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REVIEW

Costimulatory Pathways in Kidney Transplantation: Pathogenetic Role, Clinical Significance and New Therapeutic Opportunities

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Costimulatory pathways play a key role in immunity, providing the second signal required for a full activation of adaptive immune response. Different costimulatory families (CD28, TNF-related, adhesion and TIM molecules), characterized by structural and functional analogies, have been described. Costimulatory molecules modulate T cell activation, B cell function, Ig production, cytokine release and many other processes, including atherosclerosis. Patients suffering from renal diseases present significant alterations of the costimulatory pathways, which might make them particularly liable to infections. These alterations are further pronounced in patients undergoing kidney transplantation. In these patients, different costimulatory patterns have been related to distinct clinical features. The importance that costimulation has gained during the last years has led to development of several pharmacological approaches to modulate this critical step in the immune activation. Different drugs, mainly monoclonal antibodies targeting various costimulatory molecules (i.e. anti-CD80, CTLA-4 fusion proteins, anti-CD154, anti-CD40, etc.) were designed and tested in both experimental and clinical studies. The results of these studies highlighted some criticisms, but also some promising findings and now costimulatory blockade is considered a suitable strategy, with belatacept (a CTLA-4 fusion protein) being approved as the first costimulatory blocker for use in renal transplantation. In this review, we summarize the current knowledge on costimulatory pathways in the setting of kidney transplantation. We describe the principal costimulatory molecule families, their role and clinical significance in patients undergoing renal transplantation and the new therapeutic approaches that have been developed to modulate the costimulatory pathways.

Keywords: belatacept, CD28, CD40, dialysis, drug development, graft rejection, kidney transplantation

INTRODUCTION

T cells have a key role in initiating and regulating the adaptive immunity, since they are involved into the modulation of the immune response toward both native and foreign antigens.

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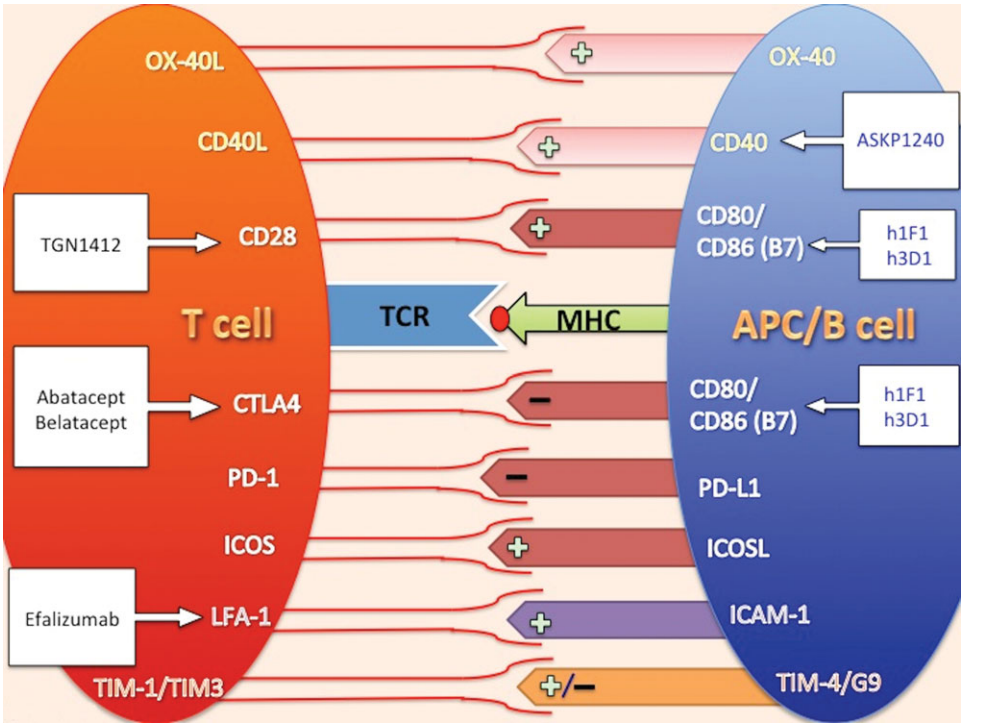

FIGURE 1. Role of costimulatory pathways in T cell activation. The first T cell activation signal is provided by the antigen-specific interaction between TCR and MHC expressed on the APCs and B cells. Costimulatory pathways may influence T cell-dependent immune response through stimulatory (+) or inhibitory (−) signals. In the figure, the different costimulatory families are represented. From the top: molecules of TNF-related family, members of CD28 family, adhesion and TIM molecules. mAbs tested in human kidney transplantation are indicated in the white boxes (for details see also text and Tables 2 and 3).

Activation of naïve T cells requires two different signals. The first one is antigen specific, and it implies the interaction between the antigen presenting cells (APCs) and the CD4+ T cells by the major histocompatibility complex (MHC) class II. The second one requires the interaction between specific receptors on the T cells and their ligands on the APCs, the so-called costimulatory pathways (Figure 1) [1].

Without co-stimulation the T cell-antigen interaction results in anergy, creating an immunologic antigen-specific tolerance [2].

However, costimulation is not always an event that activates the immune response. In fact, the costimulatory molecules expressed on APC and T cells might also have an inhibitory effect [3].

For these reasons, the costimulatory pathways seem to play a pivotal role in the balance between stimulatory and inhibitory signals, which modulate T cell-dependent immune response.

Different costimulatory families have been described. These molecules can interact with each other either up or downregulating the T cell activation [4].

Among the identified costimulatory molecules, the best characterized are the CD28:B7 and the TNF-related families. The description and the characterization of these pathways has not only highlighted an important regulatory mechanism of the adaptive immunity, but also offered the possibility to explore new treatment options in immune-mediated conditions, such as cancer or autoimmune disorders, and in solid organ transplantation [5, 6].

