Chapter 3
The TNF–TNFR Family of Co-signal Molecules

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Abstract Costimulatory signals initiated by the interaction between the tumor necrosis factor (TNF) ligand and cognate TNF receptor (TNFR) superfamilies promote clonal expansion, differentiation, and survival of antigen-primed CD4$^+$ and CD8$^+$ T cells and have a pivotal role in T-cell-mediated adaptive immunity and diseases. Accumulating evidence in recent years indicates that costimulatory signals via the subset of the TNFR superfamily molecules, OX40 (TNFRSF4), 4-1BB (TNFRSF9), CD27, DR3 (TNFRSF25), CD30 (TNFRSF8), GITR (TNFRSF18), TNFR2 (TNFRSF1B), and HVEM (TNFRSF14), which are constitutive or inducible on T cells, play important roles in protective immunity, inflammatory and autoimmune diseases, and tumor immunotherapy. In this chapter, we will summarize the findings of recent studies on these TNFR family of co-signaling molecules regarding their function at various stages of the T-cell response in the context of infection, inflammation, and cancer. We will also discuss how these TNFR co-signals are critical for immune regulation and have therapeutic potential for the treatment of T-cell-mediated diseases.

Keywords 4-1BB · CD27 · CD30 · DR3 · HVEM · GITR · OX40 · TNFR2 · TNFSF · TNFRSF

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Abbreviations

APC	antigen-presenting cell;
CAR	chimeric antigen receptor
CMV	cytomegalovirus
CTL	cytotoxic T lymphocyte
CTLA-4	cytotoxic T-lymphocyte-associated protein-4
DC	dendritic cell
DcR3	decoy receptor 3
DR3	death receptor 3
EAE	experimental autoimmune encephalomyelitis
EBV	Epstein–Barr virus
GC	germinal center
GITR	glucocorticoid-induced TNFR family-related protein
GVHD	graft-versus-host disease
HPV	human papillomavirus
HVEM	herpesvirus entry mediator
ICOS	inducible T-cell costimulator
LCMV	lymphocytic choriomeningitis virus
LIGHT	homologous to *l*ymphotoxins (LTs), *i*nducible expression, which competes with herpes simplex virus *g*lycoprotein D (HSV gD) for *H*VEM, a receptor expressed on *T* lymphocytes
mAb	monoclonal antibody
MAPK	mitogen-activated protein kinase
NF-κB	nuclear factor-kappa B
PD-1	programmed cell death-1
SLE	systemic lupus erythematosus
TCR	T-cell receptor
Tfh	T follicular helper
Th1	T-helper-1
Th17	T-helper-17
Th2	T-helper-2
Th9	T-helper-9
TL1A	TNF-like ligand 1A
TLR	Toll-like receptor
TNF	tumor necrosis factor
TNFR2	tumor necrosis factor receptor 2
TNFRSF	TNF receptor superfamily
TNFSF	TNF superfamily
TRAF	TNF receptor-associated factor
Treg cells	Foxp3$^+$ CD25$^+$ CD4$^+$ regulatory T cells

3.1   Introduction

The tumor necrosis factor (TNF) superfamily (TNFSF) of cytokines engages specific cognate cell surface receptors, the TNF receptor superfamily (TNFRSF). The TNFSF–TNFRSF interactions activate signaling pathways for cell survival, death, and differentiation that control immune function and disease, and several receptor–ligand pairs within TNF family molecules expressed by immune cells play key roles in T-cell immunity (Locksley et al. 2001; Aggarwal 2003; So et al. 2006; Croft 2009; Watts 2005; Ward-Kavanagh et al. 2016). The subset of TNFRSF molecules, 4-1BB, CD27, CD30, DR3 (death receptor 3), GITR (glucocorticoid-induced TNFR family-related protein), HVEM (herpesvirus entry mediator), OX40, and TNFR2 (tumor necrosis factor receptor 2), expressed by T cells, deliver activating signals, which are largely dependent on antigen recognition and participate in activation, clonal expansion, and differentiation of T cells. TNFRSF and TNFSF molecules that contribute to these additional signals in T cells function as a costimulatory receptor and a costimulatory ligand, respectively (Croft 2009; Watts 2005; So and Croft 2013) (Table 3.1, Fig. 3.1).

A dialogue between a naïve T cell and a dendritic cell (DC) plays an essential role for priming of naïve T cells in secondary lymph organs. The expression levels of 4-1BB, CD27, CD30, DR3, GITR, HVEM, OX40, and TNFR2 on T cells are positively or negatively controlled by activating signals through the T-cell receptor (TCR) and the costimulatory receptor CD28. At the same time, after recognition of microbes, immature DCs are activated via pattern recognition receptors and upregulate not only major histocompatibility complex (MHC) and B7 proteins but also TNFSF molecules including 4-1BBL, CD70, CD30L, TL1A (TNF-like ligand 1A), GITRL, LIGHT, OX40L, and TNF-α. The T cell-DC dialogue via each individual TNFSF–TNFRSF interaction provides additional costimulatory signals to antigen-primed naïve T cells, promotes the differentiation of naïve T cells into effector and memory T-cell populations, and supports the survival of these antigen-primed T cells in the periphery (Fig. 3.2).

In this chapter, we focus on the TNFRSF of costimulatory receptors, 4-1BB, CD27, CD30, DR3, GITR, HVEM, OX40, and TNFR2, and summarize the significance of costimulatory signals mediated by these receptors in terms of protective immunity, autoimmunity and inflammation, and cancer, in which these TNFRSF molecules play critical roles. Administration of agonistic or antagonistic agents (biologics) that can modulate the co-signaling activity of TNFRSF molecules can regulate T-cell functions in vivo, and thus these TNFSF–TNFRSF interactions have been considered as therapeutic targets for intervention in human diseases including autoimmunity and cancer.

3.2 Structure

3.2.1 *Ligand*

The costimulatory ligands of the TNFSF described in this chapter are type II (intracellular N terminus and extracellular C terminus) transmembrane proteins and have a C-terminal TNF homology domain (THD) that is responsible for receptor binding (Bodmer et al. 2002) (Table 3.1, Fig. 3.1). One TNFSF molecule assembles into a trimer on the surface of antigen-presenting cells (APCs), and its cognate monomer TNFRSF molecule on T cells is trimerized through binding to the trimeric configuration of the ligand THDs, resulting in formation of a quaternary organized hexamer complex at the T cell–APC interface (Fig. 3.2). TNFSF molecules can be produced as membrane-bound trimers or be released as soluble trimers from the cell surface after proteolytic processing in the stalk region. The membrane-bound TNFSF molecules that are engaged by their cognate receptors have been defined as inducers of signal transduction. This "reverse" signaling mediated by membrane-bound TNFSF molecules can regulate some cellular functions, such as pro-inflammatory cytokine production and cell maturation (Eissner et al. 2004; Sun and Fink 2007).

3.2.2 *Receptor*

The costimulatory receptors of the TNFRSF described in this chapter are type I (extracellular N terminus and intracellular C terminus) transmembrane proteins and have three to six cysteine-rich domains (CRDs) in their extracellular regions (Bodmer et al. 2002) (Table 3.1, Fig. 3.1). After binding to TNFSF timers via CRDs, TNFRSF molecules cluster on the T-cell membrane, which in turn initiates co-signaling cascades in antigen-recognized T cells. The cytoplasmic amino acid sequences divide TNFRSF molecules into three major groups: (1) TNF receptor-associated factor (TRAF) binding TNFRSF molecule, such as 4-1BB, CD27, CD30, DR3, GITR, HVEM, OX40, and TNFR2; (2) death domain (DD) containing TNFRSF molecule, such as DR3; and (3) decoy TNFRSF molecule lacking a functional cytoplasmic domain, such as DcR3 (decoy receptor 3). Upon binding of cognate TNFSF molecules, the TNFRSF of co-signaling molecules on T cells initiate "forward" signaling mediated by the TRAF family molecules, and this TRAF-mediated signaling contributes to activation of co-signaling cascades in antigen-primed T cells (Figs. 3.1 and 3.2). The extracellular domains of membrane-bound TNFRSF molecules can be cleaved to release soluble TNFRSF forms in some contexts.

