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Review

The cybernetics of TNF: Old views and newer ones

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ABSTRACT

The proinflammatory cytokine tumor necrosis factor (TNF) orchestrates complex multicellular processes through a wide variety of changes that it induces in cell functions. At various stages of the study of TNF, attention has been drawn to one of three different modes of its action. The work that led to the discovery of this cytokine addressed situations in which it inflicts massive damage to tissues through a mode of action that appeared to be unrestricted. In the years that followed, attention was drawn to the existence of negative feedback mechanisms that do restrict TNF formation and function, and of reciprocal mechanisms for negatively regulating TNF-induced gene activation and of cell death. Most recently, the discovery of the critical role of TNF in chronic inflammatory diseases directed attention to the ability of TNF also to act with no apparent time restriction. Major gaps still remain in our knowledge of the cellular and molecular basis for these three modes of TNF action.

Contents

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1. Introduction

Tumor necrosis factor (TNF), a cytokine generated by a variety of different cells—most effectively by mononuclear phagocytes—in response to a wide range of immune stimuli and stress conditions [1], is one of the major regulators of inflammation. Studies have shown that this cytokine initiates and orchestrates a wide range of different functional changes in cells, thereby contributing to phenotypically different phases in the inflammatory process. Throughout its history, research on TNF has periodically focused on different forms of inflammation dictated by different modes through which this cytokine was found to coordinate cell function: a form of inflammation where TNF formation and function are restricted both temporally and locally; an acute form that results in devastating destruction of tissues; and a chronic form in which the inflamed tissue responds continuously to TNF and also keeps on generating it without any extraneous inducer (Fig. 1). This essay is a brief discourse on the state of our knowledge of the networks of cellular and signaling mechanisms that dictate these three different cybernetic patterns.

2. TNF and its receptors

TNF is generated by cells as a type II transmembrane protein [2] that can be proteolytically processed by TACE/ADAM17, a metalloproteinase of the adamalysin family [3], to yield a soluble form of this cytokine. Both in its membrane-integrated and in its soluble forms TNF assembles into homotrimers, and in both it can trigger signaling. It acts through binding to one of two single transmembrane receptors, TNFR1 (TNFRSF1A) or TNFR2 (TNFRSF1B), whose signaling activities differ.

Both TNF and its receptors belong to large families of related ligands and receptors, most of whose members serve immunoregulatory roles by activating a set of proximal signaling proteins that the different members share. Although this essay focuses on TNF, the modes of regulation described here also bear on the functioning of other members of the TNF families.

The N-terminal part of the membrane-integrated form of TNF extends into the interior of the TNF-expressing cell. Similarly to the intracellular domains of the TNF receptors, this intracellular domain of the TNF molecule can be induced to associate with signaling proteins. Binding of TNF to receptors on target cells thus results not only in 'forward' signaling by TNF through the receptors of those cells, but also in 'reverse' signaling within the TNF-producing cells [4].

3. Major signaling mechanisms activated by TNF and bacterial lipopolysaccharide

To accommodate the highly pleiotropic function of TNF, the TNF receptors orchestrate a complex set of molecular interactions that are dynamically modulated by multiple contextual cues. The current knowledge of these signaling mechanisms and of the mechanisms that regulate them has been thoroughly overviewed in other articles [5–9]. Some of their principal features will be illustrated here through a few examples. Figs 1 and 2 present diagrammatic illustrations of the modes of regulation of TNF signaling discussed in this essay and of some of their contributory mechanisms.

The TNF receptors have no intrinsic enzymatic function. Signaling is initiated by their imposed juxtaposition, which provides new scaffolds for recruitment of a limited set of adapter proteins to the receptors through a few protein interaction motifs (such as TRAF-binding domains, the death domain, or the RIP homotypic interaction motif). Some of those initially recruited proteins (e.g., the TRAFs and the cIAPs) possess ubiquitin ligase activities. These recruited proteins conjugate polyubiquitin chains to the proteins with which they interact, and the links between the ubiquitins in these chains, rather than being located in the lysine corresponding to the 48th residue in the ubiquitin chain (as occurs in the ubiquitin chains that dictate proteasomal degradation), are kinds (such as linkage in lysine 63, or linear linkage) that impose interactions with signaling proteins. As a result, protein interactions are fostered in the initiating complexes, and additional signaling proteins are recruited and activated, through interaction of ubiquitin-binding motifs in those proteins with these newly formed ubiquitin chains.

Several of the signaling pathways acting downstream of these initiating complexes are comprised of protein kinase cascades and serve mainly for gene activation. Thus, for example, the three-tiered ERK, p38, and JNK MAP kinase cascades activate transcription factors such as AP1. Such signaling pathways also activate dimeric complexes of members of the NF-κB transcription factor family by phosphorylating inhibitory proteins of the IκB family that associate with these dimers. This phosphorylation targets the IκB proteins for proteasomal degradation. TNFR1 activates the 'canonical' NF-κB pathway, in which the protein kinase IKK2 mediates activation of the NF-κB dimer p65:p50. TNFR2 also triggers the 'alternative' NF-κB pathway, in which the protein kinase IKK1 that acts downstream of the protein kinase NIK mediates generation of a RelB:52 NF-κB complex.

Two other TNFR1-activated pathways serve to induce death. In the 'extrinsic cell-death pathway' the proximal signaling protein is caspase-8. This protease, through proteolytic processing and hence activation of other members of the caspase cysteine protease family, triggers apoptotic cell death. A recently discovered additional pathway triggers programmed necrosis of cells (necroptosis). Its core components are the protein kinases RIPK1 and RIPK3 and a pseudokinase activated by RIPK3, called MLKL.

As mentioned in Section 2, when TNF is in its membrane-integrated form, besides activating signaling in its target cells through the two TNF receptors it also activates signaling within the TNF-expressing cells. Molecules that interact with the TNF molecule's intracellular domain, as well as a free, cleaved form of that intracellular domain, seem to contribute to this signaling [4,10,11].

There is much similarity between the signaling mechanisms activated by the TNF receptors and those activated by receptors for pathogen components that induce TNF synthesis. Among the latter is Toll-like receptor (TLR)4, to which bacterial endotoxin (alias lipopolysaccharide, LPS) binds, and which is well known to be capable of inducing TNF synthesis. This similarity is not coincidental. TNF acts as an immune-defense mediator that alerts remote cells to the pathogenic stimuli that induce its synthesis. TNF would therefore indeed be expected to activate, within its target cells, functional changes similar to those by which the cells have chosen to respond initially to these pathogenic stimuli.

4. TNF as mediator of the acute tissue-damaging inflammation observed in a tissue's 'hypersensitive' response to pathogen components

4.1. Phenomena

TNF has been rediscovered several times over. On the first two occasions it was discovered during explorations of mechanisms underlying the swift, devastating damage that tissues undergo in hypersensitive immune-response processes. One well-known example of such destructive processes is the 'Koch phenomenon', a necrotizing hypersensitive reaction unleashed by adaptive immune mechanisms that are triggered by dual exposure to components of *Mycobacterium tuberculosis* [12]. Other examples are the local

