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The role of TNF superfamily members in T-cell function and diseases

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Abstract

Interactions that occur between several tumour necrosis factor (TNF)–TNF receptors that are expressed by T cells and various other immune and non-immune cell types are central to T-cell function. In this Review, I discuss the biology of four different ligand– receptor interactions — OX40 ligand and OX40, 4-1BB ligand and 4-1BB, CD70 and CD27, and TL1A and death receptor 3 — and their potential to be exploited for therapeutic benefit. Manipulating these interactions can be effective for treating diseases in which T cells have an important role, including inflammatory conditions, autoimmunity and cancer. Here, I explore how blocking or inducing the signalling pathways that are triggered by these different interactions can be an effective way to modulate immune responses.

A recognized triumph in immunotherapy has been the development of neutralizing antibodies and Fc fusion proteins that inhibit the binding of tumour necrosis factor (TNF) to one or both of its receptors — TNF receptor 1 (TNFR1; also known as TNFRSF1A) and TNFR2 (also known as TNFRSF1B). As a primary function of the TNF superfamily molecules is to regulate cell survival, inhibition of these interactions prevents the activation of signalling pathways downstream of the TNFRs, thereby minimizing the pro-inflammatory programme they initiate in immune cells and decreasing the pathology of autoimmune and inflammatory diseases. Based on the success of these therapies, increasing attention is now focused on other related molecules in the TNF superfamily, of which there are 19 ligands and 30 receptors. Several of the interactions that occur between TNF molecules and their receptors have gained prominence based on studies of animal models of immune function and disease. These studies indicate that interactions between TNF–TNFR molecules positively regulate T-cell responses and mediate crosstalk between T cells and other cell types. The interactions between OX40 ligand (OX40L; also known as CD252 and TNFSF4) and OX40 (also known as CD134 and TNFRSF4), 4-1BBL (also known as TNFSF9) and 4-1BB (also known as CD137 and TNFRSF9), CD70 (also known as TNFSF7) and CD27 (also known as TNFRSF7), and TL1A (also known as TNFSF15) and death receptor 3 (DR3; also known as TNFRSF25) (TABLE 1), have been the most extensively studied in terms of their direct effects on $CD4^+$ and $CD8^+$

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DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene 4-1BB | 4-1BBL | CD27 | CD70 | DR3 | OX40 | OX40L | TL1A | TNF | TNFR1 | TNFR2

FURTHER INFORMATION

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SUPPLEMENTARY INFORMATION

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T cells. In support of the importance of these interactions in controlling T-cell function, recent results have shown that blocking or promoting each one of these interactions in animal models of disease markedly affects the outcome of the disease.

In this Review, I focus on the activity of these molecules in regulating the function of T cells and other immune cells and discuss how this relates to studies of inflammation, autoimmunity and cancer, in which these molecules are promising candidates for therapy. There are two main approaches for therapy based on targeting TNF–TNFR interactions: first, to block one or more of these interactions to reduce pathogenic immune responses in autoimmune and inflammatory diseases and, second, to enhance signalling that is triggered by the TNF–TNFR interactions to stimulate a more robust immune response and promote antitumour immunity. The molecules reviewed here can also have important roles in responses to intracellular pathogens and acute viral infection, and persistent expression of these molecules may be related to the pathology that is linked to chronic viral infection. Although this is also a growing area of importance in terms of potential therapies, these roles will not be discussed here.

Overview and signalling

Expression

A feature of the molecules that form these four ligand–receptor pairs is that they are not ubiquitously expressed (TABLE 1). The finding that the expression of several of these molecules is increased following immune-cell activation suggests that they have a central role in modulating immune responses. Indeed, studies of TNFRs expressed by conventional T cells and their ligands expressed by antigen-presenting cells (ApCs) has led to the hypothesis that antigen recognition by T cells results in the engagement and bidirectional activity of the TNF–TNFR pair, which promotes the effector responses of T cells and of other immune cells[1-7] Because the expression of OX40 and 4-1BB is induced in response to antigen stimulation, these TNFRs have been proposed to be markers of effector T cells (which are pathogenic in autoimmunity or protective in infection and cancer). Although CD27 and DR3 can be constitutively expressed by conventional T cells, their expression is also strongly upregulated following T-cell activation, possibly in parallel with the upregulation of OX40 and 4-1BB expression. In addition, several stimuli can affect the level and kinetics of expression. The induction or upregulation of OX40, 4-1BB and DR3 expression occurs within 24 hours following the recognition of antigen by and activation of naive T cells, and much more rapidly by memory T cells; the expression of these receptors can last for several hours or even days[7-12].

The expression of the TNF ligands CD70, TL1A, OX40L and 4-1BBL is induced in professional ApCs, although the extent to which expression levels vary between dendritic cells (DCs), B cells and macrophages is not clear. various stimuli can promote the expression of the different ligands by ApCs, including innate signals (such as those induced by Toll-like receptor (TLR) ligands) and adaptive signals (such as those induced by interferon-γ (IFNγ))[7,12-20]. Furthermore, both $CD4^+$ and $CD8^+$ T cells can express both TNF ligands and their receptors, which suggests that T-cell–T-cell interactions can also influence the development of effector T-cell populations[21,22].

Importantly, OX40L, 4-1BBL, CD70 and TL1A can also be expressed by non-immune cells (TABLE 1), such as smooth muscle cells and endothelial cells[12,23-25], during conditions of inflammation. This implies that TNF–TNFR interactions between T cells and non-immune cells in the periphery can further contribute to the function of effector T cells and also promote an inflammatory response in non-immune cells, which ultimately will propagate tissue inflammation in a disease context. In addition, several mouse studies have shown immunomodulatory roles for CD70–CD27 and 4-1BBL–4-1BB interactions in the bone

marrow when expressed by haematopoietic progenitor cells[26,27], suggesting that these molecules might contribute to inflammatory conditions that require haematopoiesis.

Finally, other immune-cell types, such as natural killer (NK) and NKT cells, can also express one or more of these molecules, the activation of which can increase their effector function. In addition, it has been reported that regulatory T (T_{Reg}) cells can express TNFRs, and some evidence suggests that engaging these receptors on the surface of these cells helps to amplify immune responses by inhibiting the generation and/or activity of this suppressive T-cell subset (see below).

So, there is not a simple set of rules that determines when and where these molecules are expressed, and hence in which context they are important for T-cell responses. These TNF–TNFR interactions occur mainly during an ongoing immune response rather than at the initiation of a response, as their expression in resting or recently activated cells is limited. It is probable that there are instances in which each of the four ligand–receptor interactions are spatially separated (owing to differential expression levels by different cell types) or temporally separated (owing to expression at distinct intervals) during the course of an immune response. Conversely, it is possible that during other types of immune response, T cells or NK and NKT cells can express all four receptors, which would allow simultaneous engagement by the four ligands and an additive or synergistic cellular response. The expression of some of these molecules in normal and disease states has also been described in humans, and several reports suggest a correlation between their expression levels and disease status, and sometimes with therapy outcome (for examples, see REFS [28-30]). unfortunately, these studies are too numerous to describe in detail here. Given the diversity in experimental approaches used in some of these analyses, this is an area that warrants much greater attention despite the caveat that human clinical samples are limited throughout the course of any disease. It is probable that there are differences in the expression patterns of these molecules between mice and humans; for example, mouse T_{Reg} cells constitutively express several TNFR molecules, whereas the expression of these molecules is inducible on human T_{Reg} cells. However, given the hypothesis that the expression of both TNF ligands and receptors will be transient in many cases, understanding when and where they are expressed in humans should aid the diagnosis and the timing of any clinical treatment.

Signalling

The intracellular regions of OX40, 4-1BB, CD27 and DR3 associate with TNFR-associated factors (TRAFs), which are adaptor molecules that link receptor activation to inflammatory signalling pathways. In particular, TRAFs can complex with inhibitor of NF-κB, α subunit (IκBα), IκB kinase-β (IKKβ) and NF-κB-inducing kinase (NIK), thereby allowing activation of both canonical and non-canonical nuclear factor-κB (NF-κB) signalling pathways, which are known to be important for cell survival. Signalling pathways that are triggered by the activation of OX40, 4-1BB and CD27 in $CD4^+$ and $CD8^+$ T cells increase the expression of anti-apoptotic molecules, including BCL-2 (B-cell lymphoma 2), BCL-XL (also known as BCL2L1) and/or BFL1 (also known as BCL2A1)[9,31,32], which correlates with the promotion of T-cell survival by these receptors. For OX40 and 4-1BB, this has been linked to the activation of NF-κB, phosphoinositide 3 kinase and protein kinase B (also known as AKT)[31,33,34]. Another downstream effect of 4-1BB ligation[35] that might also be common to the other receptors is inhibition of the expression of the pro-apoptotic molecule BIm (BCL-2-interacting mediator of cell death) through the activation of extracellular-signal-regulated kinase (eRK). CD27 and DR3 are also strong activators of NF-κB[12,36-38], and DR3 signalling can result in the increased accumulation of T cells[7] and resistance to apoptosis[38] (FIG. 1). Indeed, although DR3 was initially named death receptor 3 owing to its intracellular death domain that can lead

to apoptosis, more recent functional data suggest that the activity of DR3 is mainly pro-inflammatory.

