Volume 285, number 2, 199–212 FEBS 09976 July 1991

ADONIS 001457939100694Z

Review

Tumor necrosis factor

Characterization at the molecular, cellular and in vivo level

Walter Fiers

Laboratory of Molecular Biology, State University, Ledeganckstraat 35, 9000 Gent, Belgium

Received 24 May 1991

TNF was originally characterized as an antitumor agent and a factor cytotoxic for many malignant cells. It is now clear that it plays an important role in the defense against viral, bacterial and parasitic infections, – and in (auto-)immune responses. Natural induction of TNF is protective, but its overproduction may be detrimental and even lethal to the host. The structure of TNF and its interaction with the two types of cellular receptor are becoming better understood. TNF elicits a variety of events in different cell types. It subverts the electron transport system or the mitochondria into production of oxygen radicals, which can kill the (malignant) cells when these do not contain or produce protective enzymes. Furthermore, TNF induces a set of genes and at least part of this transcriptional activation is mediated by NFκB. The prospects of TNF as an antitumor drug can be improved on the one hand by agents such as LI^+, which synergizes, and on the other hand by inhibitors of the systemic toxicity which do not interfere with the antitumor efficacy. Also, in tumor-bearing animals which have been rendered tolerant by administration of small doses of TNF, an effective and complete elimination of the tumors can be obtained by the combined action of TNF plus interferon.

Tumor Necrosis Factor; Septic shock; Cancer therapy; Lithium chloride; NFκB; Oxygen radicals; Mitochondria

1. INTRODUCTION

Already at the end of the 18th century, physicians noted that, occasionally, cancer patients who went through a severe infection, experienced a shrinkage and even an elimination of their tumor. Observations led to experimentations, and around the turn of the century, William B. Coley, a New York physician, used preparations derived from Gram-negative and Gram-positive bacteria in the treatment of a variety of cancer patients. Although his descriptions have to be seen in the context of that period, his extensive series of well-documented cases of successful cancer therapy is most impressive, even to this day [1]. This clinical testing led to well-controlled, fundamental research with animal model systems. In 1975, Lloyd Old and co-workers [2] could demonstrate unambiguously that treatment of mice or rabbits with 'bacille Calmette-Guérin' (BCG) for 10–14 days (stimulation of the reticuloendothelial system), followed by injection of lipopolysaccharide (LPS), led to the release into the circulation of a protein, which they called Tumor Necrosis Factor or TNF. The biological activity of this factor could be determined, on the one hand, by an in vivo antitumor test; treatment of mice carrying a transplantable, methylcholanthrene-induced sarcoma with the TNF-containing serum led to a rapid hemorrhagic necrosis and regression. On the other hand, the factor could also be shown to have a selective cytotoxic effect on a number of malignant cells in tissue culture. Remarkably, the selective killing of transformed cells was often much more pronounced when the treatment with TNF was combined with interferon (IFN) [3]. There was some evidence that the cells responsible for synthesis of TNF in vivo were macrophages, which, in the activated state, become highly responsive to LPS stimulation [4,5]. Indeed, appropriate stimulation of activated macrophages or monocytic cell lines leads to synthesis and release of TNF into the culture medium. A key observation was also that, unlike for example IFN activity which depends on cellular protein synthesis, TNF action is not abolished by co-treatment with transcription or translation inhibitors [6]; quite on the contrary, most cells become highly sensitive to TNF in the presence of actinomycin D or cycloheximide. This indicates that cytotoxicity is a nucleus-independent process originating in the cytoplasm. It was later shown that not only macrophages are able to synthesize TNF, but also, after appropriate induction, NK-cells, some T-lymphocyte subpopulations (such as $CD4^+$ TH1-cells), and even some tumor cell lines [7].

On the basis of amino acid sequence data derived from purified human or rabbit TNF, Pennica et al. [8] as well as a number of other groups cloned the human TNF (hTNF) cDNA gene (reviewed in [9]). Subsequent-

Correspondence address: W. Fiers, Laboratory of Molecular Biology, State University, Ledeganckstraat 35, 9000 Gent, Belgium

ly, the TNF cDNA genes for pig, cow, rabbit, cat, rat and mouse have been reported [9,10]. Both the human and the murine TNF cDNA gene could be expressed at very high efficiency in *Escherichia coli*. Unlike many other heterologous proteins, the recombinant TNF remained soluble in the bacterial cells and therefore could be purified from extracts without denaturation-refolding steps. Hence, highly purified hTNF and mTNF became available for physico-chemical, biological, biochemical and preclinical research, as well as for clinical application. The human genomic TNF gene is interrupted by three introns. Quite remarkably, it is located on the short arm of chromosome 6, about 200 kbp centromeric of HLA-B in the major histocompatibility complex (MHC) region [11].