**Table 3.1** The receptor and ligand system responsible for TNFR co-signaling

Receptor	Symbol	Synonyms	Ligand	Symbol	Synonyms
OX40	TNFRSF4	CD134, ACT35, TXGP1L	**OX40L**	TNFSF4	CD134L, CD252, gp34, OX-40L, TXGP1
4-1BB	TNFRSF9	CD137, ILA	**4-1BBL**	TNFSF9	CD137L
CD27	CD27	S152, Tp55	**CD70**	CD70	CD27L,CD27LG, TNFSF7
DR3	TNFRSF25	APO-3, DDR3, LARD, TNFRSF12, TR3, TRAMP, WSL-1, WSL-LR	**TL1A**	TNFSF15	MGC129934, MGC129935, TL1, VEGI, VEGI192A
CD30	TNFRSF8	D1S166E, KI-1	**CD30L**	TNFSF8	CD30LG, CD153
GITR	TNFRSF18	AITR, CD357	**GITRL**	TNFSF18	AITRL, TL6
TNFR2	TNFRSF1B	CD120b, p75, TNF-R-II, TNF-R75, TNFBR, TNFR80	**TNF-α**	TNF	DIF, TNFA, TNF-alpha, TNFSF2
			LT-α	LTA	LT, LTα$_3$, TNFB, TNFSF1
HVEM	TNFRSF14	ATAR, CD270, HVEA, LIGHTR, TR2	**LIGHT**	TNFSF14	CD258, HVEM-L, LTg
			LT-α	LTA	LT, LTα$_3$, TNFB, TNFSF1
			BTLA	BTLA	BTLA1, CD272
			CD160	CD160	BY55, NK1, NK28
DcR3	TNFRSF6B	DCR3, M68, TR6	**TL1A**	TNFSF15	MGC129934, MGC129935, TL1, VEGI, VEGI192A
			LIGHT	TNFSF14	CD258, HVEM-L, LTg
			FASL	FASLG	APT1LG1, CD178, TNFSF6
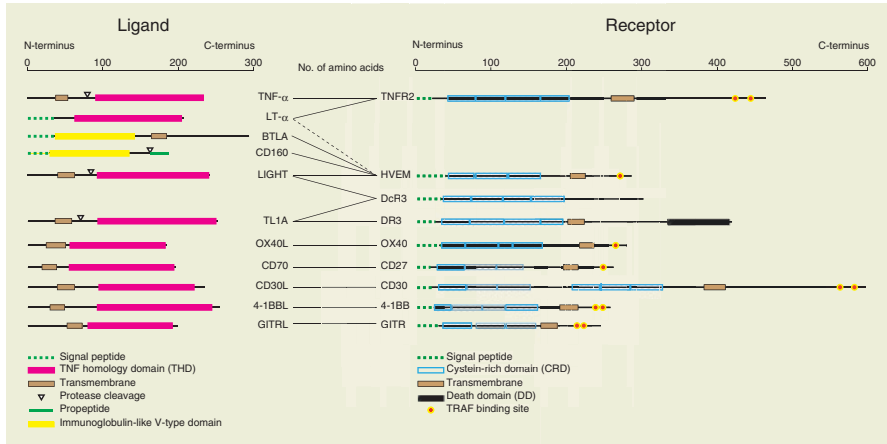
Fig. 3.1 Protein domain organization in the TNF–TNFR family of co-signaling molecules. Data are based on UniProt (www.uniprot.org). TNF-α, P01375; LT-α, P01374; BTLA, Q7Z6A9; CD160, O95971; LIGHT, O43557; TL1A, O95150; OX40L, P23510; CD70, P32970; CD30L, P32971; 4-1BBL, P41273; GITRL, Q9UNG2; TNFR2, P20333; HVEM, Q92956; DcR3, O95407; DR3, Q93038; OX40, P43489; CD27, P26842; CD30, P28908; 4-1BB, Q07011; GITR, Q9Y5U5. LT-α shows low affinity for HVEM (dashed line)

3.2.3 TRAF

After binding to cognate ligands of the TNFSF family molecules, the TNFRSF of co-signaling molecules recruits various intracellular TRAF molecules to their cytoplasmic tails, leading to activation of canonical and noncanonical nuclear factor-kappa B (NF-κB), mitogen-activated protein kinase (MAPK), and serine/threonine-protein kinase Akt (protein kinase B) cascades (Brenner et al. 2015; So and Croft 2013; Hayden and Ghosh 2014; Karin and Gallagher 2009) (Fig. 3.2). Thus, TRAFs mediate the link between receptor-proximal and cytosolic signaling events. Six TRAFs, TRAF1 to TRAF6, which share a conserved C-terminal TRAF-C domain, function both as ubiquitin E3 ligases and as adaptor molecules to potentiate signal transduction. Except for TRAF1, the N-terminal of TRAF contains a really interesting new gene (RING) and zinc-finger domains that are responsible for ubiquitin E3 ligase activity to promote the activation of downstream signaling cascades. The C-terminal of TRAF contains a coiled-coil (TRAF-N) domain and a TRAF-C domain that contributes to intermolecular interactions and sequence-specific interactions with receptor cytoplasmic tails, respectively (Ha et al. 2009; So et al. 2015).
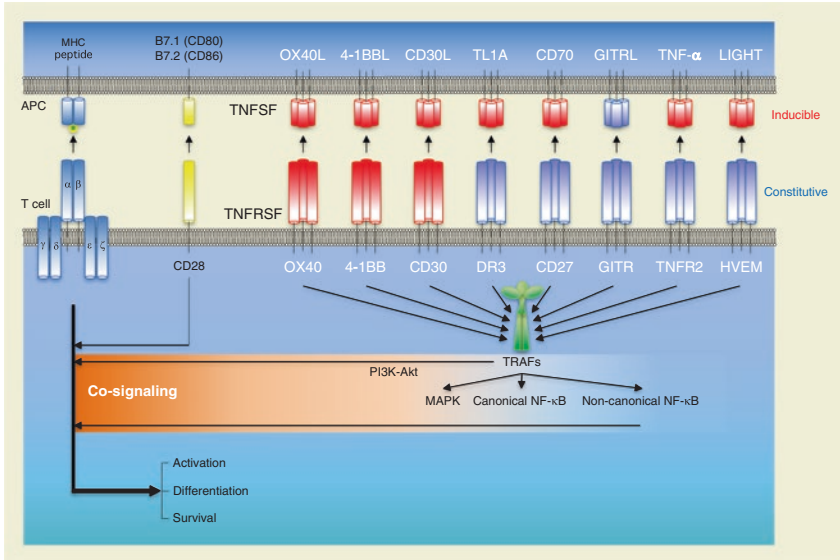
Fig. 3.2 T-cell signaling regulated by the TNF–TNFR family of co-signaling molecules. T cells are activated firstly by recognition of antigen by the T-cell receptor (TCR)/CD3 complex when antigenic peptides are presented by class I or class II molecules of the major histocompatibility complex (MHC) on antigen-presenting cell (APCs), such as dendritic cells, B cells, and macrophages. The second costimulatory signal is delivered via CD28 by interacting its ligands B7.1 (CD80) and/or B7.2 (CD86). After recognition of trimeric TNF ligand superfamily (TNFSF) molecules on APCs, TNF receptor superfamily (TNFRSF) molecules on T cells are trimerized and recruit TNF receptor-associated factors (TRAFs) to their cytoplasmic regions, which results in activation of canonical and noncanonical nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways. The TNFSF–TNFRSF interaction promotes costimulatory signaling pathways, which include phosphoinositide 3-kinase (PI3K)-Akt (protein kinase B), NF-κB, and MAPK pathways, initiated by the TCR and CD28, and plays important roles for T-cell activation, differentiation, and survival, at both early and later stages of the T-cell response. TNFSF and TNFRSF molecules constitutively and inducibly expressed by cells are indicated in blue and red, respectively

3.3 TNFR Co-signaling

3.3.1 OX40L-OX40

3.3.1.1 Expression and Function

Naïve $CD4^+$ and $CD8^+$ T cells do not express OX40. OX40 is induced on activated T cells upon antigen recognition and is downregulated after antigen clearance. Resting and memory T cells rapidly reexpress OX40 after antigen reencounter. CD28 co-signaling and pro-inflammatory cytokines including IL-1, IL-2, IL-4, and TNF-α can promote the expression of OX40. $Foxp3^+$ $CD25^+$ $CD4^+$ regulatory T cells (Treg cells) constitutively express OX40 in mice. OX40L is induced on

activated T cells and professional APCs, such as DCs, B cells, and macrophages. Activating signals via CD40, B-cell receptor (BCR), Toll-like receptors (TLRs), and cytokines, such as TSLP, type I IFN, and IL-18, and prostaglandin E2 promote the expression of OX40L on APCs. Mice deficient in *Ox40* or *Ox40l* show no abnormality in organization of lymphoid tissues and early development of the immune system. Upon interaction with OX40L, OX40 recruits TRAF2 and TRAF3 and promotes the activation of NF-κB, PI3K-Akt, and NFAT pathways in antigen-primed T cells (Table 3.1, Figs. 3.1 and 3.2).

A genetic deficiency of *Ox40* or *Ox40l* in mice leads to defective survival of antigen-primed CD4$^+$ and CD8$^+$ T cells during an active immune response and causes decreased accumulation of antigen-specific memory T cells in the later stages of the primary immune response. The interaction between OX40 on a T cell and OX40L on an APC provides critical costimulatory signals to activated T cells and controls not only cell survival but also other functional activities of effector/memory T cells. A number of studies indicate that OX40 has a more dominant role in CD4$^+$ rather than CD8$^+$ T-cell immunity because of its higher expression on CD4$^+$ T cells. OX40–OX40L interactions regulate the expansion, differentiation, and activity of Treg cells in both positive and negative manner depending on the context (Sugamura et al. 2004; Croft et al. 2009; Ishii et al. 2010; Croft 2010) (see Sect. 3.9).