In the transplant field, since standard immunosuppressive therapies induce a number of adverse effects, including nephrotoxicity and increased cardiovascular risk, inhibition of costimulatory pathways has been thought to be a new alternative strategy to avoid graft rejection.

Therefore, different approaches have been developed and tested in both experimental and clinical studies [7]. Although the results of these studies have generated some criticisms, their promising findings have shown that the costimulatory blockade could be a suitable strategy in human solid organ transplantation [8]. This review focuses on the current "state of the art" in new therapeutic opportunities for the modulation of the costimulatory pathways in kidney transplantation (KT).

COSTIMULATORY PATHWAYS

The CD28:B7 Family

The CD28:B7 family includes the following receptor–ligand pairs: CD28/CTLA4: B7.1/B7.2, ICOS:ICOSL, PD-1:PDL1/ PD-L2. Furthermore, B7-H3 and B7-H4 have also been isolated, although up to now no human receptors have been identified for such molecules [9].

The pathways involving the B7:CD28 family play a key role in the modulation of T cell activity, providing both positive and negative signals.

The CD28 molecule is a disulfide-bound molecule that belongs to the immunoglobulin superfamily and is constitutively expressed on T cells. Its interaction with B7.1 (CD80) and B7.2 (CD86) molecules expressed on the surface of APCs leads to the full activation of naïve T cells [10].

The upregulation of CTLA4 (cytotoxic T-lymphocyte-associated antigen 4/CD152) follows the activation of T cells [11]. CTLA4 is structurally homologous to CD28, but it has higher avidity for the CD80 and CD86 ligands competing with the CD28 [12]. It acts as a negative regulator of the T cells, inducing the dissociation of the CD28–CD80/86 interaction and eliciting an inhibitory effect also through the indoleamine 2,3-dioxygenase (IDO) upregulation [13, 14].

The induced costimulatory molecule (ICOS) is another member of the CD28/B7 family.

It is not expressed on naïve T cells but is induced in activated T cells, and its expression persists in memory and effector T cells [15]. By binding to B7h (B7 homolog)/B7Rh (a B7-related homolog) expressed on APCs, ICOS provides a positive signal for the T cell activation, involved in the activation of the T cell to B-cell functions, including immunoglobulin production [16].

On the contrary, the programmed death-1 (PD-1) (which is expressed on peripheral T cells, NK cells, B cells and monocytes), after binding to two ligands, PD-L1 (B7-H1) and PD-L2 (B7- DC), induces a potent inhibitory signal in the early stages of T cell activation leading to a decreased cytokine production and cell cycle arrest in the G0/G1 phase [17].

Finally, B7-H3 and B7-H4, which are B7 homologs, are both molecules inducible in immune and hematopoietic cells with unidentified putative receptors in humans. Their role has not been well defined, because they exert both stimulatory and inhibitory effects on cytokine secretion, cell growth and differentiation [18].

The TNF-Related Family

The tumor necrosis factor (TNF) superfamily comprises several members that influence T cell-mediated immune response, and it includes the following receptor–ligand pair: CD40:CD40L, OX40:OX40L, CD30:CD30L, CD27:CD70, CD137:CD137L,

glucocorticoid-induced TNF receptor-related protein (GITR):GITRL and herpes virus entry mediator (HVEM): LIGHT [19].

All these molecules share similar structural characteristics and the ability, upon ligation, to recruit the TNF receptor-associated factors (TRAFs), which mediate their intracellular effects, through activation of transcription factors, such as mitogen-activated protein kinases (MAPKs) and nuclear factor kappa B (NFκB) [20].

Different kinds of TRAFs have been described and associated with distinct immunological functions [21].

CD40: CD40L are the best-studied members of the costimulatory TNF family.

CD40 is mainly expressed on B-cells, but also on monocytes, dendritic cells, endothelial cells, smooth muscle cells and fibroblasts [22]. Its ligand, CD40L (CD154), is a 34–39 kDa type II integral membrane protein expressed on activated T cells, platelets and inflammatory cells [23]. The engagement of CD40 leads to B cell clonal expansion, germinal center formation, isotype switching, affinity maturation and generation of long-lived plasma cells [24, 25]. Moreover, CD40:CD40L interaction is decisive in the regulation of APCs and dendritic cells maturation and functions [26]. The CD40/CD40L pathway has also been known to induce CD80 and CD86 expression, resulting in the activation of the CD28 pathway [27].

Interestingly, soluble forms of CD40L and CD40 have also been characterized. These seem to act respectively as agonist (sCD40L) and antagonist (sCD40) of CD40:CD40L ligation [28].

OX-40 is expressed on activated T cells, whereas its ligand, OX40L, is mainly present on APCs and B cells. Their interaction induces B cell proliferation and differentiation, and it plays an important role in survival and clonal expansion of effector and memory T cells [29].

Other members of the TNF family present different expression profiles, resulting in the modulation of various immune processes, such as IL-2 production, Th polarization and long-term maintenance of T cell responses [30–32]. Of note, the interaction between GITR–GITRL molecules, which are widely expressed by several cell types, initiates a number of concurrent processes, including coactivation of effector T cells and NK cells, inhibition of Tregs, activation of macrophages and modulation of dendritic cells function [33].

Adhesion Molecules

T cell adhesion molecules and their cognate ligands on APCs and target cells play a major role in T cell activation. In fact, these molecules, besides facilitating cell adhesion, may present potent costimulatory properties mediating different cell activation patterns [34].

β2 integrins (CD11/CD18) are a family of adhesion molecules, which members share a common β-chain (CD18) associated with different α-subunits (CD11). They are responsible for cell-to-cell interaction between leukocytes and between leukocytes and endothelial cells [35].

