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Minireview

Molecular mechanisms of tumor necrosis factor-induced cytotoxicity

What we do understand and what we do not

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Abstract

Although TNF plays an important role in several physiological and pathological conditions, the hallmark of this important cytokine has been its selective cytotoxic activity on tumor cells. Since its cloning in 1984, understanding of how TNF selectively kills tumor cells has been the subject of research in many laboratories. Here we review TNF-induced post-receptor signaling mechanisms which seem to be involved in the pathway to cytotoxicity.

Key words: TNF; Cytotoxicity; Phospholipases; Mitochondria; Phosphorylation; Signal transduction

1. Introduction

TNF is a cytokine which is mainly produced by activated macrophages, although several other cell types can produce small amounts (reviewed in [1–3]). The original interest in TNF was based on its antitumoral activity [4]. After cloning of the gene and purification of the recombinant protein, it soon became clear that TNF also exerts biological effects on different normal cell types. This implicates an important role for TNF in several physiological and pathological conditions, for example as a crucial mediator in septic shock and cerebral malaria (reviewed in [3,5,6]). Although host-mediated effects, i.e. actions of TNF on normal cells, are certainly also involved in the antitumoral activity of TNF in vivo, the direct cytotoxic activity of TNF on many types of malignant cells has been studied most extensively. The therapeutic value of TNF in the treatment of cancer is limited by toxic side effects occurring at high TNF doses, and by a wide variation in TNF sensitivity of tumor cells. Indeed, on some tumor cells TNF at most exerts a 'cytostatic' activity, while some other tumor cells do not respond to TNF at all. The TNF sensitivity of tumor cells is not correlated with the type or the histological origin of the cells. As a rule, untransformed cell lines are resistant to the cytotoxic effect of TNF, although embryonal fibroblastic cells may be an exception [7]. A large number of tumor cells which are resistant to TNF as such, do become sensitive when TNF is used in combination with interferon-γ or some chemotherapeutic agents [8–10]. However, such combination treatments may also result in an enhanced toxicity for the host [11]. Understanding of the signaling pathways leading to TNF cytotoxicity in vitro can greatly contribute to our search for strategies to improve the therapeutic value of TNF. Results may benefit the development of combination treatments with an improved therapeutic index, and may also contribute to the development of agents liable to mimic the cytotoxic activity of TNF, but in a more selective manner.

In this overview we discuss the signaling mechanisms which presumably lead to TNF cytotoxicity in vitro. We will mainly focus on results which have been obtained in the murine fibrosarcoma cell line L929. Because of its high TNF sensitivity, this cell line has been used in many laboratories as a prototype in the study of TNF cytotoxicity. However, it should be stressed that some TNF-induced signaling mechanisms seem to be cell type-dependent.

2. Necrosis versus apoptosis

Dependent on the type of target cell and on the pres-

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ence of metabolic inhibitors, TNF can induce necrotic or apoptotic cell death [12,13]. Necrosis is characterized by cell swelling, destruction of cell organelles and cell lysis. In apoptosis, the cell shrinks, apoptotic bodies are formed, and in most cases specific internucleosomal DNA fragmentation is observed. It is still unclear whether DNA fragmentation is the cause or simply the result of TNF-induced cell death. TNF-treated L929 cells die of necrosis [13,14], although internucleosomal DNA fragmentation can be observed at late time points after TNF addition [15].

3. TNF receptors

The trimeric TNF exerts its effect by binding to and clustering specific high-affinity receptors which are present on the plasma membrane of almost all cell lines, the number varying between 100 and 10,000 [16]. Although it is a prerequisite, the presence of TNF receptors is not sufficient in order to be sensitive to TNF cytotoxicity [17]. So far, two TNF receptors with a molecular mass of 55 kDa (p55) and 75 kDa (p75) have been identified and cloned (reviewed in [3,18]). The two receptor types consist of an extracellular domain which binds TNF and is 28% homologous, a transmembrane region, and an intracellular part which is totally different and does not contain any recognizable structure which can be associated with a particular function. The remarkable absence of homology between the intracellular regions of the two TNF receptors suggests that they are involved in different functions or signal-transducing pathways. L929 cells contain about 350 p55 and 750 p75 receptors [19]. Stimulation of L929 cells (transfected with human p55 and p75 receptors) with agonistic antibodies or TNF mutants specific for human p55 or p75 receptors has shown that TNF cytotoxicity in this cell line, as in most other cell lines, is mainly or only induced by the p55 receptor. Occasionally, a more important role for p75 in TNF cytotoxicity in some particular cell lines was proposed [20,21], but this has been contradicted [22]. Although p75 is not essential for cytolytic activity, it may play a helping role. Tartaglia and coworkers [23] proposed a model in which the p75 'presents' TNF to the p55 receptor in non-lymphoid cells and at low TNF doses. In addition to TNF cytotoxicity, the p55 receptor also seems to mediate many other activities of TNF, such as cytokine induction, fibroblast proliferation and prostaglandin synthesis [24]. So far, a direct role for p75 has only been demonstrated in T lymphocytes and derived cell lines, where TNF induces cytokines and/or cell proliferation [25,26]. After binding of TNF to its receptors, the complex is rapidly internalized and degraded [27]. Whether internalization and degradation are necessary in order to induce cytotoxicity is still under debate [28,29]. The receptor is not recycled and new protein synthesis is required to maintain cell surface receptor number [30].

4. Post-receptor mechanisms

As mentioned in the previous section, structural and functional heterogeneity in TNF receptors, each linked to distinct intracellular messenger systems, cannot fully explain variations in TNF response. Response heterogeneity is certainly also brought about by a diversification of post-receptor signal-transducing pathways.

