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The Significance of OX40 and OX40L to T cell Biology and Immune Disease

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Summary

OX40 (CD134) and its binding partner, OX40L (CD252), are members of the TNFR/TNF superfamily and are expressed on activated CD4 and CD8 T cells as well as a number of other lymphoid and non-lymphoid cells. Costimulatory signals from OX40 to a conventional T cell promote division and survival, augmenting the clonal expansion of effector and memory populations as they are being generated to antigen. OX40 additionally suppresses the differentiation and activity of Treg, further amplifying this process. OX40 and OX40L also regulate cytokine production from T cells, antigen-presenting cells, NK cells, and NKT cells, and modulate cytokine receptor signaling. In line with these important modulatory functions, OX40/OX40L interactions have been found to play a central role in the development of multiple inflammatory and autoimmune diseases, making them attractive candidates for intervention in the clinic. Conversely, stimulating OX40 has shown it to be a candidate for therapeutic immunization strategies for cancer and infectious disease. This review provides a broad overview of the biology of OX40, and the intracellular signals from OX40, that impact many aspects of immune function, and have promoted OX40 as one of the most prominent costimulatory molecules known to control T cells.

Keywords

OX40; OX40L; CD4; CD8; T cells; Treg; costimulation; immune disease

OX40 and OX40L are Induced on Lymphoid and Non-lymphoid Cells

OX40 (CD134, TNFRSF4) was initially identified as a T cell activation marker (1) and later after cloning shown to be a member of the NGFR/TNFR superfamily with costimulatory function (2-5). OX40 can be induced on both activated CD4 and CD8 T cells, but is not found on resting naïve T cells or most resting memory T cells (1-3). Although it was thought for a long time that OX40 expression was restricted to activated conventional T cells, it has now been visualized on activated regulatory T cells (6), NKT cells, NK cells, and neutrophils. While functional activities of OX40 signaling to NKT cells (7,8), NK cells (9,10), and neutrophils (11), have been described, they are not extensive, and therefore this review we will focus on regulation of conventional T cells and regulatory T cells.

The ligand of OX40 (OX40L, CD252, TNFSF4) belongs to the TNF superfamily and was first identified as gp34 a protein on HTLV-I transformed cells (12,13) and later found to bind OX40 (4,14). OX40L is also not constitutively expressed but can be induced on professional antigen-presenting cells (APC) such as B cells (15), dendritic cells (16) and macrophages (17), in line

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with its action in controlling the extent of T cell priming following recognition of antigen (5, 18). Other cell types such as Langerhans cells (19), endothelial cells (20), smooth muscle cells (21), mast cells (22,23), and NK cells (10) can be induced to express OX40L, suggesting OX40-OX40L interactions can be involved in many aspects of physiological responses between T cells and lymphoid and non-lymphoid cells. T cells can also express OX40L (24) that is functional during T cell-T cell interactions, creating an additional mechanism to further amplify T cell responsiveness (25).

Following antigen stimulation OX40 is transiently induced on activated naive CD4 and CD8 T cells (5,26-28). Little expression is seen within the first hours of activation, and both in vitro and in vivo, OX40 can peak anywhere from 24 hr up to 4-5 days after initial stimulation. Although TCR signals are sufficient for inducing OX40, CD28-B7.1/2 interactions augment and sustain its expression (26,29), and T cell and APC-derived cytokines like IL-1, IL-2, and TNF may further modulate the extent and length of expression. The use of OX40 is obviously dictated by the availability of OX40L, which can be induced within 24 hr of activation of APC such as DC and B cells after ligation of CD40 or from Toll-like receptor stimulation (16,30). The extent of CD28/B7 interactions, that control CD40L induction and then CD40 signaling, also determine the kinetics and extent of OX40L expression (26), as can innate cytokines like IL-18 (31), and TSLP (32).

OX40 Promotes Effector T cell Expansion and Survival

The T cell response to any acute antigenic stimulation has three distinct phases: expansion, contraction and memory generation. After antigen encounter, naïve T cells that exist at very low frequencies proliferate and differentiate into an expanded population of heterogeneous effector T cells. This effector population is much larger than the precursor population (33-35), a feature important not only for combating infectious organisms, but also required for effective anti-tumor activity and development of autoimmune disease, although the latter is obviously a deleterious consequence of overt immune activity. OX40 plays a major role in this process, regulating both CD4 and CD8 T cell clonal expansion. Our initial studies of OX40-OX40L interactions many years ago revealed that OX40L when co-expressed with B7.1 could provide a synergistic costimulatory signal to an antigen-reacting naive CD4 T cell to prolong T cell proliferation, as well as augment production of several cytokines (5). This general principle regarding the action of OX40 was furthered when we demonstrated that OX40 knockout animals generated far fewer primary effector CD4 T cells after immunization, a finding substantiated by studies which revealed that in vivo treatment with an agonist antibody to OX40 could strongly enhance the generation of high frequencies of antigen-specific effector T cells in wild-type mice (18,36) and prevent the induction of T cell tolerance (37). Studies of OX40 knockout animals, or CD4 T cells deficient in OX40, in different model systems have further illustrated the important role for OX40-OX40L interactions in allowing efficient clonal expansion of naive T cells and generating effective primary CD4 T cell responses (38-41). Studies of OX40L-deficient mice, or with blocking antibodies to OX40L, also revealed reduced expansion or functional priming of CD4 T cells (17,29,30,42-44). Accordingly, transgenic mice in which OX40L was constitutively expressed by CD11c+ dendritic cells (45), or by T cells (46), exhibited greatly increased numbers of effector-like CD4 T cells.

Using a deficiency in OX40 in both *in vitro* and *in vivo* systems when naïve TCR transgenic CD4 T cells are stimulated with high affinity peptide (strong TCR signals), we have shown that OX40 does not control initial activation or proliferation but provides signals that maintain late proliferation and prolong T cell survival through the effector phase (25,26,40,41,47). As indicated later, this corresponds with the molecular targets of OX40 that have been defined that include molecules regulating cell cycle progression and molecules suppressing apoptosis. When CD4 T cells are sub-optimally activated with antigen (weak TCR signals), an OX40

deficiency can also markedly lessen early proliferation of CD4 T cells, a finding possibly related to increased generation of adaptive Treg or a defect in IL-4 production as discussed below.

Similarly, OX40 signals also directly promote proliferation and survival of CD8 T cells after antigen encounter, and we have demonstrated this by tracking OX40-deficient CD8 T cells in systems with immunization of protein in adjuvant, delivered by adenoviral vector, or expressed on tumor cells (27,28,48). The timing of action of OX40 is likely different on CD8 T cells because OX40 expression is much more transient than on CD4 T cells (28). In addition, other factors may influence the use of OX40 by CD8 T cells as illustrated by data showing that initial priming of naive CD8 T cells to a number of viruses is independent of OX40, such as with influenza, vesicular stomatitis virus, and mouse cytomegalovirus (38,42,49), whereas certain primary CD8 responses such as that to vaccinia virus are strongly dependent on OX40 (50). The reason for this is unknown, but might relate to production of inflammatory cytokines that provide similar signals and hence bypass a requirement for OX40. Injection of agonist antibodies to OX40 have also revealed a further mechanism to promote CD8 T cell priming, through augmenting helper activity of CD4 T cells, revealed both in virus systems and tumor systems (49,51).

In naïve CD4 T cells, OX40 engagement can preferentially lead to Th2 cell generation driven by early autocrine production of IL-4 (52-54). However, antigen and IL-12 signals can overcome this action thereby allowing OX40 to also promote Th1 responses (18,32,55,56). Although regulation of cytokine production is certainly a feature of OX40 co-signaling, because innate signals to T cells or non-T cells can result in production of cytokines such as IL-4, IL-12, and IL-6 that can strongly skew differentiation into distinct T cell lineages, the action of OX40 in regulating T cell division and survival is arguably the dominant activity of this molecule. Thus, OX40 costimulation enhances an ongoing immune response regardless of the type of polarized response, further illustrated in studies mentioned below where the absence of OX40/OX40L interactions suppresses immune disease development that is driven by Th1, Th2, Th17, or CD8 T cells.