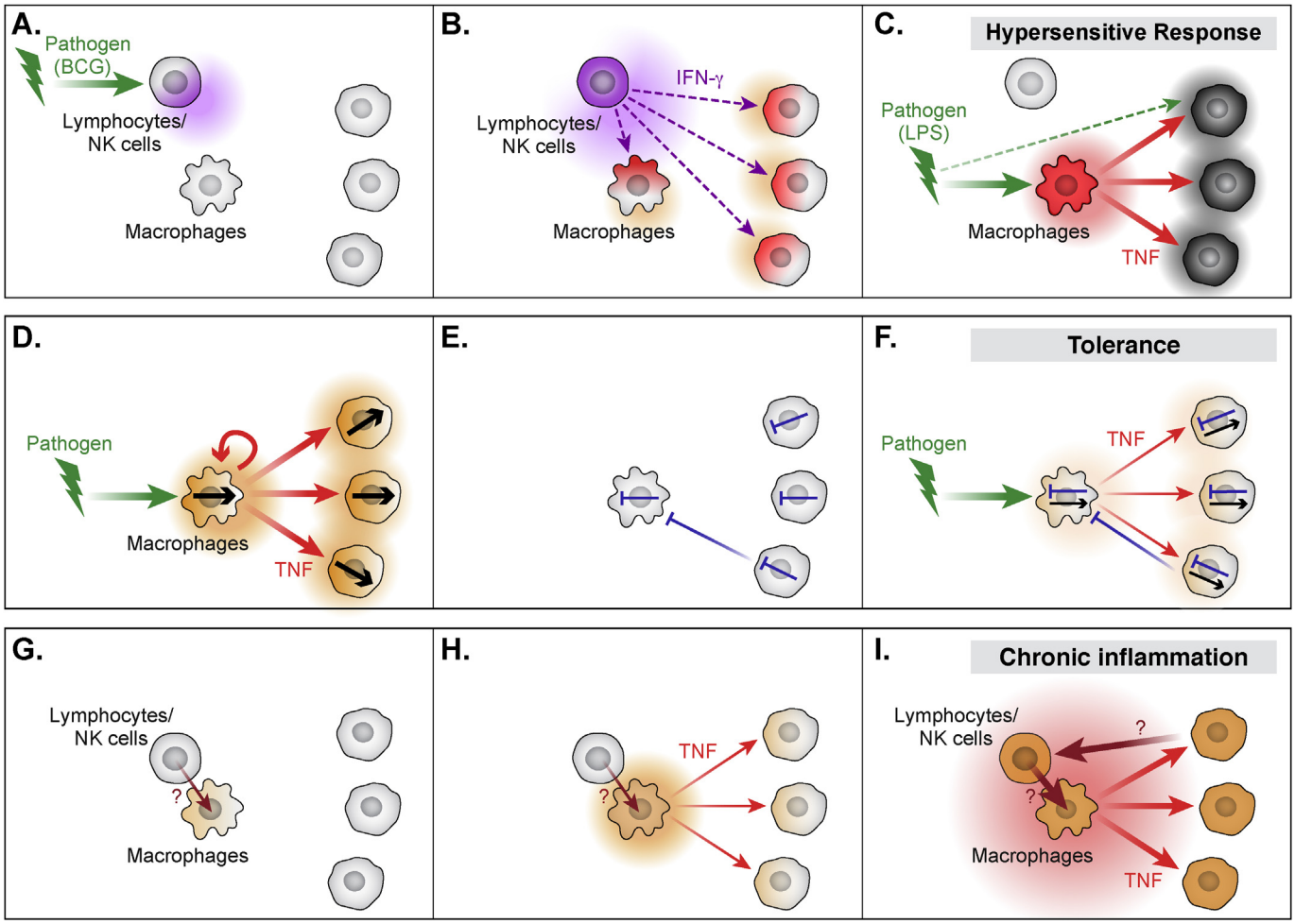

Fig. 1. Differing patterns of inflammation orchestrated by TNF. (A–C) Hypersensitive response. Cellular activities contributing to this response, in the mouse experimental model in which TNF was discovered [16]. (A and B) Priming. Initial exposure of the mouse to a pathogen (BCG, green arrow) primes the macrophages to yield enhanced inducibility of TNF through effects of IFN-γ and other mediators generated by T cells and NK cells (magenta arrows). IFN-γ also primes cells to yield an enhanced response to TNF. (C) Elicitation. Re-exposure to a pathogen component (LPS) triggers massive generation of TNF in the primed macrophages (red arrows). Transfer of TNF-containing serum from primed to unprimed mice mediates selective hemorrhagic necrosis of tumors in the unprimed mice, hence the name 'tumor necrosis factor'. However, in the LPS-treated primed mice such selectivity was not observed. Because various target cells were also primed in them and also because of synergistic effects of TNF and LPS on these target cells (broken green arrow) the induction of TNF in these mice was fatal. (D–F) Tolerance. (D) Exposure of cells to LPS or other pathogen components induces generation of TNF, which in turn triggers signaling in various target cells (black arrows) and (in an autocrine manner) also in the TNF-producing cells. (E) Numerous negative feedback mechanisms (blue arrows) are activated both in the TNF-producing cells and in the cells exposed to TNF. Some mechanisms are intracellular whereas others operate through effects of induced extracellular mediators (long blue arrow). (F) Owing to effects of the negative feedback mechanisms, re-exposure to a TNF-inducing pathogen component or to TNF triggers considerably weakened TNF generation and TNF-induced signaling. (G–I) Chronic inflammation. Hypothetical sequence of events in emergence of chronic inflammatory diseases, based on the findings that in experimental animals mere chronic expression of TNF incites chronic inflammatory diseases and mere inhibition of TNF function can yield prolonged remission associated with arrest of the chronic generation of TNF. (G and H) A hypothetical unknown agent (dark red arrow) incites chronic generation of TNF (red arrows), eliciting (I) a chronic type of response that perpetuates TNF generation. This generation apparently occurs through induced changes in lymphocytes and in NK cells, which in turn trigger the generation of TNF, mainly in macrophages, through direct cell–cell contact (dark red arrows). BCG, *Mycobacterium* bacillus Calmette–Guérin; IFN-γ, interferon-γ; NK, natural killer; LPS, lipopolysaccharide. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and systemic Shwartzman reactions, which are mediated by the innate immune mechanisms triggered as a result of dual exposure to bacterial components [13]. The first two reports of TNF activity described the cell-killing activity both of this cytokine and of the closely related cytokine lymphotoxin in tracking mechanisms for the massive tissue destruction initiated by lymphocyte activation, as observed in the Koch phenomenon [14,15]. (One of these two studies employed an ex vivo model of the 'delayed-type hypersensitivity' that accounts for the Koch phenomenon [15].)

Seven years later TNF was rediscovered in a study in which a dramatic tissue-destructive effect, seen in mice exposed to a priming effect of the *Mycobacterium* bacillus Calmette–Guérin (BCG) and then challenged with LPS, was recognized as a consequence of TNF induction [16]. The name 'tumor necrosis factor' was intended to highlight apparent tumor specificity in the hemorrhagic necrosis that was seen to be induced in the particular experimental model used. Subsequent studies clarified, however, that the same kind of damage is also inflicted on normal granulation tissue [17] and that the selective nature of its elicitation is apparently dictated by the particular type of integrin expressed by angiogenic endothelial cells found in these tissues [18]. TNF was found to inflict massive damage in other ways as well [19]. The harmful potential of this cytokine seems to be accounted for by its ability to induce in cells a wide range of functional changes with destructive potential. These include induction of cell 'suicide' [20], dissolution and growth arrest of connective tissue, cartilage and bone [21,22], enhanced coagulation of the serum that can result in clogging of blood vessels [23], and others.

