Published in final edited form as:

Cytokine. 2007 February ; 37(2): 101–107.

Three is Better Than One: Pre-Ligand Receptor Assembly in the Regulation of TNF Receptor Signaling

Francis Ka-Ming Chan

Immunology and Virology Program, Department of Pathology, University of Massachusetts Medical School, Room S2-125, 55 Lake Avenue North, Worcester, MA 01655, Phone: (508)-856-1664, Fax: (508)-856-1665, francis.chan@umassmed.edu

Abstract

The tumor necrosis factor (TNF) family of cytokines and their receptors regulates many areas of metazoan biology. Specifically, this cytokine-receptor family plays crucial roles in regulating myriad aspects of immune development and functions. Disruption of ligand-receptor interaction or downstream signal transduction components in the TNF family often leads to pathological conditions. Historically, the members of the TNF receptor family (TNFRs) were thought to exist as monomeric receptor chains prior to stimulation. Binding of the trimeric ligand then induces the trimerization of the receptors and activation of downstream signaling. However, recent evidence indicates that many TNFRs exist as pre-assembled oligomers on the cell surface. Pre-ligand assembly of TNFR oligomers is mediated by the pre-ligand assembly domain (PLAD), which resides within the membrane distal cysteine-rich domain of the receptors. Growing evidence indicates that PLAD-mediated receptor association regulates cellular responses to TNF-like cytokines, especially in cells of the immune system. Thus, targeting pre-ligand assembly may offer new possibilities for therapeutic intervention in different pathological conditions involving TNF-like cytokines.

Keywords

Pre-ligand assembly domain; PLAD; TNF; TRAIL; apoptosis

1. Introduction

Historically, ligand-induced receptor oligomerization is regarded as a common mechanism for activation of cell surface receptor signaling. This is exemplified by the classical studies on receptor tyrosine kinases (RTKs), where ligand binding causes dimerization, cross phosphorylation and activation of the receptor chains (1). By contrast, a growing number of cell surface receptors have now been shown to function as pre-assembled receptor complexes. In addition, there are cell surface receptors that can function both as monomers or pre-assembled oligomers. Receptor oligomerization often serves the purpose of increasing ligand binding affinity, as in the case of EGF receptor (2). For cytokine receptors, oligomerization of different receptor subunits can have the additional effect of altering ligand specificity. In fact, for many cytokine receptors, receptor oligomerization is essential for function because the ligand binding subunit is different from the signaling subunit (3).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Receptors in the TNFR superfamily (TNFRs) were once thought to signal through ligand-induced receptor trimerization. However, emerging evidence now indicates that many members within the TNFR family in fact exist as pre-assembled oligomers prior to ligand stimulation (4). In this review, I will discuss how pre-ligand assembly contributes to ligand binding, receptor activation, cellular sensitivity to TNF ligands, and susceptibility to pathogenic mutations. I will also discuss the potential of targeting pre-ligand receptor assembly as a novel therapeutic strategy in diseases involving TNF-like cytokines.

2. Pre-ligand assembly in the regulation of TNFRs biology

2.1. TNFR structures and the ligand-induced trimerization model

TNFRs are type I membrane receptors that are distinguished by one to six cysteine-rich domains (CRDs) in the extracellular region of the receptor (5). They can be further divided into three groups: the death receptors, which mediate cell death through their cytoplasmic death domains (DDs); the non-death receptors, which signal mostly through one or more of the TNF receptor associated factors (TRAFs); and the decoy/soluble receptors (Fig. 1). For the most part, TNF-like cytokines act as trimers. Structural analyses reveal that this trimeric scaffold is preserved in the ligand-receptor complex (6–9). These results thus led to the notion that receptor activation begins with ligand-induced trimerization of monomeric receptor chains on the cell surface.

2.2. Ligand-induced trimerization causes interference of receptor signaling

Several lines of evidence, however, raise questions about the ligand-induced receptor trimerization model. For example, ligand-induced receptor oligomerization may cause signal interference for TNF-like ligands that can bind to multiple receptors on the cell surface. In the case of TNF α , which binds both TNFR-1 and TNFR-2, ligand binding may induce formation of mixed trimers containing both TNFR-1 and TNFR-2 receptor chains. Because TNFR-1, but not TNFR-2, contains a DD in the cytoplasmic tail and that efficient transduction of the cell death signal requires three intact DDs (10), this mixed trimer will be expected to interfere with apoptotic signaling via TNFR-1. However, we and others have shown that in cells expressing both TNF receptors, TNFR-2 facilitates rather than interferes with TNFR-1 mediated cell death (11–16). A similar enhancing effect on NF- κ B activation was also observed in cells expressing both TNF receptors (15). Hence, these results suggest that some sorting mechanism for the receptors must be in place to avoid formation of mixed, abortive receptor complexes.

