REVIEW

Therapeutic strategies for the costimulatory molecule OX40 in T-cell-mediated immunity

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Abstract   The T cell co-stimulatory molecule OX40 and its cognate ligand OX40L have attracted broad research interest as a therapeutic target in T cell-mediated diseases. Accumulating preclinical evidence highlights the therapeutic efficacy of both agonist and blockade of the OX40−OX40L interaction. Despite this progress, many questions about the immuno-modulator roles of OX40 on T cell function remain unanswered. In this review we summarize the impact of the OX40−OX40L interaction on T cell subsets, including Th1, Th2, Th9, Th17, Th22, Treg, Tfh, and CD8+ T cells, to gain a comprehensive understanding of anti-OX40 mAb-based therapies. The potential therapeutic application of the OX40−OX40L interaction in autoimmunity diseases and cancer immunotherapy are further discussed; OX40−OX40L blockade may ameliorate autoantigen-specific T cell responses and reduce immune activity in autoimmunity diseases. We also explore the rationale of targeting OX40−OX40L interactions in cancer immunotherapy. Ligation of OX40 with targeted agonist anti-OX40 mAbs conveys activating signals to T cells. When combined with other therapeutic treatments, such as anti-PD-1 or anti-CTLA-4 blockade, cytokines, chemotherapy, or radiotherapy, the anti-tumor activity of agonist anti-OX40 treatment will be further enhanced. These data collectively suggest great potential for OX40-mediated therapies.

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Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

1. Introduction

The immune system is finely-tuned with various highly differentiated immune cells and organs working together. As one of the major types of lymphocytes, T cells play essential roles in different stages of immune responses. Naïve T cells can quickly proliferate and differentiate into heterogeneous effector T cells after encountering antigens through particular cytokine-environment stimulation. T cells are classified into CD4$^+$ T cells and CD8$^+$ T cells according to the glycoprotein types on their membrane surface. CD4$^+$ T cells, which are also called Th (T helper) cells, can differentiate into different subsets according to the cytokine profiles[1]. The widely studied subsets of CD4$^+$ T cells are Th1, Th2, Th9, Th17, Th22, Treg (regulatory T cells), and Tfh (follicular helper T cells)[1]. CD8$^+$ T cells, known as cytotoxic T lymphocytes, play important roles in immune defense against intracellular pathogens or infected cells. They engage and kill their target through several mechanisms, such as secreting cytokines such as tumor necrosis factor-α (TNF-α) and interferon-γ (INF-γ), releasing cytotoxic granules such as perforin and granzymes, and initiating FAS/FASL interaction.

In addition to antigen stimulation through T cell receptors (TCR) and cytokine receptors, T cell activation also requires costimulatory molecular signals, such as constitutive CD27 or CD28 and inductive glucocorticoid-induced tumor necrosis factor receptor (GITR), and induced costimulatory molecules (ICOS) such as OX40 or 4-1BB[2]. Among those costimulatory molecules, OX40 is widely studied for its beneficial functions. Ligation OX40 signals with agonist anti-OX40 mAbs to convey activating signals to T cells, resulting in cytokine production, survival, expansion, and homeostasis of effector or memory T cells[3,4]. OX40 is a member of the tumor necrosis factor receptor (TNFR) family, and it is also known as CD134 or TNFRSF4[4]. OX40 was first discovered on the surface of activated rat CD4$^+$ T cells in 1987. Subsequent studies revealed that OX40 was expressed on both activated CD4$^+$ and CD8$^+$ T cells, neutrophils, and nature killer (NK) cells. OX40 has important co-stimulatory functions during T cell activation, mediating the survival and expansion of both CD4$^+$ and CD8$^+$ T cells in multiple animal models of autoimmunity, infectious disease, and cancer[5]. OX40 is also involved in controlling effector and memory T cell responses[4].

The mechanism by which OX40−OX40L signaling affects T cell function is complicated. Under certain conditions OX40 can strongly promote the induction of Th1, Th2, and Th9 cells, and under other conditions it can inhibit the generation of forkhead box p3 (Foxp3) Tregs[6]. Previous studies showed that OX40−OX40L signals can augment the immune response in T cells subsets through NF-κB, mitogen-activated protein kinase (MAPK), nuclear factor of activated T cells (NFAT), and BCL-2/XL-dependent anti-apoptotic pathways[7,8]. These signaling pathways jointly contribute to the expression of pro-survival molecules and elevated cytokine production and augment the generation of both effector and memory T cell subsets[5]. Some reports focusing on the immunity role of OX40−OX40L signals on T cell biology considered the CD4$^+$ T cells as a whole[9], leaving details of the OX40 influence on different T cell subsets neglected. Here in this review we first discuss the biology of OX40 and OX4OL and then present the detailed impact of OX40−OX40L signals on T cell subsets, including Th1, Th2, Th9, Th17, Th22, Treg, Tfh, and CD8$^+$ T cells, to provide a comprehensive understanding of its biology.

As a crucial T cell activation stimulator, either activation or blockade of OX40−OX40L signals can yield therapeutic efficacy. On one hand, blocking the OX40−OX40L pathway using OX40 or OX40L binding agents seems to inhibit autoreactive T cells and ameliorate autoimmunity disease, as in experimental autoimmune encephalomyelitis (EAE) models[8]. On the other hand, augmentation of OX40 signaling using OX40 agonist antibodies appears to enhance T cell-mediated anti-tumor immunity, contributing to tumor regression and prolonged survival. Here, OX40 agonists may increase T cell infiltration into tumors and enhance antitumor immune responses in conventional CD4$^+$ and CD8$^+$ T cells, thus improving survival rates in several preclinical cancer models, including B16 melanoma, Lewis lung carcinoma, colon cancer, breast cancer and 4T-1 breast cancer[10,11]. Therefore, agonists of the OX40−OX40L pathway potentially can be adopted for cancer immunotherapy.

2. The biology of OX40 and OX40L

OX40 is a type 1 transmembrane glycoprotein composed of about 275 amino acids with three full cysteine-rich domains (CRDs) and a partial C-terminal CRDs[7]. The apparent molecular weight of OX40 is 50 kDa[5]. OX40, along with other TNFRSF members such as 4-1BB, CD27, CD30, and CD40, are T cell costimulatory molecules acting at different stages to modulate and control the immune response[6]. Unlike other constitutive T cell costimulatory receptors, such as CD28 and CD27, OX40 is not expressed on naïve T cells. OX40 is transiently induced by signaling following TCR engagement after antigen (Ag) recognition, peaking 48−72 h in activated T cells, including activated CD4$^+$ and CD8$^+$ T cells, as well as in neutrophils, natural killer cells (NK) and natural killer T cells (NKTs)[5]. The only exception is that OX40 is constitutive in mouse FOXP3$^+$ Treg cells. Many factors are involved in the kinetics of OX40 expression, including the cytokine milieu, the persistence of antigen, the inflammatory environment, and the influence of other co-stimulation pathways[5]. OX40-deficient T cells might normally proliferate and differentiate into effector T cells at 2−3 days after TCR engagement, but they showed notable reduction of survival after 12−13 days[12,13]. That is, OX40 signaling may not affect the initial activation phase of T cells, but may play an essential role in maintaining late proliferation and survival in the T cell effector phase.

The ligand for OX40 (OX40L, also known as CD252), is also a member of the TNFR superfamily. OX40L is a type II glycoprotein with a 23-amino-acid cytoplasmic tail and a 133-amino-acid extracellular domain[7]. Murine OX40L can stimulate both human and mouse T cells; however, human OX40L can only stimulate human T cells[14]. OX40L is mainly expressed on activated antigen-presenting cells (APCs), such as dendritic cells (DCs), activated B cells, and macrophages. The types of cells that can be induced to express OX40L is much broader. Apart from APCs, OX40L also expressed on hematopoietic cells such as activated NK cells, mast cells or the responding CD4$^+$ T cells, and non-hematopoietic cells, such as endothelial cells or smooth muscle cells (summarized in Table 1)[4], which provides the foundation for the two-step OX40−OX40L costimulation model (Fig. 1).

Molecule	Expression level	Expressed by
OX40	Inducible	CD4$^+$ and CD8$^+$ T cells
		Neutrophils, NK cells, NKT cells
		Mouse Treg cells (constitutive expression)
OX40L	Inducible	DCs, macrophages, B cells
		NK, mast, activated T cells smooth muscle, endothelial cells

More specifically, the first step of OX40−OX40L signaling is the interaction between OX40 and OX40L after 2−3 days, when activated T cells bind to professional APCs, mainly DCs, since OX40L expression on the APC surface is inducible 1−3 days following initial antigen encounter. After that, the OX40-expressing activated T cells can encounter other OX40L-expressing cells during the effector phase after leaving the initial DCs. For example, OX40L expressed by CD4$^+$ T cells can also bind to OX40 on other CD4$^+$ T cells to enhance T cell survival[15]. OX40L$^+$ B cells[16], OX40L$^+$ NK cells, OX40L$^+$ mast cells[17] and OX40L$^+$ endothelial cells can interact with OX40$^+$-activated T cells to support T-cell function during the effector phase, which may result in multi-channel activation of T cells.

3.   Effect of the OX40−OX40L interaction on different T cell subsets

As for the impact of the OX40−OX40L interaction on T cell subsets, Table 2 displays a brief summary and is also illustrated in Fig. 2. Regarding CD4$^+$ T cell subsets, OX40−OX40L signals can enhance the Th1-mediated immune response, promote the generation and maintenance of Th2, favor Th9 differentiation through the non-canonical NF-κB pathway, augment Tfh development, and antagonize Treg generation and Treg-mediated suppression. As for CD8$^+$ T cell subsets, OX40 promotes the survival and expansion of CD8$^+$ T cells and the recall response of CD8$^+$ memory T cells *in vivo*. Nevertheless, studies on the effect of OX40 on Th22 are relatively rare at present.