Lymphocyte function-associated antigen 1 (LFA-1, CD11a/CD18) is present on T cells, B cells, macrophages and neutrophils [36]. Through its interaction with intracellular adhesion molecule 1 (ICAM-1, CD54) on APCs, it plays an important role in the initial phases of T cell activation, since it results necessary for the formation of the immune synapse [37].

In particular, while the LFA-1:ICAM interaction is required to stabilize the T cell:APC contacts allowing an optimal T cells activation, it seems that LFA-1 is able per se to transmit positive costimulatory signals, resulting in cytokine release and T cell activation and differentiation [38, 39].

CD2:LFA-3 are other adhesion molecules involved into costimulatory pathways that belong to the immunoglobulin superfamily.

LFA-3 (CD58) is expressed on hematopoietic and nonhematopoietic cells, including leukocytes and endothelial cells. Its ligand, CD2 is expressed on T cells, NK cells and dendritic cells [40]. CD58:CD2 ligation has been shown to mediate T cell adhesion and activation and cytokine production [41].

TIM molecules

The T cell Ig and mucin domain (TIM) proteins are type I transmembrane proteins, with extracellular Ig and mucin domains, which have been recently described as a novel group of costimulatory molecules, expressed on a wide variety of innate and adaptive immune cells [42]. The human TIM gene family has three members TIM-1, TIM-3 and TIM-4 proteins.

TIM-1:TIM-4 is a receptor–ligand pair expressed respectively on activated T cells and APCs [43]. Engagement of TIM-1 by TIM-4 in the presence of T cell receptor (TCR) stimulation provides a potent positive costimulatory signal to T cells, enhancing proliferation, survival and cytokine production, including high levels of IL-17, necessary for T helper type 17 cell (Th17) differentiation [44].

TIM-3 with its ligand galectin-9 (G9) is predominantly expressed on the Th1 cell subset [45]. The role of TIM-3 in the regulation of immune processes has not been clarified yet, because it has been shown that its activation could both promote or inhibit the immune response. In fact, both the induction and the blockage of TIM-3 signaling have been associated to increased inflammation and T cell activation [46, 47].

COSTIMULATORY PATHWAYS IN DIALYSIS PATIENTS

A functional impairment of the immune system cells, such as polymorphonuclear leukocytes, monocytes, natural killer cells and T lymphocytes, has been observed in patients with chronic kidney disease (CKD), in particular those on renal replacement therapy (RRT – including hemodialysis-HD, and peritoneal dialysis-PD) [48, 49]. This condition is responsible for the increased susceptibility to infections, malignancies and the unresponsiveness to vaccination that characterize CKD patients [50]. It has been hypothesized that a defect of the costimulatory signal could play an important role in this condition of acquired immunodeficiency [51].

Girndt et al. [52] evaluating the expression of CD28 ligands, CD80 and CD86, in monocytes isolated from peripheral blood of HD patients, observed that lower levels of CD86 membrane expression were correlated to an altered proliferative capacity of T cells and reduced response to vaccination. Also an alteration of the CD40:CD40L interaction has been observed in patients on RRT [53]. Recently, we described the presence of a whole imbalance of CD40 pathway in this population, characterized by a modified CD40/sCD40 pattern, with elevated sCD40 serum levels associated to a reduced CD40 membrane expression [54]. Different mechanisms were evaluated to explain this phenomenon, and we found that the reduced renal clearance was the main cause of elevated sCD40 levels in HD patients [55]. The presence of these alterations, in particular the high levels of sCD40, a natural antagonist of the CD40/CD40L interaction, could have an immunodepressant effect. This hypothesis was supported by the finding that HD patients presenting higher sCD40 serum levels were less responsive to HBV vaccine, whereas a reduction in sCD40 serum levels, obtained by dialytic treatment, was associated to a significant increase of the response to vaccination [56]. These data thus seem to support the idea that high sCD40 levels could be involved in the immune dysfunction associated with renal failure. As sCD40, also the serum levels of soluble CD30, another member of TNF costimulatory family, were found elevated in HD and

CKD patients, when compared with healthy subjects [57]. These data clearly indicate that impaired expression of costimulatory pathways may be an important feature of the cellular immune defect in HD patients.

However, the role of these alterations on the clinical course of HD patients, and the mechanisms by which they might influence the outcome after KT, is unknown, so far.

COSTIMULATORY PATHWAYS IN KIDNEY TRANSPLANTATION

Role of Costimulatory Pathways on Mechanisms Involved in Organ Rejection

Immune regulation during organ transplantation is an active process involving multiple steps, including cytokine release and interactions among different cell types, such as T and B cells, natural killer cells, monocytes, etc. [58]. In particular, CD4+T helper cell-mediated response to alloantigens is a central event in the activation of the transplant rejection processes.

Upon interaction with their cognate antigen via the TCR, naıve CD4+T cells can differentiate into various lineages, including the T helper type 1 (Th1) and type 2 (Th2) subsets, Th17, and regulatory T cell populations (Treg). These subsets are functionally distinct and are characterized by specific cytokine production profiles [59].

Costimulatory pathways, providing both positive and negative regulatory signals, could modulate the functional differentiation of T helper cells influencing the immune response toward graft rejection or, on the contrary, to a state of tolerance (Figure 2) [4, 60].

