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TNF biology, pathogenic mechanisms and emerging therapeutic strategies

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Abstract

TNF is a pleiotropic cytokine with important functions in homeostasis and disease pathogenesis. Recent discoveries have provided insights into TNF biology that introduce new concepts for the development of therapeutics for TNF-mediated diseases. The model of TNF receptor signalling has been extended to include linear ubiquitination and the formation of distinct signalling complexes that are linked with different functional outcomes, such as inflammation, apoptosis and necroptosis. Our understanding of TNF-induced gene expression has been enriched by the discovery of epigenetic mechanisms and concepts related to cellular priming, tolerization and induction of 'short-term transcriptional memory'. Identification of distinct homeostatic or pathogenic TNF-induced signalling pathways has introduced the concept of selectively inhibiting the deleterious effects of TNF while preserving its homeostatic bioactivities for therapeutic purposes. In this Review, we present molecular mechanisms underlying the roles of TNF in homeostasis and inflammatory disease pathogenesis, and discuss novel strategies to advance therapeutic paradigms for the treatment of TNF-mediated diseases.

Forty years have passed since the description of a serum factor inducing tumour necrosis, 30 years since the cloning and purification of TNF, and almost 20 years since the approval of the first drug that targets TNF[1]. The initial concept of TNF as a potential drug for the treatment of cancer was followed by the opposite concept of TNF as a drug-target for inflammatory diseases[2,3]. Currently, five biologic agents targeting TNF are approved for the treatment of rheumatoid arthritis (RA), inflammatory bowel disease (IBD; for example, Crohn disease and ulcerative colitis), psoriasis, psoriatic arthritis, ankylosing spondylitis, juvenile idiopathic arthritis (JIA) and, most recently, hidradenitis suppurativa[4,5] (TABLE 1). Notably, lower-cost biosimilar TNF-inhibitors have already been developed and introduced in the clinic[6]. In addition to the approved indications, TNF-blockade is also used, off-label, in Behçet disease, non-infectious ocular inflammation, and pyoderma gangrenosum, as well as in patients with TNF-receptor associated periodic fever syndrome (TRAPS), adult-onset Still disease and systemic-onset JIA[7]. In this Review, we focus on the latest discoveries

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about the biology of TNF, and outline new concepts that have been introduced in therapeutics for TNF-mediated diseases.

TNF-induced signal transduction

TNF receptors

Newly synthesized TNF is expressed initially as a transmembrane protein, which requires proteolytic cleavage by TNFα-converting enzyme (TACE, also named ADAM17) to release soluble TNF[8]. TACE-dependent release of soluble TNF has been implicated in TNF-mediated inflammatory pathology in disease models[9]. TNF exerts versatile bioactivities via binding to, and activation of, two distinct receptors: TNF receptor 1 (TNFR1) and TNFR2 (REF. 10). TNFR1 is expressed ubiquitously, bears conserved death-domain motifs, and is activated by both soluble and transmembrane TNF. Expression of TNFR2 is restricted to specific cell types, such as neurons, immune cells and endothelial cells. TNFR2 lacks a death domain and thus is unable to induce programmed cell death directly; this receptor has been proposed to be activated primarily by transmembrane TNF[11]. One model of TNFR signalling proposes that TNFR1 primarily promotes inflammation and tissue degeneration, whereas TNFR2 mediates local homeostatic effects, such as cell survival and tissue regeneration[12] (FIG. 1). This model suggests that selective therapeutic blockade of TNFR1 would keep homeostatic TNFR2 signalling intact; indeed, such a strategy is under development for the treatment of TNF-mediated diseases[12].

Homotrimers of TNF bind to homotrimeric TNFRs to induce signalling[10]. Ligand-binding to TNFR1 results in the recruitment of the adaptor molecule TNFR1-associated death domain protein (TRADD) and the assembly of distinct signalling complexes, termed complexes I, IIa, IIb and IIc, which lead to distinct functional outcomes[13,14] (FIG. 1a).

TNF receptor signalling

TNFR signalling via complex I—Upon TNF binding to TNFR, complex I is assembled at TNFR cyto plasmic domains at the plasma membrane and comprises TRADD, receptor-interacting serine/threonine-protein kinase 1 (RIPK1), TNFR-associated factor 2 (TRAF2), cellular inhibitor of apoptosis protein 1 (cIAP1) or cIAP2, and linear ubiquitin chain assembly complex (LUBAC)[14] (FIG. 2). LUBAC consists of haem-oxidized IRP2 ubiquitin ligase-1 (HOIL-1), HOIP (HOIL-1 interacting protein, also known as E3 ubiquitin-protein ligase RNF31) and Sharpin. The current TNFR signalling model postulates the building of a scaffolding ubiquitin network in a step-wise process[15]. Initially, TRAF2 and cIAP1 or cIAP2 mediate Lys63-linked ubiquitination of complex I components. Subsequently, the LUBAC complex adds Met1-linked linear ubiquitin chains that strengthen the ubiquitin network, resulting in the stabilization of complex I and amplification of signalling[16,17]. Additional ‘atypical’ ubiquitin chains, such as Lys11-linked chains, can also facilitate signal transduction through TNFRs[18].

The scaffolding ubiquitin network enables the recruitment and activation of two signalling complexes: the transforming growth factor (TGF)-β-activated kinase 1 (TAK1) complex, comprising TAK1, TAK1-binding protein 2 (TAB2) and TAB3, and the inhibitor of κB (IκB) kinase (IKK) complex, comprising NFκB essential modulator (NEMO, also known as

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IKKγ), IKK subunit-α (IKKα) and IKKβ[14] (FIG. 2). TAK1 triggers mitogen-activated kinase (MAPK) signalling cascades that lead to activation of downstream JUN N-terminal kinase (JNK) and p38, as well as AP1 transcription factors, whereas IKKβ activates the canonical nuclear factor κB (NFκB) pathway (FIG. 2). Thus, induction of signalling complex I leads to expression of NFκB and AP1 target genes that are important in inflammation, host defence, and cell proliferation and survival[1,14].

TNFR signalling via complexes IIa, IIb and IIc—In contrast to complex I, complexes IIa, IIb and IIc are assembled in the cytoplasm and have distinct signalling and functional outcomes[13,14,19–21] (FIG. 1a). Complexes IIa and IIb lead to activation of a caspase cascade that results in TNF-induced cell death via apoptosis[21,22]. A highly controlled process, apoptosis is implicated in the turnover of cells during development and organogenesis, epi thelial homeostasis, inflammation, immunity, and disease pathogenesis. Apoptotic cells remain intact and are rapidly phagocytized by macrophages, a process which has suppressive effects and diminishes inflammatory cytokine production[14].

Complex IIc activates the necroptosis effector mixed lineage kinase domain-like protein (MLKL) by a RIPK3-dependent mechanism[13,19,23]. In contrast to apoptosis, necroptosis results in plasma-membrane rupture, which releases intracellular contents and triggers local inflammation[13]. TNF-induced necroptosis of cells at barrier surfaces such as the skin and intestinal mucosa can compromise barrier function and thereby contribute to inflammation[24–26]. The implications of TNF-induced necroptosis in the pathogenesis of inflammatory diseases are the focus of active investigations[19], and the RIPK1 and RIPK3 kinases that control these processes are considered potential therapeutic targets[25–27].

Fine-tuning of TNFR-signalling

Fine-tuning the magnitude and kinetics of complex I signalling—TNF-induced complex I signalling requires precise control to prevent the development of deleterious excessive or chronic inflammation. Several studies support the concept that negative regulators are used to fine-tune the magnitude, amplitude and kinetics of TNFR signalling. Additionally, it has been suggested that specific negative modulators might set cell-type-specific thresholds for TNFR activation. These ‘signalling brakes’ operate by targeting receptor-proximal signalling events, by inhibiting IKK-complex activity, or by regulating downstream checkpoints. The mech anisms utilized by these negative regulators include cleavage or internalization of TNFRs[10], destabilization of signalling complexes[28], displacement of signalling molecules from complexes by antagonists[29–34], as well as degradation (proteasomal or lysosomal)[31,35–37], inactivation (for example, dephosphory lation)[38–41], or seques tration of signalling factors[42–44]. Although the expression and activity of negative regulators can be constitutive, induction by TNF is consistent with a model of ‘applying the brakes’ as part of inducible inhibitory-feedback loops[42]. The three major classes of negative regulators of TNF-induced complex I signalling include regulators of ubiquitination, phosphatases, and IκBs (summarized in TABLE 2).

