

## CHAPTER 5

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# The Role of OX40 (CD134) in T-Cell Memory Generation

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### Abstract

Memory T-cell generation is limited by activation-induced cell death during the effector T-cell stage. Cell surface proteins are known to transmit signals that either accentuate or limit T-cell death after activation. This chapter will focus on the TNF-receptor family member OX40, which is expressed on effector T cells and when engaged greatly enhances survival of T cells leading to increased memory T-cell generation. Targeting OX40 *in vivo* can alter the fate of T-cell survival. Enhancing OX40 signaling during Ag priming through agonists increases memory T-cell development, while blocking OX40 signaling decreases the memory T-cell pool. These two opposing outcomes provide therapeutic tools for blocking inflammation in autoimmune conditions and enhancing immunity in hosts harboring cancer or chronic pathogens. OX40 agonists and antagonists are in the first stages of human clinical trials and their therapeutic potential will soon be realized.

### Introduction

The generation of functional immunologic memory via long-lived T- and B-cell responses is paramount to protective immunity against recurrent pathogen infections and is the goal of current vaccine strategies.<sup>1</sup> The coordination of long-lived CD4, CD8 and B-cell responses is a hallmark of the adaptive arm of immunity and is an irreplaceable part of protective immunity. The quality of the long-term adaptive immunity is directly related to the amount of Ag-specific memory T and B cells that are generated following an initial Ag challenge.<sup>1</sup> Hence, understanding the mechanisms that regulate the generation and maintenance of immune cells could lead to improved vaccine strategies and also help hosts harboring chronic pathogens or cancer tip the balance towards immune clearance and host survival. In particular, this chapter will explore the contributions of the TNF-receptor family member, OX40, to T-cell memory generation and how to exploit OX40-specific pathways for clinical benefit in patients with autoimmunity, cancer and chronic pathogens.

There has been a number of T-cell surface molecules linked to the biologic function of memory T-cell generation and function. The list includes several TNF-receptors, 4-1BB, FAS, LT- $\beta$ R, CD27 and OX40 as well as other proteins such as CD28, ICOS, ICAM and PD-1. While all of these cell surface proteins have been linked to the function of memory T cells, it is clear that OX40 plays a seminal role in the generation of both CD4 and CD8 T-cell memory.<sup>2</sup> T-cell responses to cognate Ag are characterized by an early expansion phase, a contraction phase, followed by the generation and persistence of long-term memory cells.<sup>1</sup> The generation of large

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numbers of long-term memory T cells is limited by activation-induced cell death (apoptosis) during the contraction phase. Recently, it has been shown that engagement of OX40 during Ag priming *in vivo* diminishes T-cell activation-induced cell death leading to increased numbers of long-lived antigen-specific CD4 and CD8 T cells.<sup>3-7</sup> This chapter will review the events that lead to OX40 enhanced T-cell memory generation through natural OX40 ligand engagement (endogenous OX40 activation) or via exogenous administration of OX40 agonists (OX40 ligand:Ig or anti-OX40) *in vivo*.

## Background

OX40 has a unique pattern of expression; it is for the most part restricted to lymphoid tissue<sup>8</sup> and mainly expressed on activated CD4 and CD8 T cells.<sup>9</sup> More recently, it has been shown that OX40 is also constitutively expressed on mouse T regulatory cells.<sup>10</sup> OX40 expression on recently activated naïve CD4 T cells peaks within 24-48 hr after TCR engagement by peptide Ag in the context of MHC class II and returns to baseline levels 120 hr later.<sup>11</sup> Effector CD4 T cells upregulate OX40 expression more rapidly than naïve T cells within 4 hr after Ag stimulation.<sup>11</sup> The transient expression of OX40 on activated effector cells is observed both *in vitro* and *in vivo*.<sup>11,12</sup> OX40 expression on naïve CD8 T cells starts 24 hr after Ag stimulation, peaks at 48-72 hr and declines thereafter.<sup>5</sup> OX40<sup>+</sup> T cells are found preferentially at sites of inflammation and not normally in the peripheral blood. In animal models for both autoimmunity and cancer OX40<sup>+</sup> T cells found within the site of disease are enriched for the recently stimulated auto- or tumor Ag-specific T cells.<sup>13-15</sup> Therefore, OX40 represents a convenient target by which the function of Ag-specific T-cell responses can be modulated in various disease models, even without prior knowledge of the specific Ag(s) involved.<sup>16</sup> In essence, manipulation of OX40<sup>+</sup> T cells *in vivo* targets the ongoing “endogenous” immune responses, but does not affect the remainder of the peripheral T-cell repertoire. OX40<sup>+</sup> T cells have been detected at the inflammatory site in several human autoimmune diseases and in the following human cancers: melanoma, breast, colon, head and neck and more recently prostate cancer, bladder cancer, lung cancer and sarcoma<sup>16-18</sup> and data not shown. Therefore, manipulation of OX40<sup>+</sup> T cells in patients with a variety of diseases could have a wide range of clinical benefits.

The original description of the OX40 monoclonal antibody (Ab) showed that this antibody bound activated CD4 T cells and augmented their proliferation during the later stages of *in vitro* stimulation.<sup>9</sup> When the biologic effects of anti-OX40 were originally described, the field of costimulation was in its infancy. CD28 was the first costimulatory molecule described on T cells; it was shown to augment T-cell stimulation when administered in combination with TCR signaling.<sup>19-21</sup> The CD28 interaction with its ligands (CD80/86) is essential to achieve optimal activation of naïve T cells; if a signal is delivered through the TCR receptor in the absence of CD28 ligation, the T cell becomes anergic or dies prior to becoming small resting T cells.<sup>21</sup> CTLA-4 is expressed after TCR engagement and competes with CD28 for binding CD80/86 and when engaged provides a negative signal that puts the brakes on T-cell proliferation. OX40 was originally shown to have costimulatory activity on an Ag-specific CD4<sup>+</sup> T-cell line *in vitro* of similar potency to that of CD28.<sup>22</sup> While interaction of B7/CD28 is required for the optimal stimulation of naïve T cells,<sup>23</sup> OX40-specific costimulation appears to be most important for the stimulation of effector T cells.<sup>11,24</sup> Both CD28 and OX40 appear to play important but distinct costimulatory roles in the development of Ag-activated peripheral T cells and both signals are required for the optimal generation of memory CD4<sup>+</sup> T cells.<sup>7</sup> The remainder of this book chapter will focus on the mechanistic details of how OX40 functions to increase effector T-cell survival/function ultimately leading to increased generation of a functional memory response. Ultimately, manipulation of OX40 signaling could beneficially alter the course of several diseases and this concept will be further dissected within this book chapter.

