and one study found that administration of CTLA-4-lg was associated with an increase in the Treg population via effects on dendritic cells [47].

The effects of CTLA-4-lg on Treg cells in patients with RA are thought to be complex. Analysis of peripheral blood by flow cytometry and functional assays revealed a lower frequency of CD4+CD25brightFoxp3+ Treg cells in CTLA-4-lg-treated patients than in controls (patients with RA who did not receive CTLA-4-lg and healthy controls). However, the ex vivo function of Treg cells (as measured by inhibition of lymphocyte proliferation) in patients treated with CTLA-4-lg was found to be enhanced in comparison with patients not treated with CTLA-4-lg [48]. There is a potential concern that therapies that modulate CD28 co-stimulation may interfere with Treg homeostasis, given that CD28 signaling has been found to induce survival of Tregs in mice [49]. Encouragingly, belatacept, a second-generation CTLA-4-lg, has also been shown not to have any short- or long-term deleterious effects on circulating Treg numbers or function in patients undergoing renal transplantation [50,51].

3.3. Monocytes and macrophages

There is evidence that CTLA-4-lg may directly affect the phenotype and function of monocytes. Proportions of circulating CD14+ monocytes (purified from peripheral blood mononuclear cells [PBMC]) increased significantly during treatment with CTLA-4-lg over 1 and 2 weeks [52]. This increase was accompanied by significant decreases in the expression of adhesion molecules (CD106+ and CD15+), and a significantly decreased migratory capacity through the endothelial cell layer (both in vitro and in vivo) that was found to be CD80/CD86 dependent. The rise in circulating monocytes may be due to a decreased capacity to migrate into the tissues, and a reduction in the capacity of monocytes to migrate into the synovial tissue might contribute to the reduction in inflammation that is seen during the clinical use of CTLA-4-lg.

The aberrant production of proinflammatory cytokines by macrophages themselves is one of the major pathways underlying the pathologic process in RA, and biologic DMARDs have targeted proinflammatory cytokines such as TNF-α, IL-1 and IL-6 [53]. There is evidence that CTLA-4-lg directly binds to macrophages and modulates their function, including the production of proinflammatory cytokines [24]. Using immunofluorescence microscopy, primary cultures of macrophages obtained from synovial tissue of patients with RA have been shown to express CD86; a reduction in immunofluorescent staining in macrophages incubated with CTLA-4-lg provides evidence for interaction of CD86 on macrophages with CTLA-4-lg. Furthermore, CTLA-4-lg was found to downregulate the production of inflammatory cytokines, such as IL-6 and TNF-α, by synovial macrophages from patients with RA in co-cultures with concanavalin-A-activated T cells (Fig. 2) [24]. Similarly, CTLA-4-lg was demonstrated to be capable of reducing proinflammatory cytokine (IL-12 and IFN-γ) production by cultured monocyte-derived macrophages stimulated using either cytokine-activated T cells or toll-like receptor ligands [54]. Investigation into the timing of CTLA-4-lg and synovial macrophage interaction found that significant CTLA-4-lg-mediated downregulation of TNF-α, IL-6, IL-1β and transforming growth factor-β1 gene expression occurs between 3 and 12 h of in vitro administration [55]. The fast effect of CTLA-4-lg on reducing cytokine production appears to be mediated by direct binding of CTLA-4-lg to CD80 and CD86. Therefore, although it has previously been shown that CTLA-4-lg does not alter gene expression in B cells or dendritic cells [52,56], it is possible that the interaction, or co-crosslinking, between CD80/CD86 and perhaps Fc receptors could alter signaling pathways in cell types other than B and dendritic cells.

The transcription factor NF-κB is an intracellular signaling molecule essential for the expression of a variety of immune response genes, including those related to proinflammatory cytokines [57]. Recently, it was observed that CTLA-4-lg reduces inflammatory cytokine gene expression in human macrophages and promotes downregulation of the intracellular signaling pathway linked to NF-κB, including increased expression of the cytoplasmic inhibitor IκBα, both at the gene and protein level [58]. These data provide additional evidence of the potential direct effect of CTLA-4-lg on signaling in APCs.

