Regulation of tumour necrosis factor signalling: live or let die

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Abstract | Tumour necrosis factor (TNF) is a pro-inflammatory cytokine that has important roles in mammalian immunity and cellular homeostasis. Deregulation of TNF receptor (TNFR) signalling is associated with many inflammatory disorders, including various types of arthritis and inflammatory bowel disease, and targeting TNF has been an effective therapeutic strategy in these diseases. This Review focuses on the recent advances that have been made in understanding TNFR signalling and the consequences of its deregulation for cellular survival, apoptosis and regulated necrosis. We discuss how TNF-induced survival signals are distinguished from those that lead to cell death. Finally, we provide a brief overview of the role of TNF in inflammatory and autoimmune diseases, and we discuss up-to-date and future treatment strategies for these disorders.

Tumour necrosis factor (TNF) is one of the most intensively studied cytokines of the immune system. Decades of work has established that TNF is a central player within a complicated network of cytokines, and that it regulates not only pro-inflammatory responses but also processes as diverse as cellular communication, cell differentiation and cell death. It is due to these far-reaching and important regulatory functions of TNF that alterations to its biology have been associated with multiple diseases, including autoimmunity and cancer.

TNF activity was described by Carswell in 1975, and the molecule was later cloned, purified and initially characterized by Aggarwal and colleagues in the mid-1980s[1–3]. These important discoveries were the first steps in characterizing two prominent protein superfamilies: the TNF superfamily (TNFSF) and the TNF receptor superfamily (TNFRSF). To date, 19 ligands and 29 receptors of the TNFSF and TNFRSF, respectively, have been identified[2]. A characteristic hallmark of members of the TNFSF is their ability to promote pro-inflammatory signalling[3].

Of all of the members of the TNFSF and TNFRSF, TNF and its two receptors — TNFR1 and TNFR2 — are the best characterized. TNF is initially expressed as a trimeric type II transmembrane protein. This can be cleaved by the metalloproteinase TNF-converting enzyme (TACE; also known as ADAM17), which is controlled by inactive rhomboid protein 2 (iRHOM2), to give rise to soluble extracellular TNF[4–9]. The expression of membrane-bound TNF and the generation of its soluble form are tightly regulated processes that occur in response to diverse stimuli. Soluble TNF can bind to either TNFR1 or TNFR2, which differ in their structure and expression pattern, as well as in the signalling pathways that they induce once they are engaged[10]. Although TNFR1 is expressed by almost every mammalian cell type, TNFR2 expression is essentially restricted to immune cells and endothelial cells[10]. The binding of TNF to either TNFR1 or TNFR2 can ultimately activate the transcription factor nuclear factor-κB (NF-κB), but the signalling cascades that lead from each receptor to NF-κB activation are markedly different. These complexities, together with the diverse regulation of the expression of TNF itself, result in finely tuned, cell type-specific responses to TNF. In this Review, we summarize the current state of knowledge of TNFR signalling and explain how this knowledge can be used to design novel therapies.

TNF signalling in NF-κB activation

TNFR1 and TNFR2 signalling elements. The extracellular domains of both TNFR1 and TNFR2 are rich in cysteine and able to bind to the same TNF ligand. However, their intracellular domains are strikingly different. TNFR1 contains a cytoplasmic 'death domain', which is a conserved sequence of 80 amino acids that forms a distinctive fold[11,12]. This death domain enables TNFR1 to recruit the adaptor molecule TNFR1-associated death domain protein (TRADD), which is a crucial component of the TNFR1 signalling complex[13] (FIG. 1). By contrast, TNFR2 lacks the cytoplasmic death domain sequence and recruits TNFR-associated factor 1 (TRAF1) and TRAF2 rather than TRADD[12–14]. Both TNFR1–TRADD signalling and TNFR2 signalling through TRAF1 and TRAF2 can lead to NF-κB activation, but whereas TNFR2 engagement

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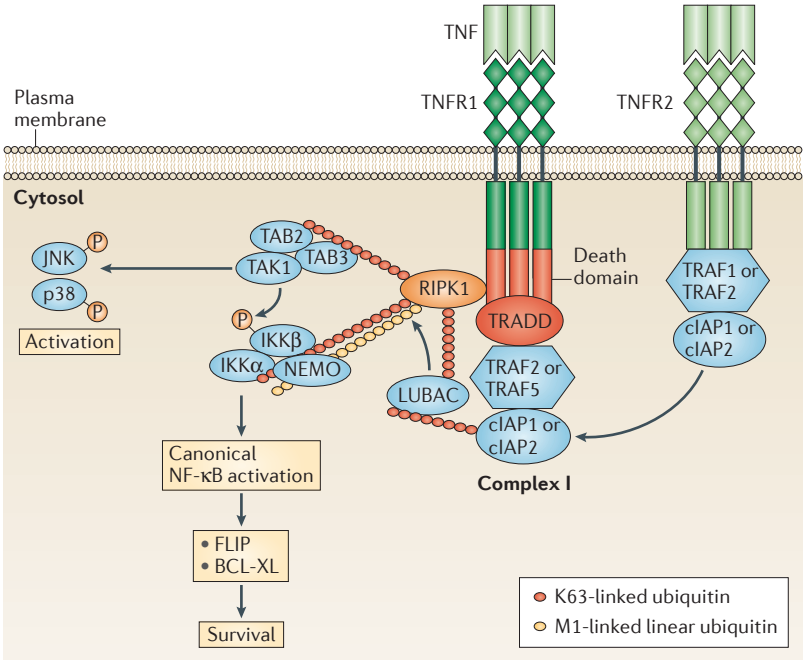

Figure 1 | **TNFR1 complex I contains ubiquitylated RIPK1 and activates nuclear factor-κB, JNK and p38 signalling.** Following the binding of tumour necrosis factor (TNF) to TNF receptor 1 (TNFR1), TNFR1 binds to TNFR1-associated death domain protein (TRADD), which recruits receptor-interacting serine/threonine-protein kinase 1 (RIPK1), TNFR-associated factor 2 (TRAF2) or TRAF5 and cellular inhibitor of apoptosis protein 1 (cIAP1) or cIAP2 to form TNFR1 signalling complex I. TNFR2 binds to TRAF1 or TRAF2 directly to recruit cIAP1 or cIAP2. Both cIAP1 and cIAP2 are E3 ubiquitin ligases that add K63-linked polyubiquitin chains to RIPK1 and other components of the signalling complex. The ubiquitin ligase activity of the cIAPs is needed to recruit the linear ubiquitin chain assembly complex (LUBAC), which adds M1-linked linear polyubiquitin chains to RIPK1. K63-polyubiquitylated RIPK1 recruits TGFβ-activated kinase 1 and MAP3K7-binding protein 2 (TAB2) and TAB3 and TGFβ-activated kinase 1 (TAK1), which activate signalling mediated by JUN N-terminal kinase (JNK) and p38, as well as the IκB kinase (IKK) complex. The IKK complex then activates nuclear factor-κB (NF-κB) signalling, which leads to the transcription of anti-apoptotic factors — such as the long isoform of FLICE-like inhibitory protein ($FLIP_L$) and BCL-XL (also known as BCL2L1) — that promote cell survival. NEMO, NF-κB essential modulator.

promotes cell survival via this pathway, TNFR1–TRADD signalling can result in either cell survival or cell death depending on downstream signalling events and cellular context. It is still not entirely clear how TNFR1 and TNFR2 signalling are regulated, apart from through the differential expression patterns of TNFR1 and TNFR2 themselves. In many cases, immune cells that express TNFR2 also express TNFR1, and this makes predicting the outcome of TNF-mediated signalling a challenge. It has been shown that the relative levels of TNFR1 and TNFR2 on the surface of such cells and their activation status in a specific context have an important role in determining cell fate[15,16]. However, crosstalk between these receptors is also possible, and the past decade has seen the formulation of several new concepts that have broadened our view of the effects of TNF-induced signalling.

Upon engagement by TNF, TNFR1 translocates to lipid rafts in the plasma membrane, and this is crucial for NF-κB activation[17]. The binding of TNF to the pre-assembled TNFR1 induces a conformational change in the cytoplasmic domain of the receptor that enables the recruitment of TRADD, which in turn recruits receptor-interacting serine/threonine-protein kinase 1 (RIPK1)[13,18,19]. Once bound together, TNFR1, TRADD and RIPK1 initiate the assembly of TNFR1 complex I, which directs downstream signalling events.

The next step in complex I formation is the recruitment of TRAF2, which binds to the amino-terminal TRAF-binding domain of TRADD[20]. At this point, the signalling cascades downstream of TNFR1–TRADD–RIPK1–TRAF2 and TNFR2–TRAF2 become similar again, but the binding of TRAF2 to TNFR2 is much weaker than that of TRAF2 to TRADD[21]. This difference suggests that an affinity-based regulatory mechanism may exist to control these two TNF-induced pathways. It is tempting to speculate that, at a certain TNF concentration, TNFR2 might act as a signalling dampener to attenuate or alter the strength or outcome of signalling by TNFR1 in the same cell[21–23]. Downstream of this, TRAF2 binds to cellular inhibitor of apoptosis protein 1 (cIAP1; also known as BIRC2) and cIAP2 (also known as BIRC3). However, studies in mice suggest that TRADD, TRAF2, RIPK1 and the cIAPs are all important, but not indispensable, for TNFR1-induced NF-κB activation[24–30].

Role of ubiquitylation. Post-translational modification of the proteins involved in TNF signalling cascades has a major role in determining TNF-induced outcomes. The primary modification relevant to our discussion here is ubiquitylation, which involves the covalent linkage of the highly conserved ubiquitin protein to a target protein. The molecular details of ubiquitylation have been reviewed extensively elsewhere[31,32]. Briefly, it is a hierarchical three-step process involving E1 ubiquitin-activating, E2 ubiquitin-conjugating and E3 ubiquitin ligase enzymes attaching defined strings of ubiquitin molecules to target proteins to form polyubiquitylated conjugates[33]. The length of the attached ubiquitin chain may alter the fate of the target protein, as may the nature of the covalent bond linkage between ubiquitin proteins itself. These polyubiquitin linkages usually involve specific lysine or methionine residues. For TNF-induced signalling, polyubiquitylation via K11, K48 or K63 branched linkages, or alternatively via M1 linear linkages, has a predominant role in determining target protein fate. For example, proteins that are attached to K48-linked polyubiquitin chains are targeted for degradation[31]; such destruction of a signalling mediator contributes to the shutdown of intracellular signalling. By contrast, K63- and M1-linked polyubiquitylation events reinforce protein scaffolding and cellular activation. Both degradative and activating functions have been described for K11-linked polyubiquitin chains[32].

With respect to TNF-induced NF-κB activation, it was originally thought that the creation of a K63-linked ubiquitylated scaffold containing RIPK1 was crucial for recruiting downstream signalling mediators. Both cIAP1 and cIAP2 were shown to add K63-linked polyubiquitin chains to RIPK1 at its acceptor site K377 (REFS 34,35),

and TRAF2 and TRAF5 were also thought to function as E3 ligases that attached K63-linked polyubiquitin chains to RIPK1 (REF. 36). However, whether TRAF2 truly has an E3 ligase function is the subject of much debate[37–39], leaving cIAP1 and cIAP2 as the most likely candidates. Interestingly, some cells (for example, mouse embryonic fibroblasts) can recruit RIPK1 to TNFR1 independently of TRADD, but RIPK1 cannot be ubiquitylated because recruitment of the ubiquitin ligases cIAP1 and cIAP2 via TRAF2 requires TRADD[26,27].

In addition to controversy about the enzyme responsible, the relevance of the K63 linkage has been questioned. An elegant ubiquitin-replacement study showed that K63-linked polyubiquitin chains cannot be solely responsible for TNF-induced NF-κB activation, implying the involvement of other linkages or mechanisms[40]. Indeed, it was subsequently demonstrated that, in addition to K63-linked ubiquitin chains, RIPK1 simultaneously contains K11-, K48- and M1-linked polyubiquitin chains[41–43].