2.3. ALPS – the case against ligand-induced trimerization

Perhaps the strongest evidence against the ligand-induced oligomerization model comes from studies of human patients with Autoimmune Lymphoproliferative Syndromes (ALPS). ALPS is a systemic, lupus-like autoimmune disease characterized by the production of autoantibodies, lymphoproliferation and accumulation of a unique population of T-cells that are Thy1⁺CD3⁺B220⁺CD4⁺CD8⁺. These phenotypes are reminiscent of that found in the *lpr* and *gld* mice, which harbor mutations in Fas and Fas ligand (FasL), respectively (17). Thus, it is not surprising that the majority of ALPS patients possess heterozygous mutations in the TNFR-like death receptor CD95/Fas/APO-1. Interestingly, several of the ALPS mutations target the extracellular domain of the receptor, causing either premature termination of the receptor chain or internal deletion. In many of these cases, the mutations completely abolished ligand binding (18). According to the ligand-induced trimerization model, these non-ligand-binding receptors will not be recruited into the signaling receptor complex and therefore will not interfere with wild type Fas receptor signaling. However, patients who inherited these mutations clearly developed autoimmune symptoms due to defective Fas-induced apoptosis (19). These results suggest that the non-ligand-binding mutants must exert some kind of dominant interfering effect on the wild type receptor.

2.4. Identification of the pre-ligand assembly domain (PLAD)

Interestingly, all of the pathogenic Fas mutations found in ALPS have preserved the membrane-distal CRD, which had no previously ascribed function and is not involved in ligand binding. These results prompted us to examine whether the membrane-distal first CRD might contribute to receptor function by associating with wild type Fas receptor. Indeed, biochemical analyses show that the first CRD of Fas, as well as that of TNFR-1 and TNFR-2, is essential for the formation of homotypic, ligand-independent receptor complexes (19–21). The interaction of TNFRs via the first CRD, termed the pre-ligand assembly domain (PLAD), explains the unliganded structure of TNFR-1, which adopts a parallel dimeric conformation at neutral pH with extensive contacts in the membrane-distal first CRD (22–24). Interestingly, ligand binding causes a conformational change in the pre-assembled receptor complex as measured by fluorescence resonance energy transfer (FRET) (25), which supposedly represents a conformational change of the pre-assembled complex that facilitates downstream signal transduction (Fig. 2A). More recently, PLAD-mediated pre-ligand assembly has been observed in TRAIL receptors and viral TNFR homologs (26,27). Interestingly, in the case of TRAIL receptors, the PLAD facilitates homotypic as well as heterotypic receptor associations as a means to modulate cellular response to TRAIL stimulation (26), a subject that we will discuss further in section 3.2.

2.5. Are the pre-assembled complexes dimers, trimers or oligomers?

As I have mentioned in section 2.4, the unliganded structure of TNFR-1 is a dimer. In addition, the TNFR members CD27 and CD40 have been shown to exist as dimers through intermolecular disulfide bonds. On the other hand, the ligand-bound structures of TNFR-1, TRAIL-R2, BAFF-R3 and many other TNFRs reveal a trimer to trimer stoichiometry (6–9, 28). Moreover, structural analyses of the receptor cytoplasmic tails of TNFR-1, TNFR-2, Fas, CD40 and their interacting signal adapters reveal a requirement for trimeric symmetry for downstream signal transduction (29–34). These conflicting observations thus raise questions about the stoichiometry of the pre-assembled receptor complex. However, the discrepant results can be reconciled if multiple copies of pre-assembled dimers form macro-molecular aggregates upon ligand binding (Fig. 2B). In this model, the trimeric symmetry of the ligand-receptor complex is preserved in these higher order structures. Indeed, in the case of Fas, formation of macro-molecular aggregates is an essential intermediate step during receptor activation (35,36).

2.6. Biological implications of pre-ligand assembly

Does signaling via pre-assembled receptors offer any biological advantages over ligand-induced trimerization? One possibility is that pre-assembled receptors can bind ligands with higher affinity than monomeric receptors. In fact, deletion of the PLAD in TNFR-1, TNFR-2, TRAIL-R2 and TRAIL-R4 severely compromised their ability to bind ligands (20,26). Since most TNF-like cytokines are present at low concentrations physiologically, pre-assembled receptors may facilitate rapid cellular responses to cytokine stimulation. In addition, sorting of receptors that share the same ligand into pre-assembled homotypic complexes, such as TNFR-1 and TNFR-2, circumvents the potential for signal interference that may otherwise occur if the receptors were assembled via ligand-induced trimerization.