OX40 Blocks Natural Regulatory T cell Activity

An action on regulatory T cells has recently revealed another aspect to the biology of OX40-OX40L interactions that will further allow expansion and functional activity of effector T cell populations (6). An immune response needs to be controlled to prevent autoreactivity and this is in part mediated by suppressive activities of specialized subsets of T cells termed Treg (57). Several subtypes of Treg have been described, which fall into two major groups, namely nTreg or naturally occurring CD4+CD25+Foxp3+ cells, and iTreg or inducible or adaptive regulatory T cells (58,59).

nTreg develop in the thymus, and the transcription factor Foxp3 is selectively expressed by these cells and is required for their development and suppressive function (60). OX40-deficient mice have reduced numbers of nTreg in very young mice but normal numbers in adulthood, showing that OX40 has little role in their development and homeostasis (61). However, nTreg express high levels of OX40 (constitutive in mice, rapidly induced in humans) and several groups have reported impaired suppressive function of nTreg when OX40 is ligated, either in vitro, or in vivo in models of inflammatory bowel disease (61), rejection of allogenic bone marrow (62) or skin transplants (63). It has been proposed that the effect of OX40 is due to blocking the inhibitory activity of nTreg, although in some cases a stimulatory action on effector T cells making them resistant to suppression could have explained the results. However, using OX40-deficient CD25-CD4+ T cells responding to antigen and cocultured with wild-type or OX40-deficient antigen-specific nTreg in vitro, we have confirmed that

OX40 signaling directly to a Treg can block its regulatory activity (So, unpublished). This action would then further amplify the direct effect on OX40 in promoting division and survival of effector T cells, allowing greater numbers and functional activity of this population of cells. nTreg still express Foxp3 after OX40 is ligated, and so the molecular explanation for any reduced suppressive capacity is currently unknown. Multiple mechanisms have been proposed to explain how Tregs function. One study showed that nTreg might utilize CTLA-4 to down-regulate B7.1 and B7.2 expression on dendritic cells, thus affecting the capacity of antigen-presenting cells to activate conventional T cells (64). OX40 has been reported to down-regulate CTLA-4 expression (65), but whether this might explain the action on blocking nTreg function has not been investigated.

OX40 Antagonizes Generation of Inducible Regulatory T cells

Perhaps as significant as blocking Treg activity is to inhibit the generation of new Treg. Naive T cells in the periphery can also acquire Foxp3 and become iTreg, driven primarily by TGFβ and potentiated by IL-2 and retinoic acid. As well, other iTreg can be induced from naive T cells, including IL-10-secreting Foxp3-cells, called Tr1 cells, promoted by IL-10 or dexamethasone/vitamin D3 (66).

OX40 signals have recently been shown to antagonize Foxp3 induction in naïve CD4 T cells (63,67). We found that T cells converted to a greater extent into Foxp3+ cells when OX40 was not expressed, and accordingly, an agonist antibody to OX40 also markedly suppressed CD4 T cells differentiating into Foxp3+ iTreg in response to TGF-β (67). Using a mouse model of tolerance to inhaled antigen, we have confirmed this activity in vivo, showing that OX40 signals, cooperating with innate signals from TLR4, suppressed the induction of tolerance by blocking the generation of Foxp3+ iTreg and concomitantly promoting the development of effector T cells, leading to susceptibility to developing airway disease (68). Intranasal antigen induced antigen-specific CD4+Foxp3+ Treg that outnumbered IL-4- and IFN-γ-producing effector CD4 T cells by 100:1 or greater. Inhaled LPS, given with intranasal antigen, altered the ratio of Treg to IL-4+ or IFN-γ+ T cells in favor of effector cells via inducing OX40L, and consequently blocking OX40L-OX40 interaction prevented loss of tolerance and resulted in the generation of greater numbers of iTreg. This antagonistic role might be due to the capacity of OX40 signals to directly interfere with TGFβR signals in some cases (67), but can also be explained by a feedback inhibition brought about by OX40-induced T cell cytokines such as IL-4 and IFN-γ that synergize with APC-derived cytokines like IL-6 to prevent the continued expression of Foxp3 (68).

Additionally, it has been reported that OX40 signals inhibit the generation of IL-10-producing iTreg. OX40 blocked upregulation of IL-10 in vitro in both responding naïve and memory CD4 T cell populations, and ligation by OX40L also inhibited IL-10 expression in differentiated Tr1-like iTreg (69). Whether this is a major activity in vivo is not yet clear, although we found a modest effect of OX40 signals, provided by injection of an agonist antibody, in blocking a similar IL-10-producing CD4 T cell population that develops in the salivary glands of mice infected with cytomegalovirus (70).

OX40 Promotes the Generation of Memory CD4 T cells

After the primary effector T cell response, when antigen is no longer available, a contraction phase ensues whereby most antigen-specific T cells die, both to reduce the potential of immune pathologic tissue damage and to maintain space for new T cell populations to develop to other antigens. However, a subpopulation of the activated primary cells survive, called memory T cells, and these can confer protective immunity to the host. The memory T cells have a capacity to persist, and acquire a set of functions including the ability of self-renewal after antigen removal and rapid effector responses upon antigen re-exposure. The overall size of an initial

memory T cell pool might be determined by several factors, that impact either naive T cell activation and differentiation, the extent of primary clonal expansion, or the extent of death during the contraction phase of the primary response. After antigen removal, common γ chain cytokines such as IL-7 and IL-15 support the maintenance of memory T cells.

Work with OX40-deficient mice has clearly demonstrated that OX40 can have a great impact on the ability to generate high frequencies of memory CD4 T cells (18,47,71,72), substantiated by other studies using in vivo treatment with agonist anti-OX40 that can strongly promote more memory CD4 T cells to develop (18,36,65,73). Exactly when OX40 acts to promote memory generation is not clear, nor the cell type that might drive this through expression of OX40L. Recent two-photon imaging of CD4 T cell behavior in the lymph nodes showed that the interaction of a naïve CD4 T cell with a DC might last 24-48 h after antigen priming (74,75). Although OX40 on T cells and OX40L on dendritic cells can be upregulated during this time period, expression of both OX40 and OX40L on CD4 T cells can occur later than 48 hr after initial antigen encounter, and OX40L can also be present on other APC at times potentially after T cell-DC interactions are terminated. It is likely in many cases that OX40L will be provided initially by an activated DC, however OX40L expressed by other cell types may also provide OX40 signals to activated daughter T cells. In line with this, some studies suggest that OX40L on $CD4^+CD3^-$ lymphoid tissue inducer cells (LTi), B cells, and responding T cells themselves, can support T cell survival at the late effector phase (25,76,77). According to this model, OX40-OX40L interactions in either early or late priming phases may control the accumulation of effector T cells at the peak of immune responses, which then by default concomitantly translates into controlling the magnitude of the memory pool. This assumes that an effector T cell can convert to a memory T cell, and that a set percentage of effectors will always survive the contraction phase of the primary response and become memory cells (18, 36,65). These conclusions have been reinforced by the analysis of transgenic mice that express OX40L on DC or CD4 T cells (45,46), in which constitutive OX40 signals lead to increased numbers of polyclonal ($CD44^{hi}$ $CD62L^{lo}$) effector/memory CD4 T cells and induce development of interstitial pneumonia and inflammatory bowel disease (46).

OX40 Differentially Controls Development of Subsets of Memory CD4 T cells

Although some studies support a simple effector to memory transition, as described above, other data suggest that distinct subsets of memory CD4 T cells are generated by alternate pathways. Some will develop following the effector to memory pathway, while the commitment of others might occur during early priming of naive T cells and not involve an effector state. There is heterogeneity in memory T cell populations, which have been categorized into two primary subsets, effector memory (Tem) and central memory (Tcm) T cells (78). Tcm are identified by expression of L-selectin (CD62L) and CCR7, preferentially home in secondary lymphoid tissues, and contribute to long-term protection upon re-exposure to Ag. However, Tem do not express CCR7 and CD62L, reside in extra-lymphoid tissues, and upon recall response induce rapid effector function (78-81). Due to the sizable numbers of memory CD8 T cells and appropriate techniques to detect them, much is known about the generation and lineage development of CD8 Tcm and Tem cells, but there is little progress regarding how and when CD4 Tcm and Tem cells actually form (82,83).