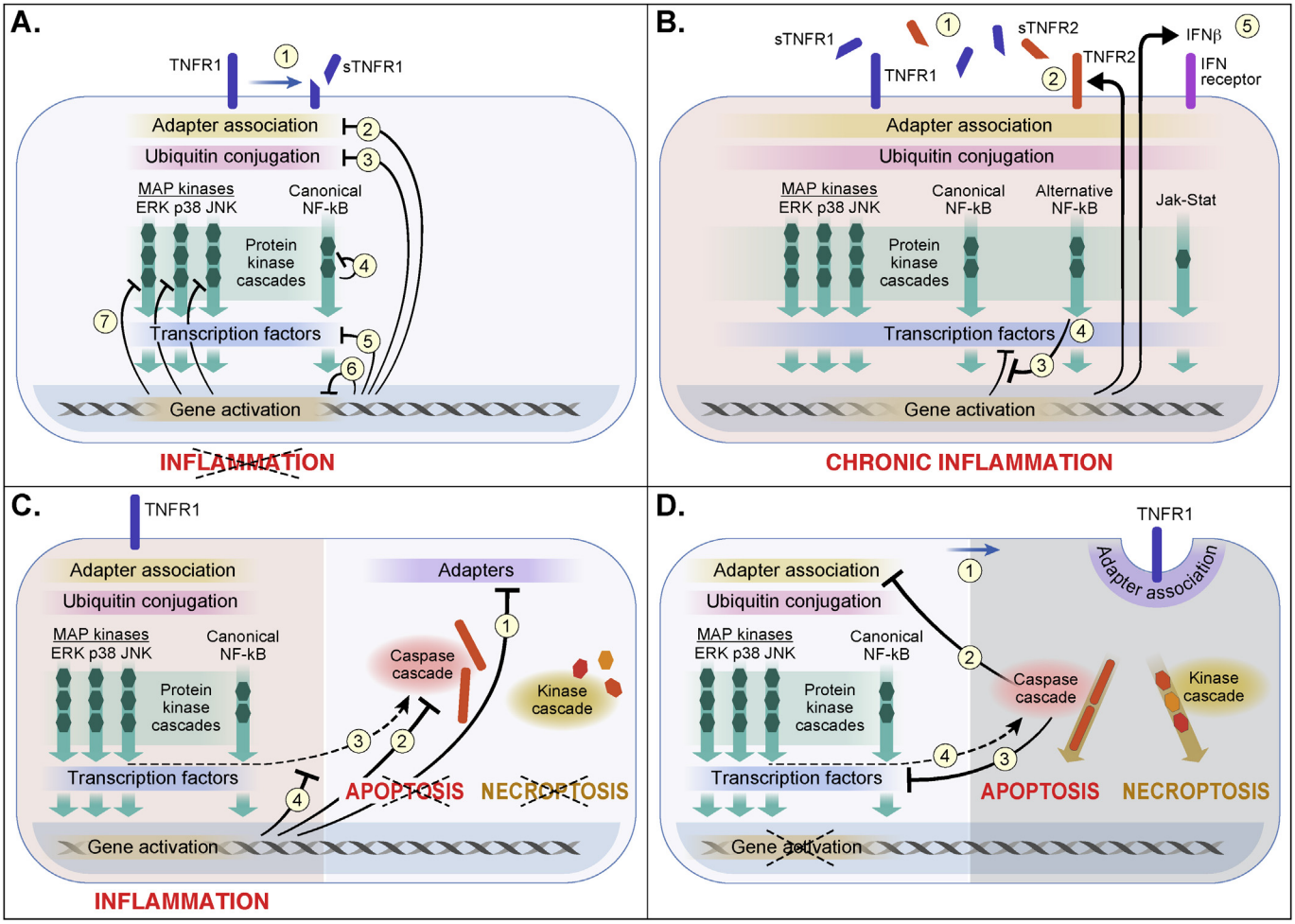

Fig. 2. Diagrammatic representation of signaling mechanisms contributing to different modes of TNF action. The diagram presents a highly simplified illustration of the major signaling pathways activated by TNF and some examples of their modulation in the different modes of TNF action discussed in this essay. Hexagons represent protein kinases and protein kinase analogs; quadrilaterals represent cysteine proteases of the caspase family. (A) Examples of the kinds of negative feedback mechanisms that restrict the proinflammatory function of TNF. (1) Receptor shedding. (2) Induced synthesis of proteins that dissociate the proximal signaling complexes. (3) Induced synthesis of ubiquitin chain-modifying enzymes. (4) Inhibitory self-phosphorylation of protein kinases. (5) Induced synthesis of proteins and of lncRNAs that bind transcription factors and arrest their function. (6) Induced synthesis of proteins that arrest activation of genes by associating with their DNA or chromatin components. (7) Induced synthesis of phosphatases that dephosphorylate MAP kinases. (B) Examples of mechanisms believed to contribute to a chronic response to TNF. (1) Soluble TNF receptors accumulating at sites of chronic inflammation might act as buffering agents, extending the duration of TNF action. (2) Triggering of TNFR1 induces TNFR2 synthesis. (3) TNFR2 signals for activation of the alternative NF-κB pathway, which may facilitate a chronic response to TNF in two ways. First, by cleaving induced p100 and p105 molecules it can arrest their inhibitory effects on the function of canonical NF-κB complexes. (4) Secondly, the activities of some of the genes that were activated by the canonical pathway can be maintained by RelB:p52 dimers generated by the alternative pathway, while being unaffected by inhibitors of the canonical NF-κB pathway. (5) Prolonged stimulation by TNF yields generation of IFN-β. Stat1-activated by IFN-β, acting together with TNF-induced NF-κB, constitutively turns on various 'interferon signature' genes characteristic of chronic inflammation. (C and D) Mechanisms contributing to reciprocal negative regulation of gene activation and cell-death induction by TNF. (C) Mechanisms contributing to arrest of death induction upon induction of gene activation. (1) Proteins upregulated via NF-κB activation block the assembly of signaling complexes that initiate both the extrinsic caspase-mediated cell-death pathway to apoptosis and the kinase cascade leading to necroptosis. (2) Other NF-κB-activated genes block downstream events in the extrinsic cell-death pathway. (3 and 4) Chronic activation of JNK, which enhances death induction by TNF, is also withheld by NF-κB activated genes. (D) Mechanisms contributing to the arrest of signaling for gene activation upon induction of apoptotic cell death. (1) Whereas the activation of NF-κB and some other signaling pathways contributing to TNF-induced inflammation is mediated by signaling complexes that associate with the TNF receptor shortly after TNF binds to it (while the receptor is still expressed on the cell surface), signaling for cell death is initiated by cytoplasmic complexes that are generated only later, after uptake of the TNF receptors into the cell. This time gap allows the proinflammatory and anti-death signaling pathways to prevail in the absence of contextual cues that would arrest the latter mechanisms. (2) Caspases activated via the extrinsic cell-death pathway cleave proteins that participate in initiation of the signaling pathways that lead to NF-κB activation. Besides abolishing the signaling activity of these proteins, cleavage of some of the proteins also yields fragments inhibitory to signaling. (3) Some of the distal signaling proteins in the pathways that initiate inflammation are also cleaved and are thus inactivated by caspases. (4) Chronic activation of JNK enhances death induction by TNF. IFN-β, interferon β; lnc, long non-coding; TNFR1 and TNFR2, TNF receptor 1 and 2.

4.2. Mechanisms and physiological relevance: fragmentary knowledge and open questions

In view of the disappointing results of the clinical studies prompted by the tissue-destructive anti-cancer effects of TNF [24] and by its contribution to acute inflammatory conditions [25] in experimental animal models, research interest in hypersensitive responses involving TNF declined, leaving many open questions about their mechanisms. What we did manage to learn until then was that when TNF elicits hypersensitive responses it does so in cooperation with other specific inducing agents such as interferon gamma (IFN γ) [26] (Fig. 1a) whose production can occur secondarily to that of IL-12 [27]. Other such agents are IL-1 [28] and TLR ligands such as LPS [29]. While there is quite detailed knowledge of various mechanisms that contribute to the priming effects of IFN γ [30–32], very little is known of the mechanisms by which TNF function synergizes with those of IL-1 and of TLR ligands.

As described in Section 5, both the induction of TNF and its cellular effects are restricted by multiple negative feedback mechanisms. IFN-γ, besides enhancing cell responsiveness to TNF inducers and TNF effects—for example, by upregulation of the TNF receptors [33]—also counteracts the inhibitory effects of these antagonizing mechanisms. As an example, IFN-γ reverses the gene remodeling arrest dictated by negative feedback mechanisms [34].

Our knowledge of the exact effector mechanisms by which TNF elicits its dramatic tissue-destructive effect is even more fragmentary. The direct cell-killing activity (by which TNF was detected in the early studies that led to its discovery [14,15]) seems to contribute little to the tissue-damaging effects observed in the subsequent study in which its name was coined [16]. The evidence suggests that in the latter case the damage derives from the coordinated action of several different effector immune functions [17,18,35–37]. The details of this coordination, however, are unclear. It is not known exactly how these various activities are orchestrated by TNF to yield dramatic rapid destruction of tissue, or to what extent the various distinct phenomena of massive tissue destruction that prompted the research leading to the discovery of TNF involve the same or different cellular activities and the same mode of coordinating them.