Signals from OX40, 4-1BB, CD27 and DR3 also synergize with T-cell receptor (TCR)-induced signals to allow cell cycle progression (and thereby promote T-cell division) and cytokine production by T cells. Ligation of OX40 enhances the expression of survivin and aurora B kinase[34,39], which function together to promote the activity of cyclin-dependent kinases and allow S phase progression and mitosis in T cells[40]. Ligation of 4-1BB can also influence the expression of cyclins[41]. Other events that are reportedly triggered by OX40, 4-1BB, DR3 and CD27 ligation include the activation of JuN N-terminal kinase (JNK) and activator protein 1 (Ap1), the activation of the mitogen-activated protein kinases p38 (REFS [38,42,43]) and eRK, and the nuclear accumulation of nuclear factor of activated T cells (NFAT)[44]. These molecules are involved in promoting the production of cytokines, including interleukin-2 (IL-2), IL-4, IL-5 and IFNγ (FIG. 1). In addition, ligation of TNFRs can lead to the upregulation of cytokine receptor expression, such as IL-2 receptor α-chain (IL-2Rα) and IL-12Rβ, which further amplifies the immune response by increasing the sensitivity of T cells to these growth factors[32,45-47].

Regulatory T (T_{Reg}) cell

A specialized T cell that suppresses the effector immune responses of other immune cells and is crucial for the maintenance of peripheral tolerance. One $CD4^+$ T_{Reg}-cell subset is characterized by the expression of the transcription factor forkhead box P3 (FOXP3), whereas other types of T_{Reg} cell are normally characterized based on their expression of immunosuppressive cytokines such as interleukin-10 and transforming growth factor-β.

The ability of the TNF ligands to activate signalling pathways in professional APCs probably also contributes to their function. In particular, bidirectional signalling affects B-cell function and antibody responses and consequently has a strong effect on this arm of the immune response, although an in-depth discussion of this is outside the scope of this Review. Cross-linking of OX40L and CD70 results in the production of pro-inflammatory cytokines (including TNF, IL-1, IL-6 and IL-12) and the proliferation of DCs and B cells when they are also stimulated through TLRs, CD40 or membrane-expressed immunoglobulin molecules[17,48]. Binding of 4-1BBL to its receptor can mediate similar effects, such as increased cell division and increased production of TLR-induced pro-inflammatory cytokines by macrophages and DCs[49,50]. The differentiation of myeloid progenitors that express 4-1BBL is suppressed following ligation of its receptor[27], which might be due to the production of cytokines by the cell expressing 4-1BBL that in this case inhibit myeloid-cell development. Whether TL1A can signal has not yet been investigated. Although some of the signalling intermediates that are involved in TNF ligand-induced pathways have been described (FIG. 1), limited data are available to directly link these pathways with distinct cellular responses.

Functional effects in immune cells

Each of the four TNF–TNFR interactions can stimulate conventional T cells and APCs, mediate communication between $CD4^+$ and $CD8^+$ T cells and promote immune responses. These ligand–receptor pairs also mediate interactions between NK cells and T cells, between NKT cells and APCs presenting lipid antigens, and between T cells and other types of immune or tissue cell.

Co-stimulation of CD4+ and CD8+ T cells

Interactions between individual TNF–TNFR pairs control T-cell responses in two ways. First, they regulate the frequency of effector and/or memory $CD4^+$ or $CD8^+$ T cells that can be generated from naive T cells in response to antigen stimulation by providing proliferative and survival signals either directly to the T cells or to the APCs with which they interact. These molecules also regulate the frequency of effector memory T cells that are generated in recall responses. Second, they control T-cell function directly by promoting the production of cytokines such as IL-4 and IFNγ, or indirectly through stimulating the production of pro-inflammatory cytokines, such as IL-1 and IL-12, by professional or non-professional APCs[1-7,51-53]. It still not clear whether these interactions contribute to the acquisition of cytotoxic function by $CD8^+$ T cells, which has been indicated by some studies[11]; however, the regulation of T-cell expansion[54] might be a more important function to the overall activity of cytotoxic T cells. The interactions between these TNF superfamily members are primarily thought to deliver co-stimulatory signals, as their effects largely depend on antigen recognition and TCR signalling.

It has been directly shown in specific studies of immunity[55], or implied in studies of immune-mediated diseases (see below and TABLE 2), that several of these interactions collectively contribute to the overall response of a T cell. However, how the different effects of TNF–TNFR interactions are integrated in the control of T-cell responses is not clear, although a greater understanding of this is important for the design of new therapies that target TNF–TNFR interactions. Several models for how these interactions might contribute to T-cell responses are shown in FIG. 2. In the first scenario, the individual TNFRs act sequentially to promote continued T-cell proliferation, survival and effector functions over the course of an immune response. In this case, CD27, DR3, OX40 and 4-1BB would all sequentially contribute to the generation of large populations of effector T cells. Inhibiting any one of these molecules could then be effective for the treatment of autoimmune or inflammatory conditions, although establishing the optimal stage or time at which to target a given receptor during the disease process would be important. A second, more complex scenario involves the synergistic activity and temporal expression of these molecules. In this scenario, the sustained T-cell response would depend on both a signalling threshold that would be mediated by several different ligand–receptor interactions and multiple signals imparted by TNFRs at distinct time points. Blocking of a single interaction would markedly suppress the response, but the appropriate interaction to target would depend on both the type and stage of disease. Another complex scenario involves a situation in which many antigens (or autoantigens) are presented to several different T-cell populations that express and use different sets of TNFRs. In this case, an effective therapy to block such a heterogenous T-cell response would need to target at least two ligand–receptor interactions to inhibit the associated disease.

As discussed below, there are some examples in which blocking individual interactions in the same disease model can inhibit pathology and others in which a therapeutic effect is only observed if more than one interaction is neutralized. These data suggest that, although each molecule could be a good target for blocking a T-cell response, different molecules may be involved in different diseases, or the stage of disease when each molecule is relevant may vary. Systematic approaches that target each of these molecules at various points during disease progression are required to understand which of the models discussed above explain the proliferation and function of T-cell subsets in the context of a given inflammatory condition. It is probable that the involvement of the different TNFRs varies with the nature of the antigen being presented, the presence of pathogen-derived products and the innate inflammatory milieu that results when foreign or self antigen is recognized. Interestingly, most of the studies on CD27 and 4-1BB have examined the regulation of $CD8^+$ T-cell responses by these molecules, whereas more studies on OX40 and DR3 have been carried out with $CD4^+$ T cells. However,

it should also be noted that both $CD4^+$ and $CD8^+$ T cells have been reported to express all of the TNFRs, and the perceived dichotomy in the integration of TNF–TNFR interactions referred to above does not translate to all immune responses that have been examined so far. Therefore, there is not a single model describing the use of these molecules that applies to all immune responses.

Amplifying inflammatory responses through non-T cells

The functions of other immune-cell types, in addition to T cells, are also controlled by the TNF–TNFR interactions (FIG. 3, TABLE 1), which probably contribute to the amplification of the immune response. The neutralization of these molecules will therefore compound the effects of therapeutic targeting beyond that of T-cell inhibition. It has been reported that OX40L and OX40[56,57], CD70 and CD27 (REF. [58]), DR3 (REF. [59]) and 4-1BB[60] are involved in either directly enhancing NK-cell effector function (that is, cytotoxic ability and cytokine production) or in NK-cell-mediated help for the activation or differentiation of conventional T cells. Similarly, activated NKT cells express OX40 (REF. [61]), 4-1BB[62] and DR3 (REF. [51]), and signals transmitted through these receptors can directly increase NKT-cell activity by promoting either cell expansion or survival and by enhancing cytokine production. Through the production of IFNγ, NKT cells can also promote CD70 expression by DCs that can subsequently prime a conventional T-cell response[63], and a similar positive feedback mechanism that involves induction of IFNγ expression by NKT cells probably occurs if other TNFRs expressed by NKT cells are engaged.

The expression of CD27, CD70 (REF. [64]) and OX40L[65] is induced on most B cells and can promote B-cell proliferation and differentiation to antibody-secreting cells. In addition, the expression of OX40, OX40L, 4-1BB, 4-1BBL and CD70 can be induced on activated mast cells. OX40L expressed by mast cells can stimulate conventional T cells[66,67], and there is evidence (mainly from studies of 4-1BB) to support the idea that TNFR signalling in mast cells has a role in increasing the production of pro-inflammatory mediators by these cells[68]. One study also reported that neutrophils express OX40 and 4-1BB, ligation of which might contribute to tissue inflammation by increasing cell survival or the production of pro-inflammatory mediators[69]. Interestingly, adult lymphoid-tissue inducer cells have been shown to constitutively express DR3, and DR3 stimulation induced the expression of OX40L by these cells. This was proposed to be another amplification loop that might sustain T-cell responses or help to maintain the longevity of memory T cells[70,71].

