TNFR1 and TNFR2 signalling and its clinical implications

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Introduction

Tumour necrosis factor (TNF) is a potent inflammatory mediator exerting effects on many different pathological and physiological functions. The discovery of TNF can be traced back over a century, with the observation by P. Brunes that some of his cancer patients showed tumour regression following acute bacterial infection. This finding led to the practice of injecting cancer patients with bacteria or bacterial products by William B. Coley[1]. These products have been referred to as Coley's toxin. In 1962, O'Malley et al. reported tumour necrotizing activity in the serum obtained from mice after injection of *Serratia marcescens* polysaccharide[2]. The name "TNF" was first used by E.A. Carswell et al. to name the substance in the serum of mice treated with endotoxin, which resulted in haemorrhagic necrosis of tumours[3]. In 1984 and 1985, two different proteins that caused lysis of tumour cells were purified, structurally identified and cDNA cloned[4-6]. They were named TNF-α and TNF-β. These two proteins show around 50% sequence homology and interact with the same membrane receptors but are produced from different cells and exhibit different physiological functions[4-6]. Beutler et al. reported a high degree homology exists between mouse cachectin and human TNF-α [7]. The advancement in our understanding of TNF as well as improvements in molecular biology capabilities at that time resulted in the generation of recombinant TNF (rTNF) which led to investigations into the clinical utility of TNF.

TNF was first identified as a nonspecific anti-tumour therapy. Early studies demonstrated the efficacy of purified TNF against various kinds of murine tumours and human tumours heterotransplanted into nude mice[8]. However, all phase I clinical trials of intravenous, subcutaneous or intramuscular-administered rTNF showed toxicities, including fever, chills, headache, fatigue, dyspnea, tachycardia and hypotension, without significant clinical efficacy in advanced cancer patients[9-12]. Although the hope of TNF being an effective treatment for cancer were dashed, several studies indicated TNF is a potent mediator of inflammation via induction of

IL-6 production[13,14]. Subsequently, in a highly informative series of experiments it was shown that blockade of TNF in cultures of synovial cells from rheumatoid arthritis (RA) patients prevented the expression of IL-1 and other pro-inflammatory cytokines, suggesting that TNF was responsible for driving the production of multiple mediators of inflammation[15]. The importance of TNFα in the pathogenesis of RA was finally confirmed in clinical trials in which intravenous administration of chimeric anti-TNF mAb (infliximab, Remicade®) caused clear reductions in the level of disease activity and radiographic progression of disease[16-18]. Similar findings were subsequently reported for soluble TNF receptor-Fc fusion protein (etanercept, Enbrel®)[19,20] (Hasler, 1996;). Anti-TNF therapy was finally approved for treatment, in combination with methotrexate, of RA patients in 1998. In addition to RA, anti-TNF therapy has now been approved for the treatment of ankylosing spondylitis (AS), psoriasis, psoriatic arthritis (PsA), juvenile idiopathic arthritis (JIA), inflammatory bowel disease (IBD), and most recently, hidradenitis suppurativa and noninfectious intermediate and posterior uveitis and panuveitis [21,22]. However, clinical trials with anti-TNF therapy in multiple sclerosis (MS) patients resulted in disease exacerbation[23,24]. Moreover, it has been reported that the use of anti-TNF therapy in RA caused the development of autoimmune disease of systemic lupus erythematosus (SLE) and MS[25,26]. The paradoxical effects of anti-TNF therapy suggest that TNF plays both proinflammatory and regulatory roles in the immune system. In this review, we will focus on the biology and pathophysiologic role of TNF and on the therapeutic implications of modulating TNF receptor signalling.

TNFR1 and TNFR2 signalling

TNF is a pleiotropic pro-inflammatory cytokine, which is mainly produced by activated monocytes/macrophages, but also by various cell types such as NK cells, T lymphocytes and eosinophils in response to internal and external stimuli[27]. TNF is first synthesized as a type II transmembrane protein of 233 amino acids, and expressed on the cell surface as a trimer. Subsequent cleavage by TNF-converting enzyme (TACE, also named ADAM17) liberates the trimer into the circulation. Both the membrane form of TNF (mTNF) and the soluble form of TNF (sTNF) exert physiologic functions by binding to two structurally and functionally distinct receptors on target cells, TNF receptor 1 (also named as TNFR1, CD120a, p55 and

TNFRSF1A) and TNF receptor 2 (also named as TNFR2, CD120b, p75 and TNFR12A) on target cells.

TNFR1 is expressed on most cells of the body, bears a cytoplasmic death domain, and is activated by both mTNF and sTNF[28]. The death domain enables TNFR1 to recruit TNFR1 associated death domain (TRADD), which mainly leads to apoptosis and inflammation[29]. By contrast, TNFR2 is exclusively on a more restricted range of cell types such as $CD4^+$ and $CD8^+$ lymphocytes, endothelial cells, astrocytes, oligodendrocytes, thymocytes, myocytes, and human mesenchymal stem cells[30-36], and is preferentially activated by mTNF[28]. Because of the lack of a death domain, TNFR2 recruits TNFR associated factor 1 (TRAF1) and TRAF2 rather than TRADD[29,37,38]. In contrast to TNFR1-TRADD signaling, TNFR2 signaling through TRAF1 and TRAF2 mediates a homeostatic effect, including cell survival and tissue regeneration[39]. However, further studies reveal some degree of receptor crosstalk and overlapping function between TNFR1 and TNFR2, which seems to depend on many factors, such as cell type, intracellular or extracellular environment, stimuli and age [40,41].