TNF cytotoxicity does not require RNA or protein synthesis [31]. On the contrary, in the presence of the transcription inhibitor actinomycin D or the translation inhibitor cycloheximide, the cytotoxic activity of TNF is considerably increased and most TNF-resistant cells become even TNF-sensitive. This suggests the presence and/or induction by TNF of so-called 'TNF resistance proteins'. Indeed, some TNF-induced proteins have been identified which can partially inhibit TNF cytotoxicity when overexpressed in particular cell lines [32–35]. The observation that at least two phenotypically different sublines can be derived from L929 cells, suggests the existence of different resistance mechanisms within one single cell line [36]. Interestingly, transfection of TNF-sensitive L929 cells with the TNF gene under a constitutive promoter induces TNF resistance by downregulation of the receptors; how the latter effect is brought about, is still under investigation [19,37].

One can distinguish early, fairly fast (within minutes), and late (within hours) biochemical effects of TNF. In contrast to some other hormones and cytokines, TNF has to be continuously present with the cells for several hours in order to induce a maximal cytotoxic effect. This fact makes it difficult to determine the exact role of early and late TNF-induced effects in the signaling to cell death. Although the early effects might be involved in priming the cells, they cannot fully explain TNF cytotoxicity, as most of them are already reduced to background levels within less than 1 h.

4.1. G protein activation

It has been reported by several laboratories that pertussis toxin inhibits TNF cytotoxicity [38–40]. Pertussis toxin ADP-ribosylates the G proteins G_t, G_i, and G_o, and uncouples them from receptors. Branellec and coworkers [41] found that pertussis toxin-catalyzed ADP ribosylation of G proteins is considerably decreased in TNF-treated cell membranes from MCF-7 cells. Furthermore, Imamura et al. [40] reported that TNF promotes guanine nucleotide binding to the membranes of L929 and HL-60 cells. This increase in GTP binding is associated with a parallel increase in GTPase activity, which is inhibited by pertussis toxin pretreatment. Such reports suggest that a pertussis toxin-

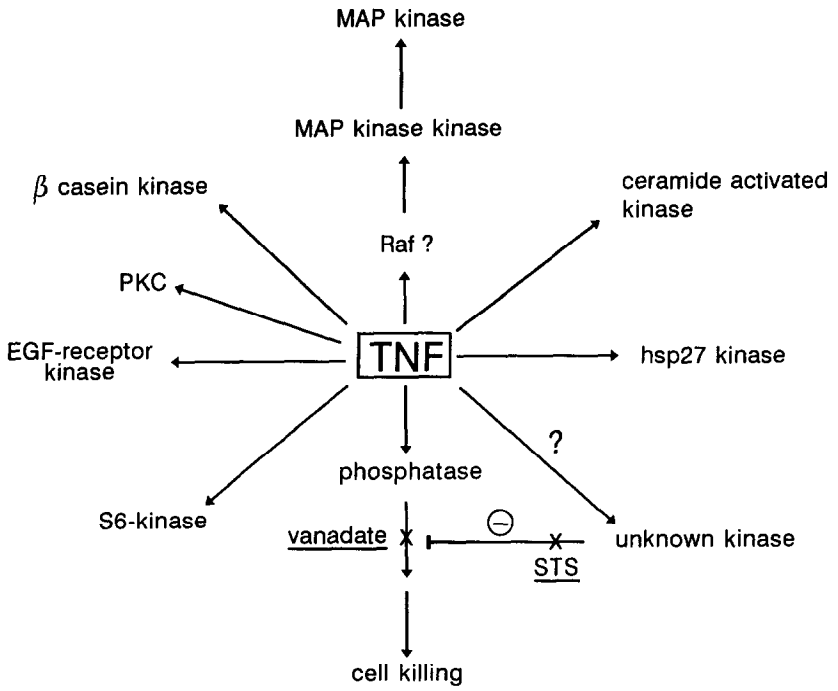

Fig. 1. TNF-activated protein kinases and phosphatases. TNF treatment of cells leads to early stimulation of several kinases. It should be mentioned that not every kinase is activated in the same cell line; for example, the activation of mitogen-activated protein (MAP) kinase, S6-kinase, β-casein kinase and heat-shock protein (hsp) 27 kinase has been demonstrated in L929 cells. Moreover, for most of these kinases a direct role in TNF cytotoxicity seems unlikely. Instead, TNF sensitization observed with the general protein kinase inhibitor staurosporine (STS) suggests that a sofar unidentified protein kinase, which might be TNF-activated, counteracts the TNF signal. In addition, the protective effect of vanadate on TNF cytotoxicity for L929 cells suggests a role for a TNF-activated phosphatase in the signaling to cell death. PKC, protein kinase C; EGF, epidermal growth factor.

sensitive G protein might be involved in TNF cytotoxicity. Although receptors coupled to G proteins usually consist of a seven-transmembrane domain structure [42], it has been reported that such a configuration is not universal. Indeed, the insulin-like growth factor II receptor, containing a single transmembrane domain, has been demonstrated to couple directly to G_{i2} in a manner similar to that of conventional G protein-coupled receptors [43].

4.2. NF-κB activation

TNF binding leads rapidly, i.e. within minutes, to activation of the transcription factor NF-κB, which means that the heterodimer p55–p65 is released from IκB and can move from the cytoplasm to the nucleus, where it binds to specific loci on the DNA. However, there is no evidence that NF-κB activation is involved in TNF cytotoxicity, although the signaling mechanisms leading to TNF cytotoxicity and NF-κB activation seem to partially overlap. For example, protease inhibitors have been shown to inhibit both TNF cytotoxicity and NF-κB activation [44,45]. However, it is not known whether the relevant protease system is the same in both types of biological effects. On the other hand, some phospholipase inhibitors only protect against TNF cytotoxicity [46].