Effects on T_{Reg} cells

The expression of OX40, CD27, 4-1BB and DR3 is either constitutive (in mice) or rapidly induced (in humans) on natural and inducible $CD4^+$ or $CD8^+$ T_{Reg} cells. Natural T_{Reg} cells express forkhead box P3 (FOXP3) and are selected in the thymus, whereas inducible T_{Reg} cells can differentiate from naive $CD4^+$ or $CD8^+$ T cells in the periphery in response to antigen and may or may not express FOXP3. Increasing the numbers or activity of T_{Reg} cells results in the suppression of immune responses, which is beneficial for the treatment of autoimmune and inflammatory conditions. By contrast, decreasing the numbers and function of T_{Reg} cells can enhance both innate and adaptive immune responses, which is beneficial for the treatment of cancer. Studies using mouse T_{Reg} cells have shown that ligation of the TNF superfamily members OX40 and 4-1BB affect these T-cell subsets (see below); these studies have been reviewed in detail elsewhere[72]. Regulation of T_{Reg} cells is therefore an important consideration for immunotherapy, although similar studies using human T_{Reg} cells are at present limited.

Lymphoid-tissue inducer cell

A cell that is present in developing lymph nodes, Peyer's patches and nasopharynx-associated lymphoid tissue. Lymphoid-tissue inducer cells are required for the development of these lymphoid organs. Adult lymphoid-tissue inducer cells have been proposed to orchestrate the development of tertiary lymphoid structures during inflammatory responses.

The effects of triggering OX40 and 4-1BB on T_{Reg} cells can result in either of two main outcomes that allow greater overall immune responsiveness (FIG. 4). First, signalling triggered by OX40 has been found to inhibit the development of $FOXP3^+$ T_{Reg} cells that differentiate from naive $CD4^+$ T cells in the presence of transforming growth factor-β (TGFβ)[73-75] and to suppress the differentiation of IL-10-producing ($FOXP3^-$) $CD4^+$ T_{Reg} cells from naive $CD4^+$ T cells[76]. Furthermore, ligation of OX40 negatively affects the stability of these cell populations, as it can lead to the downregulation of FOXP3 and IL-10 expression in newly differentiated inducible T_{Reg} cells and consequently result in their conversion to effector T cells (M.C., unpublished observations, and REF. [76]). So, ligation of OX40 has a dual effect on promoting T-cell responses: it enhances the proliferation of effector T cells and concomitantly blocks the generation of inducible T_{Reg} cells. However, whether signalling through CD27, 4-1BB or DR3 also affects the induction of peripheral T_{Reg} cells is not yet known but is of obvious interest. In addition, binding of OX40 and 4-1BB to their ligands has been shown to block the suppressive activity of T_{Reg} cells in culture systems that contained both inducible and natural T_{Reg} cells. This seems to be due to both a direct effect by blocking T_{Reg}-cell function and an indirect effect on effector T cells that renders them resistant to suppression[74,75,77-82]. Although the mechanistic explanation for both effects is currently unknown, the ability of TNFRs to overcome T_{Reg}-cell inhibition is in line with their activating effects on other immune-cell types. No studies on the effect of CD27 and DR3 on T_{Reg}-cell activity have been reported.

By contrast, other studies have shown that OX40 and 4-1BB can promote the proliferation or survival of $CD4^+$ and $CD8^+$ T_{Reg} cells[80,83-86]. These results were mainly obtained in experiments that used exogenous stimulation with agonists, so whether this effect occurs *in vivo* when endogenous ligands bind OX40 or 4-1BB is not clear. Although these results seem to conflict with the studies above on the effect of TNFRs on T_{Reg} cells, they are not necessarily mutually exclusive. The enhancement of cell proliferation and survival by TNFRs has been described in some studies of human natural T_{Reg} cells *in vitro* (see below), and this effect could be useful for clinical exploitation, as discussed below.

Therapeutic implications

Inflammatory and autoimmune diseases

To decrease immunopathology and inhibit disease progression, therapies for inflammatory or autoimmune diseases should aim to suppress the immune responses of T cells, ApCs, NK cells and NKT cells. Secondary and complementary to this aim, therapy should ideally allow the function, maintenance or generation of T_{Reg} cells to aid the long-term control of disease. Therefore, when considering TNFRs as therapeutic targets, a logical option would be to prevent their interactions with their TNF ligands, which would therefore decrease the expansion and survival of pathogenic cell populations and/or decrease their production of pro-inflammatory cytokines. In support of this concept, analysis of the development of autoimmune and inflammatory diseases in animals that are deficient for individual TNF ligands or TNFRs has revealed that elimination of these interactions decreases the severity of the disease (TABLE 2).

Preclinical studies have analysed the activity of neutralizing antibodies that are specific for TNF ligands, or of Fc fusion proteins that contain a TNFR that binds to the ligand and thereby

blocks the endogenous interaction. The effects of blocking each of the four ligand–receptor interactions discussed in this Review have been assessed in models of inflammatory disease (including allergy, asthma, transplantation, graft-versus-host disease (GvHD) and atherosclerosis) and autoimmune disease (including experimental autoimmune encephalomyelitis (EAE), diabetes, colitis, adjuvant- or collagen-induced arthritis, and systemic lupus erythematosus (SLE)) (TABLE 2). These studies have shown that neutralizing any one of these TNF–TNFR interactions can result in strong suppression of disease symptoms, which in many cases is specifically linked to decreased activity of $CD4^+$ or $CD8^+$ T cells, or in some cases to impaired NK- and NKT-cell function. Although most of the TNFRs or their ligands have been shown to be expressed by cells from patients with active autoimmune or inflammatory diseases, the relevance of some of the TNF–TNFR interactions that are being targeted in experimental disease models has not been investigated in human diseases (TABLE 2).

Another therapeutic approach by which to dampen inflammation is through the administration of depleting antibodies. These therapies target the ligand or receptor and directly eliminate the pathogenic cells that express a specific molecule. This approach involves either coupling of specific antibodies to toxins or the development of antibodies that have increased intrinsic mechanisms of antibody-dependent cell-mediated cytotoxicity or complement-dependent cytotoxicity. molecules such as OX40 and 4-1BB, and possibly DR3 and CD70, may be particularly suited to this approach given that their expression is limited and that they might be reliable markers of pathogenic cells. Studies in experimental models of auto-immunity, in which the depletion of OX40-expressing T cells led to a positive outcome, support this idea[87,88]. Furthermore, as NK and NKT cells might also express these molecules during disease, their depletion could improve the therapeutic outcome. However, it should be noted that a potential side effect of this type of therapy is increased susceptibility to infectious diseases.

Given that an important function of signalling induced by the ligation of OX40, 4-1BB, CD27 and DR3 is to promote cell survival, it follows that neutralizing antibodies that are specific for these molecules could also lead to apoptosis of immune cells (including T cells) that rely on these signals for survival during an immune response. Therefore, neutralization of these receptors should provide the same end result as the direct depletion strategies mentioned above. The finding that both depleting and non-depleting OX40–immunoglobulin fusion proteins were effective in suppressing disease in a model of colitis provides evidence that both approaches can produce the same overall result[89]. However, an important issue when considering depletion versus neutralization strategies that target TNFRs is whether they affect T_{Reg} cells. Accumulating evidence suggests that naturally occurring and inducible T_{Reg} cells are essential for the induction of immunological tolerance associated with transplantation and autoimmunity and for the protection against other inflammatory diseases such as asthma. As described above, recent studies of TNFRs suggest that blocking some of these molecules might result in greater differentiation of inducible T_{Reg} cells and/or increased functional activity of pre-existing naturally occurring and inducible T_{Reg} cells. By contrast, depleting reagents might eliminate both activated T_{Reg} cells and pathogenic cells, as the expression of these molecules by human T_{Reg} cells is upregulated following activation. Although depletion should still result in strong short-term benefits and inhibition of disease, the lack of T_{Reg}-cell activity might lead to re-established disease in the long term, as newly emerging pathogenic cells would not be controlled. Therefore, depletion versus neutralization should be carefully considered before clinical therapy.

Graft-versus-host disease

(GVHD). A disease that results from the immunological attack by donor allogeneic T cells that are transferred along with the allograft (such as bone marrow, liver or gut allografts) of target recipient organs or tissues (such as the skin and gut). GVHD occurs in graft recipients that cannot eliminate the host-reactive donor T cells owing to immunosuppression, immunological immaturity or tolerance of the recipient.

Experimental autoimmune encephalomyelitis

(EAE). An animal model of the human autoimmune disease multiple sclerosis. EAE is induced in experimental animals by immunization with myelin or peptides derived from myelin. The animals develop a paralytic disease with inflammation and demyelination in the brain and spinal cord.

Systemic lupus erythematosus

(SLE). An autoimmune disease in which autoantibodies that are specific for DNA, RNA or proteins associated with nucleic acids form immune complexes that damage small blood vessels, especially in the kidneys. Patients with SLE generally have abnormal B- and T-cell function.