Upon engagement by homotrimers of TNF, TNFR1 translocates to lipid rafts in the plasma membrane and forms a homotrimer[42,43]. The binding of TNF to TNFR1 induces a conformational change in the cytoplasmic death domain, which results in the recruitment of TRADD and receptor-interacting serine/threonine-protein kinase I (RIPKI)[37,44,45]. The co-localisation of TFNR1, TRADD and RIPKI initiates the assembly of distinct complexes, named complexes I, IIa, IIb and IIc, which leads to activation of distinct downstream signaling pathways[46,47]. The formation of these three complexes is mainly determined by the ubiquitylation status of RIPK1. Complex I is composed of TRADD, RIPK1, TRAF2 or TRAF5, cellular inhibitor of apoptosis protein 1 (cIAP1) or cIAP2 and the linear ubiquitin chain assembly complex (LUBAC)[37,44,46,48-50]. Initially, both TRAF2/5 and cIAP1/2 are E3 ubiquitin ligases that mediate K63-linked ubiquitination of RIPKI[51-53]. Subsequently, the LUBAC complex, which consists haemi-oxidized IRP2 ubiquitin ligase 1 (HOIL1), HOIL1-ineracting protein (HOIP) and SHANK-associated RH domain-inertracting protein (SHARPIN), attaches an M1-linked polyubiquitin chain to RIPK[49,54-56]. Both K63- and M1-linked polyubiquitination events stabilize complex I and facilitate further signaling. Ubiquitin chains attached to RIPK1 enable the recruitment and

activation of two signaling complexes, the transforming growth factor (TGF)-activated kinase I (TAKI) complex and the inhibitor of κB (IκB) (IKK) complex[49,54]. The TAKI complex, which is composed of TAKI, TAKI-binding protein 2 (TAB2) and TAB3, phosphorylate mitogen-activated kinase (MAPK) and consequently lead to the activation of the JUN N-termianl kinase (JNK), p38 and AP1 transcription factor[57,58]. The IKK complex, comprising nuclear factor κB (NFκB) essential modulator (NEMO), IKK subunit-α (IKKα) and IKKβ, activate NFκB pathway signaling[59,60]. Induction of NFκB and AP1 target genes plays an indispensable role in inflammation, host defense, cell proliferation and survival[46].

In contrast, the formation of complex IIa and IIb depends on non-ubiquitylated RIPK1. For the formation of complex IIa, the attached K63- and M1- linked polyubiquitin chain are removed from RIPK1 in complex I by cylindromatosis (CYLD) and result in dissociation of the deubiquitylated RIPK1 from membrane bound complex I[61-63]. The released deubiquitylated RIKP1 interacts with cytosolic TRADD, FAS-associated death domain protein (FADD), pro-caspase 8 and the long isoform of FLICE-like inhibitory protein ($FLIP_L$) to form complex IIa[64,65]. Alternatively, complex IIb is formed when the RIPK1 is not ubiquitylated due to the depletion or degradation of cIAPs[51]. The non-ubiquitylated RIPK1 dissociates from membrane bound complex I and interacts with RIPK3, pro-caspase 8 and $FLIP_L$ to from complex IIb. Both complex IIa and IIb generate active caspase 8 from pro-caspase 8 and lead to activation of the downstream caspase cascade and thus induce cell death via apoptosis. At the same time, the deubiquitylated RIPK1 and RIPK3 must be cleaved by the pro-caspase 8- $FLIP_L$ heterodimer or active caspse 8 to prevent cells from necroptosis[64,66-69]. The formation of complex IIc is similar to complex IIa and complex IIb formation in that the released deubiquitylated or non-ubiquitylated RIPK1is caused by deubiquitylation mediated by CYLD or depletion of cIAPs. However, under some circumstances, RIPK1 and RIPK3 cannot be cleaved due to inactivation of caspase and aggregate to from complex IIc (also named as necrosome). Complex IIc activates mixed lineage kinase domain-like protein (MLKL) and thus leads to the induction of necroptosis[37,45,70]. In contrast to apoptosis which is a form of highly controlled programmed cell death, necroptosis results in plasma membrane rupture, which leads to leakage of intracellular contents and local inflammation[37,38].

TNFR2 lacks the TRADD, but instead binds to TRAF1 or TRAF2 directly to recruit cIAP1 or cIAP2. The binding of TRAF2 to TNFR2 is much weaker in comparison with that of TRAF2 to TRADD[71]. The aggregation of TRAF1/2 and cIAP1/2 triggers the formation of complex I and downstream MAPK and NFκB signaling. TNFR2-TRAF signaling mediates a homeostatic effect including tissue generation, cell proliferation and cell survival[39]. The interaction between TNFR1 and TNF2 has not been fully elucidated. The difference of binding affinity between TRAF2-TNFR2 and TRAF2-TRADD suggests TNFR2 might have a regulatory effect on TNFR1 signaling in the same cell[70-72].

It has previously been proposed that TNFR2 is needed for TRAF2 degradation, leading to activation of the alternative NFκB pathway and to contribute to MAPK and classical NFκB signaling. Ruspi et al studied the signalling functions of TNFR2 in primary macrophages[73]. TNF was found to induce TRAF2 degradation which was blocked in TNFR2$^{-/-}$ macrophages. However, TRAF2 degradation was not required for TNF-induced tolerance of p38 MAPK activation. It was also found that the alternative NFκB pathway was not activated in primary macrophages by TNF or lipopolysaccharide. As expected, TNFR2 was required for activation of p38 MAPK and NFκB pathways by TNF. Of interest was the finding that, although TNFR2 was not required for p38 MAPK activation and IκBα degradation at high concentrations of TNF, it did play a role in activation of these pathways at lower concentrations of TNF. These findings suggest that TNFR2 plays an additional, or sensitising, role to TNFR1 in activation of p38 MAPK and NFκB pathways[73].

Role of TNF in health and disease

TNF is required for development of the immune system, defense against pathogens and cancer, development of germinal centers and granuloma formation[74,75]. However, uncontrolled production or function of TNF has been proved to be associated with the pathogenesis of many inflammatory diseases, including RA, AS, PsA, JIA and IBD. In addition, TNF triggers hyperalgesia by inducing peripheral and central sensitization in experimental arthritis, which is partly independent of inflammation. Anti-TNF therapy could reverse the effect and result in analgesia[76-80]. TNF is also potentially implicated in the pathogenesis of atherosclerosis and Dupuytren disease[81,82].

