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## Costimulation and T cells as therapeutic targets

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### Abstract

Full activation and differentiation of resting T cells into effector T cells requires at least two signals, the first through engagement of the T cell antigen receptor (TCR) by the antigen-MHC complex on antigen presenting cells (APC), and the second by engagement of co-stimulatory molecules such as CD28, on T cells by ligands such as CD80/86 on APCs. Effector T cell differentiation is associated with proliferation, secretion of cytokines, and expression of additional surface molecules. These inducible structures may have stimulatory (ICOS, OX40, and 4-1BB) or inhibitory (CTLA-4) potential. To the extent that T cells have a role in particular immune-mediated diseases, interruption of T cell co-stimulation is a potentially worthwhile approach to treatment of those conditions. This article summarizes experience in treating rheumatological disease by perturbation of T cell co-stimulation, and also describes structures that could be future targets for this type of therapeutic approach.

### CD28:CTLA-4 (CD152)/CD80:CD86

CD28 is the prototypic TCR for co-stimulatory signals. CTLA-4, which is up-regulated on activated T cells, also binds to the CD28 ligands CD80/86 (B7.1 and B7.2), and this interaction inhibits further T cell activation (1–4).

The ligation of CD28 by CD80/86 not only sends co-stimulatory signals into the T cell but can also send activating signals into the APC (5). Maturation and activation of APCs is accompanied by expression of other co-stimulatory molecules, as well as secretion of cytokines such as IL-6. Induction of IL-6 secretion from APCs via CD80/86 is of interest since IL-6 is one of the cytokines associated with the pathogenesis of rheumatoid arthritis (RA). IL-6 is necessary for the development of Th17 cells, which contribute to several autoimmune diseases including RA, and IL-6 also has downstream roles in tissue damage. This is only one example of the many ways in which co-stimulatory receptor-ligand engagement is linked to cytokine pathways in RA and other diseases. Therapeutic blockade of such interactions would be expected, therefore, to have secondary effects on key disease-related cytokines.

In addition to interfering with the binding of CD28 to CD80/86, thereby interrupting the co-stimulatory signal into T cells via CD28, CTLA-4 can also send inhibitory signals directly into the T cell that further limit T cell activation (6, 7). Likewise, the ligation of CD80/86 by CTLA-4 can also deliver regulatory signals to the APC. The interaction of CD80/86 with

CTLA-4 leads to the induction of indoleamine dioxygenase (IDO) in APCs. IDO is thought to be critical in inducing anergy in T cells, during the T cell-APC interaction, via the depletion of tryptophan, which is necessary for full T cell activation (8, 9). These findings suggest that CTLA-4 can actively dampen T cell activation both by direct effects on the T cell and also by effects on APCs.

Thus, the interaction of CD28 with CD80/CD86 is critical in the regulation of T cell activation and is an important target in autoimmune diseases associated with aberrant activation of T cells such as RA. However, use of antibodies against CD28 itself would be hazardous if agonistic effects occurred. Indeed, administration of TGN1412, an anti-CD28 monoclonal antibody, to healthy human volunteers resulted in acute onset of cytokine storm and associated multi-organ failure (10). This led to suspension of further development of this drug or of other antibodies directly targeting CD28.

CTLA-4Ig which is composed of CTLA-4 fused with the constant portion of the human IgG, binds to CD80/CD86 on APCs and thereby interferes with the co-stimulation of T cells induced by the interaction of CD80/CD86 with CD28. CTLA-4Ig was developed as a therapeutic intervention for RA and transplant rejection, two diseases associated with T cell activation. Preclinical studies with CTLA-4Ig showed promise in an animal model of RA (11). CTLA-4Ig (abatacept) was approved for treatment of RA in 2007.

### Clinical studies of CTLA-4Ig (abatacept) in RA

After a successful Phase I study where 50% of the patients achieved ACR 20 responses without any significant adverse events, a phase II study was done in patients with active disease despite standard doses of methotrexate. In this study, abatacept was administered concurrently with stable doses of methotrexate for 6 months. The ACR 20/50/70 responses were 60%, 36%, and 16% in the group receiving abatacept plus methotrexate compared to 25%, 12%, and 2% in the placebo plus methotrexate arm (12, 13). The patients in this trial continued to improve beyond the initial 6 month period, in an open label extension trial with abatacept and methotrexate for additional 6 months. The percentage of patients achieving ACR50 and ACR70 increased to 41.7% and 20.9 % respectively (14). The same cohort of patients continued to show improvement in clinical efficacy with an acceptable safety profile in a 5 year extension study. The ACR20/50/70 responses at 5 years were 82.7%, 65.4%, and 40% respectively (15).

