

Review

Modulating co-stimulation: a rational strategy in the treatment of rheumatoid arthritis?

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Abstract

Rheumatoid arthritis (RA) is a common destructive inflammatory disease that affects 0.5–1% of the population in many countries. Even though several new treatments have been introduced for patients with RA, a considerable proportion of patients do not benefit from these, and the need for alternative treatment strategies is clear. This review explores the potential for a therapy targeting the adaptive immune system by modulating co-stimulation of T cells with a CTLA4-Ig fusion protein (abatacept).

Introduction

A large body of evidence has accumulated during recent years describing the benefit of early control of inflammation in order to avoid long-term disability and to maintain good function in patients with rheumatoid arthritis (RA) [1–3]. The introduction of tumour necrosis factor (TNF)-blocking agents, in particular when they are given together with methotrexate, has further improved the situation in terms of disease activity, joint destruction and function for many patients [4–6]. This success of therapies targeting TNF using monoclonal antibodies or recombinant receptor constructs has set the scene for the introduction of additional 'biological' therapies that target key structures of the immune system. This has also introduced a need to elucidate the roles played by various immune events in the pathogenesis of RA in different groups of patients with this disease, especially those who do not benefit from TNF-blocking agents.

Blocking innate immune responses

Innate immune responses are rapid ways in which the organism may eradicate pathogens. Cells that participate include neutrophils, macrophages and natural killer cells. Common for these cells is the ability to secrete inflammatory mediators upon activation by rather unspecific stimuli from microbial and other agents. In conditions such as RA, which are characterized by chronic inflammation, these cells contribute substantially to the (immune) pathology. Indeed, during the 1990s blocking the proinflammatory cytokine TNF

was demonstrated to be beneficial in experimental arthritis [7] and later also for human disease (see above). Furthermore, blockade of IL-1, IL-6 and IL-15 has been tested in both experimental arthritis in rodents [8,9] and in human RA [10–12], with promising results.

Taken together, these data have led to a general belief that innate immune responses are crucial to the manifestations of RA, and that adaptive immune responses may be less important in the pathogenesis of the disease and more difficult to target. However, that belief over-simplifies this complex disorder, and there are old as well as recent indications that the adaptive immune system is also of major pathophysiological importance in RA, and it may also be an efficient target for RA therapy [13,14].

Blocking adaptive immune responses

Following the rapid immune reactions by cells of the innate immune system, adaptive immune responses are mounted as part of the normal immune response to pathogens. These responses are characterized by their high specificity for the antigen, and under normal conditions they are sequentially upregulated and downregulated. Cells characteristic of the adaptive immune system are B cells, T cells and professional antigen-presenting cells (APCs; i.e. dendritic cells, macrophages and B cells).

B cells

B cells perform important functions as antibody-secreting cells but they can also function as APCs and cytokine producers. In RA, a role for B cells in the pathogenesis of the disease has long been discussed [15–17]. First, rheumatoid factor (i.e. anti-IgG Fc antibodies) is frequently present in sera of patients with RA [18,19] and has even been used as a prognostic marker for the development of an erosive disease course [20]. Second, anti-citrullin antibodies are frequently detected in RA patients [21–23]. These antibodies are very specific for RA; they can appear before the onset of disease

and so can be used as a prognostic marker for disease development [24,25]. Both of these RA-associated antibody responses are initiated with the help of activated T cells.

New therapies for RA are emerging that focus on the adaptive arm of the immune system, one of them being rituximab. Rituximab targets the CD20 molecule, which is selectively expressed on B cells and depletes these cells [26]. This treatment approach has yielded good responses in the majority of rheumatoid factor positive RA patients treated thus far, but more clinical research is necessary before it can be widely applied clinically [17].

