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MINIREVIEWS

Targeting CD40L: a Promising Therapeutic Approach

Dimitris Daoussis, Andrew P. Andonopoulos, and Stamatis-Nick C. Liossis*

Division of Rheumatology, Department of Internal Medicine, Patras University Hospital, University of Patras Medical School, Patras, Greece

The activation of lymphocytes is a central event of the adaptive immune response. Physiologically, this activation is carefully controlled. Productive stimulation of T cells is necessary for all T-cell-dependent immune responses and requires two distinct intracellular signals.

The first signal is antigen specific; it is delivered via the antigen-specific T-cell surface receptor (TCR) when antigen is properly presented to the T cell in the context of major histocompatibility complex molecules, found on the cell membrane of antigen-presenting cells (APC). APC are not just passive antigen presenters but they are also responsible for providing the second signal. This second signal is necessary for full and productive T-cell activation, and the process is referred to as costimulation. Occupancy of the TCR alone without a costimulatory signal does not lead to productive T-cell activation. Such T cells are unable to sustain proliferation and often undergo apoptosis, fail to produce cytokines, and become unresponsive to subsequent activation, entering a state called anergy. Initially, it was thought that soluble factors, such as cytokines, were the key transmitters of costimulatory signals. Later, it became apparent that costimulation is a cognate process. Costimulatory signals are delivered through the interaction of several receptor-ligand pairs of cell surface molecules between the T cell and the APC.

Costimulation is a fail-safe mechanism of the immune system to prevent unnecessary lymphocyte activation and works at multiple levels. It allows full activation, prevents anergy or apoptosis, induces differentiation to effector or memory status, sustains cell proliferation, and allows cell-cell cross talk and cooperation. This cross talk between the T cell and the APC is accomplished by receptor-ligand pairs on their cell surfaces, allowing bidirectional communication between participating cells. The first cell surface pair of molecules shown to have costimulatory function was the CD28-B7 pair. Several other pairs of cell surface molecules including CD40-CD40 ligand (CD40L), CD2-CD58, CD11-CD18/ICAM-1, and VLA4-VCAM were described later (17). In this report, we review the importance of the costimulatory signals delivered via the CD40-CD40L pair of molecules.

CD40L is a member of the tumor necrosis factor (TNF) family of cell surface interaction molecules. It is a 261-amino-acid type II membrane glycoprotein, and its expression is mainly confined to the $CD4^+$-T-cell subset. CD40L expression is induced shortly after T-cell activation and represents an early activation marker of T lymphocytes. CD40 is constitutively expressed mainly on B cells, macrophages, and dendritic cells (10). The CD40-CD40L pathway has been extensively investigated and has been shown to play multiple functional roles in the healthy immune system. It enhances the antigen-specific T-cell response through the activation of dendritic cells and the induction of interleukin 12 (IL-12) production by these cells to focus the immune response on the antigen that has engaged the TCR (6, 16, 24, 50, 57). It sustains this response for as long as the antigen remains in the system, and it induces effector functions of interacting $CD40^+$ target cells. For example, engagement of CD40 on endothelial cells by activated T cells expressing CD40L leads to upregulation of adhesion molecules such as ICAM-1, VCAM-1, and E-selectin, which results in increased leukocyte margination (47, 67). Activation of APC by CD40-CD40L interaction induces the production of inflammatory cytokines, chemokines, NO, and metalloproteinases. Interaction of $CD4^+$ $CD40L^+$ T cells with CD40 on B cells leads to B-cell differentiation, proliferation, immunoglobulin (Ig) isotype switching, and formation of memory B cells. The physiological function of CD40L is underscored in patients with congenital deficiencies of the CD40L gene. This X-linked inherited immunodeficiency, the hyper-IgM syndrome, is characterized by the absence of mature antibody isotypes and persistence of high titers of circulating IgM, confirming that the interaction between CD40 on B cells and CD40L on activated T cells is crucial for Ig isotype switching (1).