The Abatacept Trial in Treatment of Anti-TNF Inadequate Responders (ATTAIN), a 6 month phase III trial, was done in patients with RA who had inadequate clinical response or adverse effects on anti-TNF agents (16). A significant proportion of patients demonstrated a favorable therapeutic response at 6 months. The percentage of patients achieving ACR20/50/70 responses were 50%, 20.3%, and 10.2% in the study arm, in comparison to 19.5%, 3.8%, and 1.5% in the placebo arm. This study was then continued as an open label extension for additional 18 months with continued improvement, with 56% achieving ACR20, 33% achieving ACR50, and 16% achieving ACR70 responses (17). These findings were confirmed in another trial involving patients with inadequate responses to anti-TNF agents. This study showed that a washout period, in previous anti-TNF non-responders, was not necessary for subsequent improvement with abatacept (18).

The sustained clinical efficacy of abatacept was shown in another trial in which abatacept was administered to patients who had inadequate responses to methotrexate, Abatacept in Inadequate Responders to Methotrexate (AIM) (19). This study was designed to evaluate clinical improvement as well as radiographic progression. In this 1 year study patients continued to show clinical improvement similar to previous studies with ACR 20/50/70 responses being 73%, 48.3% and 28.8%, in comparison to 39.7%, 18.2% and 6/1% in the

placebo group, respectively. Further, there was a 50% reduction in the progression of the Genant Modified Sharp scores in the abatacept group. As in the previous studies, patients in this study were continued for an additional 12 months in an open label extension study. There was continued clinical and radiographic improvement into the end of the second year.

Abatacept was directly compared with infliximab or placebo in the Tolerability, Efficacy and Safety in Treating Rheumatoid Arthritis (ATTEST) trial. This 12 month study demonstrated that patients treated with infliximab had more rapid clinical improvement than patients on abatacept during the initial 6 months, but patients on abatacept continued to have the same rate of clinical response throughout the 1 year period of the study and the clinical response at 1 year was therefore significantly greater than in the infliximab group (DAS28 scores changed by  $-2.88$  in the abatacept group vs  $-2.25$  in the infliximab group) (18). It is important to point out that while abatacept was used at 10 mg/kg, which is the optimal dosage used in clinical practice, infliximab was used at 3 mg/kg, which is on the lower end of the recommended dosage of 2 mg/kg-8 mg/kg. The therapeutic efficacy of abatacept in comparison to higher dosages of infliximab or to other anti-TNF agents remains to be evaluated. In a recent study, involving patients with early RA, administration of abatacept in combination with methotrexate was associated with a significantly increased rate of remission compared to administration of methotrexate alone, at 1 year of follow up. Forty-one percent in the abatacept plus methotrexate group achieved DAS28 defined remission (DAS28 $<2.6$ ) compared to 23.3% in the methotrexate only group (22).

Abatacept demonstrated a favorable safety profile in all of the above trials. Two trials were designed to assess the safety of abatacept in combination with other agents. The Abatacept Study of Safety in Use with other RA Therapies (ASSURE) trial was designed to evaluate the safety of abatacept for use in patients receiving DMARDs, as well as biologics (23). Patients receiving abatacept along with other non-biologic DMARDs demonstrated a favorable safety profile. On the other hand, combination of abatacept with other biologics was associated with an increased rate of infections (23). In another trial abatacept was administered along with etanercept (24). Patients on the combination did not show significant clinical improvement over and above those treated with a single biologic agent, but had more infections. These studies demonstrate that abatacept has a favorable safety profile when administered with non-biologic DMARDs, but the combination of abatacept with another biologic agent is not recommended.

The above studies illustrate the efficacy of abatacept in the treatment of RA, in various scenarios including early RA, methotrexate non-responders, and anti-TNF failures. Additionally it has been shown to be consistently safe in multiple studies, except in combination with other biologic agents.

Although CTLA-4Ig (abatacept) was developed to reduce the activating signal into the T cell via CD28, the precise mechanisms of action underlying the therapeutic response in RA remain to be elucidated. In one study serum IL-6 was reduced in RA patients on CTLA-4Ig, suggesting that interruption of the activation/maturation signal into APCs via CD80/86 might be important (25). In another study, levels of IFN- $\gamma$ , IL-6, IL-1 $\beta$ , and MMPs were reduced in the RA synovium of patients receiving abatacept. In the same patients, the levels of RANK/RANKL, which interact to induce osteoclastogenesis, were lowered, while OPG (osteoprotegerin), a decoy receptor which inhibits bone damage by interfering with RANK/RANKL, tended to increase (26). Thus it is possible that abatacept has direct as well as indirect effect on both T cells and other immune system cells in RA.

### **Belatacept – a modified CTLA-4Ig**

Belatacept, the second generation modified version of abatacept, is composed of an altered extracellular domain of CTLA-4 fused to an altered Fc portion of human IgG. The Fc portion contains two mutations that reduce its ability to bind to Fc receptors. The CTLA-4 component of belatacept bears two mutations that increase binding affinity to CD80 and CD86. Belatacept binds to both CD80 and CD86, but with a greater affinity for CD80. However the binding to both CD80 and CD86 on APCs is required for the full suppressive effect of belatacept (27, 28). This drug has shown promise in clinical trials in renal transplantation (29). A phase II trial has been completed in RA and results are pending (NCT00279760).