Intriguingly, TNF suppresses excessive immune responses and protects tissue from damage[39]. Faustamn et al demonstrated that administration of low dose TNF selectively killed autoreactive T cells, leading to reversal of type I diabetes[83]. A subsequent study showed that a TNFR2 agonist killed autoreactive CD8+ T cells and exhibited less systemic toxicity compared with administration of TNF. Other than TNFR2 signaling induced apoptosis of autoreactive T cells, TNF exerts immune suppressive function via regulatory T cells (Treg). Thus, TNFR2 is highly expressed on Treg and plays an indispensable role in maintaining the stabilization of the Treg phenotype in the inflammatory environment[84,85]. Furthermore, $TNFR2^+$ Tregs exhibit higher suppressive function than $TNFR2^-$ Tregs[86-88]. In synergy with IL-2, TNF was found to increase the expression of CD25 and Foxp3 on Tregs. However, the stimulatory effect was not seen in $TNFR2^{-/-}$ mice[89]. Various defects in TNFR2, including polymorphisms in the TNFR2 gene or TNFR2 shedding have been implicated in the pathogenesis of several autoimmune diseases[90-92]. These findings suggest that TNFR2 plays an immunoregulatory role in the immune system. In addition to its immunoregulatory role, TNF critically contributes to tissue regeneration, such as neuronal remyelination, cardiac remodeling and cartilage regeneration[93-95]. These results suggest TNF plays an indispensable role in maintaining homeostatic status, which is attributed to TNFR2 signaling[39].

Anti-TNF therapy in disease

The development of TNF inhibitors has revolutionized the management of autoimmune disease. To date, there are five anti-TNF agents, infliximab (Remicade), etanercept (Enbrel), adalimumab (Humira), certolizumab (Cimzia) and golimumab (Simponi) that have been approved for use in RA patients. The indication of anti-TNF therapy has extended to a variety of rheumatic disease, including AS, PsA, psoriasis, JIA, IBD, hidradenitis suppurativa, noninfectious intermediate and posterior uveitis and panuveitis.

Despite the great success of anti-TNF therapy, there are still drawbacks. In clinical trials or post marketing registry studies, around 10-30% patients are resistant to anti-TNF therapy (primary failure) and more patients exhibited a decrease clinical response to anti-TNF therapy after sustained treatment (secondary failure)[20,96-98]. The reasons for a lack of response are still elusive. In addition, the use of TNF inhibitors

may increase the risk of serious infection and malignancies in patients[99]. Moreover, some patients under anti-TNF therapy developed additional autoimmune diseases, including type I diabetes, SLE, MS and psoriasis[100-102]. A clinical trial of using lenercept (a soluble TNFR-Fc fusion protein) for the treatment of relapsing remitting MS had to be discontinued owing to unexpected aggravation of the disease in the lenercept treatment group[24]. These unmet needs result in the necessities of developing new anti-TNF therapy.

The unexpected adverse effect of the lenercept clinical trial might be explained by the dual role of TNF, including proinflammation and immunoregulation, which is attributed to TNFR1 and TNFR2 signaling. Because most of the proinflammatory effects of TNF are mediated by TNFR1 signaling, it might be beneficial to selectively block TNFR1 signaling and leave TNFR2 signaling unaffected. This therapeutic strategy may preserve the homeostatic function of TNFR2 signaling and offer advantages over conventional anti-TNF therapy, which blocks both TNFR1 and TNFR2, in the treatment of inflammatory/autoimmune disease, especially demyelinating disease. There are at least two strategies to specifically block the TNFR1 signaling: (1) selective inactivation of sTNF and (2) blocking TNFR1 by TNFR1 antagonists or TNFR1 antibodies.

TNFR1 signalling can be activated by mTNF or sTNF, whereas TNFR2 is preferentially activated by mTNF[28]. Selective inactivation of sTNF, which leaves mTNF intact, reduces functional TNFR1 signaling, but also maintains the homeostatic effect mediated by TNFR2. In addition, mTNF is sufficient to support TNF-mediated formation of lymphoid germinal centers and granulomas, which provide protection against pathogens[103-105]. Dominant-negative TNF (dnTNF) is synthesized based on mutations in sTNF, which prevent the TNF mutein from binding to TNFR. These TNF muteins could rapidly exchange subunits with endogenous TNF, thereby forming mixed TNF heterotrimers, which are unable to activate TNFR. The process of exchanging subunits between dnTNF and endogenous TNF is effective for sTNF, but not for mTNF. XPro1595, the first dnTNF to be generated, was shown to have comparable efficacy with etanercept in treating various animal disease models, including acute hepatitis and inflammatory arthritis without suppressing immunity to infection[106,107]. In the experimental autoimmune encephalitis (EAE) model, XPro950

significantly improved EAE clinical score, whereas etanercept had no therapeutic effect. Subsequent studies showed the beneficial effect of dnTNFs in treating animal models of neurodegenerative disease, such as spinal cord injury (SCI), MS, and Parkinson's disease (PD)[108-111].

Specific blocking of TNFR1 can be achieved by TNFR1 antagonists or TNFR1 specific antibodies. R1antTNF, a TNFR1 specific antagonist with impaired affinity to TNFR2, is generated by amino sequence modification. In treating with EAE or collagen induced arthritis (CIA), the results showed significant therapeutic effect of R1antTNF, without affecting host immunity, as judged by the viral clearance rate[112,113]. Regarding TNFR1 specific antibody, a monovalent domain antibody (DMS5540) specific for TNFR2 was compared with etanercept in treating CIA. Both DMS5540 and etanercept were similar effective in suppressive CIA progression. However, increased effector T cell activity and decreased Treg number was observed in etanercept treatment group but not in the DM5540 treatment group, suggesting an immunoregulatory role for TNFR2. In addition, TNFR1 inhibition, but not TNFR2 inhibition, expanded and activated Tregs. It was also observed that there was a marked increase in the expression of FoxP3 and TNFR2 in the joints during the period of remission, which adds further weight to the concept that TNFR2 plays a physiologic role in the resolution of inflammation.
In another study, TNF receptor one silencer (TROS), assembled by linking two anti-TNFR1 nanobodies (single domain antibodies), was found to exert a therapeutic effect in suppressing acute TNF-induced liver inflammation and colitis[114]. In addition, a TNFR1 specific antibody was demonstrated to ameliorate EAE. Taken together, these findings suggest a potential therapeutic strategy of TNFR1 specific inhibition, which might be superior to conventional anti-TNF therapy because of its sparing of TNFR2 immunoregulatory signalling.