4.3. Protein phosphorylation

In some cells rapid phosphorylation occurs after TNF treatment [47,48] (Fig. 1). In L929 cells as well as in some other cell lines, a rapid activation of several cytosolic Ser/Thr protein kinases, with a maximum already at 10 min post TNF treatment, has been demonstrated. Among these are casein kinase-2 as well as the two extracellular signal-regulated kinases erk-1 and erk-2, also known as MAP2 kinases [49,50]. These kinases are thought to play an important role in the phosphorylation cascades initiated by growth factors [51]. Activation of these kinases might be essential for priming the cells to TNF cytotoxicity, but their activation lacks the specificity needed to explain the unique property of TNF to cause tumor cell cytotoxicity. Nevertheless, an essential role for protein phosphorylation/dephosphorylation in TNF cytotoxicity is suggested by the remarkable synergy between TNF and staurosporine [52]. The latter is a potent inhibitor of many protein kinases, suggesting that a protein phosphorylation step is counteracting the TNF signal (Fig. 1). This is further suggested by the observation that in L929 cells and in other cell lines, the tyrosine phosphatase inhibitor vanadate can inhibit TNF cytotoxicity ([53]; our own unpublished results). The nature of the kinase(s) and phosphatase(s) involved is still not clear. However, experiments with protein kinase inhib-

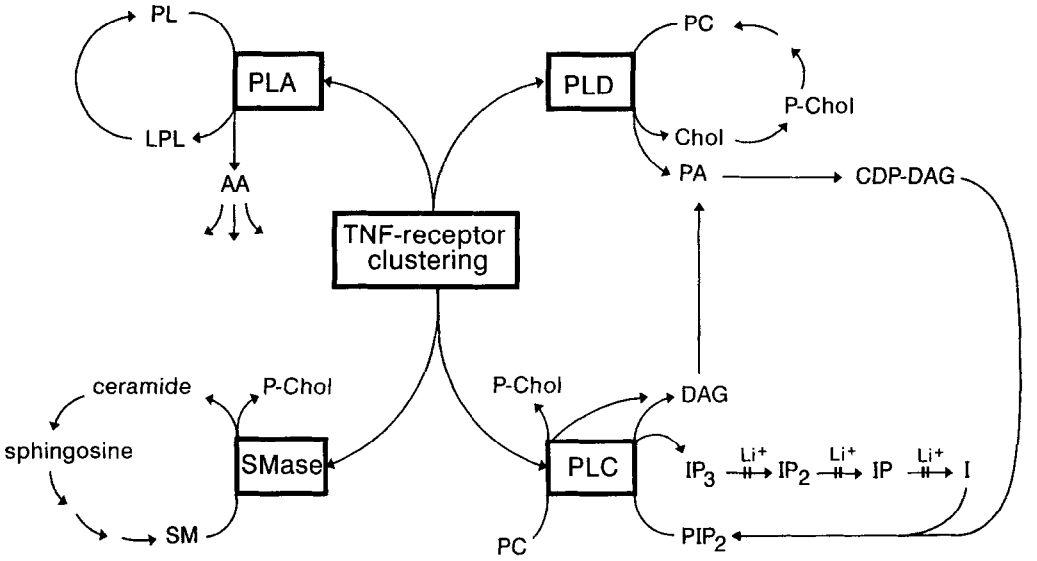

Fig. 2. TNF-induced lipid hydrolysis and formation of second messengers. TNF-induced clustering of TNF receptors leads to activation of four different phospholipases. Phospholipase (PL) A_2 hydrolyses phospholipids (PL) with the formation of lysophospholipids (LPL) and arachidonic acid (AA). The latter can be further metabolized to prostaglandins, leukotrienes and thromboxanes. PLC-mediated hydrolysis of either phosphatidylcholine (PC) or phosphatidylinositol 4,5-diphosphate (PIP_2) leads to the formation of the protein kinase C activator diacylglycerol (DAG) on the one hand, and of phosphoryl choline (P-Chol) or the Ca^{2+}-mobilizing agent inositol triphosphate (IP_3), respectively, on the other hand. The latter is converted to inositol (I) by a number of inositol phosphate phosphatases, some of which are inhibited by Li^+ ions. PLD-mediated hydrolysis of PC results in the formation of phosphatidic acid, which can be converted to cytidine diphosphate (CDP)-DAG, which reacts with I, leading to the re-synthesis of PIP_2. Finally, sphingomyelinase (SMase) converts sphingomyelin (SM) to P-Chol and the second messenger ceramide.

itors other than staurosporine have shown that TNF sensitization by the latter agent cannot be explained on the basis of inhibition of any of the major protein kinases known [52].

4.4. *Phospholipase activation*

Several inhibitors of phospholipase A_2 (PLA_2) reduce TNF cytotoxicity in L929 cells and in most other tumor cell lines studied. The observation that TNF induces the release, after 2 h treatment, of arachidonic acid in TNF-sensitive, but not in TNF-resistant L929 cells, further supports a role for PLA_2 [39,54,55] (Fig. 2). Recently, Hayakawa et al. [56] showed that a particular TNF-resistant subline of L929 has decreased PLA_2 activity; more interestingly, they could reinduce TNF sensitivity in this cell line by transfection with the gene coding for a recently identified, high-molecular mass, cytosolic, arachidonic acid-specific PLA_2. However, a decreased PLA_2 activity does not seem to be a general mechanism of reduced TNF cytotoxicity, as other TNF-resistant L929 sublines could be derived which did not display these characteristics ([56]; our own unpublished results). Metabolites of arachidonic acid are not involved in TNF cytotoxicity, since several inhibitors of arachidonic acid metabolization do not affect TNF cytotoxicity [39]. Moreover, there is no reason to believe that arachidonic acid itself plays a role in TNF-induced cell lysis, as release of the fatty acid occurs equally well by serum treatment of the cells [55]. The question how PLA_2 is involved in TNF cytotoxicity remains unanswered. Possibly, lysophospholipids, formed as a result of PLA_2 activity, are cytotoxic for the cells. PLA_2 activity can be regulated by lipocortins. However, TNF has no effect on the level or phosphorylation of these proteins [57]. Recently, it has been shown that phosphorylation and gene expression of the high-molecular mass cytosolic arachidonic acid-specific PLA_2 are increased by TNF treatment of HeLa cells [58]. However, in the latter cell line, TNF only induces a clear arachidonic acid release in the presence of Ca^{2+} ionophore. Moreover, the observation that phosphorylation and activation of this PLA_2 can be inhibited by staurosporine [59], while the latter drug drastically increases TNF cytotoxicity [52], makes a direct role for this particular PLA_2 in TNF cytotoxicity rather unlikely.