### **OX40 (CD134)/OX40L (CD252)**

OX40 is predominantly expressed on activated CD4 and CD8 T cells, following stimulation via TCR and CD28, and its ligand, OX40L is expressed on dendritic cells, B cells, macrophages, and endothelial cells. (30). Pro-inflammatory cytokines IL-1, IL-2 and TNF can further augment the expression of OX40. OX40L is induced on APCs after stimulation via Toll Like Receptors as well as CD40 (31, 32). There is bidirectional activation of T cells and APCs via the OX40-OX40L pathway. Stimulation of OX40 on T cell induces proliferation and cytokine secretion and signaling via OX40L into APCs induces the secretion of pro-inflammatory cytokines by the APCs. (31). Targeting of this interaction with anti-OX40L antibody or liposomes coated with anti-OX40 antibody has been shown to reduce severity of arthritis in animal models of RA (33–35).

In RA there is increased expression of OX40 on peripheral blood CD4 T cells with a trend towards a positive correlation with serum CRP levels. OX40 as well as OX40L expressing cells are also present in increased numbers in RA synovium. OX40 is expressed on both CD4 and CD8 positive synovial T cells, and the levels of expression correlate with disease activity (34, 36–38).

The interaction of OX40 with OX40L is important in RA in the context of T cell interactions with endothelial cells as well as B cells. Ligation of OX40 on activated T cells by OX40L on endothelial cells is thought to facilitate egress of activated T cells from the peripheral circulation into the target tissue (39), while ligation of OX40 by OX40L on B cells induces proliferation and immunoglobulin secretion by the B cells (40, 41).

Although there is increased expression of OX40 and OX40L in RA synovium, and preclinical studies in animal models, involving abrogation of this interaction, have shown promise, much remains to be elucidated regarding the role of OX40/OX40L in mediating immune events underlying RA. Currently there is an ongoing phase II trial involving anti-OX40L antibody in patients with asthma (NCT00983658).

### **ICOS (CD278)/ICOSL (CD275)**

ICOS/ICOSL belongs to the CD28 family of co-stimulatory molecules. ICOS is predominantly expressed on CD4 and CD8 T cells following activation by CD28. ICOSL is predominantly expressed on B cells. Studies in mice as well as in humans have shown that ICOS signaling into the T cells induces IL-4 and IL-10 secretion from T cells.

ICOS deficient mice have attenuated IgM and IgG responses, as well as reduced levels of the inflammatory cytokine IL-17, and are resistant to inflammatory arthritis, suggesting that this pair can also mediate bi-directional signaling, into the B cell as well as the T cell (42). In keeping with this finding, administration of anti-ICOSL antibody resulted in attenuation

of CIA with associated reduction in anti-collagen antibody responses and reduced production of pro-inflammatory cytokines TNF $\alpha$ , IL-1, and IL-6 (43).

ICOS expression is increased on CD4 memory T cells in the synovial fluid and ICOSL is expressed in the synovial tissue in RA. Stimulation of the synovial fluid T cells via ICOS is associated with increased cytokine production (44, 45). Monocytes from RA synovial fluid have augmented expression of ICOSL as well as ICOS (45). Binding of ICOSL on monocytes to ICOS on T cells, within the inflamed joint, could provide costimulatory signals to the T cells. Efficacy of blocking ICOS-ICOSL interactions in RA remains to be established, but an anti-ICOSL antibody is currently undergoing a phase I trial in patients with SLE (NCT00774943).

#### **4-1BB (CD137)/4-1BBL(CD137L)**

4-1BB is induced on CD4 and CD8 T cells following stimulation via the TCR and CD28. The ligand for 4-1BB, 4-1BBL is expressed on activated B cells, dendritic cells and macrophages. Additionally, under certain circumstances dendritic cells express 4-1BB and T cells express the 4-1BBL (46).

The functional consequences of ligation of this pair are complex. The duration of expression of these molecules can be transient or sustained, depending on the cell type and the immune microenvironment. The effects of signaling via 4-1BB into CD4 T cells are distinct from signaling into CD8 T cells, and the *in vitro* effects often do not corroborate with *in vivo* effects. Most studies agree that ligation of 4-1BB sends a co-stimulatory signal into the CD4 T cell and is associated with induction of cytokine production and enhanced T cell activation and survival, especially in the absence of CD28 signaling. Similar to its effects on the CD4 T cell, ligation of 4-1BB also sends co-stimulatory signal into the CD8 T cell (46).

Interestingly, administration of an agonistic anti-4-1BB antibody resulted in amelioration of inflammatory arthritis with associated reduction of B cell responses, and expansion of an IFN- $\gamma$ -producing CD8 T cell population in the murine model of CIA (47, 48).