A major step in clarifying the role of ubiquitylation in TNF signalling was the identification and characterization of the linear ubiquitin chain assembly complex (LUBAC)[41,44]. LUBAC consists of three proteins: haeme-oxidized IRP2 ubiquitin ligase 1 (HOIL1; also known as RBCK1), HOIL1-interacting protein (HOIP; also known as RNF31) and SHANK-associated RH domain-interacting protein SHARPIN[41,42,45–47]. Interestingly, the recruitment of LUBAC to complex I depends on the K63-polyubiquitylation activity of cIAP1 and cIAP2. LUBAC association with the TNFR signalling complex is not strictly RIPK1 dependent (RIPK1 is a prominent target of cIAP1 and cIAP2), but is increased in the presence of RIPK1. Once recruited, LUBAC stabilizes complex I by catalysing the attachment of a linear M1-linked polyubiquitin chain, usually to RIPK1 (REFS 18,41,45). The relevance of K63-linked as opposed to M1-linked linear polyubiquitylation events in TNFR1 signalling remains under debate. It is clear that both processes are involved in TNF-induced signal transduction, but their specific and relative contributions are not entirely defined. Nevertheless, it is currently generally accepted that RIPK1 is a central molecular switch in complex I, and that all downstream signalling by this complex depends on the ubiquitylation status of RIPK1 (see below).

Role of NF-κB essential modulator. Ubiquitin chains attached to RIPK1 by LUBAC recruit the IκB kinase (IKK) complex to the TNFR1 signalling core[41,44]. The IKK complex consists of three subunits: two kinases called IKKα and IKKβ, and the regulatory subunit NF-κB essential modulator (NEMO; also known as IKKγ)[48,49]. Once activated, IKK phosphorylates NF-κB inhibitor-α (IκBα), which binds to NF-κB during steady-state conditions and keeps it inactive in the cytoplasm. Upon its phosphorylation, IκBα is targeted for K48-linked ubiquitylation followed by degradation, which releases NF-κB from suppression. Newly freed NF-κB translocates to the nucleus and activates the transcription of its numerous target genes, which are involved in cell survival and proliferation[48,49]. The details of IKK-driven NF-κB activation have been previously summarized in two comprehensive reviews and will not be discussed further here[48,49].

Membrane-proximal recruitment of NEMO to ubiquitylated RIPK1 is crucial for IKK activation and thus NF-κB activation, as has been particularly well demonstrated for the T cell receptor-dependent pathway of NF-κB activation in T cells[50]. Accordingly, mutations in the ubiquitin-binding domain of NEMO blunt NF-κB activation in response to TNF[34,51]. In binding to ubiquitylated RIPK1, NEMO shows significantly higher affinity for M1-linked linear polyubiquitin chains than for K63-linked polyubiquitin chains[52,53]. In addition, NEMO can bind to K11-linked polyubiquitin chains attached to RIPK1 (REF. 43). As a result, NEMO recruitment might be modulated by the relative concentrations of the various types of polyubiquitylated chains present within complex I. The various types (K11-, K48-, K63- and M1-linked) and concentrations of polyubiquitin chains attached to RIPK1 may permit multiple cooperative or parallel possibilities for recruiting NEMO. This mechanism opens up interesting options for fine-tuning ubiquitin-dependent signalling to modulate the activity of recruited targets, as one ubiquitin linkage might be more dominant in a specific pathway. Such factors might explain why K63-linked polyubiquitin chains seem to be dispensable for TNF-mediated NF-κB activation but not for interleukin-1β (IL-1β)-mediated NF-κB activation[40].

NEMO itself may undergo M1-linked linear polyubiquitylation, but it is unclear when or where this modification occurs[42,44]. Based on signalling hierarchy, it seems unlikely that M1-linked linear polyubiquitylation of NEMO precedes its recruitment to the TNFR1 signalling core. In any case, the polyubiquitin chains present at NEMO and RIPK1 are bound by the ubiquitin-binding protein TGFβ-activated kinase 1 and MAP3K7-binding protein 2 (TAB2) or TAB3, which interact with TGFβ-activated kinase 1 (TAK1). This binding is a crucial step in IKK activation because TAK1 is the direct initiator of this process[34,54,55]. TAK1 also phosphorylates mitogen-activated protein kinase kinases (MAPKKs), which trigger the activation of the JUN N-terminal kinase (JNK) and p38 pathways[54–56].

Intriguingly, the TAB–TAK1 complex binds to K63-linked polyubiquitin chains of RIPK1 (FIG. 1) with higher affinity than to M1-linked polyubiquitin chains[34,57]. To make matters more complex, there is recent evidence from studies of IL-1β-dependent signalling that hybrid K63- and M1-linked polyubiquitin chains may exist, which can recruit NEMO using the M1-linked polyubiquitin portion and simultaneously recruit TAB–TAK1 using the K63-linked polyubiquitin portion[58]. Such a mechanism would allow the IKK complex to efficiently colocalize with and become activated within TNFR1 complex I by binding to the same polyubiquitin chain. However, to date, these hybrid polyubiquitin chains have only been detected in the IL-1β-dependent signalling pathway[58]. It will be interesting to determine whether this mechanism operates in other pathways — particularly in TNF-mediated signalling — and whether hybrid polyubiquitin chains can in fact be attached to RIPK1.

TNF signalling in apoptosis

TNF is a very versatile cytokine with pleiotropic functions in immunity, inflammation and cell death[3]. Interestingly, the mechanism by which these various functions of TNF are mediated is not just a simple matter of binding to different receptors. In fact, the binding of TNF to TNFR1 can induce either cell survival or different forms of cell death; therefore, the regulation of TNF signal transduction is a constant balancing act between these opposing functions.

Classical apoptosis. As described above, TNF-induced TNFR1 signalling leading to the NF-κB activation that supports cell survival and inflammation is mediated by the membrane-bound TNFR1–TRADD–RIPK1–TRAF2 signalling core known as complex I[59]. However, based on its structure, TNFR1 is classified as a death receptor and classical apoptosis was shown to occur upon TNF binding to TNFR1 (REFS 2,3). The signalling mediators of the classical apoptotic pathway were first defined by studying the death receptor FAS (also known as CD95 and TNFRSF6)[11]. The death domain fold in the cytoplasmic portion of the FAS protein was shown to bind to FAS-associated death domain protein (FADD); this is the first step in the formation of a membrane-bound death-inducing signalling complex (DISC). The death effector domain of FADD then recruits pro-caspase 8 to form a homodimer that autocatalytically removes its pro-domains, stabilizing an active conformation and resulting in the release of an activated caspase 8 homodimer into the cytoplasm. This activated caspase 8 molecule uses its proteolytic activity to target and cleave numerous intracellular substrates, including the executioner caspases that drive classical apoptosis[11,60]. Depending on the situation, this cytoplasmic apoptotic signalling can be amplified or modified by the mitochondrial cell death pathway, which has been reviewed elsewhere[61].

In contrast to FAS-mediated classical apoptosis, the apoptotic cascade initiated by TNF binding to TNFR1 is much more complicated. The process can be mediated by either one of two TNFR1 signalling complexes known as TNFR1 complex IIa and complex IIb. The initial assembly of these protein complexes upon TNFR1 stimulation occurs proximal to the membrane, but they translocate to the cytosol to continue further complex formation. As well as the apoptosis mediators mentioned above, the cytosolic complexes IIa and IIb contain additional components (FIG. 2).

RIPK1 as a central molecular switch. An important molecular switch that determines whether TNF–TNFR1 signalling mediates cell survival or apoptosis seems to be the ubiquitylation status of RIPK1. Not only is the polyubiquitylation of RIPK1 bound to TRADD essential for NF-κB activation (as discussed above), but it also prevents the formation of complex IIa and complex IIb and thus death induction, such that the cell survives[34,35,62,63]. Conversely, when RIPK1 is not ubiquitylated, TNF-driven NF-κB signalling is turned off so that TNF-driven apoptotic signalling dominates and the cell dies. Non-ubiquitylated RIPK1 leads to dissociation of a RIPK1-containing complex from the membrane and the formation of protein complexes that promote cell death.

Several ubiquitin-modifying proteins can act on RIPK1. Both the ubiquitin-modifying enzyme A20 (also known as TNFAIP3) and the deubiquitylating enzyme Cezanne (also known as OTUD7B) can remove K63-linked polyubiquitin chains from RIPK1 (REFS 64,65). Subsequently, A20 and/or the E3 ubiquitin ligase caspase regulator 2 (CARP2; also known as RFFL) add K48-linked polyubiquitin chains to RIPK1, which triggers its degradation and thus the suppression of TNF-induced NF-κB signalling[65,66]. In addition, A20 can use its carboxy-terminal zinc finger 7 domain to bind to M1-linked polyubiquitin chains, thereby preventing the binding of LUBAC to NEMO and blocking TNF-induced LUBAC-mediated NF-κB activation[67].

Another deubiquitylating enzyme that acts on RIPK1 and is crucial for shutting down TNF-induced NF-κB activation is cylindromatosis (CYLD)[68,69]. CYLD associates with complex I via TRAF2 and removes both K63- and M1-linked polyubiquitin chains from target proteins[32,57,68,70]. Recent evidence suggests that CYLD removes K63- and M1-linked polyubiquitin chains from RIPK1, causing the deubiquitylated RIPK1 to dissociate from the membrane-bound TNFR1 signalling core[68]. This freed and deubiquitylated RIPK1 then assembles in the cytosol with TRADD, FADD, a pro-caspase 8 homodimer, and a heterodimer of pro-caspase 8 and the long isoform of FLICE-like inhibitory protein ($FLIP_L$; also known as the long isoform of CFLAR) to form complex IIa (FIG. 2). It is important to note that although RIPK1 initiates the assembly of complex IIa, RIPK1 is inactivated by a $FLIP_L$–pro-caspase 8 heterodimer in order to proceed with the apoptotic programme (see below). Once complex IIa is assembled, pro-caspase 8 undergoes autocatalytic cleavage and activation, thereby releasing active caspase 8 into the cytosol to trigger the execution of the classical apoptotic programme[59,62].

Role of cIAPs and formation of complex IIb. An alternative independent complex IIb that also promotes classical apoptosis is assembled under conditions in which the E3 ligases cIAP1 and cIAP2 are depleted[68] (FIG. 2). The degradation or depletion of cIAPs reduces or prevents RIPK1 ubiquitylation[36,68]. This non-ubiquitylated RIPK1 soon dissociates from membrane-bound complex I. In a process that depends on the kinase activity of RIPK1 but does not involve TRADD recruitment, non-ubiquitylated RIPK1 assembles with RIPK3, pro-caspase 8 and $FLIP_L$ to form complex IIb, which then induces apoptosis in a manner similar to complex IIa[68,71]. As complex IIb is nucleated by RIPK1 and does not require TRADD, it is also known as the ripoptosome. Similarly to complex IIa, cleavage and inactivation of cytosolic RIPK1 or RIPK3 (that is not incorporated into complex IIb) is essential for the apoptotic cascade (see below).