REGULATION OF CD40L EXPRESSION

CD40L expression is normally tightly regulated. TCR ligation initiates the induction of CD40L expression on the surface of the activated T cells. Additional costimulatory or cytokine signals enhance CD40L upregulation. CD40L mRNA expression peaks 1 to 2 h after T-cell stimulation, and cell surface CD40L protein is fully expressed within 4 to 6 h. Rapid disappearance from the cell surface follows, as CD40L is barely detectable by 16 h (18). This transient CD40L expression gives the antigen-activated T cell a brief opportunity to deliver helper signals to interacting B cells, macrophages, or dendritic cells. Other cell surface accessory molecules have been found to help CD40L expression, including CD28, LFA-3, and ICOS (11, 44, 59). Specific cytokines, such as IL-2, IL-12, and IL-15,

* Corresponding author. Mailing address: Division of Rheumatology, Dept. of Internal Medicine, Patras University Hospital, University of Patras Medical School, 26500 Rion, Patras, Greece. Phone: 30 2610 999693. Fax: 30 2610 993982. E-mail: sliossis@med.upatras.gr.

TABLE 1. Nonrheumatic diseases in which CD40-CD40L interactions may play a role

Disease	Explanation
AIDS	CD40L is necessary for immune defense against human immunodeficiency virus and opportunistic infections (31, 32); this may overlie the similarity of clinical manifestations of AIDS to those of the congenital CD40L deficiency (hyper IgM syndrome)
Cancer	Efforts are being made to enhance antitumor immunity; an orally administered CD40L gene therapy has been tried recently against lymphoma (34)
Chronic lymphocytic leukemia	Malignant cells express both CD40 and CD40L, and their interaction may contribute to tumor growth, making CD40L a therapeutic candidate target (51)
Hodgkin's disease	The CD40L survival pathway is augmented in patients with B-cell malignancies (9)
Pulmonary fibrosis	CD40-CD40L interaction mediates fibroblast activation and production of the profibrotic cytokine transforming growth factor β (55); human lung fibroblasts from normal and scarred lung express CD40L; this expression is augmented by the profibrotic cytokine IL-13 and is downregulated by gamma interferon, a cytokine with antiscarring properties; fibroblast cell lines from human idiopathic pulmonary fibrosis tissue express high levels of CD40L compared to fibroblasts from nonscarred lungs (30)
Alzheimer's disease	CD40-CD40L is a critical enhancer of microglial cell activation (60)

upregulate CD40L expression while others, such as IL-4 and IL-10, downregulate CD40L (23, 49, 56).

Solid evidence has been accumulated indicating that free ionized cytoplasmic calcium (Ca^{2+}) is the principal second messenger, leading eventually to CD40L gene transcription. Nuclear factor of activated T cells (NFAT) dephosphorylation by calcineurin, which is a Ca^{2+}-dependent phosphatase, leads to NFAT activation and translocation to the nucleus. Activated NFAT, with the cooperation of activating protein 1 (AP-1), plays a key role in CD40L transcriptional regulation. Cyclosporine (CsA), a powerful inhibitor of the calcineurin pathway, has been previously shown to eliminate CD40L expression on normal T cells. More specifically, only 4% of T cells from healthy subjects express CD40L upon activation, in the presence of CsA at concentrations of 100 ng/ml, compared to 62% in the absence of CsA (18).

CD40L expression is also regulated at the posttranscriptional level. Stress-activated mitogen-activated protein (MAP) kinases are thought to mediate CD40L mRNA stability. Finally, regulation is also achieved at the posttranslational level either by endocytosis or by metalloproteinase-mediated enzymatic cleavage of the cell surface CD40L, leading to the formation of soluble CD40L (sCD40L) (10).