In addition to PLA_2, we have also provided evidence for a role of phospholipase C (PLC) activation and inositol lipid hydrolysis in TNF cytotoxicity (Fig. 2). This is based on the finding that a remarkable synergy between TNF and lithium ions can be observed in L929 and in some other cell lines [60]. One of the better known effects of Li^+ is the inhibition of two different inositol phosphate phosphatases [61]. In agreement with this, we found that in L929 cells treated with TNF in the presence of lithium ions, inositol mono-, di- and triphosphates as well as CDP-diacylglycerol accumulated to appreciable levels within 2 h after TNF treatment [62]. TNF-resistant cells or cells which are not sensitive to the TNF-sensitizing effect of LiCl did not reveal an accumulation of inositol phosphates upon treatment with a combination of TNF and LiCl, suggesting a role for TNF-induced inositol

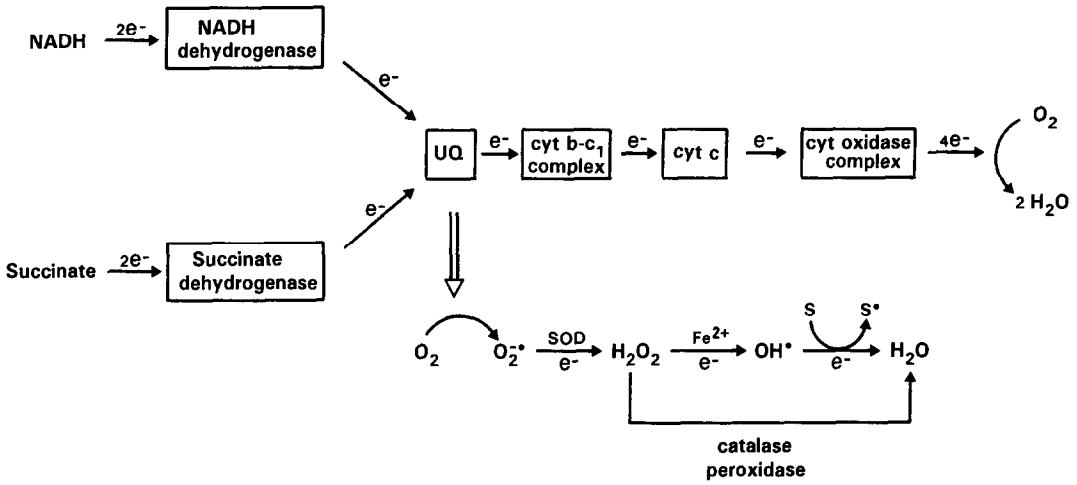

Fig. 3. Production of superoxide radicals by the mitochondrial respiratory chain. Succinate and NADH are oxidized by succinate dehydrogenase and NADH dehydrogenase, respectively. The two electrons (e^-) are then passed on to ubiquinone (UQ). From UQ, e^- pass through different cytochromes (cyt). Finally, reduced cytochrome c is re-oxidized by the multi-enzyme complex cytochrome oxidase. For every four e^- taken in by this complex, one oxygen molecule becomes fully reduced to two molecules of water. 1 to 5% of oxygen leaks from this pathway, mainly at the UQ site, and undergoes stepwise univalent reduction. The one electron reduction of molecular oxygen generates the superoxide radical (O_2^-•). This can subsequently be reduced to hydrogen peroxide (H_2O_2), most commonly in a dismutation reaction catalyzed by superoxide dismutase (SOD). H_2O_2 can be directly detoxified to water by a catalase or peroxidase-catalyzed reduction. Alternatively, H_2O_2 can be reduced, usually in an iron-catalyzed reaction, to the hydroxyl radical (OH•), which can react with almost every type of organic substrate (S) in living cells. By the use of specific mitochondrial inhibitors we found that interference of the electron transport at the NADH and succinate dehydrogenase level markedly protected the cells against TNF. On the other hand, electron transport inhibition behind the ubiquinone region potentiated TNF cytotoxicity. These results suggest that oxidative events generated in the mitochondria, and not inhibition of energy-coupled processes, are crucial in TNF-induced cytotoxicity.

lipid hydrolysis in TNF cytotoxicity. The mechanism by which an accumulation of inositol phosphates enhances TNF cytotoxicity is still unknown. Although a role of Ca^{2+} ions in TNF-induced cytotoxicity is somewhat controversial [63,64], it seems quite possible that an inositol triphosphate-induced Ca^{2+} release from intracellular stores enhances the biochemical reactions leading to cell death. This is further suggested by the observation that several Ca^{2+}-lowering agents partially protect against TNF cytotoxicity in L929 cells [62]. Some time ago, Schütze and coworkers [65] demonstrated that TNF could also rapidly activate a phosphatidylcholine-specific PLC in some cell lines, resulting in an increase in diacylglycerol levels and protein kinase C activation. However, it seems rather unlikely that this activity is involved in TNF cytotoxicity, since this particular PLC is activated only transiently for 10 min, while TNF cytotoxicity requires the presence of TNF for hours. Moreover, TNF does not seem to activate this phosphatidylcholine-specific PLC in L929 cells (our own unpublished observations).

In addition to PLA_2 and PLC, also PLD becomes activated upon TNF treatment of L929 cells [66] (Fig. 2). PLD activity results in the formation of phosphatidic acid. Whether the latter plays an essential role in TNF cytotoxicity is unknown. Since phosphatidic acid can increase cytosolic Ca^{2+} levels [67], this second messenger may further boost the Ca^{2+} rise induced by inositol triphosphate formed after PLC activation by TNF.