3.1.   OX40 enhances the Th1-mediated immune response

T helper 1 (Th1) cells are characterized by the secretion of interferon-γ (IFN-γ), as well as a series of other cytokines including tumor necrosis factor alpha (TNF-α), interleukin-2
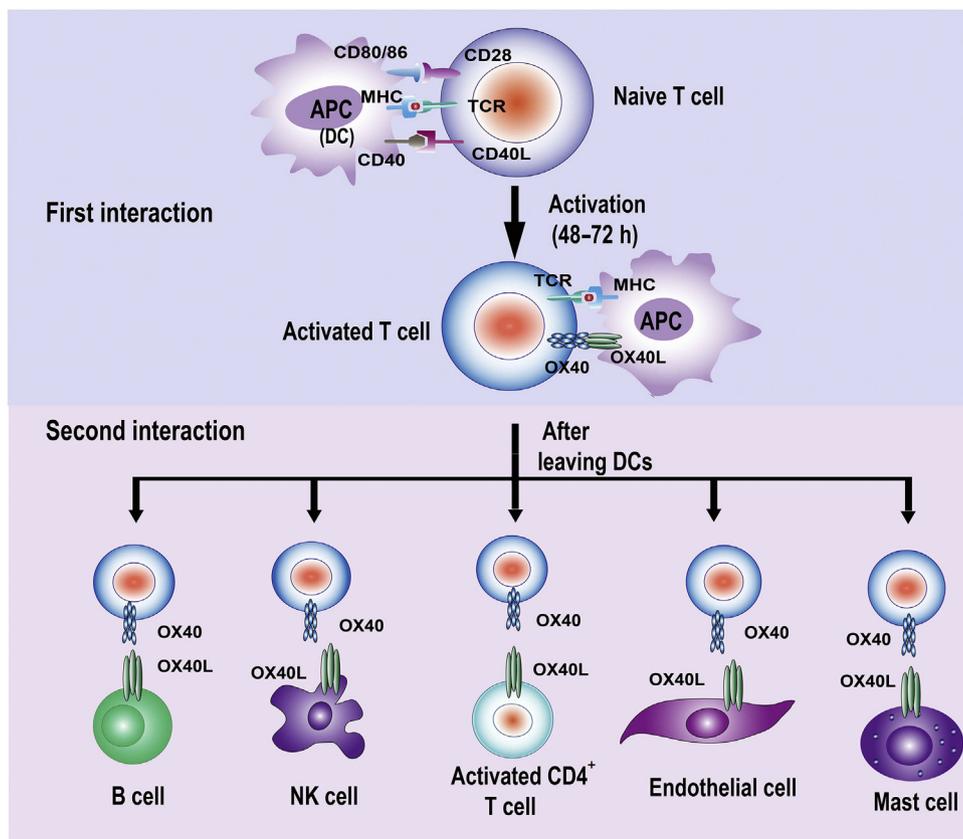
Figure 1   Schematic diagram of the two-step OX40L costimulation model. The OX40 signals to activated OX40-expressing T cells are first provided by professional APCs during 48−72 h after antigen recognition. After the first interaction the OX40-expressing T cells may leave DCs and interact with other OX40L-expressing cells during the effector phase, such as B cells, nature killer (NK) cells, activated CD4$^+$ T cells, endothelial cells, or mast cells, which results in the multi-channel activation of T cells through OX40−OX40L signaling.

Table 2 Effect of OX40 on different T cell subsets.

T cell	The impact of OX40 stimulatory signal	Condition	Ref.
CD4$^+$	Inhibiting FOXP3 expression and Tregs induction *via* AKT−mTOR pathway	*In vitro*	18
	Enhancement of helper CD4 T cell activity and humoral immunity	Rodent malaria	19
	Triggering larger memory Th1 and Tfh CD4 T cell	Plasmodium infections	20
	Promoting CD4 T cell proliferation and survival	Graves' disease	21
	Inducing splenic CD4 T cell activation, and splenic Tfh cell accumulation	Systemic lupus erythematosus	22
	Promoting Th1 cell differentiation and proliferation, and attenuating Treg suppressive activity	Obesity mice	23
	Orientation of CD4$^+$ cells toward Th1 responses	Glioma-bearing mice	24
	Enhancement of tumoral CD4$^+$ effector T cell responses	*In vitro*	25
	OX40-deficient mice are reduction of both Th1 and Th2 cytokines	*In vivo*	26
	Promoting IL-17 production	Rheumatoid synovium	27
Th2	Contribution of OX40L to the development of Th2-mediated pulmonary inflammation	Murine model of asthma	28
Th9	Induction of Th9 cells	Airway inflammation	29
Th17	Inhibiting IL-17 expression and Th17 cell-mediated autoimmunity	*In vitro*	30
	Augmentating Th17 cytokine expression	Uveitis	31
Tfh	Promoting BLIMP-1 expression and diverting cells away from Tfh cell differentiation	Lymphocytic choriomeningitis virus	32
	Promoting the differentiation of human Th cells toward the Tfh lineage	Systemic lupus erythematosus	33
	Amplification of Tfh cell development cooperating with ICOS	Vaccinia virus infection	34
Treg	OX40 expression in Tregs was greater than in conventional CD4 and CD8 T cells	Head and neck cancer	35
	Reversing the suppressive effects of Tregs	Cutaneous squamous cell carcinoma	25
	Blockade of OX40L decreased Tregs proliferation	Crescentic glomerulonephritis	36
	Treg cells suppress mast cell degranulation through OX40−OX40L interaction	Allergies	37
	Induction of Treg activation and their suppressive function	*In vitro*	38
	Supporting Treg cell development, homeostasis, and suppressive activity	Inflammatory bowel disease mice model	39
	Blocking Tregs inhibitory activity, and restored effector T-cell proliferation	Graft-*versus*-host disease mice model	40
	Blocking inducible and natural regulatory T cell function	Human OX40 antibodies	41
Tregs	OX40 inhibits TGF-β- and antigen-driven conversion of naive CD4 T cells into CD25$^+$FOXP3$^+$ T cells	*In vitro*	42
	OX40 ligand shuts down IL-10-producing Treg cells	*In vitro*	43
	OX40 costimulation turns off FOXP3$^+$Tregs	*In vitro*	44
	OX40 expression by T reg cells was indispensable for suppression colitogenic T cell responses	Mouse models of colitis	45
CD8$^+$	OX40 can control survival of primed CD8 T cells	Adoptive T cell transfer	46
	OX40 supports CD8 T cells expansion and confer CTL-mediated protection against tumor growth.	Adoptive transfer of CD8 cells	47
	Cooperation between CD4 and CD8 T cells for antitumor activity is enhanced by OX40 signals	Adoptive transfer of CD8 cells	48
	OX40-deficient mice are competent in generating B cell and CTL responses after virus infection	LCMV and influenza virus	49
	OX40L costimulated memory CD8 T cell responses largely through indirect effects	*In vitro*	50
	OX40 signals directly augment activation, cytokine secretion, proliferation of human CD8$^+$ T cells	*In vitro*	51
	Synergy with anti-PD-L1 in the initial reversal of CD8$^+$ T cell exhaustion	*In vitro*	52
	OX40 agonism enhance CD8$^+$ memory	*In vitro*	53
CD4$^-$CD8$^-$	Control CD4$^-$CD8$^-$ survival by regulation on BCL-2, BCL-XL, and BCL2L11	*In vitro*	54
CD4$^+$CD28$^-$	An alternative costimulator of CD4$^+$CD28$^-$ T cells	Autoimmune arthritis	55

(IL-2), granulocyte-macrophage colony-stimulating factor (GM-CSF) and lymphotoxin[1]. Th1 cells are mainly implicated in cell-mediated antiviral and antimicrobial immunity to intracellular pathogens. As a matter of fact, the effect of Th1 cells on immunology is pleiotropic and the multitude of functions of Th1 is expanding. An excessive Th1 response will promote immunopathology and exacerbate autoimmunity by enhancing the production of pathogenic autoantibodies. However, deficiency in Th1 function would be associated with immunodeficiency.
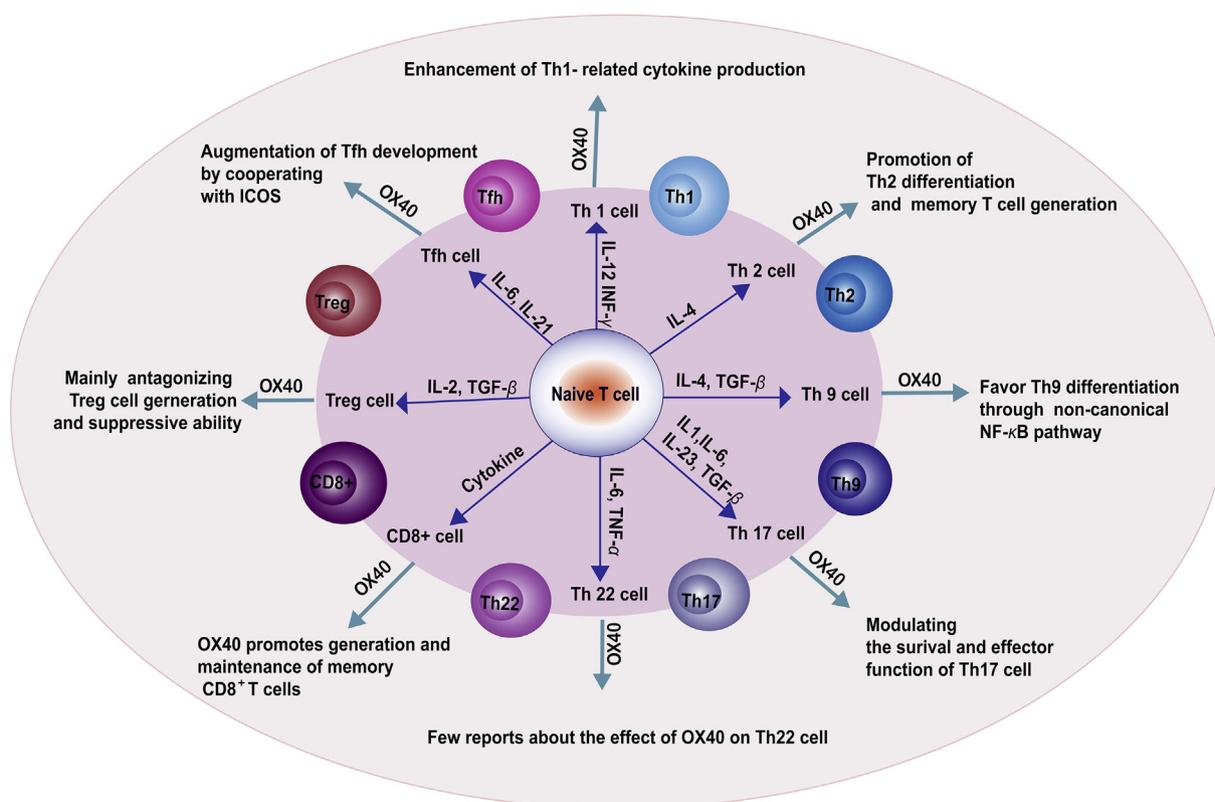
Figure 2 The summary of the impact of the OX40−OX40L interaction on T cell subsets. OX40−OX40L signals can enhance the Th1-mediated immune response, promote generation and maintenance of Th2, favor Th9 differentiation through the non-canonical NF-κB pathway, augment Tfh development, and antagonize Treg cell generation and Treg-mediated suppression. And as for CD8$^+$ T cell subsets, OX40 promotes the survival and expansion of CD8$^+$ T cells and the recall response of CD8$^+$ memory T cells *in vivo*.