3.3.1.2 Protective Immunity

Pathogen invasion results in activation of antigen-specific CD4$^+$ and CD8$^+$ T cells, and the OX40 co-signaling in primed T cells plays a key role in establishing protective immunity against infections by promoting the accumulation of antigen-specific effector/memory T cells. The OX40–OX40L interaction enhances pathogen-specific T-cell immunity in various models of infection including lymphocytic choriomeningitis virus (LCMV), influenza virus, vaccinia virus, human immunodeficiency virus, Epstein–Barr virus (EBV), cytomegalovirus (CMV), *Listeria monocytogenes*, and malaria. Although excessive stimulation of activated T cells with an agonistic monoclonal antibody (mAb) to OX40 induces adverse responses in some contexts, OX40 co-signaling generally augments both primary and memory T-cell responses to bacteria, virus, and parasite infections (Mousavi et al. 2008; Humphreys et al. 2007; Salek-Ardakani et al. 2008; Zander et al. 2015).

The cross talk and counterbalance between OX40 costimulatory and PD-1 (programmed cell death 1, CD279) co-inhibitory pathways play important roles for generation of protective CD4$^+$ T-cell immunity against malaria. OX40 is expressed by malaria-specific CD4$^+$ T cells but not CD8$^+$ T cells, and the expression is sustained after malaria infection. OX40L blockade and OX40 agonism exhibit enhanced and reduced parasite burdens, respectively. OX40 signaling supports the expansion of IFN-γ$^+$ T-bet$^+$ T-helper-1 (Th1) cells and Bcl-6$^+$ T follicular helper (Tfh) cells. OX40 signaling also enhances extra-follicular IgM$^+$ plasmablast formation, sustained Tfh responses, germinal center (GC) reactions, and T-dependent malaria-specific antibody class switching to IgG1. Both OX40 and PD-1 are expressed by malaria-

specific CD4+ T cells, and simultaneous PD-1 blockade and OX40 stimulation induce overproduction of IFN-γ, which limits Tfh cell-dependent humoral immunity that requires parasite control. This shows that OX40 co-signaling augments malaria-specific CD4+ T-cell immunity and that PD-1 co-inhibitory signaling counterbalances the OX40 signaling to inhibit Th1 effector responses, which in turn facilitates the generation of optimal Tfh response required for humoral immunity to parasite (Zander et al. 2015).

In vaccinia virus infection in mice, the OX40–OX40L interaction promotes the development of Tfh cells, which is critical for GC development, plasma cell generation, and virus-specific antibody responses. OX40 is expressed by differentiating Tfh cells, and OX40L is expressed by CD11c+ DCs in the periarteriolar lymphoid sheaths and the marginal zone bridging channels and by B220+ B cells in the follicles. OX40L+ DCs and OX40L+ B cells are in contact with OX40+ CD4+ T cells at the T/B borders in the white pulp of the spleen. OX40 and ICOS (inducible T-cell costimulatory, CD278) are co-expressed by CXCR5+ Bcl-6+ Tfh cells in GC, and deficiency in *OX40* or OX40L blockade or ICOS blockade significantly inhibits the development of both Tfh cells and GC B cells. This demonstrates that OX40 cosignaling in synergy with ICOS co-signaling maximizes Tfh responses and GC reactions, which are important for humoral immunity against vaccinia virus (Tahiliani et al. 2017).

A case study that reports a nonfunctional *OX40* mutant in a patient strongly suggests that OX40 co-signaling plays a critical role in establishing a long-term T-cell immunity to pathogens in humans. The homozygous Arg65Cys missense/loss-of-function mutation in the first of four CRDs of extracellular region in OX40 causes a low level of OX40 expression on the T-cell surface, and this residual OX40 mutant protein does not show any binding activity to OX40L. The peripheral blood from this patient contains a decreased frequency of memory CD4+ T cells. Consistent with this, in vitro recall T-cell responses to encountered antigens, including tuberculin purified protein derivatives (PPD), tetanus toxoid, EBV, CMV, and herpes simplex virus type 1, are severely impaired in this patient (Byun et al. 2013).

3.3.1.3 Autoimmunity and Inflammation

The OX40–OX40L interaction drives the development of autoimmunity and inflammation in many different animal models of asthma, colitis, graft-versus-host disease (GVHD), diabetes, multiple sclerosis, rheumatoid arthritis, atherosclerosis, and transplantation. Polymorphisms of the *OX40L* and *OX40* genes have been identified as risk factors in human diseases including systemic lupus erythematosus (SLE), rheumatoid arthritis, Sjögren syndrome, systemic sclerosis, cardiovascular disease, and narcolepsy (Table 3.2). Antigen-primed pathogenic T cells, but not naïve T cells, express OX40 or rapidly reexpress OX40 upon antigen reencounter, and thus the blockade of OX40L-OX40 interaction with a mAb or the depletion of OX40+ T cells with a mAb to OX40 has been suggested to be effective for treatment of these T-cell-mediated diseases (Sugamura et al. 2004; Croft et al. 2009; Ishii et al. 2010; Croft 2010).

Table 3.2 Human disease linkage in the TNF-TNFR family of co-signaling molecules

Gene (Symbol)	Chromosomal location	OMIM No.	Disease phenotypes, symptoms, and molecular features	References, PubMed unique identifier (PMID) No.
OX40 (TNFRSF4)	1p36.33	600315	A homozygous loss-of-function mutation in the OX40 gene. Immunodeficiency. Childhood-onset classic Kaposi sarcoma.	23897980
OX40L (TNFSF4)	1q25.1	603594	A single risk haplotype for systemic lupus erythematosus in the OX40L gene that correlates with increased cell surface OX40L expression.	18059267
			Systemic lupus erythematosus, GWAS.	18204446, 19838195, 23273568, 26502338, 26663301, 27399966, 28714469
			Rheumatoid arthritis, GWAS.	23143596, 24532676
			Asthma, GWAS.	27182965, 31036433
4-1BB (TNFRSF9)	1p36.23	602250	A homozygous loss-of-function mutation in the 4-1BB gene. Immunodeficiency. Defective immunity against EBV.	30872117
			Rheumatoid arthritis, GWAS.	24390342, 30423114
			Breast carcinoma, GWAS.	29059683
CD27 (CD27)	12p13.31	186711	Homozygous, heterozygous, and compound heterozygous mutations in the CD27 gene. Immunodeficiency. Defective immunity against EBV.	22197273, 22801960, 25843314
CD70 (CD70)	19p13.3	602840	Homozygous loss-of-function mutations in the CD70 gene. Immunodeficiency. Defective immunity against EBV.	28011863, 28011864
TL1A (TNFSF15)	9q32	604052	Crohn's disease, GWAS.	18587394, 21102463, 23266558, 26192919, 26891255, 30500874
			Inflammatory bowel disease, GWAS.	18758464, 23128233, 26192919
			Ulcerative colitis, GWAS.	20848476, 21297633, 26192919
			Leprosy, GWAS.	20018961, 25642632
			Primary biliary cirrhosis, GWAS.	23000144, 28062665
			Chronic inflammatory diseases (ankylosing spondylitis, Crohn's disease, psoriasis, primary sclerosig cholangitis, ulcerative colitis), GWAS.	26974007

Gene	Location	OMIM	Disease	PubMed IDs
TNF-α (TNF)	6p21.33	191160	Crohn's disease, GWAS.	21102463
			Oral cavity cancer, GWAS.	27749845
HVEM (TNFRSF14)	1p36.32	602746	Chronic inflammatory diseases (ankylosing spondylitis, Crohn's disease, psoriasis, primary sclerosig cholangitis, ulcerative colitis), GWAS.	26974007
LIGHT (TNFSF14)	19p13.3	604520	Multiple sclerosis, GWAS.	21833088, 24076602
LT-α (LTA)	6p21.33	153440	Cervical carcinoma, GWAS.	28806749
			Crohn's disease, GWAS.	21102463
DcR3 (TNFRSF6B)	20q13.33	603361	Glioma, GWAS.	19578366, 21531791, 22886559, 24908248, 26424050, 28346443, 29743610
			Allergic diseases (atopic dermatitis, asthma, hay fever, eczema), GWAS.	25574825, 26482879, 29083406, 29785011, 30929738
			Ulcerative colitis, GWAS.	21297633, 28067908
			Inflammatory bowel disease, GWAS.	26192919, 28067908
			Crohn's disease, GWAS.	28067908
			Colorectal cancer, GWAS.	30510241, 31089142
			Chronic inflammatory diseases (ankylosing spondylitis, Crohn's disease, psoriasis, primary sclerosig cholangitis, ulcerative colitis), GWAS.	26974007
TRAF1 (TRAF1)	9q33.2	601711	Rheumatoid arthritis, GWAS.	17804836, 20453842, 23143596, 24532676, 30891314
TRAF3 (TRAF3)	14q32.32	601896	A heterozygous dominant-negative mutation in the TRAF3 gene. A history of herpes simplex virus-1 encephalitis.	20832341
			Multiple sclerosis, GWAS.	24076602
			Asthma, GWAS.	30929738
			Systemic lupus erythematosus, GWAS.	28714469
TRAF6 (TRAF6)	11p12	602355	Rheumatoid arthritis, GWAS.	24390342, 30423114
			Myositis, GWAS.	26362759

Genome-wide association study (GWAS) data are based on the GWAS Catalog (www.ebi.ac.uk/gwas/home). EBV, Epstein-Barr virus

One human lupus study shows the correlation between the frequency of circulating OX40L$^+$ myeloid APCs in blood and the disease activity of SLE. OX40L is highly expressed in inflamed tissues including the tonsils, kidney, and skin from SLE patients. Sera from SLE patients contain immune complexes with RNA that can promote OX40L expression through TLR7 in APCs, and this OX40L activates human CD4$^+$ T cells to become Tfh-like cells possessing B-cell-helper activity. This proposes a hypothesis that excessive OX40 co-signaling through OX40L over-production on APCs causes aberrant Tfh responses that lead to SLE (Jacquemin et al. 2015).