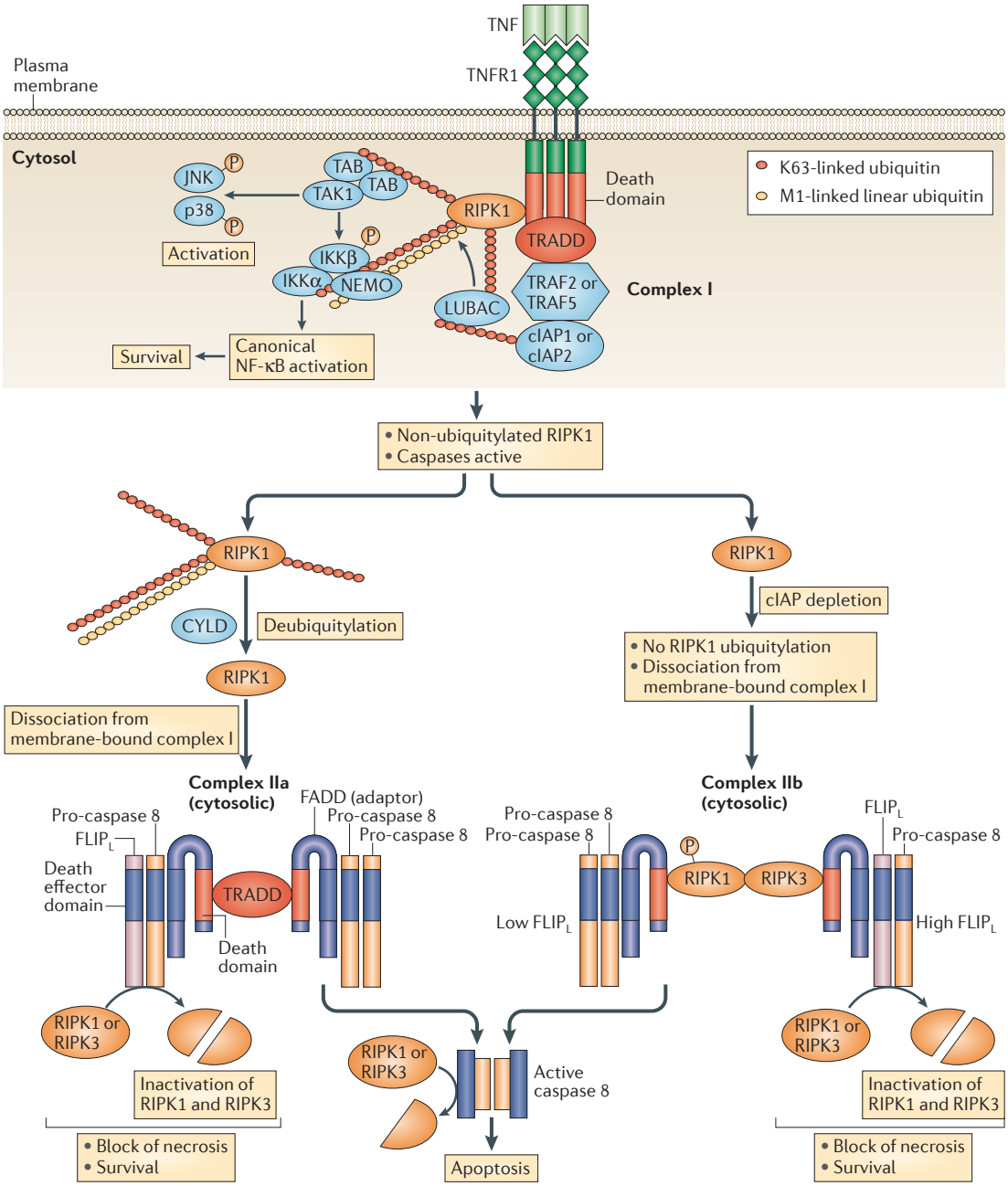

Figure 2 | **TNF-induced apoptosis requires non-ubiquitylated RIPK1 and active caspases.** The formation of cytosolic tumour necrosis factor receptor 1 (TNFR1) complex IIa and complex IIb depends on non-ubiquitylated receptor-interacting serine/threonine-protein kinase 1 (RIPK1). For the formation of complex IIa, ubiquitylated RIPK1 in complex I is deubiquitylated by cylindromatosis (CYLD). This deubiquitylated RIPK1 dissociates from the membrane-bound complex and moves into the cytosol, where it interacts with TNFR1-associated death domain protein (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. By contrast, complex IIb is formed when the RIPK1 in complex I is not ubiquitylated in the first place owing to conditions that have resulted in the depletion of cellular inhibitor of apoptosis proteins (cIAPs; which normally ubiquitylate RIPK1). This non-ubiquitylated RIPK1 dissociates from complex I, moves into the cytosol, and assembles with FADD, pro-caspase 8, $FLIP_L$ and RIPK3 (but not TRADD) to form complex IIb. Formation of complex IIb, which has also been named the ripoptosome, depends on the kinase activity of RIPK1. In both complex IIa and complex IIb, pro-caspase 8 forms both homodimers and a heterodimer with $FLIP_L$. For either complex IIa or complex IIb to prevent necroptosis, both RIPK1 and RIPK3 must be inactivated by the cleavage activity of the pro-caspase 8–$FLIP_L$ heterodimer or fully activated caspase 8. The pro-caspase 8 homodimer generates active caspase 8, which is released from complex IIa and complex IIb. This active caspase 8 in the cytosol then carries out cleavage reactions to activate downstream executioner caspases and thus induce classical apoptosis. IKK, IκB kinase; JNK, JUN N-terminal kinase; LUBAC, linear ubiquitin chain assembly complex; NEMO, NF-κB essential modulator; NF-κB, nuclear factor-κB; TAB, TGFβ-activated kinase 1 and MAP3K7-binding protein; TAK1, TGFβ-activated kinase 1; TRAF, TNFR-associated factor.

The degradation of cIAP1 or cIAP2 can be induced by the release of second mitochondria-derived activator caspase (SMAC; also known as DIABLO) from the mitochondria[72]. In an experimental setting, SMAC mimetics bind to the baculovirus IAP repeat domain of cIAP1 and cIAP2 and thereby turn on their E3 ubiquitin ligase activity[73]. This action triggers auto-ubiquitylation of cIAP1 and cIAP2, followed by their degradation[74–76]. Interestingly, this degradation of cIAP1 and cIAP2 by SMAC mimetics also leads to the stabilization of NF-κB-inducing kinase (NIK; also known as MAP3K14)[76]. NIK is best known as a central inducer of the non-canonical NF-κB signalling pathway. Consequently, stabilization of NIK and the dissociation of non-ubiquitylated RIPK1 from complex I (as a result of cIAP1 and cIAP2 depletion) shifts the balance in the cell from canonical to non-canonical NF-κB signalling[75,77]. This latter pathway also induces autocrine TNF signalling, which stimulates and facilitates the formation of complex IIb and thus induces apoptosis[68,78].

The dual survival role of FLIP$_L$. Any push towards TNF-induced apoptosis is most often balanced by TNF-induced NF-κB activation[79]. In cells with sufficient complex I and NF-κB activity, canonical NF-κB signalling induces the upregulation of FLIP$_L$, high levels of which exert potent inhibition of caspase 8 activation and, subsequently, apoptosis triggered by death receptor engagement[59,80–82]. Increased non-canonical NF-κB signalling can also lead to elevated FLIP$_L$ expression[83]. FLIP$_L$ is thus thought to be a key player that keeps the formation of the pro-apoptotic complex IIa and complex IIb in check[59,68,83]. Moreover, as FLIP$_L$ expression is induced by NF-κB signalling[82], the inhibition of NF-κB activation or the functional inactivation of its targets is needed to induce apoptosis via complex IIa[59,68]. However, it should be noted that the anti-apoptotic programme of NF-κB is unlikely to rely solely on FLIP$_L$ upregulation as NF-κB is known to control the expression of multiple anti-apoptotic factors[83]. In addition, the synthesis of FLIP$_L$ is controlled not only by NF-κB-mediated regulation of gene expression but also at the level of protein stability. The stability of FLIP$_L$ is directly affected by complex I-mediated activation of JNK; JNK activates the E3 ubiquitin ligase ITCH, which mediates K48-linked polyubiquitylation and proteasomal degradation of FLIP$_L$ (REF. 84). This mechanism influences cellular FLIP$_L$ levels in response to complex I formation and stress signalling.

Although high levels of FLIP$_L$ inhibit apoptosis, FLIP$_L$ is in fact vital for the parallel suppression of the necrotic cell death pathway. FLIP$_L$ resembles pro-caspase 8 in structure but lacks the catalytic domain[11,80]. Therefore, FLIP$_L$ can form a heterodimer with pro-caspase 8, preventing its homodimerization and inhibiting caspase 8 activation. Instead, the FLIP$_L$–pro-caspase 8 heterodimer displays enzymatic activity that is different from that of the activated caspase 8 homodimer, and it cleaves only a subset of its substrates[85]. The formation of a FLIP$_L$–pro-caspase 8 heterodimer in complex IIa and complex IIb or full activation of caspase 8 is crucial for cleaving and inactivating RIPK1 and RIPK3 (as well as CYLD in complex IIa), which inhibits the induction of the necrotic cell death pathway (see below)[85–87]. This has led to the intriguing hypothesis that the function of pro-caspase 8 is not exclusively linked with apoptosis, but that it also mediates survival by suppressing the necrotic cell death pathway[86]. However, more convincing data need to be provided to fully support a role for the FLIP$_L$–pro-caspase 8 heterodimer and its substrates in preventing necrosis.

Compared with complex IIa, the situation for complex IIb-mediated cell death seems to even be more complicated owing to the existence of and regulation by three different FLIP isoforms: FLIP$_L$, and the two short forms, FLIP$_S$ and FLIP$_R$ (REFS 81,88–90). Enforced FLIP$_L$ expression prevents the assembly of complex IIb, whereas overexpression of FLIP$_S$ (and potentially that of the closely related FLIP$_R$) facilitates complex IIb formation[71,91]. All FLIP isoforms block the apoptotic programme of complex IIb, but FLIP$_S$ actively drives another pro-inflammatory cell death pathway, which is discussed in the next section.

TNF signalling in necroptosis

Survival versus death decision making. Although FLIP$_L$ is involved in regulating apoptosis, FLIP$_S$ actively drives another TNF-triggered cell death pathway that has been named regulated necrosis, or necroptosis[91]. Necroptosis is a pro-inflammatory form of cell death in which the initiating events are caspase independent. Unlike the contained self-destruction of an apoptotic cell, a necroptotic cell displays swelling of cellular organelles, rupture of the cell membrane and uncontrolled release of cellular contents into the surrounding tissue, followed ultimately by cell death[92,93]. At a molecular level, RIPK1 is the pivotal molecule in a complex and versatile signalling pathway that decides whether TNF–TNFR1 engagement will result in NF-κB activation (cell survival), apoptosis ('tidy' programmed cell death) or necroptosis ('messy' programmed cell death). As previously mentioned, if high levels of ubiquitylated RIPK1 are present, then membrane-bound complex I is formed, NF-κB activation proceeds and the cell survives. If the cytosolic complexes IIa and IIb have assembled, and deubiquitylated RIPK1 and RIPK3 are cleaved by an active FLIP$_L$–pro-caspase 8 heterodimer, then other caspases become activated, apoptosis occurs and the cell dies a contained death[59,68,74,75,86]. However, if deubiquitylated RIPK1 is present but caspases are inactivated, such that RIPK1 and RIPK3 cannot be cleaved by a FLIP$_L$–pro-caspase 8 heterodimer, the cell death programme switches from apoptosis to necroptosis[62,94–97]. This might explain why FLIP$_S$ promotes necroptosis but FLIP$_L$ does not. Similarly to FLIP$_L$, FLIP$_S$ can also form a heterodimer with pro-caspase 8. However, based on structural differences, these FLIP$_S$–pro-caspase 8 heterodimers are completely catalytically inactive and cannot inactivate RIPK1 or RIPK3 (REFS 91,98). This would serve as a valid hypothesis as to why the enforced expression of FLIP$_S$, but not that of FLIP$_L$, is associated with the RIPK1- and RIPK3-dependent necrotic cell death pathway.