## **Role of OX40/OX40L Interaction in Memory T-Cell Generation and Function**

OX40 expression is upregulated upon TCR engagement even in the absence of a strong innate immune adjuvant, however expression of the OX40 ligand is somewhat limited, especially in the absence of innate immune signaling.<sup>25,26</sup> Hence, the biologic role of endogenous OX40/OX40L interaction has been easier to ascertain in models where a proinflammatory event occurs causing innate immune activation (e.g., viral challenge, EAE, or asthma). It is now evident that the extent of OX40L expression regulates the magnitude of OX40 signaling within activated T cells.<sup>27</sup> The OX40 ligand (OX40L) is a Type II transmembrane protein (TNF-family member) that was first identified in mice and shown to have ~67% homology to the human protein gp34.<sup>28</sup> Subsequently, studies revealed that the gp34 protein could bind to human OX40, demonstrating that gp34 is the human homologue of OX40L and to date this is the only known ligand that binds OX40.<sup>8,29</sup> OX40L is expressed on activated APCs including B-cells, macrophages, DCs, NK cells, Langerhans cells, human airway smooth muscle (ASM) cells, CD4+ CD3- accessory cells and activated vascular endothelial cells and appears to be induced in a CD40-dependent manner.<sup>25,30-39</sup> The OX40L is also expressed by T cells and confer T:T-cell interaction that may also be important for OX40 signaling.<sup>40</sup>

There were two sentinel studies describing OX40/OX40L knockout mice, which suggested that memory T-cell generation was impaired upon targeted disruption.<sup>41,42</sup> The initial study challenged OX40 knockout mice with LCMV and influenza viruses. While these investigators observed no differences in Ag-specific CD8 T-cell memory and Ab responses, there was a significant decrease in viral-specific CD4 T-cell memory. In particular, there was a significant reduction in lung infiltration of CD4<sup>+</sup> T cells in virally infected OX40 knockout mice compared to controls. The second study described defective recall responses in OX40L knockout mice. This group immunized WT and OX40L ko mice with various Ag(s) and found defective proliferation and cytokine production within the CD4 T-cell compartment nine days following immunization. They also found defective Th1 and Th2 responses following restimulation *in vitro*, suggesting that the OX40 signaling can stimulate both Type 1 and 2 responses.<sup>41</sup> This study also revealed a decline in Ag-specific Abs (all isotypes) in KLH immunized OX40L ko mice and a slight decline in CD8 T-cell cytotoxicity.<sup>41</sup> Soon to follow was a manuscript detailing a marked decrease in CD4 T-cell memory following immunization of OX40 ko mice.<sup>43</sup> This study immunized mice with KLH either *in alum* (i.p.) or CFA delivered *s.c.* Similar to the previous study they found a marked decrease in Ag-specific cytokine production seven days after priming (effector T-cell stage). This study also investigated long-term survival of Ag-specific CD4 T cells and found a profound decrease in memory T-cell frequency and Ag-specific cytokine production in the OX40 ko mice. The frequency of long-term Ag-specific memory T cells decreased 11-fold and 23-fold in the CFA and Alum immunized OX40 ko hosts, respectively. This group further reported that an OX40-specific defect that led to decreased survival of Ag-stimulated T cells was in part due to decreased expression of the anti-apoptotic proteins, Bcl-2 and Bcl-xl.<sup>7</sup>

CD4 T-cell memory responses within the lung are a critical component for the induction of asthma in mouse models.<sup>44</sup> OX40 expression by T cells appears to be an essential component of T-cell-mediated lung inflammation in asthma, as OX40 ko mice develop a tempered form of the disease.<sup>44</sup> In addition, this group found that Ag-specific long-term memory T cells (120 days post-Ag priming) were also dependent on OX40 signaling to induce asthma.<sup>45</sup> This data suggest that OX40 signaling of long-term memory T cells is critical for their effector function and blocking OX40 signaling *in vivo* may have clinical implications for individuals with asthma.

Recent literature has focused on two phenotypes of memory T cells that have separate functional properties, effector and central memory.<sup>46-48</sup> Effector memory T cells reside in both lymphoid and nonlymphoid tissue, where they elicit immediate function by producing cytokines and/or being cytotoxic with little clonal expansion upon reencountering Ag.<sup>46-48</sup> In contrast,

central memory T cells are mainly located in the secondary lymphoid tissues, where they mediate long-lasting protection through clonal expansion.<sup>46-48</sup> A recent study showed a dramatic decrease in the effector memory population after Ag stimulation of OX40 deficient CD4 T cells compared to their WT counterparts.<sup>49</sup> There was no difference in these two populations three days after activation (as defined by CD62L and CD44), however as the cells became long-term memory T cells there was a dramatic loss in the effector memory population. The data also suggested that an OX40-specific signal generated early in the immune response is important to maintain these long-term effector memory CD4 T cells.<sup>49</sup>

The importance of CD8 memory T-cell generation in the context of OX40 signaling has been studied in both viral and tumor model systems.<sup>50</sup> Initial studies used OX40 deficient T-cell receptor transgenic T cells (OT1) and compared them to WT cells upon adoptive transfer in tumor-bearing mice (EG7). These investigators found that the survival of the OX40 ko OT1 T cells was diminished compared to WT T-cell transfers into tumor-bearing mice and this also correlated with diminished anti-tumor activity. Transfecting the anti-apoptotic gene, Bcl-xl, into the OX40 ko CD8 T cells enhanced survival of these cells and increased their efficacy against an ova-expressing tumor. Another study investigated influenza-specific CD8 T-cell priming and memory T-cell expansion in the absence of OX40 signaling (OX40 ligand ko mice).<sup>51</sup> They found that primary expansion and memory CD8 T-cell survival was not affected in the OX40 ligand ko mice, however upon viral challenge the influenza-specific T cells within the OX40 ligand ko hosts showed defective recall responses. Subsequent experiments showed that the defect in secondary expansion of viral-specific CD8 T cells was conferred to the cells during the initial priming phase.<sup>51</sup>