In addition to the membrane bound forms, 4-1BB and 4-1BBL can also be present in soluble forms in the serum. Both s4-1BB and s4-1BBL levels are elevated in RA sera and those levels positively correlate with each other and with disease severity. In this same study the expression of membrane 4-1BB was not elevated in RA (49, 50). This suggests that the expression and secretion of 4-1BB and its ligand are differentially regulated and adds more complexity to the interpretation of the *in vivo* role of these structures. Currently there is an ongoing phase II study involving anti-4-1BB antibody in patients with melanoma (NCT00612664).

#### **CD4 (OKT4, Leu 3a, T4)**

CD4 is a type I transmembrane glycoprotein and a member of the Ig superfamily. It is a coreceptor in MHC class II antigen-restricted T-cell activation, involved in stabilizing the MHC/TCR complex (51). In addition to MHC class II, ligands for CD4 include viral gp120 protein of HIV retroviruses, IL-16 and gp17. CD4 is also involved in regulation of T-B lymphocyte adhesion in the absence of antigen recognition (52). CD4 is a receptor for IL-16 and binding of IL-16 induces a migratory response in Th1 CD4 T cells. CD4 is expressed on helper T cells and some myeloid cells.

Monoclonal antibodies to CD4 failed to show efficacy in RA. Administration was associated with infusion reactions, including low-grade fever, flu-like symptoms and unpredictable occurrence of a skin rash and/or vasculitis (53–55). Additionally, infusion resulted in rapid

decline of CD4 T cells which remained depressed even six months post treatment (56). Further development of these antibodies for treatment of RA was abandoned.

### **CD5/CD72 (Tp67, T1, Ly1, Leu-1)**

CD5 is a type I transmembrane glycoprotein expressed by most T cells and some B cells. The putative ligand of CD5 in humans is CD72. CD5 can transmit both inhibitory and stimulatory signals depending on the cell type and its maturation stage (51). CD5 transmits inhibitory signals in thymocytes and B1a cells but is a co-stimulatory signal receptor on mature peripheral T cells (51). Expression of CD5 regulates responsiveness of human T cells to IL-1 (52). The B1a CD5-positive population is expanded in RA and Sjogren's syndrome (52).

Anti-CD5 antibody treatment had a partial therapeutic effect in CIA in DBA/1 mice (57). When an anti-CD5 antibody was covalently linked to the A chain of ricin, binding was followed by internalization and intracellular toxin release. Early patient studies of an anti-CD5 ricin-linked immunoconjugate demonstrated rapid depletion of CD5 cells but considerable toxicity including fevers, prostration, fluid retention and myalgias (58, 59). When tested in placebo-controlled trials at a lower dose in patients with RA, T cell depletion was neither marked nor prolonged and the treatment failed to produce a clinical response (60).

### **CD40- CD40ligand (Bp50, TNF receptor 5) – ligand CD154 (CD40L)**

CD40 is a type I integral membrane glycoprotein that is expressed on APCs and is the receptor for CD40L expressed on activated CD4 T cells, CD8 T cells, mast cells, NK cells, monocytes and activated platelets. Binding of CD40L provides a co-stimulatory signal to B cells, promoting growth, differentiation and isotype switching (51). Binding of CD40L by CD40 transmits activating signals to T cells and APCs to express B7 molecules, which in turn stimulates further T-cell proliferation (61).

IDEC-131 and BG9588 are humanized monoclonal antibodies to CD40L. BG9588 was given to 28 patients with active proliferative lupus nephritis, resulting in a reduction in anti-dsDNA titers and proteinuria. The trial was stopped early due to the occurrence of thromboembolic events, including two nonfatal myocardial infarctions, which may have been due to the expression of CD40L on platelets (62, 63). IDEC-131, when tested in a placebo-controlled trial in patients with mild-to-moderate active systemic lupus erythematosus, demonstrated no thromboembolic events but no clinical activity (64). Further development of these antibodies was not pursued.

### **LFA-3 (CD58)/CD2**

LFA-3 is a member of the CD2 family within the Ig superfamily that occurs as a glycoposphatidylinositol-anchored and type I integral membrane protein (51). LFA-3 is the ligand for CD2 and mediates adhesion between killer and target cells and in cell-mediated cytotoxicity. Adhesion through CD2 occurs between APCs and T lymphocytes, thymocytes and thymic epithelial cells, and T lymphocytes and erythrocytes.

Alefacept is a dimeric fusion protein consisting of an antigen, 1-92-LFA-3 (CD58) fused with the Fc portion of IgG1. By inhibition of LFA-3/CD2 interactions, alefacept can inhibit immune processes which rely on T lymphocyte activation in a non-selective manner. Alefacept is approved for the treatment of adult patients with moderate to severe chronic plaque psoriasis who are candidates for systemic therapy or phototherapy. Alefacept should not be administered to patients infected with HIV as it can reduce CD4 T lymphocyte

counts, potentially accelerating disease progression or increasing complications of disease in these patients

(<http://www.fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/ucm150623.htm> accessed 9/23/09).

