Immunobiology of CD30+ T Lymphocytes

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CD30 (Ki-1) was originally described in 1982 as a molecule expressed on Hodgkin and Reed-Sternberg cells in Hodgkin's Disease. Shortly thereafter it was discovered that CD30 expression also marks a distinct category of non-Hodgkin's lymphoma termed CD30-positive anaplastic large cell lymphoma (ALCL). We now know that CD30 is expressed more broadly and can be found on activated B and T lymphocytes, virally-infected cells, and neoplasias of lymphoid origin. The ligand for CD30 (CD30L or CD153) is expressed by activated T cells, B cells, macrophages, neutrophils, eosinophils, and Hassal's corpuscles and epithelial cells of the thymic medulla. cDNA cloning and sequencing of CD30 and CD30L revealed that they are members of the tumor necrosis factor receptor (TNFR) and TNF families, respectively. The physiologic function of CD30 in activated T cells has recently been a topic of great interest. Engagement of CD30 on T cells by CD30L or agonist antibodies leads to activation of the TNFR-associated factors (TRAF) signaling pathway and a variety of functional outcomes ranging from enhanced proliferation to induction of apoptosis. This review will describe the structural and functional features of CD30 and discuss emerging evidence that CD30+ T cells may play a role in the regulation of immune responses.

ABBREVIATIONS:

Th2	T helper type 2
TNFR	tumor necrosis factor receptor
TRAF	TNF-receptor associated factors
NF-κB	nuclear factor kappa B
CD30L	CD30 ligand
Tg	transgenic
CsA	cyclosporine
MLR	mixed lymphocyte reaction

Introduction

CD30 was initially identified by the Ki-1 monoclonal antibody (mAb) as a membrane antigen expressed on Hodgkin and Reed Sternberg cells in patients with Hodgkin's disease.[1] Subsequently, CD30 was found to be expressed in anaplastic large cell lymphoma (ALCL) and thus has become an important marker in the clinical classification of lymphoid malignancies. CD30 is also expressed on virally infected cells including human T lymphotrophic virus I- or II-(HTLV)-transformed T cells, human immunodeficiency virus (HIV) infected T cells, and Epstein Barr virus (EBV) transformed B cells. Whether expression of CD30 is a consequence of virally-induced trans-activation of the CD30 gene or the intrinsic activation state of the cell is unclear. In normal lymphoid tissue, CD30 expression is restricted to activated T- and B-cell blasts in the extrafollicular region and B-cell blasts at the rim of germinal centers.[1,2]

Resting, naive T cells express minimal, if any, CD30. However, CD30 is upregulated in vitro on T cells upon activation by mitogen or cross-linking of the antigen receptor. The functional significance of CD30 expression on activated T cells has not been resolved. Some studies have suggested that CD30 is preferentially found on T helper-type 2 cells (Th2)[3] while other reports have clearly shown that CD30 is not an absolute marker of Th2 cells.[4] More recent efforts have focused on the possibility that CD30 acts as a costimulatory molecule in antigen-specific T cell responses.

A soluble form of CD30 (sCD30) which derives from the cleavage of membrane CD30 by a zinc-metalloprotease has also been described. Elevated levels of circulating sCD30 has been found in association with HIV infection, CD30+ neoplasias, and multiple autoimmune diseases including primary biliary cirrhosis and rheumatoid arthritis. The functional significance of sCD30 remains to be determined.

CD30 is a member of the TNFR superfamily that is comprised of over 15 transmembrane receptor molecules including CD40, Fas, TNFR1, TNFR2, 4-1BB, CD27 and OX40 (Fig. 1).[5] The extracellular regions of TNFR family members are characterized by multiple cysteine-rich motifs of which human