Reports from several studies of complex I signalling kinetics have shown that TNF induces oscillations of NFκB activity that dampen over time[42,45–47]. This observation suggests that

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the coordinated function of signalling brakes does not simply terminate TNF-induced signalling, but progressively diminishes the amplitude of waves of NFκB dimers that enter and exit the cell nucleus. The fraction of responding cells and the number of waves are related to the concentration of TNF[47]. However, the oscillatory behaviour of TNFR signalling can depend on cell type and context. For example, a single pulse with a saturating dose of TNF induces sustained activation of NFκB in fibroblast-like synoviocytes (FLS) from patients with RA[48]. Although the concentration of TNF gradually diminished over time in culture, the activity of IKK complexes, as well as the nuclear localization and binding of target genes by NFκB subunit p65 (encoded by *RELA*), were maintained for several days after the single TNF-pulse[48]. Further studies are necessary to investigate whether the sustained NFκB activity in RA FLS is attributable to functional incompetence of signalling brakes.

Fine-tuning the direction of TNFR-signalling: 'live or die?'—TNF and TNFR1 are at the crossroads of inflammation, survival, apoptosis and necroptosis[13,14]. The factors that determine whether TNFR1 engagement induces complex I, leading to inflammatory responses, or complexes IIa, IIb and IIc, leading to cell death, are not well understood. The current model of this 'live or die' decision-making suggests that the expression levels of antiapoptotic molecules such as the long form of cellular FLICE-inhibitory protein (c-$FLIP_L$), and the degree of RIPK1 ubiquitination are critical elements in determining which pathway is activated. c-$FLIP_L$ binds to caspase-8 and blocks specifically its proapoptotic effector functions[14]. Regarding the ubiquitination status of RIPK1, the model proposes that ubiquitinated RIPK1 is tethered to complex I, whereas stripping of RIPK1 from the ubiquitin cloak results in its cytoplasmic trafficking and interaction with programmed-cell-death-inducing machinery[49]. Studies published in the past 5 years have revealed the roles of Sharpin[50–54], A20 (REF. 55) and ubiquitin carboxyl-terminal hydrolase CYLD[49] in the decision to undergo cell death. Other factors related to cell differentiation and activation state, as well as additional environmental signals, are likely to affect this decision; thus, the outcome of TNFR1 signalling is context-dependent. Similarly, regulation of the balance of complex IIa/IIb versus complex IIc formation is incompletely understood, but RIPK1 and RIPK3 seem to have an important role in this process[27,56–58].

TNF-induced gene expression

Cell-type specificity and epigenetic regulation

At the cellular level, several hundred genes are regulated (induced or suppressed) by TNF in a cell-type-specific manner. For instance, in FLS, TNF induces copious amounts of IL-6, with rapid and sustained production[48]. In macrophages, lower levels of IL-6 are induced more transiently[48], whereas neutrophils have limited capacity to produce IL-6 in response to TNF[59]. Cell-type specificity of gene expression results, at least in part, from the effects of cell differentiation and cell-state transitions on the chromatin landscape[60].

The chromatin landscape refers to the genome-wide pattern of open chromatin sites that demarcate regulatory elements (promoters and enhancers) where transcription factors can readily bind to regulate gene expression[60]. During cell differentiation, lineage-specific transcription factors, termed 'pioneer factors' or 'master transcription factors', penetrate the

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barrier of histone-containing nucleosomes that maintain a 'closed' or inaccessible chromatin conformation to create lineage-specific topologies of open chromatin. According to this model, cellular patterns of gene expression in response to stimuli such as TNF are predetermined chiefly by the chromatin landscape. 'Signalling transcription factors', such as NFκB and AP1, which have limited ability to remodel chromatin, will predominantly bind to and activate genes that exhibit open chromatin at their promoters and enhancers[60].

Studies in the past 3 years have modified this concept by showing that lineage-determining transcription factors can cooperate with various environmentally activated transcription factors to remodel the enhancer repertoire of a cell, and thus alter patterns of gene expression. For example, stimulation of macrophages with diverse agonists (including Toll-like receptor (TLR) ligands or cytokines) induced cooperative recruitment of master transcription factors and stimulus-specific transcription factors in new genomic topologies, generating novel enhancers termed 'latent enhancers' (REF. 61). In the same study, acquisition of latent enhancers was shown to alter responses to subsequent stimulation. Along these lines, another study showed that TLR8 agonists remodel the chromatin at the *IL6* locus in neutro phils and uncover a latent enhancer, converting *IL6* in neutrophils into a TNF-inducible gene[59].

Evidence suggests that chronic pathological states are associated with disease-specific stable changes in gene expression that are consistent with epigenetic mechanisms. For instance, FLS derived from patients with RA have a DNA methylome that is different from that of osteoarthritis (OA) FLS[62], and FLS derived from patients with early and longstanding RA have distinct DNA methylomes[63]. Notably, TNF alters chromatin states and induces higher levels of inflammatory cytokines and chemokines in RA FLS compared with OA FLS[48]. We hypothesize that, in the context of RA, unremitting inflammation induces disease-associated chromatin modifications, potentially altering cellular gene-induction responses to TNF. Consistently, inflammatory cytokines have been shown to modulate DNA hydroxyl-methylation in FLS and chondrocytes[64,65]. Overall, accumulating evidence suggests that epi genetic mechanisms, such as chromatin modifications or DNA methylation, established during differentiation or acquired in response to homeostatic or pathological environmental stimuli, contribute to the tissue-specific and disease-associated effects of TNF.

The microenvironment can also condition cellu lar responses to TNF independently of epigenetic mechanisms. Simultaneous engagement of TLRs and prostaglandin receptors cooperates with TNF to induce transcriptomes in monocytes, macrophages and dendritic cells that resemble chronic inflammatory states[66]. In addition, RA FLS modulate expression of approximately one-third of TNF-regulated genes in macrophages, including Myc-dependent, growth-factor-inducible and interferon (IFN)-inducible genes[67]. These findings suggest that signal integration and intercellular functional coupling will shape responses to TNF in complex inflammatory environments such as RA synovitis.

Expression kinetics of TNF-inducible genes

Three distinct patterns of induction kinetics for TNF-inducible genes have been identified: early, inter mediate and late[68–70]. The accessibility of chromatin is a critical factor in determining the expression kinetics of TNF-inducible genes. Genes with accessible

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chromatin are induced more rapidly by TNF (FIG. 3a), compared with genes that require chromatin remodelling (FIG. 3b). Genes requiring *de novo* protein synthesis for their induction are also usually expressed in a delayed manner. For instance, cell-type-specific clusters of genes exist that require the prior synthesis of IFN-β or the transcription factor IκBζ for optimal induction by TNF[59,68] (FIG. 3c,3d). Regarding the IFN-β-dependent genes, TNF first induces NFκB activation and IFN-β synthesis[68,71] (FIG. 3c). Subsequently, newly synthesized IFN-β operates in an autocrine manner, activating the Janus kinase (JAK) and signal transducer and activator of transcription (STAT) signalling pathway, which cooperates with NFκB and activates IFN-stimulated genes (ISGs) such as *CXCL9* (encoding MIG) and *CXCL10* (encoding IP-10)[68]. Regarding the IκBζ-dependent genes, TNF synergizes with TLR8 agonists or cytokines to induce IκBζ[59], which binds to and promotes activation of a distinct subset of gene promoters by inducing an activating histone mark, histone H3 trimethylation at lysine 4 (H3K4me3). The need for prior histone modification results in delayed transcription of NFκB-dependent genes in a cell-type-specific manner[59]. These results suggest a model whereby TNF induces a cascade of transcription factors that sustains late-phase gene expression. This model is supported by evidence that TNF induces a cascade mediated by initial induction of AP1 that eventually leads to expression of NFATc1, which promotes osteoclastogenesis that can contribute to the bone-resorptive properties of TNF[70]. Discovery of additional transcriptional modules that mediate novel aspects of late-phase TNF-induced gene expression and function represents an important area for future research.