PARADIGMS OF THE ROLE OF CD40-CD40L INTERACTIONS IN DISEASE

Transplantation. Transplantation surgery would not have reached the degree of success seen over the last decades without the development of efficient immunosuppressive agents that minimize the risk of transplant rejection. Monoclonal antibody (MAb) against CD40L has been used as an immunosuppressive treatment in many animal transplantation models. In primates, anti-CD40L MAb administration delays allograft transplant rejection, whereas combination with CTLA4Ig, a chimeric protein consisting of the extracellular domain of the cell surface molecule CTLA-4 and the Fc region of human IgG1 which blocks the CD28-B7 pathway, has additive and synergistic effects. This combination treatment sustains kidney allograft survival in primates and increases skin graft survival in mice (53, 69). Switching to anti-CD40L MAb therapy, after 60 days of conventional immunosuppression with CsA, was an effective treatment in a renal allograft model with monkeys, even though CsA is known to downregulate CD40L expression on T cells (7). CD40L gene expression has been demonstrated to increase fourfold in cases of acute rejection. This could serve as a noninvasive method for monitoring allograft function and also for determining the biological response to classic immunosuppressive agents such as CsA and tacrolimus, both known to inhibit CD40L induction (54). In animal transplant models, calcineurin inhibitors suppress CD40L expression on T cells in vitro but not in lymphoid tissue, indicating that the CD40-CD40L pathway remains functional during treatment with these agents, which may contribute to allograft rejection in the clinical setting (63).

Atherosclerosis. The current view of atherosclerosis, the most prevalent fatal disease in the Western world, is that of a chronic, degenerative, inflammatory disease in which the immune system is thought to play an important role. CD40 and CD40L are overexpressed in experimental and human atherosclerotic lesions. CD40 ligation on atheroma-associated cell types, such as endothelial cells, smooth muscle cells, and macrophages, leads to increased expression of mediators for the development of atherosclerosis, such as cytokines, chemokines, growth factors, and metalloproteinases. Blocking CD40-CD40L interactions with anti-CD40L MAb in mice results in diminished formation and progression of mouse atheroma but also fosters such changes in lesion biology and structure, which may be important in plaque stabilization in the human disease (52).

Platelets also express CD40L and are thought to contribute significantly to the recruitment of inflammatory cells to the damaged endothelium in vivo (5). Activated T cells expressing CD40L are located within the atherosclerotic vessel wall, a fact supporting the hypothesis that activated $CD4^+$ T cells may orchestrate the atherosclerotic process (43).

Other diseases in which the CD40-CD40L pair may play a role are briefly outlined in Table 1. A brief discussion of the role of this interaction in the autoimmune diseases systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and polymyositis follows below.

TABLE 2. CD40L expression on B and T cells from patients with SLE and RA compared with healthy B and T cells

Patient type	CD40L expression on:	
	B cells	T cells
Healthy	None	Expressed shortly only after activation
SLE	High, increases with stimulation	High, increases with stimulation and correlates with disease activity
RA	ND[a]	High, increases with stimulation and correlates with disease activity

[a] ND, not done.

SLE. (i) CD40L expression in human SLE (Table 2). Desai-Mehta et al. have reported that peripheral blood mononuclear cells (PBMC) from patients with active lupus exhibit a 21-fold increase in the percentage of $CD40L^+$ $CD4^+$ cells compared to healthy subjects. On further stimulation of the PBMC with anti-CD3 MAb, the percentage of $CD40L^+$ $CD4^+$ cells increased fivefold in healthy subjects but only 1.4-fold in active-SLE patients. However, the percentage of $CD40L^+$ T cells still remained higher in the active-SLE group. PBMC from patients with SLE in remission behaved in a way similar to that of the control group. Similar observations were made concerning CD40L expression by $CD8^+$ T cells freshly isolated from active-SLE patients (22-fold-higher expression than the control group). B cells overexpress CD40L in active-SLE patients at levels comparable to those observed in the activated T cells. Normal human B cells express very low levels of CD40L and only when they are manipulated, in contrast to active-SLE B cells that express CD40L spontaneously (12).