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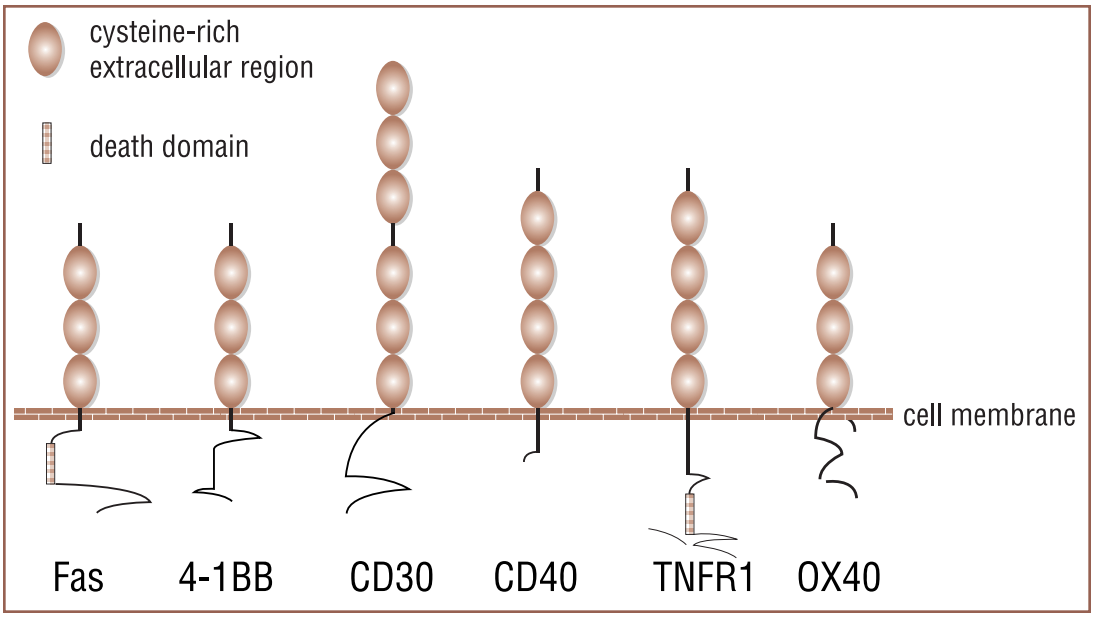

Figure 1. Schematic structure of CD30 and other members of the TNFR superfamily. Shown here are six TNFR family members, including CD30. The TNFR family is characterized by extracellular domains comprised of multiple cysteine-rich regions. Human CD30 has six of these cysteine-rich motifs whereas mouse CD30 and rat CD30 have three such regions. Unlike the extracellular region, which bears significant homology amongst TNFR family members, the intracellular region contains little homology. No intrinsic kinase activity is found in the cytoplasmic region of TNFR family members and signal transduction requires association with other cytoplasmic proteins. Fas and TNFR1 contain a 66 aa death domain which interacts with intracellular signaling molecules leading to apoptosis.

TNFR FAMILY:

A family of over 15 cell surface glycoproteins that are characterized by cysteine-rich extracellular domains. TNFR family members are signaling molecules that can influence cell growth and survival.

CD30 has six. In contrast, the large intracellular region of CD30 is quite distinct from other TNFR family members and lacks the death domain found within the Fas and TNFR1 cytoplasmic tails. Like other members of the TNFR family, ligation of CD30 leads to activation of the TRAF signal transduction pathway and NF-κB activation. On the basis of information derived from the crystal structures of other TNFR family members, CD30 has been proposed to interact with CD30L as a trimer. CD30 signaling can lead to a variety of functional effects on mature, peripheral lymphocytes including cytokine production, induction of cell surface molecule expression, proliferation, differentiation, or apoptosis. The outcome of CD30 ligation depends upon the cell type, its state of activation and differentiation and whether other cell surface receptors are concomitantly engaged. This review focuses on the expression and function of CD30 in normal, activated T lymphocytes.

Structure of CD30

CD30 is a type I 105-120 kD transmembrane glycoprotein derived from a 90 kD precursor protein which undergoes additional glycosylation. The soluble form of CD30 (sCD30) is an 88 kD molecule generated from enzymatic cleavage of the extracellular portion of membrane CD30.[6] A major mRNA species of 3.8 kb and a minor mRNA species of 2.6 kb, which arise from two polyadenylation sites in the 3′ untranslated region, have been identified for human CD30. Human,[5] rat,[7] and mouse[8] CD30 have each been cloned and sequenced. The human CD30 molecule has six cysteine-rich extracellular motifs, whereas mouse and rat have three such regions and lack the second cluster. Human CD30 is comprised of 595 amino acid (aa) residues including an 18 aa residue leader sequence, a 365 aa extracellular region, a 24 aa transmembrane region and a 188 aa cytoplastic domain. Mouse CD30 and rat CD30 are shorter than the human homologue (498 aa and 493 aa, respectively) owing primarily to the large deletion in the extracellular domain. At the protein level, excluding the gaps, the identify of human and mouse CD30 is 55% in the extracellular region and 63% in the intracellular region whereas human and rat CD30 are 51.8% and 61.2% identical in the extracellular and intracellular regions, respectively.