Targeting TNFR2 in autoimmune disease

As discussed above, TNFR2 signaling plays a regulatory role in the immune system and suppresses autoreactive T cells and activates Treg. This raises the possibility that TNFR2 agonism is a potential therapeutic strategy in autoimmune disease. TNFR2 agonism was shown to be effective in selectively killing autoreactive $CD8^+$ T cell in blood samples taken from patients with type I diabetes[115]. TNFR2 agonism is also

able to expand Treg, with superior suppressor cell function[116]. In addition to this immunoregulatory effect, one study showed that a selective TNFR2 specific agonist could rescue human neurons from oxidative stress induced cell death[117]. However, the systemic effects of TNFR2 agonists needs to be further investigated before this approach can be considered for human therapy

Conclusions

Although anti-TNF therapy has achieved great success in treating various autoimmune diseases, there are still many unmet needs and unanswered questions. In particular, the role of TNFR2 signalling in health and disease is a matter of intense interest and more comprehensive studies will be needed to fully comprehend its immunoregulatory role. Ultimately, the clinical development of TNFR1 specific inhibitors offers new possibilities for advancing the therapy of RA and other conditions.

1 McCarthy, E. F. The toxins of William B. Coley and the treatment of bone and soft-tissue sarcomas. *Iowa Orthop J* **26**, 154-158 (2006).

2 O'Malley, W. E., Achinstein, B. & Shear, M. J. Journal of the National Cancer Institute, Vol. 29, 1962: Action of bacterial polysaccharide on tumors. II. Damage of sarcoma 37 by serum of mice treated with Serratia marcescens polysaccharide, and induced tolerance. *Nutr Rev* **46**, 389-391 (1988).

3 Carswell, E. A. *et al.* An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci U S A* **72**, 3666-3670 (1975).

4 Aggarwal, B. B., Henzel, W. J., Moffat, B., Kohr, W. J. & Harkins, R. N. Primary structure of human lymphotoxin derived from 1788 lymphoblastoid cell line. *J Biol Chem* **260**, 2334-2344 (1985).

5 Aggarwal, B. B. *et al.* Human tumor necrosis factor. Production, purification, and characterization. *J Biol Chem* **260**, 2345-2354 (1985).

6 Aggarwal, B. B., Moffat, B. & Harkins, R. N. Human lymphotoxin. Production by a lymphoblastoid cell line, purification, and initial characterization. *J Biol Chem* **259**, 686-691 (1984).

7 Beutler, B. *et al.* Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. *Nature* **316**, 552-554 (1985).

8 Haranaka, K., Satomi, N. & Sakurai, A. Antitumor activity of murine tumor necrosis factor (TNF) against transplanted murine tumors and heterotransplanted human tumors in nude mice. *Int J Cancer* **34**, 263-267 (1984).

9 Feinberg, B. *et al.* A phase I trial of intravenously-administered recombinant tumor necrosis factor-alpha in cancer patients. *J Clin Oncol* **6**, 1328-1334 (1988).
10 Schwartz, J. E., Scuderi, P., Wiggins, C., Rudolph, A. & Hersh, E. M. A phase I trial of recombinant tumor necrosis factor (rTNF) administered by continuous intravenous infusion in patients with disseminated malignancy. *Biotherapy* **1**, 207-214 (1989).
11 Aulitzky, W. E. *et al.* Recombinant tumour necrosis factor alpha administered subcutaneously or intramuscularly for treatment of advanced malignant disease: a phase I trial. *Eur J Cancer* **27**, 462-467 (1991).
12 Furman, W. L. *et al.* Phase I clinical trial of recombinant human tumor necrosis factor in children with refractory solid tumors: a Pediatric Oncology Group study. *J Clin Oncol* **11**, 2205-2210 (1993).
13 Zhang, Y. H., Lin, J. X., Yip, Y. K. & Vilcek, J. Enhancement of cAMP levels and of protein kinase activity by tumor necrosis factor and interleukin 1 in human fibroblasts: role in the induction of interleukin 6. *Proc Natl Acad Sci U S A* **85**, 6802-6805 (1988).
14 Shalaby, M. R., Waage, A., Aarden, L. & Espevik, T. Endotoxin, tumor necrosis factor-alpha and interleukin 1 induce interleukin 6 production in vivo. *Clin Immunol Immunopathol* **53**, 488-498 (1989).
15 Brennan, F. M., Chantry, D., Jackson, A., Maini, R. & Feldmann, M. Inhibitory effect of TNF alpha antibodies on synovial cell interleukin-1 production in rheumatoid arthritis. *Lancet* **2**, 244-247 (1989).
16 Elliott, M. J. *et al.* Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha. *Arthritis Rheum* **36**, 1681-1690 (1993).
17 Elliott, M. J. *et al.* Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. *Lancet* **344**, 1105-1110 (1994).
18 Elliott, M. J. *et al.* Repeated therapy with monoclonal antibody to tumour necrosis factor alpha (cA2) in patients with rheumatoid arthritis. *Lancet* **344**, 1125-1127 (1994).
19 Moreland, L. W. *et al.* Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. *N Engl J Med* **337**, 141-147, doi:10.1056/NEJM199707173370301 (1997).
20 Weinblatt, M. E. *et al.* A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *N Engl J Med* **340**, 253-259, doi:10.1056/NEJM199901283400401 (1999).
21 Lee, R. A. & Eisen, D. B. Treatment of hidradenitis suppurativa with biologic medications. *J Am Acad Dermatol* **73**, S82-88, doi:10.1016/j.jaad.2015.07.053 (2015).
22 Monaco, C., Nanchahal, J., Taylor, P. & Feldmann, M. Anti-TNF therapy: past, present and future. *Int Immunol* **27**, 55-62, doi:10.1093/intimm/dxu102 (2015).