3.4. Osteoclasts

In accordance with the effects of CTLA-4-lg on cells of the monocytic/macroage lineage (from which osteoclasts are terminally differentiated) there is evidence that CTLA-4-lg has a direct effect on osteoclasts. Bone destruction in RA is mainly attributable to abnormal osteoclast activity stimulated by activated CD4+ T cells. A relationship between the bone and immune system is highlighted by experiments using human monocytes stimulated with RANKL and

![Fig. 2. Immunocytochemistry in co-cultures of synovial macrophages from patients with rheumatoid arthritis and Jurkat T cells. (A) Interleukin-6 and (B) tumor necrosis factor-α expression. *p<0.05; ***p<0.001. CTLA-4-lg, Cytotoxic T lymphocyte-associated antigen 4 immunoglobulin fusion protein. Figure from Cutolo et al., Arthritis Res Ther 2009 [24].](image-url)
macrophage colony-stimulating factor, in which osteoclasts are able to function as APCs and activate CD4+ and CD8+ T cells [59].

A recent study explored whether CTLA-4-Ig therapy impairs the differentiation of PBMC into osteoclasts in patients with RA in vivo [60]. A reduction in the frequency of osteoclast precursor cells was measured in the peripheral blood of patients with RA receiving CTLA-4-Ig monotherapy compared with untreated controls. In addition, osteoclast differentiation was impaired in patients treated with CTLA-4-Ig compared with untreated controls. Moreover, significant downregulation of key osteoclast genes, such as c-Fos and NFATc1, was found.

In the first mechanistic study of CTLA-4-Ig, changes in synovial gene expression studied after 4 months of treatment revealed a reduction in RANKL/RANK expression with concomitant osteoprotegerin upregulation [23]. Modulation of such factors that mediate osteoclastogenesis are consistent with a regulatory influence for CTLA-4-Ig on osteoclast cell differentiation and bone resorption.

In support of these data in human RA models, animal data also suggest that CTLA-4-Ig has a direct effect on osteoclasts. In a rat model of collagen-induced arthritis (CIA), prophylactic administration of CTLA-4-Ig prevented inflammation, as demonstrated by inhibition of paw swelling, cartilage damage, pannus formation and bone resorption observed in ankle and knee joints, compared with IgG-treated control rats, after injection of type II collagen. This was accompanied by a reduction in the number of osteoclasts in the knee and ankle joints of CIA rats treated with CTLA-4-Ig compared with IgG-treated controls (as detected histopathologically by staining with tartrate-resistant acid phosphatase), alongside protection against deleterious changes in bone architecture [61].

Observations that CTLA-4 binds directly to osteoclast precursor cells, inhibiting their differentiation, provides further evidence for a direct interaction between CTLA-4-Ig and osteoclasts [62]. A dose-dependent inhibition of RANKL- and TNF-mediated osteoclastogenesis was observed in vitro, after the addition of CTLA-4-Ig to both murine and human precursor cells [62] (Fig. 3A–C); this inhibitory effect did not require the presence of T cells in the culture system. Furthermore, in vivo experiments in mice showed that CTLA-4-Ig was effective in blocking TNF-induced osteoclast formation and inhibiting bone erosion in a non-T cell-dependent TNF-induced model of arthritis [62] (see Fig. 3D). That these observations occurred independently of T cells suggests a direct effect of CTLA-4-Ig on bone.

3.5. Endothelial cells

Endothelial cells in the synovial tissue are thought to play an important role in the pathogenesis of RA. They undergo activation, proliferation and changes that lead to an increase in endothelial permeability, and also express cytokines, cytokine receptors and proteases; these changes lead to migration and homing of leukocytes into the joint tissues, joint swelling, tissue degradation and angiogenesis.

Human endothelial progenitor cells, which have angiogenic potential, have been shown to possess monocyte-like antigen-presenting and T-cell co-stimulatory capacity [63]. Blocking experiments using CTLA-4-Ig identified CD28:CD80/86 as a major co-stimulatory pathway for endothelial progenitor cell-dependent T-cell activation [63].

Findings from studies of the effects of CTLA-4-Ig on endothelium in allorecognition and other T cell-mediated autoimmune conditions also suggest that the endothelium, through expression of CD80/86, may have co-stimulatory properties and be a target for immune modulation
using CTLA-4-Ig [64–66]. In a study to explore allograft rejection and the activation of alloreactive T cells by mouse vascular endothelium, CD8+ cells were found to be directly activated in a CD80-dependent fashion in co-cultures of primary endothelial cells from H-2b mice as stimulators and responder CD8+ T cells isolated from H-2k mice. Co-stimulatory blockade using CTLA-4-Ig was able to blunt direct allore cognition, as evidenced by a reduction in the proliferative response of alloreactive CD8+ T cells to vascular endothelium. This suggests that CTLA-4-Ig could be used to induce immunological tolerance in transplantation, and possibly other areas such as RA, in which immunomodulation is the goal [65]. Experiments using human pancreatic islet endothelial cells revealed that expression of CD86 was found to facilitate T-cell adhesion and migration across the endothelium and that this was also inhibited by CTLA-4-Ig [66]; the extrapolation of such findings to RA, in which migration of T cells across the synovial endothelium contributes to the pathogenic process, would be of interest. Finally, CTLA-4-Ig administered to patients with psoriatic vulgaris was found to be associated with reduced endothelial cell activation in psoriatic plaques [64].