### **Providing an Exogenous OX40 Signal (OX40 Agonists) to Enhance Memory T-Cell Generation**

The control point for OX40-dependent stimulation of T cells during an immune response appears to be at the level of OX40L expression. OX40 is expressed on all CD4 and CD8 T cells after TCR engagement. The expression of OX40L, however, is more tightly regulated. When T-cell activation via TCR engagement with peptide/MHC occurs in the absence of a strong adjuvant, the local expression of OX40L is minimal. Therefore, in the absence of adjuvant, the Ag-stimulated T cells express OX40, but because OX40L expression on APC is limiting the majority of OX40<sup>+</sup> T cells will never encounter/engage their natural ligand. This may lead to apoptosis and limit the generation of memory T cells as depicted in Figure 1. Evidence in support of this theory derives from two transgenic mouse models in which mice over express the OX40L.<sup>27,39</sup> In both models, the investigators noticed a large increase in the proportion of T cells in the lymphoid compartments as the mice aged. The OX40L transgenic mice also showed a dramatic increase in memory T-cell generation and recall responses following immunization.<sup>27</sup> Hence, one might predict that the addition of an exogenously delivered OX40 agonist (anti-OX40 or OX40L:Ig) during an ongoing immune response may increase the numbers of memory T cells generated.

The exogenous OX40 agonist hypothesis was initially tested in superAg-stimulated mice, which induces rapid *in vivo* T-cell expansion followed by deletion.<sup>3</sup> An OX40 agonist was administered at the same time as superAg to test whether this strategy might save T cells from clonal deletion. OX40 agonist administration was able to slightly increase T-cell survival in superAg (SEB) treated mice, similar to that observed with the TLR agonist, LPS.<sup>3</sup> However, combining an OX40 agonist with LPS in SEB treated mice showed dramatic synergy not only enhancing CD4 T-cell survival (greater than 2 logs), but also increasing the proliferative phase of T-cell expansion. This same dual adjuvant combination (anti-OX40/LPS) also provided increased survival of SEB specific CD8 T cells, although not as dramatic as the CD4 T-cell results. This same study also examined Ag-specific T cells stimulated by soluble Ag delivered *s.c.* in combination with anti-OX40 and/or LPS. Seven days after Ag stimulation anti-OX40 boosted the number of Ag-specific T cells 10-fold compared to controls (Ag + rat Ig) and the anti-OX40/LPS combination increased the numbers 20-fold compared to the control group. Upon inspection of long-term memory (60 days post-immunization) in the soluble Ag model, anti-OX40 increased the number of Ag-specific

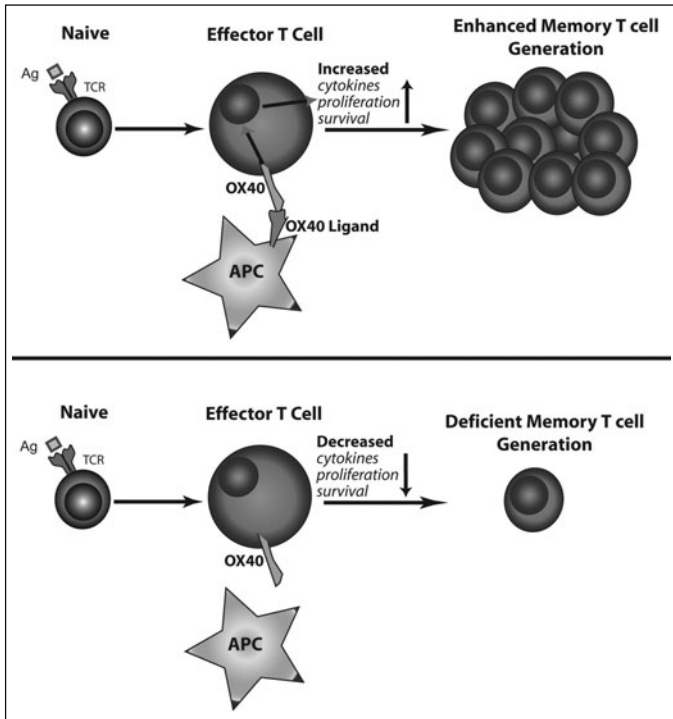


Figure 1. OX40 engagement *in vivo* enhances effector T-cell function and survival. OX40 ligation through endogenous OX40 ligand expression on antigen presenting cells (APC) stimulates effector T cells, which increases cytokine production, proliferation, and survival leading to increased memory T-cell generation. These properties are observed for both CD4 and CD8 T cells and is dependent OX40 ligand expression, which is only upregulated on activated APC. These same properties can be elicited by providing exogenous signals through OX40 agonists in hosts harboring chronic infections or cancer with positive therapeutic outcomes. Conversely, blocking endogenous OX40 ligation decreases T-cell specific inflammation and has shown great promise for autoimmune disorders.

memory CD4 T cells 15-fold and the anti-OX40/LPS combination increased CD4 T-cell memory survival 70-fold.<sup>3</sup> From this study it is clear that exogenous OX40 stimulation *in vivo* has potent adjuvant effects leading to increased generation and survival of memory T cells. Ultimately, taking advantage of this type stimulation to increase T-cell memory in hosts harboring cancer or chronic pathogens will be discussed later.