In a study published by Koshy et al., activated PBMC from healthy subjects or disease control patients showed high-intensity expression of CD40L with a gradual decrease to near baseline values at 48 h, whereas lupus PBMC continued to demonstrate significantly higher levels of CD40L at this time point. No apparent correlation of CD40L expression with disease activity was found in this study (33).

Higuchi et al. confirmed these results in both SLE and murine lupus, suggesting that ectopic CD40L expression in lupus B cells may play a crucial role in the development of SLE. Transgenic lupus-prone mice, expressing CD40L on B cells, spontaneously produce autoantibodies, and moreover, half of these mice develop glomerulonephritis with immune complex deposition (22). Devi et al. have also confirmed the high expression of CD40L on T and B lupus cells. More specifically, 45% of T cells from SLE patients express CD40L compared with only 8 to 18% of T cells from healthy subjects or RA patients. Moreover, 30% of the 48 SLE patients examined had increased expression of CD40L on B cells as well (13). However, in contrast to the above results, there is one report in which no changes in CD40L expression in lupus lymphocytes were found. Instead, these authors reported high expression of CD86 on SLE B cells, which is also an important costimulatory molecule (3). Another study has reported that monocytes from patients with active SLE express CD40L aberrantly. Twenty-three patients with active SLE were studied and compared to 16 healthy individuals. Results showed a sevenfold increase in the frequency of CD40L-expressing peripheral monocytes from SLE patients compared to healthy subjects. CD40L expression was verified at both the mRNA and protein levels and correlated significantly with disease activity (28).

CD40L is cleaved from the cell surface of activated T cells, by a matrix metalloproteinase, releasing sCD40L, a molecule of approximately 18 to 20 kDa, which forms homotrimers. A report studying the functional role of sCD40L concluded that sCD40L can induce B-cell activation and differentiation. Plasma sCD40L levels were significantly higher in active-SLE patients than in healthy donors. These levels correlated with disease activity, assessed by SLEDAI, and circulating anti-double-stranded DNA (dsDNA) autoantibody titers. It has been hypothesized that high levels of sCD40L found in active-SLE patients may play a pathogenic role in vasculitis and in the nephritis occurring in active lupus. The authors proposed that sCD40L levels could serve in the future as a predictive marker of SLE disease flares (27). Vakkalanka et al. also showed significantly higher mean concentrations of sCD40L in lupus patients than in disease controls and healthy subjects. More specifically, 66 patients with SLE were studied and compared to 30 disease control patients and 23 healthy individuals. In the healthy subjects, sCD40L was almost undetectable, whereas 38 patients with SLE had an sCD40L level above 2 ng/ml (with a mean concentration of 2.61 ± 2.15 ng/ml) and only 7 disease control patients had an sCD40l level above 1 ng/ml. Patients were divided into three groups, severe, moderate, and mild, according to clinical manifestations. Patients with severe SLE had an sCD40L mean concentration of 3.93 ± 2.86 ng/ml, those with moderate SLE had an sCD40L mean concentration of 2.81 ± 1.57 ng/ml, and finally, those with mild disease had an sCD40L mean concentration of 1.52 ± 1.06 ng/ml, indicating a correlation between sCD40L level and disease activity (62).

While CD40L induction on activated normal T cells is inhibited in the presence of CsA, it is of interest that this effect does not apply to SLE T cells. A study of CD40L expression in human lupus concluded that CsA failed to inhibit the prolonged and enhanced CD40L expression, observed in vitro on anti-CD3 MAb-activated lupus T cells. Resistance to CsA was disease activity independent. Circulating lupus monocytes also display prominent resistance to CsA inhibitory effects on CD40L expression. These results indicate that CD40L induction on SLE T cells may be regulated by a calcium/calcineurin-independent pathway (29).