A currently accepted model suggests that chromatin barriers operate as rheostats to regulate the amplitude and timing of expression of TNF-inducible genes[60]. Genes induced with a delay before peak expression are typically associated with a nonpermissive chromatin environment, such as nucleosomes that occlude regulatory elements, repressive histone marks (for example, H3K27me3 and H3K9me3) and co-repressor complexes[60] (FIG. 3). The nuclear receptor co-repressor 1 (NCoR) complex represses a cluster of TNF-inducible genes by recruiting the enzyme histone deacetylase 3 (HDAC3)[72,73]. Methyl-CpG-binding protein 2 (MeCP2), which binds to methylated CpGs, recruits the NCoR complex to gene promoters, suppressing their transcription[74] (FIG. 3e). In macrophages, lack of MeCP2 resulted in increased expression of a cluster of inflammatory genes upon TNF-stimulation[74]. Overall, the need to overcome nonpermissive chromatin states before genes can be transcribed contributes to the delayed gene-induction observed after TNF stimulation.

TNF-inducible genes can be further subclassified as transient or sustained, on the basis of the duration of their expression[48,69]. TNF induces sustained expression of inflammatory mediators in RA FLS, potentially due to prolonged NFκB signalling and sustained chromatin remodelling[48]. For a subset of TNF-inducible genes, mRNA stability is an additional determinant of the duration of their expression[69]. The mechanisms that fine-tune the expression kinetics of TNF-inducible genes, including signalling brakes and rheostat control of chromatin, help to ensure a stepwise and sequential response to pathogens and inflammatory stimuli — optimal host defence relies on a rapid and transient inflammatory response, followed by a resolution phase to attain wound healing and tissue repair.

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Altered cellular responses to other stimuli

One important function of cytokines is to change the physiological state of cells and the way they respond to environmental stimuli. This feature has been referred to as 'memory' or 'training', and includes enhanced responses to subsequent challenges (priming) or refractoriness to stimulation (desensitization or tolerance). Accumulating evidence suggests that TNF modifies cellular responses to other stimuli. For instance, TNF displays a priming effect on RA FLS, enhancing their subsequent inflammatory response to IFNs[75]. Notably, this priming was gene-specific and resulted in sensitization of RA FLS even to suboptimal concentrations of IFNs. In macrophages, by contrast, TNF desensitized the cells to the effects of lipopolysaccharide (LPS) and protected mice from LPS-induced lethality[76].

Molecular mechanisms underlying TNF-induced priming and tolerization have been revealed (summarized in FIG. 4). A common theme for both phenomena is the induction of gene-specific chromatin modifications by TNF, which alter cell responses to subsequent stimulations. In RA FLS, TNF induces an accessible chromatin state in the promoter region of *CXCL10*, enabling the immediate and robust induction of IP-10 mRNA upon re-stimulation with IFNs[75] (FIG. 4a). A genome-wide study revealed a similar capacity of TNF and other cytokines to induce faster or augmented responses to subsequent challenges, owing to a gene-specific induction of latent enhancers[61], although TNF can also suppress remodelling of chromatin at genes such as *IL6* (REF. 76).

An additional mechanism that potentially contributes to TNF-induced cell priming or tolerization is the effect of TNF on signalling events upstream of chromatin. For instance, in RA FLS, prolonged exposure to TNF increases the intracellular reservoir of STAT1, amplifying the activation of STAT1 upon secondary challenge with interferons[75] (FIG. 4a). Moreover, in macro phages, exposure to TNF induces the expression of signalling brakes, such as A20 and IκBα, which potentially restrict upstream signalling upon secondary stimulation with LPS[76] (FIG. 4b).

An intriguing finding of these studies is the sustained effect of TNF on target cells, suggesting the establishment of 'short-term transcriptional memory'. An attractive molecular explanation could be the induction by TNF of signalling molecules and chromatin modifications, which are not rapidly reversed[77]. Along these lines, TNF induces sustained expression of STAT1 (REF. 75), NFATc1 (REF. 70) and IκBδ[43]. Similarly, induction by TNF of the positive histone mark H3K4me1, which is associated with functional enhancers, is maintained for days in a large fraction of latent enhancers despite the absence of continuous stimulation[61]. Thus, short-term transcriptional memory is maintained by sequence-specific transcription factors and epigenetic mechanisms.

Role of TNF in health and disease

Homeostatic functions

Ablation of the *Tnf* gene in mice revealed that TNF has homeostatic functions in addition to its immune and inflammatory roles[78–80]. TNF is required for optimal defence against pathogens, proper lymphoid-organ architecture and germinal-centre formation, development of granulomas, resolution of inflammation and induction of tissue repair. Homeostatic

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functions of TNF are also supported by studies in mice lacking TNFRs[12] and by cell-based functional assays[76,81,82] (summarized in BOX 1). Several reports have identified molecular mech anisms that could explain some aspects of these unexpected homeostatic roles of TNF. One study showed that TNF desensitizes macrophages to the deleterious effects of secondary inflammatory challenges (tolerization)[76]. Others have described homeostatic effects of TNF in tissue regeneration, such as neuronal remyelination[83], cardiac remodelling[84] and cartilage regeneration[85]. In addition, TNF has suppressive effects on adaptive immune processes in models of autoimmunity such as autoimmune encephalitis[12]. The homeostatic potential of TNF has been suggested to be mediated, at least in part, by TNFR2 (REFS 12,86).

Pathogenic functions

Inflammatory and autoimmune diseases—TNF induces inflammation, activates vascular endothelium, orchestrates the tissue recruitment of immune cells and promotes tissue destruction[2] (the pathogenic functions of TNF are summarized in BOX 1). Uncontrolled production or function of TNF has been linked to the development of inflammatory diseases such as RA, IBD, psoriasis, PsA, ankylosing spondylitis and specific types of JIA. In contrast to the well-defined role of TNF in these diseases, its role in the pathogenesis of multiple sclerosis is a conundrum[12]. Although initial studies suggested a pathogenic function for TNF in multiple sclerosis, clinical trials of a global inhibitor of TNF were discontinued owing to unexpected aggravation of the disease[87]. In addition, patients receiving TNF blockade for other diseases sporadically developed lesions with demyelination, such as optic neuritis[88]. These observations suggest that TNF can suppress autoimmune processes and/or exert essential homeostatic functions within the micro environment of the central nervous system (CNS). Several studies suggest that the homeostatic and pathogenic activities of TNF are mediated by distinct molecular and cellular pathways[12]. TNFR2, which is expressed on regulatory T (T_{REG}) cells, oligodendro cytes and astrocytes, mediates immunoregulation, neuronal survival and re-meyelination; TNFR1, by contrast, induces CNS inflammation and neuronal demyelination[12]. Selective inhibitors of the neurotoxic TNFR1 pathway, which preserve the neuroprotective TNFR2 pathway, are being developed and could be a new avenue in therapeutics for patients with multiple sclerosis.

Hypernociception

TNF has been implicated in the hyperalgesia observed in the context of inflammation (inflammatory pain) and neuronal damage (neuropathic pain). In humans with RA and in several animal models of inflammation, neutralization of TNF induced rapid reduction of nociceptive neuronal activity in the afferent neurons, the spinal cord and the brain[89–94]. Notably, TNF blockade reverses hypernociception long before its effect on inflammation becomes obvious[94]. This observation suggests that the antinociceptive effects of TNF-blockade are distinct from their anti-inflammatory effects. At the cellular level, TNF seems to trigger hyperalgesia by inducing peripheral and central sensitization to mechanical stimulation[93]. Peripheral sensitization results from the direct action of TNF on C and Aδ nociceptive neurons, which innervate the site of inflammation[91]. Central sensitization is more complex, and potentially involves several cell types such as spinal cord nociceptive

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neurons, microglial cells and astrocytes[93]. At the molecular level, both peripheral and central sensitization are dependent on TNFR1-mediated signals[90,93].