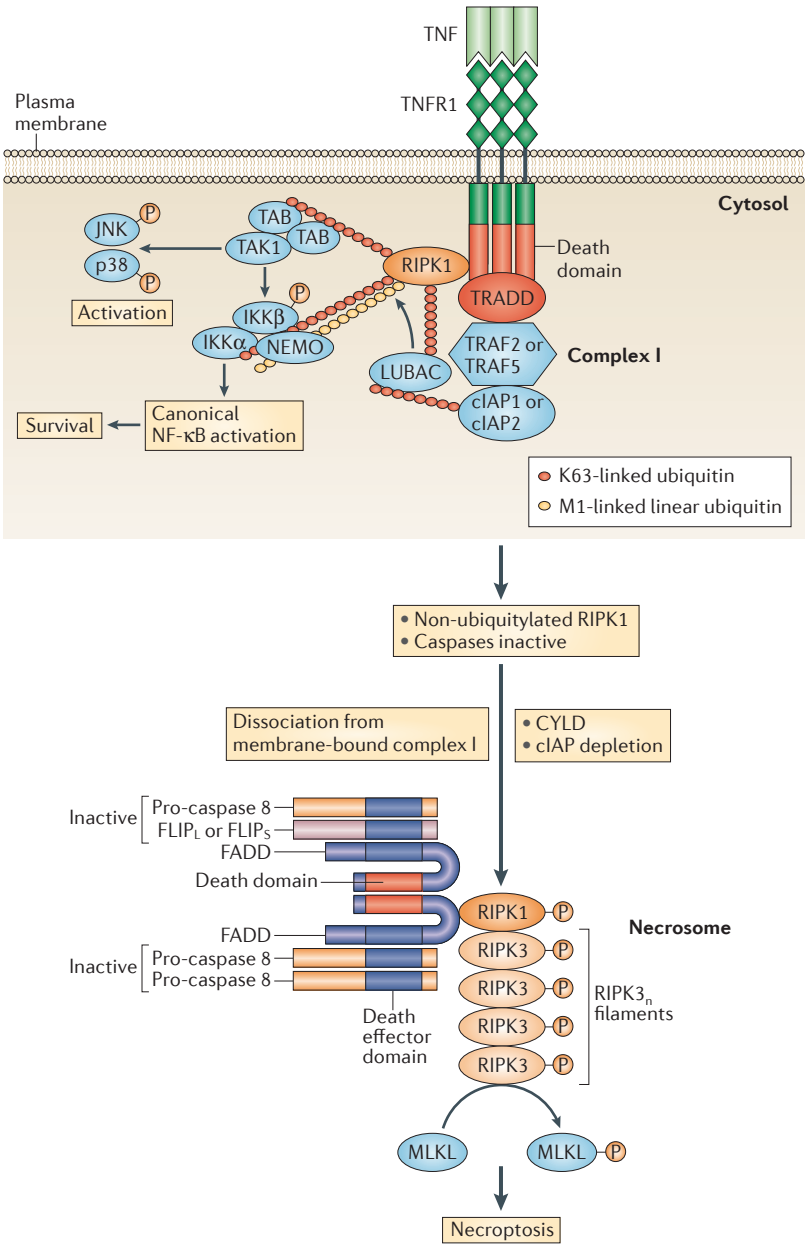

Figure 3 | **TNF-induced necroptosis requires non-ubiquitylated RIPK1 and inactive caspases.** Formation of the necrosome is similar to complex IIa and complex IIb formation in that the dissociation of receptor-interacting serine/threonine-protein kinase 1 (RIPK1) from complex I and its movement into the cytosol is initiated either by RIPK1 deubiquitylation mediated by cylindromatosis (CYLD) or by RIPK1 non-ubiquitylation due to depletion of cellular inhibitor of apoptosis proteins (cIAPs). Other components such as FAS-associated death domain protein (FADD) and pro-caspase 8 might also be present in the complex. RIPK1 recruits numerous RIPK3 molecules (denoted $RIPK3_n$ in the figure). Neither RIPK1 nor RIPK3 is cleaved owing to the prevailing state of caspase inhibition. Instead, RIPK1 and RIPK3 come together to form amyloid microfilaments called necrosomes in a reaction that is dependent on the kinase activities of RIPK1 and RIPK3. The participation of the phosphorylated form of the pseudokinase mixed lineage kinase domain-like (MLKL) is also crucial for the induction of necroptosis. $FLIP_L$, long isoform of FLICE-like inhibitory protein; $FLIP_S$, short isoform of FLICE-like inhibitory protein; IKK, IκB kinase; JNK, JUN N-terminal kinase; LUBAC, linear ubiquitin chain assembly complex; NEMO, NF-κB essential modulator; NF-κB, nuclear factor-κB; TAB, TGFβ-activated kinase 1 and MAP3K7-binding protein; TAK1, TGFβ-activated kinase 1; TNF, tumour necrosis factor; TNFR1, TNF receptor 1; TRADD, TNFR1-associated death domain protein; TRAF, TNFR-associated factor.

Roles of RIPK1 and RIPK3. Necroptosis depends mainly on the presence of significant levels of non-ubiquitylated RIPK1 and RIPK3 (REF. 99) (FIG. 3). Indeed, the level of RIPK3 expression in a cell largely determines its propensity to undergo necroptosis rather than apoptosis[62]. Similarly to RIPK1, RIPK3 belongs to the RIP family of serine-threonine kinases[100,101]. However, in contrast to RIPK1, RIPK3 is dispensable for TNF-induced NF-κB activation[29,102]. Instead, the interaction of multiple protein complexes of RIPK3 with non-ubiquitylated or deubiquitylated RIPK1 via their RHIM domains initiates the formation of a cytosolic amyloid signalling protein complex that has been named the necrosome[99,103].

Proper necrosome formation is dependent on the kinase activities of RIPK1 and RIPK3 in combination with caspase inhibition[63,103,104]. Consequently, RIPK1 and RIPK3 kinase activity is negatively regulated by caspase-mediated cleavage, which removes the kinase domains of RIPK1 and RIPK3 (REF. 105). Accordingly, pharmacological inhibition of RIPK1 and RIPK3 kinase activities with necrostatin 1 abolishes necroptosis[104]. This crucial interplay between the inhibition of caspase activity and RIPK1- and RIPK3-mediated necrotic cell death has been clearly demonstrated by the finding that genetic deletion of RIPK1 and/or RIPK3 rescues the lethality observed in caspase 8-deficient or FADD-deficient mice[86,87,106–108].

Role of SHARPIN. Necrosomes can also be formed when TNF stimulation, either in the presence of CYLD or in the absence of cIAP2, leads to the dissociation of non-ubiquitylated or deubiquitylated RIPK1 from complex I under conditions in which caspases have been inactivated. As noted above, LUBAC ubiquitylates RIPK1 via M1-linked linear linkages[41], but whether M1-linked polyubiquitin chains of RIPK1 must be removed before necroptosis can occur is not entirely clear. Recent data indicate that LUBAC negatively affects necroptosis[109]. Deficiency of SHARPIN, a component of LUBAC, leads to instability of the remaining LUBAC components HOIL1 and HOIP, and thereby limits M1-linked polyubiquitylation[42,46,47]. *In vivo*, a naturally occurring mutation that ablates SHARPIN expression in the cpdm strain of mice results in chronic proliferative dermatitis[110]. This dermatitis can be prevented by crossing cpdm mutants with $Tnf^{-/-}$ or $Tnfr1^{-/-}$ mice, which indicates that this inflammatory disease is TNF dependent[42,111]. Moreover, the absence of SHARPIN in cpdm mice increases cell death in the skin, and the cell death that occurs at this site resembles necroptosis[42]. Accordingly, the inflammatory phenotype of cpdm mice can be rescued by crossing this strain with a mutant mouse expressing a kinase-inactive RIPK1 protein or with RIPK3-null or mixed lineage kinase domain-like (MLKL)-null mice[111,112]. However, a recent report shows that caspase 8 heterozygosity delays the onset of the inflammatory disease induced by the loss of SHARPIN, which indicates that, to a certain degree, apoptotic death also seems to be involved[111]. Taken together, these findings suggest that SHARPIN and thus M1-linked linear polyubiquitylation are involved as negative regulators of the RIPK1-dependent necroptosis signalling

pathway. However, additional work on the role of linear polyubiquitylation in necroptosis is needed because SHARPIN deficiency suppresses, but does not entirely abrogate, M1-linked polyubiquitylation[42,46,47]. When M1-linked linear polyubiquitylation is completely inhibited in mice by ablation of HOIP — the catalytic subunit of LUBAC — embryonic lethality is triggered at mid-gestation[113]. Molecular analysis of these embryos has revealed the presence of an aberrant preassembled complex II that leads to a form of TNFR1-mediated cell death with both necrotic and apoptotic characteristics[113]. This result emphasizes the importance of M1-linked linear polyubiquitylation as a regulator that balances apoptosis versus necroptosis.

MLKL: a crucial effector of necroptosis. It was recently discovered that treatment of human cells with the small molecule necrosulfonamide blocks necroptosis[114]. Necrosulfonamide targets the human form of the pseudokinase MLKL[114]. A similar block in TNF-induced necroptosis can be achieved by knocking down *MLKL* expression using RNA interference[115]. The importance of MLKL in TNF signalling that leads to necroptosis has been confirmed by studies of a gene-deficient mouse model, which showed that ablation of *Mlkl* abrogates cellular necroptosis but not apoptosis[116,117]. These data further support the idea that the apoptotic and necrotic cell death pathways are separated.

Within the necrosome, serine phosphorylation of RIPK3 is crucial for recruiting MLKL, but MLKL is dispensable for the stabilization of this complex[115,118,119]. In addition, although the pseudokinase domain of MLKL binds to ATP, MLKL is catalytically inactive and serves instead as a phosphorylation target of RIPK3 that is important for downstream signalling[115,116]. Intriguingly, MLKL is the only substrate of RIPK1 and RIPK3 that is relevant for the induction of necroptosis. This conclusion is based on the ability of a constitutively active mutant MLKL protein to stimulate the necroptotic pathway either in the absence of RIPK3 or in the presence of the RIPK inhibitor necrostatin 1 (REF. 116). The intracellular location of MLKL may also be important. During the early phase of TNF-induced necroptosis, MLKL oligomers translocate to the plasma membrane where they seem to influence the location of the necrosome. Membrane localization of multimerized MLKL, which is achieved by binding to the membrane lipids phosphatidylinositol and cardiolipin, is crucial for necroptosis[120–122]. It is suggested that oligomerized MLKL forms pores that are essential for the influx of positively charged ions, such as Na^+, K^+ or Ca^{2+}, which is known to be an early event in TNF-induced necroptosis[120–122]. However, the molecular events downstream of MLKL activation are not completely defined, and substantial work is needed to unravel the signalling network that underlies this form of TNFR1-mediated cell death.

Targeting TNF in inflammatory diseases

TNF has long been associated with the clinical signs of various autoimmune and inflammatory disorders, including rheumatoid arthritis, inflammatory bowel disease (IBD), septic shock, ankylosing spondylitis, systemic lupus erythematosus (SLE), psoriasis, multiple sclerosis, respiratory diseases, vasculitis and type 1 diabetes (T1D)[123,124] (BOX 1). Owing to the complexity of TNF signalling and the various roles of TNF in different diseases, current TNF-targeted therapies have resulted in remarkable successes, but also failures, in clinical applications.

Successes in targeting TNF. TNF is a key driver in promoting and sustaining inflammatory responses involving B cells and T cells of the adaptive immune system, as well as various cell types of the innate immune system. Blocking TNF signalling using biologics (for example, monoclonal antibodies) that directly bind to either TNF itself or TNFR is currently the most effective therapeutic approach for many inflammatory diseases. At this time, five inhibitors of TNF–TNFR signalling have been approved by various regulatory bodies around the world (namely, infliximab, etanercept, adalimumab, golimumab and certolizumab pegol)[3]. As outlined below, these agents have had a profound positive impact on many patient outcomes over the last two decades. The pharmaceutical industry continues to introduce dozens of new therapeutics that modulate TNFRSF signalling, and many of these are undergoing testing in clinical trials[125].