There are a number of T-cell targeted immune adjuvants in the form of soluble Ig fusion proteins and monoclonal Abs.<sup>52</sup> Some of which have potent immune enhancing properties that lead to the eradication of tumors in cancer-bearing mice. Both anti-OX40 and anti-CTLA-4 have anti-tumor efficacy, but mediate their activity through different mechanisms.<sup>52</sup> A side by side comparison of the CD4 T-cell stimulating properties of these two Abs administered *in vivo* was tested in a soluble Ag immunization model (see Fig. 2). The study showed that both anti-OX40 and anti-CTLA-4 dramatically increased early proliferation of Ag-stimulated CD4 T cells (4 days post-immunization).<sup>4,53</sup> However, the Ag-specific T cells in the anti-CTLA-4 stimulated mice did not survive long-term and return to control levels 10 days after immunization, while the Ag-specific CD4 T cells in anti-OX40 stimulated mice maintained high numbers throughout the course of the experiment. This study also showed that OX40 agonists accentuated Ag-specific Ab responses

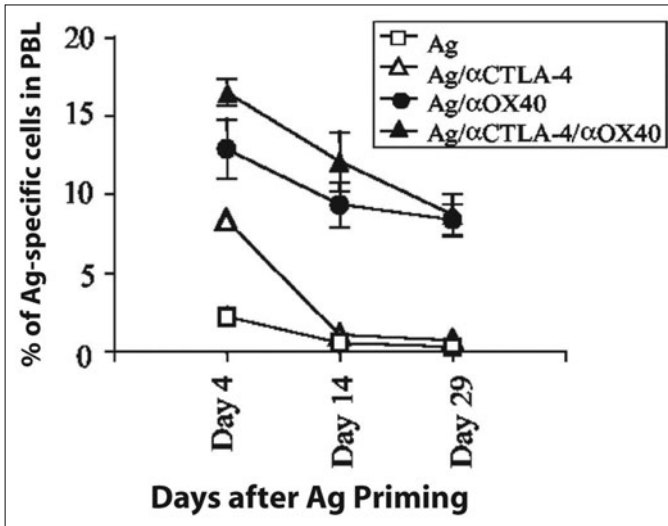


Figure 2. Administration of anti-OX40 after priming with soluble Ag greatly enhances CD4 T-cell survival. Ova-specific naive T cells were transferred into mice and were then immunized with ova (with no adjuvant) with anti-OX40, anti-CTLA-4, or anti-OX40 and anti-CTLA-4. The mice bled on days 4, 14, and 29 and assessed for the percent of ova-specific T cells via the KJ1-26 Ab. the percentage shown in the y-axis is Ag-specific cells of total CD4+ cells and the error bars represents the SE of five mice per group.

in vivo, which was not observed in anti-CTLA-4 treated mice. Subsequent studies showed that anti-OX40 downregulated CTLA-4 expression within Ag-stimulated CD4 T cells during the early priming phase.<sup>53</sup> Anti-OX40-induced CTLA-4 downregulation was shown to be important for OX40 enhanced T-cell proliferation but played no part to enhance memory T-cell survival. A subsequent report identified that a major difference between anti-OX40 and anti-CTLA-4 stimulated T cells was the upregulation of the IL-12 receptor  $\beta 2$  protein (signaling subunit).<sup>54</sup> This report went on to show that anti-OX40-mediated survival of Ag stimulated CD4 T cells was dependent on IL-12 signaling. The data also showed that there was a critical window of IL-12R upregulation, 4-7 days after antigen priming/OX40 stimulation and if the cells did not encounter IL-12 during that time frame they would undergo activation-induced cell death.<sup>54</sup>

Exogenous OX40 agonist administration delivered in vivo also affects CD8 T-cell survival and memory development. The investigators of this study used the OT1 TCR transgenic model (specific for ova) and immunized these mice with soluble ova in combination with anti-OX40 or rat Ig.<sup>5</sup> The anti-OX40 Ab increased the initial expansion phase of Ag stimulated CD8 T cells two-fold compared to the controls. OX40 agonist administration also increased the long-term survival of the CD8 T cells, 5-10-fold. The surviving CD8 T cells were mostly of central memory phenotype and were functional upon re-encountering Ag. The OX40-stimulated CD8 T cells showed a large increase in IL-2 receptor expression early during the response, which might have conferred the increase in their survival. Anti-OX40 administration also greatly enhanced CD8 T-cell recall responses. Both proliferation and survival of the recall specific T cells was increased and was in part CD4-dependent.<sup>5</sup> Although, it is clear that direct expression of OX40 on CD8 T cells also plays a key role for the OX40 agonist effect observed in both primary and secondary responses.<sup>5</sup> OX40 agonists were also shown to increase the effector/cytotoxicity function of antigen stimulated CD8 T cells by upregulating granzyme B levels, through an IL-2-dependent mechanism.<sup>55</sup>

## **Altering Memory T-Cell Generation/Function through the OX40 Axis for Therapeutic Benefit in Autoimmunity, Cancer and Hosts Harboring Chronic Pathogens**

It is clear from the studies summarized above that signaling through OX40 on either CD4 or CD8 T cells has a dramatic effect on their effector function and survival. Hence, research groups have attempted to alter the course of diseases known to have T-cell involvement through either blocking or enhancing OX40 signaling *in vivo*. What appears to make OX40 such a good target to alter T-cell function *in vivo* is its unique expression pattern, which is only upregulated after T-cell receptor engagement *in vivo* and quickly downregulated 24-48 hr after induction. Hence, *in vivo* expression at any time is extremely low, although OX40 is constitutively expressed on mouse T regulatory cells. The highest expression of OX40 is found at sites of inflammation, such as the colon in inflammatory bowel disease, the CNS in mice with EAE and in tumors/tumor-draining LNs in mice and humans with cancer. OX40<sup>+</sup> T cells sorted from these sites of inflammation are enriched for either autoimmune-specific or cancer-specific T cells.<sup>16</sup> Hence, targeting OX40 is a convenient way to home in on the relevant Ag-specific T cells without significantly affecting the peripheral T-cell repertoire.