Cancer—TNF was discovered as a serum factor that induces necrosis of tumour cells, in part by acting on tumour vasculature to compromise blood supply[95]. The cytolytic potential of TNF has been explained by its capacity to induce programmed cell death. Subsequent studies revealed that TNF also displays cytostatic effects on specific tumour cell lines[81]. In a 2013 report, TNF was shown to cooperate with IFN-γ to induce tumour-cell senescence, a cell-state characterized by permanent growth arrest[82]. The widespread use of TNF as a drug for cancer has been prevented by the systemic toxicity of TNF[96].

Accumulating evidence suggests that TNF also has the potential for opposite effects on cancer, either by inducing carcinogenesis or promoting the progression of established tumours[97]. TNF could be genotoxic by inducing *de novo* mutagenesis and suppressing the DNA-repair machinery. In the context of established tumours, TNF has the potential to promote the survival and proliferation of malignant cells, either directly by activating NFκB in tumour cells, or indirectly by inducing the production of tumour-promoting cytokines such as IL-6. In addition, TNF contributes to the escape of tumour cells from immunosurveillance by promoting the immunosuppressive capacity of myeloid-derived suppressor cells and T_{REG} cells[98,99]. Finally, TNF facilitates tumour metastasis by promoting epithelial–mesenchymal transition and by inducing the synthesis of matrix metalloproteinases (MMPs).

Cardiovascular disease—Several studies suggest that TNF is cardiotoxic for the healthy myocardium, and potentially cardioprotective for the failing myocardium. Cardiotoxicity is primarily attributable to TNF-induced cardiomyocyte apoptosis[100], whereas cardioprotection results from TNF-induced ectopic expression of keratin 8 and keratin 18 in cardiomyocytes[84]. In addition to its role in myocardial diseases, TNF is potentially implicated in the pathogenesis of atherosclerosis by affecting lipid metabolism, activating endothelial cells and inducing vascular inflammation[101].

Fibrosis and Dupuytren disease—A study published in 2013 revealed a pathogenic role for TNF during the course of Dupuytren disease[102]. Dupuytren nodules are infiltrated by classically activated macrophages that secrete TNF. Fibroblasts and myofibroblasts from Dupuytren lesions had increased expression of both TNFR1 and TNFR2, suggesting that the disease micro environment sensitizes local stromal cells to the effects of TNF. Neutralization of TNF reduced the contractile activity of myofibroblasts, suggesting that the local administration of TNF blockers might be beneficial for patients with Dupuytren disease[102].

Therapeutic targeting of TNF

Limitations of global inhibition

The approved anti-TNF agents have been a commercial success and a scientific breakthrough, improving the quality of life of millions of patients with TNF-mediated diseases. Despite these successes, the current therapeutic paradigm of global TNF blockade

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has several limitations, the three most important being low rates of disease remission, the development of adverse effects and the generation of antibodies against biologic TNF inhibitors[103]. Adverse effects include common and opportunistic infections, reactivation of latent tuberculosis, 'paradoxical' induction of autoantibodies, lupus-like symptoms, demyelination, psoriasis, sarcoidosis, as well as a potentially increased risk for specific malignancies, such as lymphomas. Development of fatal disseminated histoplasmosis and aggressive hepato splenic T-cell lymphomas in some patients undergoing treatment with TNF inhibitors resulted in the issue of 'black box' warnings for these drugs[104,105]. To overcome these limitations and improve efficacy and safety, alternative strategies are in development.

Improving TNF blockade

An attractive strategy to improve the rate of response to TNF blockade is to combine anti-TNF agents with other medications. Along these lines, combined inhibition of TNF and IL-17A with a bi-specific construct proved superior to single-cytokine blockade in inhibiting the production of inflammatory mediators in *in vitro* studies and in an arthritis model[106]. Notably, however, previous experience with combinations of biologic agents has shown that excessive immunosuppression can lead to an alarming rise in risk of infections[107,108].

Combining TNF blockade with drugs that target pathogenic pathways or cells not implicated in host defence, to improve response rates without compromising safety, is a therapeutic approach worth considering. Several attractive candidate combinations have been suggested: targeting of TNF and angiogenesis (for example, with an antibody against vascular endothelial growth factor); targeting TNF and tissue destruction (for example, with anti-MMP14 or anti-RANKL (receptor activator of NFκB ligand) which suppresses osteoclastogenesis); and targeting TNF and FLS (for example, by inhibiting cadherin 11, a cell-surface molecule that regulates FLS adhesion and inflammatory functions)[103].

Another alternative could be to shift the current therapeutic paradigm of global TNF inhibition to selective targeting of the pathogenic bioactivities of TNF, keeping the homeostatic functions of this cytokine intact. The proposed functional dichotomy between the TNFR1 pathway (primarily pathogenic) and the TNFR2 pathway (primarily homeostatic) might provide an approach to selective inhibition[12]. Next-generation TNF-blockers that selectively block the TNFR1 pathway have been developed and are described in TABLE 3 (REFS 109–117). Notably, the development of selective TNFR2 agonists provides an option for the future: to combine selective blockade of the TNFR1 axis with selective boosting of TNFR2 signalling[118].

In 2011, progranulin was found to operate as a natural antagonist of TNF by binding to both TNFR1 and TNFR2 (REF. 119). Administration of recombinant progranulin or of an engineered progranulin analogue (Atsttrin) was beneficial in several animal models of inflammatory arthritis[119]. Notably, progranulin has anabolic effects in cartilage via TNFR2-mediated activation of MAPK1 and MAPK3 (REF. 85). This observation suggests that progranulin might alleviate arthritis via dual effects on TNFR-mediated pathways, by blocking deleterious TNFR1 signalling and by engaging TNFR2 to trigger MAPK-mediated anabolic effects in chondrocytes[85].

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Reducing immunogenicity

The development of antibodies against TNF-blocking biologic agents results in allergic reactions and in progressive loss of drug effectiveness. This issue of drug immunogenicity has been only partially addressed with the development of humanized or fully human biologic agents. An alternative approach is to trigger the production of natural neutralizing anti-TNF antibodies via active immunization. This method seemed feasible in a limited 12-month phase II study in patients with RA or IBD[120], as active immunization with TNF-kinoid (composed of recombinant human TNF conjugated to the carrier protein KLH) resulted in the generation of neutralizing polyclonal antibodies against TNF. The clinical efficacy of this approach remains to be determined, and needs to be weighed against the potentially catastrophic consequences of life-long ablation of TNF.

Controlling TNF synthesis

Downregulation of the inappropriate transcription of TNF could be effective in ameliorating TNF-driven pathologies. IκBβ has been shown to function as a gene-selective co-activator, driving prolonged transcription of TNF[121]. Mice lacking IκBβ have reduced transcription of *Tnf* and are protected from LPS-induced lethality and collagen-induced arthritis. Gene-selectivity was explained by the specific interaction of the newly synthesized IκBβ with the p65–c-Rel NFκB dimer, which binds to the κB2 site in the promoter of *Tnf*[121]. Hence, blocking IκBβ might be a promising strategy to selectively inhibit the chronic phase of TNF production and treat chronic inflammatory diseases, such as RA.

As mentioned in this Review, precursor transmembrane TNF is cleaved by TACE, releasing soluble TNF. As TACE has important homeostatic functions by regulating EGFR-signalling in epithelial barriers, direct targeting of TACE raises safety concerns. However, inactive rhomboid protein 2 (iRhom2) has been identified as a myeloid-specific regulator of TACE that facilitates the shedding of membrane-bound TNF[8]. This observation suggests that therapeutic targeting of iRhom2 is a potentially attractive strategy to block the activity of TACE, specifically in macrophages, to prevent the release of pathogenic soluble TNF. This therapeutic strategy has the advantage of leaving intact the proposed homeostatic transmembrane TNF–TNFR2 pathway.

Inhibiting intracellular responses

Small molecules targeting chromatin regulators have the capacity to block specific pathogenic pathways downstream of TNFRs. In a 2014 study, pharmacologic inhibition of bromodomain and extra-terminal domain (BET) proteins suppressed TNF-induced endothelial cell activation, attenuating leukocyte rolling, adhesion and transmigration[122]. Notably, at the transcriptional level, inflammatory genes associated with TNF-induced super-enhancers were preferentially downregulated[122]. This observation suggests that BET inhibitors selectively block branches of the TNF-induced transcriptional programs.