The long intracellular region of CD30 contains no intrinsic catalytic sites but does contain several potential phosphorylation sites for tyrosine kinases and serine/threonine kinases. Approximately 100 aa residues within the carboxy-terminus of the intracellular region of CD30 are conserved within human, rat, and mouse and have been divided into

domains (D) 1, 2, and 3.[7] D2 and D3 have been shown to be critical for interaction with the TRAF 1, 2, and 5 signaling proteins, whereas D1 can mediate NF-kB activation independent of known TRAF proteins.

Expression and Regulation of CD30 on T Lymphocytes

CD30 is generally considered to be an activation-dependent marker for T lymphocytes. It should be noted, however, that very low levels of CD30 have been detected on 3-31% of freshly isolated peripheral blood T cells from healthy donors using immunofluorescent staining with enhanced sensitivity.[9] Most in vitro studies with primary T cells utilize mitogenic stimulation or cross-linking of the antigen receptor to elicit a measurable $CD30^+$ T cell population. Thus, CD30 can be induced on normal human T cells by a variety of stimuli including alloantigen,[10] PHA, and anti-CD3 mAb+IL-2.[11] Human peripheral blood T cells stimulated with alloantigen express detectible levels of CD30 within 72 hours and peak expression levels are found in day 4-5. Maximal expression after anti-CD3+IL-2 stimulation is detected at 48-72 hours indicating that CD30 is a relatively late activation marker in comparison to CD69 and CD25. The majority (>80%) of $CD30^+$ T cells elicited by anti-CD3 mAbs are found within the $CD4^+$ T cell subset although $CD8^+CD30^+$ cells are also evident. In contrast, alloantigenic stimulation yields comparable percentages of $CD4^+CD30^+$ and $CD8^+CD30^+$ cells and both subsets can be generated in the presence of cyclosporine (CsA).

Role of CD28 and Cytokines in Regulation of CD30 Expression. Several lines of evidence indicate that cytokines and the costimulatory molecule CD28 participate in the regulation of CD30 expression. Alzona et al[12] found that exogenous IL-12 increased the percentage of $CD30^+$ peripheral blood T cells, whereas exogenous IL-4 markedly decreased the percentage of $CD30^+$ T cells when used in combination with anti-CD3 mAbs. Cytokines alone, however, fail to induce CD30 expression in the absence of mitogen or TCR crosslinking. In this system it appears that IL-12 acts as a proliferation factor for $CD30^+$ T cells and helps to expand the $CD30^+$ population rather than recruiting $CD30^+$ cells from the $CD30^-$ fraction. In contrast, Annunziato et al[13] found that IL-4 significantly increased the percentage of $CD30^+$ human T cells generated from purified $CD45RA^+$ umbilical cord blood T cells stimulated with PHA+IL-2. The reasons for these discrepancies are unclear but may relate to the differences in the source of T cells (peripheral blood versus umbilical cord blood) or the mode of activation (anti-CD3 mAbs versus PHA+IL-2).

Signals through the costimulatory molecule CD28 can also influence CD30 expression. In mice, costimulation through CD28 is required for T cell expression of CD30.[14] Plate bound anti-CD3 mAbs failed to induce CD30 expression on lymph node T cells from wild-type mice. However, when lymph node cells were stimulated with anti-CD3 mAbs in the presence of CD28 costimulation, $CD30^+$ T cells were generated. Similarly, $CD30^+$ T cells could be elicited from peptide-pulsed bulk lymph node cultures of ovalbumin (OVA) TCR transgenic (Tg) mice, whereas lymph nodes from CD28-deficient mice transgenic for the OVA TCR did not yield $CD30^+$ T cells. Interestingly, the addition of IL-4 to lymph node cell cultures obtained from CD28-deficient OVA TCR Tg mice could overcome the requirement for CD28 and supported the development of $CD30^+$ T cells. Moreover, blockade of IL-4 uptake or CD28 co-stimulation with anti-IL-4 mAbs or CTLA4-Ig, respectively, prevented the development of $CD30^+$ T cells in lymph node cells from OVA TCR Tg mice. Nakamura et al[15] found that CD30 is reciprocally regulated on $CD4^+$ T cells by IL-4 and IFN-γ. Primary activation of naive $CD4^+$ T cells from TCR Tg mice with antigen alone failed to generate $CD30^+$ T cells. The addition of antigen and exogenous IL-4, however, resulted in expression of CD30 by >90% of the $CD4^+$ T cells and the induction of CD30 was antagonized by IFN-γ. These authors conclude that CD30 expression is a measure of the cell's ability to respond to IL-4.