The discovery of lymphotoxin (LT), now often referred to as TNF-β, in fact predates that of TNF [12]. Unlike TNF, LT is exclusively made by T-lymphocytes, both $CD4^+$ and $CD8^+$ cells, following appropriate stimulation in the context of class II and class I restriction, respectively. The gene for LT is closely linked (about 1200 bp distance) to the TNF gene within the MHC region. Also the LT cDNA gene can be efficiently expressed in *E. coli* [13].

Since the cloning and expression of the TNF and LT genes, proteins, probes, antibodies, assays and other related reagents have become widely available. This has transformed this previously esoteric field into one of the most rapidly expanding areas of biomedical research. Still more recent analytical (and perhaps clinical) tools relate to the membrane-bound and soluble form of two types of TNF receptor (see below).

2. THE TNF MOLECULE

The hTNF cDNA gene codes for a mature polypeptide of 157 amino acids (156 for mTNF), preceded by a 76 amino acid long presequence [14]. The latter is much longer than a classical signal sequence and furthermore is almost as strongly conserved between different mammalian species as the mature sequence, which suggests a specific, essential function [9]. Indeed, TNF can also exist in an unprocessed, membrane-bound form (26 kDa protein) [15]. In this respect, as in many others, TNF is analogous to interleukin (IL)-1 (see below).

The native structure of TNF is a trimer with a total molecular mass of 52 kDa. The trimeric structure was shown both chemically by cross-linking experiments, as well as physico-chemically by analytical ultracentrifugation and by X-ray solution scattering [14,16,17]. Lower molecular weight estimates obtained by gel filtration were presumably due to protein/matrix interaction. Each of the three subunits contains a disulfide bridge which is not essential for biological activity [9].

Well-diffracting crystals of TNF have been obtained [14,17] and the three-dimensional structure was solved at 2.6 Å resolution [18,19]. The shape of the molecule resembles a triangular cone in which each of the three subunits has a typical jelly roll-β structure. Each subunit consists of two β-pleated sheets, five antiparallel β-strands in each. The three subunits are arranged edge to face. The outside β-sheet is rich in hydrophilic residues, while the inner sheet is hydrophobic and contains the C-terminal segment, which is located close to the central axis of the trimer. Quite remarkably, the 3D-structure is clearly reminiscent of the arrangement of many viral capsids around threefold symmetry axes, such as those found in picornaviruses, in the hemagglutinin of influenza virus and in a number of eicosahedral plant viruses. In fact, the highest structural homology is with the triangular arrangement of the Satellite Tobacco Necrosis Virus (STNV) capsid protein. Up to 71% of the TNF residues are structurally equivalent to residues in STNV. It so happened that we also had elucidated the primary sequence of STNV capsid protein [20]; not unexpectedly, there is no discernible homology in primary structure. Obviously, the discovery of a similar conformational motif, on the one hand, in TNF and, on the other hand, in viral capsids, raises intriguing questions regarding convergent or divergent evolution.

The next question then is where on the TNF molecule is (are) the active site(s)? Many changes have been made by chemical methods and especially by genetic engineering approaches [9]. Especially revealing have been studies involving screening of randomly obtained mutants selecting those where loss or alteration of function was not due to a gross distortion of conformation. These mutations cluster in the lower half of the triangular pyramid, in the groove between two subunits [21,22]. This active site corresponds to the receptor-binding domain, and of course also follows the threefold symmetry.

The hLT precursor starts with a classical 34-amino acid long signal sequence followed by the mature protein, which contains 171 residues [23]. In agreement with this, there is no evidence that LT can also exist in a membrane-bound form. There is a natural minor form of the hLT protein which lacks the first 23 amino acids. hLT does not contain cysteine residues. Unlike hTNF, hLT is a glycoprotein (*N*-glycosylation of position 62), but it may be noted that also mTNF is a glycoprotein. The amino acid sequence of mature hLT is about 30% identical with that of hTNF and about 50% homologous. Mainly four regions of sequence identity/homology can be recognized and these correspond to the scaffold domains, centrally located in the trimeric structure [24]. This strongly suggests that the overall conformation of hLT is very similar to that of hTNF. hLT is relatively more resistant to proteases as compared to hTNF, but more sensitive to detergents, organic solvents and acidic conditions [25].

3. TNF RECEPTORS

TNF receptors are present on nearly all cell types with a few exceptions, such as erythrocytes and unstimulated T-lymphocytes. The number of receptors vary from about 200 up to 10000, and the binding constant is around 2×10^{-10} M [14]. Although the presence of the TNF receptor is a prerequisite for a biological effect, there is no correlation between the number of receptors and the magnitude of the response, or even the direction of response.