Antibody-dependent cell-mediated cytotoxicity

A cytotoxic mechanism by which an antibody-coated target cell is directly killed by a leukocyte that expresses Fc receptors, such as a natural killer (NK) cell, macrophage or neutrophil. A specific receptor for the Fc region of IgG is CD16, which is expressed on the surface of most NK cells. Following binding to immunoglobulin, CD16 initiates a signalling cascade that results in the release of cytotoxic granules (containing perforin and granzyme B), which induce apoptosis of the antibody-coated cell.

Another issue is whether targeting a single TNF–TNFR interaction would be effective for the treatment of human diseases. A strong reduction in disease severity has been reported when OX40 or OX40L were absent or neutralized in most of the experimental mouse models of inflammatory and autoimmune disease that have been used; similar results were observed when DR3 or TL1A were absent or neutralized in EAE, asthma, arthritis and colitis models. more encouragingly, several studies have shown that targeting OX40L or TL1A is effective for suppressing ongoing disease in several different pre-clinical studies[51,52,87,88,90-98]. Collectively, the data suggest that targeting a single TNF–TNFR interaction might yield promising results for the effective treatment of human diseases. Surprisingly, there are limited data on how the absence or neutralization of CD70–CD27 or 4-1BBL–4-1BB interactions affects disease; targeting these interactions suppresses disease in mouse models of EAE, arthritis, GVHD and allograft rejection (TABLE 2), but reports on whether this therapy is effective in other inflammatory and autoimmune conditions have not been published. Given that the manipulation of CD70–CD27 and 4-1BBL–4-1BB interactions has a marked therapeutic effect in tumour models (see later) and that these molecules are involved in T-cell function in many instances[99-104], further preclinical studies that analyse the effects of blocking these molecules in other disease models are warranted.

There are, however, some studies that show no effect or only a moderate clinical benefit when a single TNF–TNFR interaction is manipulated. This does not necessarily mean that the interaction is irrelevant or that it is not a good therapeutic target. For example, in the context of transplantation, blocking OX40L alone is effective for limiting the rejection of grafts with minor MHC mismatches[105] but has no benefit in preventing the rejection of grafts that are fully MHC mismatched. However, blocking OX40L–OX40 interactions together with CD80/CD86–CD28 and/or CD40L–CD40 interactions is effective for preventing the rejection of fully

MHC mismatched grafts[75,106,107]. Similar results have been obtained by targeting CD70 (REF. [108]) or 4-1BBL[109] together with CD28 or CD40L in mouse models of transplantation. The fact that blocking multiple TNFRs, or a TNFR and an immunoglobulin superfamily member such as CD28, was more effective than blocking a single TNFR in these studies might indicate either that the T cells that are involved in graft rejection simultaneously express several signalling receptors or that several different subsets of T cells — each regulated by different TNF–TNFR interactions — are involved in the response (FIG. 3). Thus, these data suggest that a significant effect on the immune response in some inflammatory conditions will only occur when multiple interactions are targeted.

Studies of 4-1BB have explored an alternate therapeutic strategy that relies on stimulatory rather than neutralizing reagents to inhibit the activity of pathogenic cells. In this case, agonist antibodies that are specific for 4-1BB suppress disease in mouse models of EAE, asthma, arthritis, SLE, colitis, diabetes and GvHD (TABLE 2). Although there is not yet a consensus on how stimulation of 4-1BB prevents disease, most of the evidence suggests that its effects are related to the expansion of either natural or inducible T_{Reg} cells (either $CD4^+$ or $CD8^+$ T cells), or to the hyperactivation of IFNγ-producing $CD8^+$ T cells that acquire regulatory capacity[110-114]. However, this approach has not been tested extensively and there are no reports indicating that reagents which stimulate OX40, DR3 or CD27 have similar inhibitory effects on inflammatory disease. This raises the question of whether 4-1BB is fundamentally different from other TNFRs in terms of the signalling pathways it induces or in terms of its expression profile. Alternatively, it is possible that the antibody used in these studies has specific properties, such as the ability to act as a superagonist, thereby mediating the suppression of disease.

Despite these promising results, it is counter-intuitive to suggest that reagents which enhance signalling through a co-stimulatory receptor can also suppress inflammatory and autoimmune diseases. Therefore, caution is needed when considering the use of a 4-1BB-specific agonist antibody to treat inflammatory diseases, as pathogenic effector T cells might be stimulated in addition to T_{Reg} cells. Indeed, a clinical trial that tested a superagonist antibody specific for CD28 that was expected to promote the expansion of the T_{Reg}-cell population without augmenting the activity of other T cells had disastrous results[115]. However, the finding that stimulatory TNFR reagents can expand T_{Reg} cells might be useful for cellular therapy approaches. Adoptive transfer of T_{Reg} cells is being considered as a treatment for autoimmune disease, although a limiting factor is that it is difficult to obtain sufficient numbers of functional T_{Reg} cells to suppress pathogenic cells *in vivo*. Reagents that expand T_{Reg} cells *in vitro*, such as TNFR-specific agonists and ApCs that are engineered to express OX40L, 4-1BBL and CD70, are currently being tested as strategies to achieve this; however, an important outstanding question with this approach is whether the T_{Reg} cells will retain their suppressive function once they have been adoptively transferred to a patient[85,86,116].

Complement-dependent cytotoxicity

A mechanism by which a monoclonal antibody binds complement, leading to direct cell toxicity and complement-mediated killing of the cell to which the antibody is bound. The result is a membrane attack complex that makes a hole within the cell membrane, causing cell lysis and death.

Tolerance

A term that denotes lymphocyte non-responsiveness to antigen, but implies an active process, not simply a passive lack of response.

Studies of cancer

Therapy for cancer should aim to promote the antitumour activity of T cells, ApCs, NK cells and NKT cells, to kill existing tumour cells and to promote immunological memory that protects against recurring tumours. In addition, antitumour therapy should ideally prevent the generation and/or function of T_{Reg} cells. Numerous mouse studies have investigated the effectiveness of agonists or stimulatory Fc fusion proteins that express the extracellular portion of TNF ligands and cross-link TNFRs to trigger productive signalling[117,118] (TABLE 3). As with most tumour studies, the level of protection achieved with the various approaches is highly variable, largely depending on the tumour model used. In most reports, protective antitumour responses are associated with increased effector activity of $CD4^+$ and/or $CD8^+$ T cells, as well as NK and NKT cells. Because the potential effect of stimulating TNFRs on T_{Reg} cells has only recently been recognized, it is not clear whether the approaches used in these studies also modulate T_{Reg} cells. However, a recent report of OX40 suggests that a proportion of the antitumour effect is probably mediated through the inhibition of T_{Reg} cells[81]. The effects of targeting DR3 in the context of cancer have yet to be investigated.

An important issue in antitumour therapy is determining which type of agonist will be most effective. Studies that use more stringent (that is, less immunogenic) tumour models have indicated that treatment with a single stimulatory agonist is not effective and that co-administration of a second agonist or another factor that stimulates T-cell function, such as granulocyte/macrophage colony-simulating factor (Gm-CSF) or IL-12 (REFS [119-121]), results in significantly greater antitumour reactivity. Furthermore, Fc fusion proteins may have better activity than antibodies[122]. The development of reagents that are hexameric[123,124] and/or directly linked to antibody fragments that specifically target the tumour or tumour site[124, 125], or the development of RNA aptamers[126,127], may lead to more effective therapeutic options. In addition, these reagents may have fewer safety issues as they would be less likely to induce a robust immune response against the reagent or to cause sustained immune responses that lead to side effects such as a cytokine storm. Although the development of auto-immune disease symptoms is an inherent risk of tumour immunotherapy, the benefits will probably outweigh the risks for most patients with cancer. As the expression of OX40 and 4-1BB is mainly restricted to activated immune cells, targeting these molecules is less likely to induce autoimmune side effects than targeting molecules such as CD27, which is constitutively expressed.

Additional antitumour strategies involve the transduction of tumour cells with CD70, OX40L or 4-1BBL using viral vectors that express the genes encoding these molecules. This can be carried out *ex vivo*, followed by re-implantation of the tumour cells into the host, or *in vivo* by directly injecting the viral vector into the tumour. This approach causes the tumour to become more immunogenic and leads to the stimulation of an immune response. A combined approach, such as the transduction of a TNF ligand together with Gm-CSF, IL-12 or CD80 into the tumour has also been found to be effective[128-130]. Cellular therapy with DCs engineered to express TNF ligands to boost antitumour T-cell responses is another strategy that has shown some success in mouse models[131,132]. Although cellular therapy approaches can be more complicated than treatment that involves a simple injection of an agonist, they have the potential to be more specific owing to the ability of DCs to present tumour antigens and to thereby target T cells that are specific for the tumour.

Finally, strategies to directly kill tumour cells by targeting them with antibodies specific for CD70 are currently being developed for clinical testing. In contrast to the limited expression of this molecule by normal cells, many tumours express CD70, and targeting $CD70^+$ tumours with depleting or drug-conjugated CD70-specific antibodies has shown promising results in experimental models of cancer[133,134]. However, similar to other depletion strategies, there is

the inherent potential that activated DCs, B cells and T cells may also be depleted, thereby putting the patient at risk of infection.

