Modulating Co-Stimulation

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Summary: The modulation of co-stimulatory pathways represents a novel therapeutic strategy to regulate autoimmune diseases. Auto-reactive CD4+ T cells play a critical role in initiating the immune response leading to inflammation and autoimmune diseases. Blocking co-stimulatory signals prevents T-cell activation, thus diminishing autoimmune responses and possibly preventing the progression of autoimmune disease. Blockade of several co-stimulatory pathways has been investigated in animal models and has led to clinical trials testing specific blocking agents in humans. In this review we will describe the role of co-stimulatory pathways, primarily the CD28-B7 pathway, in autoimmune diseases, and we will present *in vivo* and *in vitro* studies supporting the efficacy of co-stimulation blockade in animal models of autoimmune disease. Finally, we will discuss the clinical therapeutic efficacy of blocking monoclonal antibodies in preventing or reducing autoantigen driven T-cell activation in humans with particular attention to the CD28/B7 pathway. Inhibiting co-stimulatory molecule interactions by using monoclonal antibodies seems to be an original approach to regulate autoimmune diseases in humans. **Key Words:** T-cell activation, CD28/B7 molecules, co-stimulation blockade, CTlA-4 Ig, EAE/MS, monoclonal antibody (mAb).

INTRODUCTION

Optimal antigen-specific activation of T cells requires two separate signals (FIG. 1). The first signal is specific and mediated through the T-cell receptor (TCR) recognizing the MHC-peptide complex. The second signal is co-stimulatory and is provided by both the cell-surface molecules displayed on antigen presenting cells (APC) and the soluble factors. The absence of a second signal promotes T-cell anergy, which is a state of antigen-specific nonresponsiveness due to the regulation of interleukin (IL)-2 production, which is a fundamental growth factor for T cells.[1–5] The two-signal model oversimplifies the contribution of each signal because the strength of the TCR signal has a quantitative influence on T-cell activation and differentiation.[6,7] Thus, T-cell activation can occur in the absence of signal 2 if the TCR signal is very strong. Several co-stimulatory signaling molecules have been described, and they generally belong to two families: 1) the B7 family[8] and 2) the TNF family of receptors.[9] In this review we focus mainly on members of the B7 family (CD28:B7, PD-1:PD-1 ligands), on CD40–CD154 from the TNF family and on some other co-stimulatory molecules.

Relevance of CD28/B7 pathway in autoimmune diseases

One of the major and better-characterized T-cell co-stimulatory pathways identified is the CD28/B7 pathway. The engagement of CD28, expressed on T cells, with B7-1 and B7-2 (CD80 and CD86, respectively), expressed on APCs, is able to intensify the specific signal delivered through the TCR and promotes cell division and differentiation.[1,3] Conversely the engagement of B7 molecules with the cytotoxic T-lymphocyte associated gene-4 (CTLA-4) receptor, predominantly expressed on activated T cells, delivers an inhibitory signal.[10,11] The co-stimulation provided by CD28 seems to be essential in the induction of experimental autoimmune encephalitis (EAE), a murine model of multiple sclerosis (MS), and it is also involved in the recruitment of activated T cells to the site of the lesions after the clinical onset of the disease. Direct blockade of CD28 with anti-CD28 Fab fragments after disease onset was able to reduce disease severity. Similarly, anti-B7-1 prevented further relapses in EAE by inhibiting epitope spreading.[12–15] T cells derived from mice deficient in CD28 were not able to

