REVIEW

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Is it wise to target the late costimulatory molecule OX40 as a therapeutic target?

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Abstract

The immune response triggered following pathogen recognition, though required to clear the infection, can be detrimental if it is produced in excess or fails to resolve promptly. Excessive inflammation contributes to infectious and noninfectious pathologies in the gut (such as inflammatory bowel disease), lung (such as bronchiolitis), and in a variety of autoimmune conditions. T cells contribute significantly to pathology during inflammation. Global anti-inflammatory strategies can alleviate the consequences of exuberant inflammation by suppressing T cell activity, but may leave the patient vulnerable to opportunistic infection. More specific therapies aim to suppress only those T cells involved in the disease process, and one such approach is to target late costimulatory molecules. These are not expressed on naïve or resting memory cells. Rather, they have a specific window of expression and their ligation results in the production of abundant inflammatory cytokines. By targeting these molecules, it is hoped that inflammation will reduce, but that therapies will be specific enough to avoid, global immune suppression. This review focuses on the late costimulatory molecule OX40, compare it with other T cell costimulators, and highlight why it is a more suitable target for immune intervention than other immune suppressive strategies.

Key words: costimulatory molecules, autoimmunity, infection, OX40, immunopathology

Abbreviations: APC – antigen-presenting cell, IL – interleukin, ICOS – inducible costimulator, MHC – major histocompatibility complex, OX40L – OX40 ligand, TCR – T cell receptor, TNF – tumor necrosis factor, TNFRSF – TNF receptor superfamily, TNFSF – TNF superfamily, TRAF – TNF receptor-associated factor, Treg – regulatory T cells.

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T CELL ACTIVATION AND COSTIMULATION

T cell activation begins with the binding of an antigenic peptide to a major histocompatibility complex (MHC) molecule on the surface of an antigen-presenting cell (APC) which is then recognized by the T cell receptor (TCR). Since the TCR has no intracellular signaling domain, additional molecules are recruited (collectively denoted CD3). However, this TCR/CD3 signal, known as signal 1, is not sufficient in itself to completely activate a T cell. The TCR:peptide:MHC interaction is stabilized by co-receptors (CD4 on T helper (Th) cells, CD8 on cytotoxic T cells). A number of other costimulatory molecules are also required to enhance signal 1 and are referred to as signal 2.

Costimulatory molecules on T cells can be divided, on the basis of their structural relationships, into three groups: immunoglobulin (Ig)-like receptors, integrins, and tumor necrosis factor receptor superfamily (TNFRSF) members. The majority of these molecules are expressed following TCR engagement and provide positive signals to the T cell encouraging maturation, proliferation, survival, and cytokine production.

Ig-like receptors, including the constitutively expressed CD28 and the inducible costimulator (ICOS), interact with ligands expressed on the surface of APCs. CD28 provides strong costimulation and, in almost all cases, is necessary for full T cell activation. Signaling through CD28 enhances protein tyrosine phosphorylation [58] and promotes cytoskeletal reorganization [51] and TCR association with lipid rafts [60]. CD28 signaling also increases gene expression and stabilizes mRNA molecules from pro-inflammatory and survival genes [9, 66]. These signals continue until CD28 is displaced by cytotoxic T lymphocyte antigen-4, which arrests signaling and ends the activation process. Without the CD28 signal, T cells become anergic, i.e. they lose the ability to respond to antigen and acquire a quiescent, non-prolif-

erative phenotype [20]. This state of anergy is central to the development of peripheral tolerance and ensures that harmless antigens, encountered in the absence of other "danger" signals, do not trigger a potentially damaging autoimmune or allergic response. In contrast, T cells that receive adequate costimulation are driven to proliferate, produce inflammatory cytokines such as interleukin (IL)-6 and IL-1β, and ultimately survive and progress into the memory pool. The CD28 signal is so vital to full activation of naïve T cells that it is known as signal 2.

Integrins, such as leukocyte function-associated antigen-1, also provide T cells with activation signals following interaction with their ligands on APCs, but are reliant on the presence of other costimulators, in particular CD28, to exert this effect [14]. They also play an important part in T cell migration, interacting with adhesion molecules on endothelial cells to allow T cell diapedesis to the site of inflammation [39].