TNF treatment of lymphoid cell lines results in the rapid activation of a neutral sphingomyelinase, which causes sphingomyelin hydrolysis and generation of ceramide [68] (Fig. 2). The elevation in intracellular ceramide levels has been proposed to mediate the effects of TNF on cell differentiation. Interestingly, ceramide can mimic TNF-induced activation of erk-1 and erk-2 in HL-60 cells [69]. Similarly, Dhaibo et al. [70] found that ceramide also mimics the growth-inhibitory effect of TNF on Jurkat cells, supporting a role for sphingomyelin hydrolysis in the growth-inhibitory effect of TNF on these cells. We did not observe an effect of several ceramide analogues on TNF cytotoxicity for L929 cells, nor did we find a TNF-induced increase in ceramide formation in these cells. These results make a general involvement of the sphingomyelin pathway in TNF cytotoxicity rather unlikely.

4.5. Mitochondria and oxygen radical production

Matthews and colleagues [71] were the first to report that sensitive cells treated with TNF show abnormalities in their mitochondria: they looked swollen and had fewer cristae. Recently, we and others published evidence that TNF-induced events interfere with the normal electron flow in the mitochondria [72–74] (Fig. 3). Moreover, when L929 cells are cultured for a long time in ethidium bromide or chloramphenicol, it becomes possible to select clones which have lost functional mitochondria and which lack mitochondrial DNA. These cell clones have become virtually resistant to TNF-induced cytotoxicity, further suggesting a role of mitochondria in TNF cyto-

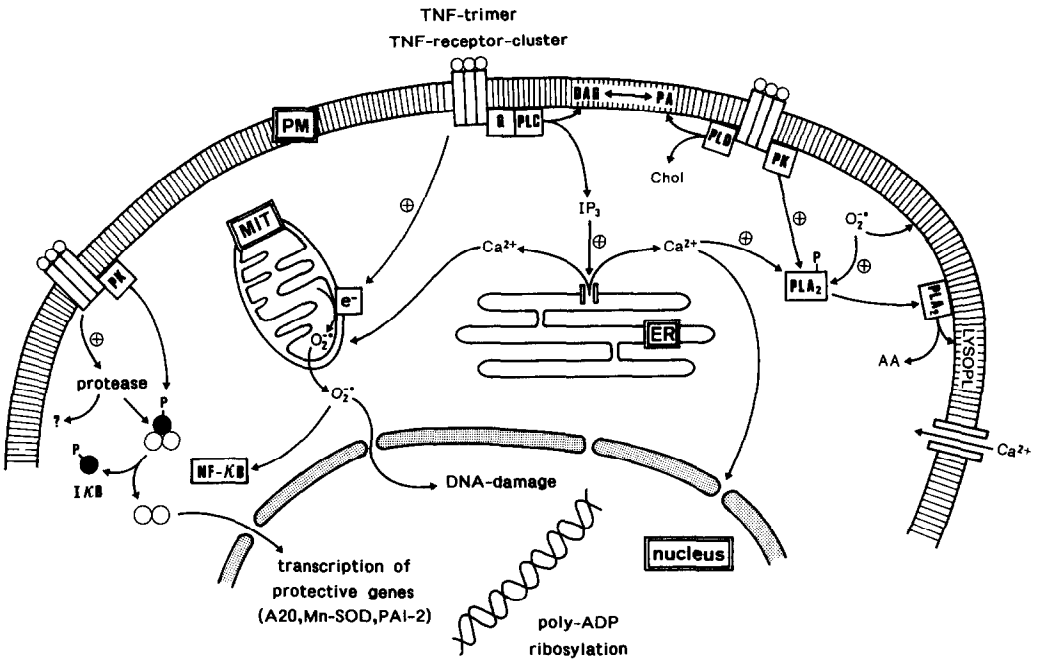

Fig. 4. Working hypothesis for TNF-induced signaling leading to cell death in L929 cells (see text for further details). AA, arachidonic acid; Chol, choline; DAG, diacylglycerol; ER, endoplasmic reticulum; G, G protein; IκB, inhibitor κB; IP_3, inositol triphosphate; LYSOPL, lysophospholipids; MIT, mitochondria; Mn-SOD, manganous superoxide dismutase; NF-κB, nuclear factor κB; O_2^- •, superoxide radical; PA, phosphatidic acid; PAI-2, plasminogen activator inhibitor type 2; PLA_2, phospholipase A_2; PLC, phospholipase C; PLD, phospholipase D; PK, protein kinase; PM, plasma membrane.

toxicity [75]. Of interest, the TNF-induced cytokine synthesis (e.g. interleukin-6) is also largely abolished in these cells, strongly suggesting that the pathways leading to cytotoxicity and gene induction are at least partially overlapping. Another finding implicating mitochondrial involvement in response to TNF was the observation that TNF induces, at least in some cells, the mitochondrial protein manganese superoxide dismutase, which confers resistance to TNF [32]. The above observations led to the hypothesis that reactive oxygen formation in the mitochondria plays an essential role in TNF cytotoxicity (Fig. 3). This is further supported by the observations that TNF cytotoxicity does not occur under anaerobic conditions and that there is direct evidence for lipid peroxidation based on the formation of malonyldialdehyde production [71]. If free radicals are involved in TNF-mediated killing, it may be predicted that free radical scavengers would inhibit cytolysis. The data indicate that some scavengers do indeed protect, but some others do not [39,76]. The finding that iron chelators inhibit killing is consistent with a role for TNF-induced free radical damage [39,74]. Iron catalyzes the Fenton reaction which produces the highly toxic hydroxyl radical from hydrogen peroxide. Direct measurements of radical production have led to rather controversial results. A 1.8-fold increase in hydroxyl radical formation was found in tumorigenic mouse fibroblast L-M cells exposed to TNF for 18 h [77]. By the use of lucigenin-enhanced chemiluminescence Hennet and coworkers [78] showed that TNF induced an increase in superoxide anion generation. In contrast, we were not able to detect an increase in radical production upon TNF stimulation of L929 cells, although radicals produced after stimulation with menadione were readily detectable with the method used (V. Goossens et al., in preparation). The reason for this discrepancy is still unclear. However, the fact that in contrast to our results [14], Hennet et al. [79] also obtained protection with the proto-oncogene bcl-2, which is known to prevent apoptosis, suggests that cell line difference might be the explanation for these divergent results. The absence of a general increase in radicals in our L929 cells is in apparent contrast with the protection obtained with some radical scavengers and with the TNF resistance observed for cells lacking functional mitochondria. A possible explanation may be based on a redistribution of mitochondria after TNF stimulation, resulting in a localized TNF-induced increase in radical production. A similar mitochondrial redistribution has already been observed in several cell types, for example in phorbol myristate acetate-activated macrophages [80].