Since OX40- or OX40L-deficient animals have shown reductions in both Th1 and Th2 cytokine responses *in vivo*, the role of OX40−OX40L interactions in regulating Th1 responses is unclear. In naive CD4$^+$ T cells, OX40−OX40L signals bias cytokine interleukin-4 (IL-4) production toward T-helper (Th) cell 2 (Th2) generation. With the help of antigen or IL-12 priming, OX40−OX40L signals may overcome this Th2 directive action and regulate the differentiation of CD4$^+$ T cells into Th1 cells[56]. It also has been demonstrated that in the absence of adjuvant, OX40−OX40L interaction is capable of inducing a Th2 response, while in the presence of adjuvants both Th1 and Th2 responses are augmented. In an OX40L-deficient murine model of allergic asthma, Arestides et al.[57] found that blockade of OX40−OX40L signaling failed to support potent CD4 T cell responses, and the levels of allergen-induced Th1 as well as Th2 cytokines were significantly reduced. Thus, OX40 appears to be involved in a variety of mechanisms to control the priming of Th2 and Th1 responses, which appears dependent on the experimental conditions adopted. And engagement of OX40 with OX40L may deliver a strong co-stimulatory signal to newly activated effector T-cells and enhance both Th1 and Th2 responses[7].

While further studies proved that OX40−OX40L interactions exerted critical costimulatory effects on the Th1-mediated immune response and contributed to immunopathology in a variety of animal models[58] including experimental inflammatory bowel disease[59], EAE[60], rheumatoid arthritis (RA)[61], colitis[62] and graft-*versus*-host-disease. Blockade of OX40−OX40L interactions ameliorated these Th1-specific immune diseases. Using a myelin oligodendrocyte glycoprotein peptide (MOG)-induced EAE murine model, Ndhlovu,

et al.[60] showed that the severity of EAE was either aggravated or alleviated depending on the expression of OX40L. OX40L-deficient mice or wild-type mice treated with anti-OX40L mAb (MGP-34) showed reduced clinical manifestations of EAE coupled with a reduction in Th1 cytokine production, including IFN-γ and IL-2[60]. Conversely, transgenic overexpression of OX40L in mice lead to a greater severity of EAE with significantly elevated levels of IL-2, IL-6, and IFN-γ[60]. Although these altered Th1 cytokine responses may have resulted from the inability of differentiation of both Th1 and Th2 effectors; noteworthy, however, is the recognition that EAE is usually regarded as a Th1-mediated disease and OX40−OX40L interactions were indeed involved in the Th1-mediated immune responses.

3.2. OX40 promotes generation and maintenance of Th2

Th2 cells are recognized for the secretion of IL-4, IL-5 and IL-13. Th2 cells are typically involved in immunity defense against extracellular pathogens and multi-cellular parasites, allergies and atopic illnesses[1,63]. Th2 cells can exert anti-inflammatory ability by suppressing cell-mediated or Th1-mediated inflammation, since IL-4 possesses strong suppression of Th1 cell generation even in the presence of IFN-γ. However, Th2 cells also play a role in several humorally-associated autoimmune diseases, in that aberrant IL-4 expression can induce activation of autoreactive B cells and thereby promote tissue inflammation and immunopathology.

Under neutral conditions the OX40−OX40L interaction preferentially promotes CD4$^+$ T cells to differentiate into Th2 effector cells through enhanced calcium/nuclear factor-activated T cell

(NFATc) signals and up-regulated IL-4 production. A major role for the OX40−OX40L interaction in Th2-mediated allergic asthma[64] and *Leishmania* infection murine models has been substantiated[65]. An impaired ability to generate a Th2 response in an OX40-deficient murine asthma model has also been demonstrated, and was characterized by high levels of immunoglobulin E, IL-5 and IL-4 due to a reduced number of antigen-specific memory T cells, thus resulting in diminished lung inflammation and attenuated airway hyperreactivity[64]. On the other hand, Hoshino et al.[28] demonstrated a critical contribution of OX40L to the development of Th2-mediated experimental leishmaniasis and pulmonary inflammation. In an OX40L-deficient murine asthma model, all asthmatic responses including increased mucus production, accumulation of eosinophils, and high levels of Th2 cytokines were greatly diminished[28]. Administration of the neutralizing anti-OX40L mAb to wild-type asthmatic mice also abolished the induction of asthmatic responses during the sensitization period, indicating a pathogenic role of OX40L in differentiation of Th2 cells and in the pathway of Th2 polarization *in vivo*[28]. In accordance with several reports, in the absence of OX40−OX40L signals Th2 cells are impaired in clonal expansion resulting in a quite low level of memory T cells, suggesting that the OX40−OX40L interaction was indispensable in Th2 cell differentiation and Th2 polarization[66,67]. What's more, the OX40−OX40L interaction was also indispensable in Th2 memory T cell generation and memory recall responses by sustaining expression of the antiapoptotic members of BCL-2 family, such as BCL-2, BCL-XL, or BFL1[66,67].

3.3. OX40 favors Th9 differentiation through a non-canonical NF-κB pathway

Th9 cells, preferentially producing IL-9, are a novel CD4$^+$ T cell subset in the immune system with proinflammatory functions[1]. Th9 cells are featured with potent anticancer ability, as well as properties to promote the development of autoimmune and allergic diseases[1]. Th9 cells are closely associated with Th2 cells, since the Th2-produced cytokine IL-4 provides one of the key signals for Th9 induction. However, even under optimal polarizing conditions *in vitro*, the differentiation frequency of Th9 cells is quite low. Their activation not only requires integration of signals from TGF-β and IL-4 cytokines but also needs the combined stimulation of costimulatory molecules, such as OX40.

OX40 ligation on activated CD4$^+$ T cells showed great potency in promoting Th9 cell induction, converting a large number of CD4$^+$ Tconv cells into Th9 cells *in vitro*, thus emphasizing the importance of OX40−OX40L costimulation[67]. OX40-mediated Th9 cell enhancement has repercussions *in vivo*, including contributions to antitumor immunity or regulation of immunopathology in allergic lung and ocular inflammation[67]. In an allergic murine model, injection of agonist anti-OX40 antibody induced Th9 cell-dependent allergic airway inflammation and increased hyper-proliferation of mucin-producing cells in the airway epithelium[67]. There was an absence of any IL-9 enhancement in OX40L-deficient T cells, indicating the specific stimulation through the OX40−OX40L pathway.

In addition, Th9 cells induced by OX40 costimulation showed very high expression of IL-9 without expression of Th2 or Th17 cytokines, such as IL-10, which seemed to be distinct from Th9 cells without OX40 ligation[68]. Subsequent study revealed that the promotion of Th9 through OX40−OX40L signals didn't depend on PU.1, which was a key transcriptional regulator for the

induction of Th9, but instead through tumor necrosis factor receptor-associated factor (TRAF) adaptor molecules, particularly TRAF6, which activated an NF-κB transcription factor complex and triggered a wide array of downstream biological functions[68]. OX40 costimulation could still increase IL-9-producing T cell differentiation under repression of the canonical NF-κB pathway, while mice lacking the non-canonical NF-κB subunit p52 were unresponsive to OX40 L[68]. Although OX40−OX40L signals can activate both the canonical and non-canonical NF-κB pathway, OX40 may act through the non-canonical NF-κB pathway to favor the potentiation of Th9 differentiation.

3.4. OX40 modulates the survival and function of Th17

Th17 cells are a newly emerged effector T helper cell subset. They can reinforce certain aspects of the adaptive immunity system such as host defense and tissue repair responses. They also can induce local tissue inflammation and promote autoimmune tissue injury *via* the secretion IL-17 family cytokines (mainly IL17). IL-17 signals can contribute to activation of innate immune cells, enhancement of B cell responses, recruitment of neutrophils, up-regulation of proinflammatory mediators such as TNF-α, IL-1β or intercellular adhesion molecule 1 (ICAM-1)[1]. In addition to IL-17, Th17 cells can also secrete IL-21, IL-22, IL-25[1]. Th17 cells also possess anti-inflammatory ability through the production of the potent anti-inflammatory cytokines IFN-γ and IL-10, thereby attenuating inflammation and pathology.

Accumulated evidence suggests that OX40 is a crucial costimulatory molecule involved in modulating the survival and function of Th17 cell[27]. In a mouse model of destructive arthritis deficient in IL-1 receptor, the authors showed that IL-17 did not induce OX40 expression, while activation of T cells through OX40 ligation enhanced IL-17 production, and blocking the OX40−OX40L pathway efficiently repressed the development of inflammatory peripheral arthritis, which could be at least partially linked to a significant reduction of IL-17 from Th17 cells in the peripheral synovial joints[69]. In an ovalbumin-induced uveitis model, Zhang, et al.[31] demonstrated that OX40-activating antibody significantly augmented the transfer of OX40-stimulated lymphocytes and elicited a more severe ocular inflammation *in vivo*, while an IL-17-neutralizing antibody attenuated OX40-mediated uveitis.

However, other studies also reported completely opposite results, with ligation of OX40 with OX40L on human T cells inhibiting IL-17 production, which was mediated through up-regulation of IFN-γ and IL-4, both of which are reported to inhibit the production of IL-17[70]. Moreover, OX40L still suppressed IL-17 production even in the presence of IL-23, which is a potent stimulator of IL-17 and differentiation factor for Th17 cells[70]. Therefore, the OX40−OX40L pathway plays a crucial role for enhancing the elaboration of Th17 cells, which may be partially dependent on the conditions.

3.5. Limited studies of the effect of OX40 on Th22

Th22 cells play a complicated role in inflammatory and autoimmune disease. Th22 cells produce predominantly IL-22 cytokine[71,72]; IL-22 seems to possess both pathogenic and protective effects according to environmental cues[71]. On one hand, IL-22 can promote inflammatory and autoimmune conditions in psoriasis, rheumatoid arthritis, Crohn's disease, and atopic dermatitis patients, suggesting its pathogenic role. On the other hand, IL-22

was down-regulated in the serum of patients with sarcoidosis and systemic lupus erythematosus[71]. However, there is little known about the effect of OX40−OX40L signals on Th22 cells. The influence of OX40−OX40L signals on Th22 cells, and the relationship between Th22 cells and other Th subsets, particularly Th17 cells, needs further investigation.

3.6. OX40 augments Tfh development

Follicular helper T (Tfh) cells are firstly recognized by their residence in B cell secondary lymphoid tissues areas. The anatomical location of Tfh cells allows them to favor the function of B cells, and the formation of germinal centers (GC). Many identifying molecules are involved in those functions, such as CXC chemokine receptor 5 (CXCR5), IL-21 and ICOS. Tfh cells can produce IL-21, and IL-21 serves as a "helper" cytokine to stimulate B cells through interacting with IL-21R. Due to the special role of Tfh cells, disruption of Tfh cell function may result in pathogenesis of either immune deficiencies or autoimmune diseases.

Many studies have explored the possibility that OX40−OX40L signals might have any impact on Tfh development and GC responses. OX40−OX40L signals have been found to promote expression of *CXCR5* mRNA in activated mouse T cells *in vitro*. Soluble OX40L treatment increased the expression of several Tfh-associated molecules on T cells in lupus patients *in vitro*, including CXCR5, BCL-6 and IL-21. These studies also showed that OX40−OX40L interactions promoted CD4 T cells to accumulate at the T/B border and in B cell follicles in murine models[73]. There was a reduction of antibody production in OX40L-transgenic mice and mice lacking OX40 or OX40 L[73]. Based on those findings, it was concluded that OX40−OX40L signals indeed exert an effect on the maintenance of the Tfh function.