Two approaches have been used for OX40-specific therapy in autoimmune disease. One involves direct deletion of OX40 positive cells through a cytotoxic Ab, while the other targets the OX40 ligand in attempt to decrease OX40-specific signaling.<sup>15,37</sup> Antibody directed deletion of OX40<sup>+</sup> T cells showed therapeutic promise in EAE, as it was able to ameliorate ongoing signs of disease.<sup>15</sup> This was accomplished using a ricin conjugated OX40-specific Ab that was shown to directly target myelin-specific T cells within the CNS of mice with EAE. This therapy led to a 2-log reduction in the myelin-specific T cells isolated from the CNS, which correlated well with a reduction in disease score. While this therapy worked well there has been some concern regarding this approach, because other cell types have more recently been identified to express OX40 including T regulatory cells and PMNs.<sup>10,56</sup> The second approach involves agents that target/bind to the OX40 ligand, thus limiting OX40-specific signals in activated T cells. The OX40 ligand is upregulated at the site of inflammation in several autoimmune models<sup>2</sup> and hence OX40 ligand blockade was a logical extension for treatment of inflammatory disorders. Initial reports showed that injection of an OX40:Ig fusion protein was effective at inhibiting clinical signs of disease in EAE when administered after disease onset.<sup>37</sup> It was also shown that OX40 ligand blockade administered during a relapse episode was effective at tempering disease; however, as soon as treatment was stopped the mice relapsed.<sup>37</sup> Therefore, it appeared that blocking OX40 signaling was able to reduce T-cell effector function, but not eliminate the cells responsible for causing the disease. OX40 ligand blockade has been used to temper a variety of autoimmune/inflammatory models including asthma, inflammatory bowel disease, viral-induced lung inflammation, graft-vs-host disease, diabetes and rheumatoid arthritis.<sup>2,57</sup> Genentech is now developing a humanized OX40 ligand Ab, which is currently being tested in a phase I clinical trial for asthma. This Ab may have far reaching potential as a potent anti-inflammatory for several human diseases in future clinical trials.

Enhancing immune responses through *in vivo* administration of OX40 agonists has shown therapeutic promise in mouse models for cancer and chronic pathogen infections.<sup>57</sup> Primarily, two agents have been used to achieve successful agonist stimulation: (1) an OX40 ligand:Ig fusion protein and (2) an OX40 agonist Ab. The initial report showed that both OX40L:Ig and anti-OX40 had similar activity to regress tumors in cancer-bearing mice.<sup>14</sup> Although, there has been a more recent report that suggests the OX40L:Ig fusion protein has better anti-tumor efficacy than the anti-mouse OX40 agonist Ab (termed OX86).<sup>58</sup> The OX40-specific anti-tumor efficacy has been observed in several tumor models, including sarcoma, melanoma, colon cancer, breast carcinoma, lung cancer, glioma, prostate cancer.<sup>2</sup> The anti-tumor efficacy generated by OX40 agonists is dependent on both CD4 and CD8 T cells and it has been shown that OX40 agonists do enhance tumor-Ag specific memory T-cell development.<sup>14,59</sup> Subsequently, another report has shown that anti-OX40 administration greatly augments the adoptive transfer of tumor-reactive

**Table 1. Potential use for OX40-specific treatment in disease**

	Treatment Schemes	
	OX40 Agonists	OX40L Blockade
Agents used	Anti-OX40 mAb OX40L-Ig fusion protein OX40L-transduced/transfected APCs Recombinant OX40L-expressing virus/tumor/bacteria OX40-specific DNA aptamers	Anti-OX40 ligand mAb OX40-Ig fusion protein
Potential diseases for therapeutic use	Cancer (all types) Persistent bacterial infections Chronic viral infections (HIV, hepatitis C)	Multiple sclerosis Rheumatoid arthritis Allergic asthma Inflammatory bowel disease Lupus Type 1 diabetes Psoriasis Atherosclerosis GVHD Pathogen-induced inflammation (influenza, SARS, West Nile virus)

T cells.<sup>60</sup> Anti-OX40 showed similar therapeutic efficacy to IL-2 in supporting tumor-reactive T-cell mediated destruction of lung-metastases. However, IL-2 in combination with adoptive immunotherapy did not support the eradication of brain metastases, while anti-OX40 showed powerful synergy to eradicate brain metastases.<sup>60</sup> It was not clear why anti-OX40 was able to augment the efficacy of T cells while IL-2 was not, but it may be linked to differential expression of T-cell surface proteins and their ability to help break the blood brain barrier.

While it is clear that OX40 agonists given as a single agent can enhance anti-tumor immunity in cancer-bearing hosts, there are models where its activity alone is not enough to cure mice of disease. Hence, there have been a number of studies that have attempted combination therapies with vaccines/cytokines and anti-OX40.<sup>54,61,62</sup> These combination therapies have included GM-CSF secreting whole tumor vaccines as well as the addition of innate cytokines, both of which showed promising synergy.<sup>54,61,62</sup> It was shown that anti-OX40 in combination with a GM-CSF secreting whole cell vaccine expressing the Her-2/neu tumor Ag was able to enhance CD8 T-cell responses and regress tumors (breast cancer model).<sup>62</sup> However, the vaccine alone showed very little anti-tumor efficacy, which correlated with a weak Her-2-specific CD8 T-cell response. It was subsequently shown that the increase in Her-2-specific CD8 T cells elicited via the combination treatment was dependent on anti-OX40 accentuating CD4 T-cell help. Another combinatorial approach that has shown great promise for tumor immunotherapy is combining anti-OX40 with innate cytokine(s), especially IL-12. One of the theoretical limitations of priming the immune system to tumor-specific Ags is the lack of "danger" signals (e.g., CpG, dsRNA, LPS, etc...) known to elicit innate cytokines when tumor Ags are presented to the immune system in vivo. As previously mentioned, IL-12 is necessary to mediate the survival of anti-OX40-stimulated CD4 T cells and the combination of anti-OX40 and IL-12 in tumor-bearing mice showed synergistic therapy.<sup>54</sup> This combination was therapeutically effective in the poorly immunogenic prostate cancer model, TRAMP-C1, where neither IL-12 nor anti-OX40 alone showed any therapeutic efficacy.<sup>54</sup> IL-12 and anti-OX40 also showed dramatic therapeutic synergy in an active immunization model using a tumor-dendritic fusion



vaccine injected directly into the spleen.<sup>54</sup> Whether IL-12 actually enhances survival of OX40 stimulated tumor-reactive T cells or increases Th1/Tc1 immunity was not directly ascertained in these models, but most likely both mechanisms are involved to enhance OX40-mediated tumor destruction.