It has been proposed that shortly after naive CD4 T cell activation, the precursors of Tcm and Tem develop, characterized as $CD44^{hi}CD62L^{hi}$ and $CD44^{hi}CD62L^{lo}$ populations respectively, with the idea that Tem precursors go through a “true” primary effector stage before transitioning to a memory population, whereas Tcm precursors are less activated and do not become “true” primary effectors. Our studies tracking these populations in one model system, using adoptive transfer of OVA-specific TCR transgenic T cells responding to cognate antigen, demonstrated that the absence of OX40 signals selectively impaired the generation of CD4 Tem, but had

little effect on Tcm, in both lymphoid and non-lymphoid tissues (47). This suggests that only Tem precursors received OX40 signals, resulting in a reduction of primary effectors and then Tem cells developing over time. Comparable expression of OX40 and other activation markers on early arising $CD62L^{hi}$ and $CD62L^{lo}$ T cells was found, indicating that both precursor subsets had been activated to some extent. Moreover, the OX40 deficiency did not promote more Tcm cells to develop, implying that the precursors of this population did not receive OX40 signals at all rather than OX40 dictating the early balance between these populations. In line with this, comparable development of both Tem and Tcm precursors was found within 3 days of naïve T cell activation regardless of the absence of OX40 signals (47). Tcm may then develop from a subset of activated CD4 T cells that are not fully differentiated, and this idea is substantiated by studies showing that naïve CD4 T cells that arrive late into the draining lymph node may encounter lower numbers of peptide-MHC complexes, divide less, and most differentiate into Tcm cells (84). In line with this, when few naïve T cells respond to antigen, and antigen is not limiting, either with endogenous naïve CD4 T cells tracked with tetramer (85), or with a very low number of adoptively transferred naïve TCR transgenic CD4 T cells (Soroosh, unpublished), most become $CD44^{hi}CD62L^{lo}$ Tem rather than $CD44^{hi}CD62L^{hi}$ Tcm. In the latter case, the frequency of total CD4 memory T cells that develop is also severely reduced in the absence of OX40. Thus, in contrast to Tem precursors, Tcm precursors may never interact with OX40L-expressing APC, or alternatively they could be refractory to OX40 signals because of some unknown negative activity that keeps them from transitioning to effector cells. This emphasizes that transient OX40 signals that occur during priming of naïve CD4 T cells imprint a long-lasting survival advantage in Tem precursors and effector CD4 T cells that contributes to optimal development of Tem (Fig. 1).

OX40 and the Reactivation of Memory CD4 T cells

At one time co-stimulatory signals were not thought to be required for reactivation and effector function of memory CD4 T cells based on the fact that memory cells could respond by proliferating fairly well to anti-CD3, and could make cytokines like IL-4 quite efficiently when antigen was presented on poorly costimulatory APC like resting B cells that were ineffective in stimulating naive T cells (86). Consistent, with this, it was found that memory CD4 T cells were less dependent on co-stimulation by B7 molecules compared to naive T cells, and independent of co-stimulation by CD40-CD40L (87). However, our studies a number of years ago in vivo showed that blocking OX40-OX40L interactions strongly suppressed the response of memory T cells that mediated lung inflammation, showing that rather than becoming co-stimulation independent, memory CD4 T cells may shift to being more reliant on inducible co-stimulatory molecules such as OX40 for their response (88).

OX40 is down-regulated and returns to base-line levels after the effector phase of a response, but is rapidly re-expressed on memory T cells following re-encounter with antigen (5,88). In a murine model of asthma, memory CD4 T cells, elicited by initial systemic immunization with antigen in alum, can induce eosinophilic lung inflammation when reactivated several weeks later by challenging mice with aerosolized antigen. In this scenario, OX40L is rapidly up-regulated on dendritic cells and at the same time, the frequency of effector CD4 T cells ($CD44^{hi}/OX40^{+}$) dramatically increases in lung draining lymph nodes, bronchoalveolar lavage (BAL), and lung tissue (88). Showing OX40L interactions were required for this recall response, a blocking anti-OX40L antibody administrated at the time of rechallenge with aerosolized antigen completely inhibited memory T cell expansion and lung inflammation. In order to show that OX40 directly controlled the expansion and effector function of the memory population, antigen-specific memory Th2 cells were generated from TCR transgenic mice deficient in OX40, and found to be strongly impaired in expanding in vivo and unable to promote lung inflammation when mice were exposed to inhaled antigen (88). Demonstrating that this was not confined to a Th2 response, similar data were obtained transferring an OX40-

deficient Th1-like memory population (Salek-Ardakani, unpublished). Confirmatory data have been obtained in a reciprocal experiment where wild-type antigen-specific memory CD4 T cells were generated by transferring naïve TCR transgenic T cells into congenic mice followed by immunization with antigen in LPS as an adjuvant. The resulting uncommitted/Th1-like memory T cells were re-isolated several weeks later and transferred into wild-type or OX40L-deficient mice. After secondary challenge with antigen, memory CD4 T cells were again markedly impaired in accumulating in the absence of OX40L (Soroosh and Ishii, unpublished data). Interestingly, the resultant secondary effector T cell population displayed a predominantly Tem phenotype ($CD62L^{lo}$) in wild-type hosts but the few effectors that accumulated in OX40L-deficient hosts were mainly Tcm phenotype ($CD62L^{hi}$). Whether this indicates that only Tem but not Tcm use OX40 for their recall response is not clear and needs to be investigated in the future.

To further confirm the important role of OX40 co-stimulatory signals during reactivation of memory CD4 T cells, polyclonal $CD25^{neg}CD44^{hi}$ memory phenotype CD4 T cells were adoptively transferred into sublethaly-irradiated lymphopenic mice. In contrast to monoclonal memory T cells that undergo a homogeneous slow rate of homeostatic proliferation, polyclonal CD4 T cell comprise two distinct populations that undergo either an extremely rapid or a slow rate of cell division (89). The rapidly dividing cells most likely respond to antigens in the environment, mirroring a conventional memory recall response. The slow dividing cells are independent of TCR signals but require homeostatic cytokines (IL-7 and IL-15) for their basal proliferation (89). We found that although the absence of OX40 signals had no effect on the slow-dividing memory cells, blocking OX40-OX40L interactions strikingly suppressed proliferation, accumulation, and IFN-γ production by the rapidly dividing memory cells (Ine, Soroosh and Ishii, unpublished data). Taken together these data indicate a novel role for OX40 as a regulator of the recall response of a memory CD4 T cell, and suggest an important clinical application for using OX40 or OX40L blocking antibodies to abrogate the pathologic consequences of Tem cells in allergic disorders, autoimmunity, and chronic disease.

OX40 Promotes Generation and Maintenance of Memory CD8 T cells

Although initial studies using mouse models of viral infection suggested that OX40 signals had a minimal impact on generating CD8 T cell responses (38,90,91), other data in contact hypersensitivity reactions and allograft rejection found CD8 T cell responses were impaired when OX40L was absent (30,42). As mentioned previously, we then showed that OX40 could directly control the response of primary CD8 T cells using OX40-deficient antigen-specific CD8 T cells from TCR transgenic mice (27,28). We also found that OX40 could augment memory generation and secondary CD8 T cell responses to tumor cells (48,51). Further confirming that OX40-OX40L interactions can control CD8 memory, using acute influenza virus infection and tracking endogenous virus-specific CD8 T cells, a study demonstrated that OX40 had little impact on initial priming of CD8 T cells, but mice deficient in OX40L exhibited diminished formation of both lymphoid and non-lymphoid resident virus-specific memory CD8 T cells (92). Moreover, the absence of OX40L during priming also led to reduced accumulation of secondary effector CD8 T cells after memory cells reencountered antigen, although in this case the authors concluded that OX40L was not needed during the recall response itself but rather programmed CD8 T cells during priming for enhanced reactivity (92). Our recent data in a murine model of vaccinia virus infection has also found that mice deficient in OX40 generate far fewer virus-specific memory CD8 populations, although in this case the primary response was also strongly compromised (50). Whether OX40 plays a role in reactivation of vaccinia-specific memory CD8 T cells has not yet been addressed. This raises a question of why OX40 has varying roles in CD8 responses to different infections. In this regard, cytokines such as IFN-I and IL-12, produced in response to pathogens, have been shown to also strongly influence the generation of CD8 T cells, perhaps imparting similar signals to

those transmitted by more classical costimulatory molecules such as OX40 (93,94). Analysis of several infections revealed differential requirements for IFN-I in controlling the initial expansion and generation of memory CD8 T cells, and interestingly that IFN-I played little role during infection with vaccinia virus (95). Thus the alternate use of OX40 might depend on the amount and type of innate cytokines induced by a virus. These might either simply replace the need for an OX40 signal, or directly control virus replication such that the action of OX40 in providing expansion or survival signals to CD8 T cells is not needed because a large effector and memory population does not need to be generated. We found another variant of the use of OX40 during infection with murine cytomegalovirus (MCMV). OX40 knockout mice in this case again showed no defect in generating acute primary CD8 T cell populations, but were impaired in accumulating high numbers of later arising, so-called inflationary memory CD8 T cell populations, whereas the lower numbers of non-inflationary memory T cells derived from the initial acute populations were unaffected (49). Thus, the differential requirement for OX40-OX40L interactions by CD8 T cells in response to varying viral infections might relate to the rate of viral replication, antigenic load, time of access to antigenic peptides, frequency of epitope-specific naïve T cells, and the specific cytokine milieu induced by each virus.