More recently, it became clear that there are in fact two types of TNF receptor, which can be differentiated by their size (ligand blotting after denaturing, but non-reducing gel electrophoresis), or by monoclonal antibody recognition [25-27]. Several groups were able to clone the two types of TNF receptor (TNF-R) cDNAs [28-30]. This was possible using probes based on partial amino acid sequence information determined either on purified soluble receptor, derived from urine or serum, or purified receptor itself. The first TNF receptor has a molecular weight of about 55 kDa, and can be referred to as TNF-R55 or TNF-RI; the second TNF receptor has a molecular weight of about 75 kDa, and can be referred to as TNF-R75 or TNF-RII. TNF-R55 seems to be ubiquitous and occurs, amongst others, on epithelial cells and on fibroblasts. TNF-R75 seems more restricted to cells of hematopoietic origin and is, for example, strongly expressed upon induction of T-cells. Both TNF-R55 and TNF-R75 bind TNF as well as LT, although the latter with lower avidity. The binding constant for hTNF of TNF-R55 is about $K_d \approx 0.5$ nM, and that of TNF-R75 about $K_d \approx 0.1$ nM. The extracellular domain of TNF-R55 is 182 amino acids long, and that of TNF-R75 235 residues. Both are *N*-glycosylated, but only the latter is *O*-glycosylated. Both sequences are related to each other, and each contains four sequence motifs, about 40 residues in length, each containing 6 (some 4) cysteine residues. This extracellular domain is significantly homologous to the extracellular part of nerve growth factor receptor (NGF-R) as well as to the CDw40 and OX40 antigens, and to an open reading frame of Shope fibroma virus. The cytoplasmic domain of TNF-R55 is 220 amino acids in length, and that of TNF-R75 174 residues. Remarkably, there is no homology in this region, and neither domain provides any hints as to its function (absence of GTP-binding sites, protein kinase sites, etc.).

Soluble TNF-binding proteins have been characterized both in the serum of cancer patients as well as in urine. There are two types, antigenically distinguishable and corresponding to the shedded extracellular domains of the two species of TNF receptor [31]. Remarkably, although binding of ^{125}I-TNF to both types of TNF receptor can be competed out by hLT, the binding of [^{125}I]TNF to the soluble binding proteins is not displaceable by excess hLT [28,29,31,32]. This is certainly true for the soluble TNF-R55, but less so for the soluble TNF-R75 [31]. The study of the two types of soluble TNF receptors in the presence or absence of the ligands (TNF and/or LT) in the circulation and in various other biological fluids, in function of disease state and in function of various treatments, is a vast virgin territory, which needs to be thoroughly explored in the coming years. The presence of soluble TNF-R in the serum may compete and inhibit TNF action on cells, but on the other hand, by binding the TNF in a dissociable form, it may dramatically affect the pharmacokinetics and the stability of TNF [33].

It has long been known that the species specificity of TNF is complex, as some activities are species-independent, while others are specific (to be discussed in section 6). With the availability of the cloned receptors it could be shown that in the mouse the TNF-R55 binds both mTNF and hTNF, but, remarkably, the TNF-R75 only binds mTNF ([34]; our own unpublished results). This discrimination by the two types of TNF-R is not true for the human homologues.

The time course of events after TNF binds to its receptor can be followed by electron microscopy using gold particle-labeled TNF [35]. The TNF/TNF-R complexes are internalized via clathrin-coated pits, endosomes, multivesicle bodies and finally end up in the secondary lysosomes, where they are degraded. Unlike the mechanism of action of a number of hormones and other cytokines, TNF needs to be continuously present for many hours in order to fully exert its effect on the cell. The two types of TNF receptor can be distinguished on the surface of human cells by means of specific monoclonal antibodies [27]. Monoclonal antibodies directed against the TNF-R55 can mimic a number of TNF effects, both nucleus-dependent and nucleus-independent, for example on fibroblasts and on endothelial cells [36]. This allows one to conclude first of all that these cellular TNF effects are mediated by the TNF-R55 receptor (discussed in section 8). Another important conclusion is that triggering of the TNF receptor by its ligand occurs by clustering. This is supported by the fact that a multivalent IgM monoclonal antibody was considerably more effective than the divalent IgG. As mentioned above, a TNF molecule has three potential interaction sites with its receptor (the clefts between the three subunits) [22]. In this way, the TNF receptors can be cross-linked. It remains to be investigated whether binding of the bona fide ligand, TNF, has additional effects than simple clustering, as mimicked by monoclonal antibodies. Also, the mechanism of triggering the TNF-R75 by TNF remains at present unknown.