Function of $CD30^+$ T Cells

Relationship of CD30 Expression and Cytokine Production. Attempts have been made to correlate CD30 expression on T cells with a particular cytokine profile but so far no clear pattern has emerged. Romagnani and colleagues[3] analyzed a large panel of human Th0, Th1, and Th2 clones specific for bacterial antigens or allergens and found that Th2 clones expressed abundant CD30

COSTIMULATORY MOLECULES:

Cell surface molecules on T cells that can provide signals which complement activation through the T cell receptor for optimal T cell responses. CD28 is the most important T cell costimulatory molecule described so far.

mRNA and membrane CD30 but Th1 clones did not. Th0 clones displayed an intermediate pattern of CD30 expression. These authors also identified $CD8^+$ T cell clones derived from the peripheral blood of individuals with HIV infection that express CD30 and secrete IL-4 and IL-5. Hence, they proposed that CD30 is preferentially expressed on cells which produce Th2 cytokines. Other groups, however, have detected CD30 expression on human $CD4^+$ Th0, Th1 and Th2 type clones indicating the CD30 is not restricted to Th2 cells.[4,16] It is possible that a cytokine secreted by Th2 cells, such as IL-4, acts to sustain CD30 expression since Bengtsson et al[16] found that Th0, Th1, and Th2 clones all expressed CD30 after anti-CD3 stimulation but only Th2 clones continued to express CD30 after seven days.

Stimulation of primary human T cells with anti-CD3+IL-2 or alloantigen elicits a $CD30^+$ T cell population that is the major source of IFN-γ and IL-5 production.[10,11] $CD30^+$ T cells can also secrete IL-10 but produce no IL-4, and relatively modest levels of IL-2, compared to activated $CD30^-$ cells. Moreover, $CD4^+CD30^+$ cells provide greater helper activity for B cell immunoglobulin production than $CD4^+CD30^-$ cells. When peripheral blood cells from a group of patients with chronic hepatitis C virus (HCV) were restimulated in vitro with immunodominant HCV core epitopes, >50% of the panel showed significant induction of $CD30^+$ T cells.[17] The majority of the $CD30^+$ cells induced by HCV peptides were $CD4^+$ cells. Intracellular cytokine staining demonstrated that the $CD30^+$ T cells produce IFN-γ and IL-10 but minimal IL-2 or IL-4. Thus, although in some cases $CD30^+$ T cells secrete IL-4, they can also produce IFN-γ, IL-10 or IL-5 in the absence of IL-4.

$CD30^+$ T Cells and Alloreactivity. Alloantigen is a robust stimuli of CD30 expression on human T cells in vitro. CD30 expression can be detected within 48-72 hours of alloactivation and by day 4-5 approximately 20-40% of T cells in mixed lymphocyte reactions (MLR) are $CD30^+$. T-cell expression of CD30 induced by primary alloactivation is sustained for >10 days. This differs from the pattern of CD30 expression on T cells activated with anti-CD3+anti-CD28 mAbs. When human T cells are activated with anti-CD3+anti-CD28 mAbs, CD30 is detected within 48 hours but thereafter the proportion of $CD30^+$ T cells rapidly declines. Preliminary experiments indicate that the loss of $CD30^+$ T cells is not due to apoptosis and is likely a result of downregulation of CD30 expression on activated T cells. Thus, stimulation of human T cells with anti-CD3+anti-CD28 mAbs results in an activated T cell population which is predominantly $CD30^-$ whereas the majority of activated T cells elicited by alloantigen are $CD30^+$. The factors that account for this appear to be independent of the strength of the T cell receptor signal and may relate to differences in costimulation or cytokine production.

Functionally, alloactivated $CD30^+$ T cells are the major source of IFN-γ and IL-5, but not IL-2, and are the predominant proliferating cell population. Allostimulation induces CD30 expression on both $CD4^+$ and $CD8^+$ T cells. While CsA diminishes the absolute number of $CD30^+$ T cells generated in MLR, the relative proportion of $CD30^+$ T cells is not altered.