In summary, targeting any of the four TNF–TNFR interactions discussed here has great therapeutic potential based on the concept that the continued activity of an effector cell (pathogenic or protective) requires signals from these molecules to maintain cell division, allow survival and promote cytokine production. The fact that several of the TNF superfamily members are inducibly expressed during the active phase (or phases) of disease or inflammation means that these molecules might be better therapeutic targets than more broadly expressed cell surface molecules.

Concluding remarks

Accumulating evidence has shown that many TNF superfamily molecules have a central role in immune regulation, immune-mediated diseases and cancer. The four interactions that are discussed here are only a part of the bigger superfamily; other co-stimulatory interactions, such as those of CD30, TNFR2, Hvem (herpes-virus entry mediator; also known as TNFRSF14), GITR (glucorticoid-induced TNFR-related protein; also known as TNFRSF18), TACI (also known as TNFRSF13B), CD40 and lymphotoxin-β receptor with their ligands, are equally noteworthy. It is clear that the interactions between OX40, 4-1BB, CD27 or DR3 with their ligands have important roles in controlling T-cell function and the interactions of T cells with other immune cells. In addition, preclinical studies in mouse models indicate that the therapeutic targeting of these interactions might have the same potential as targeting TNF, which has proven so successful.

RNA aptamer

An oligonucleotide sequence that has the ability to recognize virtually any class of target molecules with high affinity and specificity. RNA aptamers are emerging as a class of molecules that rival antibodies in terms of therapeutic and diagnostic applications. They provide some advantages over antibodies, as they can be produced by chemical synthesis, have better storage properties and are less immunogenic.

Cytokine storm

A sudden surge in the circulating levels of pro-inflammatory cytokines, such as interleukin-1 (IL-1), IL-6, tumour necrosis factor and interferon-γ.

There are still great gaps in our knowledge regarding the activity, expression characteristics and involvement of these different molecules at various stages of the immune response and over the course of disease. Importantly, it is still unresolved how similar or dissimilar each of these receptors are to one another in terms of the signalling complexes they form, the level of signalling that each one triggers and whether different TNF–TNFR interactions induce different cellular responses. In the case of therapy, it is not clear which molecule will be the best target; this is a particularly hard question to address given the difficulty in translating preclinical mouse studies to humans. To better resolve these issues, further studies in animal models of disease are warranted that mimic specific stages of human disease more closely, and targeting combinations of these family members should be carried out to determine the extent of cooperation and overlap between these four ligand–receptor pairs. So far, clinical trials are in progress to test agonist antibodies that are specific for OX40 and 4-1BB in patients with cancer, OX40L-specific neutralizing antibodies in patients with asthma and CD70-specific depleting antibodies in patients with autoimmune disease and cancer. Further research will

provide new information regarding the potential usefulness of these molecules in different clinical applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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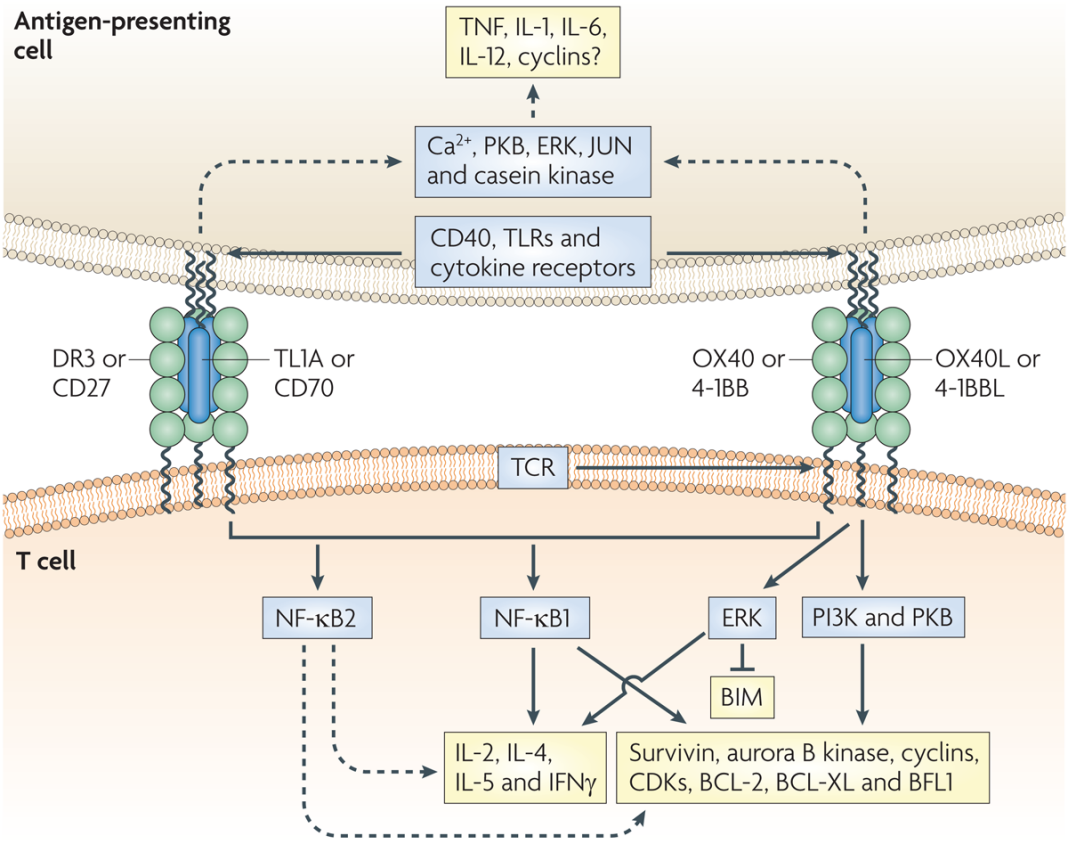

Figure 1. TnF–TnFR family interactions and molecular targets in T cells and APCs

Tumour necrosis factor receptors (TNFRs) are characterized by several cysteine-rich domains, and TNF ligands are characterized by a TNF homology domain. Both OX40 ligand (OX40L) and TL1A are homotrimers (that is, three receptor monomers bind to the trimeric ligand), and this molecular arrangement probably applies to interactions between CD70 and CD27, 4-1BBL and 4-1BB, and TL1A and death receptor 3 (DR3). During interactions between T cells and antigen-presenting cells (APCs), the expression of TNF ligands by the APC is probably induced following activating signals from either CD40 (when bound to CD40L expressed by a T cell) or from Toll-like receptor (TLR)-mediated signals. The ligation of cytokine receptors by cytokines such as TNF, interleukin-1 (IL-1), IL-6, IL-12, IL-18 and thymic stromal lymphopoietin (not shown) can also promote TNF ligand expression. The expression of OX40 and 4-1BB can be induced by activation signals from the T-cell receptor (TCR) following recognition of peptide–MHC complexes. The main common downstream signalling event triggered by TNFRs is the activation of nuclear factor-κB 1 (NF-κB1), which leads to cell division and enhanced survival and can contribute to the production of cytokines, such as IL-2, IL-4, IL-5 and interferon-γ (IFNγ). NF-κB2 can also be activated downstream of these TNFRs, although its primary function in cellular responses is not clear. Other signalling molecules that have been described to be activated following TNF-–TNFR interactions include phosphoinositide 3 kinase (PI3K), protein kinase B (PKB), extracellular-signal-regulated kinase (ERK), JUN N-terminal kinase (JNK) (not shown) and nuclear factor of activated T cells (NFAT) (not shown), which also contribute to cell division, survival and cytokine production. Triggering of any TNFR might lead to the expression of other proteins that promote proliferation, including survivin, aurora B kinase, cyclins and cyclin-dependent kinases (CDKs), as well as the expression of anti-apoptotic proteins, including BCL-2 (B-cell lymphoma 2), BCL-XL, BFL1 (BCL-2-related protein A1), and/or the downregulation of the

expression of pro-apoptotic proteins, such as BIM (BCL-2-interacting mediator of cell death). Signals downstream of the TNF ligands can promote the secretion of pro-inflammatory cytokines by APCs, such as TNF, IL-1, IL-6 and IL-12, and lead to cellular proliferation.