The TNFRSF includes several late costimulatory molecules. Typically, these molecules are not constitutive and provide positive signals to T cells inducing their survival and proliferation. In contrast to constitutive molecules, such as CD28, and early costimulators, such as ICOS, the TNFRSF members are for the most part exclusively expressed on, and as such may be used to specifically target, fully activated inflammatory T cells. Such targeting has been tested in a variety of animal models of disease and has proved effective in many cases. Several papers detail the use of T cell costimulatory blockade in models of autoimmunity, but there is evidence to suggest that these therapies have potential for the treatment of infectious diseases by reducing the inflammatory pathology associated with an over-exuberant immune response.

This review will focus on the TNFRSF member OX40 and its ligand OX40L, a member of the TNF superfamily. However, targeting other TNFRSF and TNFSF members is likely to provide similar benefits and have similar limitations.

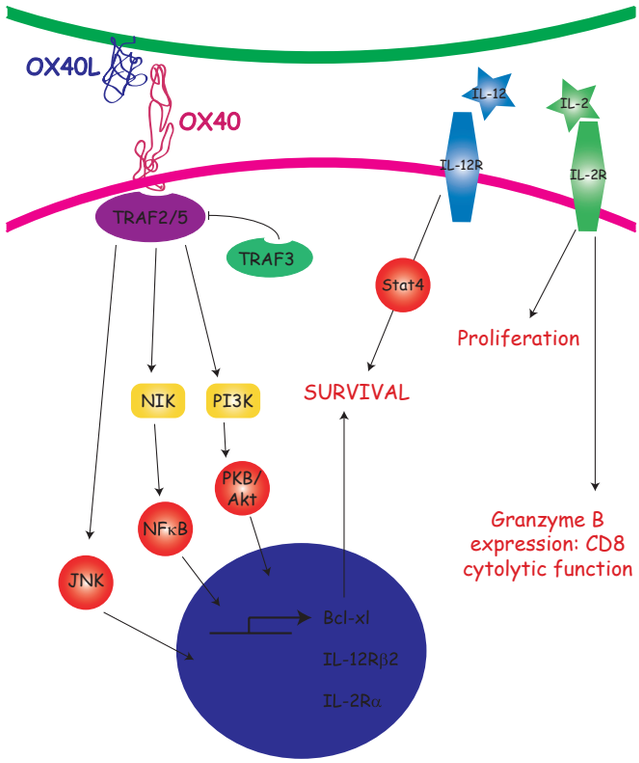

Fig. 1. OX40 signaling results in T cell activation, proliferation, and survival. Signaling is mediated by the TNF receptor-associated factor (TRAF)2 or TRAF5. The downstream signaling events culminate in the translocation of transcription factors to the nucleus and the subsequent transcription of several genes. These include the anti-apoptotic factor Bcl-xl, the β chain of the receptor for IL-12, and the α chain of the receptor for IL-2. Ligation of the IL-12R by IL-12 leads to the activation of the transcription factor Stat4, which has been linked to T cell survival. As well as being involved in T cell proliferation, IL-2 signaling is thought to regulate the OX40-related augmentation of CD8 T cell function by enhancing the production of granzyme B. Signaling is arrested by the competitive recruitment of TRAF3 to the intracellular portion of OX40.

OX40

OX40 (CD134) is a 50-kDa glycosylated protein which, as a conventional member of the TNFRSF, has three extracellular cysteine-rich domains which interact with the extracellular domains of OX40L on APCs [56]. Both molecules are membrane bound and trimerization is required for signaling, another common feature of TNF/TNFR superfamily members [10]. Following ligation, OX40-expressing T cells receive signals that result in the transcription of anti-apoptotic factors, such as certain members of the Bcl-2 family [47], and the increase in expression of cytokine receptors such as the IL-12 receptor [48] (see Fig. 1). In addition, it is now clear that signals are transmitted on both sides of the interaction [37] and that OX40L-expressing cells can increase their production of pro-inflammatory cytokines [42], Ig [54], or T cell-attracting chemokines [32] following ligation, which also induces the maturation of dendritic cells [42] and B cells [54].