Reactive nitrogen intermediates are other types of free radicals which are able to inhibit mitochondrial functions, and which are cytostatic and cytotoxic to tumor cells [81]. TNF has recently been shown to induce the synthesis of NO synthetase and the production of NO in L929 cells [82]. A direct role for reactive nitrogen intermediates in TNF cytotoxicity is unlikely, since inhibitors of NO production and NO scavengers do not protect against TNF cytotoxicity [83].

4.6. Are other biochemical pathways involved?

Inhibitors of serine-type proteases protect cells from TNF-induced lysis, suggesting that a protease may be involved in the cell-killing process [44,84]. In addition, ADP ribosylation of particular proteins is observed from 4 h on after exposure to TNF. Furthermore, inhibitors of ADP ribosylation also prevent TNF-mediated cytotoxicity [85], suggesting an important role of ADP ribosylation either in signal transduction or in mediating toxic reactions.

5. Discussion: a model of TNF-induced cytotoxicity

Considerable effort has been devoted to clarify the post-receptor mechanisms of TNF. However, the available data are difficult to interpret, since they have been obtained on a variety of cells which respond differently to TNF. In this review, we focused on those biochemical pathways which are thought to be implicated in the process of TNF-mediated cytotoxicity. Fig. 4 summarizes the biochemical pathways which are possibly involved in the induction of TNF cytotoxicity in L929 cells. Upon binding to its cell surface receptors, TNF initiates competing processes: transcription of protective genes vs. a programme of self-destruction. Receptor clustering activates protein kinases and phospholipases, possibly through the activation of a pertussis toxin-sensitive G protein. This results in the formation of arachidonic acid, inositol phosphates, diacylglycerol, phosphatidic acid and derivatives. These mediators in some way, either directly or through the release of other second messengers, activate new pathways (including mitochondrial radical production) which finally result in cell death.

Although the above described signaling pathways fit in a model of TNF cytotoxicity, there is strong evidence that at least some of these signaling pathways are also involved in the induction of other TNF-induced biological responses. For example, modulation of the inositol metabolism by LiCl not only increases TNF cytotoxicity, but also TNF-induced mitogenesis (our own unpublished results), TNF-induced inflammation [86] and TNF-induced gene activation [87]. Moreover, several other growth factors and hormones which induce totally different activities, also use pathways which at least partially overlap those stimulated by TNF. The main question therefore is: how is selectivity obtained? Furthermore, many other critical questions regarding TNF cytotoxicity remain to be answered. Among them are: what is the role of the different TNF receptors? Is there a role for receptor phosphorylation? Are TNF receptors directly associated with signaling molecules? What determines the outcome of the competing metabolic processes? How are the phospholipases activated? Is there a role for mitochondrial radical production? Are the early, transient, effects of TNF on, for example, protein phosphorylation involved in the signaling process leading to cell death? With the availability of specific antibodies against the two TNF receptors and several signaling molecules, as well as the corresponding cloned genes, it is now possible to investigate in detail questions related to the functional role of the two receptor proteins and their link to the intracellular signaling machinery. Further studies will hopefully yield a cohesive picture of the intracellular signal transduction network which links TNF cell surface receptors to TNF cytotoxicity for malignant cells.

References

[1] Fiers, W. (1991) FEBS Lett. 285, 199–212.
[2] Vilček, J. and Lee, T.H. (1991) J. Biol. Chem. 266, 7313–7316.
[3] Fiers, W. (1993) in: The Natural Immune System: Humoral Factors (Sim, E., Ed.), pp. 65–119, IRL Press, Oxford.
[4] Carswell, E.A., Old, L.J., Kassel, R.L., Green, S., Fiore, N. and Williamson, B. (1975) Proc. Natl. Acad. Sci. USA 72, 3666–3670.
[5] Beutler, B. (1992) (Ed.) Tumor Necrosis Factors. The Molecules and their Emerging Role in Medicine, Raven Press, New York.
[6] Vassalli, P. (1992) Annu. Rev. Immunol. 10, 411–452.
[7] Suffys, P., Beyaert, R., Van Roy, F. and Fiers, W. (1989) Anticancer Res. 9, 167–172.
[8] Williamson, B.D., Carswell, E.A., Rubin, B.Y., Prendergast, J.S. and Old, L.J. (1983) Proc. Natl. Acad. Sci. USA 80, 5397–5401.
[9] Fransen, L., Van der Heyden, J., Ruysschaert, R. and Fiers, W. (1986) Eur. J. Cancer Clin. Oncol. 22, 419–426.
[10] Alexander, R., Nelson, W. and Coffey, D. (1987) Cancer Res. 47, 2403–2406.
[11] Talmadge, J.E., Bowersox, O., Tribble, H., Lee, S.H., Shepard, H.M. and Liggitt, D. (1987) Am. J. Pathol. 128, 410–425.
[12] Laster, S.M., Wood, J.G. and Gooding, L.R. (1988) J. Immunol. 141, 2629–2634.
[13] Grooten, J., Goossens, V., Vanhaesebroeck, B. and Fiers, W. (1993) Cytokine 5, in press.
[14] Vanhaesebroeck, B., Reed, J.C., De Valck, D., Grooten, J., Miyashita, T., Tanaka, S., Beyaert, R., Van Roy, F. and Fiers, W. (1993) Oncogene 8, 1075–1081.
[15] Schmid, D.S., Hornung, R., McGrath, K.M., Paul, N. and Ruddle, N.H. (1987) Lymphokine Res. 6, 195–202.
[16] Fiers, W., Brouckaert, P., Devos, R., Fransen, L., Leroux-Roels, G., Remaut, E., Suffys, P., Tavernier, J., Van der Heyden, J. and Van Roy, F. (1986) in: Molecular Biology of *Homo sapiens*, Cold Spring Harbor Symposia on Quantitative Biology, vol. 51, pp. 587–595, Cold Spring Harbor Laboratory, Cold Spring Harbor.
[17] Tsujimoto, M., Yip, Y.K. and Vilček, J. (1985) Proc. Natl. Acad. Sci. USA 82, 7626–7630.
[18] Loetscher, H., Steinmetz, M. and Lesslauer, W. (1991) Cancer Cells 3, 221–226.
[19] Vanhaesebroeck, B., Decoster, E., Van Ostade, X., Van Bladel, S., Lenaerts, A., Van Roy, F. and Fiers, W. (1992) J. Immunol. 148, 2785–2794.
[20] Heller, R.A., Song, K., Fan, N. and Chang, D.J. (1992) Cell 70, 47–56.
[21] Grell, M., Scheurich, P., Meager, A. and Pfizenmaier, K. (1993) Lymphokine Cytokine Res. 12, 143–148.
[22] Tartaglia, L.A., Rothe, M., Hu, Y.-F. and Goeddel, D.V. (1993) Cell 73, 213–216.
[23] Tartaglia, L.A., Pennica, D. and Goeddel, D.V. (1993) J. Biol. Chem. 268, 18542–18548.
[24] Engelmann, H., Holtmann, H., Brakebusch, C., Shemer Avni, Y.,