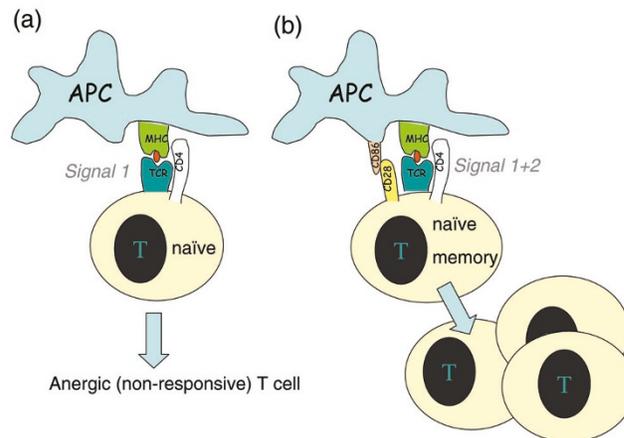
CD4⁺ T cells

T cells can be divided into CD4⁺ and CD8⁺ T cells; the former are the classic helper cells and are crucial, for example, for antibody production and activation of cytotoxic immune responses. The CD4⁺ cells are also the dominant T cells in inflammatory infiltrates in the synovia of RA patients [27,28]. The impact of this is further substantiated by the fact that MHC class II is also abundantly expressed in the rheumatoid synovium [28]; thus, T cells have the potential to become reactivated locally in the joint. The importance of T cells in arthritis was further validated in mice, in which disease can be transferred to a naïve host by injecting T cells from an affected animal [29]. Also, experimental disease can be controlled by T cell depletion before initiation [30]. Thus, therapeutic interventions targeting T cells have for some time remained an attractive option in RA. However, further studies along this line have been disappointing; in a first trial [31] targeting of CD4⁺ T cells with monoclonal antibodies did not affect disease development. A different monoclonal antibody caused more profound depletion of T cells [32], but this therapeutic approach was associated with significant adverse effects and mortality. In addition, using other available agents that interfere specifically with T cell functions, such as cyclosporin, has been only moderately successful [33], suggesting that we must find more efficient agents that interfere with T cell activation and investigate their benefit in clinical settings.

CD4⁺ T cells and co-stimulation

Both naïve and activated/memory T cells traffic in the circulation. However, at the site of inflammation (e.g. the rheumatic joint) only activated/memory T cells are found. This is because of restriction of surface molecules that are needed for homing to tissue, and these are not expressed on naïve T cells [34]. A naïve T cell becomes activated after it encounters antigen. The antigen is presented on HLA molecules of APCs, but just recognition of antigen and MHC by the T cell receptor is not sufficient for a naïve T cell to become activated (Fig. 1a). Co-stimulation is also needed; this is an interaction that sustains APC–T cell contact and amplifies signals in the T cell [35]. The best characterized co-stimulatory signal is that provided by CD28 expressed on T cells ligating to CD80/86 (B7-1 and B7-2 molecules) on APCs (Fig. 1b). CD28 ligation of naïve T cells has been

Figure 1



Activation of naïve T cells requires (a) T cell receptor (TCR)–peptide–MHC interaction (signal 1) and (b) co-stimulation (signal 2) for full activation. This can be provided by so-called professional antigen-presenting cells (APCs; i.e. dendritic cells, macrophages and B cells). In the absence of co-stimulation the T cells will become anergic.

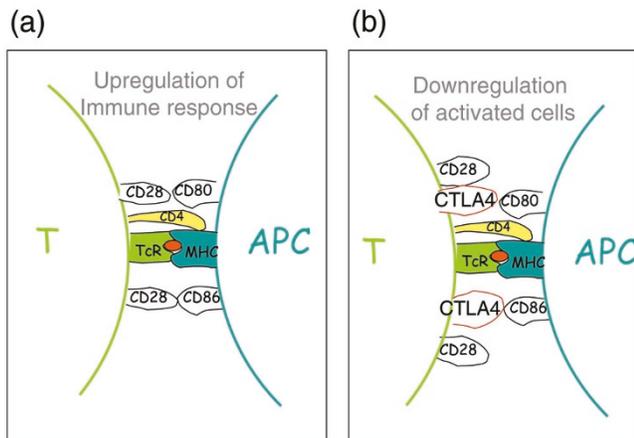
proven to be essential for IL-2 production and cell proliferation [36]. Also, most activated/memory T cells retain their surface expression of CD28, suggesting that this molecule is also involved in reactivating T cells [37].

Since the initial description of CD28 and CD80/86, the list of co-stimulatory molecules has steadily grown and includes ICOS, CD134 (Ox40) and CD27, among others [38]. They do not utilize the CD80 and CD86 molecules, and so the use of a soluble blocking cytotoxic T lymphocyte-associated antigen (CTLA4)–immunoglobulin (Ig) complex only prevents 'classic' co-stimulation mediated by CD28.

CTLA4 (CD152) is a molecule that can out-compete CD28 for ligation of the B7 molecules (Fig. 2). Its affinity for the B7 molecules is 10–20 times greater than that of CD28. Biologically, these differences in affinity result in limitation and subsequent downregulation in T cell responses. That this mechanism is needed is clearly demonstrated in CTLA4 knockout mice, which die within 4 weeks of birth from lymphoproliferative disease [39,40].