To further dissect the role of OX40 in development of memory CD8 T cells to pathogens, we performed adoptive transfer experiments with OVA-specific OX40-deficient TCR transgenic CD8 T cells, with recombinant *Listeria monocytogenes* expressing OVA (r*LM*-OVA) as an example of acute bacterial infection. There was minimal use of OX40 for the early accumulation and effector function of CD8 T cells. However, the absence of OX40 on OVA-specific CD8 T cells severely compromised the size of the memory T cell pool that was generated (96). In regard to the differentiation pathway for generation of memory CD8 T cells, it has also been proposed that early cells arising from naive populations might be precursors of separate pathways to effector or memory cells. Recently, Kaech and colleagues (97) showed that IL-7R and killer cell lectin-like receptor G1 (KLRG1) can be used to distinguish so-called short-lived effector CD8 T cells (SLEC; IL-7R^{lo} KLRG1^{hi}) from memory-precursor effector T cells (MPEC; IL-7R^{hi} KLRG1^{lo}) thought to give rise to memory populations in some cases. Using these markers, we found in response to *Listeria* that the absence of OX40 diminished the frequency of IL7R^{hi}KLRG1^{lo} MPEC that arose early in the response, correlating with the subsequent reduced size of the memory T cell pool. During the infection, the majority of KLRG1^{lo} cells converted to KLRG1^{hi} cells and died in the absence of OX40 signals. Not surprisingly, the recipient mice containing the few memory CD8 T cells that arose in the absence of OX40 were not able to clear a subsequent infection with bacteria. However, isolating comparable numbers of memory CD8 T cells from the primary hosts and transferring into a second host infected with *rLM-OVA* indicated an equivalent protective capacity of OX40-deficient memory CD8 T cells on a per cell basis (96). These data strongly illustrate that there is not one rule governing the use and function of OX40, with variations between CD4 and CD8 T cell responses, and within CD8 T cell responses to different pathogens or antigenic environments. In some cases OX40 controls the initial effector T cell population that forms the acute response and gives rise to the bulk of the memory population. In contrast, in other situations, OX40 only controls a subset of early-activated T cells that appear to give rise to memory, but it does not impact the acute response (Fig. 1).

Another aspect of memory CD8 T cell regulation that OX40 might control in some instances is the ability to persist long term, because of an action on the antigen-independent self-renewal process called basal homeostatic proliferation, that similar to CD4 T cells is thought to be largely controlled by IL-7 and IL-15 (98,99). Adoptive transfer of antigen-specific memory CD8 T cells revealed that blocking OX40-OX40L interactions, during the induction of these memory cells to *Listeria* infection, severely inhibited their subsequent homeostatic proliferation, and contributed to their decline in numbers over a period of several weeks (96). The explanation for this is not clear. It is possible that OX40 may regulate the future ability of

memory CD8 T cells to divide by altering the expression and function of genes involved in cell cycle progression (100), or more likely might impact the continued expression or functionality of cytokine receptors like IL-7R or IL-15R that are required for basal proliferation.

Collectively, the important function of OX40 signals in priming and generation of CD4 and CD8 T cell immunity, and in development of T cell memory, propels OX40 and OX40L to the forefront of potential targets for therapy of autoimmune and inflammatory disease, as well as for the design of vaccination and therapeutic adjuvant strategies for infectious disease and cancer. Mouse models of disease described briefly below and summarized in Tables I and II have reinforced this notion.

OX40 Controls inflammatory Disease and Cancer

Most of the major models of inflammatory or autoimmune disease have now been examined with respect to the involvement of OX40 and OX40L (Table I). OX40- and OX40L-deficient mice showed significantly attenuated Th2 lung inflammation in allergic asthmatic mouse models (39,101), and as mentioned above, blocking OX40L markedly suppressed memory effector T cell accumulation and therefore abolished the key features of asthmatic responses including eosinophilia, airway hyper-responsiveness, mucus production and Th2 cytokine production (88). Blocking OX40L was also recently shown to prevent allergic asthmatic disease induced by TSLP and most significantly also showed therapeutic efficacy in a pre-clinical monkey study (102), further suggesting great promise for therapy of asthma and atopy.

OX40L-OX40 interaction also plays a pivotal role in the pathogenesis of experimental allergic encephalomyelitis (EAE). Encephalitogenic myelin basic protein (MBP)-specific T cells upregulated OX40 during the onset of EAE, and deletion of OX40+ effector T cells at the site of inflammation resulted in amelioration of EAE (103). CNS-derived APC from mice with actively induced EAE express OX40L and initiate effector T cell responses. Reduced ongoing signs of disease were observed with administration of soluble OX40.Ig fusion protein at the onset of actively induced or adoptively transferred EAE (17). OX40L-deficient mice poorly developed EAE, and OX40L transgenic mice displayed enhanced EAE symptoms, further supporting the role of OX40L-OX40 interactions in controlling pathogenic T cells in this immune disorder (104). The OX40L-OX40 interaction was also found to be involved in rheumatoid arthritis progression as administration of a neutralizing anti-OX40L antibody into already sensitized mice dramatically ameliorated the severity of collagen-induced arthritis (105). Similarly, the role of OX40 has been explored in the pathogenesis of other diseases where activated CD4 or CD8 T cells predominantly orchestrate the immune response, such as in colitis (43,106); graft-versus-host disease (107,108); and diabetes in NOD mice (109,110). In all cases, the lack of OX40 or OX40L, or blocking their interaction, very significantly suppressed the induction of disease symptoms. In most, reduced disease has been associated with weak CD4 or CD8 T cell responses, although it is highly likely that decreased activities of other cell types, including NK cells, NKT cells, mast cells, and neutrophils, that all can express OX40L and/or OX40, might have accounted in part for reduced clinical symptoms. Polymorphisms in OX40L have been shown to associate with susceptibility to atherosclerosis, and mouse models have now found that OX40L plays a strong role in promoting this inflammatory disease (111,112). Polymorphisms in OX40L have also recently been reported to predict susceptibility to developing lupus (113). However, as yet, no animal studies have been carried out in lupus models to determine if disease can be blocked by preventing OX40-OX40L interactions.

As further summarized in Table I, a number of studies have also targeted OX40L in mouse transplantation models with very promising results, albeit with the caveat that blocking OX40L

alone has generally been ineffective in stringent full-MHC mismatches. However, strong activity in promoting long-term graft survival has been seen where OX40L was neutralized in combination with targeting CD28/B7 and/or CD40/CD40L interactions (114-117). Again, the major effect has been on suppressing T cells or T cell-derived cytokines, although new data has indicated that targeting OX40L might concomitantly block effector T cells and promote Foxp3+ Treg (118), which would be ideal for long-term transplantation tolerance. This may also have applied to some of the studies of autoimmunity and inflammation, although effects on Treg were not evaluated.