OX40 agonists have also been shown to enhance T-cell responses to chronic pathogens (e.g., viruses and bacteria). In particular, anti-OX40 was administered into mice harboring a chronic cytomegalovirus known to replicate in visceral organs (i.e., salivary gland).<sup>63</sup> The initial study showed that anti-OX40 enhanced viral specific effector T-cell differentiation leading decreased viral replication in the salivary gland. Subsequently this group treated mice during the initial stage of infection and found that anti-OX40 greatly enhanced CD8 T-cell responses during the early stages of infection, which led to protective immunity.<sup>64</sup> The OX40 agonist strategy was also beneficial against hosts infected with *Cryptococcus neoformans*, where the infection resides in the lungs and becomes persistent.<sup>65</sup> The persistence of this pathogen is characterized by immune deviation to a nonclearing Th2 response, leading to chronic eosinophilia in the lungs.<sup>65</sup> Administration of an OX40L:Ig fusion protein drove a cytokine switch from Th2 to Th1 and reduced the pathogen burden and reduced the eosinophilia. OX40-specific elimination of *C. neoformans* was dependent on IFN- $\gamma$ /IL-12, as injection of an OX40 agonist to IFN- $\gamma$  or IL-12 ko mice harboring the pathogen was not able to resolve the infection.

It is clear that OX40 agonists have potent immune stimulating properties in several disease models, which ultimately helps the host eradicate harmful/potentially lethal entities within the body. Recently, our group has translated these findings into a cancer patient-specific clinical trial. Initially we tested the safety/dosing of a mouse anti-human OX40-specific monoclonal Ab in nonhuman primates.<sup>66</sup> We found that OX40 agonist administration to nonhuman primates potentiated memory T-cell generation and increased Ag-specific Ab responses similar to what was observed in mice. However, in contrast to the mouse studies we found that the adjuvant affect lasted longer, up to a month after the injection. We also observed a transient in drop in lymphocyte counts in the peripheral blood seven days after the initial Ab infusion and this was followed by a rebound where the lymphocyte numbers increased over base-line values. Upon completion of the monkey studies, the FDA approved a Phase I clinical trial in patients with Stage IV cancer. So far we have treated 17 patients with relatively low toxicity and we have observed immune stimulatory effects in most of the patients post-anti-OX40 treatment. In particular we have observed an increase in cycling memory T cells starting with the CD4 T-cell population one week after Ab infusion, followed by CD8 T cells usually 2-4 weeks post-OX40 treatment. We have observed cycling T cells in both the central memory compartment (CD28<sup>+</sup>) and effector memory (CD28<sup>-</sup>) populations. The increase in cycling memory T cells usually lasts 28-days following Ab infusion, however in some patients this effect lasted during the entire two-month evaluation period. There has been some hint of anti-tumor activity with four patients showing regression of some metastatic disease; however there have been no complete responders on this trial to date.

## **Conclusion**

In summary, OX40-specific signaling within T cells plays a key role in the generation of memory T cells as well as T-cell effector function. Its biologic function in vivo appears to be limited by expression of the OX40 ligand, which is expressed mainly on activated antigen presenting cells. OX40 ligand expressing cells are found within sites of inflammation in autoimmune disease and hence strategies have evolved to temper inflammation via blockade of OX40/OX40 ligand interaction. In contrast, little to no OX40 ligand expression is observed in hosts harboring tumors and some chronic infections and therefore accentuating OX40 signaling can enhance immunity leading to the destruction of these harmful entities. It is clear that tipping the balance of T-cell immunity through the OX40 axis could have important ramifications for several human diseases. The first stages of OX40-specific clinical trials are now being performed and efficacy of these trials will be determined in the future.