Box 1 | **TNFR signalling in inflammatory disorders**

Tumour necrosis factor (TNF) was initially described as a factor that induces tumour cell death (by Carswell and Old in 1975), but it has since been shown to be heavily involved in diseases that are characterized by chronic inflammation. Prominent among these disorders is rheumatoid arthritis, which is a chronic inflammatory disease that mainly affects the peripheral joints but can also damage the lungs, skin, kidneys and heart. In rheumatoid arthritis, leukocytes infiltrate the synovial membranes of the joints and secrete chemokines, prostaglandins and pro-inflammatory cytokines, including TNF. In particular, TNF recruits osteoclast precursors from the bone marrow into synovial membranes, where these cells mediate the initial bone loss that is observed in patients. In addition, TNF-dependent signalling via receptor activator of nuclear factor-κB (RANK; also known as TNFRSF11A) and RANK ligand (RANKL; also known as TNFSF11) activates the downstream pathway mediated by TEC, Bruton's tyrosine kinase (BTK) and phospholipase C (PLC) that leads to the expression of nuclear factor of activated T cells, cytoplasmic 1 (NFATc1), and subsequent osteoclast differentiation and activation. TNF also promotes NFATc1 expression by signalling through receptor-interacting serine/threonine-protein kinase 1 (RIPK1), nuclear factor-κB (NF-κB), JUN N-terminal kinase (JNK) and p38. Finally, TNF blocks the function of osteoblasts, which are the cells responsible for regenerating bone. It is this TNF-dependent dysregulation of osteoclasts and osteoblasts that is central to the imbalanced resorption of bone tissue in the joints that results in inflammation. Another inflammatory disorder involving TNF is inflammatory bowel disease (IBD), which is a collection of maladies that includes ulcerative colitis and Crohn disease. IBD is caused by an abnormal immune response against certain bacterial species that are present among the normal intestinal flora. Serum TNF levels in patients with IBD positively correlate with the severity of clinical manifestations of ulcerative colitis or Crohn disease, indicating that increased levels of TNF are probably a risk factor for these diseases. Macrophages, monocytes and T helper 1 (T_H1) cells serve as direct sources of TNF in patients with IBD. Furthermore, T_H17 cells producing interleukin-17A (IL-17A) and IL-17F can induce macrophages to synthesize various pro-inflammatory cytokines, including TNF, IL-1 and IL-6, which intensify the immune attack against the intestinal flora.

Table 1 | **Diseases for which TNF inhibition is not approved**

Disease	Effects of TNF inhibition	Refs
Multiple sclerosis	TNF-specific antibody treatment of patients with multiple sclerosis has led almost exclusively to immune activation and disease exacerbation	173
Sepsis or septic shock	Trials showed a small but significant benefit with TNF-targeted therapeutic strategies (3% better survival; better results were observed with therapies against TNF than against TNFR)	129
Respiratory diseases	• Etanercept seems to benefit patients with severe chronic asthma • Phase II trial of infliximab in patients with mild to moderate chronic obstructive pulmonary disease failed	173,177
Vasculitis	TNF antagonists have been successfully used in patients with Behçet, rheumatoid, systemic and cryoglobulinemic vasculitis	173,178
Type 1 diabetes	Treatment of paediatric patients with new-onset type 1 diabetes with etanercept showed benefit; however, adult patients with type 1 diabetes showed no significant disease improvement	139,179
Systemic lupus erythematosus	Infliximab improved disease outcome in some patients with systemic lupus erythematosus	148

TNF, tumour necrosis factor; TNFR, TNF receptor.

As far back as 1985, Beutler and colleagues[126] showed that bacteria-induced sepsis in mice could be blocked by passive immunization with antibodies directed against TNF. Unfortunately, initial clinical trials using TNF-specific antibodies to treat patients with sepsis did not have the desired outcome[127,128]. More recent data have shown that TNF-specific antibodies can be beneficial for patients with sepsis but that success may depend on several factors, including exactly which therapeutic is used and how soon after infection the drug is administered[129,130]. With respect to rheumatoid arthritis, strong preclinical data from mouse models of arthritis supported the testing of TNF-specific antibodies in clinical trials and confirmed the effectiveness of blocking TNF-mediated signalling[131]. This approach efficiently slows or prevents the progression of bone and cartilage damage in many patients with rheumatoid arthritis[132,133]. Currently, several biologics against TNF or TNFR have been approved for therapeutic use not only for various forms of arthritis but also for psoriasis, ankylosing spondylitis and IBD[134–137]. With respect to IBD, numerous studies have demonstrated that TNF-targeted therapies can restore T cell homeostasis, inhibit inflammation and support the healing of the intestinal mucosa. In particular, TNF-targeted therapy of patients with ulcerative colitis greatly inhibits the activation and proliferation of pathogenic $CD4^+$ and $CD8^+$ T cells, and their secretion of TNF and IL-17A[138].

Failures in TNF targeting. Despite the above successes, TNF-targeted therapies sometimes have counterintuitive effects, and they have shown contradictory results in terms of efficacy (TABLE 1). For example, TNF inhibition has had a beneficial impact in children but not in older adults with T1D[139,140], and on rare occasions, it has triggered new-onset T1D in patients who received TNF-targeted therapies for other inflammatory diseases[141,142]. As another example, although numerous studies have shown that TNF expression is enhanced in active lesions in patients with multiple sclerosis[143,144], TNF inhibition in this group is detrimental[145,146]. This conundrum may be explained by the recent discovery that patients with multiple sclerosis express a novel truncated form of TNFRSF1A that is soluble and able to sequester TNF in the extracellular space, thereby antagonizing TNF signalling[147]. Treatment with TNF-specific antibodies that basically mimics the sequestration of TNF might explain why patients with multiple sclerosis deteriorate rather than improve. Similar to the role of TNF in multiple sclerosis, in the case of patients with SLE, it is hypothesized that TNF has an immunosuppressive rather than a pro-inflammatory role. Therefore, TNF inhibition would accelerate rather than slow SLE progression. Although TNF-targeted therapy has been efficacious in patients with SLE who also suffer from arthritis or nephritis, most clinical trials of TNF-targeted therapies in SLE have been discontinued owing to adverse events[148].

These negative patient outcomes following TNF-targeted therapy imply that, at least for some inflammatory diseases (such as T1D, SLE and multiple sclerosis), treatment with soluble TNF might be beneficial. Indeed, there are initial data supporting this approach based on the ability of TNF to selectively induce the death of autoreactive T cells or to induce endotoxin tolerance in macrophages[149,150]. As TNF-mediated signalling is very important for many physiological functions in the body, it should not be surprising that the use of TNF-specific and TNFR-specific therapeutics sometimes cause adverse events, including the development of lymphomas and infections[151,152]. Other severe side effects (summarized in TABLE 2) that have been related to TNF inhibition include lupus-like syndrome, diabetes, the induction of autoantibodies, psoriasis, demyelinating diseases and congestive heart failure[153–157].

New approaches in TNF targeting. The serious problems encountered using TNF-specific and TNFR1-specific antibodies to treat some patients with inflammatory conditions has spurred on the development of new strategies[158,159]. Although some efforts have turned to

Table 2 | **Summary of severe adverse events associated with TNF inhibition***

Therapeutic	FDA-approved indication	Possible adverse events	Refs
Infliximab	• Rheumatoid arthritis • Psoriatic arthritis • Psoriasis • Ankylosing spondylitis • Crohn disease • Ulcerative colitis	• Lupus-like syndrome • Cutaneous or systemic vasculitis • Interstitial lung diseases • Demyelinating neuropathies • Guillain–Barré syndrome • Infections with *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Salmonella* spp., *Legionella* spp. and *Nocardia* spp. • Nephritis • Various types of malignancies (including Hodgkin and non-Hodgkin lymphoma; hepatosplenic T cell lymphoma; acute and chronic leukaemia; melanoma; Merkel cell carcinoma; and breast, colorectal, lung, and head and neck cancer)	145,146,152–154, 156–158,171–173
Etanercept	• Rheumatoid arthritis • Psoriatic arthritis • Psoriasis • Ankylosing spondylitis • Juvenile idiopathic arthritis	• Lupus-like syndrome • Cutaneous or systemic vasculitis • Interstitial lung diseases • Type 1 diabetes (juvenile but not adult patients) • Nephritis • Demyelinating neuropathies • Significantly smaller observed rate for lymphoma and non-melanoma skin cancer, compared with other TNF inhibitors. For other malignancies, etanercept did not increase the occurrence rate compared with control arms in the controlled portions of clinical studies for all indications	139,141,142,145,14 6,153,154,156,158,1 72,173
Adalimumab	• Rheumatoid arthritis • Psoriatic arthritis • Psoriasis • Ankylosing spondylitis • Crohn disease • Juvenile idiopathic arthritis	• Lupus-like syndrome • Cutaneous or systemic vasculitis • Interstitial lung diseases • Infection gastrointestinal and urinary tract • Autoimmune hepatitis • Bronchitis and tuberculosis • Optic neuritis and demyelinating neuropathies • Various types of malignancies (including Hodgkin and non-Hodgkin lymphoma, hepatosplenic T cell lymphoma and non-melanoma skin cancer)	146,152–154, 156–158, 172–174
Golimumab	• Rheumatoid arthritis • Psoriatic arthritis • Ankylosing spondylitis	• Serious bacterial, fungal and viral infections • Various types of malignancies (including Hodgkin and non-Hodgkin lymphoma, hepatosplenic T cell lymphoma and melanoma) • Cytopaenia	154,158,175
Certolizumab pegol	• Rheumatoid arthritis • Crohn disease	• Infections with *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Salmonella* spp., *Legionella* spp. and *Nocardia* spp. • New-onset psoriasis • Cutaneous or systemic vasculitis • Various types of malignancies (including Hodgkin and non-Hodgkin lymphoma, and acute and chronic leukaemia)	154, 156–158, 175,176

*The five US Food and Drug Administration (FDA)-approved tumour necrosis factor (TNF)-specific biologics can cause various severe adverse events when used to treat patients with certain inflammatory diseases. However, the benefits of these treatments outweigh the rare occurrences of these side effects.

targeting interleukins[160], many pharmaceutical companies are focusing on improving immunosuppressive therapies that target TNF. In addition, there remains interest in blocking TNF–TNFR signalling by using small-molecule inhibitors against kinases such as spleen tyrosine kinase (SYK), Janus kinases and p38, which mediate signalling downstream of TNF; such inhibitors have shown benefits in some patients with rheumatoid arthritis[161,162]. Various types of JNK inhibitors are in early-phase clinical trials, as are inhibitors of phosphodiesterase 4 (PDE4), an enzyme that is crucial for soluble TNF generation and release. Although PDE4 blockade was very effective in inflammatory diseases, first-generation PDE4 inhibitors failed in clinical trials owing to the high prevalence of nausea and emesis[163]. These adverse events could be explained by the importance of PDE4 function in modulating the central nervous system. The latest generation of PDE4 inhibitors, including roflumilast and apremilast, shows increased specificity towards individual PDE4 isoforms and is associated with fewer side effects. Roflumilast shows efficacy in patients with chronic obstructive pulmonary disease and apremilast reduces inflammation in patients with psoriasis or psoriatic arthritis[164,165].

Another interesting concept is the development of small-molecule inhibitors that prevent TNF trimerization, which is an event that is crucial for TNF-induced signalling[166]. Finally, non-biologic interference with the binding of TNF to TNFR may be of benefit in some situations. In several mouse models of arthritis, treatment with the growth factor progranulin (PGRN) or with an engineered protein called atsttrin, which is composed

of three PGRN fragments, prevented TNF-mediated inflammation[167]. Alternatively, it may be possible to specifically activate the TNFR2 pathway in the autoreactive T cells that drive inflammatory diseases. In this case, TNF-mediated apoptosis might selectively kill these T cells but have no effect on other host cells[10,125].

It should be noted that it is essential to test all new approaches for reducing TNF-associated inflammation in clinical trials to determine whether they can outperform treatments with currently approved TNF-targeted biologics. For instance, although small-molecule inhibitors of TACE showed promising preclinical results[168], several clinical trials examining these agents were largely unsuccessful and had to be discontinued owing to severe liver toxicity[169]. Although treatment with TNF-specific antibodies occasionally results in serious adverse events, these biologics have been remarkably successful; therefore, developing improved biologics against TNF and TNFR may be the most promising strategy (for example, Atrosab (Baliopharm), which is a fully humanized monoclonal antibody that specifically blocks TNFR1 but does not interfere with TNFR2 (REF. 170)). In any case, three decades of experience with the use of TNF-targeted biologics in the clinic has clearly shown that one therapeutic will not treat all inflammatory diseases. The challenge for the future lies in creating target-specific drugs that can be used in combination and can be tailored to a particular type of inflammatory disease. Ultimately, the goal is to devise treatments that carry a reduced risk of adverse events and provide more effective therapy for patients.