In the pre-assembled TNFR complex, the signaling cytoplasmic tails are brought into close proximity with one another. Does it render the receptors more prone to inadvertent activation? For death receptors such as TNFR-1, Fas and TRAIL receptors, the consequence of unregulated receptor activation is deleterious. Different TNFRs seem to have developed different strategies to counter this potential problem. For instance, binding of the cytoplasmic tail of TNFR-1 to the silencer of death domain (SODD) maintains the receptor in its quiescent state (37). For Fas and TRAIL receptors, formation of pre-ligand complexes and therefore sensitivity to death

ligand stimulation is regulated by external cues (see section 5). Furthermore, expression of downstream signal inhibitors such as cFLIP can also prevent inadvertent receptor activation (38).

Although pre-ligand assembly ensures efficient signaling of TNFRs, it does appear to predispose TNFRs to dominant interfering mutations. This is in fact the case in ALPS. However, since genetic deficiency in TNFRs do not compromise metazoan development and survival, this susceptibility to dominant interfering mutations appears to be a small price to pay in exchange for a more dynamic and efficient receptor signaling system.

3. PLAD as a regulatory mechanism and therapeutic target

3.1. Pre-ligand assembly of Fas in CD4⁺ T-cells

Emerging evidence now indicates that pre-ligand assembly is a highly regulated process that controls cellular sensitivity to TNF-like cytokine stimulation. One such example is the response of CD4⁺ T-cells to FasL-induced apoptosis. Although cell surface Fas expression is readily detectable in naïve CD4⁺ T-cells, they are resistant to FasL-induced apoptosis. However, upon T-cell receptor (TCR) stimulation, lymphocytes gradually acquire responsiveness to FasL-induced apoptosis (39). Specifically, restimulation through the TCR causes death of the activated CD4⁺ T-cells via the death cytokines FasL and TNF α in an autocrine/paracrine fashion (40). A crucial feature of this cell death response, often termed “activation induced cell death (AICD)”, is that cell death occurs only in cells that have been re-stimulated through the TCR, but not other bystander cells. Interestingly, TCR stimulation of activated CD4⁺ T-cells causes a dramatic redistribution of Fas receptors from the detergent-soluble membrane to the detergent-insoluble lipid rafts, which promotes pre-ligand assembly of Fas receptor complexes and heightens the sensitivity of CD4⁺ T-cells to FasL-induced apoptosis (41) (Fig. 3A). Disruption of the lipid rafts hampers formation of pre-assembled Fas complexes and reduces the sensitivity to FasL-induced apoptosis (41). Thus, clonal specificity of cell death is ensured by the TCR or the “competency to die” signal (42,43). Moreover, pre-ligand assembly may critically enforce peripheral tolerance by regulating FasL-induced apoptosis of autoreactive CD4⁺ T-cells (18,44).

3.2. Pre-ligand assembly of TRAIL receptors in CD8⁺ T-cells

Another example of pre-ligand assembly as a regulatory mechanism is found in TRAIL (TNF-related apoptosis inducing ligand) receptors. TRAIL binds to five receptors, the membrane-bound TRAIL-R1/DR4/APO-2, TRAIL-R2/DR5/Killer/TRICK, TRAIL-R3/DcR1/LIT/TRID, TRAIL-R4/DcR2/TRUNDD and the soluble receptor osteoprotegerin. TRAIL-R1 and TRAIL-R2 contain cytoplasmic DDs and are responsible for apoptosis induction. On the other hand, neither the GPI-linked TRAIL-R3 nor TRAIL-R4, which contains a severely truncated DD, can induce apoptosis. Hence, TRAIL-R3 and TRAIL-R4 were considered “decoys” that inhibit TRAIL-induced apoptosis by sequestering TRAIL from the death-inducing receptors (45). By contrast, we and others have recently shown that the extracellular domains of decoy receptors and death receptors can interact in a ligand-independent manner (26,46), suggesting that pre-ligand assembly, but not ligand sequestration, may account for the inhibitory effects of TRAIL-R3 and TRAIL-R4 in certain cell types. Indeed, that appears to be the case in primary CD8⁺ T-cells.