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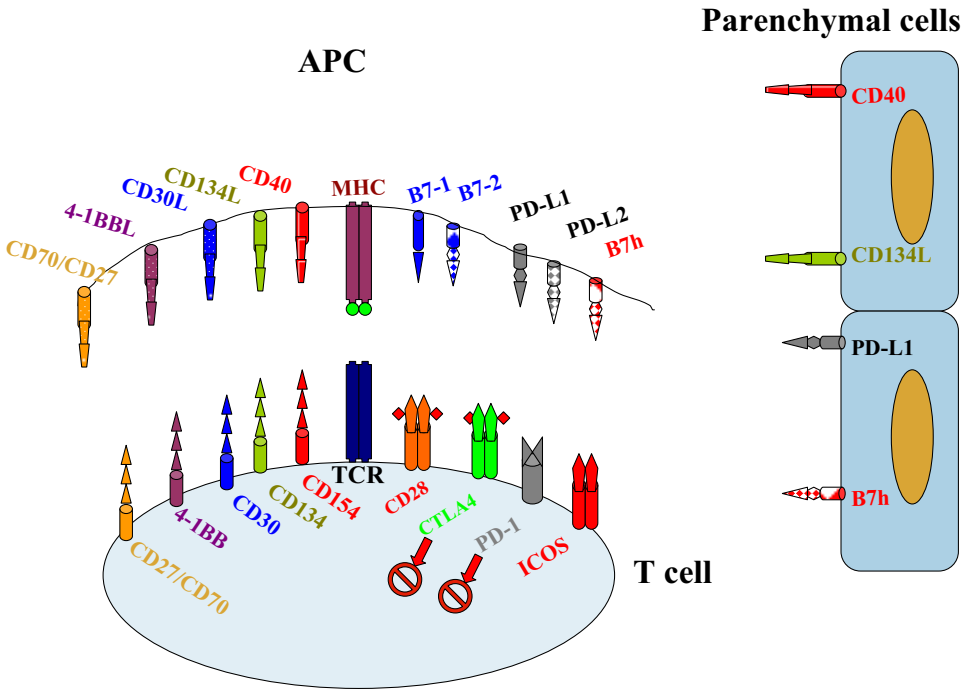

FIG. 1. Schematic representation of co-stimulatory molecules. Signal 1 is represented by the T-cell receptor (TCR) interacting with the antigen in the MHC groove. The B7 family of co-stimulatory molecules is depicted to the right of the TCR, whereas the TNF family of receptors is depicted on the left. Some of the ligands are also expressed on parenchymal cells. Signals from the CTLA4 and PD-1 are inhibitory to the T cell. (1BB, CDw137; 1BBL, CDw137 ligand; CTLA-4, cytotoxic T-lymphocyte associated gene-4; ICOS, inducible co-stimulator; PD, programmed death.)

produce IL-2 in response to stimuli, although they efficiently released interferon-γ, the prototypical T-helper 1 cytokine.

B7-1 and B7-2 are differentially expressed on APCs showing different kinetics and distinct binding affinities. Although B7-2 is constitutively expressed on monocytes, B7-1 expression can be induced on activation. The B7 molecules seem to play an important role in the effector phase of EAE. Adoptive transfer of myelin oligodendrocyte glycoprotein specific T cells to B7-1/B7-2 deficient mice resulted in reduced EAE.[16] In the CNS, B7 molecules are mostly expressed by T cells during the course of EAE[17] and they interact differently with their ligands (CD28 and CTLA-4), leading to co-stimulation on CD28 binding or down-modulation in the case of CTLA-4 binding. Although B7-1 and B7-2 seem to have redundant functions, many studies showed a possible differential role for these molecules in initiating and regulating autoimmune diseases.[18] In MS, B7-1 is preferentially expressed during disease activity,[19,20] and it is associated with MS plaques and expressed on B cells during relapses.[21] Different genetic backgrounds or the presence of different myelin antigens, as well as the avidity of T-cell interaction with the antigen-MHC complex, may determine the diverse role of B7-1 and B7-2.

CO-STIMULATORY BLOCKADE IN EAE: AN ANIMAL MODEL OF MS

The fundamental role of CD28/B7 co-stimulation has inspired the investigation of its role in animal models of organ-specific autoimmune diseases that are induced through the administration of self-proteins or single peptides. In these models, blockade of CD28 co-stimulation at the time of immunization reduced the severity of the disease or completely prevented it.[22–24] Different approaches have been used to inhibit co-stimulation by targeting each of the molecules, CD28 on T cells or B7-1 and B7-2 on APC. Blocking CD28/B7 pathway at the time of disease induction showed efficacy in the prevention of disease in animal models of EAE, lupus and diabetes.[25–27] Some of these studies concurrently showed decreased proliferative ability of the auto-reactive T cells in lymph nodes,[28] suggesting decreased expansion of T cells *in vivo*. Other mechanisms contributing to disease suppression were immune deviation by regulating the equilibrium of Th1 and Th2 cytokines, controlling T-cell migration or inducing anergy.[14,29]

EAE is an inflammatory disease of the CNS that can be induced in a number of species by immunization with myelin antigens or encephalitogenic peptides. It is the major animal model for the study of human MS, and has been studied extensively in rats, mice and guinea pigs.