Minguela et al has studied the expression of CD30 on T cells in the peripheral blood following human liver transplantation.[18] These authors found that $CD30^+$ T cells were present in very low numbers in the blood irrespective of whether graft rejection was evident. Additional studies will be necessary to determine if $CD30^+$ T cells can be identified within human allografts, although $CD30^+$ T cells have been identified within rejecting allografts in experimental animal models. In a DA→Lewis rat orthotopic liver transplant model, CD30 transcripts were detected within the inflammatory infiltrate of rejecting allografts as early as day 2 following transplantation and increase in abundance through day 8.[19] Phenotypic analysis of cells isolated from the infiltrate of rejecting allografts revealed that 10-20% of the T cells express CD30. In contrast, CD30 transcripts were not detected in isografts. Thus, the initial studies on $CD30^+$ T cells and alloreactivity indicate that alloantigen induces a functional population of $CD30^+$ T cells and that $CD30^+$ T are found within rejecting allografts. Future studies will address the function and regulation of alloreactive $CD30^+$ T cells in transplantation.

TH1 AND TH2 CELLS:

Two polarized subsets of antigen-primed T cells that can be distinguished by the cytokines they produce. Th1 cells produce IFN-γ whereas Th2 cells produce IL-4, IL-5 and IL-13.

CD30L (CD153)

The ligand for CD30, CD30L, is a 26-40 kDa type II membrane glycoprotein that was originally isolated by expression cloning of the cDNAs from a murine T-cell clone and from anti-CD3 stimulated human peripheral blood mononuclear cells.[20] The CD30L protein is 239 aa in length and shows

significant homology in the extracellular C terminal region with other members of the TNF family including TNF-α, TNF-β, and CD40L. Human and mouse CD30L are 72% identical at the aa level and CD30L is expressed primarily on activated T cells, B cells and monocytes/macrophages. CD30L is also constitutively expressed on neutrophils and has been detected on eosinophils, epithelial cells and Hassel's corpuscles in the medulla of human thymus.

Direct comparison of CD30 and CD30L expression in murine splenocytes stimulated with anti-CD3 indicates that the kinetics of expression of this receptor-ligand pair on T cells are quite different. In general, CD30 expression continues to increase following T-cell activation through day 5 of culture whereas CD30L induction is more rapid and more transient.[8] The maximal overlap in expression of CD30-CD30L occurs on day 2 of culture and by day 4 CD30L expression is minimal. Slightly different kinetics of CD30L expression were observed in a separate study when murine splenic T cells were stimulated with immobilized anti-CD3 mAbs in the absence or presence of anti-CD28 mAbs.[21] In this study CD30L was detected at 24 hours and disappeared by 48 hours following stimulation. CD28 stimulation did not effect the kinetics nor the duration of CD30L expression. CD30L was found predominantly on $CD4^+$ T cells although $CD8^+$ T cells could express low levels of CD30L.

The nature of the molecular interaction between CD30 and CD30L has not been elucidated; however, models based on the crystal structures of other members of the TNFR family indicate that the ligands trimerize when interacting with the corresponding receptor protein. Thus, it has been suggested that cross-linking induces signaling by promoting association of the cytoplasmic domains of the receptor. This view is supported by the studies of Powell et al[22] who constructed and expressed a functional, soluble form of CD30L comprised of the extracellular domain of human CD30L fused to the extracellular domain of the human CD8α chain. The sCD30L/CD8α fusion protein existed in monomeric and trimeric form and was functional in that it could bind to CD30 and, like anti-CD30 agonist mAbs, induce cell death or inhibit proliferation in $CD30^+$ cell lines.

Does CD30 Act as a Costimulatory Molecule?