23 van Oosten, B. W. *et al.* Increased MRI activity and immune activation in two multiple sclerosis patients treated with the monoclonal anti-tumor necrosis factor antibody cA2. *Neurology* **47**, 1531-1534 (1996).
24 TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group. *Neurology* **53**, 457-465 (1999).
25 Sicotte, N. L. & Voskuhl, R. R. Onset of multiple sclerosis associated with anti-TNF therapy. *Neurology* **57**, 1885-1888 (2001).
26 Shakoor, N., Michalska, M., Harris, C. A. & Block, J. A. Drug-induced systemic lupus erythematosus associated with etanercept therapy. *Lancet* **359**, 579-580, doi:10.1016/S0140-6736(02)07714-0 (2002).
27 Grivennikov, S. I. *et al.* Distinct and nonredundant in vivo functions of TNF produced by t cells and macrophages/neutrophils: protective and deleterious effects. *Immunity* **22**, 93-104, doi:10.1016/j.immuni.2004.11.016 (2005).
28 Grell, M. *et al.* The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. *Cell* **83**, 793-802 (1995).
29 Tartaglia, L. A., Ayres, T. M., Wong, G. H. & Goeddel, D. V. A novel domain within the 55 kd TNF receptor signals cell death. *Cell* **74**, 845-853 (1993).
30 Ware, C. F. *et al.* Tumor necrosis factor (TNF) receptor expression in T lymphocytes. Differential regulation of the type I TNF receptor during activation of resting and effector T cells. *J Immunol* **147**, 4229-4238 (1991).
31 Vandenabeele, P., Declercq, W., Beyaert, R. & Fiers, W. Two tumour necrosis factor receptors: structure and function. *Trends Cell Biol* **5**, 392-399 (1995).
32 Li, X., Yang, Y. & Ashwell, J. D. TNF-RII and c-IAP1 mediate ubiquitination and degradation of TRAF2. *Nature* **416**, 345-347, doi:10.1038/416345a (2002).
33 Dopp, J. M. *et al.* Expression of the p75 TNF receptor is linked to TNF-induced NFkappaB translocation and oxyradical neutralization in glial cells. *Neurochem Res* **27**, 1535-1542 (2002).
34 Grell, M., Becke, F. M., Wajant, H., Mannel, D. N. & Scheurich, P. TNF receptor type 2 mediates thymocyte proliferation independently of TNF receptor type 1. *Eur J Immunol* **28**, 257-263 (1998).
35 Irwin, M. W. *et al.* Tissue expression and immunolocalization of tumor necrosis factor-alpha in postinfarction dysfunctional myocardium. *Circulation* **99**, 1492-1498 (1999).
36 Bocker, W. *et al.* IKK-2 is required for TNF-alpha-induced invasion and proliferation of human mesenchymal stem cells. *J Mol Med (Berl)* **86**, 1183-1192, doi:10.1007/s00109-008-0378-3 (2008).
37 Hsu, H., Xiong, J. & Goeddel, D. V. The TNF receptor 1-associated protein TRADD signals cell death and NF-kappa B activation. *Cell* **81**, 495-504 (1995).
38 Rothe, M., Sarma, V., Dixit, V. M. & Goeddel, D. V. TRAF2-mediated activation of NF-kappa B by TNF receptor 2 and CD40. *Science* **269**, 1424-1427 (1995).

39 Probert, L. TNF and its receptors in the CNS: The essential, the desirable and the deleterious effects. *Neuroscience* **302**, 2-22, doi:10.1016/j.neuroscience.2015.06.038 (2015).

40 Pimentel-Muinos, F. X. & Seed, B. Regulated commitment of TNF receptor signaling: a molecular switch for death or activation. *Immunity* **11**, 783-793 (1999).

41 Naude, P. J., den Boer, J. A., Luiten, P. G. & Eisel, U. L. Tumor necrosis factor receptor cross-talk. *FEBS J* **278**, 888-898, doi:10.1111/j.1742-4658.2011.08017.x (2011).

42 Legler, D. F., Micheau, O., Doucey, M. A., Tschopp, J. & Bron, C. Recruitment of TNF receptor 1 to lipid rafts is essential for TNFalpha-mediated NF-kappaB activation. *Immunity* **18**, 655-664 (2003).

43 Locksley, R. M., Killeen, N. & Lenardo, M. J. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* **104**, 487-501 (2001).

44 Hsu, H., Huang, J., Shu, H. B., Baichwal, V. & Goeddel, D. V. TNF-dependent recruitment of the protein kinase RIP to the TNF receptor-1 signaling complex. *Immunity* **4**, 387-396 (1996).

45 Ting, A. T., Pimentel-Muinos, F. X. & Seed, B. RIP mediates tumor necrosis factor receptor 1 activation of NF-kappaB but not Fas/APO-1-initiated apoptosis. *EMBO J* **15**, 6189-6196 (1996).

46 Brenner, D., Blaser, H. & Mak, T. W. Regulation of tumour necrosis factor signalling: live or let die. *Nat Rev Immunol* **15**, 362-374, doi:10.1038/nri3834 (2015).

47 Pasparakis, M. & Vandenabeele, P. Necroptosis and its role in inflammation. *Nature* **517**, 311-320, doi:10.1038/nature14191 (2015).

48 Hsu, H., Shu, H. B., Pan, M. G. & Goeddel, D. V. TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. *Cell* **84**, 299-308 (1996).

49 Haas, T. L. *et al.* Recruitment of the linear ubiquitin chain assembly complex stabilizes the TNF-R1 signaling complex and is required for TNF-mediated gene induction. *Mol Cell* **36**, 831-844, doi:10.1016/j.molcel.2009.10.013 (2009).

50 Gerlach, B. *et al.* Linear ubiquitination prevents inflammation and regulates immune signalling. *Nature* **471**, 591-596, doi:10.1038/nature09816 (2011).

51 Bertrand, M. J. *et al.* cIAP1 and cIAP2 facilitate cancer cell survival by functioning as E3 ligases that promote RIP1 ubiquitination. *Mol Cell* **30**, 689-700, doi:10.1016/j.molcel.2008.05.014 (2008).

52 Li, H., Kobayashi, M., Blonska, M., You, Y. & Lin, X. Ubiquitination of RIP is required for tumor necrosis factor alpha-induced NF-kappaB activation. *J Biol Chem* **281**, 13636-13643, doi:10.1074/jbc.M600620200 (2006).