1. Aggarwal, B. B. *et al.* Human tumor necrosis factor. Production, purification, and characterization. *J. Biol. Chem.* **260**, 2345–2354 (1985).
2. Aggarwal, B. B. Signalling pathways of the TNF superfamily: a double-edged sword. *Nature Rev. Immunol.* **3**, 745–756 (2003).
3. Aggarwal, B. B., Gupta, S. C. & Kim, J. H. Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. *Blood* **119**, 651–665 (2012).
A comprehensive review about the TNFSF and TNFRSF, TNF signalling in cell survival and death, and clinical and therapeutic implications of targeting TNF signalling.
4. Kriegler, M., Perez, C., DeFay, K., Albert, I. & Lu, S. D. A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. *Cell* **53**, 45–53 (1988).
5. Black, R. A. *et al.* A metalloproteinase disintegrin that releases tumour-necrosis factor-α from cells. *Nature* **385**, 729–733 (1997).
6. Eck, M. J. & Sprang, S. R. The structure of tumor necrosis factor-α at 2.6 Å resolution. Implications for receptor binding. *J. Biol. Chem.* **264**, 17595–17605 (1989).
7. Jones, E. Y., Stuart, D. I. & Walker, N. P. Structure of tumour necrosis factor. *Nature* **338**, 225–228 (1989).
8. Adrain, C., Zettl, M., Christova, Y., Taylor, N. & Freeman, M. Tumor necrosis factor signaling requires iRhom2 to promote trafficking and activation of TACE. *Science* **335**, 225–228 (2012).
9. McIlwain, D. R. *et al.* iRhom2 regulation of TACE controls TNF-mediated protection against *Listeria* and responses to LPS. *Science* **335**, 229–232 (2012).
References 8 and 9 describe the role of iRHOM2 in TACE activation and in controlling immune responses.
10. Faustman, D. & Davis, M. TNF receptor 2 pathway: drug target for autoimmune diseases. *Nature Rev. Drug Discov.* **9**, 482–493 (2010).
11. Lavrik, I., Golks, A. & Krammer, P. H. Death receptor signaling. *J. Cell Sci.* **118**, 265–267 (2005).
12. 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).
13. Hsu, H., Xiong, J. & Goeddel, D. V. The TNF receptor 1-associated protein TRADD signals cell death and NF-κB activation. *Cell* **81**, 495–504 (1995).
14. Rothe, M., Sarma, V., Dixit, V. M. & Goeddel, D. V. TRAF2-mediated activation of NF-κB by TNF receptor 2 and CD40. *Science* **269**, 1424–1427 (1995).
15. Lenardo, M. J. Interleukin-2 programs mouse αβ T lymphocytes for apoptosis. *Nature* **353**, 858–861 (1991).
16. Pimentel-Muinos, F. X. & Seed, B. Regulated commitment of TNF receptor signaling: a molecular switch for death or activation. *Immunity* **11**, 783–793 (1999).
17. Legler, D. F., Micheau, O., Doucey, M. A., Tschopp, J. & Bron, C. Recruitment of TNF receptor 1 to lipid rafts is essential for TNFα-mediated NF-κB activation. *Immunity* **18**, 655–664 (2003).
18. 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).
19. Ting, A. T., Pimentel-Muinos, F. X. & Seed, B. RIP mediates tumor necrosis factor receptor 1 activation of NF-κB but not Fas/APO-1-initiated apoptosis. *EMBO J.* **15**, 6189–6196 (1996).
20. 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).
21. 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).
22. 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 (2005).
23. 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).
24. Yeh, W. C. *et al.* Early lethality, functional NF-κB activation, and increased sensitivity to TNF-induced cell death in TRAF2-deficient mice. *Immunity* **7**, 715–725 (1997).
25. Chen, N. J. *et al.* Beyond tumor necrosis factor receptor: TRADD signaling in toll-like receptors. *Proc. Natl Acad. Sci. USA* **105**, 12429–12434 (2008).
26. Ermolaeva, M. A. *et al.* Function of TRADD in tumor necrosis factor receptor 1 signaling and in TRIF-dependent inflammatory responses. *Nature Immunol.* **9**, 1037–1046 (2008).
27. Pobezinskaya, Y. L. *et al.* The function of TRADD in signaling through tumor necrosis factor receptor 1 and TRIF-dependent Toll-like receptors. *Nature Immunol.* **9**, 1047–1054 (2008).
28. Devin, A. *et al.* The distinct roles of TRAF2 and RIP in IKK activation by TNF-R1: TRAF2 recruits IKK to TNF-R1 while RIP mediates IKK activation. *Immunity* **12**, 419–429 (2000).
29. Lee, T. H., Shank, J., Cusson, N. & Kelliher, M. A. The kinase activity of Rip1 is not required for tumor necrosis factor-α-induced IκB kinase or p38 MAP kinase activation or for the ubiquitination of Rip1 by Traf2. *J. Biol. Chem.* **279**, 33185–33191 (2004).
30. Mahoney, D. J. *et al.* Both cIAP1 and cIAP2 regulate TNFα-mediated NF-κB activation. *Proc. Natl Acad. Sci. USA* **105**, 11778–11783 (2008).
31. Hershko, A. & Ciechanover, A. The ubiquitin system. *Annu. Rev. Biochem.* **67**, 425–479 (1998).
32. Komander, D. & Rape, M. The ubiquitin code. *Annu. Rev. Biochem.* **81**, 203–229 (2012).
33. Hochstrasser, M. Origin and function of ubiquitin-like proteins. *Nature* **458**, 422–429 (2009).
34. Ea, C. K., Deng, L., Xia, Z. P., Pineda, G. & Chen, Z. J. Activation of IKK by TNFα requires site-specific ubiquitination of RIP1 and polyubiquitin binding by NEMO. *Mol. Cell* **22**, 245–257 (2006).
35. Li, H., Kobayashi, M., Blonska, M., You, Y. & Lin, X. Ubiquitination of RIP is required for tumor necrosis factor α-induced NF-κB activation. *J. Biol. Chem.* **281**, 13636–13643 (2006).
36. 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 (2008).
37. Zheng, C., Kabaleeswaran, V., Wang, Y., Cheng, G. & Wu, H. Crystal structures of the TRAF2: cIAP2 and the TRAF1: TRAF2: cIAP2 complexes: affinity, specificity, and regulation. *Mol. Cell* **38**, 101–113 (2010).
38. Yin, Q., Lamothe, B., Darnay, B. G. & Wu, H. Structural basis for the lack of E2 interaction in the RING domain of TRAF2. *Biochemistry* **48**, 10558–10567 (2009).
39. Vince, J. E. *et al.* TRAF2 must bind to cellular inhibitors of apoptosis for tumor necrosis factor (TNF) to efficiently activate NF-κb and to prevent TNF-induced apoptosis. *J. Biol. Chem.* **284**, 35906–35915 (2009).
40. Xu, M., Skaug, B., Zeng, W. & Chen, Z. J. A ubiquitin replacement strategy in human cells reveals distinct mechanisms of IKK activation by TNFα and IL-1β. *Mol. Cell* **36**, 302–314 (2009).
41. 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 (2009).
42. Gerlach, B. *et al.* Linear ubiquitination prevents inflammation and regulates immune signalling. *Nature* **471**, 591–596 (2011).
43. Dynek, J. N. *et al.* c-IAP1 and UbcH5 promote K11-linked polyubiquitination of RIP1 in TNF signalling. *EMBO J.* **29**, 4198–4209 (2010).
This study was the first to describe the K11 ubiquitylation of RIPK1 in TNF signalling.
44. Tokunaga, F. *et al.* Involvement of linear polyubiquitylation of NEMO in NF-κB activation. *Nature Cell Biol.* **11**, 123–132 (2009).
References 41 and 44 led to the identification of HOIL1 and HOIP as functional components of the native TNFR1 signalling complex. The approach described in reference 44 also led to the identification of SHARPIN, the third functional component of LUBAC. The spontaneous inflammation described for cpdm mice was shown to be dependent on TNF.
45. Kirisako, T. *et al.* A ubiquitin ligase complex assembles linear polyubiquitin chains. *EMBO J.* **25**, 4877–4887 (2006).
46. Ikeda, F. *et al.* SHARPIN forms a linear ubiquitin ligase complex regulating NF-κB activity and apoptosis. *Nature* **471**, 637–641 (2011).
47. Tokunaga, F. *et al.* SHARPIN is a component of the NF-κB-activating linear ubiquitin chain assembly complex. *Nature* **471**, 633–636 (2011).
References 46 and 47 describe SHARPIN as a third component of the TNF signalling complex.
48. Israel, A. The IKK complex, a central regulator of NF-κB activation. *Cold Spring Harb. Perspect. Biol.* **2**, a000158 (2010).
49. Hoffmann, A. & Baltimore, D. Circuitry of nuclear factor κB signaling. *Immunol. Rev.* **210**, 171–186 (2006).
50. Weil, R. *et al.* Induction of the NF-κB cascade by recruitment of the scaffold molecule NEMO to the T cell receptor. *Immunity* **18**, 13–26 (2003).