Sarov, I., Nophar, Y., Hadas, E., Leitner, O. and Wallach, D. (1990) J. Biol. Chem. 265, 14497–14504.
[25] Tartaglia, L.A., Weber, R.F., Figari, I.S., Reynolds, C., Palladino Jr., M.A. and Goeddel, D.V. (1991) Proc. Natl. Acad. Sci. USA 88, 9292–9296.
[26] Vandenabeele, P., Declercq, W., Vercammen, D., Van de Craen, M., Grooten, J., Loetscher, H., Brockhaus, M., Lesslauer, W. and Fiers, W. (1992) J. Exp. Med. 176, 1015–1024.
[27] Mosselmans, R., Hepburn, A., Dumont, J.E., Fiers, W. and Galand, P. (1988) J. Immunol. 141, 3096–3100.
[28] Decker, T., Lohmann-Matthes, M.L. and Gifford, G.E. (1987) J. Immunol. 138, 957–962.
[29] Smith, M.R., Munger, W.E., Kung, H.-F., Takacs, L. and Durum, S.K. (1990) J. Immunol. 144, 162–169.
[30] Watanabe, N., Kuriyama, H., Sone, H., Neda, H., Yamauchi, N., Maeda, M. and Niitsu, Y. (1988) J. Biol. Chem. 263, 10262–10266.
[31] Ruff, M.R. and Gifford, G.E. (1981) in: Lymphokines (Pick, E., Ed.) Vol. 2, pp. 235–272, Academic Press, New York.
[32] Wong, G.H.W. and Goeddel, D.V. (1988) Science 241, 941–944.
[33] Kumar, S. and Baglioni, C. (1991) J. Biol. Chem. 266, 20960–20964.
[34] Jäättelä, M., Wissing, D., Bauer, P.A. and Li, G.C. (1992) EMBO J. 11, 3507–3512.
[35] Opipari, A.W., Hu, H.M., Yabkowitz, R. and Dixit, V.M. (1992) J. Biol. Chem. 267, 12424–12427.
[36] Vanhaesebroeck, B., Van Bladel, S., Lenaerts, A., Suffys, P., Beyaert, R., Lucas, R., Van Roy, F. and Fiers, W. (1991) Cancer Res. 51, 2469–2477.
[37] Himeno, T., Watanabe, N., Yamauchi, N., Maeda, M., Tsuji, Y., Okamoto, T., Neda, H. and Niitsu, Y. (1990) Cancer Res. 50, 4941–4945.
[38] Hepburn, A., Boeynaems, J.M., Fiers, W. and Dumont, J.E. (1987) Biochem. Biophys. Res. Commun. 149, 815–822.
[39] Suffys, P., Beyaert, R., Van Roy, F. and Fiers, W. (1987) Biochem. Biophys. Res. Commun. 149, 735–743.
[40] Imamura, K., Sherman, M.L., Spriggs, D. and Kufe, D. (1988) J. Biol. Chem. 263, 10247–10253.
[41] Branellec, D., De Cremoux, P., Barreau, P., Calvo, F. and Chouaib, S. (1992) Eur. J. Immunol. 22, 963–967.
[42] Iismaa, T.P. and Shine, J. (1992) Curr. Opin. Cell Biol. 4, 195–202.
[43] Murayama, Y., Okamoto, T., Ogata, E., Asano, T., Iiri, T., Katada, T., Ui, M., Grubb, J.H., Sly, W.S. and Nishimoto, I. (1990) J. Biol. Chem. 265, 17456 17462.
[44] Suffys, P., Beyaert, R., Van Roy, F. and Fiers, W. (1988) Eur. J. Biochem. 178, 257–265.
[45] Henkel, T., Machleidt, T., Alkalay, I., Krönke, M., Ben-Neriah, Y. and Baeuerle, P.A. (1993) Nature 365, 182–185.
[46] Patestos, N.P., Haegeman, G., Vandevoorde, V. and Fiers, W. (1993) Biochimie 75, 1007–1018.
[47] Robaye, B., Hepburn, A., Lecocq, R., Fiers, W., Boeynaems, J.M. and Dumont, J.E. (1989) Biochem. Biophys. Res. Commun. 163, 301–308.
[48] Guy, G.R., Chua, S.P., Wong, N.S., Ng, S.B. and Tan, Y.H. (1991) J. Biol. Chem. 266, 14343–14352.
[49] Van Lint, J., Agostinis, P., Vandevoorde, V., Haegeman, G., Fiers, W., Merlevede, W. and Vandenheede, J.R. (1992) J. Biol. Chem. 267, 25916–25921.
[50] Vietor, I., Schwenger, P., Li, W., Schlessinger, J. and Vilček, J. (1993) J. Biol. Chem. 268, 18994–18999.
[51] Blenis, J. (1993) Proc. Natl. Acad. Sci. USA 90, 5889–5892.
[52] Beyaert, R., Vanhaesebroeck, B., Heyninck, K., Boone, E., De Valck, D., Schulze-Osthoff, K., Haegeman, G., Van Roy, F. and Fiers, W. (1993) Cancer Res. 53, 2623–2630.
[53] Totpal, K., Agarwal, S. and Aggarwal, B.B. (1992) Cancer Res. 52, 2557–2562.