5. Restricted TNF formation and function: lessons form endotoxin tolerance

5.1. Phenomena

It has long been known that whereas in some situations exposure to immune stimuli primes the immune system to respond more vigorously upon second stimulation, in other situations such pre-exposure has the opposite effect, resulting in decreased responsiveness. Such desensitization was first noticed in studies of activation of adaptive immunity by antigens [38,39] and later it was also observed when activation of innate immune responses by bacterial toxins was assessed. The latter phenomenon, 'endotoxin tolerance', was found to affect proinflammatory effects of the toxins while not affecting or even enhancing other induced changes, including some leading to suppression of inflammation [40].

The discovery of TNF and its role in mediating many of the proinflammatory functions of LPS [19,41,42] shed new light on the mechanisms of endotoxin tolerance. TNF generation was found to be among the LPS effects that were most effectively suppressed after pre-exposure to pathogen components [43–45]. Furthermore, TNF itself was found to render cells unresponsive to various LPS effects including TNF induction. Evidence was presented to suggest that TNF can also induce in cells 'tolerance' to some of its own effects [45] (Fig. 1b). The ability of both LPS and TNF to suppress the response to subsequently applied inducers of proinflammatory functions was also shown to affect innate immune responses to various other potentially damaging challenges, such as ischemic reperfusion [46–48] and hyperthermia [49].

5.2. Mechanisms

5.2.1. Intracellular mechanisms restricting induction and proinflammatory functions of TNF

The complexity of signaling mechanisms acting downstream of the TNF receptors is mirrored by a similar complexity of feedback mechanisms controlling those functions. A complex set of mechanisms, similar to those that regulate TNF function, also controls the signalling by receptors for pathogen components such as LPS that induce TNF formation. These mechanisms have been listed in great detail elsewhere (e.g. [50–53]). A few examples are provided here to illustrate the kinds of mechanisms involved (Fig. 2a).

Some of the feedback mechanisms are exerted by pre-existing proteins, enabling rapid arrest, while others are mediated by proteins newly generated upon gene activation by TNF receptors and the LPS receptor TLR4. Binding of ligands by the receptors triggers downregulation of the latter, which occurs both via their uptake into the cell and by their proteolytic cleavage [54–59]. Mutational ablation of the proteolytic cleavage of TNFR1 was convincingly shown to facilitate inflammation [60].

Further downstream, assembly of the proximal signaling complexes is downregulated by induced synthesis of enzymes that reverse the polyubiquitin-chain generation fostering this assembly. One example of such an enzyme is CYLD, a deubiquitinase that specifically disassembles K63-linked polyubiquitin links. Another is A20, a multifunctional protein that besides catalyzing deubiqitination can also block the function of polyubiquitin chains merely by binding to them, and can catalyze the generation of K48-linked polyubiquitin chains that dictate protein degradation [61]. TLR4 induces functional arrest of its proximal signaling complexes via induction of proteins such as SOCS1, IRAK-M and SHIP-1, which displace crucial components of these complexes [51,53]. SODD, a protein that seems to bind specifically to TNFR1, was suggested to serve a similar inhibitory role in restricting signaling activation by this receptor, except that SODD seems to act constitutively to arrest signaling, and that TNF was claimed to transiently abate its inhibitory effect [62].

Of the various signaling mechanisms activated by LPS and TNF, the pathways leading to NF-κB activation play a particularly important role in the induction of genes that participate in the inflammatory process. These pathways can be temporally restricted at multiple mechanistic levels. Self-phosphorylation of IKK2 [63] and phosphorylation of NIK by IKK1 once these kinases have been activated [64] serve to downregulate their kinase activities in a protein synthesis-independent manner. The NF-κB p65:p50 dimers, after mediating gene activation, are destroyed through effects of ubiquitin ligases such as PLIM2 [65] or of proteins (e.g., MURR1/COMMD1) that coordinate the function of such ligases [66–68]. NF-κB function is also arrested by induced inhibitory proteins of the IκB family [69–74]. Moreover, accessibility of NF-κB regulated genes to the activated NF-κB proteins is decreased by induced association of these genes with dimers of the p50 or p52 NF-κB proteins that have transcriptional inhibitory effects [75,76].

Both the activation of transcription by LPS and its activation by TNF require chromatin remodeling. The tolerization by both agents to such activation is reflected in specific arrest of the remodeling of genes that mediate inflammation [77]. In the case of TNF (but not of LPS), induction of this arrest in macrophages depends on the function of the kinase GSK3 [78].

The stimulatory effect of other signaling pathways through which TNF stimulates gene activation, such as the ERK- and p38 MAP-kinase cascades, can also be suppressed by their prior exposure to TNF and LPS. This occurs, for example, by activation of the genes for phosphatases that dephosphorylate and thus reverse the activation of the kinases that participate in these pathways. Of particular importance for the emergence of endotoxin tolerance is the induction by p38-kinase of the gene encoding a phosphatase (MAP kinase phosphatase-1) that dephosphorylates this kinase, thereby inactivating it. One of the consequences of p38 activation is inhibition of the function of proteins that destabilize various short-living mRNAs, including that of TNF. Arrest of the function of p38 kinase therefore results in post-transcriptional arrest of TNF synthesis [7,79].