The physiologic function of the $CD30^-$CD30L interaction has not been elucidated. It has been clearly demonstrated, however, that signal transduction through membrane CD30 can lead to multiple biologic effects in T cells ranging from cellular proliferation to cell death. CD30L-mediated signaling of $CD30^+$ cells can be mimicked by anti-CD30 mAbs, and clones M44 and M67, in particular, have been useful in studying the effects of CD30 signal transduction in human T cells. Ligation of CD30 by agonist anti-CD30 mAbs or $CD30L^+$ transfectants induced IFN-γ production[11] and proliferation[20] in anti-CD3 stimulated normal T cells, NF-κB activation and viral expression in an HIV infected human T-cell line,[23] Ca^{++} flux in Jurkat cells,[24] and enhanced proliferation in a "T-cell-like" Hodgkin's disease cell line.[25] These data indicate that crosslinking of CD30 results in signal transduction and alteration of human T-cell function. Since much of this work has been done using transformed T cells or T-cell lines, however, the role of CD30 in normal human T cell activation and function is not yet established. Several lines of evidence also indicate that CD30 signaling can modulate cytokine production and cellular proliferation in murine T cells. Triggering of CD30 induced IL-5 production in an allospecific murine $CD8^+$ CTL line[8] and enhanced proliferation in anti-CD3+anti-CD28 stimulated lymph node cells.[14] Taken together, these data indicate that CD30 may act as a costimulatory molecule in concert with signals emanating from the T-cell receptor.

CD30 and Other Members of the TNFR Superfamily Which Possess Costimulatory Function. Given the relatively late appearance of CD30 following T-cell activation it is possible that CD30 acts to further modulate T-cell function following the CD28-mediated second signal. We have observed that human T-cell activation by alloantigen results in a reduction in CD28 levels concomitant with an increase in CD30. In addition, CD30 appears to have a more prominent role in costimulation when the TCR signal is suboptimal. Thus, CD30 might be viewed as similar to other TNFR family members such as 4-1BB and OX-40 (Table 1). These molecules, in general, are preferentially expressed on T cells, and can function as costimulatory molecules after CD28 signaling, and may be important in sustaining the immune response. 4-1BB is expressed on both $CD4^+$ and $CD8^+$ T cells, while its ligand, 4-1BBL, is found on activated B cells, macrophages and dendritic cells. The 4-1BB/4-1BBL interaction has been shown to be

CD30L:

The ligand for CD30, CD30L, also known as CD153, is a cell surface molecule and a member of the TNF superfamily. It is expressed on activated T cells, B cells and monocytes/macrophages as well as neutrophils, eosinophils and epithelial cells in the thymus.

Table 1 | **COMPARISON OF CD30 WITH OTHER TNF RECEPTOR FAMILY MEMBERS EXPRESSED ON ACTIVATED T CELLS**

MOLECULE	MW	MAXIMAL EXPRESSION[a]	SIGNALING MOLECULES[b]	COSTIMULATORY FUNCTION[b]	LIGAND	EXPRESSION OF LIGAND
CD30	105-120 kD	72-120 hrs	TRAF 1, 2, 3, 5 activates NF-κB	Cytokine production, apoptosis	CD153	Activated T cells and monocytes
OX-40 (CD134)	47-51 kD	48-72 hrs	TRAF 2, 3, 5 activates NF-κB and JNK	Proliferation, cytokine production	OX40L	B cells, dendritic cells and vascular endothelium
4-1BB (CD137)	30 kD monomer 55 kD dimer	48-72 hrs	TRAF 1, 2, 3 activates NF-κB and JNK	Proliferation, cytokine production, CTL generation	4-1BBL	Activated B cells, dendritic cells and macrophages
CD27	55 kD homodimer	72-96 hrs	TRAF 2, 3, 5 activates NF-κB and JNK	Proliferation, cytokine production, CTL generation	CD70	Activated T and B cells

[a] time of peak protein expression following stimulation with antigen or anti-CD3 mAbs.
[b] cytoplasmic signaling molecules associated with ligation of TNFR family member.
[c] T cell functions known to be induced or enhanced by signaling through the TNFR family member.

APOPTOSIS:

Programmed cell death that can be induced by a variety of stimuli including cell surface molecules such as the TNFR family member Fas.

particularly important in CD8-mediated responses to alloantigen,[26] virus[26,27] and tumors.[28] OX-40, in contrast, appears to play a more important role in $CD4^+$ T cell function.[29] It is unlikely that CD30, 4-1BB, or OX-40 can completely replace CD28 as a costimulatory molecule. Rather, it seems that these TNFR family members may serve as regulatory molecules on particular cell subsets at different stages of T cell activation to modulate discrete effector functions.