## CD11a/CD18

CD11a is a type I integral membrane glycoprotein and a member of the integrin  $\alpha$ -chain family. CD11a complexes with CD18 to form leucocyte function associated antigen 1 (LFA-1). Ligands for LFA-1 are CD54 (ICAM-1), CD102 (ICAM-2), CD50 (ICAM-3), ICAM-4, ICAM-5 and JAM-1 (CD321). LFA-1 is an important adhesion and signal transduction molecule involved in inflammation (51).

Efalizumab is a recombinant humanized IgG<sub>1</sub> monoclonal antibody that binds to CD11a, the  $\alpha$ -subunit of LFA-1. LFA-1 binds to ICAM-1, facilitating the migration of T lymphocytes through the endothelial lining during inflammation. Binding of efalizumab to LFA-1 prevents lymphocyte migration through the endothelium, decreasing lymphocyte trafficking to target organs during inflammation. Efalizumab was approved for the treatment of moderate to severe plaque psoriasis in 2003 in the United States and in 2004 in Europe. In February 2009, the FDA issued a Public Health Advisory to notify healthcare professionals of three confirmed, and one possible report of progressive multifocal leukoencephalopathy (PML) caused by reactivation of latent JC virus infection that had occurred in patients receiving efalizumab. Three patients died. Simultaneously, the EMEA recommended suspension of marketing approval of efalizumab. In April 2009, the manufacturer began the voluntarily withdrawal of efalizumab from the worldwide market citing an unfavorable balance between the increased risk of PML and the benefits of treatment of plaque psoriasis ([http://www.gene.com/gene/products/information/pdf/raptiva\\_withdrawal\\_dhcp.pdf](http://www.gene.com/gene/products/information/pdf/raptiva_withdrawal_dhcp.pdf) accessed 9/23/2009).

## CD49d (Integrin alpha-4-chain, VLA-4-alpha chain)

CD49d is a type I transmembrane glycoprotein which non-covalently associates with CD29 molecules to form VLA-4 (integrin  $\alpha$ 4/ $\beta$ 1) and with the  $\beta$ 7 integrin subunit to form the  $\alpha$ 4/ $\beta$ 7 integrin. CD49d regulates multiple inflammatory responses by enhancing adhesion to and rolling of lymphocytes on vascular endothelium by binding to VCAM-1, allowing lymphocyte migration from circulation into tissue (51). CD49d, when associated with  $\beta$ 7 integrin, is involved in the homing of T cell subsets to gut mucosal tissue via interaction with MAdCAM-1.

Natalizumab is a humanized IgG<sub>4</sub> monoclonal antibody that binds to the  $\alpha$ 4 subunit of  $\alpha$ 4 $\beta$ 1 and  $\alpha$ 4 $\beta$ 7 integrins and prevents binding to their respective endothelial receptors, VCAM-1 and MAdCAM-1. The inhibition of binding to VCAM-1 is believed to prevent the movement of T lymphocytes across the endothelial-cell basal lamina and into the CNS parenchyma, an important step in the initiation of lesions in multiple sclerosis (65). Presumably, inhibition of binding to MAdCAM-1 prevents T cell extravasation to the gut, decreasing inflammation in the inflammatory bowel disease. Natalizumab is approved for the treatment of relapsing multiple sclerosis and Crohn's disease in the United States and relapsing multiple sclerosis in Europe. The biological effect seen with natalizumab in patients with RA receiving methotrexate was not sufficient to warrant further development for RA (NCT00083759).

Natalizumab was approved in November 2004 by the FDA and was withdrawn from the market by its manufacturer in 2005 after it was linked to two cases of PML when administered in combination with interferon beta-1a in the treatment of multiple sclerosis

and one case of PML in a patient with Crohn's disease (66–68). The drug returned to the market in 2006 under a special risk management plan, called the TYSARBI-TOUCH Prescribing Program. All patients receiving natalizumab therapy in the United States must be enrolled and closely monitored for the occurrence of PML and other serious opportunistic infections. From July 2006 to September 8, 2009, 13 reported cases of natalizumab related PML were confirmed worldwide in patients being treated for multiple sclerosis and the risk for developing PML appears to increase with the number of natalizumab infusions received.

(<http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm107198.htm> accessed 9/23/2009). Physicians and patients need to be aware of the potential for immunosuppression and risk of PML. All cases of PML and serious infections should be reported to MedWatch, the FDA safety information and adverse event reporting program, to identify risk factors for PML and other serious infection associated with natalizumab.

### **CD97/CD55/chondroitin sulfate**

CD97 is a transmembrane protein belonging to the secretin receptor superfamily, G-protein-coupled receptor family and the epidermal growth factor-seven-span transmembrane family (EGF-TM7) of molecules. CD97 is expressed on activated T and B cells and constitutively by monocytes, macrophages, dendritic cells and granulocytes. CD97 binds CD55 and chondroitin sulfate (51). EGF domains mediate CD97 interactions with different ligands, with CD55 interacting with EGF domains 1 and 2, and chondroitin sulfate interacting with EGF domain 4 (51). Increased levels of soluble CD97 have been found in RA synovial fluid by ELISA, and binding of CD97 to CD55 has been observed in RA synovial tissue utilizing binding assays with multivalent fluorescent probes (69–71). A monoclonal, neutralizing antibody to CD97, mAb 1B2, was effective in preventing murine collagen-induced arthritis (72). Inhibition of CD97 has not been attempted in human clinical trials.