## References

1. Dutton RW, Bradley LM, Swain SL. T-cell memory. *Annu Rev Immunol* 1998; 16:201-23.
2. Redmond WL, Weinberg AD. Targeting OX40 and OX40L for the treatment of autoimmunity and cancer. *Crit Rev Immunol* 2007; 27(5):415-36.
3. Maxwell JR, Weinberg A, Prell RA et al. Danger and OX40 receptor signaling synergize to enhance memory T-cell survival by inhibiting peripheral deletion. *J Immunol* 2000; 164(1):107-12.
4. Evans DE, Prell RA, Thalhofer CJ et al. Engagement of OX40 enhances antigen-specific CD4(+) T-cell mobilization/memory development and humoral immunity: comparison of alphaOX-40 with alphaCTLA-4. *J Immunol* 2001; 167(12):6804-11.
5. Ruby CE, Redmond WL, Haley D et al. Anti-OX40 stimulation in vivo enhances CD8(+) memory T-cell survival and significantly increases recall responses. *Eur J Immunol* 2007; 37(1):157-66.
6. Pippig SD, Pena-Rossi C, Long J et al. Robust B-cell immunity but impaired T-cell proliferation in the absence of CD134 (OX40). *J Immunol* 1999; 163(12):6520-9.
7. Rogers PR, Song J, Gramaglia I et al. OX40 promotes Bcl-xL and Bcl-2 expression and is essential for long-term survival of CD4 T-cells. *Immunity* 2001; 15(3):445-55.
8. Baum PR, Gayle RB 3rd, Ramsdell F et al. Molecular characterization of murine and human OX40/OX40 ligand systems: identification of a human OX40 ligand as the HTLV-1-regulated protein gp34. *EMBO J* 1994; 13(17):3992-4001.
9. Paterson DJ, Jefferies WA, Green JR et al. Antigens of activated rat T-lymphocytes including a molecule of 50,000 Mr detected only on CD4 positive T blasts. *Mol Immunol* 1987; 24(12):1281-90.
10. Takeda I, Ine S, Killeen N et al. Distinct roles for the OX40-OX40 ligand interaction in regulatory and nonregulatory T-cells. *J Immunol* 2004; 172(6):3580-9.
11. Gramaglia I, Weinberg AD, Lemon M et al. Ox-40 ligand: a potent costimulatory molecule for sustaining primary CD4 T-cell responses. *J Immunol* 1998; 161(12):6510-7.
12. Weinberg AD, Celnik B, Vainiene M et al. The effect of TCR V beta 8 peptide protection and therapy on T-cell populations isolated from the spinal cords of Lewis rats with experimental autoimmune encephalomyelitis. *J Neuroimmunol* 1994; 49(1-2):161-70.
13. Buenafe AC, Weinberg AD, Culbertson NE et al. V beta CDR3 motifs associated with BP recognition are enriched in OX-40+ spinal cord T-cells of Lewis rats with EAE. *J Neurosci Res* 1996; 44(6):562-7.
14. Weinberg AD, Rivera MM, Prell R et al. Engagement of the OX-40 receptor in vivo enhances antitumor immunity. *J Immunol* 2000; 164(4):2160-9.
15. Weinberg AD, Bourdette DN, Sullivan TJ et al. Selective depletion of myelin-reactive T-cells with the anti-OX-40 antibody ameliorates autoimmune encephalomyelitis. *Nat Med* 1996; 2(2):183-9.
16. Weinberg AD. OX40: targeted immunotherapy—implications for tempering autoimmunity and enhancing vaccines. *Trends Immunol* 2002; 23(2):102-9.
17. Ramstad T, Lawnicki L, Vetto J et al. Immunohistochemical analysis of primary breast tumors and tumor-draining lymph nodes by means of the T-cell costimulatory molecule OX-40. *Am J Surg* 2000; 179(5):400-6.
18. Vetto JT, Lum S, Morris A et al. Presence of the T-cell activation marker OX-40 on tumor infiltrating lymphocytes and draining lymph node cells from patients with melanoma and head and neck cancers. *Am J Surg* 1997; 174(3):258-65.
19. Martin PJ, Ledbetter JA, Morishita Y et al. A 44 kilodalton cell surface homodimer regulates interleukin 2 production by activated human T-lymphocytes. *J Immunol* 1986; 136(9):3282-7.
20. Jenkins MK, Mueller D, Schwartz RH et al. Induction and maintenance of anergy in mature T-cells. *Adv Exp Med Biol* 1991; 292:167-76.
21. Harding FA, McArthur JG, Gross JA et al. CD28-mediated signalling costimulates murine T-cells and prevents induction of anergy in T-cell clones. *Nature* 1992; 356(6370):607-9.
22. Kaleeba JA, Offner H, Vandenbark AA et al. The OX-40 receptor provides a potent costimulatory signal capable of inducing encephalitogenicity in myelin-specific CD4+ T-cells. *Int Immunol* 1998; 10(4):453-61.
23. Lenschow DJ, Walunas TL, Bluestone JA. CD28/B7 system of T-cell costimulation. *Annu Rev Immunol* 1996; 14:233-58.
24. Weinberg AD, Vella AT, Croft M. OX-40: life beyond the effector T-cell stage. *Semin Immunol* 1998; 10(6):471-80.
25. Stuber E, Neurath M, Calderhead D et al. Cross-linking of OX40 ligand, a member of the TNF/NGF cytokine family, induces proliferation and differentiation in murine splenic B-cells. *Immunity* 1995; 2(5):507-21.
26. Stuber E, Strober W. The T-cell-B-cell interaction via OX40-OX40L is necessary for the T-cell-dependent humoral immune response. *J Exp Med* 1996; 183(3):979-89.