TRAIL has recently been shown to control the expansion of memory CD8⁺ T-cells during recall response and the development of functional CD8⁺ T-cell during homeostatic proliferation (47,48). Exposure to a CD4⁺ T-cell “help” signal during initial priming is essential for proper memory CD8⁺ T-cell proliferation during secondary challenge. CD8⁺ T-cells do not receive CD4⁺ T-cell help proliferated normally during initial priming. However, their

expansion during memory response is blunted due to TRAIL-induced apoptosis (48). We found that TRAIL-R4 is essential in protecting CD8⁺ T-cells from TRAIL-induced apoptosis, since RNAi knock-down of TRAIL-R4 expression sensitizes them to TRAIL. The resistance of CD8⁺ T-cells to TRAIL also correlates with ligand-independent association between TRAIL-R4 and TRAIL-R2, presumably through the PLAD. Strikingly, the interaction between TRAIL-R2 and TRAIL-R4 is abolished rapidly upon phorbol ester treatment, which mimics partial T-cell activation in the absence of CD4⁺ T-cell help. This coincides with enhanced response to TRAIL-induced apoptosis (26). Thus, pre-ligand assembly modulates TRAIL-induced apoptosis in CD8⁺ T-cells and may affect their memory responses (Fig. 3B). Similar to the situation of Fas in CD4⁺ T-cells, pre-ligand assembly and TRAIL-mediated apoptosis may be an important mechanism to prevent the expansion of autoreactive T-cells. In this regard, it is intriguing that TRAIL has been implicated to contribute to the pathogenesis of several autoimmune diseases including type I diabetes and experimental autoimmune encephalomyelitis (49–54). Furthermore, these results may also explain why cancer cells that express decoy receptors are not always resistant to TRAIL-induced apoptosis. In this scenario, the balance between homotypic death receptor complexes versus heterotypic mixed TRAIL receptor complexes may determine cellular response to TRAIL.

3.3. Viral PLAD as an immune evasion mechanism

The importance of PLAD-mediated pre-ligand assembly in regulating TNF-like cytokine signaling is further highlighted by the recent findings that viral TNFR homologs also exhibit PLAD-like functions. Viral TNFR homologs were thought to inhibit TNF-induced inflammation and/or cell death by binding to and sequestering host-produced TNF (55). However, intracellularly expressed M-T2 from Myxoma virus can inhibit TNF-induced cell death (56), suggesting that mechanisms other than ligand sequestration is involved. Strikingly, the inhibitory effect was abolished when the PLAD sequence in M-T2 was deleted. Indeed, biochemical and co-localization studies using fluorescently tagged receptors showed that M-T2 interacts with host TNFR-1 and TNFR-2 via the PLAD (27). Hence, M-T2 targets the PLAD to disrupt TNFR-1 signaling. Given the fact that all T2-like proteins encoded by poxviruses contain a PLAD-like motif (27), it is tantalizing to speculate that viral inhibition of TNFR signaling via the PLAD is a common immune evasion strategy employed by many viruses.

3.4. PLAD as a therapeutic target

As I have discussed in previous paragraphs, all dominant interfering mutations of Fas found in ALPS patients have preserved the PLAD. In fact, mutation that causes severe truncation of the receptor and leaves only an intact PLAD is still pathogenic (18). Also, the fact that viral T2 proteins target the PLAD as an immune evasion mechanism highlights the possibility of targeting the PLAD in therapeutic settings (57). Indeed, we recently showed that PLAD peptides derived from TNFR-1 and TNFR-2 could potently inhibit TNF-induced cell death and NF- κ B activation. Moreover, the PLAD peptide from TNFR-1 was highly effective against arthritis induced by intra-articular injection of TNF and collagen-induced arthritis (58). Although PLAD peptide mimetics are unlikely to be useful clinically due to stability issues, these results do provide rationale for the design of future therapeutics that target the PLAD of various TNFRs in different diseases.

4. Concluding remarks

The discovery of pre-ligand assembly has revealed unexpected aspects of TNFR biology and opened up new possibilities for therapeutic intervention. However, it is important to point out that not all TNFRs may be regulated by pre-ligand assembly. In fact, even for receptors that form pre-ligand complexes, their association appears to be regulated differently in different cell types and during distinct cell developmental stages. Thus, a better understanding of how

pre-ligand assembly is regulated is required before we can fully harness the potential of targeting this biological interaction in therapeutic settings.

Acknowledgements

The author would like to thank members of the lab past and present for discussion and contributions to the work discussed here. The work discussed here is supported by CA113786. The author is a recipient of an investigator award from the Cancer Research Institute and the Smith Family Foundation.