Genes activated by LPS or TNF are also found to be downregulated by micro-RNAs induced upon pre-exposure to these agents. These micro-RNAs associate with the transcripts encoded by these genes and enhance their degradation or block their translation [80,81]. Recent evidence indicates that certain induced

long non-coding RNAs (lncRNAs) also contribute to endotoxin tolerance. One such lncRNA, called 'Lethe', whose synthesis is enhanced by TNF, binds to the NF-κB p65/p50 dimer and thus blocks activation of the canonical NF-κB pathway [82].

Reverse signaling by cell-surface TNF provides another level of control allowing activities of the TNF-producing cells, including the expression of TNF, to be adjusted to the effectiveness of TNF function. One of the main functions of the 'reverse signals' emanating from TNF upon its binding to its receptors appears to be inhibition of TNF synthesis [4,83,84].

5.2.2. Reciprocal negative regulation of gene-activation and cell-death-induction pathways

Among the numerous functional changes induced in cells by TNF and TNF-inducing agents, the induction of cell death is exceptional not only in its functional connotation but also in the mode of its restriction. This mode of restriction was noticed before the restriction of any other function of TNF was detected. In a study of cybrids of cells with differing grades of sensitivity to TNF-induced death, cellular resistance to death was found to be a dominant feature, suggesting the existence of some 'anti TNF-induced death mechanisms' in cells [85]. Treatment of cells with inhibitors of protein synthesis has long been known to sensitize cells to TNF cytotoxicity. Conversely, their treatment with TNF or IL-1 under conditions allowing protein synthesis enhances cellular resistance to TNF-induced death [20,86,87]. These findings indicated, firstly, that TNF signals for cell death through pathway(s) that are independent of protein synthesis. Secondly, unlike the various phenomena described in Section 5.2.1, where a signaling pathway is restricted by activities and proteins operating within the same pathway, the TNF-activated cell-death pathways are restricted by other pathways. Although the cell-death pathways are themselves independent of protein synthesis, they are inhibited by TNF-activated pathways that do signal for protein induction.

Indeed, neither the function of the extrinsic apoptotic pathway nor necroptosis requires protein synthesis and both can be blocked by certain proteins synthesized as a consequence of the TNF-mediated activation of pathways that lead to NF-κB activation. Both the extrinsic cell-death pathway and necroptosis are blocked by the NF-κB-induced protein cFLIP(L) [88–90]. NF-κB-induced proteins also act to restrict the duration of JNK activation by TNF, thereby preventing the strong enhancement of TNF-induced apoptotic death by chronically activated JNK that is apparently exerted through functional arrest of various anti-death proteins [91].

An additional mechanism by which NF-κB activation restricts the extrinsic cell-death pathway is through S-nitrosylation of caspases subsequently to NF-κB-mediated induction of NO synthetase [92]. Several other anti-apoptotic mechanisms acting further downstream in the extrinsic pathway [93] are also controlled by NF-κB (Fig. 2c). Some proximal events in the TNF-induced pathway that leads to NF-κB activation are themselves inhibitory to proximal events in cell-death induction [94–96].

Conversely, caspase activation in the induction of apoptotic cell death arrests the activation of NF-κB through cleavage of signaling proteins (such as RIPK1) and generation of their inhibitory fragments [97–99] (Fig. 2d). Such reciprocal negative regulation, mediated via the inhibitory effects of proximal signaling enzymes, is also observed between the extrinsic apoptotic and the necroptotic death pathways [89,100,101].

NF-κB is activated by TNFR1 shortly after its stimulation, before TNFR1 is taken up and before the TNF-induced activation of JNK reaches the duration at which it enhances death induction. In contrast, induction of the extrinsic cell-death pathway and necroptosis are triggered only after uptake of the receptor and generation of cytosolic signaling complexes [102]. Death is therefore normally induced only when specific contextual cues arrest the anti-death mechanisms associated with the NF-κB pathway.

Induction of hepatocyte death in mice in vivo results in massive damage to the liver and consequently in death of the mice. Such death is the basis for a test that is widely used to assess the effectiveness of LPS and TNF functioning in vivo [103]. As with the death-inducing effect of TNF ex vivo, this deadly effect in vivo depends on arrest of the synthesis of NF-κB-activated genes, and as in the ex vivo effect it can be withheld by pre-exposure to TNF or IL-1 [104,105].

The relevance of the cross-regulation of gene activation and cell death induction for the control of inflammation depends on the particular mode of death induced in the particular situation. Necroptosis and chronic activation of JNK are thought to contribute to inflammation and their arrest might therefore contribute to withholding of TNF induced inflammation. Apoptotic death, on the other hand, is believed to have anti-inflammatory consequences [106], and its inhibition by pre-exposure to TNF or LPS might therefore promote rather than weaken their proinflammatory functions.

5.2.3. Induced intercellular interactions restricting TNF formation and function

Within multicellular organisms, the response of individual cells to an extracellular inducer can be modulated by products of the responses of other cells to the same inducer. A major regulatory process restricting both the formation and the function of TNF in vivo through effects of mediators generated in remote cells is the one activated by the hypothalamic–pituitary–adrenal axis [107]. TNF, like various other pro-inflammatory mediators, on reaching high enough levels in the blood triggers pituitary release of adrenocoticotropin, and this circulating hormone then triggers the formation of corticosteroids in the adrenal gland and their release from it. TNF also induces corticosteroid formation in the intestine [108]. These corticosteroids block both TNF transcription and the TNF-evoked induction of various proteins [45].

An additional molecule that restricts TNF formation in a manner that involves multicellular interactions is cyclic AMP. Once TNF is formed it stimulates prostaglandin generation through the induction of cyclooxygenase-2 and the induction and activation of phospholipase A2 (e.g. [109]). Binding of the induced prostaglandins to receptors on TNF-producing cells triggers the generation of cyclic AMP, which effectively blocks TNF transcription [110,111]. This inhibition is attenuated, however, by TNF-mediated induction of cyclic AMP phosphodiesterase [112]

6. TNF as a mediator of chronic self-perpetuating inflammation

6.1. Phenomena

The view of TNF as an immune mediator whose formation and function are always temporally restricted has been challenged by studies demonstrating constitutive generation of TNF in certain chronically inflamed tissues [113–119]. Subsequent studies in mice showed that chronic TNF generation imposed, among other ways, by mutation of a regulatory region in the TNF transcript [120] dictates pathological changes characteristic of chronic human inflammatory diseases. Those findings, and the dramatic attenuation of the activity of certain chronic inflammatory diseases in humans injected with TNF-blocking agents, pointed to a causal role for TNF in these diseases [121,122]. In many patients blocking of TNF was found to have a durable curative effect that was maintained long after the inhibition was terminated [123], implying that this causal role is preeminent.

Some auto-inflammatory diseases are caused by inborn mutations [124]. In contrast, there seems to be no such role for inborn mutations in the autoimmune diseases driven by TNF. Both the chronic formation and the chronic action of TNF in these diseases must therefore be attributable to mechanisms that are dictated by the normal genotype.

6.2. Mechanisms

Studies of the impact of chronic exposure of ex vivo cultured cells to TNF provided some clues to mechanisms that might contribute to the ability of this cytokine to exert prolonged proinflammatory effects (Figs. 1c and 2b), as detailed below.

6.2.1. Prolonged exposure to TNF induces expression of genes with longer half-life

Short TNF stimuli induce genes whose transcription is rapid and whose half-life is short, resulting in cellular changes that are swift but transient. Longer stimuli yield expression of genes that are induced slowly, owing largely to a delay in splicing [125], and whose half-life is long [126], resulting in cellular responses that evolve more slowly but are maintained for longer.

6.2.2. Protracted activation of NF-κB

Activation of NF-κB in cells treated by TNF for different durations was found to yield two distinct modes of response. Stimulation by TNF for up to a certain length of time activates NF-κB for a fixed short duration. With longer stimulation times this response is prolonged, and is maintained throughout the period of exposure to TNF [127].

The shift from transient to chronic activation is assisted by redundancy at several mechanistic levels. There is redundancy of the proximal mechanisms for NF-κB activation, where different phases in the process benefit from linkages of branched and of linear polyubiquitin chains, from phosphorylation of TFAF2, and from RIPK1-dependent and RIPK1-independent activation of NF-κB [128,129].

There is also redundancy in the kinds of NF-κB-induced proteins that bind to the NF-κB dimers and block their functions. Phosphorylation of the NF-κB inhibitor IκBα by IKK2 is followed by NF-κB-mediated activation of the IκBα gene, constituting a delayed type of feedback that yields an oscillating pattern of NF-κB activation [130,131]. This oscillation is dampened by the more slowly induced IκBβ and IκBε [130]. NF-κB activation also yields generation of the NF-κB proteins p100 and p105, which contain IκB-like ankyrin repeat moieties and can therefore also block the function of the canonical NF-κB complex [69–74]. However, one of the genes activated by TNFR1 is the other, less frequently expressed TNF receptor, TNFR2 [132]. Triggering of TNFR2 by chronically applied TNF can activate the alternative NF-κB pathway, in which proteolytic processing of p100 and p105 [73,74] can relieve inhibition of the canonical pathway by these proteins. Activation of the alternative NF-κB pathway also yields RelB:p52 NF-κB dimers, which have been shown to replace the canonical p65:p50 complexes at the promoters of various TNF-induced genes in cells exposed to TNF for lengthy periods [133]. This late shift to NF-κB dimers that are insensitive to the effects of NF-κB-induced IκB proteins and of other inhibitors of the canonical NF-κB pathway contributes to the chronicity of the inflammatory response.