51. Wu, C. J., Conze, D. B., Li, T., Srinivasula, S. M. & Ashwell, J. D. Sensing of Lys 63-linked polyubiquitination by NEMO is a key event in NF-κB activation [corrected]. *Nature Cell Biol.* **8**, 398–406 (2006).
52. Lo, Y. C. *et al.* Structural basis for recognition of diubiquitins by NEMO. *Mol. Cell* **33**, 602–615 (2009).
53. Rahighi, S. *et al.* Specific recognition of linear ubiquitin chains by NEMO is important for NF-κB activation. *Cell* **136**, 1098–1109 (2009).
54. Wang, C. *et al.* TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature* **412**, 346–351 (2001).
55. Kanayama, A. *et al.* TAB2 and TAB3 activate the NF-κB pathway through binding to polyubiquitin chains. *Mol. Cell* **15**, 535–548 (2004).
56. Shim, J. H. *et al.* TAK1, but not TAB1 or TAB2, plays an essential role in multiple signaling pathways *in vivo*. *Genes Dev.* **19**, 2668–2681 (2005).
57. Komander, D. *et al.* Molecular discrimination of structurally equivalent Lys 63-linked and linear polyubiquitin chains. *EMBO Rep.* **10**, 466–473 (2009).
58. Emmerich, C. H. *et al.* Activation of the canonical IKK complex by K63/M1-linked hybrid ubiquitin chains. *Proc. Natl Acad. Sci. USA* **110**, 15247–15252 (2013).
59. Micheau, O. & Tschopp, J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. *Cell* **114**, 181–190 (2003).
This groundbreaking paper gave the first description of the TNFR1 complex I and complex II.
60. Wilson, T. R. *et al.* Procaspase 8 overexpression in non-small-cell lung cancer promotes apoptosis induced by FLIP silencing. *Cell Death Differ.* **16**, 1352–1361 (2009).
61. Brenner, D. & Mak, T. W. Mitochondrial cell death effectors. *Curr. Opin. Cell Biol.* **21**, 871–877 (2009).
62. He, S. *et al.* Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-α. *Cell* **137**, 1100–1111 (2009).
63. Cho, Y. S. *et al.* Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. *Cell* **137**, 1112–1123 (2009).
64. Enesa, K. *et al.* NF-κB suppression by the deubiquitinating enzyme Cezanne: a novel negative feedback loop in pro-inflammatory signaling. *J. Biol. Chem.* **283**, 7036–7045 (2008).
65. Wertz, I. E. *et al.* De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-κB signalling. *Nature* **430**, 694–699 (2004).
66. Liao, W. *et al.* CARP-2 is an endosome-associated ubiquitin ligase for RIP and regulates TNF-induced NF-κB activation. *Curr. Biol.* **18**, 641–649 (2008).
67. Verhelst, K. *et al.* A20 inhibits LUBAC-mediated NF-κB activation by binding linear polyubiquitin chains via its zinc finger 7. *EMBO J.* **31**, 3845–3855 (2012).
68. Wang, L., Du, F. & Wang, X. TNF-α induces two distinct caspase-8 activation pathways. *Cell* **133**, 693–703 (2008).
69. Hitomi, J. *et al.* Identification of a molecular signaling network that regulates a cellular necrotic cell death pathway. *Cell* **135**, 1311–1323 (2008).
70. Kovalenko, A. *et al.* The tumour suppressor CYLD negatively regulates NF-κB signalling by deubiquitination. *Nature* **424**, 801–805 (2003).
71. Tenev, T. *et al.* The Ripoptosome, a signaling platform that assembles in response to genotoxic stress and loss of IAPs. *Mol. Cell* **43**, 432–448 (2011).
72. Du, C., Fang, M., Li, Y., Li, L. & Wang, X. Smac, a mitochondrial protein that promotes cytochrome *c*-dependent caspase activation by eliminating IAP inhibition. *Cell* **102**, 33–42 (2000).
73. Wu, G. *et al.* Structural basis of IAP recognition by Smac/DIABLO. *Nature* **408**, 1008–1012 (2000).
74. Vince, J. E. *et al.* IAP antagonists target cIAP1 to induce TNFα-dependent apoptosis. *Cell* **131**, 682–693 (2007).
75. Varfolomeev, E. *et al.* IAP antagonists induce autoubiquitination of c-IAPs, NF-κB activation, and TNFα-dependent apoptosis. *Cell* **131**, 669–681 (2007).
76. Yang, Q. H. & Du, C. Smac/DIABLO selectively reduces the levels of c-IAP1 and c-IAP2 but not that of XIAP and livin in HeLa cells. *J. Biol. Chem.* **279**, 16963–16970 (2004).
77. Zarnegar, B. J. *et al.* Noncanonical NF-κB activation requires coordinated assembly of a regulatory complex of the adaptors cIAP1, cIAP2, TRAF2 and TRAF3 and the kinase NIK. *Nature Immunol.* **9**, 1371–1378 (2008).
78. Petersen, S. L. *et al.* Autocrine TNFα signaling renders human cancer cells susceptible to Smac-mimetic-induced apoptosis. *Cancer Cell* **12**, 445–456 (2007).
79. Van Antwerp, D. J., Martin, S. J., Kafri, T., Green, D. R. & Verma, I. M. Suppression of TNF-α-induced apoptosis by NF-κB. *Science* **274**, 787–789 (1996).
80. Muzio, M. *et al.* FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex. *Cell* **85**, 817–827 (1996).
81. Golks, A., Brenner, D., Fritsch, C., Krammer, P. H. & Lavrik, I. N. c-FLIPR, a new regulator of death receptor-induced apoptosis. *J. Biol. Chem.* **280**, 14507–14513 (2005).
82. Micheau, O., Lens, S., Gaide, O., Alevizopoulos, K. & Tschopp, J. NF-κB signals induce the expression of c-FLIP. *Mol. Cell. Biol.* **21**, 5299–5305 (2001).
83. Karin, M. & Lin, A. NF-κB at the crossroads of life and death. *Nature Immunol.* **3**, 221–227 (2002).
84. Chang, L. *et al.* The E3 ubiquitin ligase itch couples JNK activation to TNFα-induced cell death by inducing c-FLIP$_L$ turnover. *Cell* **124**, 601–613 (2006).
85. Pop, C. *et al.* FLIP$_L$ induces caspase 8 activity in the absence of interdomain caspase 8 cleavage and alters substrate specificity. *Biochem. J.* **433**, 447–457 (2011).
86. Oberst, A. *et al.* Catalytic activity of the caspase-8-FLIP$_L$ complex inhibits RIPK3-dependent necrosis. *Nature* **471**, 363–367 (2011).
87. Dillon, C. P. *et al.* Survival function of the FADD-CASPASE-8-cFLIP$_L$ complex. *Cell Rep.* **1**, 401–407 (2012).
88. Irmler, M. *et al.* Inhibition of death receptor signals by cellular FLIP. *Nature* **388**, 190–195 (1997).
89. Shu, H. B., Halpin, D. R. & Goeddel, D. V. Casper is a FADD- and caspase-related inducer of apoptosis. *Immunity* **6**, 751–763 (1997).
90. Rasper, D. M. *et al.* Cell death attenuation by 'Usurpin', a mammalian DED-caspase homologue that precludes caspase-8 recruitment and activation by the CD-95 (Fas, APO-1) receptor complex. *Cell Death Differ.* **5**, 271–288 (1998).
91. Feoktistova, M. *et al.* cIAPs block Ripoptosome formation, a RIP1/caspase-8 containing intracellular cell death complex differentially regulated by cFLIP isoforms. *Mol. Cell* **43**, 449–463 (2011).
References 71 and 91 describe a novel protein complex — the ripoptosome — in which FLIP isoforms act as a rheostat that influences RIPK3-dependent necroptosis or caspase-dependent apoptosis.
92. Kroemer, G. *et al.* Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. *Cell Death Differ.* **16**, 3–11 (2009).
93. Galluzzi, L. *et al.* Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. *Cell Death Differ.* **19**, 107–120 (2012).
94. Vercammen, D. *et al.* Inhibition of caspases increases the sensitivity of L929 cells to necrosis mediated by tumor necrosis factor. *J. Exp. Med.* **187**, 1477–1485 (1998).
95. Holler, N. *et al.* Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. *Nature Immunol.* **1**, 489–495 (2000).
96. Zhang, D. W. *et al.* RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. *Science* **325**, 332–336 (2009).
References 62, 63 and 96 identify RIPK3 as an essential component in the necrotic pathway, which depends on the kinase activity of RIPK3. They also show that RIPK1 recruits RIPK3.
97. Zheng, L. *et al.* Competitive control of independent programs of tumor necrosis factor receptor-induced cell death by TRADD and RIP1. *Mol. Cell. Biol.* **26**, 3505–3513 (2006).
98. Kavuri, S. M. *et al.* Cellular FLICE-inhibitory protein (cFLIP) isoforms block CD95- and TRAIL death receptor-induced gene induction irrespective of processing of caspase-8 or cFLIP in the death-inducing signaling complex. *J. Biol. Chem.* **286**, 16631–16646 (2011).
99. Vandenabeele, P., Galluzzi, L., Vanden Berghe, T. & Kroemer, G. Molecular mechanisms of necroptosis: an ordered cellular explosion. *Nature Rev. Mol. Cell Biol.* **11**, 700–714 (2010).
100. Meylan, E. & Tschopp, J. The RIP kinases: crucial integrators of cellular stress. *Trends Biochem. Sci.* **30**, 151–159 (2005).
101. Zhang, D., Lin, J. & Han, J. Receptor-interacting protein (RIP) kinase family. *Cell. Mol. Immunol.* **7**, 243–249 (2010).
102. Newton, K., Sun, X. & Dixit, V. M. Kinase RIP3 is dispensable for normal NF-κBs, signaling by the B-cell and T-cell receptors, tumor necrosis factor receptor 1, and Toll-like receptors 2 and 4. *Mol. Cell. Biol.* **24**, 1464–1469 (2004).
103. Li, J. *et al.* The RIP1/RIP3 necrosome forms a functional amyloid signaling complex required for programmed necrosis. *Cell* **150**, 339–350 (2012).
This paper describes amyloid-like RIPK1 and amyloid-like RIPK3 aggregates that are crucial for necroptosis.
104. Degterev, A. *et al.* Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. *Nature Chem. Biol.* **1**, 112–119 (2005).
This paper describes necrostatin 1 as an inhibitor of necroptosis that inhibits RIPK1 activity.
105. Feng, S. *et al.* Cleavage of RIP3 inactivates its caspase-independent apoptosis pathway by removal of kinase domain. *Cell. Signal.* **19**, 2056–2067 (2007).
106. Kaiser, W. J. *et al.* RIP3 mediates the embryonic lethality of caspase-8-deficient mice. *Nature* **471**, 368–372 (2011).
107. Zhang, H. *et al.* Functional complementation between FADD and RIP1 in embryos and lymphocytes. *Nature* **471**, 373–376 (2011).
108. Kaiser, W. J. *et al.* RIP1 suppresses innate immune necrotic as well as apoptotic cell death during mammalian parturition. *Proc. Natl Acad. Sci. USA* **111**, 7753–7758 (2014).
References 86 and 108 are crucial papers demonstrating the interplay between the apoptotic and necrotic cell death pathways. They show that deletion of *Ripk3* can rescue lethality caused by genetic ablation of caspase 8.
109. Vanlangenakker, N., Bertrand, M. J., Bogaert, P., Vandenabeele, P. & Vanden Berghe, T. TNF-induced necroptosis in L929 cells is tightly regulated by multiple TNFR1 complex I and II members. *Cell Death Dis.* **2**, e230 (2011).
110. Seymour, R. E. *et al.* Spontaneous mutations in the mouse Sharpin gene result in multiorgan inflammation, immune system dysregulation and dermatitis. *Genes Immun.* **8**, 416–421 (2007).
111. Rickard, J. A. *et al.* TNFR1-dependent cell death drives inflammation in Sharpin-deficient mice. *eLife* **3**, e03464 (2014).
112. Berger, S. B. *et al.* Cutting edge: RIP1 kinase activity is dispensable for normal development but is a key regulator of inflammation in SHARPIN-deficient mice. *J. Immunol.* **192**, 5476–5480 (2014).
113. Peltzer, N. *et al.* HOIP deficiency causes embryonic lethality by aberrant TNFR1-mediated endothelial cell death. *Cell Rep.* **9**, 153–165 (2014).
114. Zhao, J. *et al.* Mixed lineage kinase domain-like is a key receptor interacting protein 3 downstream component of TNF-induced necrosis. *Proc. Natl Acad. Sci. USA* **109**, 5322–5327 (2012).
115. Sun, L. *et al.* Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. *Cell* **148**, 213–227 (2012).
116. Murphy, J. M. *et al.* The pseudokinase MLKL mediates necroptosis via a molecular switch mechanism. *Immunity* **39**, 443–453 (2013).
117. Wu, J. *et al. Mlkl* knockout mice demonstrate the indispensable role of Mlkl in necroptosis. *Cell Res.* **23**, 994–1006 (2013).
References 114–117 identified MLKL as a phosphorylation target of RIPK3 and as the crucial substrate in the necrotic cell death pathway.
118. Xie, T. *et al.* Structural insights into RIP3-mediated necroptotic signaling. *Cell Rep.* **5**, 70–78 (2013).
This report shows the structure of the RIPK3 kinase domain bound to MLKL.
119. Chen, W. *et al.* Diverse sequence determinants control human and mouse receptor interacting protein 3 (RIP3) and mixed lineage kinase domain-like (MLKL) interaction in necroptotic signaling. *J. Biol. Chem.* **288**, 16247–16261 (2013).
120. Cai, Z. *et al.* Plasma membrane translocation of trimerized MLKL protein is required for TNF-induced necroptosis. *Nature Cell Biol.* **16**, 55–65 (2014).