CD28 signaling has been shown to play an important role in the activation of the immune response through the stimulation of interleukin-2 (IL-2) production and the differentiation of naïve T cells toward Th1 or Th2 subset. CD28 signaling depends of several factors, such as the strength of TCR signaling and IL-4 levels [61, 62]. On the contrary, CTLA-4 inhibits T cell proliferation, IL-2 production and both Th1 and Th2 differentiation [3]. Notably, CTLA-4 is also highly expressed on Treg cells that, because of their suppressive functions, are fundamental for the maintenance of peripheral tolerance [63]. Experimental models of mice deficient for CD28 showed a severe disruption of T-cell homeostasis (T-effector cell/Treg balance) [64], whereas CTLA-4 stimulation antagonized CD28-mediated immune response prolonging graft survival in both experimental and clinical settings (see below).

Also the other members of CD28 family may influence transplant-related immune response.

Activation of the negative costimulatory pathway PD-1 has shown the ability to decrease T cell proliferation by the reduction of IFNγ and IL-2 production and the induction of Treg suppressive activity [65, 66]. Consequently, administration of an agonistic fusion protein for PD-1 has been demonstrated to prolong cardiac allograft survival in experimental models [67].

On the contrary, ICOS, which has a key role in B cell maturation, induces Th differentiation toward Th2 phenotype stimulating the expression of IL-4, IL-5 and IL-10 [68]. Interestingly, TNF-related costimulatory molecules seem to affect transplant immune regulation differently compared with CD28 family. In fact, CD40/CD40L engagement, beyond T cell activation, promotes a potent enhancement of antigen presentation and productive immune responses via APC and dendritic cell stimulation [69].

Moreover, other members of this family, such as CD30 or OX-40, being abundantly expressed on activated but not resting T cells, have been considered as main regulators in the effector and memory phases of the immune response, rather than the initial phase of T cell priming [70, 71].

Adhesion molecules with costimulatory properties could also have a role in the modulation of processes implied in transplant immunology. ICAM/LFA-1 interactions

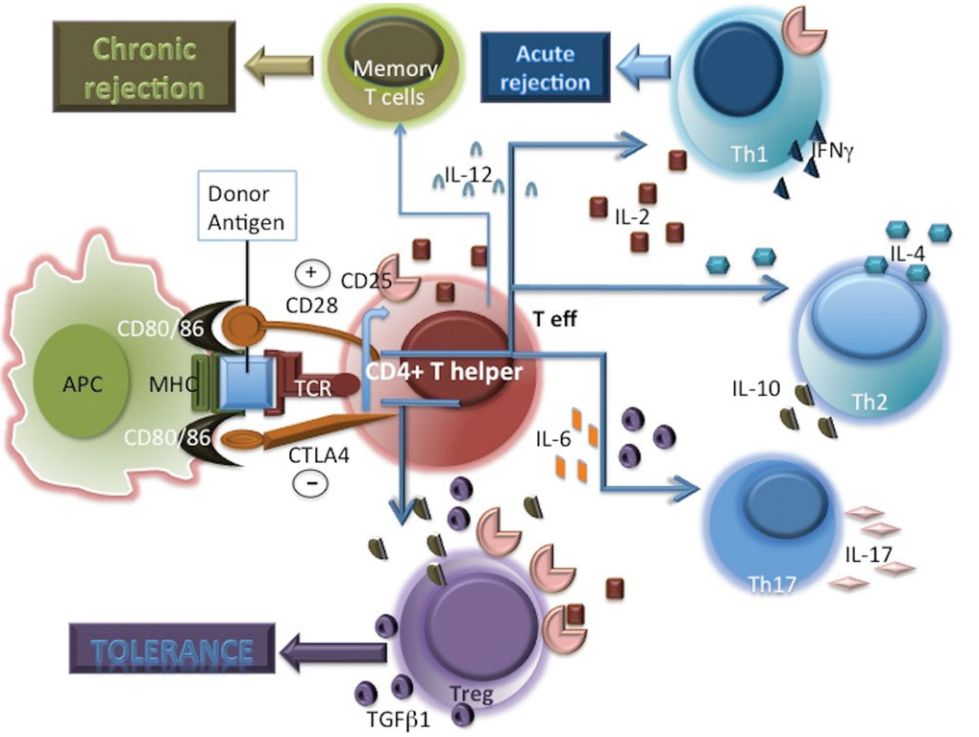

FIGURE 2. $CD4^+$ T cell differentiation in transplant recipients. Upon interaction with donor antigen via the TCR, the naïve CD4+T helper cell may differentiate toward functional subsets promoting inflammatory and graft rejection (T effector cells-Teff- or T memory cells) or a suppressive response (regulatory T cells, Treg), each of which is characterized by specific cytokine patterns. Costimulatory molecules may provide both positive (+) and negative (−) signals to T cell, directing CD4+T helper cell differentiation also by the regulation of cytokine release. In the figure, only molecules of CD28 family have been represented (for more details see text and references [3, 4 and 60]).

influence T helper cell differentiation predominantly supporting Th1 induction, at the expense of Th2, whereas costimulation of TCR with CD2 specifically induces the differentiation of Treg cells [72, 73].

Finally, also the members of TIM family may be involved in a wide array of transplant-related immune responses [74]. In fact, TIM molecules can direct the differentiation of multiple T cell subsets, including Th1, Th2 and Th17 cells, as well as regulate the activity of Foxp3 + Tregs and contribute to activation of APCs [75, 76].

Costimulation in Human Kidney Transplantation

The most studied costimulatory molecules in KT are the CD28, its antagonist CTLA-4, the CD28 receptors CD80 and CD86, and the CD40/CD40L pair.

Histological studies, performed by immunohistochemistry, interestingly show a differential expression of costimulatory molecules in renal biopsies of allograft recipients undergoing acute or chronic rejection [77].