4. Discussion

It is well established that CTLA-4-Ig competes with CD28 for binding to CD80 and CD86 receptors on APCs, modulating the co-stimulatory signal required for full T-cell activation early in the inflammatory cascade [12,21]. Recent studies overviewed here reveal that CTLA-4-Ig also impacts different T- and B-cell types involved in RA disease processes and pathology [31–36,47,48], and may be involved in directly downregulating cytokine production by macrophages [24,54]. Furthermore, CTLA-4-Ig has protective effects on bone via the inhibition of osteoclast differentiation, which may be independent of inflammation [61,62] (Fig. 1B). Through a literature search and from our own expertise and knowledge, recent publications relevant to the mechanism of action of CTLA-4-Ig in RA have been identified and discussed in this review. Some of the findings on the potential mechanisms by which CTLA-4-Ig mediates its effects support observations reported in clinical studies, thereby providing a mechanistic basis for the clinical efficacy of this biologic in the treatment of RA; others are still more speculative, but promising, and require further study.

The T cell-centric mechanism of action for CTLA-4-Ig has been previously demonstrated in a large number of studies [21]. However, reduced proinflammatory cytokine expression in the synovial tissue following CTLA-4-Ig treatment has been reported in patients with RA [23,34], supporting a role for CTLA-4-Ig in decreasing downstream activation of cytokine production at the site of inflammation. In particular, these studies have demonstrated a pronounced reduction in production of the cytokine IFN-γ with CTLA-4-Ig treatment, supporting a role for CTLA-4-Ig at the level of the T cell. Effector (CD28+) T cells in particular produce large amounts of IFN-γ. Interestingly, reductions or low numbers of CD28− T cells were found to correlate with improved disease activity or remission with CTLA-4-Ig treatment [31,32], which supports a mechanism of action for CTLA-4-Ig via CD28 modulation. This simple laboratory measure may represent a potential opportunity as a predictive biomarker, and its clinical validity and utility should be explored further. Surprisingly, one study reviewed here suggested that CTLA-4-Ig may not act directly on synovial T cells, but more likely prevents systemic T-cell activation, suggesting that CTLA-4-Ig has the potential to act early in the disease by preventing the initiation of pathogenic processes in secondary lymphoid organs [34]. This is consistent with findings from a randomized clinical trial of CTLA-4-Ig in patients with undifferentiated arthritis or very early RA [67], in which CTLA-4-Ig monotherapy delayed progression to clinically definite RA in some patients, and delayed radiographic and magnetic resonance imaging progression; outcomes that were maintained for more than 6 months after treatment stopped.

The effects of CTLA-4-Ig on other lymphocyte subsets have been explored and demonstrated reductions in synovial B-cell expression and markers of B-cell expression in patients with RA following CTLA-4-Ig treatment [23,43]. Findings from murine models discussed here reveal that CTLA-4-Ig may suppress T-cell dependent B-cell responses [33], and that this may prevent the breach of B-cell tolerance and the emergence of self-reactivity in an RA model [33]. The potential of CTLA-4-Ig to prevent the breach of tolerance in patients with RA may allow the immune system to be ‘reset’. However, the associated risks of anergying beneficial immune responses must be considered. Recent congress reports using a model of antigen-specific tolerance and priming have suggested that, although CTLA-4-Ig does significantly modulate T-cell activation in vivo, it does not induce a phenotype and functional state of T-cell tolerance after primary antigen encounter [68]. The potential of CTLA-4-Ig to reduce the production of antibodies against self-antigens, however, is consistent with previous clinical trial observations showing anti-CCP and RF seroconversion in patients with early RA [69]. CTLA-4-Ig may also inhibit the propagation or homing of pathogenic effector memory cells (and monocytes), which can mediate inflammation at peripheral tissue sites, possibly by direct interaction with endothelial cells as discussed above [35,36,66]. Useful memory T-cell responses (such as those stimulated during vaccination), however, can also be potentially modulated by CTLA-4-Ig, as evidenced by a reduced response against tetanus toxoid in healthy subjects who were vaccinated after receiving CTLA-4-Ig [70]. While CTLA-4-Ig may have reduced vaccine-elicited mean geometric titers of tetanus toxoid antibody, it did not inhibit the ability of subjects to develop at least a two-fold protective response to tetanus toxoid [70]. Finally, preliminary data exploring the effects of CTLA-4-Ig on Treg cells, as discussed here, have revealed a complex action on this cell type [48].