OX40L blockade in conjunction with traditional costimulation blockade is effective in preventing alloreactive T-cell responses that hinder long-term graft function and survival. In a murine allogeneic skin graft model, a blocking mAb to OX40L, but not to ICOSL (ICOS ligand, CD275), CD70, RANKL (receptor activator of NF-κB ligand, TNFSF11, CD254), or 4-1BBL, significantly improves allograft survival when combined with traditional blockade with anti-CD40L and CTLA-4 (cytotoxic T-lymphocyte-associated protein-4, CD152)-immunoglobulin (CTLA-4-Ig). This combined blockade effectively suppresses not only primary but also memory allogeneic T-cell responses. In a nonhuman primate renal transplantation model, treatment with combined humanized anti-OX40L mAb and belatacept (CTLA-4-Ig) exhibits a synergistic suppressive effect in prolonging allograft survival. This demonstrates that successful acceptance of allografts can be achieved by the simultaneous blockade of multiple costimulatory pathways and suggests that in this setting blockade of OX40 co-signaling is effective for dampening memory T-cell responses against allografts (Kitchens et al. 2017).

3.3.1.4   Cancer

Agonistic agents against OX40, such as anti-OX40 mAb and OX40L-Ig, provide potent costimulatory signals that can augment the expansion and proliferation of CD4$^+$ and CD8$^+$ T cells that recognize tumor antigens. In addition, the transduction of tumor cells or DCs with a vector that expresses OX40L can effectively boost antitumor T-cell responses. Although augmenting OX40 co-signaling can induce tumor regression and delay tumor growth, combination therapies of OX40 agonists with additional immune modulations, such as other mAbs to costimulatory/co-inhibitory receptors, cytokines, vaccination, chemotherapy, and radiotherapy, have been suggested to be more effective in comparison to OX40 monotherapies (Moran et al. 2013; Linch et al. 2015).

OX40 agonism can effectively eradicate tumor cells in the presence of an immune checkpoint inhibitor and vaccination. Combination of mAb treatment of mice with anti-OX40 agonist and anti-CTLA-4 antagonist promotes the expansion and differentiation of tumor antigen-specific effector/memory CD8$^+$ T cells. In murine models of mammary carcinoma and spontaneous prostate adenocarcinoma, additional vaccination targeting to cross-presenting DCs using an anti-DEC205 (CD205) mAb conjugated with HER2, a tumor-associated antigen that is overexpressed in a subset

of breast cancers, promotes the induction of IFN-γ-producing CD4^{+} and CD8^{+} T cells that infiltrate into the tumor, which leads to extensive tumor destruction and improved mice survival. This demonstrates that OX40 agonism in synergy with checkpoint inhibitor blockade and tumor antigen vaccination augments durable T-cell immunity that is effective for tumor eradication (Linch et al. 2016).

3.3.2 4-1BBL-4-1BB

3.3.2.1 Expression and Function

As with OX40, 4-1BB is not expressed by naïve or resting CD4^{+} and CD8^{+} T cells but is transiently upregulated on activated T cells upon TCR triggering. 4-1BBL is transiently induced on activated professional APCs by signals from CD40 and TLRs. A genetic deficiency of *4-1bb* or *4-1 bbl* in mice displays no abnormality in the development of lymphocytes and lymphoid organs. Co-signaling via 4-1BB promotes T-cell proliferation, cytokine production, and cell survival. 4-1BB plays more predominant effects on CD8^{+} T-cell responses because of its higher expression on CD8^{+} T cells in comparison with CD4^{+} T cells. Upon binding with 4-1BBL, 4-1BB recruits TRAF1 and TRAF2, which leads to activation of NF-κB, Akt, and MAPK pathways (Snell et al. 2011; Wortzman et al. 2013a; Croft 2009) (Table 3.1, Figs. 3.1 and 3.2).

4-1BB is expressed on Treg cells. Divergent expression of 4-1BB and CD40L can distinguish antigen-specific human Treg cells from conventional activated CD4^{+} T cells. In the course of short-term activation of human peripheral blood mononuclear cells (PBMCs) with antigens in vitro, Treg cells and conventional CD4^{+} T cells upregulate 4-1BB and CD40L, respectively. Purified 4-1BB^{+} Treg cells express Treg marker genes including *FOXP3*, *CD25*, *HELIOS*, and *CTLA4*. 4-1BB^{+} Treg cells display a complete demethylation of the Treg-specific demethylated region (TSDR) at the *FOXP3* gene locus and exhibit a potent antigen-specific suppressive activity. This indicates that the extremely low frequency of antigen-specific Treg cells can be isolated from human PBMCs based on the surface expression of 4-1BB (Bacher et al. 2016).

3.3.2.2 Protective Immunity

The predominant role for 4-1BB co-signaling in CD8^{+} T-cell immunity has been demonstrated in various murine infection models including LCMV, influenza virus, CMV, murine gammaherpesvirus 68, vesicular stomatitis virus, and *Listeria monocytogenes*. The requirement of 4-1BB co-signaling for both CD4^{+} and CD8^{+} T-cell responses depends on the antigen load, the persistence of the infection, and the nature of pathogens. The levels of 4-1BB on T cells can be sustained under conditions where the infection persists. In general, mice lacking *4-1bb* or *4-1 bbl* show

defective pathogen clearance and impaired antigen-specific CD8^{+} T-cell memory formation, especially in chronic or highly infectious disease context with the higher antigenic load. On the other hand, in a situation where antigens are rapidly cleared or the infection induces minimal inflammation, the deficiency in *4-1bb* or *4-1 bbl* does not display defective antigen-specific T-cell responses (Snell et al. 2011; Wortzman et al. 2013a).

3.3.2.3 Cancer

Ligation of 4-1BB with agonistic antibodies or engineered 4-1BBL on the surface of tumor cells or DCs promotes 4-1BB co-signaling that leads to expansion of tumor antigen-specific effector CD4^{+} and CD8^{+} T cells. Anti-4-1BB agonists can induce significant tumor regressions in various established tumor models, although the associated immunopathology has been reported. The use of anti-4-1BB agonists in combination with other cancer therapeutics, such as checkpoint inhibitors, vaccination, anticancer drugs, radiation, cytokines, and adoptive T-cell therapy, has been shown to exert synergistic antitumor activity (Sanchez-Paulete et al. 2016; Bartkowiak and Curran 2015; Vinay and Kwon 2014).

4-1BB agonism combination with vaccination can induce durable antitumor T-cell immunity against vaginally implanted tumor cells. In a mouse model of human papillomavirus (HPV) syngeneic tumor, mice administered with anti-4-1BB agonistic mAb combination with a peptide vaccine, which is composed of peptides from HPV-16 E6/E7 oncoproteins and α-galactosylceramide, display stronger tumor regression, which is accompanied with expansion of HPV-specific cytotoxic CD4^{+} and CD8^{+} T cells and with robust infiltration of these cytotoxic T lymphocytes (CTLs) into the tumor microenvironment. Neither checkpoint inhibitors (anti-CTLA-4 or -PD-1) nor anti-TNFR agonists (anti-OX40, -CD40, or -GITR) cannot recapitulate the therapeutic effect of the 4-1BB agonism. This indicates that 4-1BB co-signaling plays a predominant role for induction of robust antitumor T-cell immunity against cervical neoplasia (Bartkowiak et al. 2015).

The cytoplasmic tail of 4-1BB has been utilized for a critical component of the chimeric antigen receptors (CARs) for adoptive T-cell therapy against B-cell leukemia, lymphoma, and myeloma. A T cell transduced to express a CAR that comprises of an extracellular tumor antigen-binding single-chain antibody fragment and intracellular 4-1BB and TCR CD3ζ signaling domains exhibits a potent cytotoxic activity after recognition of target antigens on the surface of tumor cells. This 4-1BB-CAR, but not CD28-CAR, ameliorates T-cell exhaustion or anergy that is induced by persistent activation of the CD3ζ domain (Long et al. 2015). After recognizing target antigens, human CD8^{+} T cells expressing 4-1BB-CAR display a phenotype with CD45RO^{+} CCR7^{+} central memory T cells that have an enhanced respiratory capacity, increased fatty acid oxidation, and increased mitochondrial biogenesis. In contrast, CD28-CAR promotes the induction of CD45RO^{+} CCR7^{−} effector memory T cells with an enhanced aerobic glycolysis (Kawalekar et al. 2016). These studies demonstrate that the 4-1BB signaling domain is superior over the CD28 signaling

domain in terms of inducing beneficial T-cell signaling activity and suggest that 4-1BB-CAR promotes a better T-cell response that is required for tumor regression.