Kinase inhibitors have also been described to suppress intracellular responses to TNF. For instance, tofacitinib, a JAK inhibitor approved for use in the treatment of RA, suppressed the late phase of TNF-induced STAT activation and cytokine production in macrophages[123].

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Conclusions

Important advances in the understanding of TNF biology and pathogenic functions are summarized in BOX 2. These advances have important therapeutic implications as they identify therapeutic targets — such as LUBAC components, mediators of necroptosis, epigenetic regulators (for example, BET proteins[124] and HDACs[125]) and novel transcription factors — whose inhibition might have more-selective effects on disease-specific pathogenic mechanisms,possibly achieving comparable efficacy without the toxic effects associated with global TNF blockade. Recognition of the homeostatic functions of TNF has led to new concepts for the treatment of rheumatic diseases, presenting the possibility of strengthening mechanisms of tolerization and tissue repair as a complement to suppression of inflammatory pathways.

Despite decades of research, however, many important challenges and gaps remain in our understanding of the basic science of TNF and in how to translate this knowledge into better treatments. Important directions for future basic research include a deeper understanding of the molecular underpinnings and functional consequences of the alternative outcomes of 'life' versus 'death' signalling, of the epigenetic regulation of TNF responses, of mechanisms that restrain TNF responses, and of homeostatic functions that could be manipulated to alter the pathogenic profile of TNF. An intriguing research direction related to epigenetic mechanisms is the effect of TNF on the expression of non-coding RNAs, including microRNAs[126,127] and long non-coding RNAs[128]. Another future research priority is the harnessing of basic science and the power of new high-throughput sequencing technologies and genome-wide approaches to study patients before and after TNF blockade therapy to identify markers predictive of clinical response and mech anisms associated with resistance to TNF blockade[129]. The identification of such markers and mechanisms is a major unmet need that will enable replacement of the current trial-and-error therapeutic approach with a stratified and increasingly precise strategy, in which the decision to introduce TNF blockade will be based on validated biomarkers that predict response and not on empirical criteria[130]. Such translational studies also offer hope for the discovery of alternative pathogenic pathways that can be targeted in patients who are resistant to therapy with TNF inhibitors.

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Key points

- TNF is a pleiotropic cytokine that exerts homeostatic and pathogenic bioactivities
- A new concept in therapeutics of TNF-mediated diseases is the selective inhibition of the pathogenic effects of TNF with preservation of its homeostatic functions
- TNF-induced necroptosis is a new pathway potentially implicated in TNF-mediated pathologies
- TNF induces cellular priming, tolerization, and short-term transcriptional memory in a context-dependent manner
- Combining TNF-blockade with drugs that target pathogenic pathways or cells not implicated in host defence is an attractive approach to improve effectiveness without compromising safety

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Box 1 | Functions of TNF

Homeostatic functions

- Defence against pathogens
- Organogenesis and development: lymphoid organ architecture
- Tissue regeneration: neuronal remyelination; cardiac remodelling; cartilage regeneration
- Immunoregulation: tolerization (desensitization) of macrophages, apoptosis of inflammatory cells
- Inhibition of tumorigenesis: necrosis, senescence (cytostatic effect)

Pathogenic functions

- Inflammation: induction of inflammatory mediators (cytokines, lipid mediators); recruitment of inflammatory cells (induction of chemokines and adhesion molecules, endothelial cell activation); survival of inflammatory cells; necroptosis
- Autoimmunity: inhibition of T_{REG} cells
- Tissue degeneration: induction of tissue-destructive enzymes; apoptosis; osteoclastogenesis
- Hypernociception: peripheral neuronal sensitization (effects on C and Aδ neurons); central neuronal sensitization (effects on spinal cord nociceptive neurons, microglial cells and astrocytes)
- Tumorigenesis: genotoxic effects (induction of *de novo* mutagenesis, impairment of DNA repair); induction of tumour-cell survival and proliferation; facilitation of tumour escape from immunosurveillance (effects on MDSCs and T_{REG} cells); facilitation of tumour metastasis (induction of EMT and MMPs)
- Atherogenesis: inflammation; endothelial cell activation; effect on lipid metabolism

EMT, epithelial–mesenchymal transition; MDSC, myeloid-derived suppressor cell; MMP, matrix metalloproteinase; T_{REG} cell, regulatory T cell.

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Box 2 | Recent advances in TNF biology and function

- Implication of the LUBAC complex and linear ubiquitination in TNF signalling, inflammatory responses and human diseases
- Appreciation of the induction of complex IIc and the functions of RIPK3 and MLKL in TNF-induced necroptosis, which could have an important role in rheumatic disease pathogenesis
- Recognition of the ability of TNF to induce pathogenic epigenetic modifications, which promote heightened inflammatory responses even to minor environmental challenges
- Discovery of mechanisms, including induction of transcription modules mediated by novel TNF-induced transcription factors, that sustain cellular responses to TNF; these unremitting responses to TNF are probably important in sustaining chronic inflammation
- Improved understanding of the importance of homeostatic functions of TNF, which include tolerization of macrophages, promotion of tissue repair possibly related to TNFR2 signalling, and suppressive effects on adaptive immunity

LUBAC, linear ubiquitin chain assembly complex; MLKL, mixed lineage kinase domain-like protein; RIPK3, receptor-interacting serine/threonine-protein kinase 3; TNFR2, TNF receptor 2.

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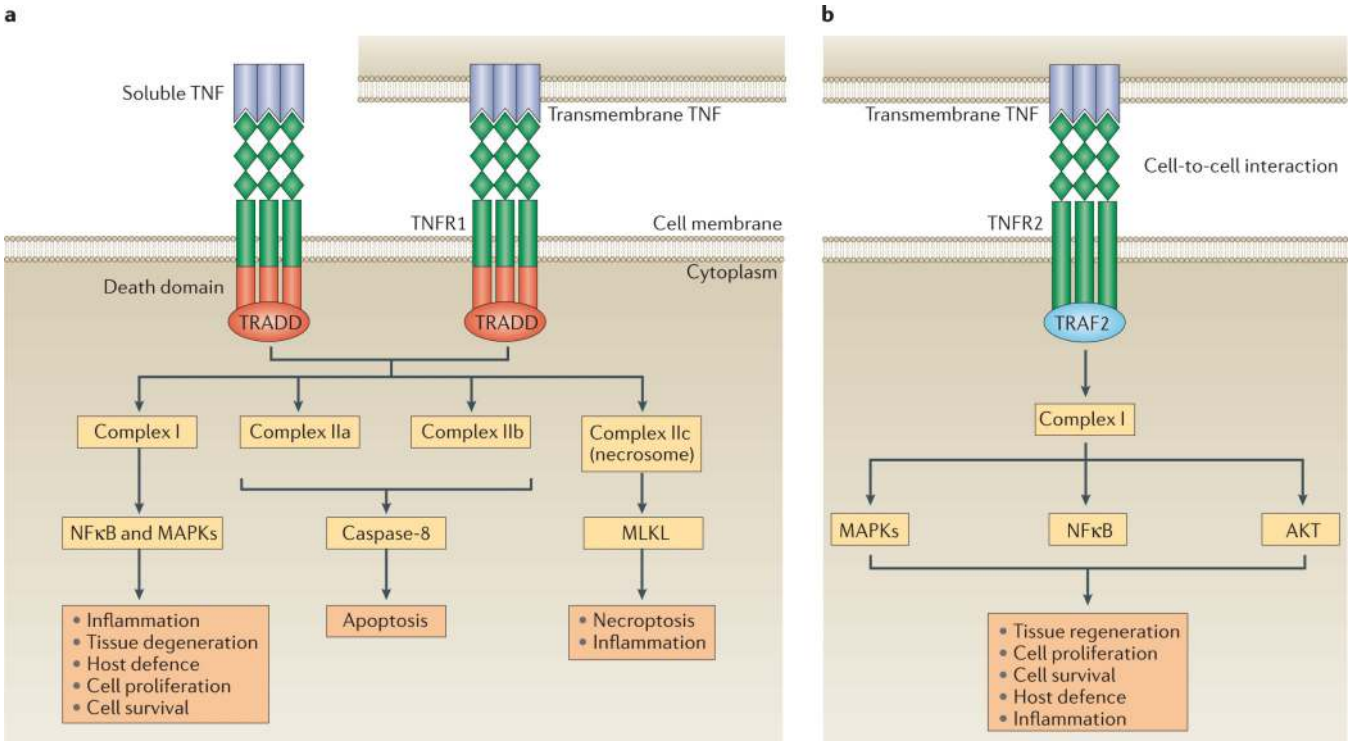