27. Murata K, Nose M, Ndhlovu LC et al. Constitutive OX40/OX40 ligand interaction induces autoimmune-like diseases. *J Immunol* 2002; 169(8):4628-36.
28. Miura S, Ohtani K, Numata N et al. Molecular cloning and characterization of a novel glycoprotein, gp34, that is specifically induced by the human T-cell leukemia virus type I transactivator p40tax. *Mol Cell Biol* 1991; 11(3):1313-25.
29. Godfrey WR, Fagnoni FF, Harara MA et al. Identification of a human OX-40 ligand, a costimulator of CD4+ T-cells with homology to tumor necrosis factor. *J Exp Med* 1994; 180(2):757-62.
30. Imura A, Hori T, Imada K et al. The human OX40/gp34 system directly mediates adhesion of activated T-cells to vascular endothelial cells. *J Exp Med* 1996; 183(5):2185-95.
31. Calderhead DM, Buhlmann JE, van den Eertwegh AJ et al. Cloning of mouse Ox40: a T-cell activation marker that may mediate T-B-cell interactions. *J Immunol* 1993; 151(10):5261-71.
32. Ohshima Y, Tanaka Y, Tozawa H et al. Expression and function of OX40 ligand on human dendritic cells. *J Immunol* 1997; 159(8):3838-48.
33. Sato T, Ishii N, Murata K et al. Consequences of OX40-OX40 ligand interactions in langerhans cell function: enhanced contact hypersensitivity responses in OX40L-transgenic mice. *Eur J Immunol* 2002; 32(11):3326-35.
34. Zingoni A, Sornasse T, Cocks BG et al. Cross-talk between activated human NK cells and CD4+ T-cells via OX40-OX40 ligand interactions. *J Immunol* 2004; 173(6):3716-24.
35. Akiba H, Oshima H, Takeda K et al. CD28-independent costimulation of T-cells by OX40 ligand and CD70 on activated B-cells. *J Immunol* 1999; 162(12):7058-66.
36. Burgess JK, Carlin S, Pack RA et al. Detection and characterization of OX40 ligand expression in human airway smooth muscle cells: a possible role in asthma? *J Allergy Clin Immunol* 2004; 113(4):683-9.
37. Weinberg AD, Wegmann KW, Funatake C et al. Blocking OX-40/OX-40 ligand interaction in vitro and in vivo leads to decreased T-cell function and amelioration of experimental allergic encephalomyelitis. *J Immunol* 1999; 162(3):1818-26.
38. Kim MY, Gaspal FM, Wiggert HE et al. CD4(+)CD3(-) accessory cells costimulate primed CD4 T-cells through OX40 and CD30 at sites where T-cells collaborate with B-cells. *Immunity* 2003; 18(5):643-54.
39. Brocker T, Gulbranson-Judge A, Flynn S et al. CD4 T-cell traffic control: in vivo evidence that ligation of OX40 on CD4 T-cells by OX40-ligand expressed on dendritic cells leads to the accumulation of CD4 T-cells in B follicles. *Eur J Immunol* 1999; 29(5):1610-6.
40. Soroosh P, Ine S, Sugamura K et al. OX40-OX40 ligand interaction through T-cell-T-cell contact contributes to CD4 T-cell longevity. *J Immunol* 2006; 176(10):5975-87.
41. Murata K, Ishii N, Takano H et al. Impairment of antigen-presenting cell function in mice lacking expression of OX40 ligand. *J Exp Med* 2000; 191(2):365-74.
42. Kopf M, Ruedl C, Schmitz N et al. OX40-deficient mice are defective in Th cell proliferation but are competent in generating B-cell and CTL Responses after virus infection. *Immunity* 1999; 11(6):699-708.
43. Gramaglia I, Jember A, Pippig SD et al. The OX40 costimulatory receptor determines the development of CD4 memory by regulating primary clonal expansion. *J Immunol* 2000; 165(6):3043-50.
44. Jember AG, Zuberi R, Liu FT et al. Development of allergic inflammation in a murine model of asthma is dependent on the costimulatory receptor OX40. *J Exp Med* 2001; 193(3):387-92.
45. Salek-Ardakani S, Song J, Halteman BS et al. OX40 (CD134) controls memory T helper 2 cells that drive lung inflammation. *J Exp Med* 2003; 198(2):315-24.
46. Kaech SM, Wherry EJ, Ahmed R. Effector and memory T-cell differentiation: implications for vaccine development. *Nat Rev Immunol* 2002; 2(4):251-62.
47. Wherry EJ, Ahmed R. Memory CD8 T-cell differentiation during viral infection. *J Virol* 2004; 78(11):5535-45.
48. Wherry EJ, Teichgraber V, Becker TC et al. Lineage relationship and protective immunity of memory CD8 T-cell subsets. *Nat Immunol* 2003; 4(3):225-34.
49. Soroosh P, Ine S, Sugamura K et al. Differential requirements for OX40 signals on generation of effector and central memory CD4+ T-cells. *J Immunol* 2007; 179(8):5014-23.
50. Song A, Tang X, Harms KM et al. OX40 and Bcl-xL promote the persistence of CD8 T-Cells to recall tumor-associated antigen. *J Immunol* 2005; 175(6):3534-41.
51. Hendriks J, Xiao Y, Rossen JW et al. During viral infection of the respiratory tract, CD27, 4-1BB and OX40 collectively determine formation of CD8+ memory T-cells and their capacity for secondary expansion. *J Immunol* 2005; 175(3):1665-76.
52. Melero I, Hervas-Stubbs S, Glennie M et al. Immunostimulatory monoclonal antibodies for cancer therapy. *Nat Rev Cancer* 2007; 7(2):95-106.
53. Prell RA, Evans DE, Thalhofer C et al. OX40-mediated memory T-cell generation is TNF receptor-associated factor 2 dependent. *J Immunol* 2003; 171(11):5997-6005.

54. Ruby CE, Montler R, Zheng R et al. IL-12 is required for anti-OX40-mediated CD4 T-cell survival. *J Immunol* 2008; 180(4):2140-8.
55. Redmond WL, Gough MJ, Charbonneau B et al. Defects in the Acquisition of CD8 T-Cell Effector Function after Priming with Tumor or Soluble Antigen Can Be Overcome by the Addition of an OX40 Agonist. *J Immunol* 2007; 179(11):7244-53.
56. Baumann R, Yousefi S, Simon D et al. Functional expression of CD134 by neutrophils. *Eur J Immunol* 2004; 34(8):2268-75.
57. Sugamura K, Ishii N, Weinberg AD. Therapeutic targeting of the effector T-cell costimulatory molecule OX40. *Nat Rev Immunol* 2004; 4(6):420-31.
58. Sadun RE, Hsu WE, Zhang N et al. Fc-mOX40L fusion protein produces complete remission and enhanced survival in 2 murine tumor models. *J Immunother* 2008; 31(3):235-45.
59. Kjaergaard J, Tanaka J, Kim JA et al. Therapeutic efficacy of OX-40 receptor antibody depends on tumor immunogenicity and anatomic site of tumor growth. *Cancer Res* 2000; 60(19):5514-21.
60. Kjaergaard J, Peng L, Cohen PA et al. Augmentation versus inhibition: effects of conjunctive OX-40 receptor monoclonal antibody and IL-2 treatment on adoptive immunotherapy of advanced tumor. *J Immunol* 2001; 167(11):6669-77.
61. Gri G, Gallo E, Di Carlo E et al. OX40 ligand-transduced tumor cell vaccine synergizes with GM-CSF and requires CD40-Apc signaling to boost the host T-cell antitumor response. *J Immunol* 2003; 170(1):99-106.
62. Murata S, Ladle BH, Kim PS et al. OX40 costimulation synergizes with GM-CSF whole-cell vaccination to overcome established CD8+ T-cell tolerance to an endogenous tumor antigen. *J Immunol* 2006; 176(2):974-83.
63. Humphreys IR, de Trez C, Kinkade A et al. Cytomegalovirus exploits IL-10-mediated immune regulation in the salivary glands. *J Exp Med* 2007; 204(5):1217-25.
64. Humphreys IR, Loewendorf A, de Trez C et al. OX40 costimulation promotes persistence of cytomegalovirus-specific CD8 T-Cells: A CD4-dependent mechanism. *J Immunol* 2007; 179(4):2195-202.
65. Humphreys IR, Edwards L, Wälzl G et al. OX40 ligation on activated T-cells enhances the control of *Cryptococcus neoformans* and reduces pulmonary eosinophilia. *J Immunol* 2003; 170(12):6125-32.
66. Weinberg AD, Thalhofer C, Morris N et al. Anti-OX40 (CD134) administration to nonhuman primates: immunostimulatory effects and toxicokinetic study. *J Immunother* 2006; 29(6):575-85.