References

- Schlessinger J. Common and distinct elements in cellular signaling via EGF and FGF receptors. *Science* 2004;306:1506–1507. [PubMed: 15567848]
- Ozcan F, Klein P, Lemmon MA, Lax I, Schlessinger J. On the nature of low- and high-affinity EGF receptors on living cells. *Proc Natl Acad Sci U S A* 2006;103:5735–5740. [PubMed: 16571657]
- Leonard WJ. Cytokines and immunodeficiency diseases. *Nat Rev Immunol* 2001;1:200–208. [PubMed: 11905829]
- Chan KF, Siegel MR, Lenardo MJ. Signaling by the TNF receptor superfamily and T cell homeostasis. *Immunity* 2000;13:419–422. [PubMed: 11070160]
- Locksley RM, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* 2001;104:487–501. [PubMed: 11239407]
- Banner DW, D'Arcy A, Janes W, Gentz R, Schoenfeld HJ, Broger C, Loetscher H, Lesslauer W. Crystal structure of the soluble human 55 kd TNF receptor-human TNF beta complex: implications for TNF receptor activation. *Cell* 1993;73:431–445. [PubMed: 8387891]
- Cha SS, Sung BJ, Kim YA, Song YL, Kim HJ, Kim S, Lee MS, Oh BH. Crystal structure of TRAIL-DR5 complex identifies a critical role of the unique frame insertion in conferring recognition specificity. *J Biol Chem* 2000;275:31171–31177. [PubMed: 10893238]
- Hymowitz SG, Christinger HW, Fuh G, Ultsch M, O'Connell M, Kelley RF, Ashkenazi A, de Vos AM. Triggering cell death: the crystal structure of Apo2L/TRAIL in a complex with death receptor 5. *Mol Cell* 1999;4:563–571. [PubMed: 10549288]
- Mongkolsapaya J, Grimes JM, Chen N, Xu XN, Stuart DI, Jones EY, Screaton GR. Structure of the TRAIL-DR5 complex reveals mechanisms conferring specificity in apoptotic initiation. *Nat Struct Biol* 1999;6:1048–1053. [PubMed: 10542098]
- Boldin MPI, Mett L, Varfolomeev EE, Chumakov I, Shemer-Avni Y, Camonis JH, Wallach D. Self-association of the “death domains” of the p55 tumor necrosis factor (TNF) receptor and Fas/APO1 prompts signaling for TNF and Fas/APO1 effects. *J Biol Chem* 1995;270:387–391. [PubMed: 7529234]
- Heller RA, Song K, Fan N, Chang DJ. The p70 tumor necrosis factor receptor mediates cytotoxicity. *Cell* 1992;70:47–56. [PubMed: 1320461]
- Haridas V, Darnay BG, Natarajan K, Heller R, Aggarwal BB. Overexpression of the p80 TNF receptor leads to TNF-dependent apoptosis, nuclear factor-kappa B activation, and c-Jun kinase activation. *J Immunol* 1998;160:3152–3162. [PubMed: 9531270]
- Weiss T, Grell M, Siemienski K, Muhlenbeck F, Durkop H, Pfizenmaier K, Scheurich P, Wajant H. TNFR80-dependent enhancement of TNFR60-induced cell death is mediated by TNFR-associated factor 2 and is specific for TNFR60. *J Immunol* 1998;161:3136–3142. [PubMed: 9743381]
- Declercq W, Denecker G, Fiers W, Vandenabeele P. Cooperation of both TNF receptors in inducing apoptosis: involvement of the TNF receptor-associated factor binding domain of the TNF receptor 75. *J Immunol* 1998;161:390–399. [PubMed: 9647248]
- Chan FK, Lenardo MJ. A crucial role for p80 TNF-R2 in amplifying p60 TNF-R1 apoptosis signals in T lymphocytes. *Eur J Immunol* 2000;30:652–660. [PubMed: 10671223]
- Chan FK, Shisler J, Bixby JG, Felices M, Zheng L, Appel M, Orenstein J, Moss B, Lenardo MJ. A role for tumor necrosis factor receptor-2 and receptor-interacting protein in programmed necrosis and antiviral responses. *J Biol Chem* 2003;278:51613–51621. [PubMed: 14532286]
- Rao VK, Straus SE. Causes and consequences of the autoimmune lymphoproliferative syndrome. *Hematology* 2006;11:15–23. [PubMed: 16522544]