EAE is a T-cell-mediated disease, which is initiated by antigen-specific encephalitogenic CD4+ T cells.[30,31] Alternatively, EAE can be caused by adoptive transfer of T cells specific for myelin antigens, confirming the autoimmune nature of the disease.[32,33] *In vivo* studies have shown a great variability among different strains of mice in the development of EAE after immunization[34] with myelin antigens such as myelin basic protein (MBP) and proteolipid protein (PLP), the principal constituents of the myelin sheath and myelin oligodendrocyte glycoprotein.[35,36] T cells that are able to induce EAE are not deleted in the thymus, but they are part of the normal cell repertoire, suggesting that these typically quiescent cells may become pathogenic and cross the blood-brain barrier where they initiate an autoimmune response in the CNS. One of the factors driving activation of auto-reactive cells seems to be the co-stimulatory pathway.

Co-stimulatory molecules are differentially expressed during the various phases of EAE, which are likely due to genetic influences because there is also differential expression in various mouse strains. In the EAE model induced by myelin basic protein immunization, the expression of B7-2 was predominant during the acute phase or relapse, whereas B7-1 was found exclusively during remission.[17,37] CD28 was highly expressed during acute disease as well as during remission. In PLP-induced EAE, B7-1 was the molecule predominantly expressed during acute episodes of disease.[37] Some studies have suggested that B7-1 and B7-2 might have distinct functions and favor Th1 or Th2 cytokines, respectively; although later studies did not confirm these findings. The discrepancy might be due to differential expression of B7 molecules at different times after activation or to differential expression on several cell types.

CTLA-4 is expressed on the T-cell surface after activation and binds B7-1 and B7-2 with higher avidity than CD28. CTLA-4Ig, a fusion protein comprising the extracellular domain of CTLA-4 with an Ig tail, blocks the interaction of CD28 with B7 molecules. When administered after adoptive transfer of pathogenic T cells, CTLA-4Ig delayed disease onset and decreased the severity of the clinical symptoms under certain experimental conditions.[15,26] In other studies, CTLA-4Ig administration exacerbated the disease,[10,38,39] demonstrating the complexity of the CD28/B7 system. It is possible that CTLA-4Ig is unable to enter the CNS and the inhibition of the CD28/B7 interaction should occur at the site of inflammation. One study demonstrated that whereas systemic treatment with CTLA-4Ig had only marginal effects on EAE, the local delivery of CTLA-4Ig by adenoviral vectors was effective in ameliorating disease.[40] Other studies showed that anti-CD28 or CTLA4Ig used after the first clinical episode of disease, prevented additional relapses, thus suggesting that this approach may be useful even after the autoimmune disease is already established.[41,42]

The contradictory results derived from studies on co-stimulation highlight the complexity of the fine interactions among the different molecules at different stages of the disease and in different models of the disease.

Other co-stimulatory pathways

CD40/CD40L. Another recently characterized T-cell co-stimulatory pathway is the CD40/CD40L (CD154). CD40 molecules are expressed on the surface of APCs and B cells, and CD154 are expressed on the surface of activated T cells.[43,44] CD40 may provide a direct co-stimulatory signal for full T-cell activation. There is also evidence that engagement of CD40 and CD154 leads to upregulation of B7 expression on APCs.[45,46] The mechanisms of EAE inhibition by blockade of the CD28-B7 co-stimulatory pathway by the fusion protein CTLA4Ig or anti-B7 monoclonal antibodies (mAbs) have been investigated by several laboratories.[12,14,26,47,48] Blockade of CD28-B7 or CD40-CD154 pathways was successful in preventing or ameliorating ongoing disease in numerous other autoimmune disease models.[27,42,49–52] Furthermore, the approach of co-stimulatory signal blockade was successful in preventing transplant rejection.[53,54]

CTLA-4 plays a negative role in T-cell activation and represents a major regulator in the maintenance of peripheral tolerance. In fact, mice deficient for CTLA-4 develop fatal lymphoproliferative disorders.[10,11] The administration of anti-CTLA-4Ab favors T-cell expansion and autoimmune reactivity. Studies both, *in vivo* and *in vitro*, have demonstrated that CTLA-4 has a role in T-helper regulation favoring Th2 differentiation and promoting the secretion of high levels of IL-4 and IL-5. Furthermore, CTLA-4 seems to promote the expression of anti-apoptotic factors such as Bcl-x_L, playing a role in T-cell survival.[55] Therapeutic approaches using activating antibodies may be considered in the future to exploit the natural down-regulatory activity of CTLA-4.