Historically, a T cell-centric view of the mechanism of action of CTLA-4-Ig has been proposed. While T-cell modulation likely contributes to a large part of the mechanism of action of CTLA-4-Ig, recent studies indicate direct and indirect effects of CTLA-4-Ig on other cell types. Observations discussed have shown that CTLA-4-Ig directly binds to macrophages and can downregulate their production of proinflammatory cytokines [24]. This has the potential to reduce the inflammation and structural damage caused by macrophage-derived cytokines such as TNF-α, IL-6 and IL-1, but the mechanism of action of the direct effect on this cell type requires further investigation.

Studies have also demonstrated that CTLA-4-Ig binds directly to osteoclast precursor cells, inhibiting their differentiation [62], suggesting that it could have a direct protective effect on bone that could prevent structural damage in RA, independent of effects on inflammation. Clinical study data reviewed here show that changes in the expression of gene encoding factors involved in osteoclast differentiation and activation [23] are consistent with a direct regulatory influence for CTLA-4-Ig on osteoclast cell differentiation and bone resorption. These findings support observations from a randomized clinical trial that demonstrated reductions in MRI-assessed osteitis, with structural and synovial benefits, at Month 4 of treatment that were sustained to Month 12 in patients who received CTLA-4-Ig plus MTX. For patients originally randomized to placebo plus MTX, improvements in osteitis, and structural and synovial benefits, were seen following the addition of CTLA-4-Ig [71].

In addition, observations from studies of the effects of CTLA-4-Ig on the endothelium in allore cognition and other T cell-mediated autoimmune conditions [64–66] suggest that the endothelium may have co-stimulatory properties and be a target for immune modulation in RA using CTLA-4-Ig. However, these observations were not made in RA patients or models, and studies on cultured endothelial cells are needed to support the extrapolation of such conclusions (research ongoing).

Finally, as well those discussed in this review, it is possible that additional cell types involved in RA may be identified as targets for CTLA-4-Ig. Studies suggest that both fibroblast-like synoviocytes and dermal fibroblasts have the potential to act as activators of T cells. Fibroblast-like synoviocytes have recently been explored as a target for a CTLA-4-FasL fusion protein [72] and dermal fibroblasts have been shown to induce maturation of dendritic cells, as demonstrated by expression of CD80/86 and other molecules [73]. A potential role of CTLA-4-Ig in modulating
this fibroblast activity is unknown, however, and may warrant further research.

The different biologics available for the treatment of RA act through different mechanisms [10,74]. It is hypothesized that patients who have characteristically inflammatory RA may respond better to cytokine inhibitors, whereas patients with B cell–driven disease may respond better to therapies that have direct effects on B cells. Data overviewed here indicate that CTLA-4-Ig reduces synovial inflammation by modulating multiple aspects of the immunological response, via both the T and B lymphocyte routes, suggesting that it could target disease driven by either lymphocyte subset and could potentially be useful in treating a wide spectrum of patients, particularly those with early RA given the role of adaptive immunity in some of the earliest stages of the disease [18]. CTLA-4-Ig directly interacts with different cell populations according to their state of activation and expression level of CD80/86 (B7.1/B7.2 complex) co-stimulatory molecules, resulting in targeted effects. As a consequence, CTLA-4-Ig is likely to play a role in different stages of RA disease pathology. Given the potential to act on multiple targets in the immune system, CTLA-4-Ig may also be a suitable treatment for other conditions in which the immunopathology involves the interplay of several components of the immune system. CTLA-4-Ig is currently under investigation for use in additional indications including lupus nephritis [75,76] and psoriatic arthritis [77].

In clinical trials, CTLA-4-Ig is well tolerated and associated with consistent safety [78–81]. We postulate that the favorable safety and tolerability profile of CTLA-4-Ig is due to the targeted nature of its mechanism of action. CD80/86 co-stimulatory molecules, the target for CTLA-4-Ig, are overexpressed in several cell types in relation to their state of activation, and as such the drug concentrates its effects specifically on cells involved in the RA disease process, avoiding ‘random’ targets. Furthermore, by exerting its effects on multiple cell types, treatment with CTLA-4-Ig results in improvements in both clinical and structural aspects of disease. As such, the targeted upstream mechanism of CTLA-4-Ig CD80/86 co-stimulation modulation, together with its broad effects on multiple cell types, results in a well-balanced safety and efficacy profile.