3.3.3 CD70–CD27

3.3.3.1 Expression and Function

Unlike OX40 and 4-1BB, CD27 is constitutively expressed on naïve CD4+ and CD8+ T cells and Treg cells. CD70, the ligand for CD27, is induced on activated DCs and B cells, and signals from TLRs, CD40, and BCR enhance the expression of CD70. Activated T cells also express CD70. Mice deficient in *Cd27* or *Cd70* show normal development of lymphoid organs and lymphocytes. The CD27–CD70 interaction promotes the expansion of antigen-specific effector/memory CD4+ and CD8+ T cells, which contributes to the establishment of T-cell immunity. Upon binding with CD70, CD27 recruits TRAF2 and TRAF5 to activate NF-κB and MAPK pathways (Nolte et al. 2009; Bullock 2017) (Table 3.1, Figs. 3.1 and 3.2).

3.3.3.2 Protective Immunity

A population of APCs exclusively localized in the gut lamina propria expresses higher levels of CD70. This CD70+ APC population provides a critical CD27 co-signaling into T cells, which supports proliferation and differentiation of gut muco-sal T cells after oral *Listeria monocytogenes* infection (Laouar et al. 2005).

Optimal CD27 co-signaling plays an important role for protective T-cell immunity against infections. Critical roles for CD27 co-signaling in pathogen clearance have been shown in murine infection models including influenza virus, LCMV, and CMV. *Cd27*−/− mice display impaired primary CD4+ and CD8+ T-cell responses to influenza virus, and a memory T-cell response to a secondary challenge with influenza is also greatly attenuated (Hendriks et al. 2000). Mice deficient in *Cd70* or injected with an anti-CD70 blocking mAb display diminished primary effector CD8+ T-cell responses to acute LCMV infection and delayed virus clearance (Penaloza-MacMaster et al. 2011; Munitic et al. 2013). By contrast, the prolonged and excessive CD27–CD70 interactions with higher antigenic load during chronic virus infection induce pathological cytokine responses and immunopathology, which promotes activation-induced T-cell death and limits viral clearance. Blockade of this adverse signaling can restore protective CD8+ T-cell responses (Penaloza-MacMaster et al. 2011; Wensveen et al. 2012; Clouthier and Watts 2015). These studies suggest that an optimal level of CD27 co-signaling is required for the differentiation of effector/memory T cells that are important for pathogen clearance.

Loss of function of *CD27* or *CD70* in humans is associated with higher susceptibility to EBV infection and EBV-related diseases. Homozygous mutations in

CD70 in patients abolish surface expression of CD70 on B cells or binding to its cognate receptor CD27 on T cells, which results in impaired T-cell proliferation, cytotoxic responses, and IFN-γ production toward CD70-negative EBV-infected B cells (Izawa et al. 2017; Abolhassani et al. 2017). These studies suggest that CD70–CD27 interactions are important for generating stable T-cell immunity against EBV.

3.3.3.3 Autoimmunity and Inflammation

CD27 co-signaling promotes intestinal inflammation mediated by CD4$^+$ T cells. Adoptive transfer of CD45RB^{high} CD4$^+$ T cells into lymphocyte-deficient *Rag$^{-/-}$* recipient mice leads to weight loss, diarrhea, and severe colitis. In this T-cell-dependent murine colitis model, either deficiency of *Cd27* in donor CD4$^+$ T cells or administration of a blocking mAb to CD70 into recipient mice prevents the development of colitis and suppresses the differentiation of effector CD4$^+$ T cells producing pro-inflammatory cytokines. CD70 blockade also suppresses Th1 cell-driven trinitrobenzene sulfonic acid (TNBS)-induced colitis, indicating that CD27–CD70 interactions enhance the accumulation of pathogenic T cells in the gut mucosa and play an important role for development of T-cell-driven colitis (Manocha et al. 2009).

CD27–CD70 interactions facilitate allograft rejection mediated by CD8$^+$ T cells. In a murine fully mismatched cardiac allograft model, CD70 blockade inhibits the expansion of alloreactive effector/memory CD8$^+$ T cells and prevents CD8$^+$ T cell-driven allograft rejection. Cardiac allografts can survive longer in *Cd28$^{-/-}$* recipient mice, and this allograft survival is further prolonged in the presence of CD70 blockade, indicating that CD27 co-signaling plays a dominant role for the development of alloimmune responses even in the absence of CD28. Adoptive transfer of allograft-specific memory CD8$^+$ T cells into syngeneic recipient mice lacking secondary lymphoid organs induces rapid rejection of cardiac allografts, and CD70 blockade inhibits this memory recall response. This demonstrates that the CD27–CD70 pathway promotes the development of acute and chronic allograft rejection mediated by alloreactive effector/memory CD8$^+$ T cells (Yamada et al. 2005).

3.3.3.4 Cancer

Enhancing CD27 signaling via administration of CD27 agonists, transgenic expression of CD70 on APCs, or enforced expression of CD70 on tumor cells is effective for evoking and augmenting antitumor immunity (Bullock 2017).

Ligation of CD27 in combination with PD-1 blockade is effective at eradicating tumor cells expressing HPV antigens. CD4$^+$ T-cell-mediated help is critical for inducing optimal CD8$^+$ T-cell responses against HPV antigens, and CD70 blockade abrogates this helper activity and abolishes the therapeutic effect of tumor vaccination. Contrary to this, CD27 agonism mimics this help and enhances antitumor responses. Although in the absence of help from CD4$^+$ T cells, PD-1 blockade alone cannot improve tumor control, a combination of PD-1 blockade and CD27 agonism aug-

ments the therapeutic efficacy of vaccination. Thus, CD27 agonism provides a potent help to the endogenous T-cell immunity against tumor and enhances antitumor immunity either alone or in combination with PD-1 blockade (Ahrends et al. 2016).

Human T cells expressing a tumor antigen-specific CAR, which contains an intracellular TCR CD3ζ and a CD27 signaling domain, produce higher amounts of IFN-γ and exhibit increased cytotoxicity against tumors in vitro. Cognate recognition of tumor antigens supports the expansion of CD27–CAR-expressing T cells in vivo, and adoptive transfer of CD27–CAR T cells into highly immunodeficient mice that have been inoculated with human ovarian cancer cells expressing a specific antigen leads to increased regression of established tumors in recipient mice. This indicates that human T cells bearing CD27 costimulatory module in a CAR can exert enhanced antigen-specific CTL activity toward tumor cells (Song et al. 2012).

3.3.4 TL1A-DR3

3.3.4.1 Expression and Function

DR3 is constitutively expressed by naïve or resting CD4+ and CD8+ T cells, and its expression is upregulated following T-cell activation. DR3 is also expressed by Treg cells. TL1A, the ligand for DR3, is expressed on activated T cells and transiently induced on DCs and macrophages by pro-inflammatory stimuli, such as TLR and FcγR signaling. In humans, DcR3 binds to TL1A and inhibits the interaction between DR3 and TL1A. Upon binding to TL1A, DR3 provides costimulatory signals to T cells in concert with TCR signaling, which enhances pro-inflammatory cytokine production and promotes the clonal expansion and differentiation of effector T cells. Although $Dr3^{-/-}$ mice show a partial impairment in the negative selection of thymocytes, mice deficient in $Dr3$ or $Tl1a$ show normal development of lymphoid organs and lymphocytes. After interaction with TL1A, DR3 directly binds to TRADD. TRADD then recruits TRAF2 and RIP1 to the DR3 signaling complex and activates NF-κB and MAPK signaling pathways (Meylan et al. 2011; Pobezinskaya et al. 2011) (Table 3.1, Figs. 3.1 and 3.2).

3.3.4.2 Protective Immunity

DR3 co-signaling is critical for optimal expansion of IFN-γ producing CD4+ T cells that facilitate killing of intracellular bacteria. Infection of mice with *Salmonella enterica* Typhimurium induces sustained expression of TL1A on F4/80+ macrophages, and TL1A–DR3 interactions expand IFN-γ-producing Th1 cells, which leads to efficient clearance of *S. enterica* Typhimurium. Clonal expansion of $Dr3^{-/-}$ CD4+ T cells is significantly impaired, and this reduced T-cell response results in compromised control of the bacterial burden in the spleens of $Dr3^{-/-}$ mice (Buchan et al. 2012).

DR3 co-signaling is critical for inducing antiviral immunity against murine CMV and vaccinia virus. $Dr3^{-/-}$ mice cannot mount effective antiviral CD4^{+} and CD8^{+} T-cell immunity during acute virus infection, which is associated with impaired control of virus replication and increased morbidity and mortality. The impaired T-cell response in $Dr3^{-/-}$mice is caused by defects in the capacity of CD4^{+} and CD8^{+} T cells to divide and produce IFN-γ in response to cognate viral antigens (Twohig et al. 2012).

3.3.4.3 Autoimmunity and Inflammation

TL1A–DR3 interactions enhance immunopathology in a number of T-cell-dependent animal models of multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, and allergic lung diseases (Richard et al. 2015a; Meylan et al. 2011). Indeed, increased expression of both TL1A and DR3 is observed in patients with ulcerative colitis and Crohn's disease, and TL1A is one of the definitive susceptible genes to Crohn's disease in humans (Strober and Fuss 2011) (Table 3.2).