CD2/LFA3. Several other co-stimulatory molecules have been described. CD2, the T-cell rosette receptor expressed on mature T cells, is able to bind leukocyte function-associated antigen 3 (LFA-3) or CD58, providing a co-stimulatory signal. Many attempts have been made to interrupt this signal to induce immune suppression. A soluble LFA-3-IgG1 fusion protein binds to CD2 preventing its interaction with LFA-3 expressed on APCs. This drug has been tested in clinical trials in patients with psoriasis and was well tolerated and efficacious in the treatment of this disease.[56]

LFA-1/ICAM. LFA-1 is another cell-adhesion molecule expressed on the surface of monocytes, macrophages, neutrophils and lymphocytes that bind several intercellular adhesion molecules (ICAM-1, ICAM-2,

ICAM-4 and ICAM-5) expressed on various cell subsets. The interactions between these molecules plays a key role in delivering co-stimulatory signals, recruiting cells to the site of inflammation and stabilizing the APC-T cell interaction. Trials on patients with psoriasis have shown that the monoclonal antibodies directed against LFA-1 ligands, such as ICAM-1 and ICAM-3, are effective in the treatment of the disease.[57–61]

VLA-4. The very late antigen 4 (VLA-4), an adhesion molecule expressed on most leukocytes, comprises two different polypeptides (i.e., CD49d and CD29). Their binding to cell surface proteins such as MadCAM-1 and ICAM-4 favors the recruitment of cells to the site of inflammation and provides co-stimulatory signal to the T cells. Several different inhibitors of VLA-4 have been evaluated. The most successful was natalizumab, a humanized antibody directed against α_4 integrin (CD49d) tested in MS, as well as Crohn's disease.[62–64] Natalizumab was approved by the United States Food and Drug Administration for the treatment of MS based on its ability to significantly reduce the relapse rate in patients with relapsing–remitting MS. However, progressive multifocal leucoencephalopathy (PML) developed in two patients receiving natalizumab in combination with interferon-beta-1a.[65,66] A third case of PML was identified in the Crohn's disease trial after a mistaken diagnosis of fatal astrocytoma. The analysis of frozen serum samples showed the presence of JC virus DNA three months after the initiation of the trial, and two months before the appearance of symptomatic PML.[67] The use of natalizumab has been resumed as monotherapy in patients with MS who are intolerant or not responding to other treatments.

PD-1/PDL-1. Programmed death 1 (PD-1) and its ligands (PDL-1 and PDL-2) are new members of the B7 family of co-stimulatory molecules. PD-1 is expressed on activated CD4+, CD8+, B cells and myeloid cells but not on naïve cells.[68] PDL-1 is constitutively expressed on freshly isolated splenic T cells, B cells, macrophages and dendritic cells. PDL-1 has also been detected in nonlymphoid organs in both mice and humans.[69,70] The engagement of PD-1, with its ligand PDL-1, causes inhibition of anti-CD3-induced activation of T cells particularly in suboptimal co-stimulatory condition and when weak signals are provided to the TCR.[71] The engagement of PDL-2 is also capable of reducing CD4+ T-cell activation and cytokine production after TCR stimulation.[72] However, other reports indicate that the PD-1 pathway can provide a positive co-stimulatory signal inducing T-cell proliferation as well as cytokine production.[73,74] The reasons for this discrepancy are unknown but could possibly be related to the existence of another ligand that delivers a positive signal to the T cell. It has been demonstrated that PD-1-deficient mice show increased T- and B-cell proliferation and higher predisposition to develop autoimmune diseases.[75] In the spontaneous model of diabetes (nonobese diabetic [NOD], mouse) the blockade of PD-1 and PDL-1 caused precipitation of the disease, whereas PDL-2 did not have any effect.[76] PD-1 blockade also triggered the onset of diabetes in male NOD mice that are normally resistant to the disease. PDL-1, but not PDL-2, was found expressed on inflamed pancreatic islets in NOD mice, suggesting a key role of this pathway in the induction and progression of autoimmune diabetes.