53 Ea, C. K., Deng, L., Xia, Z. P., Pineda, G. & Chen, Z. J. Activation of IKK by TNFalpha requires site-specific ubiquitination of RIP1 and polyubiquitin binding by NEMO. *Mol Cell* **22**, 245-257, doi:10.1016/j.molcel.2006.03.026 (2006).

54 Tokunaga, F. *et al.* Involvement of linear polyubiquitylation of NEMO in NF-kappaB activation. *Nat Cell Biol* **11**, 123-132, doi:10.1038/ncb1821 (2009).

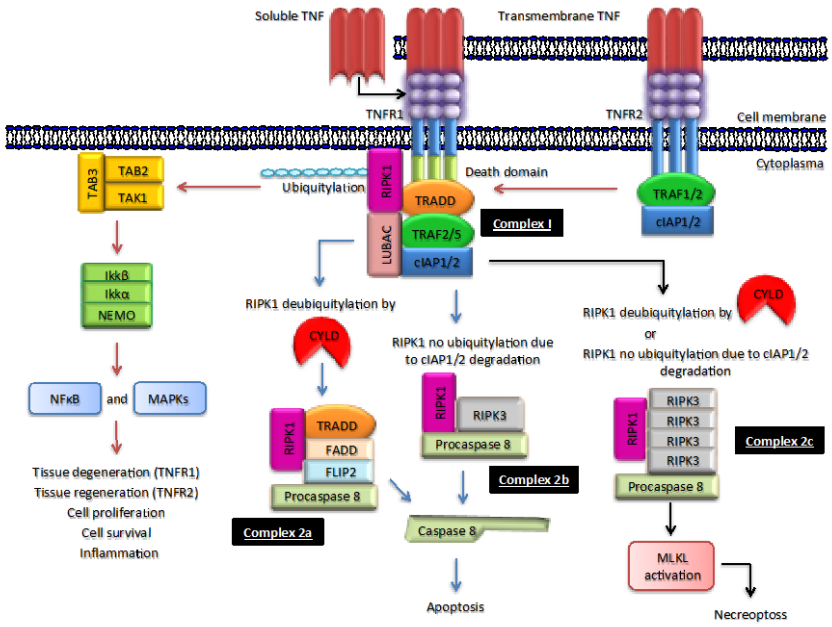
55 Tokunaga, F. *et al.* SHARPIN is a component of the NF-kappaB-activating linear ubiquitin chain assembly complex. *Nature* **471**, 633-636, doi:10.1038/nature09815 (2011).
56 Ikeda, F. *et al.* SHARPIN forms a linear ubiquitin ligase complex regulating NF-kappaB activity and apoptosis. *Nature* **471**, 637-641, doi:10.1038/nature09814 (2011).
57 Wang, C. *et al.* TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature* **412**, 346-351, doi:10.1038/35085597 (2001).
58 Kanayama, A. *et al.* TAB2 and TAB3 activate the NF-kappaB pathway through binding to polyubiquitin chains. *Mol Cell* **15**, 535-548, doi:10.1016/j.molcel.2004.08.008 (2004).
59 Israel, A. The IKK complex, a central regulator of NF-kappaB activation. *Cold Spring Harb Perspect Biol* **2**, a000158, doi:10.1101/cshperspect.a000158 (2010).
60 Hoffmann, A. & Baltimore, D. Circuitry of nuclear factor kappaB signaling. *Immunol Rev* **210**, 171-186, doi:10.1111/j.0105-2896.2006.00375.x (2006).
61 Komander, D. & Rape, M. The ubiquitin code. *Annu Rev Biochem* **81**, 203-229, doi:10.1146/annurev-biochem-060310-170328 (2012).
62 Komander, D. *et al.* Molecular discrimination of structurally equivalent Lys 63-linked and linear polyubiquitin chains. *EMBO Rep* **10**, 466-473, doi:10.1038/embor.2009.55 (2009).
63 Kovalenko, A. *et al.* The tumour suppressor CYLD negatively regulates NF-kappaB signalling by deubiquitination. *Nature* **424**, 801-805, doi:10.1038/nature01802 (2003).
64 Micheau, O. & Tschopp, J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. *Cell* **114**, 181-190 (2003).
65 He, S. *et al.* Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. *Cell* **137**, 1100-1111, doi:10.1016/j.cell.2009.05.021 (2009).
66 Wang, L., Du, F. & Wang, X. TNF-alpha induces two distinct caspase-8 activation pathways. *Cell* **133**, 693-703, doi:10.1016/j.cell.2008.03.036 (2008).
67 Vince, J. E. *et al.* IAP antagonists target cIAP1 to induce TNFalpha-dependent apoptosis. *Cell* **131**, 682-693, doi:10.1016/j.cell.2007.10.037 (2007).
68 Varfolomeev, E. *et al.* IAP antagonists induce autoubiquitination of c-IAPs, NF-kappaB activation, and TNFalpha-dependent apoptosis. *Cell* **131**, 669-681, doi:10.1016/j.cell.2007.10.030 (2007).
69 Oberst, A. *et al.* Catalytic activity of the caspase-8-FLIP(L) complex inhibits RIPK3-dependent necrosis. *Nature* **471**, 363-367, doi:10.1038/nature09852 (2011).
70 Fotin-Mleczek, M. *et al.* Apoptotic crosstalk of TNF receptors: TNF-R2-induces depletion of TRAF2 and IAP proteins and accelerates TNF-R1-dependent activation of caspase-8. *J Cell Sci* **115**, 2757-2770 (2002).
71 Park, Y. C. *et al.* A novel mechanism of TRAF signaling revealed by structural and functional analyses of the TRADD-TRAF2 interaction. *Cell* **101**, 777-787 (2000).