DR3 co-signaling in CD4^{+} T cells exacerbates inflammatory responses in the target organs of autoimmune and inflammatory diseases. Although CD4^{+} T cells from the spleen and lymph nodes of antigen-immunized $Dr3^{-/-}$ mice proliferate normally in response to recall antigen stimulation in vitro, $Dr3^{-/-}$ mice display reduced inflammation in experimental autoimmune encephalomyelitis (EAE) and allergic airway inflammation with decreased effector CD4^{+} T-cell accumulation in the local tissues (Meylan et al. 2008).

TL1A expression in DCs supports the expansion and differentiation of inflammatory IL-17-producing helper T cells (Th17 cells), and thus $Tl1a^{-/-}$ mice show reduced clinical severity in EAE that is dependent on autoimmune Th17 cells (Pappu et al. 2008).

TL1A promotes the differentiation of IL-9-producing helper T cells (Th9 cells) by augmenting signals through DR3 on CD4^{+} T cells, and endogenous TL1A enhances allergic pathology mediated by Th9 cells in murine models of experimental eye inflammation and allergic airway disease (Richard et al. 2015b).

3.3.4.4 Cancer

DR3 co-signaling augments antitumor CD8^{+} T-cell immunity. Engagement of DR3 with soluble TL1A enhances both primary and secondary expansion of antigen-specific CD8^{+} T cells in vivo. Mice inoculated with TL1A-expressing tumor cells generate protective antitumor CD8^{+} CTL responses and effectively eradicate the tumor cells in a CD8^{+} T-cell-dependent manner (Slebioda et al. 2011).

3.3.5 CD30L–CD30

3.3.5.1 Expression and Function

CD30 is expressed by activated CD4^{+} and CD8^{+} T cells and Treg cells. CD30 is also well recognized as a marker for malignant tumors including Hodgkin's lymphoma and anaplastic large cell lymphoma. CD30L is induced on activated T cells and professional APCs. CD30–CD30L interactions promote the expansion and survival of effector and memory T cells. *Cd30*^{−/−} mice have no obvious defects in peripheral T-cell or B-cell lineages. The phenotype of immune homeostasis of *Cd30l*^{−/−} mice has not been described so far. CD30 utilizes TRAF2 and TRAF3 to activate NF-κB and MAPK pathways (Watts 2005; Ward-Kavanagh et al. 2016) (Table 3.1, Figs. 3.1 and 3.2).

3.3.5.2 Protective Immunity

CD30–CD30L interactions establish optimal T-cell immunity against *Mycobacterium avium*. During infection with *M. avium*, CD30L blockade leads to increased mycobacterial burdens. *Cd30*^{−/−} mice display higher susceptibility to *M. avium* infection, and the defective antibacterial response is associated with a decreased expansion of IFN-γ-producing CD4^{+} T cells specific for bacterial antigens (Florido et al. 2004). *Cd30l*^{−/−} mice are susceptible to *Mycobacterium bovis* Bacillus Calmette–Guérin (BCG) infection and exhibit higher bacterial burden. The numbers of antigen-specific IFN-γ-producing CD4^{+} T cells in the spleen, lung, and peritoneal cavity of *Cd30l*^{−/−} mice are greatly diminished after BCG infection (Tang et al. 2008).

CD30 signaling promotes the differentiation of memory CD8^{+} T cells and is required for protective immunity against *Listeria monocytogenes*. Following *L. monocytogenes* infection, *Cd30l*^{−/−} mice can generate a normal antigen-specific effector CD8^{+} T-cell response at an early stage of infection but display a defective recall memory CD8^{+} T-cell response against reinfection (Nishimura et al. 2005).

Double knockout mice deficient in both *Cd30* and *Ox40* exhibit defective Th1 immunity against *Salmonella typhimurium*. Antigen-specific *Cd30*^{−/−}*Ox40*^{−/−} donor CD4^{+} T cells fail to persist after transfer into infected *Rag*^{−/−} recipient mice, and these recipient mice exhibit an impaired capacity to control *S. typhimurium* infection, indicating that both CD30 and OX40 signals promote Th1 immunity against *Salmonella* (Gaspal et al. 2008).

3.3.5.3 Autoimmunity and Inflammation

The CD30–CD30L interaction augments allergic airway inflammation mediated by T-helper-2 (Th2) cells. *Cd30*^{−/−} or *Cd30l*^{−/−} mice primed with ovalbumin (OVA) antigen and challenged with OVA display diminished eosinophilic infiltration into

the airway and pulmonary inflammation. In these mice, the levels of pro-inflammatory cytokines IL-5 and IL-13 in bronchoalveolar lavage fluids (BALF) are reduced, and lung-draining lymph node CD4$^+$ T cells produce lesser amount of IL-5 and IL-13 in response to OVA in vitro. Thus, CD30 signaling contributes to allergic lung inflammation through accumulation of pathogenic effector/memory Th2 cells during the course of airway inflammation (Polte et al. 2006; Nam et al. 2008; Fuchiwaki et al. 2011).

CD30 signaling in CD4$^+$ T cells enhances the development of pathogenic IL-17- and/or IFN-γ-producing CD4$^+$ T cells in autoimmune neuroinflammation. EAE disease is ameliorated in *Cd30$^{-/-}$* and *Cd30l$^{-/-}$* mice. CD4$^+$ T cells from draining lymph node of *Cd30$^{-/-}$* or *Cd30l$^{-/-}$* mice exhibit reduced proliferative and cytokine responses to recall antigen in vitro. Infiltration of inflammatory cells including CD4$^+$ T cell, macrophage/microglia, and granulocyte in the spinal cords is significantly attenuated in these knockout mice. CD30 blockade in the course of disease development ameliorates EAE in wild-type mice. Accordingly, adoptive transfer of antigen-specific wild-type CD4$^+$ T cells into *Cd30l$^{-/-}$* mice induces significantly attenuated EAE (Shinoda et al. 2015).

Signals through both CD30 and OX40 promote autoimmune tissue damage mediated by effector CD4$^+$ T cells in the context of *Foxp3* deficiency. Although *Foxp3$^{-/-}$* mice develop lethal autoimmune disease, mice with combined deficiency in *Foxp3*, *Cd30*, and *Ox40* develop and grow normally. Pathogenic CD62L^{low}CD44^{high}CD4$^+$ effector/memory T cells, which reside in *Foxp3$^{-/-}$* mice and secrete higher amounts of IL-4 and IFN-γ, are substantially decreased in *Foxp3$^{-/-}$* *Cd30$^{-/-}$Ox40$^{-/-}$* mice. Accordingly, blockade of both CD30 and OX40 signaling can prevent the development of lethal autoimmune responses that are mediated by autoreactive T cells existing in *Foxp3$^{-/-}$* mice (Gaspal et al. 2011).

3.3.6   GITRL–GITR

3.3.6.1   Expression and Function

GITR is expressed on naïve or resting CD4$^+$ and CD8$^+$ T cells at basal levels and is upregulated after T-cell activation. GITR is highly expressed by Treg cells. GITRL is expressed at low levels on APCs and is transiently upregulated after cell activation. GITR provides costimulatory signals to CD4$^+$ and CD8$^+$ T cells to enhance proliferation, effector function, and cell survival. *Gitr$^{-/-}$* mice show normal development of lymphoid organs and homeostasis of T cells. A study regarding *Gitrl$^{-/-}$* mice has not been reported so far. GITR interacts with TRAF2 and TRAF5 and activates NF-κB and MAPK pathways (Snell et al. 2011; Clouthier and Watts 2014) (Table 3.1, Figs. 3.1 and 3.2).

GITR co-signaling plays critical roles in both conventional CD4^{+} and CD8^{+} T cells and Treg cells, and the effect of GITR co-signaling on conventional T cells versus Treg cells depends on the context of the immune response (see Sect. 3.9).

3.3.6.2 Protective Immunity

GITR co-signaling plays a critical role in control of virus infection. GITR is required for primary and recall CD8^{+} T-cell responses and for survival of antigen-specific CD8^{+} T cells during viral infection, and GITR signaling protects mice from death during severe respiratory influenza infection (Snell et al. 2010).

T-cell-intrinsic GITR co-signaling increases the frequency and number of LCMV-specific CD8^{+} T cells, which results in decreased viral load. GITRL is transiently induced on APCs at early time points of LCMV infection and is downregulated to below baseline levels thereafter. GITR is also transiently upregulated on CD8^{+} T cells but is sustained at low but above baseline levels at chronic stages of LCMV infection. Thus, GITR on T cells is not efficiently engaged by endogenously expressed GITRL on APCs at the chronic stage of infection. However, an agonistic mAb to GITR can overcome the insufficiency of GITRL and activates LCMV-specific CD8^{+} T cells, which leads to lower viral load without causing overt immune pathology (Clouthier et al. 2014).