### **CD52**

CD52, a highly N-glycosylated protein, C-terminally anchored in the membrane via glycosylphosphatidylinositol, is highly expressed on the surface of thymocytes, CD4 and CD8 lymphocytes, B lymphocytes, monocytes, macrophages, NK cells, dendritic cells and eosinophils (51). Alemtuzumab (Campath-1H) is a humanized IgG<sub>1</sub> kappa monoclonal antibody to CD52 and is FDA approved for the treatment of B-cell chronic lymphocytic leukemia. In 2005, the FDA issued an alert as three patients in a clinical study of alemtuzumab in multiple sclerosis developed severe idiopathic thrombocytopenic purpura, one of whom died. Alemtuzumab is not approved for use in multiple sclerosis. Alemtuzumab was tested in RA and caused a dose-dependent cytokine release syndrome and persistent CD4 T cell lymphopenia (73, 74). Additionally, impairment of innate immunity resulted from monocyte and NK cell depletion and was associated with two fatal opportunistic infections in patients with RA (75, 76). Alemtuzumab is not FDA approved for use in RA.

### **CD27/CD70 (CD27 ligand)**

CD70 is a type II transmembrane glycoprotein member of the tumor necrosis factor superfamily. CD70 is expressed on activated B cells, T cells, NK cells and dendritic cells. CD70 binds CD27 which is constitutively expressed on resting T cells and is further up-regulated upon T cell activation. CD70-CD27 plays an important role in T cell priming by inducing proliferation of co-stimulated T cells, enhancing the generation of cytolytic and memory T cells.



Increased numbers of circulating CD70 positive CD4 T cells have been observed in RA and systemic lupus erythematosus patients. CD70 over-expression on T cells in SLE patients is due to hypomethylation of DNA sequences that flank the CD70 promoter leading to an inability to down-regulate CD70 expression once it is induced by activation of T cells (77–79). An anti-CD70 antibody, used before or after the onset of arthritis in the murine collagen induced arthritis model resulted in improvement in disease severity and reduction of autoantibodies (80). Aberrant CD70 has been detected on a number of hematologic malignancies and some carcinomas, and is being explored as a target for therapeutic antibodies in preclinical trials (81).

## Summary

Inhibition of T cell co-stimulation is a promising but complex approach to the treatment of immune-mediated diseases. Many of the co-stimulatory molecules discussed are not specific for T cells and are expressed on other cell lineages. For this reason, interference with these molecules could lead to off-target effects, such as induction of thromboembolic events in patients with SLE by anti-CD40L due to CD40L expression on platelets. Multiple arms of host defense mechanisms are required to combat some infections and weakening them can lead to emergence of opportunistic pathogens, for example the occurrence of JC virus/PML following use of various biologics.. Targeting of diverse molecules and pathways has led to cases of PML in patients receiving rituximab (anti-CD20), natalizumab (anti-CD49d) and efalizumab (anti-CD11a), all presumably through different mechanisms but with the same deleterious outcome. The specific infections that will occur due to inhibition of various co-stimulatory pathways are not currently accurately predictable, and therefore careful observation of all patients treated with biologics that block co-stimulatory pathways is of paramount importance.

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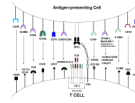
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**Figure 1.**

Co-stimulatory receptor-ligand pairs present on T cells and antigen presenting cells. On the T cell CD4, CD2, CD5, CD28, CD11a-CD18, CD49d-CD29/CD49d- $\beta$ 7 and CD27 are constitutively expressed. CTLA-4, OX40, ICOS, 4-1BB, CD40L (CD154) and CD97 are upregulated on the T cell surface following activation via the T cell receptor.

**Table 1**

Cellular distribution of costimulatory molecules on T cells and their corresponding receptor or ligand on accessory cells.