Figure 1. Signalling modalities and bioactivities downstream of TNF receptors

a | TNF receptor 1 (TNFR1) signalling is activated by both soluble and transmembrane TNF. TNFR1 bears a death domain that recruits the adaptor protein TNFR1-associated death domain protein (TRADD). Ligation of TNFR1 by soluble TNF or transmembrane TNF leads initially to the assembly of complex I, which activates nuclear factor κB (NFκB) and mitogen-activated protein kinases (MAPKs). TNFR1–complex I signalling induces inflammation, tissue degeneration, cell survival and proliferation, and orchestrates the immune defence against pathogens. Alternative signalling modalities, associated with programmed cell death, can also be activated downstream of TNFR1. The formation of the complexes IIa and IIb (also known as ripoptosome) results in apoptosis, whereas complex IIc (necrosome) induces necroptosis and inflammation. **b** | TNFR2 is proposed to be fully activated primarily by transmembrane TNF, in the context of cell-to-cell interactions. TNFR2 recruits TNFR-associated factor 2 (TRAF2) via its TRAF domain, triggering the formation of complex I and the downstream activation of NFκB, MAPKs and AKT. TNFR2 mediates primarily homeostatic bioactivities including tissue regeneration, cell proliferation and cell survival. This pathway can also initiate inflammatory effects and host defence against pathogens. MLKL, mixed lineage kinase domain-like protein.

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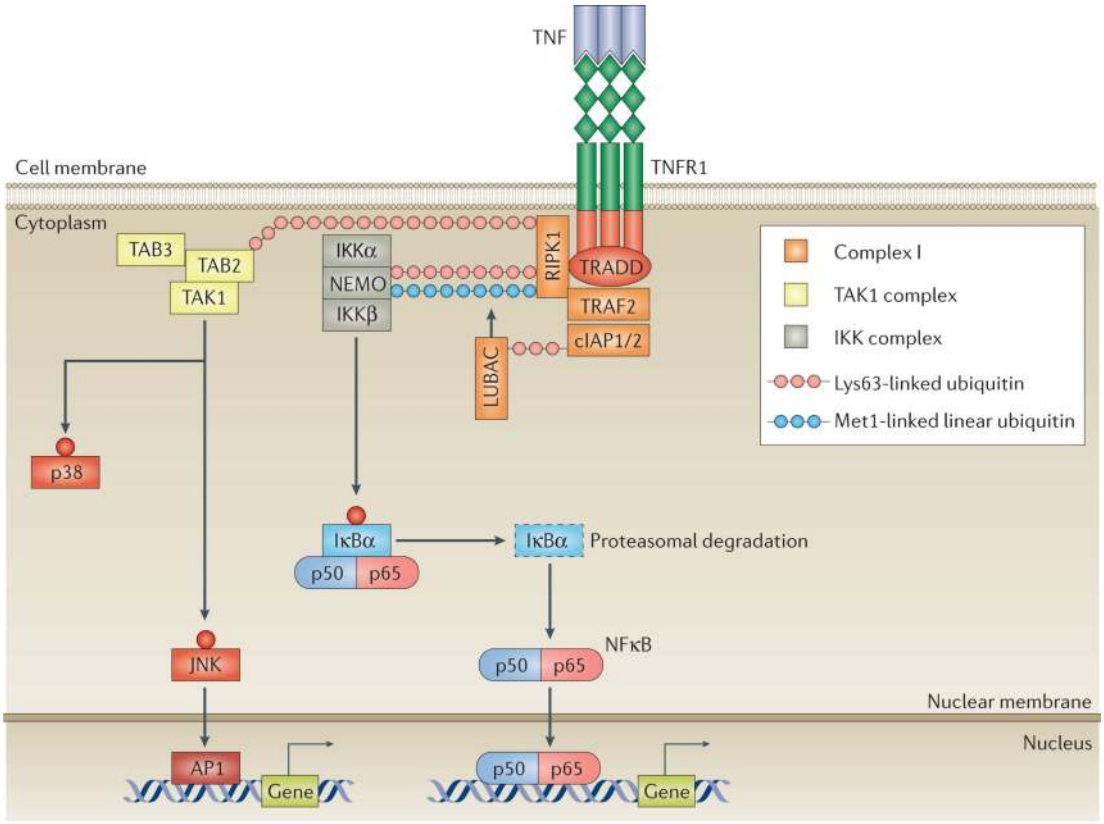

Figure 2. A model of TNFR–complex I signalling

The binding of homotrimeric TNF to homotrimeric TNF receptors (TNFRs) induces the formation of complex I, comprising TNFR1-associated death domain protein (TRADD), receptor-interacting serine/threonine-protein kinase 1 (RIPK1), TNFR-associated factor 2 (TRAF2), cellular inhibitor of apoptosis protein 1 (cIAP1) or cIAP2, and linear ubiquitin chain assembly complex (LUBAC). cIAPs and LUBAC decorate RIPK1 with scaffolding Lys63-linked and Met1-linked polyubiquitin chains, inducing the recruitment of transforming growth factor (TGF)-β-activated kinase 1 (TAK1) and inhibitor of κB (IκB) kinase (IKK) complexes. TAK1 activates p38 and JUN N-terminal kinase (JNK), leading to the transcription of AP1-target genes. IKKβ phosphorylates IκB, inducing its proteasomal degradation and the release of nuclear factor κB (NFκB). Free NFκB translocates to the nucleus, where it induces the expression of target genes. NEMO, NFκB essential modulator; TAB, TAK1-binding protein.

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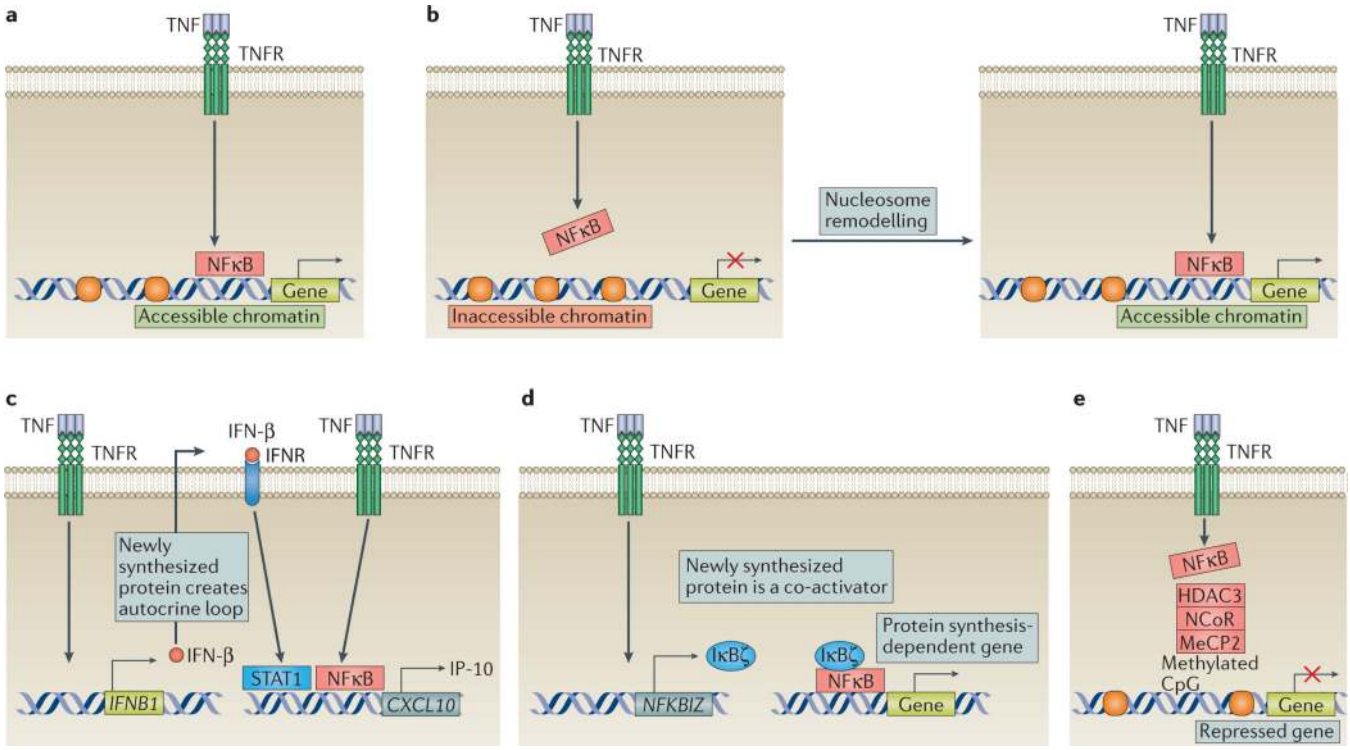