In the myelin oligodendrocyte glycoprotein-induced EAE model, blockade of PD-1, as well as PDL-2, provoked exacerbation of the disease and increased T-cell infiltration in the brain.[77] Little is known about the PD-1 pathway in humans. Studies conducted on patients with rheumatoid arthritis (RA) have shown that PD-1 is highly expressed on the CD4+ T cells isolated from synovial fluid. These cells express high levels of CTLA-4 and are able to secrete high levels of IL-10.[78] Several studies have demonstrated that PD-1 inhibition in CD28-deficient mice, normally resistant to EAE, induced worse disease than CTLA-4 blockade.[79]

In MS patients, the memory T lymphocytes subset had lower expression of PD-1.[80] PD-1 is markedly upregulated on the surface of exhausted virus-specific CD8+ T cells during chronic viral infections, such as human immunodeficiency virus (HIV)-1 and chronic lymphocytic choriomeningitis virus. In treatment naïve HIV patients, upregulated PD-1 expression on HIV-specific CD8+ T cells correlated with impaired HIV-specific CD8+ T-cell function. PD-1 expression on these cells correlated with plasma viral load and inversely correlated with CD4+ T-cell count, which are predictors of disease progression.[81] The correlations between PD-1 expression and clinical outcome were recently strengthened by the finding that long-term HIV nonprogressors exhibited functional HIV-specific memory CD8+ T cells with markedly lower PD-1 expression, whereas typical progressors showed upregulated PD-1 expression correlating with a reduction in CD4 T-cell number and increased plasma viral load.[82] Furthermore, PD-1 upregulation was associated with reduced perforin and interferon-gamma production, as well as decreased HIV-specific effector memory CD8+ T-cell proliferation in progressors. Blockade of this pathway reinvigorates the exhausted T cells, allowing them to expand and produce effector cytokines and reduce viral load.[82–85]

Given the essential role of co-stimulation in the activation of pathogenic T cells, the development of therapeutic strategies aiming at the selective blockade of co-stimulatory molecules has been a focal point of studies of autoimmune diseases. Blockade of co-stimulation would in fact have the advantage of targeting those T cells that have already received the first signal regardless of the specific antigen triggering the TCR.

TABLE 1. *Antibody Therapies in Humans*

Treatment	Target Molecule	Results	References
Abatacept	B71/B72	Improves RA and psoriasis	Moreland, et al. 2002 (106) Kremer, et al. 2005 (112) Abrams, et al. 2000 (114)
Belatacept	B71/B72	Prevents acute renal transplant rejection	Vincenti, et al. 2005 (115)
Natalizumab	VLA-4	Improves MS and Crohn's disease	Gordon, et al. 2001 (62) von Andrian, et al. 2003 (63) Polman, et al. 2006 (64)
Anti-PD-1	PD-1	Reinvigorates exhausted T cells in LCMV and HIV	Freeman, et al. 2006 (83) Trautmann, et al. 2006 (84) Petrovas, et al. 2006 (85) Zhang, et al. 2007 (82)
Anti-CD154	CD40-L	SLE and MS trials halted because of thromboembolic events	Nakamura, et al. 2006 (123)
SGN-40	CD40	Currently in clinical trial for the treatment of B-cell neoplasias	Tai, et al. 2005 (125) Kelley, et al. 2006 (124)

Abbreviations: PD, programmed death; LCMV, lymphocytic choriomeningitis virus; MS, multiple sclerosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SGN-40, humanized anti-CD40; VLA, very late antigen.