In particular, in acute rejection a prevalence of interstitial infiltration of CD80+, CD86+ and CTLA-4+ cells has been observed, whereas CD40 and CD40L are rarely expressed. On the contrary, during chronic rejection, CD40 is expressed on graft-infiltrating T cells. CD40L+CD4+ cells are present in both glomeruli and tubules, whereas only a few CD80+, CD86+ or CTLA-4+ cells are detectable [78]. These different expression patterns may indicate that activation of costimulatory pathways is a

TABLE 1. Costimulatory molecules membrane expression on peripheral blood cells in renal transplanted patients.

Clinical/histological feature	CD4+CD40L+	CD4+CTLA-4+	CD4+CD28+	CD8+CD28+
Acute rejection[a]	+	−	N/A	−
Chronic rejection[a]	+	−	+	−
Stable graft function	−	+	−	+
Long-term survival	−	+	−	+

[a]Confirmed by kidney biopsies.
Definitions: stable graft function = uneventful course without acute rejection episodes; long-term survival = as stable graft function and absence of clinical or biologic signs of chronic rejection for at least 2 years; + or − = increased or reduced expression compared with healthy subjects and other clinical features, N/A = no data available (for details see also Refs. 80–84).

stepwise process, but the real meaning of these observations and its impact on the KT clinical outcome remains unclear.

Other interesting findings come from studies evaluating the expression of costimulatory molecules on peripheral blood cells. Also in this case, different expression of costimulatory molecules has been described in relation to distinct clinical course of KT (see Table 1).

In fact, Kosmaczewska et al. [79] observed that KT recipients presented an increased percentage of peripheral CD4+ T cells expressing CD40L and CTLA-4 compared with healthy controls, probably reflecting a state of chronic CD4+ T cell activation.

Among KT patients, CD4+ T cells drawn from those with stable graft function, when compared with patients with acute or chronic graft dysfunction, showed higher CTLA-4 and lower CD40L expression. Moreover, these cells exhibited a higher potential to express CTLA-4 and CD40L and to downregulate CD28 in response to the stimulation with IL-2. These findings indicate a relationship among the expression of costimulatory molecules on CD4+ cells, their function and the clinical course of KT. In addition, a decreased percentage of circulating CD4+CD28+, accompanied by an increased expression of CD40L and CTLA-4 on CD4+ and CD3+ cells has been observed in patients with long-term surviving kidney compared with short-term graft survival patients [80].

The reduction of CD28 expression on CD4+ cells has also been confirmed by other studies in patients with successful KT [81]. Kato et al. [82] examined the rate of CD4+CD28- T cells and their functions during a 4-year follow-up study. They found that in patients with a good renal function there was a higher number of CD4+CD28- cells, compared with patients with chronic rejection and graft dysfunction. When functional activity of these cells were evaluated, the authors found that CD28- cells showed a lower response to donor-specific antigens in a mixed lymphocyte reaction, suggesting that this specific cell population might attenuate immune response to foreign antigens and participate in the long-term acceptance of KT.

Interestingly, opposite data come from studies that evaluated CD8+ T cell subset. Analyzing the phenotypic and functional characteristics of CD8+ T cell, Baeten et al. [83] found that KT patients with chronic rejection, compared with those with drug-free tolerance, presented an increased number of CD8+ CD28- cells. This CD8+ CD28- cell population resulted also less sensitive to apoptosis and exhibited a cytotoxic profile.

These observations emphasize the complexity of the immune cells interactions and functions, suggesting that a similar expression pattern (CD28-) on different cell types (CD4+ vs CD8+) accounts for opposite immune effects, i.e. tolerogenic versus activation of immune response.

Expression of costimulatory molecules was also recently studied in Tregs (CD4+CD25+FoxP3+) and CD4-CTL (CD4+GzmA+) cells. Giaretta et al. [84] described that patients with a good graft function presented a higher number of Tregs expressing surface CTLA-4 compared with KT patients with antibody-mediated chronic rejection.

Moreover, the latter group of patients showed an increased expression of CD27, a positive costimulation molecule of the TNF family, on both Treg and CD4-CTL cells. These results suggest, once more, that costimulatory profile can influence clinical outcome of KT [84].

Other costimulatory molecules studied in the clinical setting of KT were ICOS and OX-40, which respectively belong to CD28 and TNF families.

Gene polymorphism and expression analysis on peripheral cells revealed that the transcript levels of these molecules were elevated in patients with acute graft rejection [85, 86].

Similarly, more recently, posttransplant sCD30 serum levels were found significantly increased in pediatric KT recipients presenting biopsy-proven acute rejection [87]. Furthermore, the linear combination of the urinary mRNA levels of OX40, OX40L, PD-1 and Foxp3, as well as TIM-3 mRNA serum and urinary concentrations, were found higher in patients with acute rejection, allowing an accurate differentiation with other causes of allograft dysfunction and nonrejecting controls [88, 89].

Taken together all these evidences highlight the role of costimulatory pathways in the regulation (both positive and negative) of immune response in KT and its potential influence on the clinical course of KT. Important considerations may be drawn from these data.

First of all, the presence of different expression patterns in patients with distinct histological and clinical features may indicate that the evaluation of costimulatory pathway may be useful to differentiate overlapping conditions and that it may provide valuable markers for acute/chronic allograft nephropathy. Furthermore, since the lack of cells expressing costimulatory molecules has been observed in patients with cyclosporine nephrotoxicity, it is reasonable that assessment of renal expression of costimulatory pathways could be of help to discriminate episodes of rejection from calcineurin inhibitors (CIN) nephrotoxicity [77].

The evaluation of the costimulatory molecules may also be potentially useful for the follow-up of KT patients. In fact, monitoring the activation or the inhibition of the immune response by the changes in the expression pattern of costimulatory molecules could guide the clinical decisions aimed at tailoring the immunosuppressive therapy to the needs of each single patient [90, 91].

For example, recipients showing a pattern of costimulatory expression associated with long-term graft survival (i.e. prevalence of CD4+CTLA-4+cells) may be candidates for CIN minimization or withdrawal.