It is not clear why CD40L expression is higher in patients with SLE and whether this overexpression has pathogenetic implications. It has been proposed that SLE T lymphocytes, when activated by TCR engagement, favor an NFAT-dominant profile instead of a balanced NFAT–AP-1 transcription factor profile. This panel of intracellular mediators promotes transcription and mRNA stability of certain genes, including the CD40L gene. Early expression of CD40L on SLE T cells requires the CsA-sensitive NFAT pathway, but prolonged expression is more dependent on a specific MAP kinase, i.e., the extracellular signal-regulated kinase. Increased and persistent

extracellular signal-regulated kinase activity in lupus T cells could contribute to CD40L overexpression via activation of AP-1 transcription factor and possibly by stabilizing CD40L mRNA (10, 68). Cytoplasmic Ca^{2+} fluxes are supranormal in circulating T and B cells from patients with SLE, and it has been proposed that this abnormality may represent the molecular background for the abnormal CD40L expression in lupus (38, 40, 64). There is also evidence that the deficiency of the TCR ζ chain, which has been described in SLE patients, may be in part responsible for the supranormal Ca^{2+} response mentioned above (39, 61). This correlation has been reinforced by the recent report that transfectional correction of the TCR ζ chain deficiency in lupus T cells leads to normalization of the cytoplasmic Ca^{2+} fluxes (48).

(ii) Use of anti-CD40L MAb in lupus models and in SLE. The multiple functions of CD40/CD40L in the immune response have made it an attractive target for therapeutic intervention in autoimmune diseases. MAbs against CD40L were tested initially in murine lupus models. Treatment of New Zealand Black × New Zealand White (NZB×NZW) F_1 mice with continuous anti-CD40L MAb infusions resulted in a delay of disease onset for an average of 4 months, a decrease in IgG anti-dsDNA autoantibody levels, a delay in the accumulation of T cells bearing the activated memory phenotype, a decrease in the number of B cells in the spleen, and a suppression of Ig class switching and somatic mutations. Serum levels of IgG anti-dsDNA antibodies were 4- to 10-fold lower than in untreated age-matched controls. Upon cessation of treatment, the T-cell phenotype of the treated mice became indistinguishable from that of the controls and B cells attained a fully activated phenotype with elevation of IgG anti-dsDNA titers and development of proteinuria and progressive renal disease (65).

Another study employed combination treatment with anti-CD40L antibody and CTLA4Ig. Short-term combination treatment of NZB×NZW F_1 prenephritic mice resulted in a delayed onset of renal dysfunction for approximately 6 months. After the onset of proteinuria, a repeat course of treatment was able to induce remission in previously treated mice but not in previously untreated mice. There was a decrease in the level of IgG anti-dsDNA autoantibodies, and murine spleens were markedly depleted of B cells, even 16 to 20 weeks after infusion. It has been postulated that CTLA4Ig and anti-CD40L MAb treatment may act synergistically to block antiapoptotic signals and, hence, facilitate apoptosis of B cells during early B-cell activation. This treatment did not cause long-term global immunosuppression (66).

The promising results obtained in experimental animal studies were not confirmed in SLE. In human lupus, cyclophosphamide remains the "gold standard " for the treatment of major organ involvement. Cyclophosphamide is an alkylating agent which causes nonspecific inhibition of the immune response and clinically significant immunosuppression. It has significant side effects, including bladder cancer and gonadal failure, thus making the need for new therapeutic interventions a necessity. Cyclophosphamide administration has been an established treatment for lupus nephritis, a common and fearful complication of SLE and one of the leading causes of morbidity and mortality.

Two studies of anti-CD40L MAb administration in human lupus have been published. In the first, a phase 2, double-blind, placebo-controlled, multicenter study, 85 patients with mild to moderately active SLE were enrolled and received 6 injections of IDEC-1 (anti-CD40L MAb) or placebo over the course of 16 weeks. Efficacy was assessed at week 20, primarily by using SLEDAI. Results showed that treatment with IDEC-1 was safe and well tolerated but failed to demonstrate efficacy compared to the placebo (19, 26).