Evidence that CD30 Mediates Induction of Apoptosis. Despite the lack of a known death domain in the cytoplasmic region of CD30 several studies have shown that CD30 ligation can induce programmed cell death. In the thymus CD30, may participate in negative selection since mice deficient in CD30 have an enlarged thymus and elevated numbers of thymocytes.[30] Activation induced cell death with anti-CD3 mAbs is impaired in the thymocytes of $CD30^{-/-}$ mice both in vitro and in vivo. Furthermore, thymocytes from mice that over-express CD30 (CD30Tg) undergo apoptosis following CD30 ligation through a caspase 1- and caspase 3-dependent pathway that can be inhibited by bcl-2.[31] CD30Tg $CD4^+CD8^+$ double positive thymocytes also undergo enhanced negative selection when exposed to cognate peptide.

Because CD30 lacks the death domain found in Fas and TNFR1 it is likely that other signaling pathways participate in the induction of apoptosis following CD30 ligation. Using chimeric molecules composed of the extracellular and transmembrane regions of CD8 linked to the cytoplasmic portion of CD30, it was shown that multimerization of the receptor induced Fas-independent apoptosis in T-cell hybridomas, but only in conjunction with a signal through the TCR.[32] TRAF-1 and TRAF-2 appear to play an important role in the cell death pathway in this system. Induction of apoptosis with anti-CD30 mAbs in the Karpas-299 human lymphoma cell line could be partially inhibited by anti-TNF Abs.[33] These findings suggest that CD30 signaling can induce production of endogenous TNF which mediates cell death via the TNF-R1 in an autocrine pathway.

The CD30-CD30L interaction can also induce reverse signaling through CD30L. Ligation of CD30L on human T cells simultaneously stimulated with suboptimal anti-CD3 mAbs resulted in increased proliferation and IL-6 production.[34] Furthermore, engagement of CD30L expressed on neutrophils induced IL-8 production and rapid oxidative burst. Thus, the interaction between CD30 and CD30L can lead to bidirectional signal transduction.

Signaling Pathways of CD30

The initial reports on the ability of CD30 to mediate signal transduction demonstrated that ligation of CD30 on Jurkat cells increased intracellular Ca^{2+} levels.[24] Furthermore, engagement of CD30 on Th clones, a T cell-like Hodgkin's line, and an HIV-infected T cell line led to rapid activation of NF-κB involving the p50 and p65 RelA subunits.[23,25] The cytoplasmic tail of CD30 lacks any intrinsic kinase activity and does not possess a classic death domain. Therefore, it was postulated that other signaling molecules could be associated with, or recruited to, the cytoplasmic tail of CD30 to mediate signal transduction. To identify proteins that interact with the cytoplasmic domain of CD30 several groups utilized yeast two hybrid screening. Gedrich et al[35] identified three independent cDNA encoding for TRAF1, TRAF2, and TRAF3, all of which interact specifically with the cytoplasmic portion of CD30 (Fig. 2). The TRAF-binding domain of CD30 was mapped to the terminal 36

aa residues. Deletion mutant mapping revealed two independent TRAF binding sites. One site was characterized by the PEQET sequence and could bind TRAF1, TRAF2, and TRAF3 whereas a second, downstream site (EEEGKE) could bind TRAF1 and TRAF2 but not TRAF3. Similarly, Ansieau et al[36] demonstrated TRAF1, TRAF2, and TRAF3 interaction with the distal portion of the cytoplasmic region of CD30 and TRAF-dependent activation of NF-κB after CD30 cross-linking. Watanabe and colleagues[7] have identified three subdomains, D1, D2, and D3, located within a 100 aa portion of the C-terminal cytoplasmic tail that are highly conserved between rat, human, and mouse CD30. TRAF2 and TRAF5 elicit NF-κB activation through binding to D2 and D3, respectively. However, D1 can also mediate NF-κB activation independent of any known TRAF molecules.[37]

The regulation of TRAF-signaling and the factors that may lead to differential functions following CD30 ligation are areas of great interest. TRAF-interacting protein (TRIP) is an additional component of the CD30 signaling complex which inhibits TRAF2-mediated NF-κB activation and acts as a receptor-proximal negative regulator.[38] Thus, TRIP may influence the signals that ultimately lead to cellular activation/proliferation or cell death. Another determinant in the cell fate decision following CD30 signaling may be the bifurcation of signaling pathways downstream of TRAF2. TRAF2 can stimulate NF-κB activation through an NF-κB inducing kinase (NIK) pathway and c-jun through MEKK1 and SAPK/JNK. Future studies in this area are likely to address the interplay of TRAF proteins, other signal transduction molecules that can participate in CD30 signaling, the factors that determine the biologically diverse outcomes following CD30 ligation, and potential cross-talk between signaling pathways of other TNFR family members.