18. Siegel RM, Chan FK, Chun HJ, Lenardo MJ. The multifaceted role of Fas signaling in immune cell homeostasis and autoimmunity. *Nat Immunol* 2000;1:469–474. [PubMed: 11101867]
19. Siegel RM, Frederiksen JK, Zacharias DA, Chan FK, Johnson M, Lynch D, Tsien RY, Lenardo MJ. Fas preassociation required for apoptosis signaling and dominant inhibition by pathogenic mutations. *Science* 2000;288:2354–2357. [PubMed: 10875918]
20. Chan FK, Chun HJ, Zheng L, Siegel RM, Bui KL, Lenardo MJ. A domain in TNF receptors that mediates ligand-independent receptor assembly and signaling. *Science* 2000;288:2351–2354. [PubMed: 10875917]
21. Papoff G, Hausler P, Eramo A, Pagano MG, Di Leve G, Signore A, Ruberti G. Identification and characterization of a ligand-independent oligomerization domain in the extracellular region of the CD95 death receptor. *J Biol Chem* 1999;274:38241–38250. [PubMed: 10608899]
22. Naismith JH, Brandhuber BJ, Devine TQ, Sprang SR. Seeing double: crystal structures of the type I TNF receptor. *J Mol Recognit* 1996;9:113–117. [PubMed: 8877801]
23. Naismith JH, Devine TQ, Brandhuber BJ, Sprang SR. Crystallographic evidence for dimerization of unliganded tumor necrosis factor receptor. *J Biol Chem* 1995;270:13303–13307. [PubMed: 7768931]
24. Naismith JH, Devine TQ, Kohno T, Sprang SR. Structures of the extracellular domain of the type I tumor necrosis factor receptor. *Structure* 1996;4:1251–1262. [PubMed: 8939750]
25. Chan FK, Siegel RM, Zacharias D, Swofford R, Holmes KL, Tsien RY, Lenardo MJ. Fluorescence resonance energy transfer analysis of cell surface receptor interactions and signaling using spectral variants of the green fluorescent protein. *Cytometry* 2001;44:361–368. [PubMed: 11500853]
26. Clancy L, Mruk K, Archer K, Woelfel M, Mongkolsapaya J, Screaton G, Lenardo MJ, Chan FK. Preligand assembly domain-mediated ligand-independent association between TRAIL receptor 4 (TR4) and TR2 regulates TRAIL-induced apoptosis. *Proc Natl Acad Sci U S A* 2005;102:18099–18104. [PubMed: 16319225]
27. Sedger LM, Osvath SR, Xu XM, Li G, Chan FK, Barrett JW, McFadden G. Poxvirus tumor necrosis factor receptor (TNFR)-like T2 proteins contain a conserved preligand assembly domain that inhibits cellular TNFR1-induced cell death. *J Virol* 2006;80:9300–9309. [PubMed: 16940541]
28. Gordon NC, Pan B, Hymowitz SG, Yin J, Kelley RF, Cochran AG, Yan M, Dixit VM, Fairbrother WJ, Starovasnik MA. BAFF/BLyS receptor 3 comprises a minimal TNF receptor-like module that encodes a highly focused ligand-binding site. *Biochemistry* 2003;42:5977–5983. [PubMed: 12755599]
29. Huang B, Eberstadt M, Olejniczak ET, Meadows RP, Fesik SW. NMR structure and mutagenesis of the Fas (APO-1/CD95) death domain. *Nature* 1996;384:638–641. [PubMed: 8967952]
30. Martin DA, Zheng L, Siegel RM, Huang B, Fisher GH, Wang J, Jackson CE, Puck JM, Dale J, Straus SE, Peter ME, Krammer PH, Fesik S, Lenardo MJ. Defective CD95/APO-1/Fas signal complex formation in the human autoimmune lymphoproliferative syndrome, type Ia. *Proc Natl Acad Sci U S A* 1999;96:4552–4557. [PubMed: 10200300]
31. Park YC, Burkitt V, Villa AR, Tong L, Wu H. Structural basis for self-association and receptor recognition of human TRAF2. *Nature* 1999;398:533–538. [PubMed: 10206649]
32. Ye H, Park YC, Kreishman M, Kieff E, Wu H. The structural basis for the recognition of diverse receptor sequences by TRAF2. *Mol Cell* 1999;4:321–330. [PubMed: 10518213]
33. McWhirter SM, Pullen SS, Holton JM, Crute JJ, Kehry MR, Alber T. Crystallographic analysis of CD40 recognition and signaling by human TRAF2. *Proc Natl Acad Sci U S A* 1999;96:8408–8413. [PubMed: 10411888]
34. Park YC, Ye H, Hsia C, Segal D, Rich RL, Liou HC, Myszkowski DG, Wu H. A novel mechanism of TRAF signaling revealed by structural and functional analyses of the TRADD-TRAF2 interaction. *Cell* 2000;101:777–787. [PubMed: 10892748]
35. Siegel RM, Muppidi JR, Sarker M, Lobito A, Jen M, Martin D, Straus SE, Lenardo MJ. SPOTS: signaling protein oligomeric transduction structures are early mediators of death receptor-induced apoptosis at the plasma membrane. *J Cell Biol* 2004;167:735–744. [PubMed: 15557123]
36. Feig C, Tchikov V, Schutze S, Peter ME. Palmitoylation of CD95 facilitates formation of SDS-stable receptor aggregates that initiate apoptosis signaling. *Embo J* 2007;26:221–231. [PubMed: 17159907]
37. Jiang Y, Woronicz JD, Liu W, Goeddel DV. Prevention of constitutive TNF receptor 1 signaling by silencer of death domains. *Science* 1999;283:543–546. [PubMed: 9915703]