The majority of TNFRSF members signal through TNFR-associated factor (TRAF) molecules 2 and 5, which promote the activation of NFκB-inducing kinase (NIK) and IκB kinases α and β which, in turn, phosphorylate and degrade IκBα allowing its dissociation from NFκB. The NFκB constituents then move to the nucleus to act as transcription factors enhancing the expression of a variety of genes, including anti-apoptotic Bcl family members [30]. OX40 signals in this way to enhance T cell survival and may also down-regulate the expression of pro-apoptotic molecules, such as Bad and Bim, via phosphatidylinositol-3 kinase, further ensuring T cell survival and progression into the memory pool [13, 47]. These signals are eventually arrested by the competitive recruitment of TRAF3 to the intracellular part of OX40, which displaces TRAF2 and prevents signaling to NIK [55] (see Fig. 1).

Many TNFRSF members rely on TRAF molecules to initiate intracellular signaling pathways. TRAF molecules initiate phosphorylation cascades which result in the activation and translocation to the nucleus of transcription factors such as NFκB and c-Jun N-terminal kinase. Ruby et al. [48] describe a number of genes that are affected by OX40 signaling, including the β chain of the IL-12 receptor, which signals through the transcription factor Stat4 and is required for OX40-mediated T cell survival. Furthermore, the reported up-regulation of the IL-2 receptor's α chain [45] would augment IL-2 signaling. It is also suggested that IL-2 signaling is required for OX40-dependent enhancement of $CD8^+$ T cell effector function.

OX40L

OX40L is present on the surface of a number of cell types and its expression can be constitutive or induced by the presence of Toll-like receptor ligands and/or inflammatory cytokines [5, 7, 28, 42]. The downstream signaling pathways of OX40L have been less extensively studied than those of OX40, but c-fos and c-jun mRNA levels increase in endothelial cells following OX40L ligation [37] and there are a number of functional outcomes of ligation depending on the type of cell studied. Dendritic cells respond to OX40L ligation by increasing the production of pro-inflammatory cytokines [42], B cells receive maturation signals for differentiation into plasma cells and increase Ig production [53], and activated endothelial cells produce RANTES (CCL5), a T cell-attracting chemokine, suggesting a role for the OX40-OX40L interaction in T cell migration to inflammatory sites [32].

The expression kinetics of OX40 and OX40L are complimentary. Both molecules are up-regulated following MHC:peptide:TCR interaction, peak 2–3 days later, and are lost after 5–6 days. OX40 is absent on resting memory T cells, but is up-regulated in as little as 4 h following re-activation [7, 21, 22].

As well as its role in the activation and subsequent survival of naïve T cells, OX40 provides a number of other signals that contribute to the inflammatory environment. Regulatory T cells (Treg) may express OX40 to a high level [57]. However, signaling through OX40 reduces the regulatory activity of Treg, preventing their production of IL-10 [29] and reducing levels of the transcription factor Foxp3 [61]. In addition, OX40 signaling breaks T cell anergy [34] and leads to the activation of auto-reactive T cells that can then go on to cause extensive tissue damage and potentially initiate autoimmunity.

REDUNDANCY AMONG THE COSTIMULATORS

Several costimulatory molecules show similar expression kinetics and many exhibit redundancy of function to a certain extent. Even CD28 signaling can be substituted in part with signaling through 4-1BB [49], which can induce naïve T cells to produce IL-2 in the presence of a strong TCR signal. It is well known that many costimulatory molecules have similar downstream effects, but their expression kinetics vary and their mere presence suggests that there is a requirement for a succession of signals to ensure full T cell activation and memory formation. Indeed, mice deficient in any one of these molecules exhibit some form of immune dysregulation, implying that no one molecule is entirely redundant (see Table 1).

INDUCED COSTIMULATORY MOLECULES IN INFECTION

The provision of positive signals by induced costimulatory molecules plays an important role in pathogen clearance. For example, 4-1BB- and ICOS-deficient mice show delayed bacterial clearance during *Listeria monocytogenes* infection and *Salmonella enterica* infection, respectively [35, 59], and CD40L-deficient mice have impaired $CD8^+$ T cell memory responses to viruses [4].