The capacity of OX40 to regulate both CD4 and CD8 T cells makes it also a promising candidate for tumor therapy. Tumor infiltrating T cells express OX40, and injection of agonist OX40L.Ig fusion proteins, OX40 antibodies, RNA aptamers that bind OX40, or transfection of tumor cells or dendritic cells with OX40L, has been effective in suppressing tumor growth in many animal models (Table II). Of note, the therapeutic efficacy of OX40 agonists might be influenced by many factors including tumor burden, the intrinsic immunogenicity of the tumor, as well as the histological site of tumor growth (119). Again, the reported effects either involve a direct action on regulating CD8 T cell survival (48), and/or promoting CD4 T cell help for CD8 T cells (51). Augmentation of NK cell activity most likely additionally plays a role, and some data have also suggested that the anti-tumor activity elicited by targeting OX40 can involve NKT cells (8). More recently, a study showed that tumor infiltrating Foxp3+ Treg express high levels of OX40, and intratumor triggering of OX40 suppressed the activity of these Treg. Whether the inhibition of Treg allowed enhanced anti-tumor activity of preexisting CD8 CTL or resulted in the generation of new CTL was not clear (120). However, again this concomitant effect on augmenting anti-tumor T cell effector activity and suppressing Treg makes OX40 a very attractive target for inducing long-term protection against future tumor growth.

Finally in terms of therapeutics, targeting OX40 in a positive manner also has application for treatment of infectious disease. Antibodies to OX40 when administered alone, or with other agonists such as anti-4-1BB, can boost T cell responses to nominal antigens given in vaccine-type regimens (121). In line with using anti-OX40 as an adjuvant for vaccination, we showed in a mouse cytomegalovirus model that treatment with anti-OX40 can strongly limit virus replication by altering the ratio of IFNγ-producing effector CD4 T cells to IL-10-producing suppressive T cells in the salivary glands (70). Furthermore, anti-OX40 can strongly augment the efficiency of viral peptide immunization to promote the development of CD8 T cells (50) or CD4 T cells (Salek-Ardakani, unpublished) that fully protect against a future challenge with a normally lethal dose of virus.

OX40 Targets Intracellular Components of Division, Survival, and Cytokine Pathways

Lastly, and just as importantly, are the intracellular signals and targets that account for the functional activities of OX40-OX40L interactions. Although OX40L can undoubtedly signal to APC (15,16) and most likely other cell types that express it, arguably the primary signaling component of the interaction is OX40. This is illustrated by the profound activity of agonists to OX40 that provide reciprocal phenotypes in terms of T cell reactivity to a deficiency in either OX40 or OX40L. We have identified several downstream targets of OX40, which regulate T cell division, survival, and cytokine production from antigen-activated T cells.

OX40 activates both PI3K (PI-3-kinase)/PKB (protein kinase B/Akt) and NF-κB1 pathways. Sustained activation of these pathways by OX40 leads to up-regulation of several anti-apoptotic Bcl-2 family members, including Bcl-xL, Bcl-2, and Bfl-1, and molecules that regulate cellular division such as survivin and aurora B kinase (26,40,41,100). OX40-deficient T cells cannot

maintain PKB and IKK (IκB kinase) activity over time, and this drop in activity coincides with the period before these cells undergo extensive cell death. Using a gene-complementation approach, we found that both active PKB and IKKβ independently restored the defective proliferation and survival of OX40-deficient T cells, with a concomitant effect on promoting expression of Bcl-2, Bcl-xL, Bfl-1, survivin, and aurora B kinase (40,100). Survivin and aurora B complex with mTOR and somehow impact cyclin expression and hence cyclin-dependent kinase activity that allows T cells to progress through S phase of the cell cycle (122). However whether the action of OX40 on regulating these proteins is separate from the activity in promoting anti-apoptotic molecules is not clear, nor whether the effects of OX40 on division are truly separable from its effects on survival. An important question is how the OX40-PKB/NF-κB1 axis controls biological activities and gene expression levels of the aforementioned molecules. It is reasonable that OX40 regulated PKB and NF-κB1 synergize and coordinately condition their catalytic activities and mRNA/protein levels (123,124). Transcriptional activities of NF-κB1 might be critical for transactivation of Bcl-xL and Bfl-1 (125-128). However, it is still important to evaluate the exact functional targets of PKB and NF-κB1 to determine the extent of cooperation between these pathways in mediating the actions of OX40 on T cells or non-T cells. Although not investigated, other potential targets of OX40 driven PKB and NF-κB1 include IL-2Rα and IL-12Rβ that can be upregulated by OX40 signaling (129,130), and CTLA-4 and Foxp3 that are downregulated by OX40.

OX40 signals also connect to the machinery that regulates IL-2 and IL-4 production in T cells. With naïve T cells, OX40 costimulatory signals have a minimal role in IL-2 production, which is primarily controlled by costimulation from CD28 (5,26). In contrast, in effector T cells, OX40 greatly promotes IL-2 in response to antigen (5). This suggests that signaling in naïve and effector/memory T cells is different, perhaps related to alternate gene signatures (131-133) and/or membrane microenvironments (134,135). OX40 signaling can induce IL-4 from recently activated CD4 T cells, which as mentioned previously can be important for Th2 polarization and Th2 type immune responses (5,54). We reported that, under physiological conditions of antigen stimulation, OX40 signals promote nuclear localization of NFATc1 (54), a molecule important for IL-4 transactivation and Th2 development (136,137). Although agonist stimulation of OX40 with an antibody does not affect calcium mobilization in T cells activated with anti-CD3 (54), we have found that OX40-OX40L interactions can augment antigen-induced calcium influx (unpublished data), which is a primary step leading to nuclear entry of NFAT. Secondly, the OX40-PKB axis promotes phosphorylation of GSK3β (40) leading to its inactivation, and one of the reported effects of this is to suppress nuclear export of NFAT (138). Control of both nuclear import and export by OX40 is then critical for cytokine mRNA transcription by NFAT. It is also possible that PDK1 activation through OX40-PI3K controls NFAT-dependent IL-4 transcription (139), although this has not been investigated. At present, it is not clear how OX40 positively controls calcium signaling. It has been reported that other TNFR family members, 4-1BB (140) and HVEM (141), induce calcium influx through PLC-γ activation. OX40 signaling might then play a role in upregulation of TCR-dependent protein tyrosine kinase activities, which is an important step for PLC-γ recruitment into the membrane.

OX40 Forms a Co-signaling Complex that Includes PI3K/PKB and NF-κB1

How OX40 connects to the signaling pathways described above has not been clear until recently. The X-ray crystal structure of OX40 bound to OX40L shows that three copies of OX40 engage the trimeric OX40L to form a quaternary organized hexamer complex. OX40 uses residues from cysteine-rich domain 1 (CRD1), CRD2, and CRD3 to bind OX40L (142). OX40 can recruit TNFR-associated factors (TRAF) 2, 3, and 5 to its cytoplasmic tail through a short stretch of conserved acids, which contain a QEE motif (143-145)(Fig. 2). Structural analysis reveals that each carboxy-terminal TRAF domain (TRAF-C domain) forms a trimer,

which provides three symmetrical receptor-binding sites for the cytoplasmic tails of 3 individual TNFR family monomers (146). The TRAF molecules are adaptor proteins thought to directly link to downstream kinases, and thus their trimerization or oligomerization might be needed for effective signaling. Normally, this will be achieved when OX40 binds OX40L leading to OX40 forming a complex with several TRAF molecules. Forced expression of OX40, without OX40L binding, in HEK293T cells was found to result in a weak increase in NF-κB-dependent luciferase expression in transient transfection systems (147), suggesting that over-expression of OX40 can cause its ligand-independent trimerization and allow some signaling. However, in other transfection systems, we have found that normal expression of OX40 only leads to signaling when OX40L can cause aggregation of the receptor (So, unpublished).

TRAF2 appears to play a dominant role in mediating some of the functional effects of OX40. Weinberg and his colleagues evaluated TCR transgenic CD4 T cells obtained from a cross with a dominant negative (DN) TRAF2 transgenic mouse (65). The DN TRAF2 transgene strongly blocked antigen and OX40 driven effector and memory T cell generation in vivo, indicating a critical role for TRAF2 in expansion and survival driven by OX40 signals. Whether TRAF2 is required for all of the actions of OX40 is unknown, and it has not been evaluated whether TRAF3 and TRAF5 are required for any of these processes.