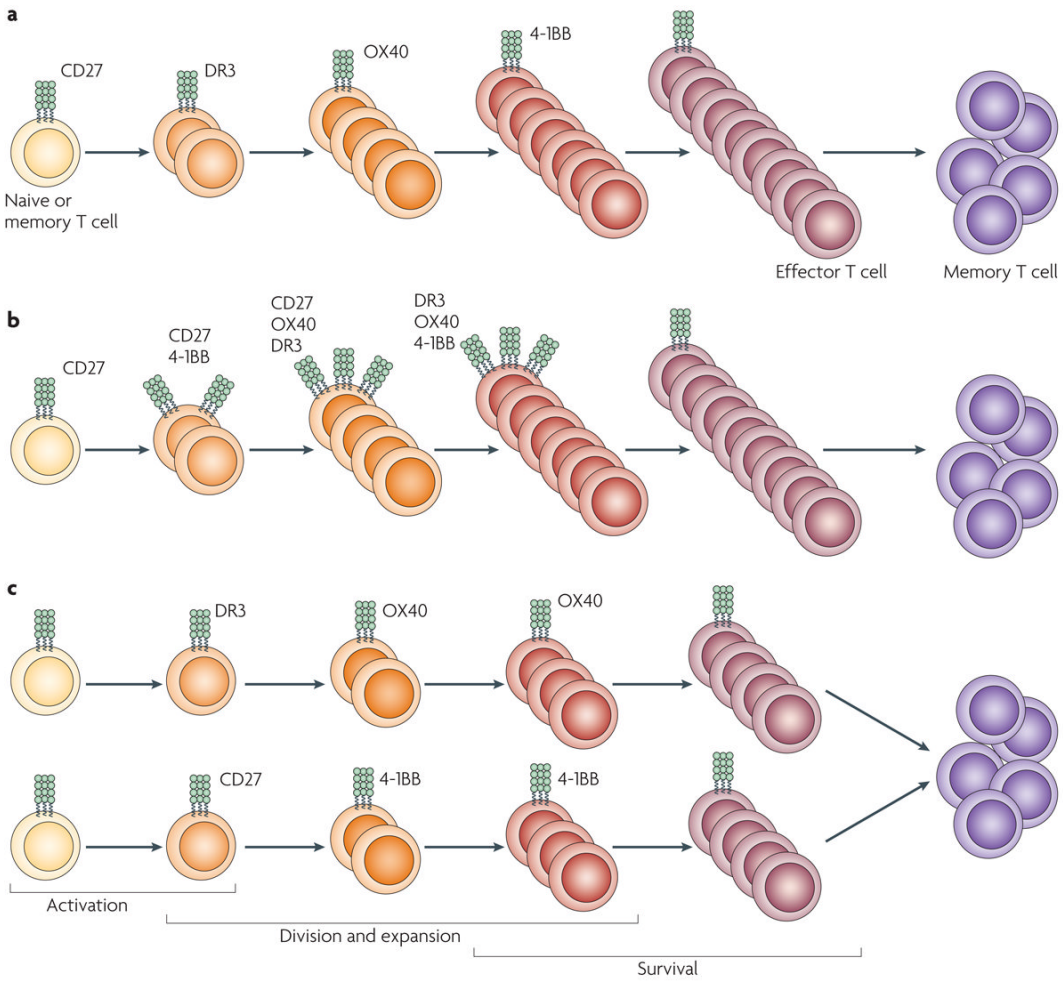

Figure 2. Control of T-cell proliferation by cooperative and sequential TNF–TNFR interactions

A hallmark of T-cell co-stimulation by the tumour necrosis factor receptors (TNFRs) OX40, 4-1BB, CD27 or DR3 is the expansion of the effector T-cell population (during the primary response and/or the secondary and memory response). However, the extent of cooperation between these individual ligand–receptor pairs over the course of most T-cell responses is not clear. Three non-mutually exclusive models that represent what might occur are shown. The involvement of the TNFRs probably varies depending on the inflammatory environment in which antigen recognition takes place and the nature and number of antigens recognized. **a**| Step-wise involvement of TNFRs, whereby the temporal activity of individual receptors increases and sustains T-cell survival and proliferation. In this case, the interactions of all of the different TNFRs with their ligands are crucial for generating large effector T-cell populations, and inhibiting any one will markedly suppress the response. **b**| A more complex scenario that involves synergistic action of different TNFRs, whereby several ligand–receptor interactions can function simultaneously, as well as sequentially. In this case, continued T-cell survival and proliferation depend on a threshold level of signalling imparted by multiple receptors. Removing any single interaction would, again, markedly reduce the response. **c**| A scenario that might explain the involvement of TNFRs in promoting a T-cell response when many antigens or autoantigens are expressed. Several populations that express different TNFRs would be involved in the response (including $CD4^+$ and $CD8^+$ T cells specific for various epitopes). If only one population is involved, blocking a single TNF–TNFR interaction would suppress the response, although which interactions are involved probably varies. However,

when several T-cell populations are active, pronounced suppression would require targeting two or more interactions. DR3, death receptor 3.

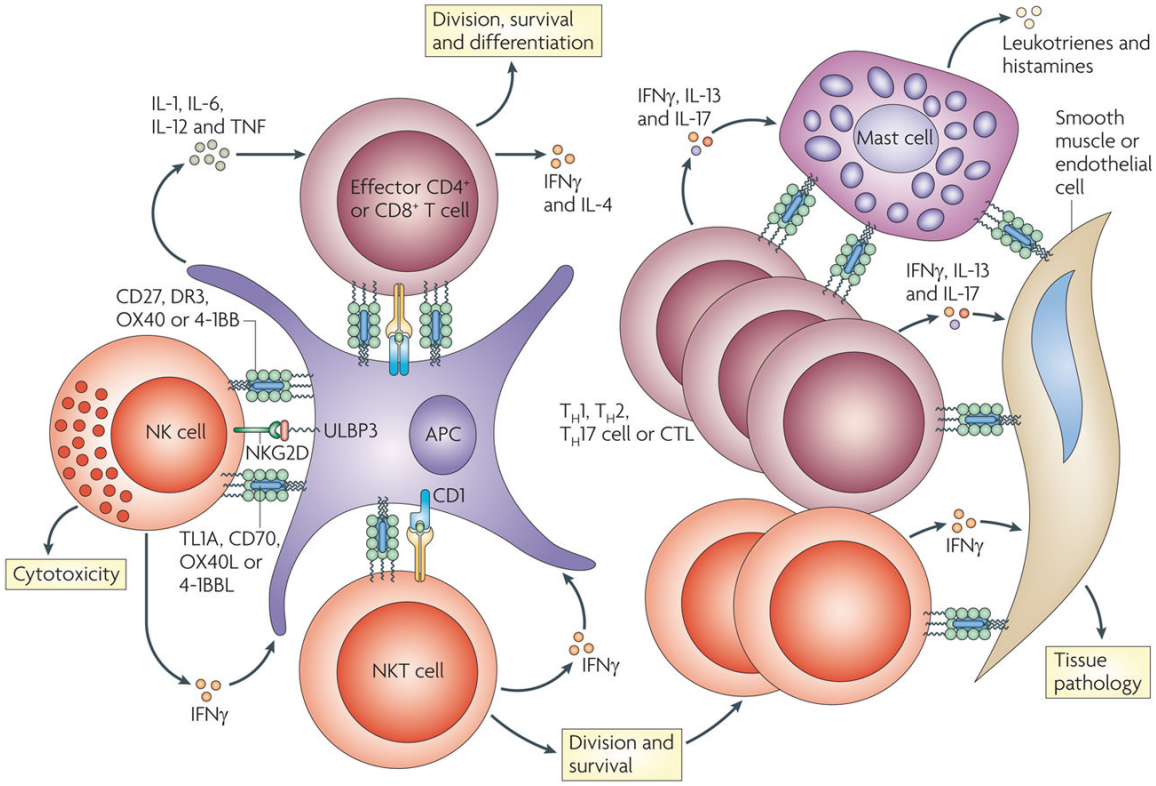

Figure 3. TNF–TNFR family interactions regulate many cell types of amplify inflammation

Effector T cells receive signals for division, survival and cytokine production following the activation of the tumour necrosis factor receptors (TNFRs) OX40, 4-1BB, CD27 and death receptor 3 (DR3) by their ligands. In addition, natural killer (NK) and NKT cells can also receive signals through TNFRs that amplify division, survival and cytokine production. The initiation of inflammatory responses can involve cooperation between NK and NKT cells with effector T cells, which might occur directly or indirectly through antigen-presenting cells (APCs). Feedback mechanisms can occur through NK- or NKT-cell-derived interferon-γ (IFNγ), which enhances APC activation in many ways, including promoting the expression of TNF ligands. In addition, activated mast cells can express many ligands, including OX40 ligand (OX40L) and 4-1BBL, which can co-stimulate effector T cells and NKT cells. The production of pro-inflammatory cytokines, such as IFNγ, interleukin-13 (IL-13) and IL-17, by T cells also can promote the expression of one or several TNF ligands on tissue cells such as endothelial, epithelial and smooth muscle cells. Through additional bidirectional signals with effector T cells, NK cells or NKT cells, these interactions probably further amplify tissue pathology, for example, by inducing the production of additional pro-inflammatory mediators such as leukotrienes and histamine. CTL, cytotoxic T cell; NKG2D, NK group 2, member D; T_H, T helper; ULBP3, cytomegalovirus UL16-binding protein.