[54] Neale, M.L., Fiera, R.A. and Matthews, N. (1990) Int. J. Cancer 45, 203–208.
[55] Suffys, P., Beyaert, R., De Valck, D., Vanhaesebroeck, B., Van Roy, F. and Fiers, W. (1991) Eur. J. Biochem. 195, 465–475.
[56] Hayakawa, M., Ishida, N., Takeuchi, K., Shibamoto, S., Hori, T., Oku, N., Ito, F. and Tsujimoto, M. (1993) J. Biol. Chem. 268, 11290–11295.
[57] Beyaert, R., Suffys, P., Van Roy, F. and Fiers, W. (1990) FEBS Lett. 262, 93–96.
[58] Hoeck, W.G., Ramesha, C.S., Chang, D.J., Fan, N. and Heller, R.A. (1993) Proc. Natl. Acad. Sci. USA 90, 4475–4479.
[59] Lin, L.-L., Lin, A.Y. and Knopf, J.L. (1992) Proc. Natl. Acad. Sci. USA 89, 6147–6151.
[60] Beyaert, R., Vanhaesebroeck, B., Suffys, P., Van Roy, F. and Fiers, W. (1989) Proc. Natl. Acad. Sci. USA 86, 9494–9498.
[61] Berridge, M.J. and Irvine, R.F. (1989) Nature 341, 197–205.
[62] Beyaert, R., Heyninck, K., De Valck, D., Boeykens, F., Van Roy, F. and Fiers, W. (1993) J. Immunol. 151, 291–300.
[63] Hasegawa, Y. and Bonavida, B. (1989) J. Immunol. 142, 2670–2676.
[64] Bellomo, G., Perotti, M., Taddei, F., Mirabelli, F., Finardi, G., Nicotera, P. and Orrenius, S. (1992) Cancer Res. 52, 1342–1346.
[65] Schütze, S., Berkovic, D., Tomsing, O., Unger, C. and Krönke, M. (1991) J. Exp. Med. 174, 975–988.
[66] De Valck, D., Beyaert, R., Van Roy, F. and Fiers, W. (1993) Eur. J. Biochem. 212, 491–497.
[67] Huang, J.M., Xian, H.H. and Bacaner, M. (1992) Proc. Natl. Acad. Sci. USA 89, 6452–6456.
[68] Dressler, K.A., Mathias, S. and Kolesnick, R.N. (1992) Science 255, 1715–1718.
[69] Raines, M.A., Kolesnick, R.J. and Golde, D.W. (1993) J. Biol. Chem. 268, 14572–14575.
[70] Dhaibo, G.S., Obeid, L.M. and Hannun, Y.A. (1993) J. Biol. Chem. 268, 17762–17766.
[71] Matthews, N., Neale, M.L., Jackson, S.K. and Stark, J.M. (1987) Immunology 62, 153–155.
[72] Lancaster Jr., J.R., Laster, S.M. and Gooding, L.R. (1989) FEBS Lett. 248, 169–174.
[73] Higuchi, M., Shirotani, K., Higashi, N., Toyoshima, S. and Osawa, T. (1992) J. Immunother. 12, 41–49.
[74] Schulze-Osthoff, K., Bakker, A.C., Vanhaesebroeck, B., Beyaert, R., Jacob, W.A. and Fiers, W. (1992) J. Biol. Chem. 267, 5317–5323.
[75] Schulze-Osthoff, K., Beyaert, R., Vandevoorde, V., Haegeman, G. and Fiers, W. (1993) EMBO J. 12, 3095–3104.
[76] Brekke, O.-L., Shalaby, M.F., Sundan, A., Espevik, T. and Bjerve, K.S. (1992) Cytokine 4, 269–280.
[77] Yamauchi, N., Kuriyama, H., Watanabe, N., Neda, H., Maeda, M. and Niitsu, Y. (1989) Cancer Res. 49, 1671–1675.
[78] Hennet, T., Richter, C. and Peterhans, E. (1993) Biochem. J. 289, 587–592.
[79] Hennet, T., Bertoni, G., Richter, C. and Peterhans, E. (1993) Cancer Res. 53, 1456–1460.
[80] de Carvalho, T.U. and de Souza, W. (1989) J. Leukocyte Biol. 45, 498–502.
[81] Stuehr, D.J. and Nathan, C.F. (1989) J. Exp. Med. 169, 1543–1555.
[82] Hauschildt, S., Scheipers, P., Bessler, W.G. and Mülsch, A. (1992) Biochem. J. 288, 255–260.
[83] Fast, D.J., Lynch, R.C. and Leu, R.W. (1993) J. Interferon Res. 13, 235–240.
[84] Ruggiero, V., Johnson, S.E. and Baglioni, C. (1987) Cell. Immunol. 107, 317–325.
[85] Agarwal, S., Drysdale, B.-E. and Shin, H.S. (1988) J. Immunol. 140, 4187–4192.
[86] Beyaert, R., De Potter, C., Vanhaesebroeck, B., Van Roy, F. and Fiers, W. (1991) Am. J. Pathol. 138, 727–739.
[87] Beyaert, R., Schulze-Osthoff, K., Van Roy, F. and Fiers, W. (1991) Cytokine 3, 284–291.