72 Grech, A. P. *et al.* Tumor necrosis factor receptor 2 (TNFR2) signaling is negatively regulated by a novel, carboxyl-terminal TNFR-associated factor 2 (TRAF2)-binding site. *J Biol Chem* **280**, 31572-31581, doi:10.1074/jbc.M504849200 (2005).
73 Ruspi, G. *et al.* TNFR2 increases the sensitivity of ligand-induced activation of the p38 MAPK and NF-kappaB pathways and signals TRAF2 protein degradation in macrophages. *Cell Signal* **26**, 683-690, doi:10.1016/j.cellsig.2013.12.009 (2014).
74 Pasparakis, M., Alexopoulou, L., Episkopou, V. & Kollias, G. Immune and inflammatory responses in TNF alpha-deficient mice: a critical requirement for TNF alpha in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. *J Exp Med* **184**, 1397-1411 (1996).
75 Marino, M. W. *et al.* Characterization of tumor necrosis factor-deficient mice. *Proc Natl Acad Sci U S A* **94**, 8093-8098 (1997).
76 Inglis, J. J. *et al.* Collagen-induced arthritis as a model of hyperalgesia: functional and cellular analysis of the analgesic actions of tumor necrosis factor blockade. *Arthritis Rheum* **56**, 4015-4023, doi:10.1002/art.23063 (2007).
77 Boettger, M. K. *et al.* Antinociceptive effects of tumor necrosis factor alpha neutralization in a rat model of antigen-induced arthritis: evidence of a neuronal target. *Arthritis Rheum* **58**, 2368-2378, doi:10.1002/art.23608 (2008).
78 Richter, F. *et al.* Tumor necrosis factor causes persistent sensitization of joint nociceptors to mechanical stimuli in rats. *Arthritis Rheum* **62**, 3806-3814, doi:10.1002/art.27715 (2010).
79 Konig, C., Zharsky, M., Moller, C., Schaible, H. G. & Ebersberger, A. Involvement of peripheral and spinal tumor necrosis factor alpha in spinal cord hyperexcitability during knee joint inflammation in rats. *Arthritis Rheumatol* **66**, 599-609, doi:10.1002/art.38271 (2014).
80 Hess, A. *et al.* Blockade of TNF-alpha rapidly inhibits pain responses in the central nervous system. *Proc Natl Acad Sci U S A* **108**, 3731-3736, doi:10.1073/pnas.1011774108 (2011).
81 Skeoch, S. & Bruce, I. N. Atherosclerosis in rheumatoid arthritis: is it all about inflammation? *Nat Rev Rheumatol* **11**, 390-400, doi:10.1038/nrrheum.2015.40 (2015).
82 Verjee, L. S. *et al.* Unraveling the signaling pathways promoting fibrosis in Dupuytren's disease reveals TNF as a therapeutic target. *Proc Natl Acad Sci U S A* **110**, E928-937, doi:10.1073/pnas.1301100110 (2013).
83 Kodama, S., Davis, M. & Faustman, D. L. The therapeutic potential of tumor necrosis factor for autoimmune disease: a mechanistically based hypothesis. *Cell Mol Life Sci* **62**, 1850-1862, doi:10.1007/s00018-005-5022-6 (2005).
84 Chen, X. *et al.* TNFR2 is critical for the stabilization of the CD4+Foxp3+ regulatory T. cell phenotype in the inflammatory environment. *J Immunol* **190**, 1076-1084, doi:10.4049/jimmunol.1202659 (2013).

85 Annunziato, F. *et al.* Phenotype, localization, and mechanism of suppression of CD4(+)CD25(+) human thymocytes. *J Exp Med* **196**, 379-387 (2002).
86 Govindaraj, C., Scalzo-Inguanti, K., Scholzen, A., Li, S. & Plebanski, M. TNFR2 Expression on CD25(hi)FOXP3(+) T Cells Induced upon TCR Stimulation of CD4 T Cells Identifies Maximal Cytokine-Producing Effectors. *Front Immunol* **4**, 233, doi:10.3389/fimmu.2013.00233 (2013).
87 Chen, X. *et al.* Co-expression of TNFR2 and CD25 identifies more of the functional CD4+FOXP3+ regulatory T cells in human peripheral blood. *Eur J Immunol* **40**, 1099-1106, doi:10.1002/eji.200940022 (2010).
88 Chen, X. *et al.* Cutting edge: expression of TNFR2 defines a maximally suppressive subset of mouse CD4+CD25+FoxP3+ T regulatory cells: applicability to tumor-infiltrating T regulatory cells. *J Immunol* **180**, 6467-6471 (2008).
89 Chen, X., Baumel, M., Mannel, D. N., Howard, O. M. & Oppenheim, J. J. Interaction of TNF with TNF receptor type 2 promotes expansion and function of mouse CD4+CD25+ T regulatory cells. *J Immunol* **179**, 154-161 (2007).
90 Barton, A., John, S., Ollier, W. E., Silman, A. & Worthington, J. Association between rheumatoid arthritis and polymorphism of tumor necrosis factor receptor II, but not tumor necrosis factor receptor I, in Caucasians. *Arthritis Rheum* **44**, 61-65, doi:10.1002/1529-0131(200101)44:1<61::AID-ANR9>3.0.CO;2-Q (2001).
91 Komata, T., Tsuchiya, N., Matsushita, M., Hagiwara, K. & Tokunaga, K. Association of tumor necrosis factor receptor 2 (TNFR2) polymorphism with susceptibility to systemic lupus erythematosus. *Tissue Antigens* **53**, 527-533 (1999).
92 Pierik, M. *et al.* Tumour necrosis factor-alpha receptor 1 and 2 polymorphisms in inflammatory bowel disease and their association with response to infliximab. *Aliment Pharmacol Ther* **20**, 303-310, doi:10.1111/j.1365-2036.2004.01946.x (2004).
93 Arnett, H. A. *et al.* TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination. *Nat Neurosci* **4**, 1116-1122, doi:10.1038/nn738 (2001).
94 Papathanasiou, S. *et al.* Tumor necrosis factor-alpha confers cardioprotection through ectopic expression of keratins K8 and K18. *Nat Med* **21**, 1076-1084, doi:10.1038/nm.3925 (2015).
95 Zhao, Y. P. *et al.* Progranulin protects against osteoarthritis through interacting with TNF-alpha and beta-Catenin signalling. *Ann Rheum Dis* **74**, 2244-2253, doi:10.1136/annrheumdis-2014-205779 (2015).
96 Lipsky, P. E. *et al.* Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. *N Engl J Med* **343**, 1594-1602, doi:10.1056/NEJM200011303432202 (2000).
97 Weinblatt, M. E. *et al.* Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. *Arthritis Rheum* **48**, 35-45, doi:10.1002/art.10697 (2003).