CTLA4–Ig fusion protein

The use of a CTLA4 fusion protein as a means to block B7 molecules has been well documented in experimental autoimmunity [41–43]. Administration of CTLA4–Ig at the time of immunization prevented collagen-induced arthritis. Interestingly, administration after disease onset also ameliorated disease [44]. Similar effects were obtained when a combination of anti-CD80 and anti-CD86 antibodies were used to block the co-stimulation, indicating the need to block both pathways in the APC. Studies conducted by Tellander

Figure 2

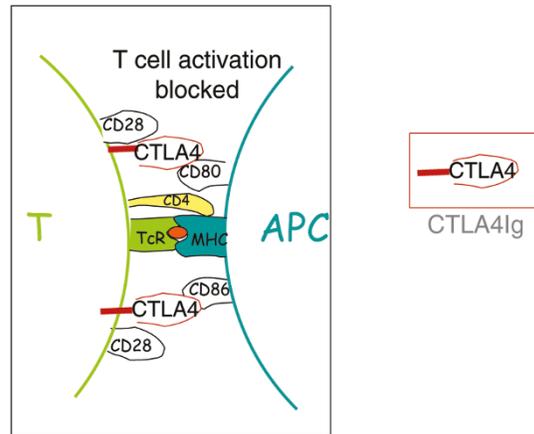
CD28 is constitutively expressed on T cells and CD86 is expressed on antigen-presenting cells (APCs). **(a)** Upon activation CD80 is also expressed on the APC and CD86 is further upregulated. **(b)** Cytotoxic T lymphocyte-associated antigen (CTLA4) expression is induced later during activation, and out-competes CD28 for the interactions, thereby inducing a downregulation in immune response.

and coworkers [45] indicate that antibody titres are also decreased when CD80/CD86 are blocked, indicating the importance of this co-stimulation pathway in B cell help.

These and several other experimental studies have led to the development of the drug abatacept – a recombinant fusion protein comprising the extracellular domain of human CTLA4 fused with a fragment of the Fc portion of human IgG₁ (Fig. 3). A first study was conducted in patients with psoriasis [46], among whom 46% achieved a 50% or greater sustained improvement in clinical disease activity. More recently, abatacept has also been administered to patients with RA, with beneficial effects [47,48] (see also the article by Ruderman and Pope in this supplement).

Levels of co-stimulation molecules in rheumatoid arthritis

In addressing the potential mechanism of action of abatacept in RA patients, it is of interest to examine whether levels of CD28, CTLA4 and CD80/86 vary among cells in the circulation and in different inflammatory compartments. A comparison of CD28 levels on the surface of peripheral blood cells of healthy individuals and RA patients [49] clearly demonstrated the highest levels of CD28 in patients with active disease. Also, CD28 expression is augmented in T cells of the synovial tissue as compared with cells from peripheral blood. This indicates that T cells in patients with active disease have strong potential to interact with and activate APCs, which in turn upregulate their CD80 and CD86 expression and activate more T cells. It is well known that the levels of CD80 and CD86 vary with the degree of activation of the APC.

Figure 3

CTLA4-Ig (cytotoxic T lymphocyte-associated antigen 4-immunoglobulin fusion protein) blocks the T cell-antigen presenting cell (APC) interaction by binding to both CD80 and CD86, thus preventing CD28 interaction. CTLA4 has a higher affinity for the B7 molecules than does CD28. TCR, T cell receptor.

Analysis of CTLA4 levels in peripheral blood [49] indicated a higher baseline level in RA patients than in healthy control individuals. Upon *in vitro* activation the cell surface levels of CTLA4 were similar in the two groups. Among RA patients more cells with surface expression of CTLA4 were found in synovial fluid than in peripheral blood [50].

Patients with co-stimulation deficient T cell populations

There is one subgroup of patients in which CTLA4-Ig and B7 blockade can be assumed to be inefficient, and this is the group with an expanded T cell population consisting of CD28null cells [51]. These cells, lacking CD28 on their cell surface, do not rely on co-stimulation for reactivation [52], and as potent producers of proinflammatory cytokines they may be at an advantage if the rest of the T cell pool, expressing CD28, is suppressed by abatacept. Because these patients are easily identified by flow cytometry, there are two principal options. They can simply be excluded from treatment with abatacept and receive alternative therapy instead, or they may be identified in retrospect in order to investigate whether the heterogeneity in response to CTLA4-Ig among RA patients can be explained by the presence of this unusual cell population. Heterogeneities in treatment response have also been observed for most other antirheumatic drugs including methotrexate and TNF-blocking agents. In all of these cases, there is a need to identify those patients in whom each drug has optimal efficacy and fewest adverse events.