Using a vaccinia virus (VacV) infection murine model, Tahiliani et al.[34] showed that the number of Tfh cells was about 70% fewer in OX40-deficient mice compared to WT mice on Day 8−15 after VacV infection. A similar decrease was also found in the level of VacV-specific IgG Abs. This study also demonstrated that OX40−OX40L interactions were involved in maintaining the Tfh and GC Tfh pool after the Tfh phenotype was initially acquired. So whether OX40 is expressed on transitional Tfh cells or on mature Tfh cells, it always can play a strong role to maximize the Tfh response. OX40−OX40L interactions were found to cooperate with ICOS−ICOSL interactions to maintain the Tfh response and amplify the ongoing Ab response at a late stage after infection[34]. Since OX40 was co-expressed with ICOS on both transitional and mature Tfh cells, it may be active in controlling Tfh cells at the same time.

In another study, OX40−OX40L signals contributed to an aberrant Tfh cell response in human systemic lupus erythematosus (SLE)[33]. OX40−OX40L signals promoted the expression of multiple Tfh-related molecules including CXCR5, IL-21 and CD40L in both naive and memory Tfh cells, and subsequently increased the generation of CXCR5+ Tfh cells while down-regulating the transcription repressor PR domain zinc finger protein 1 (PRDM1) which inhibits Tfh generation[33]. OX40L−OX40 signals were sufficient to render both naive and memory Tfh cells to become efficient B cell helpers, mainly mediated by TCR signal enhancement. One of the hallmarks of autoimmune diseases like SLE is autoreactive antibody production. This may suggest that the pathogenic contribution of Tfh cells in human SLE is *via* the OX40L−OX40 pathway, thus providing a rationale to target this pathway as a therapeutic modality.

However, there also are some contradictory results on the OX40−OX40L interaction in the regulation of Tfh cell responses. This study showed that the absence of OX40 signals did not affect Tfh differentiation, antibody generation, or even the CXCR5 expression[74]. Furthermore, Boettler et al.[32] showed that agonistic anti-OX40 mAb suppressed the expression of Bcl-6 while enhancing the expression of the Tfh-specific molecule BLIMP-1. As for the dual roles of OX40, one possible reason is that OX40 signals may collaborate with other factors to regulate Tfh differentiation, such as the microenvironment, the APCs, and Th cells themselves.

3.7. OX40 mainly antagonizes Treg-mediated suppression

To prevent autoreactivity, immune responses need to be properly controlled, which is partially dependent on the suppressive ability of Treg cells[75]. Treg cells are characterized by the expression of FOXP3, CD4, and CD25, and they are composed of two major subtypes, natural thymic derived Tregs (nTregs) and inducible Tregs (iTregs) post thymic maturation. The most commonly associated cytokines with Tregs are transforming growth factor-β (TGF-β) and IL-10. Generally, Treg cells are immunosuppressive and responsible for maintaining tolerance to self-antigens. Treg cells are also known to act as immunoregulators in many inflammatory and autoimmune disease by modulating anti-inflammatory cytokines secretion[75].

In physiological immune responses, OX40 supports the survival and proliferation of activated CD4+ T cells. It has been proposed that the potent costimulatory effect of OX40 is due to the blocking of the inhibitory activity of Tregs, although it could also be explained by the stimulatory effect on effector T cells in enhancing their resistant to suppression. Over last half-decade evidence has accumulated that OX40 signals are critical for controlling the function of FOXP3+ Treg T cells. OX40 may act as negative regulator of Treg function, which indirectly boosts effector CD4+ T cells by relieving them from Treg cell-mediated suppression. OX40 signals can counteract Treg-mediated suppression when they are delivered directly to antigen-engaged naive T cells *in vivo*[39].

OX40 is constitutively expressed in mouse Treg cells, and rapidly induced in human Treg cells. T cells were converted into FOXP3+ cells to a greater extent when OX40 was not expressed and, accordingly, an agonist antibody to OX40 also markedly suppressed CD4+ T cell differentiation into FOXP3+ iTregs even in presence of TGF-β[42]. In addition to antagonizing inducible Treg generation, some studies revealed that OX40 also blocks natural Treg activity in certain situations[40]. Voo et al.[41] developed an anti-human OX40 agonistic mouse mAb that could promote effector CD4+ and CD8+ T cell proliferation while blocking natural Treg-suppressive function. However, Vu et al.[44] pointed out that OX40 stimulation did not significantly affect naturally arising CD4+FOXP3+ Tregs, but strongly antagonized the induction of new inducible FOXP3+ Tregs from T effector cells.

Ligation of OX40 on the FOXP3+ Treg cells abrogated their ability to suppress T effector cell proliferation, IFN-γ production, and T effector cell-mediated allograft rejection. Triggering the OX40−OX40L interaction has also been proven to shut down the generation of IL-10-producing type 1 Tregs (Tr1)[43], turn off FOXP3+ Treg proliferation[44], and inhibit TGF-β- and antigen-driven conversion of naive CD4+ T cells into CD25+FOXP3+ T

cells[42]. Valzasina et al.[40] found that agonist anti-OX40 mAb could abrogate Treg-mediated suppression of T-cell proliferation, and restored *IL-2* gene transcription, cytokine production, and effector T cell proliferation in a model of graft *versus* host disease *in vivo*. Many studies have tried to identify the mechanisms by which OX40 costimulation suppresses Foxp3 expression and iTreg induction[8]. Possible pathways were through phosphorylation and nuclear exclusion of FOXO1/3[8]. Moreover, OX40 can upregulate basic leucine zipper ATF-like transcription factor 3 (BATF3) in activated CD4^{+} T cells, which produced a closed chromatin configuration to repress Foxp3 expression in a SIRT1/7-dependent manner[18].

Although the OX40−OX40L interaction potently counteracts Treg-mediated suppression in multiple experimental settings, paradoxically, some reports claimed that the OX40−OX40L axis may play a necessary role in the homeostasis of Treg cells, and OX40 agonists can enhance Treg proliferation and potentiate Treg cell suppressive activity. mRNA profiling experiments revealed that compared to non-regulatory T cells, CD25^{+}CD4^{+} Treg cells had high expression of OX40[76]. They also reported a reduced number of Treg cells in the spleen of young OX40-deficient mice while there was an elevated number in the thymus of mice that overexpress OX40L, suggesting that abnormal OX40−OX40L signals would disturb Treg cells development[76]. Griseri et al.[45] reported that OX40 was required by Treg cells for their accumulation in the colon under steady-state conditions in an adoptive T cell transfer model of colitis. While under inflammatory conditions, OX40-deficient Treg cells showed reduced accumulation in the colon and peripheral lymphoid organs, resulting in their inability to keep pace with the effector response, indicating that OX40 delivers an important survival signal to Treg cells[45].

Consistent with those results, Ikuo Takeda et al. found that Treg cells from OX40L-over-expressed mice showed more effective suppression than those from wild-type mice *in vitro*, suggesting that the suppressive function of Treg cells could be potentiated by OX40−OX40L interactions[39]. Valzasina et al.[40] did not observe differences in Treg cell number in the OX40-deficient mice described by Takeda et al.[39] They speculated that it was possibly obscured by the genetic background difference or environmental factors. OX40-deficient mice only showed a reduced number of Treg cells in their early life, implying that OX40 is either augmented or unnecessary in the homeostasis of Treg cells[39,77].

3.8. OX40 promotes the generation and maintenance of memory CD8^{+} T cells

CD8^{+} T cells play an important role in eradication of viruses, intracellular parasites, and suppression of tumors. To differentiate into effector cytotoxic T cells (CTL), CD8^{+} T cells require both antigenic stimulation and costimulatory signals. In addition to CD28, TNF receptor superfamily molecules also are involved in mediating costimulatory signals for proliferation and differentiation of CD8^{+} T cells[78].

OX40−OX40L signals were initially found to be dispensable in CD8^{+} T cell functions in a viral-infection murine model[49]. Although naive CD8^{+} T cells can initially differentiate into CTL independently of OX40−OX40L interactions *in vivo*, OX40-deficient CD8^{+} cells were not maintained at high numbers after they initially expanded. There was a significantly reduced accumulation of CTLs at the peak of primary responses, thus emphasizing the critical involvement of OX40[47]. Additionally, OX40-deficient CD8^{+} T cell expansion was impaired in response to antigen in several models, including contact hypersensitivity reactions and allograft rejection[26].

Considering that OX40L-deficient mice or agonist anti-OX40 mAb can also influence the function of non-CD8^{+} T cells, it was doubted whether OX40 signals could influence CD8^{+} T cell functions directly. Lena Serghides et al. investigated the co-stimulatory effects of OX40L with B7.1 and 4-1BBL[50]. OX40L co-stimulation increased numbers of antiviral memory CD8^{+} T cells when cultured with total T cells. However, when cultured with purified CD8^{+} T cells, OX40L barely activated memory CD8^{+} T cells, and synergistic co-stimulatory effects were detected when OX40L was combined with B7.1 or 4-1BBL. Thus, OX40L was speculated to promote memory CD8^{+} T cell responses in an indirect way, likely in co-operation with B7.1 or 4-1BBL[50].

Nevertheless, Song et al.[48] reported that anti-OX40 agonist treatment was essential for the expansion and anti-tumor activity of CD8+ T cell. As OX40−OX40L signals could not only enhance the expansion of tumor-specific CD4^{+} and CD8^{+} cells, but also promote the cooperation between CD8^{+} T cells and non-CD8^{+} T cells, mainly the CD4^{+} T cell, to enhance tumor rejection following adoptive transfer of the *in vitro*-primed CD8^{+} cells into tumor-bearing hosts. Accordingly, the tumor suppression effects of OX40 agonist antibody were greatly dependent on the participation of both CD4^{+} and CD8^{+} T cells during tumor-specific priming[10,79]. Using human CD8^{+} T cells, Fujita et al.[51] demonstrated that OX40 signals directly augmented proliferation of human CD8^{+} T cells, as well as cytokine secretion, such as INF-γ. Using primed tumor-specific or generated effector CD8^{+} T cells from OX40-deficient mice, Song et al.[46] demonstrated that the OX40−OX40L interaction was indispensable in both the CD8^{+} priming process and the recall response when primed CD8^{+} T cells encounter tumor-expressed antigen, which contributed to the survival and expansion of CD8^{+} T cells and the recall response of CD8^{+} memory T cells *in vivo*.

4. Therapeutic benefit of OX40 in autoimmunity disease

Autoimmune disease is typically characterized by autoantibodies reactive against self-antigens due to the failure of the immune system to maintain self-tolerance against autoantigen. There are some well-known autoimmune diseases with high morbidity due to poorly understood pathogenesis. These include experimental autoimmune encephalomyelitis (EAE), systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA), colitis, autoimmune experimental uveitis (AEU), type 1 diabetes mellitus, and multiple sclerosis (MS).