Costimulatory Pathways as a New Therapeutic Target in Kidney Transplantation

The insights regarding the role of the costimulatory molecules in the clinical outcome of KT have provided novel targets for therapeutic interventions. Different strategies have been developed to inhibit costimulatory signals, mainly based on monoclonal antibodies (mAbs) designed to interrupt these critical T cell activation pathways (Figure 1) [7, 91].

CD28 pathway

CD28/CTLA-4 and their cognate ligands CD80 and CD86 have been extensively studied as potential therapeutic targets in experimental and clinical KT [92] (see Table 2).

TABLE 2. Therapeutic approaches involving CD28 costimulatory family developed for experimental and clinical kidney transplantation.

CD28 family				
Target	mAbs	Experimental KT (Ref)	Human KT (Ref)	Current status (Ref)
CD80/CD86	Blocking mAbs: h1F1, h3D1	Delayed renal allograft rejection [149]	Effective in combination maintenance therapy [93]	N/A
CD28	Blocking mAb: sc28AT	Prolonged graft survival and increased Treg [94]	Not tested	N/A
	Blocking bivalent mAb (agonistic *in vitro*): JJ319	Prevent chronic rejection and induce donor-specific tolerance [150]	Not tested	N/A
	Super-agonistic mAb: TGN1412	Induced donor-specific tolerance [151]	Life-threatening cytokine-storm [97]	Suspended
CD80/CD86: CTLA-4 interaction	Fusion protein: CTLA-4 Ig (Abatacept)	Unsatisfactory [152]	Not tested	Approved for rheumatic arthritis
	Fusion protein: belatacept	Significant prolonged graft survival [102]	Good clinical results: BENEFIT, BENEFIT-EXT studies [106, 107]. Higher rate of PTLD.	Approved for KT in 2011

KT = kidney transplantation; mAb = monoclonal antibody; Treg = T regulatory cells; PTLD = posttransplant lymphoproliferative disorder; N/A = no data available.

Anti-CD80 and CD86 mAbs (h1F1 and h3D1) have been developed and tested in phase I clinical trials, showing a good safety profile when administrated in combination with standard maintenance immunosuppressive regimens [93].

However, despite these early favorable results, research on this strategy has not progressed to the following phases.

Direct inhibition of CD28-mediated signal has also been considered as a suitable way to prevent rejection in KT. Administration of an anti-CD28 antagonist mAb (sc28AT) was associated to a prolonged graft survival and increased Treg numbers in models of organ transplantation in nonhuman primates (NHP) [94]. Therefore, this approach appears promising and worthy of pursuing, even if no data on human subjects have been reported, so far.

Interestingly, in experimental models of graft versus host disease it was noticed that not only CD28 inhibition, but also its agonism might result in attenuation of the alloimmune response by selectively depleting alloantigen-activated donor T cells [95].

These findings have led to the development of "super-agonistic" anti-CD28 mAbs, which are able to induce a complete activation of T cells even in the absence of TCR stimulation [96]. Although experimental studies have demonstrated the safety of this approach, a phase I clinical trial presented dramatic results. In fact, six healthy volunteers receiving TGN1412, a humanized superagonistic anti-CD28mAb, developed life-threatening systemic inflammatory syndromes attributable to massive cytokine release (the so-called "cytokine-storm") [97]. Following studies revealed that CD28 agonists might activate human CD4+ effector memory T cells inducing the secretion of inflammatory cytokines [98]. Understandably, on the basis of this data, the clinical applications of CD28 agonism were abandoned.

However, the most studied and successful approach in blocking the CD28-mediated costimulatory signals is represented by the CTLA-4 immunoglobulin (Ig) fusion proteins.

These molecules, binding CD80 and C86 with high avidity, prevent CD28 ligation, acting as potent inhibitors of T cell activation [99].

Abatacept (CTLA-4 Ig, Orencia) was the first CTLA-4-related fusion protein (extracellular portion of human CTLA4 plus an Fc part of human IgG1) approved for clinical use in the treatment of rheumatoid arthritis. Although effective, this molecule presents some limitations; in fact, it binds CD80 with much higher affinity than CD86; however, it is not able to completely block T cell activation and proliferation [100]. Nevertheless, giving the potential utility of this approach, further studies were performed, by mutagenesis and screening strategies, aiming to identify new molecules.

Belatacept (LEA29Y, Nulojix) was characterized as a second generation of CTLA4-Ig, with two amino acid substitutions compared with abatacept, resulting in an increased affinity for CD86 and an enhanced ability to inhibit the T cell activation *in vitro* [101].

Early studies performed in KT in NHP gave positive results, being betalacept therapy associated to a marked prolongation of the survival of renal allografts. Therefore, clinical trials on KT in human subjects were designed [102, 103].

A phase II multicenter clinical trial showed that belatacept (at both low and high dose) was comparable with cyclosporine in terms of safety and efficacy, and it was associated with a better renal function [104]. Subsequently, 3-year phase III large randomized clinical trials were performed in KT from both standard (namely, Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression Trial – BENEFIT) and extended criteria donors (BENEFIT-EXT). These trials compared different belatacept regimens to cyclosporine in KT recipients, who received basiliximab induction and were maintained on mycophenolate mofetil and corticosteroids (for more explicative details, see [105, 106]).

Belatacept regimens were noninferior to cyclosporine in terms of patient and graft survival, and they were associated to a better graft function and a reduced incidence of chronic nephropathy. Of note, betalacept was also associated to reduced blood pressure, lower lipid levels and reduced incidence of new onset diabetes after transplant, improving the cardiovascular risk profile [107].