CO-STIMULATION DEPENDENCE OF NAÏVE AND MEMORY T CELLS

Several studies have shown that naïve T cells derived from TCR transgenic mice are fully dependent on the CD28/B7 co-stimulation to proliferate against specific antigens.[86–89] Conversely, inhibiting CD28/B7 interactions result in decreased proliferation and decreased number of activated cells divisions.[90,91] CD28, in fact, is important for the transcription of IL-2,[5,92] an essential T-cell growth factor; it promotes the expression of Bcl-x_L, an anti-apoptotic protein-sustaining cell survival both *in vivo* and *in vitro*,[93–96] and reduces the threshold of T-cell activation.[6] T-cell surface molecules are also adhesion molecules playing an important role in the stabilization of the immunological synapse.[97]

Unlike naïve T cells, primed as well as memory T cells are less dependent on co-stimulation,[98–101] probably due to the expression of lck, which favors a lower threshold for T-cell activation. It has been shown that the expansion of auto-reactive T cells in MS is independent of exogenous B7 co-stimulation. It has also been demonstrated that in patients with MS, myelin basic protein reactive T cells are less dependent on CD28 co-stimulation indicating a different state of activation of potentially pathogenic T cells.[101] The dependency on CD28 co-stimulation is also related to the strength of the signal delivered to the cells. T-cell activation induced by weak agonists or antigens provided at low concentration is dependent on co-stimulation.[102] Conversely, if strong agonists are available or high amounts of specific antigens are provided, the requirement for co-stimulation notably decreases.[98,101,103]

CO-STIMULATORY BLOCKADE TREATMENTS IN HUMAN AUTOIMMUNE DISEASES

In humans, one of the better characterized co-stimulatory blocking agents is CTLA-4Ig (see Table 1). CTLA-4Ig is able to bind B7-1 and B7-2 on APC, thus inhibiting their engagement to CD28 on T cells. In preclinical studies CTLA-4Ig was effective in preventing or reducing the severity of different autoimmune diseases in animal models as well as in humans[27,104–106] and in inducing islet transplant tolerance.[107] Despite these beneficial effects, CTLA-4Ig has also been shown to increase immunity under some circumstances in animals. CTLA-4Ig might in fact prevent the engagement of the negative regulator CTLA-4 promoting increased T-cell activation.[108] An additional reason explaining the dual effect of CTLA-4Ig is related to regulatory cells. Regulatory T cells are indeed dependent on CD28 for their development and survival, thus the use of CTLA-4Ig can induce a drastic reduction of this cell population and exacerbate the autoimmune process.[109,110] Despite the intricacies of co-stimulatory modulation, CTLA-4Ig has been developed and tested in patients with autoimmune diseases.

CTLA-4Ig was evaluated in humans for the treatment of rheumatoid arthritis (RA). A placebo-controlled study evaluated two different doses (2 or 10 mg/kg of body weight) of the fusion protein in more that 100 patients per group during a six-month period. CTLA-4Ig was efficacious in improving the signs and symptoms of RA and inhibiting the radiographic progression of the disease. According to criteria established by the American College of Rheumatology, significant improvements

were found only in the group treated with the highest dose of CTLA-4Ig, whereas the group treated with 2 mg of CTLA-4Ig was not considerably different from the placebo group.[106] A phase III study has also been conducted on patients with RA receiving methotrexate. The combination of methotrexate and CTLA-4Ig treatment showed significant, dose-dependent improvement in signs and symptoms of RA, as well as in quality of life of patients with active disease.[111,112] In light of these favorable results, CTLA-4Ig (abatacept) has been approved by the United States Food and Drug Administration for the treatment of RA.

CTLA-4Ig has been tested in patients with psoriasis, a T-cell-mediated skin disorder, in a phase I, open-label, dose-escalation trial.[113] Its effect on both T-cell co-stimulation and humoral responses to T-cell-dependent antigens was evaluated. Four infusions of CTLA-4Ig were given to 40 patients with stable disease. Forty-six percent of the patients, mainly in the highest dose cohorts, showed sustained clinical improvement. Reduction of epidermal hyperplasia correlating with a decrease in T-cell infiltrates and diminished epidermal proliferation were also observed.[114] No increase in T-cell apoptosis was identified in the skin lesions, suggesting that the decreased number of T cells was due to reduced proliferation and recruitment of T cells. CTLA-4Ig therapy did not induce long-term tolerance to T-cell dependent neo-antigens. Patients treated with CTLA-4Ig and immunized with two different neo-antigens (i.e., bacteriophage X174 and KLH immune activator) showed suppression of antibody titers only after the first two immunizations. Immune responses developed in most of the patients at the following immunizations, showing lack of permanent tolerance to these neo-antigens.