Due to the pivotal role of T cells in controlling the immune response and costimulatory effect of OX40−OX40L interaction with T cells, targeting the OX40−OX40L interaction might ameliorate the autoantigen-specific T-cell responses. Therefore, experiments have been conducted to verify whether targeting the OX40−OX40L interaction by blockade of OX40 or OX40L had the therapeutic benefit for autoimmune diseases, including EAE, SLE, RA, colitis, AEU, type 1 diabetes mellitus, MS, graft-*versus*-host disease (GVHD) and inflammatory bowel disease (IBD). Table 3 summarizes the effect of the OX40−OX40L interaction on several diseases. Overall, it shows that OX40 is upregulated at the sites of autoimmunity and correlates with disease severity. More specifically, there is no significant increased expression of OX40 in peripheral blood in localized diseases such as colitis, while a typical increase of peripheral OX40^{+}CD4^{+} T cells can be detected

Table 3 Therapeutic effect of OX40−OX40L in autoimmunity disease.

Autoimmunity	Model	Intervention and effect	Ref.
Experimental autoimmune encephalomyelitis (EAE)	Activated induced EAE murine model	A neutralizing OX40L mAb (RM134L) reduced mononuclear cell infiltration into the spinal cord	80
	Adoptive transfer EAE murine model	OX40 immunotoxin injection resulted in amelioration of EAE	81
	EAE murine model	Soluble OX-40R treatment ameliorated both actively induced and adoptively transferred EAE disease	82
	OX40L-deficient mice (OX40L$^{-/-}$)	Abortive T cell priming greatly reduced EAE clinical manifestations	60
	OX40L transgenic mice (OX40L-Tg)	OX40L-Tg mice developed a greater severity of EAE despite a delayed onset	60
	EAE Lewis rats	OX40 antibody enhances the autoantigen specific Vβ8.2$^+$ T cells	83
Systemic lupus erythematosus (SLE)	SLE patients	An increased frequency of peripheral CD4$^+$OX40$^+$ T cells in SLE patients	84
	SLE patients	OX40L−OX40 axis contributes to the aberrant Tfh cell response	33
	SLE patients	The serum level of OX40L or OX40 expression on CD4$^+$ T cells may act as markers of SLE	85
	SLE patients	Renal biopsies of SLE patients showed infiltration of OX40$^+$ T cells	86
	SLE patients	OX40 mAb could inhibit expression of perforin and hemolysis activities to ameliorate SLE	87
	SLE patients	Whole genome association (WGA) studies of OX40L	88
Rheumatoid arthritis (RA)	Collagen-induced arthritis (CIA) murine model	OX40 blockade reduced the proinflammatory responses and ameliorated RA development	55
	CIA murine model	Anti-OX40L mAb ameliorated RA disease and suppressed IFN-γ and anti-CII IgG2a production	61
	CIA murine model and HTLV-I Tg mouse model.	Low OX40 expression on T cells inIL-1-deficient mice resulted in impaired suppression of RA	89
	RA patients	OX40 plasma levels were higher than control group treatment of methotrexate and adalimumab	90
	CIA murine model	Antigen inhibition of CIA is associated with induction of OX40 on T cells	91
	IL-1R deficient (IL-1Ra$^{-/-}$) mice	Anti-OX40 Ab accelerated the production of IL-17	69
Colitis	Dextran sulfate sodium induced murine model	OX40-IgG treatment resulted in a dose-dependent reduction of intestinal inflammation	92
	T cell transfer model of colitis	OX40 regulated the homeostasis of intestinal FOXP3$^+$ Treg cells and suppressed colitis	45
	Crohn's disease murine model	Combination of anti-TNF-α and anti-OX40L mAbs improved the therapeutic effect of chronic colitis	93
	Biopsy specimens of patients	Positive of OX40 staining in all biopsies of patients with ulcerative colitis	94
	T cell-restored SCID mice	Anti-OX40L mAb completely blocked development of colitis	62
Autoimmune experimental uveitis (AEU)	Ovalbumin-induced AEU	Anti-OX40L antibody substantially inhibited the antigen-specificocular inflammation	31
	IRBP161-180 induced AEU	OX40-activating Ab prolonged and exacerbated the disease course of EAU	95
Type 1 diabetes mellitus	Type 1 diabetes patients	Co-expression of CD25 and OX40 receptors delineates autoreactive T-cells in type 1 diabetes	96
	Non-obese diabetic (NOD) mice	Inhibiting OX40−OX40L interactions at a late stage prevented diabetes development	97
	NOD mice	An OX40 agonist antibody (OX86) treatment reduced type 1 diabetes (T1D) incidence	98
Multiple sclerosis (MS)	Systemic sclerosis (SSc) patients	Serum soluble OX40 levels correlate with the early-onset of SSc disease	99
	MS patients	CD4$^+$ OX40$^+$ T cells were not increased in clinically active MS	100
	MS patients	OX40 is upregulated in the CNS of MS patients	101
	MS patients	*TNFSF4* polymorphisms can affect systemic sclerosis genetic susceptibility	102
	SSc patients	Polymorphisms in the *TNFSF4* gene region are associated with susceptibility to SSc	103

in more systemic conditions like SLE. OX40−OX40L blockade *in vivo* generally ameliorates autoimmunity, mainly by preventing migration, moderating T cell polarization, altering cytokine production, and preventing proliferation of active CD4$^+$ T cells[8]. Below we discuss the role of the OX40−OX40L interaction on EAE, SLE, and RA.

4.1. Blocking OX40−OX40L engagement ameliorates EAE

EAE is a well-established animal model for the human demyelinating disease of the central nervous system (CNS), multiple sclerosis (MS)[104]. MS is an autoimmune disorder characterized by variable pathological manifestations, including destruction of the myelin sheath, demyelinating plaques and axonal damage. There are several well studied EAE models and they are induced by different myelin-derived antigens: proteolipid protein (PLP)-induced EAE, myelin basic protein (MBP)-induced EAE, myelin oligodendrocyte glycoprotein (MOG)-induced EAE, and adoptively transferred EAE model. They play a crucial role in testing novel treatments for modulating the course of MS[105].

The possible therapeutic benefit of OX40 to autoimmune disease was first found in an EAE rat model, as both OX40 and OX40L are expressed on the surface of CNS cells in EAE mice; autoantigen-specific CD4+ T cells from CNS had a significantly higher level of OX40 than those from peripheral blood. OX40 or OX40L deficiency can potentially reduce the ongoing signs of EAE. OX40L-deficient mice showed decreased clinical manifestations of actively induced EAE and decreased production of proinflammatory cytokines IFN-γ, IL-2, and IL-6, which was mainly due to impaired APC capacity and abortive T cell priming[60]. In contrast, transgenic mice expressing OX40L developed a greater severity of EAE despite a delayed onset, with a markedly enhanced T cell response to protein antigen[60].

To further verify the therapeutic effect of the OX40−OX40L interaction blockade on the development of EAE, Nohara et al.[80] used PLP 139−151 peptide to build the active EAE model and an adoptively transferred EAE model, and treated those mice with various doses of neutralizing OX40L mAb (RM134L). RM134L treatment ameliorated the clinical signs of EAE in a dose-dependent manner in both actively induced EAE and the adoptively transferred EAE model, thus indicating a substantial contribution of OX40 L[87]. In this study RM134L treatment greatly inhibited the accumulation of OX40-expressing CD4+ T cells and the migration of pathogenic T cells into the CNS, but had little effect on the proliferation of IFN-γ producing Th1 cells in DLN[80].

In another study Weinberg et al.[82] also reported an improvement in clinical signs of EAE by blocking OX40−OX40L engagement. The blockade of the OX40−OX40L interaction was achieved by administration of a soluble human OX40 immunoglobulin fusion protein with the ability to bind to murine OX-40L. OX40-blockade in mice inhibited T cell proliferation by 50%−70%, resulting in a marked reduction in the severity of EAE and quicker recovery from EAE disease. However, the frequency and severity of relapses was not improved, suggesting that the effect may be short-lived[82]. As adoptive transfer of wild-type donor T cells to OX40L-deficient mice efficiently transferred EAE disease, while OX40L-deficient donor T cells failed to transfer EAE disease to wild-type recipient mice, this suggested that OX40−OX40L interaction may be involved in the T cell priming process in the occurrence of EAE disease[60].

4.2. OX40 expression may correlate with SLE severity

SLE is a chronic multisystem complex autoimmune disorder. It is characterized by pathogenic autoantibodies in association with the dysfunction of the cellular immune, humoral immune and complement system[106]. The etiological cause of SLE is still incompletely understood, while the pathogenesis is mainly owing to the formation of immune complexes and the loss of immune tolerance. The defective B cell suppression and excess T cell helper cells are clearly involved in these processes through complex interactions among T cells, B cells, and APCs[106].

To seek possible SLE biomarkers, Patschan et al.[84] analyzed the expression of CD70, CD80, CD86, CD137, CD137L, OX40, CD152, CD154 and ICOS of CD4+ T cell populations in the peripheral blood of SLE patients. Among all these co-stimulatory markers, CD80, CD86, and OX40 showed obviously elevated levels compared to a healthy group, which also correlated with the systemic lupus erythematosus disease activity index (SLEDAI)[84]. This was consistent with the results reported by Farres et al.[85] They investigated the percentage of CD4+ T cell expressing OX40 and serum OX40L levels in SLE patients with lupus nephritis (LN). They found that there was a significantly elevated percentage of OX40 expression CD4+ T cells in SLE patients. The significantly increased percentage of OX40 expression CD4+ T cells can also be found in SLE patients with LN. The same increase also can be noted in the serum OX40L levels. They suggested that OX40 expression CD4+ T cells and serum OX40L levels are an indicator of disease activity in SLE[85].

Some studies also investigated the pathogenesis of SLE through the identification of genetic components[88]. Multiple predisposing genetic factors are implicated in SLE pathogenesis, including OX40L which showed genetic linkage with SLE and also acts as components of B cell pathways[107]. Cunninghame Graham et al.[107] pointed out OX40L as a lupus susceptibility gene, as they showed the upstream region of OX40L contained a single risk haplotype for SLE. An over-expressed haplotype is correlated with increased expression of OX40L, which may influence the functional consequences of T cell activation via OX40 and destabilize peripheral tolerance[107]. In another study it was found that the OX40−OX40L interaction is bidirectional. The reverse signaling pathway via OX40L may augment the B cell differentiation and hyperactivity to aggravate SLE pathology, which is in keeping with the hallmark of SLE disease, autoreactive antibody[88].