In conclusion, this review has overviewed recent mechanistic studies describing the effects of CTLA-4-Ig on T cells, B cells, macrophages, osteoelastic and endothelial cells, cell types that express co-stimulatory molecules such as CD80/86 once activated. Findings suggest that several possible pathways could contribute to the overall mechanism of action of CTLA-4-Ig. As these probably act simultaneously or even sequentially, to what extent various mechanisms contribute to the efficacy of CTLA-4-Ig remains an open question. By further characterizing the mechanisms by which CTLA-4-Ig mediates its effects in humans, we may be able to better understand and identify appropriate patient populations for different biologic therapies, and identify clinical predictors of response, which is important as we shift towards an age of personalized medicine. Such developments would ultimately lead to improved patient outcomes — the ultimate goal of the management of RA.

Disclosure statement

M. Cutolo has received financial support from Bristol-Myers Squibb for laboratory research (funds to the University). S. Nadler is an employee of Bristol-Myers Squibb.

Take-home messages

• CTLA-4-Ig is a biologic (abatacept) used for the treatment of RA and, in initial studies, was found to selectively modulate T-cell activation by binding to the co-stimulatory molecules CD80/CD86. Overall, data suggest that CTLA-4-Ig has a unique mechanism of action involving broad but targeted immunomodulatory effects, which result in a global dampening of a variety of immune mechanisms involved in RA.

• Through direct interactions with the co-stimulatory molecules CD80/CD86 on other cells, CTLA-4-Ig may target other cell types, in addition to T cells, that are involved in the pathogenesis of RA, including B lymphocytes, macrophages and endothelial cells.

• T cell-dependent B-cell responses are modulated by CTLA-4-Ig, which results in reduced production of autoantibodies, and pathogenic and inflammatory cytokines in RA.

• Furthermore, CTLA-4-Ig can directly bind osteoclast precursor cells and inhibit their differentiation; this is consistent with CTLA-4-Ig having an anti-osteoclastogenic effect that is independent of a T cell–mediated mechanism.

• Given the potential to act on multiple targets in the immune system, CTLA-4-Ig may also be a suitable treatment for other conditions in addition to RA, for which the immunopathology involves the interplay of several components of the immune system. CTLA-4-Ig is currently under investigation for use in additional indications, including lupus nephritis and psoriatic arthritis.

Acknowledgments

Professional medical writing and editorial assistance was provided by Eve Guichard, BSc (Hons), and MaiLee Wong, PhD, of Caudex Medical and funded by Bristol-Myers Squibb.

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The mechanisms by which environmental toxicants alter developmental processes predisposing individuals to adult onset chronic disease are not well-understood. Transplacental arsenic exposure promotes atherogenesis in apolipoprotein E-knockout (ApoE(-/-)) mice. Because the liver plays a central role in atherosclerosis, diabetes and metabolic syndrome, States JC, et al. (PLoS One 2012;7:e38713) hypothesized that accelerated atherosclerosis may be linked to altered hepatic development. This hypothesis was tested in ApoE(-/-) mice exposed to 49 ppm arsenic in utero from gestational day (GD) 8 to term. GD18 hepatic arsenic was 1.2 µg/g in dams and 350 ng/g in fetuses. The hepatic transcriptome was evaluated by microarray analysis to assess mRNA and microRNA abundance in control and exposed pups at postnatal day (PND) 1 and PND70. Arsenic exposure altered postnatal developmental trajectory of mRNA and microRNA profiles. Authors identified an arsenic exposure related 51-gene signature at PND1 and PND70 with several hubs of interaction (Hspa8, IgM and Hnf4a). Gene ontology (GO) annotation analyses indicated that pathways for gluconeogenesis and glycolysis were suppressed in exposed pups at PND1, and pathways for protein export, ribosome, antigen processing and presentation, and complement and coagulation cascades were induced by PND70. Promoter analysis of differentially-expressed transcripts identified enriched transcription factor binding sites and clustering to common regulatory sites. SREBP1 binding sites were identified in about 16% of PND70 differentially-expressed genes. Western blot analysis confirmed changes in the liver at PND70 that included increases of heat shock protein 70 (Hspa8) and active SREBP1. Plasma AST and ALT levels were increased at PND70. These results suggest that transplacental arsenic exposure alters developmental programming in fetal liver, leading to an enduring stress and proinflammatory response postnatally that may contribute to early onset of atherosclerosis. Genes containing SREBP1 binding sites also suggest pathways for diabetes mellitus and rheumatoid arthritis, both diseases that contribute to increased cardiovascular disease in humans.

Prenatal arsenic exposure alters gene expression in the adult liver to a proinflammatory state contributing to accelerated atherosclerosis.