98 Finckh, A., Simard, J. F., Gabay, C., Guerne, P. A. & physicians, S. Evidence for differential acquired drug resistance to anti-tumour necrosis factor agents in rheumatoid arthritis. *Ann Rheum Dis* **65**, 746-752, doi:10.1136/ard.2005.045062 (2006).
99 Bongartz, T. *et al.* Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. *JAMA* **295**, 2275-2285, doi:10.1001/jama.295.19.2275 (2006).
100 Kollias, G., Kontoyiannis, D., Douni, E. & Kassiotis, G. The role of TNF/TNFR in organ-specific and systemic autoimmunity: implications for the design of optimized 'anti-TNF' therapies. *Curr Dir Autoimmun* **5**, 30-50 (2002).
101 Tack, C. J., Kleijwegt, F. S., Van Riel, P. L. & Roep, B. O. Development of type 1 diabetes in a patient treated with anti-TNF-alpha therapy for active rheumatoid arthritis. *Diabetologia* **52**, 1442-1444, doi:10.1007/s00125-009-1381-0 (2009).
102 Bloom, B. J. Development of diabetes mellitus during etanercept therapy in a child with systemic-onset juvenile rheumatoid arthritis. *Arthritis Rheum* **43**, 2606-2608, doi:10.1002/1529-0131(200011)43:11<2606::AID-ANR31>3.0.CO;2-X (2000).
103 Ruuls, S. R. *et al.* Membrane-bound TNF supports secondary lymphoid organ structure but is subservient to secreted TNF in driving autoimmune inflammation. *Immunity* **15**, 533-543 (2001).
104 Saunders, B. M. *et al.* Transmembrane TNF is sufficient to initiate cell migration and granuloma formation and provide acute, but not long-term, control of Mycobacterium tuberculosis infection. *J Immunol* **174**, 4852-4859 (2005).
105 Fremond, C. *et al.* Membrane TNF confers protection to acute mycobacterial infection. *Respir Res* **6**, 136, doi:10.1186/1465-9921-6-136 (2005).
106 Zalevsky, J. *et al.* Dominant-negative inhibitors of soluble TNF attenuate experimental arthritis without suppressing innate immunity to infection. *J Immunol* **179**, 1872-1883 (2007).
107 Olleros, M. L. *et al.* Soluble TNF, but not membrane TNF, is critical in LPS-induced hepatitis. *J Hepatol* **53**, 1059-1068, doi:10.1016/j.jhep.2010.05.029 (2010).
108 McCoy, M. K. *et al.* Blocking soluble tumor necrosis factor signaling with dominant-negative tumor necrosis factor inhibitor attenuates loss of dopaminergic neurons in models of Parkinson's disease. *J Neurosci* **26**, 9365-9375, doi:10.1523/JNEUROSCI.1504-06.2006 (2006).
109 McCoy, M. K. *et al.* Intranigral lentiviral delivery of dominant-negative TNF attenuates neurodegeneration and behavioral deficits in hemiparkinsonian rats. *Mol Ther* **16**, 1572-1579, doi:10.1038/mt.2008.146 (2008).
110 Brambilla, R. *et al.* Inhibition of soluble tumour necrosis factor is therapeutic in experimental autoimmune encephalomyelitis and promotes axon preservation and remyelination. *Brain* **134**, 2736-2754, doi:10.1093/brain/awr199 (2011).

111 Novrup, H. G. *et al.* Central but not systemic administration of XPro1595 is therapeutic following moderate spinal cord injury in mice. *J Neuroinflammation* **11**, 159, doi:10.1186/s12974-014-0159-6 (2014).
112 Shibata, H. *et al.* The treatment of established murine collagen-induced arthritis with a TNFR1-selective antagonistic mutant TNF. *Biomaterials* **30**, 6638-6647, doi:10.1016/j.biomaterials.2009.08.041 (2009).
113 Nomura, T. *et al.* Therapeutic effect of PEGylated TNFR1-selective antagonistic mutant TNF in experimental autoimmune encephalomyelitis mice. *J Control Release* **149**, 8-14, doi:10.1016/j.jconrel.2009.12.015 (2011).
114 Steeland, S. *et al.* Generation and characterization of small single domain antibodies inhibiting human tumor necrosis factor receptor 1. *J Biol Chem* **290**, 4022-4037, doi:10.1074/jbc.M114.617787 (2015).
115 Ban, L. *et al.* Selective death of autoreactive T cells in human diabetes by TNF or TNF receptor 2 agonism. *Proc Natl Acad Sci U S A* **105**, 13644-13649, doi:10.1073/pnas.0803429105 (2008).
116 Okubo, Y., Mera, T., Wang, L. & Faustman, D. L. Homogeneous expansion of human T-regulatory cells via tumor necrosis factor receptor 2. *Sci Rep* **3**, 3153, doi:10.1038/srep03153 (2013).
117 Fischer, R. *et al.* A TNF receptor 2 selective agonist rescues human neurons from oxidative stress-induced cell death. *PLoS One* **6**, e27621, doi:10.1371/journal.pone.0027621 (2011).


Soluble TNF
Transmembrane TNF
TNFR1
TNFR2
Cell membrane
Cytoplasma
TAB3
TAB2
TAK1
Ubiquitylation
RIPK1
Death domain
TRADD
TRAF2/5
LUBAC
cIAP1/2
Complex I
TRAF1/2
cIAP1/2
IkkB
Ikkα
NEMO
NFκB
and
MAPKs
Tissue degeneration (TNFR1)
Tissue regeneration (TNFR2)
Cell proliferation
Cell survival
Inflammation
RIPK1 deubiquitylation by
CYLD
RIPK1
TRADD
FADD
FLIP2
Procaspase 8
Complex 2a
RIPK1 no ubiquitylation due to cIAP1/2 degradation
RIPK1
RIPK3
Procaspase 8
Complex 2b
Caspase 8
Apoptosis
RIPK1 deubiquitylation by
CYLD
or
RIPK1 no ubiquitylation due to cIAP1/2 degradation
RIPK1
RIPK3
RIPK3
RIPK3
RIPK3
Procaspase 8
Complex 2c
MLKL activation
Necreoptoss