38. Budd RC, Yeh WC, Tschopp J. cFLIP regulation of lymphocyte activation and development. *Nat Rev Immunol* 2006;6:196–204. [PubMed: 16498450]
39. Peter ME, Kischkel FC, Scheuierpflug CG, Medema JP, Debatin KM, Krammer PH. Resistance of cultured peripheral T cells towards activation-induced cell death involves a lack of recruitment of FLICE (MACH/caspase 8) to the CD95 death-inducing signaling complex. *Eur J Immunol* 1997;27:1207–1212. [PubMed: 9174612]
40. Zheng L, Fisher G, Miller RE, Peschon J, Lynch DH, Lenardo MJ. Induction of apoptosis in mature T cells by tumour necrosis factor. *Nature* 1995;377:348–351. [PubMed: 7566090]
41. Muppidi JR, Siegel RM. Ligand-independent redistribution of Fas (CD95) into lipid rafts mediates clonotypic T cell death. *Nat Immunol* 2004;5:182–189. [PubMed: 14745445]
42. Hornung F, Zheng L, Lenardo MJ. Maintenance of clonotype specificity in CD95/Apo-1/Fas-mediated apoptosis of mature T lymphocytes. *J Immunol* 1997;159:3816–3822. [PubMed: 9378969]
43. Combadiere B, Reis e Sousa C, Trageser C, Zheng LX, Kim CR, Lenardo MJ. Differential TCR signaling regulates apoptosis and immunopathology during antigen responses in vivo. *Immunity* 1998;9:305–313. [PubMed: 9768750]
44. Lenardo M, Chan KM, Hornung F, McFarland H, Siegel R, Wang J, Zheng L. Mature T lymphocyte apoptosis--immune regulation in a dynamic and unpredictable antigenic environment. *Annu Rev Immunol* 1999;17:221–253. [PubMed: 10358758]
45. Almasan A, Ashkenazi A. Apo2L/TRAIL: apoptosis signaling, biology, and potential for cancer therapy. *Cytokine Growth Factor Rev* 2003;14:337–348. [PubMed: 12787570]
46. Lee HW, Lee SH, Lee HW, Ryu YW, Kwon MH, Kim YS. Homomeric and heteromeric interactions of the extracellular domains of death receptors and death decoy receptors. *Biochem Biophys Res Commun* 2005;330:1205–1212. [PubMed: 15823571]
47. Hamilton SE, Wolkers MC, Schoenberger SP, Jameson SC. The generation of protective memory-like CD8(+) T cells during homeostatic proliferation requires CD4(+) T cells. *Nat Immunol* 2006;7:475–481. [PubMed: 16604076]
48. Janssen EM, Droin NM, Lemmens EE, Pinkoski MJ, Bensinger SJ, Ehst BD, Griffith TS, Green DR, Schoenberger SP. CD4+ T-cell help controls CD8+ T-cell memory via TRAIL-mediated activation-induced cell death. *Nature* 2005;434:88–93. [PubMed: 15744305]
49. Hilliard B, Wilmen A, Seidel C, Liu TS, Goke R, Chen Y. Roles of TNF-related apoptosis-inducing ligand in experimental autoimmune encephalomyelitis. *J Immunol* 2001;166:1314–1319. [PubMed: 11145715]
50. Lamhamedi-Cherradi SE, Zheng S, Tisch RM, Chen YH. Critical roles of tumor necrosis factor-related apoptosis-inducing ligand in type 1 diabetes. *Diabetes* 2003;52:2274–2278. [PubMed: 12941766]
51. Lamhamedi-Cherradi SE, Zheng SJ, Maguschak KA, Peschon J, Chen YH. Defective thymocyte apoptosis and accelerated autoimmune diseases in TRAIL^{-/-} mice. *Nat Immunol* 2003;4:255–260. [PubMed: 12577054]
52. Mi QS, Ly D, Lamhamedi-Cherradi SE, Salojin KV, Zhou L, Grattan M, Meagher C, Zucker P, Chen YH, Nagle J, Taub D, Delovitch TL. Blockade of tumor necrosis factor-related apoptosis-inducing ligand exacerbates type 1 diabetes in NOD mice. *Diabetes* 2003;52:1967–1975. [PubMed: 12882912]
53. Song K, Chen Y, Goke R, Wilmen A, Seidel C, Goke A, Hilliard B, Chen Y. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is an inhibitor of autoimmune inflammation and cell cycle progression. *J Exp Med* 2000;191:1095–1104. [PubMed: 10748228]
54. Cretney E, McQualter JL, Kayagaki N, Yagita H, Bernard CC, Grewal IS, Ashkenazi A, Smyth MJ. TNF-related apoptosis-inducing ligand (TRAIL)/Apo2L suppresses experimental autoimmune encephalomyelitis in mice. *Immunol Cell Biol* 2005;83:511–519. [PubMed: 16174101]
55. Benedict CA, Norris PS, Ware CF. To kill or be killed: viral evasion of apoptosis. *Nat Immunol* 2002;3:1013–1018. [PubMed: 12407409]
56. Macen JL, Graham KA, Lee SF, Schreiber M, Boshkov LK, McFadden G. Expression of the myxoma virus tumor necrosis factor receptor homologue and M11L genes is required to prevent virus-induced apoptosis in infected rabbit T lymphocytes. *Virology* 1996;218:232–237. [PubMed: 8615027]
57. Chan FK. The pre-ligand binding assembly domain: a potential target of inhibition of tumour necrosis factor receptor function. *Ann Rheum Dis* 2000;59(Suppl 1):i50–53. [PubMed: 11053089]