We have recently gained evidence that TRAF2 works as the primary signaling adaptor that links OX40 to NF-κB1. Activation of the NF-κB1 pathway is initiated by signal-dependent phosphorylation, ubiquitination, and subsequent degradation of IκBα, which allows cytoplasmic NF-κB1(p50)-RelA complexes to stably translocate to the nucleus and activate gene transcription. IκBα phosphorylation is catalyzed by the IκB kinase (IKK) complex, that contains two homologous catalytic subunits, IKKα and IKKβ, and the regulatory subunit IKKγ (148,149). Activation of IKKβ is essential for the NF-κB1 pathway. In relation to TNF signaling through TNFR1, it has been reported that the IKK complex is activated after being recruited to the TNF-TNFR1 complex by TRAF2. The ring finger domain of TRAF2 is critical for recruiting IKKα and IKKβ through their leucine zipper motifs (150). Some weak interaction between TRAF2 and IKKα/β occurs before TNF stimulation, and this interaction is strengthened by TNF interacting with TNFR1 (151). Quite similar to this model for TNFR1, we have now observed the OX40L-dependent recruitment of endogenous TRAF2, IKKα, and IKKβ into a complex with OX40 in T cells (Fig. 3). Furthermore, short hairpin RNA mediated knockdown of TRAF2 caused reduced association of IKKα and IKKβ with OX40 and a substantial reduction in NF-κB1 activation, suggesting that the OX40-NF-κB1 axis is mainly controlled by TRAF2 (So, unpublished data).

After binding of OX40 with OX40L, OX40 moves into detergent-insoluble cholesterol- and sphingolipid-rich plasma membrane microdomains, termed lipid rafts. In effector-like T cells, a proportion of membrane-expressed OX40 resides in the lipid raft fractions when OX40 is not engaged, but dramatic recruitment of OX40 into rafts is seen following contact with APC expressing OX40L (So, unpublished). The cytoplasmic tail of OX40 contains three lysine (K) residues (Fig. 2), which might be targets for ubiquitination. Indeed, OX40L interaction with OX40 results in ubiquitination of OX40, which is sensitive to cholesterol depletion by methyl-β-cyclodextrin that disrupts lipid rafts. The exact meaning of this is not yet known, but it is likely this will enhance the molecular association of OX40 with certain unknown signaling molecules that could mediate or enhance the ability to connect with downstream pathways. Importantly, the recruitment of TRAF2, IKKα, and IKKβ, as well as the p85 subunit of PI3K, and PKB, into lipid rafts is promoted by OX40L binding to OX40, which results in phosphorylation of IKKα/β and PKB (So, unpublished). This demonstrates that the lipid raft is an important platform for OX40 to be functionally active and to signal.

OX40 is induced by antigen, and has largely been thought to function as a classic costimulatory molecule in transmitting intracellular signals that synergize with antigen (TCR) signals. However, it has never been elucidated how much of the activity of OX40 truly represents a co-signal. As mentioned above, triggering OX40 on T cells is responsible for prolonged activation of the PI3K-PKB pathway, which in turn contributes to the high rate of clonal expansion of recently activated T cells. This cellular and molecular function regulated by the OX40-PKB axis is quite similar to that targeted by the OX40-NF-κB1 axis (100), suggesting that PI3K-PKB and NF-κB1 synergistically maintain T cell division and survival over time. TNF and PDGF can facilitate the molecular association of PKB with IKKα/β (152,153), suggesting PKB likely complexes with OX40 through binding IKK associated with TRAF2. The cytoplasmic domain of mouse OX40 contains a PNTP amino acid sequence containing a PXXP motif, which has the potential to recruit SH3 domain containing proteins, such as p85, Lck, and Itk (Fig. 2). However, human OX40 does not contain this sequence. We have found that activation (phosphorylation) of PKB after OX40L engagement with OX40, in T cell-APC cultures, is strongly dependent on antigen/TCR signals. In contrast, OX40-OX40L interactions can result in strong activation of NF-κB1 in the absence of antigen, and little synergy in promoting this pathway occurs when antigen is presented (So, unpublished data). This implies that OX40 is quantitatively important for enhancing and sustaining PKB signals provided by the TCR, whereas OX40 can activate the NF-κB1 pathway in a qualitative manner (Fig. 3). OX40 however recruits p85 (PI3K) and PKB into a signaling complex with TRAF2 and IKKα/β independent of antigen (So, unpublished data). This shows that OX40 provides an important platform for both PI3K-PKB and NF-κB1 pathways, but raises the issue of why PKB activity is dependent on TCR co-ligation. Phosphatidylinositol (PI) 3,4-biphosphate (PIP2) and PI3,4,5-triphosphate (PIP3), produced by antigen-dependent activation of PI3K facilitate the recruitment of many pleckstrin homology (PH) domain-containing proteins, including PDK1, into plasma membrane/lipid rafts, and PDK1 can regulate PKB phosphorylation (154-156). Therefore, it is possible that OX40 collaborates with TCR-dependent membrane events by primarily allowing OX40 bound PKB to localize with PDK1, resulting in a quantitatively greater amount of PKB that can be activated by PDK1.

Concluding Remarks

Evidence from mouse models of immune function and immune disease have now shown that OX40/OX40L interactions are among the more important costimulatory pathways that have been described, and determine the extent of CD4 and CD8 T cell immunity in multiple inflammatory situations. Studies of human T cells, although much more limited, have similarly found that OX40 is a central costimulatory molecule that determines T cell reactivity (4,10, 16,22,32,52,157-160). OX40 co-signaling is not only important for the effectiveness of antigen recognition to lead to activation of TCR signaling pathways, but also for independent signaling attributes that are distinct from those transmitted via the TCR. An important and long-lasting argument in the T cell costimulation field is whether the TCR and costimulatory receptors induce distinct signals (qualitative model) or whether the signaling routes employed by both receptor systems are entirely overlapping (quantitative model) (161,162). In reality, these models are not mutually exclusive. In the case of CD28, quantitative mechanisms appear to predominate. Strong support for a quantitative view of costimulatory signaling comes from microarray analysis, showing that TCR-induced expression of several thousands of genes in primary T cells is amplified (or suppressed) to varying degrees by CD28 costimulation, but no new genes are induced by CD28 (163). OX40 signaling synergizes with both TCR and CD28 pathways (5,26) and certainly potentiates global T cell signaling. OX40 and antigen signals cooperatively augment PI3K-PKB (40) and Ca^{2+}/NFAT (54) pathways, and thus OX40 costimulation can also be viewed as quantitative. However, OX40 builds a functional signalosome for activation of NF-κB in the absence of TCR signals, and the level of NF-κB activity induced through this receptor is far greater than generated by either ligation of the TCR

or molecules like CD28, arguing for both quantitative and qualitative costimulation. All of the TNFR family members can activate NF-κB signaling by recruiting TRAF molecules, making them fundamentally different from other costimulators such as CD28 or ICOS. Thus, OX40 and OX40L, and some of their related family members, serve as an important signaling machinery in T cells that is unlikely to be redundant or easily bypassed, perhaps in part explaining their central role in T cell immunity.