GITR signaling in CD4^{+} T cells is required for antiviral CD8^{+} T-cell responses and humoral immunity that are important for control of chronic LCMV infection. *Gitr*^{−/−} mice display impaired accumulation of IL-2^{+} IFN-γ^{+} Th1 cells as well as Tfh cells and have decreased IgG responses to LCMV. CD8^{+} T cells from *Gitr*^{−/−} mice express higher levels of exhaustion markers PD-1 and Tim-3. GITR-mediated priming of Th1 cells promotes cell-mediated immunity as well as humoral immunity to optimally regulate chronic LCMV infection (Clouthier et al. 2015).

3.3.6.3 Autoimmunity and Inflammation

GITR agonism exacerbates CD4^{+} T-cell-dependent murine models of autoimmune/inflammatory diseases, such as asthma, collagen-induced arthritis (CIA), diabetes, and EAE. Treatment of mice with an agonistic mAb to GITR promotes migration, proliferation, and activation of diabetogenic T cells within the pancreatic islets and exacerbates T-cell-driven destructive insulitis in nonobese diabetic (NOD) mice (You et al. 2009).

Gitr^{−/−} mice develop significantly attenuated CIA. Draining lymph node CD4^{+} T cells from type II collagen (CII)-immunized *Gitr*^{−/−} mice secrete lower amounts of IFN-γ in response to CII *in vitro*. Donor splenic T cells from CII-immunized *Gitr*^{−/−} mice fail to induce sufficient disease symptoms of CIA in severe combined immunodeficient (SCID) recipient mice, suggesting that GITR signaling is required for CD4^{+} T cells to attain full pathogenic potential to promote CIA (Cuzzocrea et al. 2005).

3.3.6.4 Cancer

GITR signaling boosts antitumor immunity by enhancing effector function of tumor antigen-specific T cells (Schaer et al. 2012).

Although either GITR agonism or PD-1 blockade alone exhibits little antitumor effect, a combination of GITR agonism and PD-1 blockade enhances a potent antitumor immunity against ovarian cancer. Anti-GITR/PD-1 mAbs increase the frequency of IFN-γ-producing CD4$^+$ and CD8$^+$ T cells with cytotoxic activity against tumors. Combined treatment of anti-GITR/PD-1 mAbs and chemotherapeutic drugs further enhances the antitumor responses (Lu et al. 2014). Treatment of mice bearing B16 melanoma with anti-GITR/PD-1 mAbs in combination with tumor antigen vaccination leads to markedly better inhibition of tumor growth and prolonged mice survival. The tumor regression correlates with the induction of tumor-infiltrating antigen-specific effector/memory CD8$^+$ T cells producing IFN-γ and TNF-α, and depletion of these CD8$^+$ T cells abrogates the beneficial effects provided by the therapy (Villarreal et al. 2017).

Anti-GITR agonist facilitates antitumor immunity by promoting the differentiation of IL-9-producing Th9 cells. Administration of anti-GITR mAb into CT26 colon tumor- or B16F10 melanoma-bearing mice inhibits growth of tumors, which is mediated by GITR signal-driven differentiation of Th9 cells. Neutralization of IL-9 prevents the tumor regression, and IL-9 from Th9 cells promotes the development of tumor antigen-specific cytotoxic CD8$^+$ T cells through activation of tumor-associated DCs (Kim et al. 2015).

3.3.7 TNFα-TNFR2

3.3.7.1 Expression and Function

A low level of TNFR2 is expressed by resting or naïve CD4$^+$ and CD8$^+$ T cells, and its expression is further upregulated after T-cell activation. TNFR2 binds to cognate ligands TNF-α and LT-α (lymphotoxin-α). TNF-α is produced by activated macrophages, T cells, and many other cell types during inflammation and exists as a transmembrane trimer whose proteolysis by TNF-converting enzyme (TACE or ADAM17) leads to a soluble form. LT-α is produced as a soluble form. TNFR2 is more efficiently engaged by transmembrane TNF-α than by soluble TNF-α. Interaction of TNF-α with TNFR2 is costimulatory to TCR-mediated T-cell activation and promotes the activation, differentiation, and survival of T cells. *Tnfr2$^{-/-}$* mice show normal development of peripheral lymphoid organs and lymphocytes. Unlike TNFR1, TNFR2 lacks an intracellular DD and directly recruits TRAF1, TRAF2, and TRAF3 to activate NF-κB and MAPK pathways (Kim et al. 2006; Calzascia et al. 2007; Faustman and Davis 2010; Mehta et al. 2016) (Table 3.1, Figs. 3.1 and 3.2).

Mutations in *TNFR2* that lead to enhanced NF-κB signaling have been found in patients with T-cell lymphoma (Ungewickell et al. 2015).

TNFR2 is expressed on Treg cells. Similar to other TNFRSF molecules, such as GITR and OX40, the effect of TNF-α on conventional CD4⁺ and CD8⁺ T cells versus Treg cells depends on the context of inflammatory environments (see Sect. 3.9).

Although the TNF-α-TNFR2 interaction provides costimulatory signals into T cells, signals from TNFR2 limit activity of effector CD8⁺ T cells in some contexts (Wortzman et al. 2013b; Punit et al. 2015).

3.3.7.2 Protective Immunity

TNF-α-TNFR2 interactions are critical for generating cytotoxic CD8⁺ T-cell responses against adenovirus in the liver. Intrahepatic lymphocytes isolated from adenovirus-infected *Tnfa⁻/⁻* or *Tnfr2⁻/⁻* mice display decreased CTL responses against antigen-expressing target hepatocytes (Kafrouni et al. 2003).

TNFR2 signaling plays an important role for T-cell-mediated immunity against *Listeria monocytogenes*. After *L. monocytogenes* infection, *Tnfr2⁻/⁻* mice display reduced expansion and survival of antigen-specific CD4⁺ and CD8⁺ T cells producing IFN-γ and delayed bacterial clearance upon challenge with higher dose of bacteria. Antigen-specific donor *Tnfr2⁻/⁻* CD8⁺ T cells display a marked reduction in clonal expansion and survival in infected recipient mice (Kim et al. 2006).

3.3.7.3 Autoimmunity and Inflammation

In a model of murine GVHD, TNFR2 signaling in both donor CD4⁺ and CD8⁺ T cells is required for induction of acute GVHD responses mediated by alloantigen-specific CD8⁺ CTL. Recipient mice transferred with donor *Tnfr2⁻/⁻* T cells exhibit a reduced cytotoxic phenotype and a decreased elimination of host spleen cell populations mediated by allo-specific donor CD8⁺ T cells. Whereas TNFR2 signaling in donor CD4⁺ T cells is critical for their gaining of optimal helper activity for donor CD8⁺ T cells, TNFR2 signaling in donor CD8⁺ T cells promotes the maturation of fully competent KLRG1⁺ CCR7⁻ CD8⁺ T cells, termed short-lived effector cells (SLECs). This indicates that induction of optimal donor CD8⁺ CTL activity requires TNFR2 signaling in both donor CD4⁺ and CD8⁺ T-cell populations (Soloviova et al. 2013).

TNF-α blockers are well established for treatment of human inflammatory diseases, such as rheumatoid arthritis and Crohn's disease. Although T cells might not be the main target cells in the therapy, blockade of TNF-α-TNFR2 interactions promotes apoptosis of mucosal CD4⁺ T cells from patients with inflammatory bowel disease (IBD). CD14⁺ macrophages and CD4⁺ T cells isolated from the lamina propria of IBD patients express higher levels of membrane-bound TNF-α (mTNF-α) and TNFR2, respectively. Clinically effective anti-TNF-α mAbs in IBD induce

apoptosis of TNFR2$^+$ CD4$^+$ T cells when these T cells are cocultured with mTNF-α$^+$ CD14$^+$ macrophages, suggesting that disruption of mTNF-α-TNFR2 interactions with anti-TNF-α leads to suppression of gut inflammation in IBD through inducing apoptosis of pathogenic CD4$^+$ T cells in the intestine (Atreya et al. 2011).

TNFR2 signaling in CD4$^+$ T cells expands pathogenic effector T cells that promote colitis in mice. Adoptive transfer of donor naïve CD4$^+$ T cells from *Tnfr2*$^{-/-}$ mice fails to induce severe colitis in lymphopenic *Rag1*$^{-/-}$ mice. *Tnfr2*$^{-/-}$ CD4$^+$ T cells are defective in clonal expansion and differentiation into IFN-γ-producing colitogenic effector T cells in the lymphopenic environment (Chen et al. 2016).

3.3.8 LIGHT–HVEM

3.3.8.1 Expression and Function

HVEM, a member of the TNFRSF, binds to four different molecules, TNFSF molecules LIGHT and LT-α and immunoglobulin superfamily molecules BTLA and CD160, in distinct configurations, and these molecular networks activate both inflammatory and anti-inflammatory signaling pathways. Ligation of HVEM on T cells by the canonical TNF-related ligand LIGHT provides costimulatory signals to T cells. HVEM is expressed on naïve or resting CD4$^+$ and CD8$^+$ T cells and Treg cells. LIGHT is expressed on activated lymphoid cells including APC populations and T cells either as a membrane-bound or a soluble form. In human, DcR3 binds to LIGHT and inhibits the interaction between HVEM and LIGHT. HVEM co-signaling promotes the activation, differentiation, and survival of T cells. *Hvem*$^{-/-}$ and *Light*$^{-/-}$ mice have no significant abnormalities in the development of lymphoid organs and lymphocytes. HVEM recruits TRAF2 and TRAF3 to activate NF-κB pathway (Ward-Kavanagh et al. 2016) (Table 3.1, Figs. 3.1 and 3.2).