On the opposite, there was a higher rate of acute rejection episodes and an increased incidence of posttransplant lymphoproliferative disorder (PTLD) compared with the cyclosporine group (mainly during intensive treatment).

Currently, the reasons underlying these harmful effects remain obscure, being potentially involved also the regulation of specific T cell subpopulations, such as Tregs and Th17 [108, 109].

However, the good results shown in clinical trials have led to the approval of belatacept by the Food and Drug Administration and the European Medicines Agency in 2011 as the first costimulatory blocker for use in renal transplantation [110].

TNF-related pathway

Different approaches have been tested to modulate the CD40/CD40L interaction, already identified as a promising strategy to treat numerous inflammatory and immune-related diseases, such as lupus nephritis. In fact, it has been shown that CD40 and CD40L expression and release can be modified by the administration of estradiol and by targeting the redox status [111, 112].

Subsequent studies have been focused on the development of anti-CD40L (CD154) mAbs (ruplizumab, torelizumab and ABI 793) to block the CD40/CD40L interaction in the specific setting of KT (see Table 3). While these mAbs prevented allograft rejection in experimental models [113], their administration in NHP undergoing KT was associated with increased thromboembolic events [114], mainly attributed to the role of CD154 expressed on platelets in the stabilization of thrombi [115]. Therefore, due of the concern about thrombotic complications, clinical development of anti-CD154 mAbs has been suspended.

Direct CD40 inhibition through the synthesis of anti-CD40 mAbs was believed to be an alternative way to interrupt the CD40/CD40L pathway without perturbing the hemostasis.

Chimeric anti-CD40 mAbs (ch5D12 and chi220) developed and evaluated first in animal experimental models proved their safety and efficacy in prolonging beta cell islets and KT survival [116]. These promising results have led to the generation of a human anti-CD40 mAb lacking of antibody-dependent cell-mediated and complement-dependent cytotoxicity (ASKP1240). Because of its great efficacy in models of NHP KT, this molecule is currently under investigation in human subjects as well [117]. A recent phase I randomized study in healthy subjects showed that ASKP1240 administration was well tolerated, with no occurrence of adverse effects, such as cytokine release syndrome or thromboembolic events [118], whereas results from clinical studies in de novo KT are still not available (ClinicalTrials.gov: NCT01279538).

So, while waiting for more reliable information, CD40 antagonism seems to be a promising approach, especially considering that the experimental data suggest that CD40 blockade may act synergistically with CD28 pathway antagonism prolonging graft survival [119, 120].

Finally, Ripoll et al. [121] have evaluated the effect of intragraft CD40 gene silencing with an anti-CD40 siRNA in an acute vascular rejection model of KT. In this interesting study, CD40 silencing reduced the development of donor-specific antibodies, graft complement deposition and immune-inflammatory mediators, by shifting the type of rejection from humoral to cellular. This completely different approach to modulate the CD40/CD40L pathway was also associated with an increased graft survival. Even if

TABLE 3. Therapeutic approaches involving TNF-related and adhesion molecules costimulatory families developed for experimental and clinical kidney transplantation.

Target	mAbs	Experimental KT (Ref)	Human KT (Ref)	Current status (Ref)
		TNF-related family		
CD154	Blocking mAbs: hu5C8	Prevented acute rejection, but associated to thrombosis [113]	N/A	N/A
	Humanized blocking mAbs: ruplizumab, torelizumab and ABI 793	N/A	Not tested	Suspended. Studies in SLE showed high rate of thrombosis [153]
CD40	Blocking chimeric mAbs (Chi 220, Ch5D12)	Prolonged graft survival in NHP [116]	Not tested	N/A
	Blocking mAb: ASKP1240	Prolonged graft survival in NHP and suppressed T cell response [117]	Under evaluation in clinical trial (NCT01279538)	Phase I study completed [118]
		Adhesion molecules		
LFA-1	Blocking mAbs: efalizumab	N/A	Effective in phase I/II trials, but high incidence of PTLD [125]	Withdrawn in 2009 (episodes of PML in psoriasis-126)
CD2	Anti-CD2 fusion protein: alefacept	N/A	Unsatisfactory [128, 129]	Withdrawn in 2011

Note: Therapeutic approaches based on TIM pathway interference have not been reported, so far (see text).
KT = kidney transplantation; mAb = monoclonal antibody; Treg = T regulatory cells; SLE = systemic lupus erithematosus; PTLD = posttransplant lymphoproliferative disorder; PML = progressive multifocal leukoencephalopathy; N/A = no data available.

not fully supported by clinical data these early findings represent a promising road for the development of future different therapeutic strategies targeting the costimulatory pathways.

Adhesion Molecules

Adhesion molecules of both integrin and Ig families, as reported earlier, have a key role in cell-to-cell interactions and mediation of costimulatory signals.

In particular, because these molecules, such as LFA-1 and CD2, are widely expressed on memory cells, the inhibition of these pathways was considered to have an effective role in modulating long-term memory T cell responses [122]. Therefore, interventions on adhesion molecules have been developed and investigated also in clinical trials (see Table 2). Anti LFA-1 mAbs, when administrated in combination with other immunosuppressive drugs, have shown the capacity to inhibit humoral responses, specifically targeting CD8 memory cells, which may be resistant to CD28 blockade [123, 124]. Efalizumab (Raptiva) is a humanized anti-CD11a mAb, approved by the FDA for use in psoriasis in 2003. In a phase I/II randomized open clinical trial Vicenti et al. [125] tested different dose regimens of efalizumab in combination with cyclosporine in de novo renal transplantation. The treated groups presented a good graft and patient survival, but the higher dose group was burdened with an elevated rate of PTLD. These data highlighted the need of further investigations, but, unexpectedly, few years later, in 2009, efalizumab was withdrawn because of some cases of progressive multifocal leukoencephalopathy that occurred in psoriatic patients [126].