The frequency of blood active-phenotype Tfh cells is increased in active SLE patients. Jacquemin et al.[33] reported that OX40L could promote SLE disease through promoting a Tfh cell response. As Tfh cells were specialized for provision of help to B cells, there may be certain interactions between autoantibody-producing B cells and pathogenic Tfh cells through the OX40−OX40L axis. The OX40L signals in SLE patients can induce the proliferation of Tfh cells from Th cells and assist them to become functional B cell helpers[33]. Although the role of the OX40−OX40L axis in SLE disease is widely investigated, the mechanisms of the OX40−OX40L axis involved in SLE patients still needs further study. In the future, OX40−OX40L blockade may developed into a possible therapeutic approach to ameliorate SLE disease and alleviate autoantibody production, like the well-characterized T cell activation pathway, CD28−CD80/CD86 interaction.

4.3. OX40 blockade may ameliorate RA development

RA is a symmetric poly-articular autoimmune disorder. It is characterized by joint pain and progressive damage to the synovial tissue, especially in the hands, feet, and wrist, and may eventually result in joint destruction and bone erosion[89]. Although the etiological cause of RA is not totally understood, T cell-mediated autoimmune responses are definitely involved in the pathogenesis of RA. Administration of either anti-T cell antibodies or

immunosuppressive drug targeting to T cells was effective in suppression of the progression of established RA murine disease. Saijo et al.[89] found that either IL-1α- or IL-1β-deficient mice correlated with lower expression of OX40 and CD40L, and showed reduced incidence of RA, suggesting that those factors collectively contributed to the lower responsiveness of T cells. The existence of OX40L can be detected on activated synovial dendritic cells or macrophages in synovial tissue and inflamed joints from RA patients. Additionally, the expression of OX40 on infiltrating activated T cells could be enhanced by locally produced pro-inflammatory cytokines in RA synovium, such as TNF-α or IL-1β. Therefore, it is likely that OX40−OX40L signaling acts through cellular contact-dependent interaction between infiltrating activated T cells and their nearby APC cells in the RA synovium under the dysregulated pro-inflammatory cytokine environment in the rheumatoid synovium[89].

Yoshioka et al.[61] investigated the contribution of the OX40−OX40L interaction on the pathogenesis of RA by administration of a neutralizing anti-OX40L mAb to a type II collagen (CII)-induced arthritis (CIA) murine model. Interestingly, they found that administration of anti-OX40L mAb had the potential to ameliorate the severity of RA through suppression of the production of IFN-γ and anti-CII IgG2a; however, it did not suppress the activation of CII-reactive T cells. They speculated that the OX40−OX40L interaction may exert its contribution in subsequent steps after CII-reactive T cell activation[61]. That is, OX40 may act as an accelerator, but not an initiator, in the pathological development of RA.

Jiang et al.[55] reported the pathogenic role of CD4$^+$CD28$^-$OX40$^+$ T cells in RA pathogenesis. Abnormal accumulation of CD4$^+$CD28$^-$ OX40$^+$ T cells was found in both RA patients and CIA mice. Those CD4$^+$CD28$^-$ OX40$^+$ T cells showed high production of pro-inflammatory cytokines, including IFN-γ, TNF-α and IL-4, which may be correlated with clinical pathological features of RA patients and CIA mice. Blockade of the OX40−OX40L interaction resulted in inhibition of pro-inflammatory cytokine production in vitro, and also ameliorated arthritis progression in vivo[55]. A similar therapeutic benefit of OX40−OX40L blockade was found in another study, which resulted from a significant reduction in IL-17 from Th17 cells in peripheral synovial joints[27]. This evidence suggested that the regulatory role of OX40−OX40L signaling in autoimmune arthritis, which may represent an effective approach for immunomodulatory therapy.

5. Rationale for targeting OX40−OX40L interactions in cancer immunotherapy

Cancer immunotherapy is an emerging cancer treatment that utilizes the immune system of the cancer host to destroy cancer cells. Many studies have demonstrated the rationale of targeting OX40−OX40L interactions in cancer immunotherapy, as ligation of OX40 signals can promote conventional (non-regulatory) CD4$^+$ and CD8$^+$ T cell survival, sustain anti-apoptotic protein expression like BCL-XL, BCL-2 and survivn, enhance cytokine production like IL-2, IL-4, IL-5 and IFN-γ, boost effector tumor-specific T cell immune responses, and augment tumor-specific memory T cell generation following antigen challenge, as summarized in Fig. 3[4,10,11]. Therefore, OX40−OX40L interaction possesses potent immunological effects and fuels interest as a target to improve tumor immunotherapy, as summarized in Table 4.

However, some studies have revealed that the antitumor activity of OX40 agonists may be altered according to the immunogenicity of different tumor types. More specifically, OX40 agonists are likely to enhance antitumor immunity against immunogenic tumors such as MCA303 sarcoma tumors, CT26 colon carcinoma tumors, or SM1 breast cancer[10]. However, they may have less immunological protection and be insufficient to induce tumor eradication with poorly immunogenic tumors, such as B16/F10 melanoma tumors[10]. Since poorly immunogenic tumors may not provide the priming required by OX40−XO40L signals. Novel combining strategies that employ OX40 agonists in conjunction with other therapeutic treatments have been widely investigated, including combining OX40 agonists with the immunotherapy checkpoint inhibitors PD-1 or CTLA-4, immunotherapy costimulatory molecules like 4-1BB, certain cytokines like IL-2 or IL-12, chemotherapy, or radiotherapy, as summarized in Table 4.

5.1. OX40 agonists and combination immunotherapy

The well-known "checkpoint inhibitor" programmed cell death protein 1 (PD-1) and CTL-associated antigen 4 (CTLA-4) can curtail immune responses and attenuate anti-tumor immunity. Administration of antagonist monoclonal antibodies (mAbs) targeting PD-1 or CTLA-4 show substantial promise to boost T cells anti-tumor immunity. Pembrolizumab is the first anti-PD-1 mAb approved by the U. S. Food and Drug Administration (FDA) to treat advanced metastatic melanomas, neck squamous cell carcinoma and metastatic non-small cell lung cancer. Two another anti-PD-1 mAbs (nivolumab and cemiplimab), and three anti-PD-L1 mAbs (atezolizumab, avelumab, and durvalumab) are also approved by the FDA for cancer treatment. On the other hand, ipilimumab is the first anti-CTLA-4 mAb for the treatment of melanoma and renal cell carcinoma. Indeed, blockade of "checkpoint inhibitors" PD-1 or CTLA-4 has demonstrated improved survival in both preclinical and clinical trials. However, clinical results also revealed that blockade PD-1 or CTLA-4 alone was insufficient to induce tumor eradication and only a subset of patients showed objective clinical responses. In order to overcome the therapeutic limitations of checkpoint blockade monotherapy, many investigators studied the combination immunotherapy. OX40 plays a co-stimulatory role in T cell activation, and if combined with PD-1 or CTLA-4 blockade it is supposed to further overcome tumor immune tolerance and ultimately generate a robust therapeutic immune response, as summarized in Table 4.

Indeed, combination of OX40 agonists with PD-1/PD-L1 blockade may have complementary immunological effects. As OX40−OX40L interaction can increase CD4$^+$ and CD8$^+$ T cells populations which express the PD-1 receptor, and enhance production of cytokines like IFN-γ that can up-regulate PD-L1 expression within the tumor microenvironment. Guo et al.[108] demonstrated that a combination of PD-1 blockade and OX40 stimulation synergistically protects against poorly immunogenic ovarian tumor growth, whereas neither PD-1 blockade nor OX40 stimulation alone had an effect on tumor growth and ascites formation in an ID8 murine ovarian cancer model. However, combined anti-PD-1/anti-OX40 mAb treatment significantly increased the overall survival of mice and achieved 60% tumor-free mice. The combined treatment showed elevated ratios of both effector CD4$^+$ and CD8$^+$ T cells to Treg cells and myeloid-derived suppressor cells (MDSC), and elicitation of an antigen-specific CTL
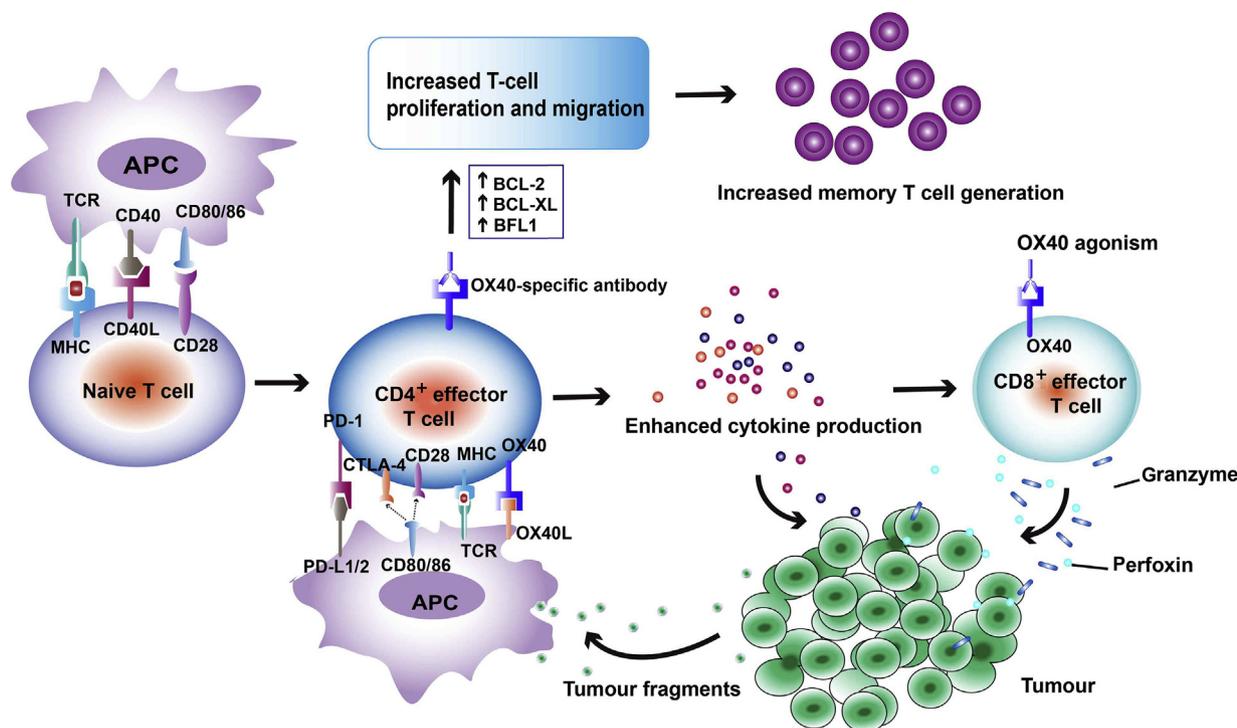
Figure 3 Schematic diagram of the rationale for targeting OX40−OX40L interactions in cancer immunotherapy. As ligation of OX40 signals can promote conventional (non-regulatory) CD4$^+$ and CD8$^+$ T cell survival, sustain anti-apoptotic protein expression (BCL-XL, BCL-2 and BFL1), enhance cytokine production of IL-2, IL-4, IL-5 and IFN-γ, boost effector tumor-specific T cell immune responses, and augment tumor-specific memory T cell generation following antigen challenge.

response owing to the formation of a local immunostimulatory microenvironment[108].