TRAF:

TNF receptor associated factors comprise a group of cytoplasmic proteins that function as signaling molecules in conjunction with TNFR cell surface proteins.

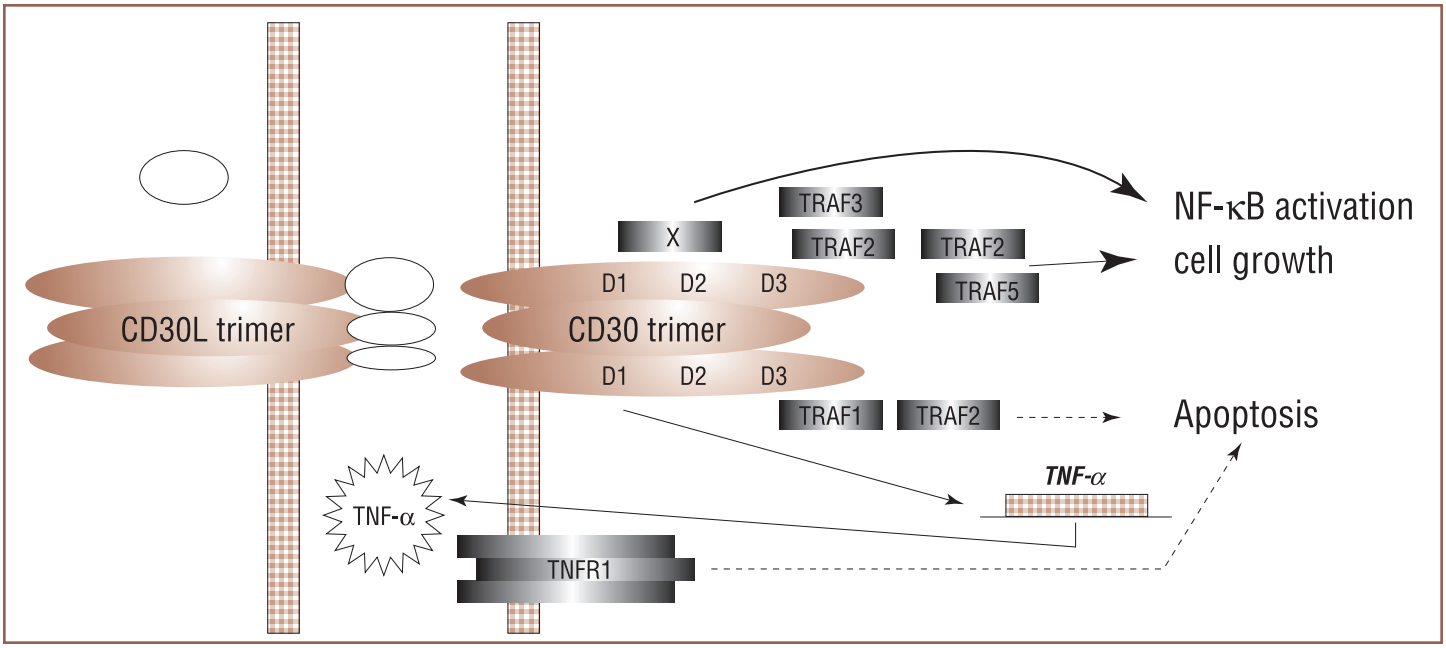

Figure 2. CD30 signaling pathways. Signal transduction through CD30 is thought to involve trimerization of the receptor-ligand pair. Several members of the TNFR family, including CD30, can bind the TNFR-associated factors (TRAF) proteins which are critical mediators of signal transduction. CD30 binds to TRAF1, TRAF2, TRAF3, and TRAF5. Three discreet cytoplasmic domains within the 100 C-terminal amino acids of CD30, termed D1, D2, and D3 have been defined. TRAF2 binds to D2 and D3, while TRAF5 binds to D3 and these interactions can lead to NF-κB activation. An unidentified protein, X, can also lead to NF-κB activation through interaction with D1. A regulatory cytoplasmic protein, TRAF-interacting protein (TRIP), can associate with TRAF2 and prevent NF-κB activation. CD30 does not possess a death domain; however the interaction of TRAF1 and TRAF2 with CD30 can lead to apoptosis. It is also possible that engagement of CD30 can induce an autocrine death pathway mediated by TNF-α production and binding to the TNFR1.

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