As for LFA-1/ICAM, also the LFA-3/CD2 interaction has been matter of research (see Table 3).

An anti-CD2 fusion protein (alefacept, Amevive), consisting of LFA-3 plus the Fc portion of human IgG1, was approved for use in psoriasis 2003 [127]. Also this drug was tested in clinical trials in KT (ClinicalTrials.gov: NCT00543569 and NCT00617604), but results were unsatisfactory and currently alefacept is no longer available because it was withdrawn from the market in 2011 [128, 129].

TIM Molecules

Therapeutic approaches based on the modulation of TIM pathways have been reported only in experimental models. In fully MHC-mismatched vascularized mouse cardiac allografts, a short course of anti TIM-1-specific antibody allowed a prolongation of the graft survival, which was associated with inhibition of alloreactive Th1 responses and preservation of Treg population [130].

On the opposite, TIM-3 blockade accelerated allograft rejection in murine transplant models, while the administration of soluble Galectin-9 promoted allograft survival in models of skin and cardiac transplantation [131, 132]. Therefore, interference with the TIM pathways may be a promising way of promoting transplant tolerance. However, this hypothesis should be evaluated by specific designed studies also in KT setting.

NOT "CONVENTIONAL" EFFECTS OF COSTIMULATORY PATHWAYS

Beyond immunological mechanisms, it is now well accepted that other nonimmunological factors may influence the course of KT.

These factors imply: donor age, sex, viral infections, CIN nephrotoxicity and cardiovascular risk factors (hypertension, diabetes), etc [133].

In particular, atherosclerosis plays a crucial role, also considering that death of graft recipients by cardiovascular diseases is one of the major causes of graft failure [134].

As known, atherosclerosis represents a chronic inflammatory disease, involving cytokine production and release, immune cell activation and endothelial damage [135]. Both the innate and the adaptive immune systems participate in the pathogenesis of atherosclerosis, as proved by infiltration of macrophages and T cells (with different subsets) in atherosclerotic plaques [136, 137]. Also in this setting, costimulatory molecules might have a key regulatory role. Almost all molecules of the different costimulatory families have been found in the vessel wall such as on atheroma-related cells [138, 139]. Dendritic cells of patients suffering from cardiovascular disease display an increased expression of CD80 and CD86, whereas expression of PD-1 and PD-L1 (potent inhibitors of T cell activation processes) is significantly decreased [140, 141].

Particular attention has been focused on the CD40/CD40L pathway because of the observation that CD40 and CD40L are widely expressed on inflammatory cells. Recent studies have shown that activated platelets express and release CD40L, which, in turn, mediates lymphocytes activation, endothelial inflammation and enhanced cytokine production [142].

In this regard, Lievens et al. [143] demonstrated, in a mouse model, that platelet CD40L presents potent proatherogenic effects promoting both leukocyte and platelet adhesion to the endothelium. Repeated intravenous injection of activated CD40L-/- platelets prevented the profound increase of atherosclerosis and the alteration of T cell homeostasis, which were instead observed after injection of CD40L+/+ platelets.

Moreover, CD40/CD40L interactions on endothelial cell surface result also in adhesion molecule expression leading to plaque instability [144]. Therefore, it is now clear that CD40/CD40L interaction may be involved in both the early and late stages of atherosclerosis, acting as a molecular link between platelets, inflammation, thrombosis and atherogenesis [145].

The clinical counterpart is constituted by the observation that sCD40L levels may predict an increased risk of cardiovascular events in patients with unstable coronary artery disease [146]. In addition, increased CD40, CD40L and sCD40L levels have also been observed in type 1 and 2 diabetic patients, where sCD40L levels are related to disease severity [147]. All together, these findings indicate that costimulatory pathways, beyond having strictly immune effects, regulate multiple pathological processes, including atherogenesis and inflammation, which might potentially influence clinical outcomes in KT patients. This evidence casts new light on the potentiality offered by the development of pharmacological treatments to modulate costimulation [148].

CONCLUSIONS AND FUTURE PERSPECTIVES

In this review, we have summarized the current knowledge on the interesting and constantly developing field of costimulatory pathways, focusing on the specific clinical setting of KT.

We described the principal costimulatory molecule families, their function and how they are represented in patients undergoing RRT and KT, also considering the effects of these molecules in the modulation of the atherosclerotic process. Finally, we briefly described the main clinical approaches that have been studied in the attempt to modulate the costimulation.

The history of development of therapeutic strategies has proved to be arduous, complex and time consuming, studded by failures. In fact, even if numerous efforts and investments have been made, to date, only one drug, belatacept, has been approved for clinical transplantation.

Nevertheless, we have acquired new knowledge on immunity, inflammation and cell biology leading to development of new promising agents that are under evaluation.

However, many questions remain unsolved as: (i) the role of other costimulatory molecules, different from CD28, CTLA-4, CD40 and CD40L, that could be potential therapeutic targets; (ii) the impact of the costimulatory status of patients in RRT on the subsequent KT, or, in other words, if modulating pre-transplantation immunity we could improve KT outcome; (iii) if the modulation of costimulatory pathways may influence atherosclerosis and other cardiovascular risk factors in KT recipients, (iv) the potential role of platelets as inflammatory cells and their involvement, together with microthrombosis, in graft rejection and (v) if the drugs currently used in clinical practice may also be employed to modulate costimulatory pathways.

Finally, we think that it could be noteworthy to investigate the effects of a multiperspective approach, including new therapeutic solutions in combination with the more conventional ones, in improving graft survival and reducing drug-related adverse events.

Although these questions appear challenging, the prominent role that costimulation has gained in the last years in many important biological processes, warrants the need for future studies addressed to evaluate these specific issues.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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