58. Deng GM, Zheng L, Chan FK, Lenardo M. Amelioration of inflammatory arthritis by targeting the pre-ligand assembly domain of tumor necrosis factor receptors. *Nat Med* 2005;11:1066–1072. [PubMed: 16170321]

Fig 1.

Schematic diagram of the structures of the three different subclasses of TNFRs. (A) The death receptors are characterized by the DDs in the signaling cytoplasmic tails. (B) The majority of TNFRs signal through binding to one of six TRAFs. (C) The decoy receptors lack intact cytoplasmic signaling tails and can exist as either soluble or membrane-bound forms. The blue ovals represent the cysteine-rich domains (CRDs). Receptors with four CRDs are shown for illustration purpose only. TNFRs can have one to six CRDs.

Fig 2.

The pre-ligand assembly model. **(A)** Upon ligand binding, the PLAD interaction is replaced by the more stable ligand-receptor interaction. A pre-assembled trimer is shown for illustration purpose. **(B)** Aggregation of pre-assembled dimers maintains the three-fold symmetry of the ligand-bound receptor complex. The receptor dimers are represented by the blue hexagons from a top-down view. Upon ligand binding, ligand-receptor aggregates formed on the membrane maintains a trimeric symmetry, as shown by the circle.

Fig 3.

(**A**) Regulation of FasL-induced apoptosis in CD4⁺ T-cells. Naïve or activated T-cells are relatively resistant to FasL because the majority of Fas are monomeric. TCR restimulation redistributes Fas to the lipid rafts, facilitates pre-ligand assembly and upregulates expression of FasL. This results in enhanced FasL-induced apoptosis. This process may mimic elimination of autoreactive T-cells that are repeatedly stimulated with autoantigens. (**B**) CD8⁺ T-cells are resistant to TRAIL-induced apoptosis due to pre-assembled TRAIL-R2 (blue) and TRAIL-R4 (brown) complexes. They are protected from TRAIL-induced apoptosis when restimulated through their TCR. This mimics the state of CD8⁺ T-cells that have received CD4⁺ T-cell help. However, if CD8⁺ T-cells receive no CD4⁺ T-cell help during initial priming (partial activation), the TRAIL-R2/TRAIL-R4 complex dissociates. These cells succumb to TRAIL-induced apoptosis upon restimulation of the TCR. This then results in failed memory CD8⁺ T-cell proliferation.