Belatacept (LEA29Y) differs from abatacept by substitution of two amino acids, which confers a stronger binding avidity to B7 and a greater inhibition of T-cell activation. The treatment with belatacept was as effective as cyclosporine in preventing acute rejection after renal transplant rejection and in helping preserve glomerular filtration rate, as measured by iohexol plasma clearance.[115]

MS is another T-cell-mediated disease in which co-stimulation seems critical to the initiation, as well as progression of the inflammatory process. The disease is characterized by the presence of perivascular, inflammatory infiltrates that lead to demyelination, traits also characteristic of EAE.[116,117]

Auto-reactive T cells are present in the peripheral circulation of both patients with MS and healthy individuals,[118] but only in the patients these cells are able to cross the blood-brain barrier and initiate the inflammatory process. MBP-reactive T cells derived from patients with MS are less dependent on B7-costimulation compared to cells from healthy individuals, and this can be expanded *in vitro* in the presence of blocking anti-CD28 monoclonal antibodies. The different requirement for B7 co-stimulation suggests that memory cells that are chronically exposed to CNS auto-antigens are in a different state of activation compared to cells from healthy individuals.[101]

A phase I, dose-escalation study to evaluate the safety and tolerability of CTLA-4Ig in patients with relapsing–remitting MS was completed at the Partners Multiple Sclerosis Center (Boston, MA). We evaluated the disease-specific safety of a single infusion of CTLA-4 Ig in patients with relapsing–remitting MS, as well as the effects on immune functions of these patients. The drug was well tolerated by patients at the different doses of the fusion protein. A group of subjects received four doses of CTLA4Ig that were also well tolerated.

Blockade of CD40-CD154 interaction is effective in preventing the induction of several autoimmune diseases in animal models of thyroiditis, arthritis, diabetes and oophoritis.[49,119–121] Both the initiation and the progression of EAE were inhibited by anti-CD154 controlling the expansion or the migration of Th1 pathogenic cells.[122] Unfortunately, studies in humans have not achieved the same success. Anti-CD154 trials were initiated in several autoimmune diseases, including a phase I study in MS, but the trials were all halted after the occurrence of thromboembolic events in lupus patients, probably due to reactivity of the monoclonal antibody with CD40L expressed on the surface of activated platelets.[123]

CD40 is expressed in B-cell malignancies and multiple solid tumors raising interest in its potential use as a target for antibody-based cancer therapy. SGN-40, a humanized monoclonal anti-CD40 antibody, inhibits B-cell tumor growth *in vitro* by mediating antibody-dependent cytotoxicity.[124]

SGN-40 has been shown to enhance the cytotoxic effect of lenalidomide against multiple myeloma (MM) cells by enhancing NK-cell-mediated lysis and upregulating CD40L on CD56(+)CD3(−) NK cells.[125,126] Stimulation through CD40 on mouse and human renal cell carcinomas promotes cytokine production and leukocyte recruitment, enhancing immune responses, and thus anti-tumor activity.[127]

CONCLUSIONS

Co-stimulatory pathways play a fundamental role in the initiation and progression of autoimmune diseases, by controlling T-cell activation. Several strategies aimed at blocking co-stimulatory pathways have been developed and tested both in animal models and humans. Unlike previous therapies targeting all T cells, co-stimulation-blocking therapies affect activated T cells and thus may target potentially pathogenic cells. These treat-

ments have shown better clinical efficacy and reduced side effects in patients with T cell-mediated autoimmune diseases. The successful results of CTLA-4Ig therapy in RA have inspired additional studies and suggest the potential use of this drug in other autoimmune disorders such as lupus and MS. Further investigations will be necessary to explore the mechanisms through which co-stimulatory blocking drugs exert their effects in humans. Animal studies have shown higher efficacy with the use of combination therapies using more than one blocking agent at once. Nevertheless, given the complexity of the co-stimulatory pathways we have to be cognizant of possible consequences of long-term therapy. It is unclear whether patients treated with co-stimulatory blocking agents will become more susceptible to opportunistic infections, particularly when used in combination therapies. However, data from the agents already in use do not suggest increased risk of infections and raise optimism for the future development of co-stimulation regulatory treatments.

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