In another study, Messenheimer et al.[110] reported that timing and sequencing are crucial to optimize the therapeutic effect of PD-1 blockade combined with anti-OX40. Anti-OX40 treatment can result in a delayed tumor progression in a preclinical oncogene-driven mammary cancer model which is refractory to PD-1 blockade. While a significant reduction in survival and tumor control was obtained with concurrent combination of anti-OX40 and anti-PD-1 blockade, suggesting that PD-1 blockade may provide an adverse effect on anti-OX40-induced therapy. Otherwise, augmented therapeutic efficacy was achieved by sequential and delaying anti-PD-1 administration, as it is more effective to give anti-PD-1 antibody after the initial T-cell boost generated by anti-OX40[110]. Although much evidence has shown that OX40 agonists are likely to synergize with PD-1 blockade, a better understanding of the immunological interplay between them is still needed.

CTLA-4 serves as a "checkpoint inhibitor" through competitively binding to B7-1 or B7-2 to downregulate the CD28 costimulatory pathway of T cells[11,122]. The therapeutic anti-tumor benefit of anti-CTLA-4 blockade is limited, especially with poorly immunogenic tumors. As OX40 and CTLA-4 are both induced to express on CD4$^+$ and CD8$^+$ T cells after TCR stimulation, many studies have been conducted to determine if combined anti-OX40 stimulation and anti-CTLA-4 blockade would augment therapeutic responses and generate broader tumor immunotherapy. Indeed, compared to monotherapy with anti-OX40 or anti-CTLA-4, the combined anti-OX40/anti-CTLA-4

immunotherapy improved tumor regression and survival of tumor-bearing hosts in some tumor models, such as the moderately immunogenic MCA-205 sarcoma tumors or the poorly immunogenic TRAMP-C1 prostate tumors[11,113]. Specifically, the therapeutic efficacy of dual anti-OX40/anti-CTLA-4 therapy is associated with decreased inhibitory effects of Treg cells and increased number and function of effector of CD4$^+$ and CD8$^+$ T cells. No therapeutic efficacy of combined therapy was obtained with pretreatment of CD4 and/or CD8 T cell depletion, suggesting a T cell-dependent mechanism[113]. Dual anti-OX40/anti-CTLA-4 therapy also resulted in enhanced cytokine production, including both Th1 (IL-2 and IFN-γ) and Th2 (IL-4, IL-5, and IL-13) cytokines, which may help to drive potent polyclonal effector T cell responses[113]. Despite the enhanced anti-tumor ability that has been achieved with combination immunotherapy, tumor immunotherapy treatment still remains challenging because of tumor tolerance and poor immunogenicity[11,112].

5.2. Combination treatment of anti-OX40 with cytokines

The combination of cytokines with anti-OX40 immunotherapy is a popular strategy to enhance the immunogenicity of tumor cells and strengthen the antitumor immune response of the host. Many cytokines, such as IL-2, IL-12, IL-4, IL-15, TNF-α, or IFN-γ, have shown potent immunomodulatory and antitumor effects in various preclinical studies[136,137]. Among them, two cytokines are of particular interest, IL-2 and IL-12. IL-2 has been approved by the FDA to treat metastatic melanoma and metastatic renal cell carcinoma. However, despite its great antitumor potency, the

Table 4 Summary of cancer immunotherapy targeting OX40–OX40L interaction alone or in combination.

Combination type	OX40 therapy	Combo	Cancer model	Effect	Ref.
Immuno- therapy	OX86	Anti-PD-1 (RMT3-2)	ID8 ovarian cancer murine model	Higher ratios of both effector CD4^{+} and CD8^{+} cells to Treg; enhanced cytolytic activity	108
	OX86	Anti-PD-1; HPV16-E7 peptide vaccine	TC-1 syngeneic mouse model	Addition of anti-PD-1 negated effect of anti-OX40/vaccine on tumor-growth inhibition	109
	OX86	Anti-PD-1 (clone G4)	MMTV PyMT mammary cancer	Anti-PD-1 significantly attenuated the therapeutic effect of anti-OX40	110
	OX86	Anti-PD-L1, STING agonist	HER-2[+] breast tumors	Primed immunity and reduced tumor growth	111
	OX86	Anti-CTLA-4 (clone 9D9)	Carcinoma model	Enhanced T cell response, expansion, effector function, and memory of CD8	112
	OX86	CTLA-4 mAb (clone 9D9)	TRAMP-C1 prostate	11/15 of tumor bearing mice rejected their tumors	113
	OX86	CTLA-4 mAb (clone 9D9)	MCA-205 sarcoma tumors	10/14 of tumor bearing mice rejected their tumors	113
	OX86	Anti-CD40 and anti-CD137 mAb	4T1 mammary carcinoma	Inhibited tumor growth when compared with anti-OX40 therapy alone	114
	OX86	Anti-CD137 and anti-B7-H1	Hepatocellular carcinoma (HCC)	Extension of survival and dense T cell infiltrates	115
	OX86	Anti-4-1BB mAb (3H3)	MethA tumor murine model	Co-stimulation mediated CD8 T cell dependent rejection	116
	OX86	Anti-4-1BB	Her-2/neu mice	Stimulating and enhancing both CD4^{+} and CD8^{+} T cells	117
Radio-therapy (RT)	OX86	Three times 20Gy SBRT	Lewis lung carcinoma (LLC)	Significant extended survival; long-term tumor free survivors; monotherapy ineffective	118
	OX86	A single dose of 20 Gy	LLC-OVA bearing mice	Enhanced therapeutic antitumor immunity; durable immunological memory	119
	OX86	A single dose of 20 Gy	Murine colorectal carcinoma	One day following radiation is optimal to administration of anti-OX40 mAb	120
	OX86	Total dose of 15, 24 or 36 Gy	Anti-PD1-resistant lung tumor model	Inhibited local and systemic antitumor growth; and improved survival rates	121
Chemo-therapy	OX86	A dose of 250 mg/ kg CTX	B16 melanoma mice model	Significant extended survival; enhanced antitumor immunity	122
	OX40 agonist	Radiation and CTX	Patients with prostatecancer	No effect on the degree of proliferation of peripheral blood lymphocytes	123
Cytokine	OX86	IL-2c	MCA-205 sarcoma mice model	Boosted tumor regression; enhanced long-term survival	124
	OX86	IL-2c	TRAMP-C1 prostate tumor mice model	Increased the survival of mice	124
	OX86	IL-12	Pulmonary metastases mice model (MCA205)	A significant reduction in pulmonary metastases; monotherapy ineffective	125
	OX86	IL-12	TRAMP-C1 prostate tumor mice model	A synergistic therapeutic effect; the enhanced anti-OX40-mediated tumor therapy by IL-12	125
Vaccine	OX86	Anti-CTLA-4 andHER2 vaccination	Mammary carcinoma model	Extensive tumor destruction; restricted Th2-cytokine production by CD4 cells; enhanced IFN-γ production	112
	OX86	Anti-CTLA-4 andHER2 vaccination	Prostate adenocarcinoma spontaneous model	Reversed anergy; enhanced the expansion and function of CD8 T cells	112
	OX86	GM-CSF expressing vaccine	FVB MMTV-neu (neu-N) mice	Breaking established CD8^{+} T cell tolerance, Prolonging the survival and effector function of CD8^{+} T cells	126

Table 4 (*continued*)

Combination type	OX40 therapy	Combo	Cancer model	Effect	Ref.
	OX86	Anti4-1BB; vaccination	Her-2/neu transgenic mice	Retard tumor growth; enhanced the immune responses; improved antitumor effect	117
	OX86	HPV16 E7 peptide vaccine	TC-1 syngeneic mouse model.	Significant ($P < 0.05$) slowdown of tumor progression, and prolonged survival	109
Others	OX86	CD4$^+$ lympho depletion	B16/F10 melanomas	Improved therapeutic efficacy for poorly immunogenic tumors	127
None	AdOX40L-modified DCs mOX-40L		B16/F10 tumors	Suppressed tumor growth; marked cytolytic activity	128
	Anti-OX-40R		B16/F10 melanoma	25% treated mice survived tumor challenge.	10
	mAbOX-86		B16/F10 melanoma	Similar therapeutic efficacy as mOX-40L:Ig	10
	mOX-40L		MCA303 sarcoma murine tumor model	Delayed tumor growth and 60% were free of tumor for >70 days	10
	mOX-40L		SM1 breast cancer line	Enhanced antitumor activity	10
	mOX-40L		CT26 tumor model	Enhanced tumor-free survival; resisted the tumor challenge	10
	hOX-40L		CT26 tumor model	No significant anti-tumor effect	10
	OX86		MCA303 sarcoma tumor model	Improved tumor-free survival and delay tumor growth in younger mice but not older	129
	OX86		CT26 colon carcinoma murine model	Age-dependent efficacy in OX40-mediated tumor-free survival	129
	OX86		CT26 colon carcinoma murine model	Induced tumor rejection in 80% of mice	130
	OX40L-Fc		MCA205 sarcoma murine model	13% of treated animals resulted in the long-term survival	131
	OX40L-Fc		CMS4(H-2d) sarcoma model	>80% of animals rejected their tumors	131
	OX86		CT26 colon carcinoma murine model	Inhibiting Treg-cell suppression and boosting effector T-cell activation	132
	OX86		CT26 tumor model	Facilitated systemic antitumor immunity	133
	mOX40L fusion protein		CT26 tumor model	A reproducible inhibition of tumor growth	134
	mOX40L fusion protein		4T1 tumor model	Dose and route dependent anti-tumor effect	134
	Fc-mOX40L		Colon 26-bearing mice	Produced complete remission	135
	Fc-mOX40L		Renal cell carcinoma-bearing mice	Produced complete remission	135

application of IL-2 in the clinic still faces several unavoidable drawbacks, such as short half-life and high dose-related toxicities[136]. IL-12 can exert potent anticancer effects through both innate and adaptive immune responses. Therefore, IL-12 often synergizes with other therapeutic agents to achieve immunomodulatory benefits.

Redmond et al.[124] investigated the antitumor efficacy of a combination of anti-OX40 therapy with IL-2 therapy. According to their study, the expression of OX40 *via* TCR stimulation also required the involvement of the IL-2 receptor complex. Exogenous rIL-2 stimulation could strongly drive OX40 up-regulation in both murine and human T cells, suggesting the dual stimulation of TCR and IL-2R on OX40 expression, which was dependent upon the activation of JAK3 and the downstream transcription factors STAT3 and STAT5[124]. Using a murine pulmonary metastases (MCA205) tumor model they demonstrated that dual anti-OX40/IL-2c immunotherapy can boost antitumor immunity with the

help of effector CD4 and CD8 T cells. Negligible anti-tumor efficacy was obtained with depletion of either CD4 or CD8 T cell subsets prior to combination treatment. Combination treatment did not affect the function of Treg cells[124]. In another study, Ruby et al.[125] demonstrated the synergistic therapeutic efficacy of a combination of anti-OX40 and IL-12 in several different mouse tumor models, including a MCA205 tumor model, the TRAMP-C1 prostate tumor model, and the CT26 colon carcinoma. IL-12 signaling is quite critical for OX40-enhanced CD4 T cell survival, which is associated with STAT4-specific signaling.