Costimulatory molecule on T cells.	Expression profile of molecule on T cell surface	Corresponding ligand/receptor	Cells which express the corresponding ligand/receptor	Expression profile of corresponding receptor/ligand
CD28/CTLA-4 (resting and activated, and memory T cells)	Constitutive/CTLA-4 only induced after activation	CD80/86	Antigen presenting cells, and on occasional activated T cells	Constitutive, further upregulated after activation/maturation.
OX40 (activated T cells, memory T cells)	Expressed following activation via TCR and CD28	OX40L	Dendritic cells, macrophages, and B cells	Expressed following activation and maturation.
ICOS (activated T cells, and CD4 memory T cells in RA)	Induced following activation via TCR and CD28	ICOSL	B cells and monocytes	Constitutive and further augmented following activation and maturation.
4-1BB (activated CD4 and CD8 T cells)	Induced following activation via TCR and CD28	4-1BBL	B cells, dendritic cells, and macrophages	Expressed following activation and maturation.
CD4 (T helper cells)	Constitutive	MHC II	Antigen presenting cells	Constitutive, further upregulated after activation
CD5 (thymocytes, T cells)	Constitutive	CD72	Antigen presenting cells	Constitutive
CD154 (CD40L) (activated T cells)	Upregulated after activation	CD40	B cells, monocytes, dendritic cells, epithelial cells, endothelial cells, fibroblasts, keratinocytes	Constitutive and upregulated following activation.
CD2 (thymocytes, T cells)	Constitutive	CD58	Leukocytes, erythrocytes, endothelial and epithelial cells, fibroblasts and smooth muscle	Constitutive, further upregulated by activation
CD11a-CD18 (LFA-1) (memory T cells)	Constitutive	CD54 (ICAM-1), CD102 (ICAM-2), CD50 (ICAM-3), CD242 (ICAM-4), ICAM-5, CD321 (JAM-1)	Activated T cells, activated B cells, monocytes, epithelial cells, endothelial cells and fibroblasts	Constitutive and further upregulated after activation
CD49d-CD29/CD49d- $\beta$ 7 integrin (activated T cells)	Constitutive, upregulated after activation	VCAM-1/VCAM-1, MadCAM-1	Endothelial cells,	Upregulated upon activation
CD97 (T cells)	Upregulated by activation	CD55	Wide expression including erythrocytes	Constitutive
CD27 (thymocytes, T cells)	Constitutive, upregulated after activation.	CD70	T cells, B cells, NK cells	Upregulated after activation



**Table 2**

The outcome of targeting co-stimulatory receptor-ligand pairs in preclinical animal models as well as in human diseases.

Receptor/ligand targeted	Human/animal studies reported	Outcome
CD28:CTLA-4/CD80:CD86	<ul style="list-style-type: none"> <li>Anti-CD28 antibody.</li> <li>Phase I trial in healthy volunteers</li> </ul>	<ul style="list-style-type: none"> <li>Infusion was associated with acute onset, severe cytokine storm and multiorgan failure. Trial suspended and drug withdrawn.</li> </ul>
	<ul style="list-style-type: none"> <li>CTLA-4Ig (Abatacept) Preclinical studies in animals and several clinical trials in patients with RA</li> </ul>	<ul style="list-style-type: none"> <li>Significant therapeutic response and acceptable risk profile.</li> <li>FDA approved for treatment of RA.</li> </ul>
	<ul style="list-style-type: none"> <li>Modified CTLA-4Ig (Belatacept)</li> <li>Phase II trial in patients with RA.</li> </ul>	<ul style="list-style-type: none"> <li>Trial completed. Results pending.</li> </ul>
OX40/OX40L	<ul style="list-style-type: none"> <li>OX40 expression is enhanced during arthritis in animal models.</li> <li>OX40 and OX40L expression on synovial T cells is increased in RA.</li> <li>Anti OX40 and anti-OX40L antibody has been tested in the animal model of RA.</li> </ul>	<ul style="list-style-type: none"> <li>Anti-OX40L antibody and liposome coated with anti-OX40 antibody has been shown to reduce severity of arthritis in animal models of RA.</li> </ul>
ICOS/ICOSL	<ul style="list-style-type: none"> <li>ICOS deficient mice have reduced arthritis.</li> <li>Expression of ICOS/ICOSL is increased in the RA synovium and synovial fluid.</li> <li>Anti ICOSL antibody has been tested in an animal model of RA.</li> <li>A phase I trial with anti ICOSL antibody is underway in patients with RA.</li> </ul>	<ul style="list-style-type: none"> <li>Anti-ICOSL antibody resulted in reduced severity of arthritis in animal models.</li> </ul>
4-1BB/4-1BBL	<ul style="list-style-type: none"> <li>Increased expression of 4-1BB in the RA synovium.</li> <li>Increased levels of s4-1BB and s4-1BB L in RA synovium and synovial fluid.</li> <li>An agonistic anti-4-1BB antibody was tested in an animal model of RA.</li> </ul>	<ul style="list-style-type: none"> <li>Administration of agonistic anti 4-1BB antibody was associated with reduced severity of arthritis in animal models of RA.</li> </ul>
CD4/MHC Class II	<ul style="list-style-type: none"> <li>Phase I and II clinical trials in patients with RA.</li> </ul>	<ul style="list-style-type: none"> <li>Failed to show therapeutic response in patients with RA.</li> </ul>
CD5/CD72	<ul style="list-style-type: none"> <li>Tested in animal model</li> </ul>	<ul style="list-style-type: none"> <li>A partial therapeutic effect in was seen in the mouse model of RA.</li> </ul>
CD40/CD40L	<ul style="list-style-type: none"> <li>Anti CD40L antibody -- BG9588 and IDEC 131 Clinical trials have been done in patients with systemic lupus erythematosus.</li> </ul>	<ul style="list-style-type: none"> <li>Failed to show response in patients with systemic lupus erythematosus.</li> </ul>
CD2/CD58	<ul style="list-style-type: none"> <li>Alefacept is a fusion protein with 1-92-LFA-3 (CD58) fused with an immunoglobulin IgG1 dimer.</li> <li>Clinical trials have been done in patients with psoriasis.</li> </ul>	<ul style="list-style-type: none"> <li>Significant therapeutic improvement. Alefacept is FDA approved for treatment of moderate to severe chronic plaque psoriasis.</li> </ul>