Figure 3. Molecular mechanisms of the differential induction kinetics of TNF-inducible genes

a | Immediate early TNF-inducible genes have an accessible chromatin state, which enables the unopposed and rapid recruitment of nuclear factor κB (NFκB). **b** | Induction of genes that have inaccessible chromatin (due to the presence of a chromatin barrier, such as nucleosomes) by TNF is delayed, as it requires chromatin remodelling to remove the chromatin barrier and enable the recruitment of NFκB. **c** | For one class of *de novo* protein synthesis-dependent genes, TNF first induces the production of IFN-β, which subsequently functions in an autocrine manner to activate signal transducer and activator of transcription (STAT) signalling. Eventually, STATs cooperate with TNF-induced NFκB for the optimal induction of genes. **d** | For another class of *de novo* protein synthesis-dependent genes dependent on IκBζ production, TNF-induced IκBζ is recruited to the promoter by NFκB and operates as a co-activator to trigger gene-induction. **e** | The induction by TNF of transcription of repressed genes is prevented by the presence of a co-repressor complex. A well-known co-repressor is nuclear receptor co-repressor 1 (NCoR), which recruits histone deacetylase 3 (HDAC3) that removes acetyl groups from neighbouring histones, creating a chromatin environment that is inaccessible to NFκB. The NCoR complex is recruited to methylated CpG motifs via the adaptor protein methyl-CpG-binding protein 2 (MeCP2). TNFR, TNF receptor.

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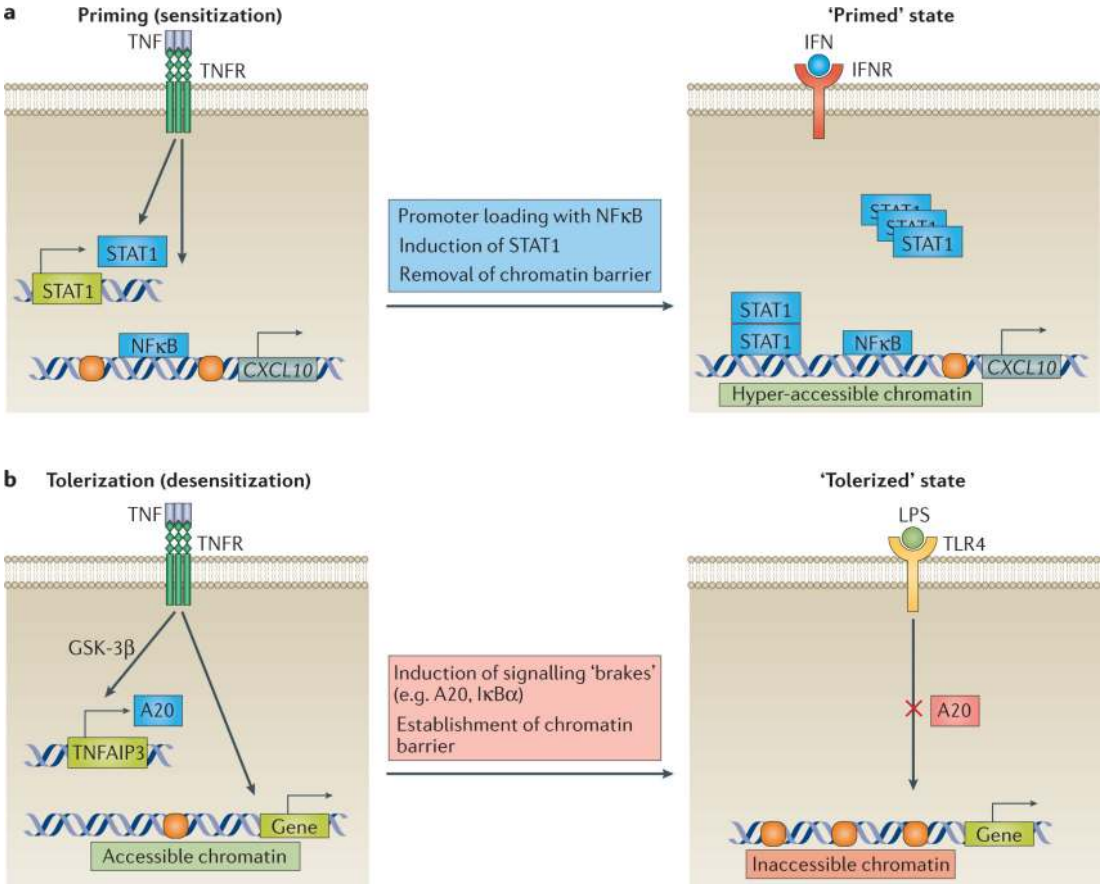

Figure 4. TNF modulates cellular responses to subsequent challenges and imposes short-term memory

a | TNF-induced cell priming (sensitization). Prolonged exposure to TNF primes the chromatin of fibroblast-like synoviocytes (FLS) in a gene-specific manner, by histone eviction, acetylation of the remaining histones, and loading of nuclear factor κB (NFκB) to specific interferon (IFN)-target genes. In addition, TNF induces the expression of signal transducer and activator of transcription 1 (STAT1), increasing the intracellular STAT1 reservoir. This 'primed' state is maintained for several days, imposing a 'short-term memory' in cells. Primed FLS display hyperresponsiveness to saturating doses and sensitization to suboptimal doses of IFNs. **b** | TNF-induced cell tolerization (desensitization). TNF induces a 'tolerized' state in macrophages by triggering the expression of A20 via a glycogen synthase kinase 3β (GSK-3β)-dependent mechanism, and by establishing a gene-specific chromatin barrier. A20 attenuates the signalling input and the chromatin barrier prevents gene-expression upon subsequent stimulation with lipopolysaccharide (LPS). TLR, Toll-like receptor.

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Table 1

TNF inhibitors and approved indications

Drug	Description	Indications
Monoclonal antibodies		
Infliximab	Chimeric (mouse and human) whole mAb against TNF	Crohn disease (adult and paediatric), ulcerative colitis (adult and paediatric), RA, PsA, plaque psoriasis, AS
Adalimumab	Human whole mAb against TNF	RA, ulcerative colitis, Crohn disease, plaque psoriasis, PsA, AS, JIA (paediatric), hidradenitis suppurativa
Certolizumab pegol	Humanized PEGylated Fab fragment of a mAb against TNF	RA, PsA, AS, Crohn's disease
Golimumab	Human whole mAb against TNF	RA, PsA, AS, ulcerative colitis
Soluble TNFR		
Etanercept	TNFR2 fused to IgG1 Fc	RA, plaque psoriasis, PsA, AS, JIA

Indications are for adult patients unless noted otherwise. AS, ankylosing spondylitis; JIA, juvenile idiopathic arthritis; mAb, monoclonal antibody; PsA, psoriatic arthritis; RA, rheumatoid arthritis; TNFR, TNF receptor.