5.3. Combining agonistic anti-OX40 mAb and RT

Radiotherapy (RT) is capable of anti-tumor effects and is widely used to treat loco-regional cancers, due to direct or indirect damage to the DNA of tumor cells[138]. RT is reported to induce bone marrow suppression and diminish T cell immunity, resulting

in immunosuppressive effects to the cancer host. While RT can also be immunostimulatory by releasing tumor-associated antigens (TAAs), or increasing major histocompatibility complex (MHC) antigen expression to enhance tumor immunogenicity, this may depend on the radiation dose, type of tumor or irradiation site[138]. Many studies are underway to evaluate the efficacy of combining RT with agonistic anti-OX40 mAbs. Since RT mainly provides therapeutic applicability to local primary tumors, the agonistic anti-OX40 mAbs may act as an immunological partner to enhance the tumor eradication of RT, and meanwhile reduce its adverse effects.

Yokouchi et al.[119] evaluated the therapeutic effect of an agonistic anti-OX40 mAb with RT to treat lung cancer in mice. Compared to monotherapy, the anti-OX40 mAb in combination with RT treatment showed an improved cure rate and prolonged survival. None of mice were cured by RT alone or anti-OX40 mAb alone, while about 75% of the mice showed complete remission after treatment with the combined therapeutic schedule, suggesting greater tumor elimination efficacy. They also found that irradiation can help augment the proportion of OX40$^+$CD4$^+$ and OX40$^+$CD8$^+$ T cells in draining lymph nodes (DLN), which may contribute to the synergistic effect of therapeutic immunity. Almost 89% of the mice treated with the anti-OX40 mAb in combination with RT remained disease free in a tumor rechallenge experiment, suggesting the durable tumor immunological memory of combination therapy[119].

Consistent with those findings, MJ Gough et al.[118] also confirmed the adjuvant role of anti-OX40 mAb to RT. They adopted the stereotactic body radiation therapy (SBRT) to a Lewis lung carcinoma (3LL)-established mouse cancer model. They found that RT initially decreased CD8 T cells within the radiation field compared to areas outside the radiation field, but later they gradually increased at the tumor site. Three treatments of RT can result in a significant increase in median survival but not durable tumor cures[118]. However, when RT was combined with a single dose of anti-OX40 mAb, not only the median survival was enhanced, tumor-free mice were obtained[118]. Anti-OX40 mAb facilitates the endogenous immune response to tumor cells mainly by increasing tumor antigen-specific CD8 T cell cytotoxic activity, which is definitely a valuable addition to RT.

In another study, Young et al.[120] identified optimal timing of combination immunotherapy with RT in advanced malignancies. They found that the optimal combination timing of anti-OX40 mAb and anti-CTLA4 mAb with RT was different, owing to their different immunotherapy mechanisms. CTLA4 is a checkpoint inhibitor, and pretreatment with anti-CTLA4 mAb showed superior tumor control. As for anti-OX40 agonist mAb, which is co-stimulatory agonist, it was optimally delivered during the tumor antigen-presentation period post-radiation. One day following radiation was suggested, which coincided with the upregulation time of OX40 on T cells[120].

5.4.   OX40 engagement and chemotherapy combination

Cancer chemotherapy routinely used in the clinic often causes lymphopenia and is easily deemed to be immunosuppressive[122]. However, studies also revealed that chemotherapy drugs, if given at proper dose, may facilitate anti-tumor potency, since the tumor cells killed by chemotherapy may be immunologically active and capable of leading to antigen cross-presentation and furtherly promote an adaptive tumor-specific immune response[139]. It is promising to combine RT with agonistic anti-OX40 mAbs treatment, since antigen presentation provided by RT-killed tumor cells can enhance subsequent effector T cell function, with participation of co-stimulatory molecules[140].

Cymerman et al.[122] reported the potent antitumor immunity of a combination of the anti-OX40 agonist mAbs (OX86) with cyclophosphamide (CTX) in well-established, poorly immunogenic B16 melanoma tumors. Approximately 75% of tumor-bearing mice survived to Day 50 after being treated with a single dose of CTX on Day 6 followed by a single dose of OX86 on Day 7, owing to both direct antitumor effects and immune-enhancing properties. Neither CTX nor OX86 alone provided superior tumor protection. They also compared the efficacy of combination of CTX and OX86 with other immunotherapy monoclonal antibodies, such as anti-CD40 (FGK45), and anti-CTLA4 (9D9)[122]. They found that the combination of CTX with OX86 showed superior potency to any of the others in poorly immunogenic tumor models. Since CTX administration can induce partial destruction of the tumor, the antigen necessary to prime a tumor-specific response is available for OX40 to enhance the function of tumor-specific T cells, resulting in increased infiltration of CD8$^+$ T cells and decrease of Treg cells. Clinical trials of combination anti-OX40 with RT or CTX have been conducted in patients with chemotherapy-resistant prostate cancer to evaluate the toxicity on peripheral blood lymphocytes (PBL)[123]. Results showed that the degree of proliferation of PBL was not affected by the administration of anti-OX40 with RT or CTX, suggesting a manageable safety and great tolerability profile for combination therapy.

5.5.   Anti-OX40 cancer therapy in human clinical trials

Currently there are six different OX40-targeted molecules used in clinical trials against cancers (Table 5). The safety and efficacy of MEDI6469 (murine anti-OX40 agonist IgG1 antibody) is widely studied in patients with metastatic colorectal cancer (NCT02559024) and in head neck cancer (NCT02274155). Consistent with mouse tumor models, the combination strategies of agonist anti-OX40 antibody in clinic trails is currently ongoing in different tumors. As shown in Table 4, there are three different kinds of ongoing combination strategies. The first one combined anti-OX40 costimulation with radiation therapy. A phase 2 trial (NCT01862900) is designed to test the safety profile and combination efficacy of MEDI6469 with SBRT in patients with metastatic breast cancer. An amplified and directed immune response is expected. The combination of anti-OX40 with co-stimulatory molecules is also being developed. A phase 1 trial (NCT02315066) is being conducted to assess the safety and maximum tolerated dose of PF-04518600, a fully human anti-OX40 agonist IgG2 antibody, alone or in combination with PF-05082566 (anti-4-1BB agonist antibody) in select advanced or metastatic carcinoma patients, and to select a recommended dose for a phase 2 study. In addition, another combination trials aims to analyze the potential of combining anti-OX40 co-stimulatory with anti-CTLA4 or anti-PDL1 co-inhibitory. For example, a phase 1 trial (NCT02410512) is proceeding to assess the safety and pharmacokinetics of MOXR0916 (humanized agonist anti-OX40 monoclonal antibody) with MPDL3280A (engineered anti-PD-L1 antibody) in a dose-escalation manner in patients with local or metastatic solid tumors. Project (NCT03241173) will explore the safety and efficacy of INCAGN01949 (fully human anti-OX40

Table 5 The ongoing (active, not recruiting) clinical trials with anti-OX40 mAbs (Date: 05/02/2019).

NCT number and title of study	Intervention	Condition
NCT01862900—Stereotactic body radiation and monoclonal antibody to OX40 (MEDI6469) in breast cancer patients with metastatic lesions	MEDI6469 (murine anti-OX40 agonist IgG1)	Metastatic breast cancer; liver and lung metastases;
NCT02274155—Anti-OX40 antibody in head and neck cancer patients	MEDI6469 (murine anti-OX40 agonist IgG1)	Head and neck cancer
NCT02559024—Anti-OX40 antibody (MEDI6469) in patients with metastatic colorectal cancer	MEDI6469 (murine anti-OX40 agonist IgG1)	Colorectal neoplasms
NCT02315066—Study of OX40 agonist PF-04518600 alone and in combination with 4-1BB agonist PF-05082566	PF-04518600 (fully human anti-OX40 agonist IgG2); PF-05082566	Neoplasms
NCT02410512—A study to assess the safety and pharmacokinetics of MOXR0916 and atezolizumab in participants with locally advanced or metastatic solid tumors	MOXR0916 (humanized anti-OX40 agonist IgG1); atezolizumab (anti-PD-L1 mAb)	Neoplasms
NCT02221960—A phase 1 study to evaluate MEDI6383 alone and in combination with MEDI4736 in adult subjects with select advanced solid tumors	MEDI6383 (OX40L fusion protein); MEDI4736 (durvalumab)	Recurrent or metastatic solid tumors
NCT02705482—A study to evaluate MEDI0562 in combination with immune therapeutic agents in adult subjects with advanced solid tumors	MEDI0562 (humanized anti-OX40 agonist IgG4); tremelimumab; durvalumab	Select advanced solid tumors
NCT02923349—A phase 1/2, open-label, dose-escalation, safety study of INCAGN01949 in subjects with advanced or metastatic solid tumors	INCAGN01949 (fully human anti-OX40 agonist antibody, IgG1)	Advanced malignancies metastatic cancer
NCT03241173—A study exploring the safety and efficacy of INCAGN01949 in combination with immune therapies in advanced or metastatic malignancies	INCAGN01949 (fully human anti-OX40 agonist antibody, IgG1); nivolumab; ipilimumab	Advanced malignancies

agonist antibody) in combination with nivolumab or ipilimumab in advanced or metastatic malignancies. There are many ongoing or completed phase 1 or phase 2 clinical trials of anti-OX40, and although phase 3 and 4 clinical trials have not yet been reported, they invariably demonstrate great promise in the development of anti-OX40 immunotherapy.

6. Conclusive remarks and future prospects

Targeting the OX40–OX40L interaction for therapeutic purposes is highly promising, as both agonistic and blockade therapies have shown great efficacy. Results from combination therapies also displayed impressive therapeutic benefits for OX40, especially OX40 agonists in combination cancer immunotherapy, in both preclinical experiments and clinical trials. However, despite these advantages, there are significant limitations to our understanding of the full spectrum of functions of the OX40–OX40L interactions and their related therapies, such as optimal timing of administration, off-target effects, the need of concurrent treatment with other therapy, and the possibility of translating therapy owing to the differences in OX40 expression between mouse and humans. Timing of OX40 agonist or blockade administration may be critical to success since OX40–OX40L interactions are a series of time-dependent checkpoints. Therefore, more investigation is urgently needed for the development of OX40-related drugs and therapies.

Author contributions

Yu Fu was responsible for writing the whole passage. Qing Lin, Zhirong Zhang, Ling Zhang were in charge of checking and revision. All figures and tables in the article were made by Yu Fu.

Conflicts of interest

The authors have no conflicts of interest to declare.

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