The second trial evaluated patients with biopsy-proven proliferative lupus nephritis by using BG9588 (anti-CD40L MAb). Twenty-eight patients with active proliferative lupus nephritis were enrolled and received 20 mg of BG9588/kg of body weight at biweekly intervals for the first three doses and at monthly intervals for four additional doses. Results showed a 50% reduction in proteinuria (where present) without worsening of renal function, disappearance of hematuria, and an increase in serum C3 concentration. Anti-dsDNA autoantibody titers declined after therapy, with mean reductions of 38.8, 50.1, and 25.3% at 1, 2, and 3 months after treatment, respectively (4). This study was terminated prematurely due to thromboembolic complications. More specifically, two cases of myocardial infarction were reported. The thrombotic effects complicating this treatment regimen raised important issues regarding future studies with humans. It should be noted that none of the patients who suffered a thrombotic event had demonstrable antiphospholipid antibodies. The role of the CD40-CD40L interaction in these thrombotic complications remains unclear and hypothetical.

A study of 5 patients with lupus nephritis who received anti-CD40L therapy showed that a short course of this treatment leads to a reduction in the number of IgG anti-DNA antibody-producing B cells. These changes persisted for several months after treatment cessation (25). A similar study of four patients with active lupus nephritis who received anti-CD40L treatment (BG9588) showed that B cells expressing CD38, CD5, and CD27, which are considered B-cell differentiation markers, disappeared from the periphery during anti-CD40L treatment. These changes were associated with a decrease in anti-dsDNA antibody levels, proteinuria, and SLE disease activity index (20).

RA. (i) CD40L expression in RA (Table 2). RA is a common autoimmune systemic rheumatic disease characterized by a chronic tissue destructive process attributed to a possible ongoing antigen-driven immune response. As activated T cells are thought to play a key role, it is not surprising that CD40-CD40L interactions have been studied in the context of RA.

Berner et al. studied the expression of CD40L on T cells from patients with RA. Sixty-two patients with RA and 20 healthy subjects were studied. CD40L was strongly expressed on >10% of T cells in 29% of RA patients but on 0% of the T cells in the healthy controls. RA patients with >10% $CD4^+$ $CD40L^+$ T cells had more active disease. Eighty-three percent of patients within this group had increased C-reactive protein levels, 89% were rheumatoid factor (RF) positive, and none of them fulfilled the American College of Rheumatology criteria for complete clinical remission, suggesting a strong correlation between disease activity and CD40L expression (2). CD40L expression at high levels may reflect augmented and prolonged activation of lymphocytes resulting in an increased and prolonged inflammatory activity. The expression of functional

CD40L at high levels on T cells from patients with RA was also demonstrated by MacDonald et al. (42).

Another study addressed the role of CD40-CD40L signaling in RF production. In the healthy immune system, high-affinity autoreactive B cells are deleted. In healthy individuals, low-affinity RF-producing B cells exist in the lymph nodes but high-affinity RF is undetectable. In RA, high-affinity RF-producing B cells accumulate within the inflamed synovia. The results of this study indicated that the interaction between CD40L on activated T cells with CD40 on RF-producing B cells is crucial, not only for the survival of these autoreactive B cells but also for RF synthesis. The two signals necessary and sufficient for induction of high-affinity RF synthesis in vivo were found to be IgG and CD40-CD40L interaction. In the absence of CD40-CD40L interaction, RF B cells were deleted (35).