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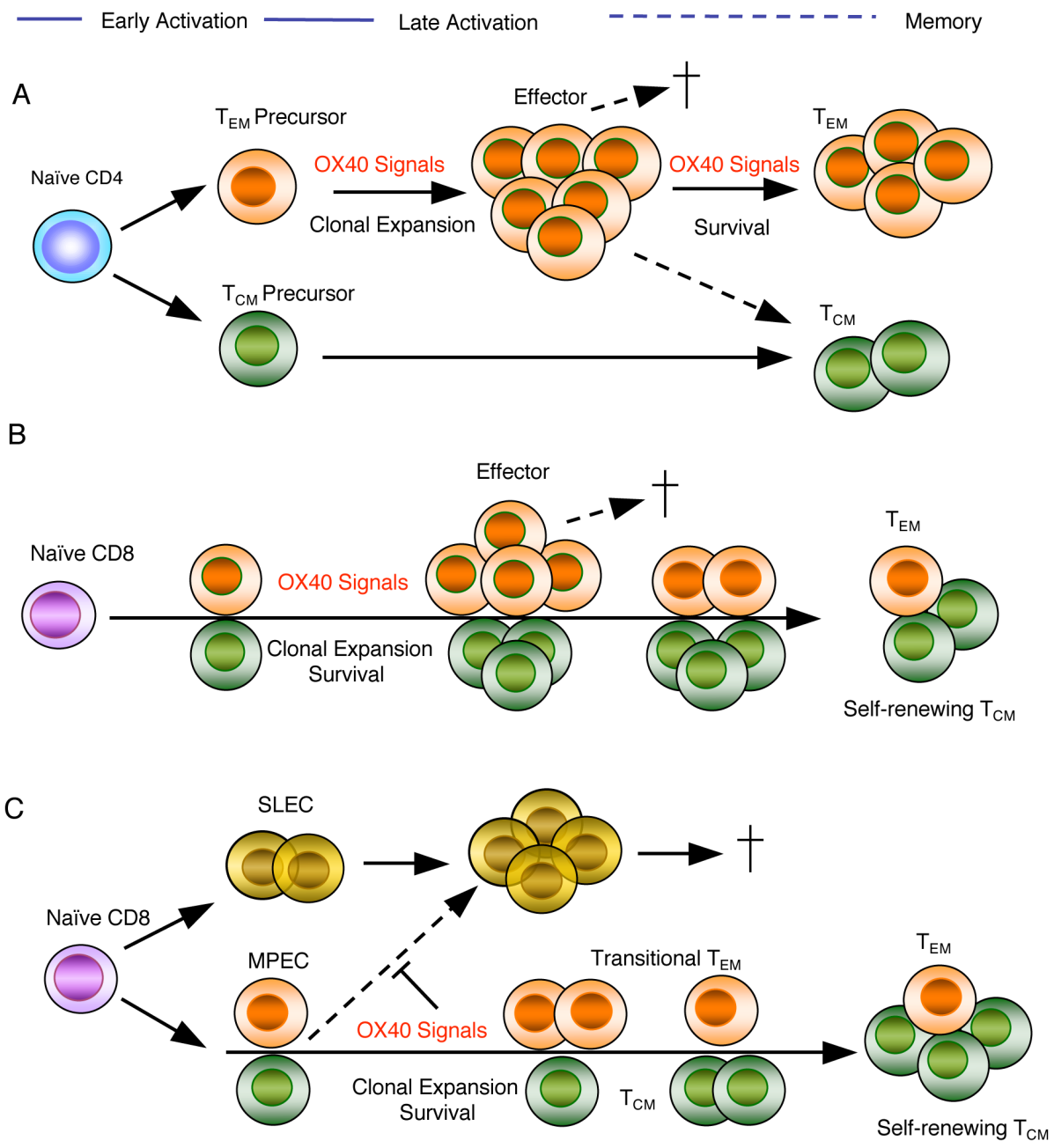

Figure 1. OX40 signals determine the size of effector and memory T cell pools

Three models for effector and memory T cell generation are depicted. A) Upon activation, naïve CD4 T cells develop into either central memory cells (Tcm) or effector memory cells (Tem). The Tcm and Tem fate decision occurs early during priming, perhaps determined by antigen access and/or dose, before OX40 is ligated on recently activated naive T cells. OX40 signals promote clonal expansion and survival of Tem precursors that differentiate in a step-wise manner into true primary effector cells and then into resting Tem after antigen is cleared. Tcm precursors expand and enter into the central memory pool in an OX40 independent fashion. OX40 is critical to CD4 T cell memory because in general Tem predominate. B) Following activation of naïve CD8 T cells, they expand and develop into effector cells that

may contain both Tcm and Tem precursors. In this context, OX40 signals promote clonal expansion and support survival of most effector CD8 T cells that enter into the memory pool regardless of their lineage potential, hence OX40 is critical to CD8 T cell memory generation. This situation might apply to responses against tumors, auto or allo-antigen, or select infectious agents. C) Activation of naïve CD8 T cells under other inflammatory conditions results in the daughter cells developing into SLEC (short-lived effector cells) and MPEC (memory precursor effector cells). During priming, OX40 promotes expansion and survival of MPEC and further inhibits conversion of MPEC into SLEC. At later times, most SLEC die, but multipotent MPEC survive and give rise to transitional Tem that progressively differentiate into long-lived Tcm. Furthermore, OX40 signals to MPEC provided during the primary effector phase impart signals to maintain the later self-renewing capacity of Tcm in the absence of antigen. This situation might apply to responses against select infectious agents, and again OX40 becomes critical for memory generation because of the control of MPEC.

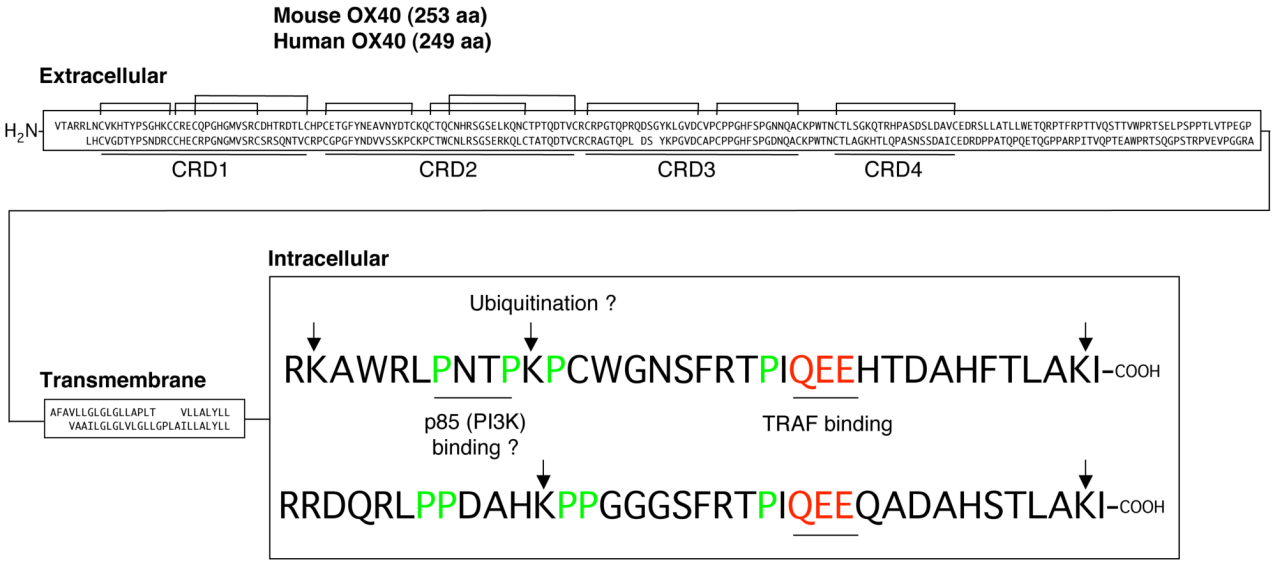

Figure 2. The cytoplasmic tail of OX40 connects to intracellular signaling pathways

The diagram depicts the extracellular, transmembrane, and intracellular regions of mouse (top) and human (bottom) OX40. The cytoplasmic region contains residues crucial for costimulatory signaling. Arguably most important is the QEE motif, characteristic of many TNFR family molecules, that mediates binding to several TNFR-associated factors (TRAF) including TRAF2, TRAF3, and TRAF5. These are adaptors allowing OX40 to link to intracellular kinases. Mouse OX40 also contains a potential PI3K binding motif (PXXP) that could directly allow connection to the kinase, PKB (Akt). Furthermore, several lysine (K) residues (shown by arrows) are present which might allow OX40 to be ubiquitinated in lipid rafts. The function of the latter action is presently unknown, but might be important for downstream kinase activity.