Table 1. Phenotypes of mice lacking costimulatory molecules

Costimulatory molecule	Superfamily	Knockout mouse phenotype	References
CD28	Ig	Impaired germinal center development; impaired antibody class switching; reduced T helper cell activity; reduced IL-2 production	16, 52
ICOS	Ig	Impaired germinal center development; impaired antibody class switching; reduced regulatory and effector memory T cell populations	6, 38
LFA-1	Integrin	Reduced neutrophil migration; recurrent bacterial infection	65
4-1BB*	TNFR	Increased Tcell proliferation; decreased cytokine production by T cells; increased myeloid cell populations	33
OX40*	TNFR	Reduced $CD4^+$ T cell proliferation; reduced T cell memory; decreased IL-2 roduction and T cell proliferation late in the primary response	21, 31
CD40L	TNFR	Impaired germinal center development; impaired antibody class switching	46, 67

*OX40 and 4-1BB knockout mice have no clear phenotype until infected.
LFA-1 – leukocyte function-associated antigen-1.

Because of the strength of signal provided by signal 2, CD28 knockout mice have increased susceptibility to parasitic [15, 40] and bacterial [36] infection. However, in the same bacterial system, ICOS-deficient mice are protected. The mechanism of this protection is the result of a skewing of the immune response from a non-protective Th2 response to a protective Th1 [36], suggesting that positive signals from costimulatory molecules do not always provide protection and can initiate inappropriate immune responses. Mice lacking the glucocorticoid-induced TNF-related protein (GITR) are more resistant to *Candida albicans* infection for similar reasons [1]. Conversely, OX40, though originally thought to be restricted to Th2 cells, can provide enhanced protection against *Cryptococcus neoformans* infection by promoting a Th1 response and increasing the production of interferon-γ [26].

INDUCIBLE COSTIMULATORY MOLECULES IN AUTOIMMUNITY AND IMMUNE PATHOLOGY

In most cases, costimulatory molecules do provide protection and help to clear pathogens effectively and efficiently. However, they can also contribute to the perpetuation of inflammatory responses which may cause damage to otherwise healthy tissues once infection has been cleared or in the context of autoimmunity.

The first study to specifically target OX40-expressing cells highlighted the potential for this therapy in the treatment of autoimmune conditions using murine experimental autoimmune encephalitis as an example [63]. In the decade since this paper was published, the therapeutic potential of OX40 targeting has been explored in several transplantation and autoimmune disease models. Transgenic mice over-expressing OX40L develop spontaneous autoimmune disease [41] which can be ameliorated with OX40L-blocking antibodies. Heart allograft survival is increased following the administration of an OX40-blocking antibody [11] as is the survival of xenoislet grafts in spontaneously diabetic mice [24] and corneal implants following anti-OX40L treatment [23].

In all these models, the efficacy of OX40 blockade is attributed to the reduced activation and increased apoptosis of activated T cells that are responsible, in part, for transplant rejection and a variety of pathologies. It is this reduction in T cell activation that makes OX40 blockade a potential therapy not only for the treatment of autoimmune conditions, but also for the reduction of pathology associated with pathogen-induced tissue damage. Indeed, blockade of OX40 in a mouse model of influenza infection reduces the pathology associated with this disease, prevents destruction of lung tissue, and reduces the number of inflammatory cells infiltrating the lung. These mice are not compromised in their ability to clear the virus and have no apparent decrease in levels of specific antibody [27].

Such therapy could also be considered with reference to other costimulatory molecules and is being explored in a number of models. GITR knockout mice have reduced lung inflammation and pleurisy [12] and blocking 4-1BB also prolongs the survival of corneal allografts [2].

Immune pathology can also be the result of reverse signaling to APCs expressing TNFSF members. For example, OX40L ligation on dendritic cells induces their maturation [42], and a recent paper suggests that an OX40L-induced enhanced maturation state in gut dendritic cells may promote the development, activation, and proliferation of T cells in a mouse model of food allergy [3]. Blocking studies in our laboratory using a mouse model of collagen-induced arthritis also suggest that the OX40L signal is important in the development of autoimmunity (Gwyer and Hussell, unpublished observations).