Possible effects of CTLA4-Ig on protective immune responses

Abatacept selectively modulates T cell activation through blocking 'classic' co-stimulation. This selective action allows

other immune pathways to remain largely intact and ensures that T cell activation is modulated rather than completely blocked. The latter scenario would lead to immune suppression and the potential for opportunistic infections. This issue was addressed in the clinical psoriasis study [46], in which immune responses against novel antigens also occurred after initiation of treatment. This selective blockade of CD28-dependent activation pathways was also observed in experimental animal studies [53]; CD28 deficient mice exhibited normal immune function in models of infection both before and after treatment with CTLA4-Ig.

Consequences at the cellular level

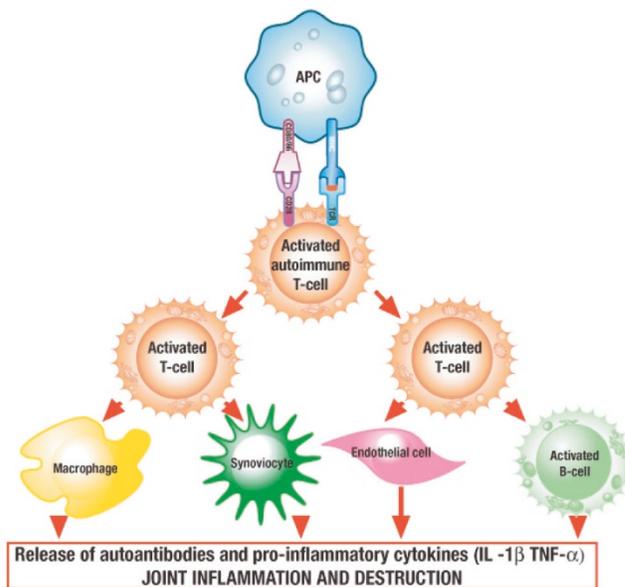
How does CTLA4-Ig work from a cellular point of view? One needs to remember that it does not target T cells directly. Rather, it blocks APCs so that they cannot co-stimulate T cells. Such blockade has direct functional consequences at the APC level; for example, after co-culturing synovial cells in the presence of CTLA4-Ig or anti-B7 antibodies, the amount of proinflammatory cytokines produced by APCs was reduced [54].

Other signalling pathways in the APC are also affected. It was recently suggested that ligation of CTLA4-Ig with CD80/86 on the APC leads to activation of the enzyme IDO (indoleamine-2,3-oxygenase); this enzyme has the potential to modulate the function of the APC similar to that which has been proposed to occur after interaction of APCs with regulatory CD25⁺CD4⁺ T cells [55,56]. Thus, we speculate that one mechanism of action of CTLA4-Ig is that it mimics a function that naturally arising regulatory T cells have on APCs. Over recent years evidence has accumulated that regulatory T cells and the pathways that activate them may be of significance for the development of RA. One such indication is the fact that regulatory T cells are enriched at the site of inflammation in the rheumatic joint, and that these T cells are also functional *in vitro* and so they probably contribute to regulation of local inflammatory reactions [57,58].

A functional consequence of blocking classic CD28-mediated co-stimulation with abatacept is that it also leads to inhibition of the proliferation of both circulating naïve and memory T cells [59]. This may assist in reducing the number of activated autoreactive T-cells available for entry into the synovium.

Interestingly, abatacept appears to have a 'physiological cousin' in mice, in which a splice variant of CTLA4 is expressed that is unable to bind the B7 molecules but nevertheless gives a negative signal to T cells by co-localizing with the T cell receptor and preventing T cell activation [60]. This is an interesting mechanism that adds to the various other means by which peripheral tolerance may decrease the chances of bystander activation of T cells. Only under optimal circumstances, which include co-stimulation, will T cell responses be elicited. Thus far, this phenomenon has not been reported in humans.

Figure 4



Interfering at an early stage in an immune response (i.e. T cell activation) is likely to block subsequent inflammatory events mediated by several different effector cells. APC, antigen-presenting cell; IL, interleukin; TNF, tumour necrosis factor.

Conclusion

T cells are key players in autoimmune diseases such as RA. Once activated, they orchestrate potentially destructive immune responses from other immune cells. Thus, by preventing the initial activation and possibly reactivation of T cells by abatacept, downstream damage mediated by macrophages, fibroblasts and B cells may be controlled (Fig. 4).

Competing interests

The author(s) declare that they have no competing interests.

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