Receptor/ligand targeted	Human/animal studies reported	Outcome
CD11a-CD18/CD54 (ICAM-1), CD102 (ICAM-2), CD50 (ICAM-3), CD242 (ICAM-4), ICAM-5, CD321 (JAM-1)	<ul style="list-style-type: none"> <li>Efalizumab is a recombinant humanized IgG<sub>1</sub> monoclonal antibody that binds to CD11a. Clinical trials have been done in patients with psoriasis.</li> </ul>	<ul style="list-style-type: none"> <li>Efalizumab was approved for moderate to severe plaque psoriasis in 2003.</li> <li>Withdrawn from the market in 2009.</li> </ul>
CD49d -CD29, CD49d-β7 integrin/VCAM-1, MAdCAM-1	<ul style="list-style-type: none"> <li>Natalizumab is a humanized IgG<sub>4</sub> monoclonal antibody that binds to the α4 subunit of α4β1 and α4β7 integrins.</li> <li>Clinical trials have been done in patients with multiple sclerosis, Crohn's disease, and RA.</li> </ul>	<ul style="list-style-type: none"> <li>Natalizumab is FDA approved for the treatment of relapsing multiple sclerosis and Crohn's disease.</li> <li>Failed to show response in patients with RA.</li> </ul>
CD97/CD55 and chondroitin sulfate	<ul style="list-style-type: none"> <li>Soluble CD97 found in synovial fluid of rheumatoid arthritis patients and binding of CD97 to CD55 has been observed in synovial tissue of patients with RA.</li> <li>Tested in animal model of RA</li> </ul>	<ul style="list-style-type: none"> <li>A monoclonal, neutralizing antibody to CD97, mAb 1B2, was effective in preventing joint inflammation in an animal model of RA.</li> </ul>
CD52 (Campath-1H)	<ul style="list-style-type: none"> <li>Alemtuzumab is a humanized IgG<sub>1</sub> kappa monoclonal antibody to CD52. Clinical trials have been done in patients with hematologic malignancies and RA.</li> </ul>	<ul style="list-style-type: none"> <li>Alemtuzumab is FDA approved for the treatment of B-cell chronic lymphocytic leukemia.</li> <li>Alemtuzumab caused dose-dependent toxicity in a trial in RA, and is not FDA approved for use in RA.</li> </ul>
CD27/CD70	<ul style="list-style-type: none"> <li>Tested in animal model</li> </ul>	<ul style="list-style-type: none"> <li>A murine anti-CD70 antibody resulted in improvement in disease severity and reduction of autoantibodies in the animal model of RA.</li> </ul>

**Table 3**

Toxicities associated with therapeutics designed to target respective receptor/ligand pair in human diseases.

Receptor/ligand targeted	Agent	Observed or anticipated toxicities
CD28:CTLA-4/CD80:CD86	TGN1412 (anti CD28 antibody)	Cytokine storm and multiorgan failure.
	Abatacept	Minimal side effect profile. Increased risk of infections in combination with anti-TNF agents.
	Belatacept	Minimal side effect profile.
CD4/MHC Class II	Zanolimab	Infusion reactions with low-grade fever, flu-like symptoms, unpredictable occurrence of a skin rash and/or vasculitis, and rapid, prolonged decline of CD4+ T cells
CD40/CD40L	BG9588	Thromboembolic events in early trials. Further development of this drug is halted.
CD2/CD58	Alefacept	Lymphopenia, possible increased risk of malignancies, infections, and liver injury.
CD11a-CD18/CD54 (ICAM-1), CD102 (ICAM-2), CD50 (ICAM-3), CD242 (ICAM-4), ICAM-5, CD321 (JAM-1)	Efalizumab	Infections, thrombocytopenia, hemolytic anemia, and progressive multifocal leukoencephalopathy. Withdrawn from the market.
CD49d -CD29, CD49d- $\beta$ 7 integrin/VCAM-1, MadCAM-1	Natalizumab	Progressive multifocal leukoencephalopathy, liver injury, infections, and hypersensitivity reactions. Withdrawn and reintroduced under a special risk management plan in patients with MS.
CD52	Alemtuzumab	Cytopenias: serious, including fatal, pancytopenia/marrow hypoplasia, autoimmune idiopathic thrombocytopenia, autoimmune hemolytic anemia, infusion reactions, and infections during trials done in RA and multiple sclerosis. Did not receive FDA approval.