6.2.3. Autocrine IFN-β signaling

Unlike TLR4 and some other pathogen-component receptors whose signaling initiates phosphorylation of the transcription factor IRF3 and, as a consequence, activation of the interferon beta (IFN-β) gene, the TNF receptors do not signal for IRF3 phosphorylation and therefore cannot activate the IFN-β gene directly. Nevertheless, prolonged TNF stimulation does result in low-level induction of IFN-β [134], probably as an outcome of its induction of IRF1, another member of the IRF family that can activate the interferon gene [135,136].

TNF induces epigenetic changes that result in increased accessibility of the promoters of various inflammatory genes to transcription factors [137]. In cells exposed over a long period to TNF this increased accessibility is maintained for a while after TNF removal, reflecting sustained gene remodeling that results in prolonged gene activation and 'innate memory' [138]. Some of the genes manifesting such sustained activation are controlled by the cooperative action of TNF-induced NF-κB and IFN-β-induced Stat1 [139]. This autocrine function of IFN-β in cells chronically exposed to TNF probably accounts for the fact that many of the genes induced in chronic inflammatory states to which TNF contributes are characteristic of the interferon response [140].

6.2.4. Soluble TNF receptors: inhibitors or buffers?

When TNF receptors are shed in response to TNF or other inducers, their cleavage occurs at the region that links their ligand-binding motif to their transmembrane domains [141]. Such cleavage thus results in the release of shed portions of the receptors that maintain the ability to bind TNF. Substantial amounts of these 'soluble TNF receptors' accumulate at regions of chronic inflammation [119,142]. When bound to these soluble receptors, TNF is incapable of binding to the TNF receptors at the cell surface [143]. Initially, therefore, the shed soluble TNF receptors were thought to serve as natural inhibitors of TNF function. More thorough characterization revealed that binding of these soluble receptors to TNF results not in irreversible inactivation of TNF but, quite the contrary, in stabilization of the trimeric (active) form of TNF and, since the binding is reversible, the soluble receptors function rather as 'buffering agents' that modify the TNF effect from an abrupt spike of strong stimulation to a mitigated but extended effect [144]. The soluble TNF receptors probably also increase local concentrations of TNF in closed body cavities by withholding its translocation through their surrounding barriers.

6.2.5. What dictates the continuous generation of TNF in chronic inflammatory diseases?

Unlike infectious diseases, in which inflammation is driven by pathogen components, the inflammatory processes in autoimmune diseases are believed to be perpetuated merely by interactions among various host cells, and to occur through direct cell–cell interactions and the various soluble mediators that these cells generate [145]. TNF-blocking agents can dictate sustained arrest of both the effects and the formation of TNF in patients with such diseases [123], implying that TNF generation in these diseases is self-perpetuating. Self-perpetuation could occur through direct TNF-mediated activation of the TNF gene. However, although the TNF gene is indeed activated by TNF, this activation seems too transient to account for the unabated generation of TNF observed in autoimmune diseases [126]. Other host-derived molecules, whose nature is still unknown, are likely to trigger this self-induced generation. They seem to be produced, at least in part, by T lymphocytes and NK cells, and to be presented to TNF-producing mononuclear phagocytes through direct cell–cell contact [146–149].

6.3. Open questions

The apparent existence of molecular mechanisms that serve specifically to allow TNF to act chronically suggests that this mode of TNF function, despite its known pathological consequences, also contributes beneficially to immune defense. There are still large gaps in our knowledge of these mechanisms. Little has been done to explore the interrelationships between this mode of TNF action and

the two others described in this essay, namely the hypersensitive unrestricted response, and the restricted response characterizing endotoxin tolerance. In mesenchymal cells, whose activation by TNF appears to be pivotal for the initiation of chronic inflammatory diseases to which TNF contributes [150], the induction of feedback mechanisms that restrict inflammatory responses seems to occur less effectively than in macrophages [137]. Still to be clarified are the extent of such heterogeneity among different cell types, the identity of the mechanisms that contribute to the heterogeneity, and the extent to which these mechanisms can be modulated by external stimuli.

7. Concluding remarks

As in Akira Kurosawa's famous film *Rashomon*, so too in the study of the highly pleiotropic function of TNF the notion of reality lies in the eye of the beholder. Scientists tend to focus on the feature of TNF function that is highlighted by the particular TNF activity that they study. Each of the three modes of regulation of TNF function addressed in this essay has in turn become the focus of attention at different historical phases of the research on this cytokine. Despite differences in the levels of attention paid to them at different times, all are likely to make important contributions both to immune defense and to immune pathology. Moreover, given the great heterogeneity of the ways in which inflammation contributes to defense, and the multiple means by which TNF affects cellular functions in inflammation, future studies are likely to reveal additional ways in which the nature, extent and duration of TNF function are controlled. The complexity of the subject is continuing to unfold and substantial effort will be required over the coming years to address the open questions. In view of the key role of the regulation of TNF function in health and especially in disease, investment of such further effort is unquestionably worthwhile.

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References

[1] Bradley JR. TNF-mediated inflammatory disease. J. Pathol 2008;214:149–60.
[2] Kriegler M, Perez C, DeFay K, Albert I, Lu SD. A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. Cell 1988;53:45–53.
[3] Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, Nelson N, Boiani N, Schooley KA, Gerhart M, Davis R, Fitzner JN, Johnson RS, Paxton RJ, March CJ, Cerretti DP. A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. Nature 1997;385:729–33.
[4] Eissner G, Kolch W, Scheurich P. Ligands working as receptors: reverse signaling by members of the TNF superfamily enhance the plasticity of the immune system. Cytokine Growth Factor Rev 2004;15:353–66.
[5] Wajant H, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling. Cell Death Differ 2003;10:45–65.
[6] Akira S, Takeda K. Toll-like receptor signalling. Nat. Rev. Immunol 2004;4:499–511.
[7] Sabio G, Davis RJ. TNF and MAP kinase signalling pathways. Semin. Immunol 2014;26:237–45.
[8] Ashkenazi A, Salvesen G. Regulated cell death: signaling and mechanisms. Ann. Rev. Cell Dev. Biol 2014;30:337–56.
[9] Hayden MS, Ghosh S. NF-kappaB, the first quarter-century: remarkable progress and outstanding questions. Genes Dev 2012;26:203–34.
[10] Domonkos A, Udvardy A, Laszlo L, Nagy T, Duda E. Receptor-like properties of the 26 kDa transmembrane form of TNF. Eur Cytokine Netw 2001;12:411–9.
[11] Friedmann E, Hauben E, Maylandt K, Schleeger S, Vreugde S, Lichtenthaler SF, Kuhn PH, Stauffer D, Rovelli G, Martoglio B. SPPL2a and SPPL2b promote intramembrane proteolysis of TNFalpha in activated dendritic cells to trigger IL-12 production. Nat. Cell Biol 2006;8:843–8.
[12] Rook GA, Stanford JL. The Koch phenomenon and the immunopathology of tuberculosis. Curr. Top Microbiol. Immunol 1996;215:239–62.
[13] Brozna JP. Shwartzman reaction. Semin. Thromb. Hemost 1990;16:326–32.
[14] Granger GA, Kolb WP. Lymphocyte in vitro cytotoxicity: mechanisms of immune and non-immune small lymphocyte mediated target L cell destruction. J. Immunol 1968;101:111–20.
[15] Ruddle NH, Waksman BH. Cytotoxicity mediated by soluble antigen and lymphocytes in delayed hypersensitivity. III. Analysis of mechanisms. J. Exp. Med 1968;128:1267–79.
[16] Carswell EA, Old LJ, Fiore N, Schwartz MK. An endotoxin-induced serum factor that causes necrosis of tumors. Proc. Natl. Acad. Sci. U. S. A 1975;72:3666–70.
[17] Kawai T, Satomi N, Sato N, Sakurai A, Haranaka K, Goto T, Suzuki M. Necrotizing activity of tumor necrosis factor: histopathological investigation using Meth A sarcoma and granulation tissue. Virchows Arch. B: Cell Pathol. Incl. Mol. Pathol 1987;53:353–8.
[18] Ruegg C, Yilmaz A, Bieler G, Bamat J, Chaubert P, Lejeune FJ. Evidence for the involvement of endothelial cell integrin alphaVbeta3 in the disruption of the tumor vasculature induced by TNF and IFN-gamma. Nat. Med 1998;4:408–14.
[19] Tracey KJ, Beutler B, Lowry SF, Merryweather J, Wolpe S, Milsark IW, Hariri RJ, Fahey TJ, Zentella A, Albert JD, Shires GT, Cerami A. Shock and tissue injury induced by recombinant human cachectin. Science 1986;234:470–4.
[20] Hahn T, Toker L, Budilovsky S, Aderka D, Eshhar Z, Wallach D. Use of monoclonal antibodies to a human cytotoxin for its isolation and for examining the self-induction of resistance to this protein. Proc. Natl. Acad. Sci. U. S. A 1985;82:3814–8.
[21] Saklatvala J. Tumour necrosis factor alpha stimulates resorption and inhibits synthesis of proteoglycan in cartilage. Nature 1986;322:547–9.
[22] Bertolini DR, Nedwin GE, Bringman TS, Smith DD, Mundy GR. Stimulation of bone resorption and inhibition of bone formation in vitro by human tumor necrosis factors. Nature 1986;319:516–8.
[23] van der Poll T, Buller HR, ten Cate H, Wortel CH, Bauer KA, van Deventer SJ, Hack CE, Sauerwein HP, Rosenberg RD, ten Cate JW. Activation of coagulation after administration of tumor necrosis factor to normal subjects. N. Engl. J. Med 1990;322:1622–7.
[24] Spriggs DR, Jaffe HS. Pathophysiologic effects of tumor necrosis factor therapy on cancer patients. In: Oppenheim JJ, Rossio JL, Gearing AJH, editors. Clinical Applications of Cytokines: Role in Pathogenesis, Diagnosis and Therapy. Oxford: Oxford University Press; 1993. p. 165–70.
[25] Couriel DR, Saliba R, de Lima M, Giralt S, Andersson B, Khouri I, Hosing C, Ippoliti C, Shpall EJ, Champlin R, Alousi A. A phase III study of infliximab and corticosteroids for the initial treatment of acute graft-versus-host disease. Biol. Blood Marrow Transplant 2009;15:1555–62.
[26] Kamijo R, Le J, Shapiro D, Havell EA, Huang S, Aguet M, Bosland M, Vilcek J. Mice that lack the interferon-gamma receptor have profoundly altered responses to infection with Bacillus Calmette–Guerin and subsequent challenge with lipopolysaccharide. J. Exp. Med 1993;178:1435–40.
[27] Wysocka M, Kubin M, Vieira LQ, Ozmen L, Garotta G, Scott P, Trinchieri G. Interleukin-12 is required for interferon-gamma production and lethality in lipopolysaccharide-induced shock in mice. Eur. J. Immunol 1995;25:672–6.
[28] Movat HZ, Burrowes CE, Cybulsky MI, Dinarello CA. Acute inflammation and a Shwartzman-like reaction induced by interleukin-1 and tumor necrosis factor Synergistic action of the cytokines in the induction of inflammation and microvascular injury. Am. J. Pathol 1987;129:463–76.
[29] Rothstein JL, Schreiber H. Synergy between tumor necrosis factor and bacterial products causes hemorrhagic necrosis and lethal shock in normal mice. Proc. Natl. Acad. Sci. U. S. A 1988;85:607–11.
[30] Hayes MP, Zoon KC. Priming of human monocytes for enhanced lipopolysaccharide responses: expression of alpha interferon, interferon regulatory factors, and tumor necrosis factor. Infect. Immun 1993;61:3222–7.
[31] Ohmori Y, Schreiber RD, Hamilton TA. Synergy between interferon-gamma and tumor necrosis factor-alpha in transcriptional activation is mediated by cooperation between signal transducer and activator of transcription 1 and nuclear factor kappaB. J. Biol. Chem 1997;272:14899–907.
[32] Schroder K, Sweet MJ, Hume DA. Signal integration between IFNgamma and TLR signalling pathways in macrophages. Immunobiology 2006;211:511–24.
[33] Israel S, Hahn T, Holtmann H, Wallach D. Binding of human TNF-alpha to high-affinity cell surface receptors: effect of IFN. Immunol. Lett 1986;12:217–24.
[34] Chen J, Ivashkiv LB. IFN-gamma abrogates endotoxin tolerance by facilitating Toll-like receptor-induced chromatin remodeling. Proc. Natl. Acad. Sci. U. S. A 2010;107:19438–43.
[35] Rothstein JL, Lint TF, Schreiber H. Tumor necrosis factor/cachectin induction of hemorrhagic necrosis in normal tissue requires the fifth component of complement (C5). J. Exp. Med 1988;168:2007–21.
[36] Van de Wiel PA, Bloksma N, Kuper CF, Hofhuis FM, Willers JM. Macroscopic and microscopic early effects of tumour necrosis factor on murine Meth A sarcoma, and relation to curative activity. J. Pathol 1989;157:65–73.
[37] Renard N, Lienard D, Lespagnard L, Eggermont A, Heimann R, Lejeune F. Early endothelium activation and polymorphonuclear cell invasion precede specific necrosis of human melanoma and sarcoma treated by intravascular high-dose tumour necrosis factor alpha (rTNF alpha). Int. J. Cancer 1994;57:656–63.
[38] Noon L, Cantab BC. Prophylactic inoculation against hay fever. Lancet 1911:1572–3.
[39] Freeman J, Oxon MD. Further observations on the treatment of hay fever by hypodermic inoculations of pollen vaccine. Lancet 1911:814–7.