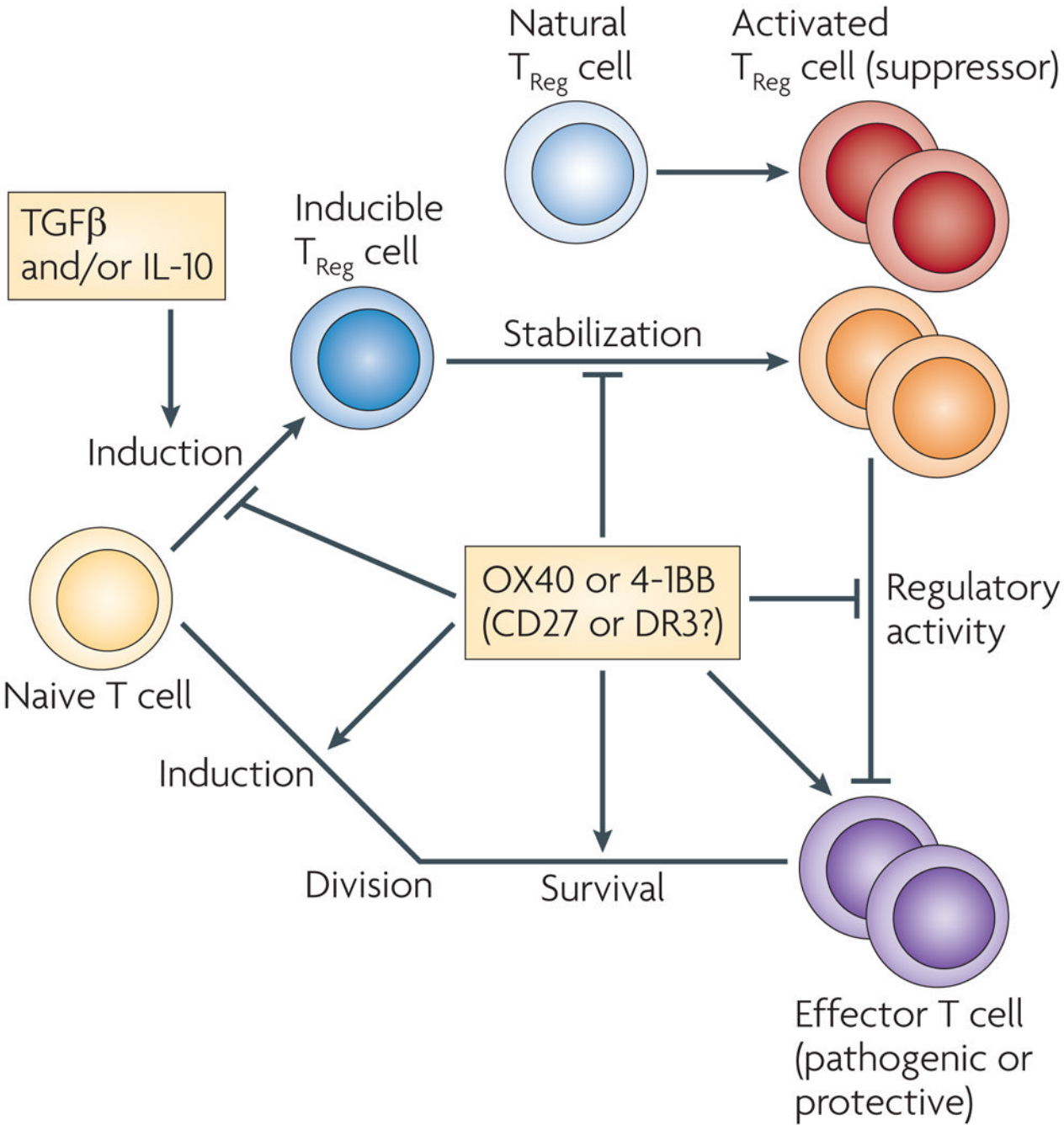

Figure 4. modulation of T_{Reg}-cell development and function by TNF–TNFR interactions

In addition to promoting the activation of effector T cells, the interaction between the tumour necrosis factor receptors (TNFRs) OX40, 4-1BB, CD27 and DR3 and their ligands might further contribute to inflammation by affecting naturally occurring or inducible regulatory T (T_{Reg}) cells. To date, only the effect of OX40 or 4-1BB ligation on T_{Reg}-cell development and function has been examined, although the fact that the different receptors can use common signalling pathways (including the nuclear factor-κB and protein kinase B pathways) means that it is possible that DR3 and CD27 have similar effects. Signals triggered following the activation of OX40 inhibit the expression of forkhead box P3 (FOXP3) and interleukin-10 (IL-10) by naive $CD4^+$ T cells that are differentiating into T_{Reg} cells by an unknown

mechanism, which might involve blocking or modulation of the signalling events downstream of transforming growth factor-β receptor (TGFβR), IL-10R or vitamin D receptor (not shown). OX40 can also reduce the stability of T_{Reg} cells, as ligation of OX40 can lead to the downregulation of FOXP3 and IL-10 expression in recently differentiated T_{Reg} cells. This may occur directly, or indirectly through promoting the production of cytokines by T helper cells, which in turn induce the expression of transcription factors such as GATA-binding protein 3 that prevent FOXP3 and/or IL-10 expression (not shown). Fully differentiated inducible $CD4^+$ and $CD8^+$ T_{Reg} cells and natural $CD4^+$ T_{Reg} cells also express OX40, 4-1BB, CD27 and DR3. OX40 and 4-1BB signals have been shown to block the suppressive function of these cells, again either directly through effects on the T_{Reg} cell itself, or indirectly by promoting the proliferation and survival of effector T cells and by rendering them resistant to T_{Reg}-cell-mediated suppression. The combined action of these TNF–TNFR interactions might lead to an increased ratio of effector T cells to T_{Reg} cells (that is, too few T_{Reg} cells to suppress the inflammatory response) and/or to greater effector T-cell activity through blocking of T_{Reg}-cell-mediated suppression. Activation of the TNFRs might also promote the expansion or survival of T_{Reg} cells (not shown), as shown in some *in vitro* systems with agonist stimulation, although studies of knockout animals do not as yet support this expansion as a physiological activity. DR3, death receptor 3.

Table 1

Expression profile of some TNF superfamily members

ligand–receptor pair	Ligand expressed by	Conditions of ligand expression	Receptor expressed by	Conditions of receptor expression
CD70–CD27	APCs (DCs and B cells)	Inducible (triggered by TLR4, TLR9, CD40, Ig or BCR, and IFNγ)	$CD4^+$ and $CD8^+$ T cells	Constitutive
	$CD4^+$ and $CD8^+$ T cells	Inducible (triggered by TCR plus CD28 cross-linking, IL-1, IL-2, TNF and IL-12)	B cells (subset)	Inducible
	Mast cells	Inducible (?)	NK cells (subset)	Constitutive
	NK cells	Inducible (?)	$FOXP3^+$ T_{Reg} cells	Constitutive (mice) or inducible (humans; triggered by TCR cross-linking)
	Smooth muscle cells	Inducible (?)	NKT cells	Constitutive
	Thymic epithelial cells	Constitutive	Haematopoietic progenitors	Constitutive
TL1A–DR3	APCs (DCs, B cells and macrophages)	Inducible (triggered by TLR4 and FcγR cross-linking)	$CD4^+$ T cells	Constitutive or inducible (triggered by TCR plus CD28 cross-linking)
	$CD4^+$ and $CD8^+$ T cells	Inducible (triggered by TCR cross-linking, IL-12 and IL-18)	$CD8^+$ T cells	Inducible (triggered by TCR plus CD28 cross-linking)
	Endothelial cells	Inducible (triggered by TNF and IL-1)	NK cells	Inducible (triggered by IL-12 plus IL-18)
			NKT cells	Constitutive
			$FOXP3^+$ T_{Reg} cells	Constitutive (mice) or inducible (humans; triggered by TCR cross-linking)
			LTi cells	Constitutive
OX40L–OX40	APCs (DCs, B cells and macrophages)	Inducible (triggered by TLR2, TLR4, TLR9, CD40, Ig or BCR, TSLP and IL-18)	$CD4^+$ and $CD8^+$ T cells	Inducible (triggered by TCR plus CD28 cross-linking, IL-1, IL-2 and TNF)
	$CD4^+$ and $CD8^+$ T cells	Inducible (triggered by TCR cross-linking and IL-12)	NK cells	Inducible (?)
	LTi cells	Inducible (triggered by DR3)	NKT cells	Inducible (triggered by TCR cross-linking)
	NK cells	Inducible (triggered by NKG2D and CD16)	$FOXP3^+$ T_{Reg} cells	Constitutive (mice) or inducible (humans; triggered by TCR cross-linking)
	Mast cells	Inducible (triggered by GM-CSF and FcεRI cross-linking)	Neutrophils	Inducible (?)
	Endothelial cells	Inducible (?)		

ligand–receptor pair	Ligand expressed by	Conditions of ligand expression	Receptor expressed by	Conditions of receptor expression
	Smooth muscle cells	Inducible (?)		
4-1BBL–4-1BB	APCs (DCs, B cells and macrophages)	Inducible (triggered by TLR4, TLR9, CD40 and Ig or BCR)	$CD4^+$ and $CD8^+$ T cells	Inducible (triggered by TCR plus CD28 cross-linking and IL-2)
	$CD4^+$ and $CD8^+$ T cells	Inducible (triggered by TCR cross-linking)	NK cells	Inducible (?)
	Mast cells	Inducible (triggered by FcεRI cross-linking)	NKT cells	Inducible (triggered by TCR cross-linking)
	NK cells	Inducible (?)	Mast cells	Inducible (triggered by FcεRI cross-linking)
	Smooth muscle cells	Inducible (?)	Neutrophils	Inducible (?)
	Haematopoietic progenitors	Constitutive	$FOXP3^+$ T_{Reg} cells	Constitutive (mouse) or inducible (human; triggered by TCR cross-linking)

APC, antigen-presenting cell; BCR, B-cell receptor; DC, dendritic cell; DR3, death receptor 3; FcγR, receptor for IgG; FcεRI, high-affinity Fc receptor for IgE; FOXP3, forkhead box P3; GM-CSF, granulocyte/macrophage colony-stimulating factor; IL, interleukin; IFNγ, interferon-γ; Ig, membrane-bound immunoglobulin; LTi, lymphoid-tissue inducer; NK, natural killer; NKG2D, NK group 2, member D; TCR, T-cell receptor; TLR, Toll-like receptor; TNF, tumour necrosis factor; TSLP, thymic stromal lymphopoietin.