121. Chen, X. *et al.* Translocation of mixed lineage kinase domain-like protein to plasma membrane leads to necrotic cell death. *Cell Res.* **24**, 105–121 (2014).
References 120 and 121 demonstrate that plasma membrane localization of MLKL is crucial for necroptosis.
122. Wang, H. *et al.* Mixed lineage kinase domain-like protein MLKL causes necrotic membrane disruption upon phosphorylation by RIP3. *Mol. Cell* **54**, 133–146 (2014).
This paper shows that MLKL can move from the cytosol to the plasma membrane and intracellular membranes to disrupt membrane integrity, resulting in necroptosis.
123. Bradley, J. R. TNF-mediated inflammatory disease. *J. Pathol.* **214**, 149–160 (2008).
124. Croft, M. *et al.* TNF superfamily in inflammatory disease: translating basic insights. *Trends Immunol.* **33**, 144–152 (2012).
This is a thorough overview of the cell specificity of various members of the TNFSF and TNFRSF and their involvement in inflammatory disease.
125. Croft, M., Benedict, C. A. & Ware, C. F. Clinical targeting of the TNF and TNFR superfamilies. *Nature Rev. Drug Discov.* **12**, 147–168 (2013).
This is a comprehensive review of clinical trial successes and failures and new biologics that are currently being tested that target members of the TNFSF or TNFRSF for immune-related diseases.
126. Beutler, B., Milsark, I. W. & Cerami, A. C. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science* **229**, 869–871 (1985).
127. Natanson, C., Hoffman, W. D., Suffredini, A. F., Eichacker, P. Q. & Danner, R. L. Selected treatment strategies for septic shock based on proposed mechanisms of pathogenesis. *Ann. Intern. Med.* **120**, 771–783 (1994).
128. Docke, W. D. *et al.* Monocyte deactivation in septic patients: restoration by IFN-γ treatment. *Nature Med.* **3**, 678–681 (1997).
129. Reinhart, K. & Karzai, W. Anti-tumor necrosis factor therapy in sepsis: update on clinical trials and lessons learned. *Crit. Care Med.* **29**, S121–125 (2001).
130. Lv, S. *et al.* Anti-TNF-α therapy for patients with sepsis: a systematic meta-analysis. *Int. J. Clin. Pract.* **68**, 520–528 (2014).
131. Keffer, J. *et al.* Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. *EMBO J.* **10**, 4025–4031 (1991).
132. Brennan, F. M., Chantry, D., Jackson, A., Maini, R. & Feldmann, M. Inhibitory effect of TNF α antibodies on synovial cell interleukin-1 production in rheumatoid arthritis. *Lancet* **2**, 244–247 (1989).
133. 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 (1997).
134. Moelants, E. A., Mortier, A., Van Damme, J. & Proost, P. Regulation of TNF-α with a focus on rheumatoid arthritis. *Immunol. Cell Biol.* **91**, 393–401 (2013).
135. Prieto-Perez, R. *et al.* Genetics of psoriasis and pharmacogenetics of biological drugs. *Autoimmune Dis.* **2013**, 613086 (2013).
136. Neurath, M. F. New targets for mucosal healing and therapy in inflammatory bowel diseases. *Mucosal Immunol.* **7**, 6–19 (2014).
137. Sieper, J. Developments in therapies for spondyloarthritis. *Nature Rev. Rheumatol.* **8**, 280–287 (2012).
138. Dahlen, R. *et al.* Infliximab inhibits activation and effector functions of peripheral blood T cells *in vitro* from patients with clinically active ulcerative colitis. *Scand. J. Immunol.* **78**, 275–284 (2013).
139. Mastrandrea, L. *et al.* Etanercept treatment in children with new-onset type 1 diabetes: pilot randomized, placebo-controlled, double-blind study. *Diabetes Care* **32**, 1244–1249 (2009).
140. Ryba, M. *et al.* Anti-TNF rescue $CD4^+Foxp3^+$ regulatory T cells in patients with type 1 diabetes from effects mediated by TNF. *Cytokine* **55**, 353–361 (2011).
141. 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-α therapy for active rheumatoid arthritis. *Diabetologia* **52**, 1442–1444 (2009).
142. Bloom, B. J. Development of diabetes mellitus during etanercept therapy in a child with systemic-onset juvenile rheumatoid arthritis. *Arthritis Rheum.* **43**, 2606–2608 (2000).
143. Hofman, F. M., Hinton, D. R., Johnson, K. & Merrill, J. E. Tumor necrosis factor identified in multiple sclerosis brain. *J. Exp. Med.* **170**, 607–612 (1989).
144. Lopez-Diego, R. S. & Weiner, H. L. Novel therapeutic strategies for multiple sclerosis — a multifaceted adversary. *Nature Rev. Drug Discov.* **7**, 909–925 (2008).
145. Robinson, W. H., Genovese, M. C. & Moreland, L. W. Demyelinating and neurologic events reported in association with tumor necrosis factor α antagonism: by what mechanisms could tumor necrosis factor α antagonists improve rheumatoid arthritis but exacerbate multiple sclerosis? *Arthritis Rheum.* **44**, 1977–1983 (2001).
146. Bosch, X., Saiz, A. & Ramos-Casals, M. Monoclonal antibody therapy-associated neurological disorders. *Nature Rev. Neurol.* **7**, 165–172 (2011).
This review is an in-depth focus on the outcomes and risks in patients receiving TNF-targeted therapies.
147. Gregory, A. P. *et al.* TNF receptor 1 genetic risk mirrors outcome of anti-TNF therapy in multiple sclerosis. *Nature* **488**, 508–511 (2012).
This article discusses a genetic variant of *TNFRSF1A* that could explain why TNF-specific therapies can promote or exacerbate certain diseases.
148. Stohl, W. Future prospects in biologic therapy for systemic lupus erythematosus. *Nature Rev. Rheumatol.* **9**, 705–720 (2013).
149. Ban, L. *et al.* Selective death of autoreactive T cells in human diabetes by TNF or TNF receptor 2 agonism. *Proc. Natl Acad. Sci. USA* **105**, 13644–13649 (2008).
150. Park, S. H., Park-Min, K. H., Chen, J., Hu, X. & Ivashkiv, L. B. Tumor necrosis factor induces GSK3 kinase-mediated cross-tolerance to endotoxin in macrophages. *Nature Immunol.* **12**, 607–615 (2011).
This article provides evidence for TNF as an anti-inflammatory agent.
151. Ali, T. *et al.* Clinical use of anti-TNF therapy and increased risk of infections. *Drug Healthc. Patient Saf.* **5**, 79–99 (2013).
152. 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 (2006).
153. Hansel, T. T., Kropshofer, H., Singer, T., Mitchell, J. A. & George, A. J. The safety and side effects of monoclonal antibodies. *Nature Rev. Drug Discov.* **9**, 325–338 (2010).
154. Thalayasingam, N. & Isaacs, J. D. Anti-TNF therapy. *Best Pract. Res. Clin. Rheumatol.* **25**, 549–567 (2011).
155. van Schouwenburg, P. A., Rispens, T. & Wolbink, G. J. Immunogenicity of anti-TNF biologic therapies for rheumatoid arthritis. *Nature Rev. Rheumatol.* **9**, 164–172 (2013).
156. Cleynen, I. & Vermeire, S. Paradoxical inflammation induced by anti-TNF agents in patients with IBD. *Nature Rev. Gastroenterol. Hepatol.* **9**, 496–503 (2012).
157. Targownik, L. E. & Bernstein, C. N. Infectious and malignant complications of TNF inhibitor therapy in IBD. *Am. J. Gastroenterol.* **108**, 1835–1842 (2013).
158. Sathish, J. G. *et al.* Challenges and approaches for the development of safer immunomodulatory biologics. *Nature Rev. Drug Discov.* **12**, 306–324 (2013).
159. Fink, M. P. & Warren, H. S. Strategies to improve drug development for sepsis. *Nature Rev. Drug Discov.* **13**, 741–758 (2014).
160. Burmester, G. R., Feist, E. & Dorner, T. Emerging cell and cytokine targets in rheumatoid arthritis. *Nature Rev. Rheumatol.* **10**, 77–88 (2014).
This review details new developments in the field of biologic agents that target cytokines involved in inflammation.
161. Palladino, M. A., Bahjat, F. R., Theodorakis, E. A. & Moldawer, L. L. Anti-TNF-α therapies: the next generation. *Nature Rev. Drug Discov.* **2**, 736–746 (2003).
162. Bonilla-Hernan, M. G., Miranda-Carus, M. E. & Martin-Mola, E. New drugs beyond biologics in rheumatoid arthritis: the kinase inhibitors. *Rheumatol.* **50**, 1542–1550 (2011).
163. Kumar, N., Goldminz, A. M., Kim, N. & Gottlieb, A. B. Phosphodiesterase 4-targeted treatments for autoimmune diseases. *BMC Med.* **11**, 96 (2013).
164. Maurice, D. H. *et al.* Advances in targeting cyclic nucleotide phosphodiesterases. *Nature Rev. Drug Discov.* **13**, 290–314 (2014).
This is an important report showing the potential of a small-molecule inhibitor to disrupt TNF trimerization and TNFR binding.
165. Schett, G., Elewaut, D., McInnes, I. B., Dayer, J. M. & Neurath, M. F. How cytokine networks fuel inflammation: toward a cytokine-based disease taxonomy. *Nature Med.* **19**, 822–824 (2013).
This report first identified that progranulin binds to TNFR, competes with TNF as an antagonist and shows anti-inflammatory potential in mouse models of arthritis.
166. He, M. M. *et al.* Small-molecule inhibition of TNF-α. *Science* **310**, 1022–1025 (2005).
167. Tang, W. *et al.* The growth factor progranulin binds to TNF receptors and is therapeutic against inflammatory arthritis in mice. *Science* **332**, 478–484 (2011).
168. Issuree, P. D. *et al.* iRHOM2 is a critical pathogenic mediator of inflammatory arthritis. *J. Clin. Invest.* **123**, 928–932 (2013).
169. Moss, M. L., Sklair-Tavron, L. & Nudelman, R. Drug insight: tumor necrosis factor-converting enzyme as a pharmaceutical target for rheumatoid arthritis. *Nature Clin. Pract. Rheumatol.* **4**, 300–309 (2008).
170. Richter, F. *et al.* Antagonistic TNF receptor one-specific antibody (ATROSAB): receptor binding and *in vitro* bioactivity. *PLoS ONE* **8**, e72156 (2013).
171. Slifman, N. R., Gershon, S. K., Lee, J. H., Edwards, E. T. & Braun, M. M. *Listeria monocytogenes* infection as a complication of treatment with tumor necrosis factor α-neutralizing agents. *Arthritis Rheum.* **48**, 319–324 (2003).
172. Ramos-Casals, M. *et al.* Autoimmune diseases induced by TNF-targeted therapies: analysis of 233 cases. *Medicine (Baltimore).* **86**, 242–251 (2007).
173. Lin, J. *et al.* TNFα blockade in human diseases: an overview of efficacy and safety. *Clin. Immunol.* **126**, 13–30 (2008).
174. van de Putte, L. B. *et al.* Efficacy and safety of the fully human anti-tumour necrosis factor α monoclonal antibody adalimumab (D2E7) in DMARD refractory patients with rheumatoid arthritis: a 12 week, Phase II study. *Ann. Rheum. Dis.* **62**, 1168–1177 (2003).
175. le Blay, P., Mouterde, G., Barnetche, T., Morel, J. & Combe, B. Short-term risk of total malignancy and nonmelanoma skin cancers with certolizumab and golimumab in patients with rheumatoid arthritis: metaanalysis of randomized controlled trials. *J. Rheumatol.* **39**, 712–715 (2012).
176. Mease, P. J. Certolizumab pegol in the treatment of rheumatoid arthritis: a comprehensive review of its clinical efficacy and safety. *Rheumatol.* **50**, 261–270 (2011).
177. Desai, D. & Brightling, C. TNF-α antagonism in severe asthma? *Recent Pat. Inflamm. Allergy Drug Discov.* **4**, 193–200 (2010).
178. Langford, C. A. Drug insight: anti-tumor necrosis factor therapies for the vasculitic diseases. *Nature Clin. Pract. Rheumatol.* **4**, 364–370 (2008).
179. van Belle, T. L., Coppieters, K. T. & von Herrath, M. G. Type 1 diabetes: etiology, immunology, and therapeutic strategies. *Physiol. Rev.* **91**, 79–118 (2011).

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