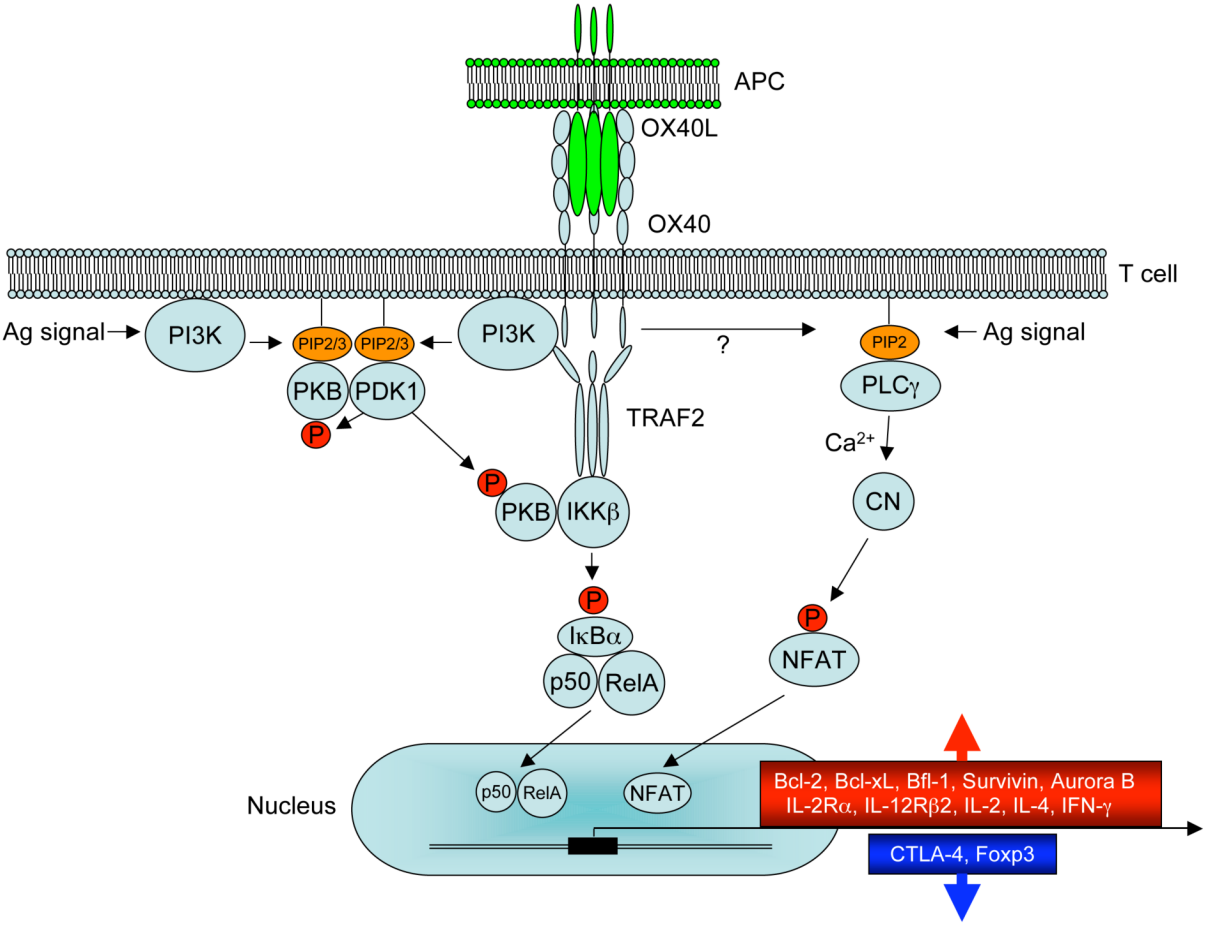

Figure 3. OX40 activates PI3k/PKB, NF-κB1, and NFAT pathways to allow both antigen-dependent and antigen-independent signaling

OX40L binding to OX40 results in recruitment of TRAF2 and the formation of a signaling complex containing IKKα and IKKβ, as well as PI3k and PKB (Akt). This complex, upon translocation into lipid rafts, is sufficient to activate NF-κB1 in an antigen independent manner, via phosphorylation and degradation of IκBα, leading to entry of p50 and RelA into the nucleus. In contrast, OX40 ligation does not effectively lead to phosphorylation of PKB, but OX40 co-operates with TCR signals brought about by antigen recognition, to augment PKB activation, possibly reflective of a requirement to recruit and activate PDK1. OX40 also synergizes with TCR signals to augment intracellular Ca^{2+}, through an unknown mechanism, that leads to enhanced nuclear import of NFAT. The downstream targets of these signaling pathways include upregulating genes that control T cell division and survival, and promoting transcription of cytokine genes, as well as expression of cytokine receptors. Suppressive events brought about by OX40 signaling include downregulation of CTLA-4 and Foxp3.

Table I

Requirement for OX40-OX40L in Inflammatory and Autoimmune Disease

Disease	Mice or Reagent	Clinical Phenotype	Immune Phenotype	Reference(s)
EAE	Anti-OX40-toxin	Strong inhibition	↓ CD4+ T cells	(103)
	OX40.Ig	Strong inhibition	↓ CD4+ T cells	(17)
	Anti-OX40L	Strong inhibition	↓ CD4+ T cells	(164)
	OX40-/-	Strong inhibition	↓ CD4+ T cells	(165)
	OX40L-/-	Strong inhibition	↓ IFNγ, IL-2, and IL-6	(104)
Colitis/IBD	OX40.Ig	Strong inhibition	↓ CD4+ T cells, CD8+ T cells	(43,166,167)
	Anti-OX40L	Strong inhibition	↓ α4β7 CD4+ T cells, CD11c+ DC	(106,168)
Asthma/Atopy	OX40-/-	Strong inhibition	↓ IL-4, IL-5, IgE	(39)
	OX40L-/-	Strong inhibition	↓ IL-13, IL-4, TNF, IFNγ	(101)
	Anti-OX40L	Strong inhibition	↓ CD4+ T cells, IL-4, IL-5, IL-13, IgE, CD11c+ DC	(88,102,169)
Diabetes	OX40L-/-	Strong inhibition	—	(109)
	Anti-OX40L	Strong inhibition	—	(110)
Arthritis (CIA/adjuvant)	Anti-OX40L	Strong inhibition	↓ IFNγ, IgG2a	(105)
	Anti-OX40-toxin	Partial inhibition	↓ CD4+ T cells	(170)
Atherosclerosis	OX40L-/-	Strong inhibition	—	(112)
	Anti-OX40L	Strong inhibition	↓ IL-4, IgG1. ↑ IgM	(111)
Transplant Rejection				
: Minor	OX40.Ig	Strong inhibition	—	(171)
: Minor	Anti-OX40L	Strong inhibition	↓ IFNγ	(117)
: Major	OX40.Ig	None		(171)
: Major	Anti-OX40L	None alone		(114)
		Strong with CD28 block	↓ IFNγ, CD3+CD44hi T cells	
: Major	Anti-OX40L	None alone		
		Strong with CD28/CD40L block	↓ CD4+ T cells, CD8+ T cells	(63,116,118)
GVHD	Anti-OX40L	Strong inhibition	↓ CD3+, CD4+ T cells, CD19 + B cells, IFNγ	(107,108)
	OX40-/-	Strong inhibition	—	(108)
	OX40L-/-	Strong inhibition	—	(108)

Table II

Tumor immunotherapy targeting OX40

Therapy	Combination Treatment	Tumor	Immune Phenotype	Reference(s)
Agonist Anti-OX40 or OX40L.Fc		Sarcoma, Melanoma Sarcoma, Glioma Colon and mammary carcinomas Thymoma Colon and renal cell carcinomas	↑ CD4+ T cells ↑ CD8+ T cells ↓ Foxp3 Treg ↑ CD8+ T cells	(73) (119) (120) (27) (172)
	Adoptive T cells	Sarcoma Thymoma Prostate tumor	↑ CD4+ T cells ↑ CD8+ T cells	(173) (51) (174)
	IL-12, anti-4-1BB	Colon carcinoma	↑ CTL	(175)
	GM-CSF	Colon and breast carcinoma	↑ CTL	(176)
	Tumor transfection with CD80	B lymphoma	↑ CTL	(177)
	DC vaccine, anti-4-1BB	Breast carcinoma	↑ CTL	(178,179)
	GM-CSF/Ag vaccine	Breast tumor	↑ CD8+ T cells	(180)
	IL-12	Sarcoma, prostate tumor	↑ CD4+ T cells	(129)
Tumor transfection with OX40L		Melanoma, Lung carcinoma Thymoma	↑ CTL ↑ CD4+ T cells, IFNγ	(181) (182)
	GM-CSF	Colon carcinoma	↑ CD4+ T cells, CD8+ T cells, IL-2	(183)
DC transfection with OX40L		Melanoma Thymoma, Melanoma	↑ CD4+ T cells ↑ CTL, NKT cells, IFNγ	(158) (8)
Agonist RNA aptamer	DC vaccine	Melanoma	↑ IFNγ	(184)