Table 2

Therapeutic targeting of TNF superfamily interactions

Model	Interaction targeted	Mice or reagent tested	Effect on disease symptom
EAE	OX40L–OX40	Toxin-conjugated OX40-specific antibody (depleting)	Substantial inhibition
		OX40–immunoglobulin fusion protein (neutralizing)	Substantial inhibition
		OX40L-specific antibody (neutralizing)	Substantial inhibition
		Ox40$^{-/-}$ or *Ox40l*$^{-/-}$ mice	Substantial inhibition
	TL1A–DR3	*Dr3*$^{-/-}$ mice	Substantial inhibition
		Tl1a$^{-/-}$ mice	Partial inhibition
	CD70–CD27	CD70-specific antibody (neutralizing)	Substantial inhibition
	4-1BBL–4-1BB	4-1BB-specific antibody (agonist)	Substantial inhibition
Colitis and IBD	OX40L–OX40	OX40–immunoglobulin fusion protein (neutralizing and depleting)	Substantial inhibition
		OX40L-specific antibody (neutralizing)	Substantial inhibition
	TL1A–DR3	TL1A-specific antibody (neutralizing)	Partial inhibition
	4-1BBL–4-1BB	4-1BB-specific antibody (agonist)	Substantial inhibition
Asthma and atopy	OX40L–OX40	*Ox40*$^{-/-}$ or *Ox40l*$^{-/-}$ mice	Substantial inhibition
		OX40L-specific antibody (neutralizing)	Substantial inhibition
	TL1A–DR3	*Dr3*$^{-/-}$ mice	Substantial inhibition
		TL1A-specific antibody (neutralizing)	Substantial inhibition
	4-1BBL–4-1BB	4-1BB-specific antibody (agonist)	Substantial inhibition
Diabetes	OX40L–OX40	*Ox40l*$^{-/-}$ mice	Substantial inhibition
		OX40L-specific antibody (neutralizing)	Substantial inhibition
	4-1BBL–4-1BB	4-1BB-specific antibody (agonist)	Substantial inhibition
Arthritis	OX40L–OX40	OX40L-specific antibody (neutralizing)	Substantial inhibition
		Toxin-conjugated OX40-specific antibody (depleting)	Partial inhibition
	TL1A–DR3	*Dr3*$^{-/-}$ mice or TL1A-specific antibody (neutralizing)	Partial inhibition
	4-1BBL–4-1BB	4-1BB-specific antibody (agonist)	Substantial inhibition
		4-1BBL-specific antibody (neutralizing)	Partial inhibition
SLE	4-1BBL–4-1BB	4-1BB-specific antibody (agonist)	Substantial inhibition
Atherosclerosis	OX40L–OX40	*Ox40l*$^{-/-}$ mice	Substantial inhibition
		OX40L-specific antibody (neutralizing)	Substantial inhibition
Minor MHC transplant mismatch	OX40L–OX40	OX40–immunoglobulin fusion protein (neutralizing)	Substantial inhibition
	4-1BBL–4-1BB	4-1BB–immunoglobulin fusion protein (neutralizing)	Partial inhibition
Major MHC transplant mismatch	OX40L–OX40	OX40–immunoglobulin fusion protein (neutralizing)	No effect
		OX40L-specific antibody (neutralizing)	Substantial inhibition with CD28 or CD28 and CD40L blockade
	CD70–CD27	CD70-specific antibody (neutralizing)	No effect alone; no effect with CD28 and

Model	Interaction targeted	Mice or reagent tested	Effect on disease symptom
			CD40L blockade; substantial inhibition with CD4 and CD28 blockade
	4-1BBL–4-1BB	*4-1bbl*$^{-/-}$ or *4-1bbl*$^{-/-}$ mice	Varying results: no effect or inhibition
		4-1BB–immunoglobulin fusion protein (neutralizing)	Partial inhibition
GVHD	OX40L–OX40	OX40L-specific antibody (neutralising)	Substantial inhibition
		Ox40$^{-/-}$ mice	Substantial inhibition
	4-1BBL–4-1BB	*4-1bb*$^{-/-}$ mice	Substantial inhibition
		4-1BB-specific antibody (agonist)	Substantial inhibition

There is an online version of this table that includes references. See Supplementary information S1 (Table). No published reports are available for the interactions that are not mentioned. 4-1BBL, 4-1BB ligand; DR3, death receptor 3; EAE, experimental autoimmune encephalomyelitis; GHVD, graft-versus-host disease; IBD, inflammatory bowel disease; SLE, systemic lupus erythematosus.

Table 3

Therapeutic targeting of TNF superfamily members in cancer

Mode of therapy	Target	Combination treatment	Tumour type
Stimulatory antibody or ligand Fc protein	OX40	NA	Sarcoma, melanoma, glioma, colon carcinoma, mammary carcinoma, thymoma and renal-cell carcinoma
		Adoptive transfer of CTLs	Sarcoma, thymoma and prostate tumour
		Administration of IL-12 and 4-1BB-specific antibody	Colon carcinoma
		Administration of GM-CSF	Colon and breast carcinoma
		Tumour transfection with CD80	B-cell lymphoma
		Administration of DC vaccine and 4-1BB-specific antibody	Breast carcinoma
		Administration of GM-CSF and tumour antigen vaccine	Breast tumour
		Administration of IL-12	Sarcoma and prostate tumor
	4-1BB	NA	Sarcoma, mastocytoma, glioma, colon carcinoma and B-cell lymphoma
		Administration of IL-12	Colon carcinoma and melanoma
		Adoptive transfer of CTLs	Plasmacytoma
		FLT3L-mediated DC mobilization	Fibrosarcoma
		HLA-DR- and CD40-specific antibodies	Renal carcinoma and mammary carcinoma
		5-fluorouracil	Renal carcinoma
	CD27	NA	B-cell lymphoma
Transfection of tumour cells	OX40L	Administration of GM-CSF	Melanoma, lung carcinomathymoma and colon carcinoma
	4-1BBL	CD80 co-transfection	Sarcoma and colon carcinoma
		CD80 and CD86 co-transfection	B-cell lymphoma
		IL-12 co-transfection	Colon carcinoma
		CD80	Squamous-cell carcinoma
		CD80, CD40L and CD48 co-transfection	T-cell lymphoma
		Adoptive transfer of LAK cells and NK cells	Adenocarcinoma
		TRANCE, CD95L and CCL21 co-transfection	T-cell lymphoma
		Soluble PD1 co-transfection	Hepatocarcinoma
	Single-chain Fv fragments specific for 4-1BB	NA	Melanoma and mammary carcinoma
	CD70	NA	Sarcoma, mastocytoma, colon carcinoma, thymoma, lymphoma, mammary adenocarcinoma and glioma
		CD80 co-transfection	Melanoma and mammary adenocarcinoma
		CD40L co-transfection	Melanoma

Mode of therapy	Target	Combination treatment	Tumour type
Transfection of DCs	OX40L	NA	Melanoma, thymoma and melanoma
	4-1BBL	NA	Colon carcinoma and adenocarcinoma
	CD70	NA	Thymoma
Stimulatory RNA aptamer	OX40	Administration of DC vaccine	Melanoma
	4-1BB	NA	Mastocytoma
Depleting antibody	CD70	NA	B-cell lymphoma, renal carcinoma and non-Hodgkin lymphoma

There is an online version of this table that includes references. See Supplementary information S1 (Table). 4-1BBL, 4-1BB ligand; CCL21, CC-chemokine ligand 21; CD95L, CD95 ligand; CTL, cytotoxic T lymphocyte; DC, dendritic cell; DR3, death receptor 3; FLT3, FMS-related tyrosine kinase 3; GM-CSF; granulocyte/macrophage colony-stimulating factor; IL, interleukin; LAK, lymphokine activated killer; NA, not applicable; NK, natural killer; PD1, programmed cell death 1; TNF, tumour necrosis factor; TRANCE, TNF-related activation-induced cytokine.