Although ligation of HVEM by BTLA activates pro-survival HVEM signaling pathways in effector and memory T cells after antigen recognition (Steinberg et al. 2013; Flynn et al. 2013; Sakoda et al. 2011), we mainly focus on the canonical HVEM–LIGHT interaction that controls HVEM co-signaling in T cells.

3.3.8.2 Autoimmunity and Inflammation

HVEM co-signaling promotes the differentiation of pathogenic effector CD4$^+$ T cells that exacerbate experimental autoimmune uveitis (EAU). Pathogenic responses and clinical scores of EAU are significantly decreased in *Hvem*$^{-/-}$ mice and draining lymph node T cells from *Hvem*$^{-/-}$ mice display reduced cell proliferation and production of Th1- and Th17-related cytokines in response to antigen restimulation in vitro. *Light*$^{-/-}$, *Btla*$^{-/-}$, and *Light*$^{-/-}$*Btla*$^{-/-}$ mice also display attenuated EAU disease phenotypes and decreased Th1-/Th17-type responses. A blocking mAb to

HVEM that can inhibit both HVEM–LIGHT and HVEM–BTLA interactions ameliorates pathogenic Th1/Th17 responses in EAU (Sakoda et al. 2016).

In a T-cell-dependent allergic lung inflammation model, the HVEM–LIGHT interaction plays a dominant role in the survival of pathogenic memory Th2 cells. After antigen recall challenges, adoptively transferred donor OVA-specific *Hvem*$^{-/-}$ Th2 cells display reduced accumulation in the airways, lungs, and draining mediastinal lymph nodes of recipient mice and induce decreased airway eosinophilia and Th2 cytokine responses. Interruption of HVEM–LIGHT interactions also inhibits the accumulation of inflammatory memory Th2 cells in the lung. Although *Light*$^{-/-}$ donor CD4$^+$ T cells cannot sustain after antigen exposure in vivo, cotransfer of wild-type and *Light*$^{-/-}$ donor T cells rescues the survival defect of *Light*$^{-/-}$ donor T cells, suggesting that HVEM induces cell survival signals into T cells through interacting with LIGHT expressing on neighboring antigen-specific T cells. This demonstrates that HVEM expressed by CD4$^+$ T cells induces cell survival signals and controls T-cell-driven allergic lung inflammation (Soroosh et al. 2011).

In a T-cell-dependent murine colitis model, HVEM signaling in donor CD4$^+$ T cells promotes intestinal inflammation and contributes to the immunopathology mediated by differentiated colitogenic T cells. *Rag*$^{-/-}$ recipient mice with donor CD45RB^{high} CD4$^+$ T cells from *Hvem*$^{-/-}$ or *Light*$^{-/-}$ mice display reduced intestinal inflammation, indicating that both HVEM and LIGHT on CD4$^+$ T cells are required for the development of inflammatory T cells within the intestine during colitis development. *Rag*$^{-/-}$ recipient mice receiving *Hvem*$^{-/-}$ CD4$^+$ T cells express lesser amounts of IL-6, TNF-α, and IL-12 mRNAs in the colon and have a decreased number of donor T cells producing IFN-γ or IL-17. In the absence of HVEM signaling, donor CD4$^+$ T cells expand normally at early time points but cannot persist at later time points in lymphopenic hosts. These results suggest that T-cell-expressing LIGHT interacting with HVEM expressed by an adjacent T cell plays a critical role for the expansion, differentiation, and survival of gut-homing pathogenic T cells driving colitis (Schaer et al. 2011; Steinberg et al. 2008).

HVEM–LIGHT interactions contribute to GVHD pathogenesis. Adoptive transfer of allogeneic T cells from *Hvem*$^{-/-}$ or *Light*$^{-/-}$ mice into recipient mice induces attenuated anti-host CTL responses, which results in prolonged survival of recipient mice. *Hvem*$^{-/-}$ and *Light*$^{-/-}$ donor T cells undergo apoptosis after transfer into recipient mice. Accordingly, administration of an anti-HVEM mAb, which interferes with HVEM–LIGHT interactions, ameliorates GVHD responses and prolongs the survival of recipient mice (Xu et al. 2007).

3.3.8.3 Cancer

HVEM co-signaling via LIGHT plays an important role for establishing robust T-cell immunity against tumors. Mice inoculated with tumor cells expressing an agonistic mAb to HVEM on the cell surface generate protective antitumor immunity in a manner dependent on both CD4$^+$ and CD8$^+$ T cells. The potent antitumor CTL activity is associated with increased IFN-γ production, and mice which have

rejected this tumor are resistant to rechallenge of parental tumors, indicating that HVEM signaling facilitates tumor-reactive T-cell responses, induction of long-term antitumor immunity, and tumor regression (Park et al. 2012).

LIGHT expression in the tumor environment elicits potent antitumor immunity leading to tumor regression (Yu and Fu 2008). Combined immunotherapy with LIGHT expression on tumors and antigen-specific vaccination is effective for generating tumor-infiltrating CD8$^+$ T-cell responses that are important for tumor eradication. Adenovirus-mediated forced expression of LIGHT in HPV16-induced tumors results in upregulation of IFN-γ and chemoattractant cytokines in the tumor, which results in induction of antigen-specific CD8$^+$ T cells in the peripheral lymphoid organs and within the tumor. However, antigen-specific T cells induced by LIGHT cannot induce complete tumor regression. More importantly, additional vaccination with HPV16 antigen boosts the frequency of antigen-specific T cells and prevents the outgrowth of tumors. This suggests that LIGHT expressed by tumors expands incoming T cells via HVEM signaling and that these LIGHT–HVEM interactions in the presence of antigenic signals play an important role for generating robust antitumor immunity that can eradicate tumors (Kanodia et al. 2010).

3.3.9 *The Role of TNFRSF Molecules in Immune Regulation Mediated by Treg Cells*

Treg cells play important roles for regulation of conventional CD4$^+$ and CD8$^+$ T-cell responses. TNFRSF molecules described in this chapter are all expressed by both Treg cells and conventional T-cell populations, and the cognate TNFSF–TNFRSF interaction regulates the balance between Treg cells and conventional T cells, which is critical for determining all aspects of the immune response. Although the cell-intrinsic effects of the TNFRSF signaling in Treg cells are still unclear (Shevach and Stephens 2006; So et al. 2008; Croft 2014; Mbanwi and Watts 2014), it has been demonstrated that signals from TNFRSF molecules influence the Treg and conventional T-cell balance through (1) promoting the development of natural or thymus-derived Treg cells, (2) antagonizing the development of induced or periphery-derived Treg cells, (3) promoting the expansion and survival of Treg cells, (4) downregulating Treg cell suppressive functions, and (5) enhancing resistance of naïve/effector/memory responder CD4$^+$ and CD8$^+$ T cells to the Treg cell-mediated suppression.

3.4 Conclusion

Five out of eight TNFRSF molecules, CD27, DR3, HVEM, GITR, and TNFR2, discussed in this chapter are constitutively expressed on most CD4$^+$ and CD8$^+$ T cells, whereas other three TNFRSF molecules, 4-1BB, CD30, and OX40, are

upregulated on activated T cells after antigen recognition. Most of the cognate ligands for these TNFRSF molecules are not highly expressed by APC populations or other cell types and are induced by antigen recognition or innate stimuli or inflammation. The co-signaling activated by the cognate TNFSF–TNFRSF interaction quantitatively and/or qualitatively augments signals that are initiated by the TCR and CD28 and controls not only in early phases of T-cell responses to enhance cell proliferation, differentiation, and survival but also in later phases of memory T-cell responses (Fig. 3.2). Thus, TNFRSF molecules expressed by T cells have central roles in infection, immune-mediated diseases, and cancer that are critically regulated by various populations of antigen-primed T cells. Understanding of co-signaling functions of TNFRSF molecules in health and disease has progressed over the past few decades, and biologics or gene engineering technologies that inhibit or stimulate co-signaling functions of TNFRSF molecules have proven to be clinically efficacious for autoimmune and inflammatory diseases and cancer (Sugamura et al. 2004; Croft et al. 2013; Moran et al. 2013; Ward-Kavanagh et al. 2016). However, there are still fundamental questions regarding signaling mechanisms of the TNF–TNFR family of co-signal molecules: (1) qualitative and/or quantitative contribution of the TNFR co-signaling to the TCR, (2) cross talk between TNFR and co-inhibitory molecules, (3) intrinsic TNFR signaling functions in Treg cells, (4) utilization patterns of individual TRAF molecules in each individual TNFR signaling complex that might explain the qualitative difference in co-signaling activity, (5) cross talk of costimulatory signals among TNFRSF molecules, (6) roles of reverse signaling mediated by membrane-bound TNFSF ligands expressed by T cells, and (7) adverse (death) signaling effects of co-stimulatory TNFRSF molecules expressed by T cells. Further research will uncover these unresolved issues and may provide novel information regarding the potential usefulness of blockade or agonism of the TNFR co-signaling pathways in human diseases (Table 3.2).

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