[40] Biswas SK, Lopez-Collazo E. Endotoxin tolerance: new mechanisms, molecules and clinical significance. Trends Immunol 2009;30:475–87.
[41] Beutler B, Milsark IW, Cerami AC. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. Science 1985;229:869–71.
[42] Michie HR, Spriggs DR, Manogue KR, Sherman ML, Revhaug A, O'Dwyer ST, Arthur K, Dinarello CA, Cerami A, Wolff SM, et al. Tumor necrosis factor and endotoxin induce similar metabolic responses in human beings. Surgery 1988;104:280–6.
[43] Takasuka N, Tokunaga T, Akagawa KS. Preexposure of macrophages to low doses of lipopolysaccharide inhibits the expression of tumor necrosis factor-alpha mRNA but not of IL-1 beta mRNA. J. Immunol 1991;146:3824–30.
[44] Zuckerman SH, Evans GF, Butler LD. Endotoxin tolerance: independent regulation of interleukin-1 and tumor necrosis factor expression. Infect. Immun 1991;59:2774–80.
[45] Lehner MD, Hartung T. Endotoxin tolerance-mechanisms and beneficial effects in bacterial infection. Rev. Physiol. Biochem. Pharmacol 2002;144:95–141.
[46] Colletti LM, Remick DG, Campbell Jr DA. LPS pretreatment protects from hepatic ischemia/reperfusion. J. Surg. Res 1994;57:337–43.
[47] Pradillo JM, Romera C, Hurtado O, Cardenas A, Moro MA, Leza JC, Davalos A, Castillo J, Lorenzo P, Lizasoain I. TNFR1 upregulation mediates tolerance after brain ischemic preconditioning. J. Cereb. Blood Flow Metab 2005;25:193–203.
[48] Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. Nat. Med 2011;17:796–808.
[49] Watanabe N, Tsuji N, Kobayashi D, Yamauchi N, Akiyama S, Sasaki H, Sato T, Okamoto T, Niitsu Y. Endogenous tumor necrosis factor functions as a resistant factor against hyperthermic cytotoxicity in pancreatic carcinoma cells via enhancement of the heart shock element-binding activity of heart shock factor 1. Chemotherapy 1997;43:406–14.
[50] Brown J, Wang H, Hajishengallis GN, Martin M. TLR-signaling networks: an integration of adaptor molecules, kinases, and cross-talk. J. Dent. Res 2011;90:417–27.
[51] Kondo T, Kawai T, Akira S. Dissecting negative regulation of toll-like receptor signaling. Trends Immunol 2012;33:449–58.
[52] Ruland J. Return to homeostasis: downregulation of NF-kappaB responses. Nat. Immunol 2011;12:709–14.
[53] Clark K. Protein kinase networks that limit TLR signalling. Biochem. Soc. Trans 2014;42:11–24.
[54] Tsujimoto M, Yip YK, Vilcek J. Tumor necrosis factor: specific binding and internalization in sensitive and resistant cells. Proc. Natl. Acad. Sci. U. S. A 1985;82:7626–30.
[55] Lanz M, Malik S, Slevin ML, Olsson I. Infusion of tumor necrosis factor causes an increase in circulating TNF-binding proteins in humans. Cytokines 1992;2:402–6.
[56] Higuchi M, Aggarwal BB. TNF induces internalization of the p60 receptor and shedding of the p80 receptor. J. Immunol 1994;152:3550–8.
[57] Aderka D, Sorkine P, Abu-Abid S, Lev D, Setton A, Cope AP, Wallach D, Klausner J. Shedding kinetics of soluble tumor necrosis factor (TNF) receptors after systemic TNF leaking during isolated limb perfusion relevance to the pathophysiology of septic shock. J. Clin. Investig 1998;101:650–9.
[58] Nomura F, Akashi S, Sakao Y, Sato S, Kawai T, Matsumoto M, Nakanishi K, Kimoto M, Miyake K, Takeda K, Akira S. Cutting edge: endotoxin tolerance in mouse peritoneal macrophages correlates with down-regulation of surface toll-like receptor 4 expression. J. Immunol 2000;164:3476–9.
[59] Zager RA, Johnson AC, Lund S, Randolph-Habecker J. Toll-like receptor (TLR4) shedding and depletion: acute proximal tubular cell responses to hypoxic and toxic injury. Am. J. Physiol. Renal. Physiol 2007;292: F304–12.
[60] Xanthoulea S, Pasparakis M, Kousteni S, Brakebusch C, Wallach D, Bauer J, Lassmann H, Kollias G. Tumor necrosis factor (TNF) receptor shedding controls thresholds of innate immune activation that balance opposing TNF functions in infectious and inflammatory diseases. J. Exp. Med 2004;200:367–76.
[61] Iwai K. Diverse ubiquitin signaling in NF-kappaB activation. Trends Cell. Biol 2012;22:355–64.
[62] Jiang Y, Woronicz JD, Liu W, Goeddel DV. Prevention of constitutive TNF receptor 1 signaling by silencer of death domains. Science 1999;283:543–6.
[63] Delhase M, Hayakawa M, Chen Y, Karin M. Positive and negative regulation of IkappaB kinase activity through IKKbeta subunit phosphorylation. Science 1999;284:309–13.
[64] Razani B, Zarnegar B, Ytterberg AJ, Shiba T, Dempsey PW, Ware CF, Loo JA, Cheng G. Negative feedback in noncanonical NF-kappaB signaling modulates NIK stability through IKKalpha-mediated phosphorylation. Sci. Signal 2010;3:ra41.
[65] Tanaka T, Grusby MJ, Kaisho T. PDLIM2-mediated termination of transcription factor NF-kappaB activation by intranuclear sequestration and degradation of the p65 subunit. Nat. Immunol 2007;8:584–91.
[66] Ganesh L, Burstein E, Guha-Niyogi A, Louder MK, Mascola JR, Klomp LW, Wijmenga C, Duckett CS, Nabel GJ. The gene product Murr1 restricts HIV-1 replication in resting CD4+ lymphocytes. Nature 2003;426:853–7.
[67] Burstein E, Hoberg JE, Wilkinson AS, Rumble JM, Csomos RA, Komarck CM, Maine GN, Wilkinson JC, Mayo MW, Duckett CS. COMMD proteins, a novel family of structural and functional homologs of MURR1. J. Biol. Chem 2005;280:22222–32.
[68] Geng H, Wittwer T, Dittrich-Breiholz O, Kracht M, Schmitz ML. Phosphorylation of NF-kappaB p65 at Ser468 controls its COMMD1-dependent ubiquitination and target gene-specific proteasomal elimination. EMBO Rep 2009;10:381–6.
[69] Rice NR, MacKichan ML, Israel A. The precursor of NF-kappa B p50 has I kappa B-like functions. Cell 1992;71:243–53.
[70] Naumann M, Nieters A, Hatada EN, Scheidereit C. NF-kappa B precursor p100 inhibits nuclear translocation and DNA binding of NF-kappa B/rel-factors. Oncogene 1993;8:2275–81.
[71] Basak S, Kim H, Kearns JD, Tergaonkar V, O'Dea E, Werner SL, Benedict CA, Ware CF, Ghosh G, Verma IM, Hoffmann A. A fourth IkappaB protein within the NF-kappaB signaling module. Cell 2007;128:369–81.
[72] Savinova OV, Hoffmann A, Ghosh G. The Nfkb1 and Nfkb2 proteins p105 and p100 function as the core of high-molecular-weight heterogeneous complexes. Mol. Cell 2009;34:591–602.
[73] Yilmaz ZB, Kofahl B, Beaudette P, Baum K, Ipenberg I, Weih F, Wolf J, Dittmar G, Scheidereit C. Quantitative dissection and modeling of the NF-kappaB p100-p105 module reveals interdependent precursor proteolysis. Cell Rep 2014;9:1756–69.
[74] Banoth B, Chatterjee B, Vijayaragavan B, Prasad MV, Roy P, Basak S. Stimulus-selective crosstalk via the NF-kappaB signaling system reinforces innate immune response to alleviate gut infection. Elife 2015:4.
[75] Kastenbauer S, Ziegler-Heitbrock HW. NF-kappaB1 (p50) is upregulated in lipopolysaccharide tolerance and can block tumor necrosis factor gene expression. Infect. Immun 1999;67:1553–9.
[76] Wedel A, Frankenberger M, Sulski G, Petersmann I, Kuprash D, Nedospasov S, Ziegler-Heitbrock HW. Role of p52 (NF-kappaB2) in LPS tolerance in a human B cell line. Biol. Chem 1999;380:1193–9.
[77] Foster SL, Hargreaves DC, Medzhitov R. Gene-specific control of inflammation by TLR-induced chromatin modifications. Nature 2007;447:972–8.
[78] Park SH, Park-Min KH, Chen J, Hu X, Ivashkiv LB. Tumor necrosis factor induces GSK3 kinase-mediated cross-tolerance to endotoxin in macrophages. Nat. Immunol 2011;12:607–15.
[79] Li L, Chen SF, Liu Y. MAP kinase phosphatase-1, a critical negative regulator of the innate immune response. Int. J. Clin. Exp. Med 2009;2:48–67.
[80] Suarez Y, Wang C, Manes TD, Pober JS. Cutting edge: TNF-induced microRNAs regulate TNF-induced expression of E-selectin and intercellular adhesion molecule-1 on human endothelial cells: feedback control of inflammation. J. Immunol 2010;184:21–5.
[81] Nahid MA, Satoh M, Chan EK. MicroRNA in TLR signaling and endotoxin tolerance. Cell. Mol. Immunol 2011;8:388–403.
[82] Rapicavoli NA, Qu K, Zhang J, Mikhail M, Laberge RM, Chang HY. A mammalian pseudogene lncRNA at the interface of inflammation and anti-inflammatory therapeutics. Elife 2013;2:e00762.
[83] Tanabe Y, Kohchi C, Kitahara-Tanabe N, Mizuno D, Soma G. Involvement of 26-kDa membrane-bound tumour necrosis factor precursor in bidirectional feedback regulation on 17-kDa tumour necrosis factor production after stimulation by lipopolysaccharide. Cytokine 1998;10:82–92.
[84] Sipos O, Török A, Kalic T, Duda E, Filkor K. Reverse signaling contributes to control of chronic inflammation by anti-TNF therapeutics. Antibodies 2015;4:123–40.
[85] Nophar Y, Holtmann H, Ber R, Wallach D. Dominance of resistance to the cytocidal effect of tumor necrosis factor in heterokaryons formed by fusion of resistant and sensitive cells. J. Immunol 1988;140:3456–60.
[86] Wallach D. Preparations of lymphotoxin induce resistance to their own cytotoxic effect. J. Immunol 1984;132:2464–9.
[87] Holtmann H, Hahn T, Wallach D. Interrelated effects of tumor necrosis factor and interleukin 1 on cell viability. Immunobiology 1988;177:7–22.
[88] Wallach D, Varfolomeev EE, Malinin NL, Goltsev YV, Kovalenko AV, Boldin MP. Tumor necrosis factor receptor and Fas signaling mechanisms. Ann. Rev. Immunol 1999;17:331–67.
[89] Oberst A, Dillon CP, Weinlich R, McCormick LL, Fitzgerald P, Pop C, Hakem R, Salvesen GS, Green DR. Catalytic activity of the caspase-8-FLIP(L) complex inhibits RIPK3-dependent necrosis. Nature 2011;471:363–7.
[90] Feoktistova M, Geserick P, Kellert B, Dimitrova DP, Langlais C, Hupe M, Cain K, Macfarlane M, Hacker G, Leverkus M. cIAPs block ripoptosome formation, a RIP1/caspase-8 containing intracellular cell death complex differentially regulated by cFLIP isoforms. Mol. Cell 2011.
[91] Liu J, Lin A. Role of JNK activation in apoptosis: a double-edged sword. Cell Res 2005;15:36–42.
[92] Li J, Bombeck CA, Yang S, Kim YM, Billiar TR. Nitric oxide suppresses apoptosis via interrupting caspase activation and mitochondrial dysfunction in cultured hepatocytes. J. Biol. Chem 1999;274:17325–33.
[93] Wallach D, Kovalenko AV, Varfolomeev EE, Boldin MP. Death-inducing functions of ligands of the tumor necrosis factor family: a Sanhedrin verdict. Curr. Opin. Immunol 1998;10:279–88.
[94] Mannick JB, Hausladen A, Liu L, Hess DT, Zeng M, Miao QX, Kane LS, Gow AJ, Stamler JS. Fas-induced caspase denitrosylation. Science 1999;284:651–4.
[95] O'Donnell MA, Legarda-Addison D, Skountzos P, Yeh WC, Ting AT. Ubiquitination of RIP1 regulates an NF-kappaB-independent cell-death switch in TNF signaling. Curr. Biol 2007;17:418–24.
[96] Hatano E, Bennett BL, Manning AM, Qian T, Lemasters JJ, Brenner DA. NF-kappaB stimulates inducible nitric oxide synthase to protect mouse