Cho et al. studied the role of CD40-CD40L interaction in the production of vascular endothelial growth factor (VEGF). In RA, the inflamed synovia has tumor-like characteristics. The perpetuation and expansion of the rheumatoid pannus depend on neovascularization, as the extensive migration of mononuclear cells into the synovia and pannus overgrowth depend on the existence of a rich vascular bed. VEGF, a heparin-binding dimeric glycoprotein, is a central mediator of angiogenesis and induces endothelial cell proliferation and capillary permeability. It was demonstrated that CD40 ligation on synovial fibroblasts by CD40L on activated T cells resulted in increased production of VEGF, which is further augmented in the presence of IL-1, TNF-α, and transforming growth factor β (8). Ligation of CD40 on RA synoviocytes by CD40L on activated T cells within the synovia significantly increases the production of TNF-α in a dose-dependent fashion (21). TNF-α is a key cytokine in RA pathogenesis. Besides TNF-α, the production of IL-10 is also augmented via CD40-CD40L interactions (15). Synovial fluid T cells from patients with RA express high levels of CD40L compared to T cells from the peripheral blood of healthy donors, and after in vitro activation, they display a prolonged, high-level expression of CD40L. Results showed 8.71% of synovial fluid T cells from RA patients to be $CD40L^+$ versus 1.74% in the control group (41). The potential role of CD40-CD40L interaction in RA pathogenesis led to efforts of pharmacologic manipulation of this axis as a therapeutic approach in patients with RA.

(ii) Use of anti-CD40L MAb in animal models of RA. Treatment with anti-CD40L MAb suppresses the development of collagen-induced arthritis, which is an animal model of RA. More specifically, development of joint inflammation was blocked, and infiltration by inflammatory cells of the subsynovial tissue and cartilage erosion were diminished (14). In K/B×N transgenic mice, a model of Ig-mediated arthritis, anti-CD40L MAb treatment significantly diminished the development of arthritis when administered a week before the onset of clinically apparent disease, but the treatment was unsuccessful when administered in established disease (36).

Dermatomyositis/polymyositis. In one study analyzing 9 patients with polymyositis and dermatomyositis, it was observed that muscle-infiltrating T cells were $CD40L^+$ in all 9 cases, suggesting a potential role of CD40-CD40L interactions in the above clinical entities (58).

CONCLUSIONS

CD40-CD40L interactions are thought to play an important role in the pathogenesis of certain diseases. Pharmacologic manipulation of this axis has been tried with promising results in some cases. One approach of interfering with CD40-CD40L cross talk is by administering a MAb against CD40L. Another way is by inhibiting CD40L expression from within by using small molecules which are able to enter the cell and inhibit specific pathways responsible for CD40L expression. A classic example of this approach is CsA, a potent calcineurin inhibitor, which is widely used in the treatment of many clinical entities, including transplantation and rheumatic diseases. More-specific inhibitors of certain intracellular molecular pathways have been developed recently. MAP kinase inhibitors are able to modify the expression of costimulatory molecules and may represent promising therapeutic weapons in the future (37). Trichostatin A, a histone deacetylase inhibitor, modulates the expression of many proteins at the transcriptional level by modifying the histone acetylation status. The in vitro use of trichostatin A on lupus T cells led to a decrease in CD40L production along with a simultaneous correction of IL-10 overproduction and upregulation of previously repressed gamma interferon gene expression (46). Trichostatin A and suberonylanilide hydroxamic acid, another histone deacetylase inhibitor, have been tried in the lupus-prone MRL-lpr/lpr mice recently, with promising results, such as a significant reduction in proteinuria and spleen weight (45).

Targeting a costimulatory molecule as a therapeutic approach raises important safety issues, as costimulation blockade interrupts the effective immune response, potentially making subjects more prone to infection. This is partially confirmed in animal models, as anti-CD40L treatment in the long-term led to global immunosuppression. In the two short-term clinical trials of anti-CD40L treatment with humans, severe immunosuppression and infections were not recorded. Promising results were obtained from animal models of RA and SLE with anti-CD40L MAb treatment, but these results await confirmation with humans.

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