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Table 2

Inhibitors of TNFR signalling

Inhibitor	Description	Mechanisms
Regulators of ubiquitination		
A20	• OTU-domain DUB • Inducible by TNF, via a GSK-3β-dependent mechanism[76] • Interacts with partners, such as Itch, TAX1BP1, RNF11, protein Ymer and ABINs, to form ubiquitin-editing complexes[36,131-133] • Polymorphisms or mutations of the gene encoding A20 (*TNFAIP3*) are associated with inflammatory diseases such as RA, SLE, psoriasis, and IBD[134]	• DUB: cleaves Lys63-linked ubiquitin chains from RIPK1 and NEMO[135] • E3 ubiquitin ligase: conjugates Lys48-linked ubiquitin chains to RIPK1, UbcH5c and Ubc13, inducing their proteasomal degradation[135] • Antagonistic ubiquitin binder: antagonizes the binding of LUBAC, NEMO, and E2 ubiquitin-conjugating enzymes[32,33] • Targets signalling effectors for lysosomal degradation[37]
Cezanne	• OTU-domain DUB • Inducible by TNF	DUB: cleaves primarily Lys11-linked ubiquitin chains from targets such as RIPK1 (REF. 136)
Ubiquitin thioesterase otulin	• OTU-domain DUB • Not inducible by TNF • Has high specificity for inhibiting events related to linear ubiquitination	• DUB: cleaves linear ubiquitin chains from RIPK1 and NEMO, destabilizing complex I[137] • Antagonistic ubiquitin binder: antagonizes the binding of NEMO to Met1-linked ubiquitin chains[34] • Regulator: interacts directly with HOIP, regulating its ligase-activity[138,139]
Ubiquitin carboxyl-terminal hydrolase CYLD	• USP-family DUB • Interacts with partners, such as Itch, p62, HOIP and optineurin[35] • Individuals with mutations of *CYLD* are predisposed to cylindromatosis[140]	Cleaves primarily Lys63-linked ubiquitin chains and secondarily linear chains from NEMO, TRAF2 and TAK1 (REF. 141)
USP4	USP-family DUB	• Cleaves Lys63-linked ubiquitin chains from TRAF2, RIPK1 and TAK1 (REF. 142)
USP11	USP-family DUB	• Cleaves Lys48-linked ubiquitin chains from IκBs, preventing their proteasomal degradation and the release of NFκB[28]
USP15	USP-family DUB	• Cleaves Lys48-linked ubiquitin chains from IκBs, preventing their proteasomal degradation and the release of NFκB[28]
USP21	USP-family DUB	• Cleaves Lys63-linked ubiquitin chains from TRAF2, RIPK1 and TAK1 (REF. 28)
USP31	USP-family DUB	• Upstream: cleaves Lys63-linked chains from TRAF2 (REF. 28) • Downstream: cleaves ubiquitin chains from p65 modifying its transcriptional activity[28]
MCPIP1	• Unclassified DUB • Inducible by TNF • Interaction with diverse adaptor proteins can instruct MCPIP1 to function as either RNase or DUB	• Upstream DUB: cleaves Lys63-linked chains from TRAF2 and RIPK1 (REF. 143) • Downstream DUB: cleaves Lys48-linked chains from IκBα[143] • RNase: promotes degradation of inflammatory mRNAs[144]
Itch	• E3 ubiquitin ligase • Interacts with A20 or CYLD to form ubiquitin-editing complexes[35,36]	Conjugates Lys48-linked ubiquitin chains to substrates, inducing their proteasomal degradation[35,36]
Optineurin	• Antagonistic ubiquitin binder inducible by TNF	Antagonizes the binding of NEMO to polyubiquitinated RIPK1 (REFS 29,30)

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Inhibitor	Description	Mechanisms
	• Mutations in *OPTN* have been identified in patients with POAG and ALS[29], including ALS-associated mutations that disrupt the ubiquitin-binding capacity of optineurin and abolish its inhibitory functions in TNFR signalling[29]	
p47	Antagonistic ubiquitin binder	Binds to polyubiquitinated NEMO directing its lysosomal degradation[31]
Phosphatases		
PP1	Phosphatase	• Targets IKK-complex[39] • Binding to IKK-complex is directed by the adaptor protein CUEDC2 (REF. 39)
PPP2CA-PPP2R1A-PPP2R5C (PP2A holoenzyme)	Phosphatase	Targets TRAF2
PPP2CB-PPP2R1A (PP2A core enzyme)	Phosphatase	Targets IKK-complex
PPP2CA-PPP2R1B (PP2A core enzyme)	Phosphatase	Removes Ser536-phosphorylation from p65, modifying its transcriptional activity
PP2Cα (PP1A)	Phosphatase	Targets IKK-complex
PP2CP (PP1B)	Phosphatase	• Targets TAK1; binding to TAK1 is directed by the adaptor protein 14-3-3E[40] • Targets IKK-complex
WIP1 (PP1D)	Phosphatase	Removes Ser536-phosphorylation from RelA/p65, modifying its transcriptional activity[41]
Inhibitors of NFκB		
IκBα	Rapidly inducible by TNF[43]	Newly synthesized IκBα enters the nucleus, dissociates NFκB dimers from chromatin, and exports NFκB to the cytoplasm[42]
IκBε	Inducible by TNF with slower kinetics (compared with IκBα)[43,44]	Functions as a back-up or fail-safe mechanism[43,44]
IκBδ (p100)	Inducible by TNF with delayed and sustained kinetics[43]	Sequesters NFκB subunits in cytoplasm

ABIN, A20-binding inhibitor of NFκB activation; ALS, amyotropic lateral sclerosis; CUEDC2, CUE domain-containing protein 2; DUB, deubiquitinating enzyme; GSK-3β, glycogen synthase kinase 3β; HOIP, HOIL-1-interacting protein (E3 ubiquitin-protein ligase RNF31); IBD, inflammatory bowel disease; IκB, inhibitor of κB; IKK, IκB kinase; LUBAC, linear ubiquitin chain assembly complex; MCPIP1, MCP-induced protein 1 (ribonuclease ZC3H12A); NEMO, NFκB essential modulator; NFκB, nuclear factor κB; OTU, ovarian tumour; POAG, primary open-angle glaucoma; RA, rheumatoid arthritis; RIPK1, receptor-interacting serine/threonine-protein kinase 1; RNF11, RING finger protein 11; SLE, systemic lupus erythematosus; TAK, transforming growth factor (TGF)-β-activated kinase 1; TAX1BP1, Tax1-binding protein 1; TNFR, TNF receptor; TRAF, TNFR-associated factor; USP, ubiquitin specific peptidase.

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Table 3

Next-generation selective inhibitors of soluble TNF-TNFR1 pathway

Class	Agent	Description
TNFR1-specific antibodies	ATROSAB[110,111]	Full-length IgG against a specific epitope of TNFR1
		Maintains the conformation of TNFR1 in an inactive state and obstructs the binding of TNF and LTα
		The Fc-region has been mutated to abolish the capacity to bind Fcγ receptor and complement, avoiding immune system activation
	MDS5541 (REFS 109,112)	Domain-antibody comprising a single variable region with specificity for TNFR1 linked to another single variable region with specificity for albumin
	TROS[113]	Trivalent nanobody comprising two distinct domains with specificy for TNFR1 (resulting in high-affinity binding to TNFR1) and an anti-albumin domain
TNF muteins[114,115]	XENP345 (REFS 145,146) and XPro1595 (REF. 147)	Dominant-negative TNF muteins that interact with native soluble TNF to form inactive heterotrimers, which have markedly reduced receptor-binding and signalling capacities
	R1antTNF[116,117]	TNFR1-selective antagonistic TNF mutein, that is, selective binding capacity for TNFR1 without signalling capacity
		Also has dominant negative TNF function, by forming inactive mixed heterotrimers with native TNF

Notes: TNF muteins are TNF molecules engineered to introduce amino acid changes; such modifications in the receptor interface domain can impair the receptor-binding and signalling capacity of these molecules. ATROSAB, antagonistic TNF receptor one-specific antibody; LT-α, lymphotoxin-α; R1antTNF, TNF receptor 1 antagonist; TNFR, TNF receptor; TROS, TNF receptor-one silencer.