hepatocytes from TNF-alpha- and Fas-mediated apoptosis. Gastroenterology 2001;120:1251–62.
[97] Lin Y, Devin A, Rodriguez Y, Liu ZG. Cleavage of the death domain kinase RIP by caspase-8 prompts TNF-induced apoptosis. Genes Dev 1999;13:2514–26.
[98] Henkler F, Baumann B, Fotin-Mleczek M, Weingartner M, Schwenzer R, Peters N, Graness A, Wirth T, Scheurich P, Schmid JA, Wajant H. Caspase-mediated cleavage converts the tumor necrosis factor (TNF) receptor-associated factor (TRAF)-1 from a selective modulator of TNF receptor signaling to a general inhibitor of NF-kappaB activation. J. Biol. Chem 2003;278:29216–30.
[99] Kim HS, Chang I, Kim JY, Choi KH, Lee MS. Caspase-mediated p65 cleavage promotes TRAIL-induced apoptosis. Cancer Res 2005;65:6111–9.
[100] Holler N, Zaru R, Micheau O, Thome M, Attinger A, Valitutti S, Bodmer JL, Schneider P, Seed B, Tschopp J. Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. Nat. Immunol 2000;1:489–95.
[101] Newton K, Dugger DL, Wickliffe KE, Kapoor N, de Almagro MC, Vucic D, Komuves L, Ferrando RE, French DM, Webster J, Roose-Girma M, Warming S, Dixit VM. Activity of protein kinase RIPK3 determines whether cells die by necroptosis or apoptosis. Science 2014;343:1357–60.
[102] Micheau O, Tschopp J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. Cell 2003;114:181–90.
[103] Galanos C, Freudenberg MA, Reutter W. Galactosamine-induced sensitization to the lethal effects of endotoxin. Proc. Natl. Acad. Sci. U. S. A 1979;76:5939–43.
[104] Wallach D, Holtmann H, Engelmann H, Nophar Y. Sensitization and desensitization to lethal effects of tumor necrosis factor and IL-1. J. Immunol 1988;140:2994–9.
[105] Freudenberg MA, Galanos C. Induction of tolerance to lipopolysaccharide (LPS)-d-galactosamine lethality by pretreatment with LPS is mediated by macrophages. Infect. Immun 1988;56:1352–7.
[106] Wallach D, Kang TB, Kovalenko A. Concepts of tissue injury and cell death in inflammation: a historical perspective. Nat. Rev. Immunol 2014;14:51–9.
[107] Turnbull AV, Rivier CL. Regulation of the hypothalamic–pituitary–adrenal axis by cytokines: actions and mechanisms of action. Physiol. Rev 1999;79:1–71.
[108] Noti M, Corazza N, Mueller C, Berger B, Brunner T. TNF suppresses acute intestinal inflammation by inducing local glucocorticoid synthesis. J. Exp. Med 2010;207:1057–66.
[109] Fournier T, Fadok V, Henson PM. Tumor necrosis factor-alpha inversely regulates prostaglandin D2 and prostaglandin E2 production in murine macrophages Synergistic action of cyclic AMP on cyclooxygenase-2 expression and prostaglandin E2 synthesis. J. Biol. Chem 1997;272:31065–72.
[110] Kunkel SL, Spengler M, May MA, Spengler R, Larrick J, Remick D. Prostaglandin E2 regulates macrophage-derived tumor necrosis factor gene expression. J. Biol. Chem 1988;263:5380–4.
[111] Renz H, Gong JH, Schmidt A, Nain M, Gemsa D. Release of tumor necrosis factor-alpha from macrophages. Enhancement and suppression are dose-dependently regulated by prostaglandin E2 and cyclic nucleotides. J. Immunol 1988;141:2388–93.
[112] Kast RE. Tumor necrosis factor has positive and negative self regulatory feed back cycles centered around cAMP. Int. J. Immunopharmacol 2000;22:1001–6.
[113] Di Giovine FS, Nuki G, Duff GW. Tumour necrosis factor in synovial exudates. Ann. Rheum. Dis 1988;47:768–72.
[114] Saxne T, Palladino Jr MA, Heinegard D, Talal N, Wollheim FA. Detection of tumor necrosis factor alpha but not tumor necrosis factor beta in rheumatoid arthritis synovial fluid and serum. Arthritis Rheum 1988;31:1041–5.
[115] Firestein GS, Alvaro-Gracia JM, Maki R. Quantitative analysis of cytokine gene expression in rheumatoid arthritis. J. Immunol 1990;144:3347–53.
[116] Cappello M, Keshav S, Prince C, Jewell DP, Gordon S. Detection of mRNAs for macrophage products in inflammatory bowel disease by in situ hybridisation. Gut 1992;33:1214–9.
[117] Braegger CP, Nicholls S, Murch SH, Stephens S, MacDonald TT. Tumour necrosis factor alpha in stool as a marker of intestinal inflammation. Lancet 1992;339:89–91.
[118] Murch SH, Braegger CP, Walker-Smith JA, MacDonald TT. Location of tumour necrosis factor alpha by immunohistochemistry in chronic inflammatory bowel disease. Gut 1993;34:1705–9.
[119] Ettehadi P, Greaves MW, Wallach D, Aderka D, Camp RD. Elevated tumour necrosis factor-alpha (TNF-alpha) biological activity in psoriatic skin lesions. Clin. Exp. Immunol 1994;96:146–51.
[120] Kawane K, Tanaka H, Kitahara Y, Shimaoka S, Nagata S. Cytokine-dependent but acquired immunity-independent arthritis caused by DNA escaped from degradation. Proc. Natl. Acad. Sci. U. S. A 2010;107:19432–7.
[121] van Dullemen HM, van Deventer SJ, Hommes DW, Bijl HA, Jansen J, Tytgat GN, Woody J. Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). Gastroenterology 1995;109:129–35.
[122] Sfikakis PP. The first decade of biologic TNF antagonists in clinical practice: lessons learned, unresolved issues and future directions. Curr. Dir. Autoimmun 2010;11:180–210.
[123] Ulfgren AK, Andersson U, Engstrom M, Klareskog L, Maini RN, Taylor PC. Systemic anti-tumor necrosis factor alpha therapy in rheumatoid arthritis down-regulates synovial tumor necrosis factor alpha synthesis. Arthritis Rheum 2000;43:2391–6.
[124] Brydges S, Kastner DL. The systemic autoinflammatory diseases: inborn errors of the innate immune system. Curr. Top. Microbiol. Immunol 2006;305:127–60.
[125] Hao S, Baltimore D. RNA splicing regulates the temporal order of TNF-induced gene expression. Proc. Natl. Acad. Sci. U. S. A 2013;110:11934–9.
[126] Hao S, Baltimore D. The stability of mRNA influences the temporal order of the induction of genes encoding inflammatory molecules. Nat. Immunol 2009;10:281–8.
[127] Poppers DM, Schwenger P, Vilcek J. Persistent tumor necrosis factor signaling in normal human fibroblasts prevents the complete resynthesis of I kappa B-alpha. J. Biol. Chem 2000;275:29587–93.
[128] Workman LM, Habelhah H. TNFR1 signaling kinetics: spatiotemporal control of three phases of IKK activation by posttranslational modification. Cell. Signal 2013;25:1654–64.
[129] Walczak H, Iwai K, Dikic I. Generation and physiological roles of linear ubiquitin chains. BMC Biol 2012;10:23.
[130] Hoffmann A, Levchenko A, Scott ML, Baltimore D. The IkappaB-NF-kappaB signaling module: temporal control and selective gene activation. Science 2002;298:1241–5.
[131] Nelson DE, Ihekwaba AE, Elliott M, Johnson JR, Gibney CA, Foreman BE, Nelson G, See V, Horton CA, Spiller DG, Edwards SW, McDowell HP, Unitt JF, Sullivan E, Grimley R, Benson N, Broomhead D, Kell DB, White MR. Oscillations in NF-kappaB signaling control the dynamics of gene expression. Science 2004;306:704–8.
[132] Winzen R, Wallach D, Kemper O, Resch K, Holtmann H. Selective up-regulation of the 75-kDa tumor necrosis factor (TNF) receptor and its mRNA by TNF and IL-1. J. Immunol 1993;150:4346–53.
[133] Saccani S, Pantano S, Natoli G. Modulation of NF-kappaB activity by exchange of dimers. Mol. Cell 2003;11:1563–74.
[134] Jacobsen H, Mestan J, Mittnacht S, Dieffenbach CW. Beta interferon subtype 1 induction by tumor necrosis factor. Mol. Cell. Biol 1989;9:3037–42.
[135] Yarilina A, Park-Min KH, Antoniv T, Hu X, Ivashkiv LB. TNF activates an IRF1-dependent autocrine loop leading to sustained expression of chemokines and STAT1-dependent type I interferon-response genes. Nat. Immunol 2008;9:378–87.
[136] Yarilina A, Ivashkiv LB. Type I interferon: a new player in TNF signaling. Curr. Dir. Autoimmun 2010;11:94–104.
[137] Lee A, Qiao Y, Grigoriev G, Chen J, Park-Min KH, Park SH, Ivashkiv LB, Kalliolias GD. Tumor necrosis factor alpha induces sustained signaling and a prolonged and unremitting inflammatory response in rheumatoid arthritis synovial fibroblasts. Arthritis Rheum 2013;65:928–38.
[138] Monticelli S, Natoli G. Short-term memory of danger signals and environmental stimuli in immune cells. Nat. Immunol 2013;14:777–84.
[139] Sohn C, Lee A, Qiao Y, Loupasakis K, Ivashkiv LB, Kalliolias GD. Prolonged tumor necrosis factor alpha primes fibroblast-like synoviocytes in a gene-specific manner by altering chromatin. Arthritis Rheumatol 2015;67:86–95.
[140] van der Pouw Kraan TC, van Gaalen FA, Kasperkovitz PV, Verbeet NL, Smeets TJ, Kraan MC, Fero M, Tak PP, Huizinga TW, Pieterman E, Breedveld FC, Alizadeh AA, Verweij CL. Rheumatoid arthritis is a heterogeneous disease: evidence for differences in the activation of the STAT-1 pathway between rheumatoid tissues. Arthritis Rheum 2003;48:2132–45.
[141] Brakebusch C, Varfolomeev EE, Batkin M, Wallach D. Structural requirements for inducible shedding of the p55 tumor necrosis factor receptor. J. Biol. Chem 1994;269:32488–96.
[142] Cope AP, Aderka D, Doherty M, Engelmann H, Gibbons D, Jones AC, Brennan FM, Maini RN, Wallach D, Feldmann M. Increased levels of soluble tumor necrosis factor receptors in the sera and synovial fluid of patients with rheumatic diseases. Arthritis Rheum 1992;35:1160–9.
[143] Engelmann H, Novick D, Wallach D. Two tumor necrosis factor-binding proteins purified from human urine Evidence for immunological cross-reactivity with cell surface tumor necrosis factor receptors. J. Biol. Chem 1990;265:1531–6.
[144] Aderka D, Engelmann H, Maor Y, Brakebusch C, Wallach D. Stabilization of the bioactivity of tumor necrosis factor by its soluble receptors. J. Exp. Med 1992;175:323–9.
[145] McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N. Engl. J. Med 2011;365:2205–19.
[146] Lacraz S, Isler P, Vey E, Welgus HG, Dayer JM. Direct contact between T lymphocytes and monocytes is a major pathway for induction of metalloproteinase expression. J. Biol. Chem 1994;269:22027–33.
[147] McInnes IB, Leung BP, Sturrock RD, Field M, Liew FY. Interleukin-15 mediates T cell-dependent regulation of tumor necrosis factor-alpha production in rheumatoid arthritis. Nat. Med 1997;3:189–95.
[148] Sebbag M, Parry SL, Brennan FM, Feldmann M. Cytokine stimulation of T lymphocytes regulates their capacity to induce monocyte production of tumor necrosis factor-alpha, but not interleukin-10: possible relevance to pathophysiology of rheumatoid arthritis. Eur. J. Immunol 1997;27:624–32.
[149] Moelants EA, Mortier A, Van Damme J, Proost P. Regulation of TNF-alpha with a focus on rheumatoid arthritis. Immunol. Cell. Biol 2013;91:393–401.
[150] Armaka M, Apostolaki M, Jacques P, Kontoyiannis DL, Elewaut D, Kollias G. Mesenchymal cell targeting by TNF as a common pathogenic principle in chronic inflammatory joint and intestinal diseases. J. Exp. Med 2008;205:331–7.