TARGETING COSTIMULATORS IN HUMANS

No therapies that target OX40 or OX40L are currently clinically available, but several studies show the presence of these molecules in sites of disease. OX40 is expressed on T cells in the brain and central nervous system of multiple sclerosis patients, concentrated around active lesions [8], and is also found on cells in synovial fluid isolated from the inflamed joints of rheumatoid arthritis patients [19, 68]. In these diseases, pathology is localized to these areas and OX40 is not found outside the diseased areas. Increases in OX40 expression on T cells isolated from the peripheral blood have so far only been observed in systemic lupus erythematosus patients [43] and HIV^+ patients [69] who experience systemic symptoms and show much more widespread T cell activation.

A similar expression pattern is observed with other costimulatory molecules in other disease states. For example, one study shows the restriction of 4-1BB expression to T cells found adjacent to hepatocellular carcinomas. 4-1BB is not found on T cells in the livers of healthy people or in the peripheral blood of either group [62]. A separate study demonstrates increased expression of ICOS on $CD4^+$ T cells in the lamina propria of ulcerative colitis and Crohn's disease patients. Again, these patients do not have elevated levels of ICOS on their peripheral blood cells and healthy people show low ICOS expression in both sites. Furthermore, disease severity in inflammatory bowel disease patients positively correlates with the amount of ICOS expressed [50]. These results suggest that costimulatory molecules in general provide a unique target for immune therapies. Expression that is restricted to effector T cells at the site of inflammation would limit the extent to which the immune system is compromised and leave the memory and naïve T cell populations intact.

CAVEATS AND LIMITATIONS

The above studies imply that blockade of the OX40-OX40L interaction has potential as a targeted

immunosuppressant and an anti-inflammatory intervention. However, it is important to consider the possible side-effects of such treatment.

The OX40 signal may play an important role in the suppression of tumors, as shown by the enhanced anti-tumor immunity provided by treatment with an agonist OX40 antibody [44, 64]. It will be important to consider, therefore, the possibility of an increase in the risk of tumors with long-term suppression of OX40 signals and further studies using tumor-prone animal models will be required to establish risk.

These treatments must also be considered for each disease separately; for example, in a murine model of *Cryptococcus neoformans* infection, OX40 blockade protected the mice from infection-induced pathology by reducing the damaging Th2 response. However, in the same model, mice were also protected by an OX40 agonist which switched the destructive Th2 response to a protective Th1 response [26]. Agonizing signals through 4-1BB, whilst exacerbating disease in some models, can alleviate the development of conjunctivitis in one mouse model by suppressing Th2 responses [17].

It is clear that blocking late costimulatory molecules provides more specific protection than generally suppressing the immune system. However, each costimulatory molecule must be viewed individually, as their redundancy and relative expression kinetics will no doubt prove critical. ICOS, for example, is expressed earlier than OX40 and, as such, is found more widely on T cells. Blockade of ICOS during flu infection does reduce inflammation, but also compromises viral clearance [25]. The central role of OX40 in the provision of survival signals and the development of T cell memory raises the question of the effects of blockade on subsequent infection. The development of $CD4^+$ T cell memory in OX40-deficient mice is severely compromised [21] and antibody production may be compromised indirectly if inadequate signals are provided to B cells during the primary immune response as a consequence of the reduced survival of T cells. This has been addressed to some extent in animal models of infection and it appears that protection and memory are maintained without OX40. Data from our laboratory show that OX40 blockade during murine influenza infection does not compromise antibody production and the animals are fully protected against re-challenge with the same virus strain [27]. Gaspal et al. [18] suggest that CD30 (another TNFRSF member) could provide signals to compensate for the loss of OX40, observing significant reduction in antibody responses to vaccination in double-knockout animals.

Though the full consequences of costimulatory blockade are yet to be established, the outlook is promising for these therapies. Their use in the treatment of autoimmunity could provide similar benefits to other immunosuppressive treatments, such as anti-TNF therapy, with fewer associated side-effects. There appears to be a window of opportunity for immune intervention of T cell costimulation. Inhibition of early activation events may compromise immunity and the formation of T cell memory, but late application of therapy will not prevent pathology. If we restrict such therapies to those molecules induced at specific stages of T cell activation, we can rely on redundant pathways to maintain enough T cells to fight infection and create memory without causing pathology.

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