4. TNF ACTION ON CELLS IN CULTURE

The original interest in TNF mainly arose from its selective toxic action on malignant cells. The classical cell line for testing TNF cytotoxicity is the murine

fibrosarcoma line L929, although nowadays other cell lines are available which are even more sensitive, such as WEHI164 clone 13 [37]. The cytotoxic action of TNF is nucleus-independent and in fact the sensitivity of the cytotoxic assay can be increased 50- to 100-fold in the presence of actinomycin D or cycloheximide [6]. It was originally reported by Williamson et al. [3] that the cytotoxic action of TNF on many cell types is considerably enhanced by IFN. This was clearly confirmed when the recombinant protein became available [14,38,39]. This difference between many malignant cells as compared to normal cells in response to the combined action of TNF and IFN is one of the most dramatic manifestations of the aberrant metabolism in the former. Usually the synergism can be obtained both with type I-IFN (IFN-α or IFN-β), as well as with type II-IFN (IFN-γ), but sometimes type I-IFNs are more effective. Interestingly, the selective toxic effect of natural IFN-γ preparations on a number of malignant cell lines could not be reproduced with the cloned, recombinant IFN-γ, and was presumably due to contamination of the former preparations with LT. Unlike IFNs, which have an anti-proliferative effect on a number of cell lines due to a cytostatic or anti-mitogenic action, TNF actually causes lysis of the target cells. But this is not the only way that TNF can kill. In some cell types TNF action leads to apoptosis [40-42]. Teleologically, cell death in vivo by apoptosis means that the shrunken body can be removed by engulfment, rather than a lysis process which releases all kinds of molecular domains which should not be seen by the immune system.

On many types of cells, even in the absence of protein synthesis, TNF causes release of arachidonic acid [14,43,44], and, when the proper enzymes are present, this leads to secretion of prostaglandins, especially PGE_2, and some other eicosanoids [45]. Treatment of, for example, endothelial cells with TNF also induces synthesis of platelet-activating factor (PAF); in this case, induction of new enzymes may be involved [46].

TNF added to neutrophils leads, within minutes, to a respiratory burst and degranulation, releasing elastase, lysozyme and other enzymes [47,48].

The addition of TNF to many cell types induces a various set of genes by transcriptional activation. This has been studied in more detail in a number of cell types, such as fibroblasts and especially endothelial cells, as these are the main targets when TNF appears in the circulation. Gene products induced by TNF can be found in the nucleus, such as c-fos and c-jun, in organelles such as Mn-superoxide dismutase (MnSOD) in the mitochondria, in the cytoplasm, on the cell membrane, or secreted in the medium. Among the new antigens appearing on the endothelial membrane, the procoagulant factor, the leukocyte adhesion molecule E-LAM-1, membrane-bound IL1, an enhancement of the lymphocyte adhesion molecule I-CAM-1 and of class I-HLA structures, etc. [reviewed in 49,50] can be mentioned. Examples of induced proteins which are secreted, are IL6, GM-CSF, M-CSF and plasminogen activator inhibitor [50,51].

On a number of cell types, especially primary fibroblasts, TNF in fact exerts a mitogenic activity [39,52,53]. Presumably, this is due to induction of proteins promoting cell cycling. On the other hand, the fact that blocking RNA or protein synthesis so strongly enhances the cytotoxicity of TNF, is often explained by assuming that TNF itself induces the synthesis of protective proteins [54], which either interfere with the generation of toxic products or else help to detoxify these. In fact the two phenomena may be linked; TNF stimulation of a cell may lead to synthesis of effectors, which are responsible for the mitogenic response, but which, when the latter is not allowed to proceed, become toxic/lethal. The activity of TNF on a number of other cell types, such as macrophages, osteoblasts (involved in bone resorption), chondrocytes, T-lymphocytes and B-lymphocytes, has been reviewed by Balkwill [55].

We have seen above that hLT binds both to the cloned TNF-R55 and to the TNF-R75, albeit with a lower binding affinity. Hence one would expect that independently of whether cells exhibit one or other or both of these receptors, they would respond similarly to either cytokine, except that higher concentrations of hLT would be needed. However, this is clearly not so; there are a number of biological systems where TNF is effective, while LT is not (reviewed in [56,57]). For example, TNF induces production of GM-CSF, M-CSF and IL1 in endothelial cells, while LT has almost no activity; on the other hand, LT has a proliferative effect on some B-lymphoblastoid cells, which is not shared with TNF. Among tumor cell lines, the relative sensitivity towards TNF as compared to LT differs very significantly [58]. As these results cannot be correlated with the relative involvement of one or other of the two TNF receptors, it seems much more likely that the TNF receptor molecules are associated with accessory proteins, and that interaction with TNF generates several signals, not all of which can be exerted by LT. Alternatively, it can still not be excluded that there is a third type of receptor.

5. TNF: INVOLVEMENT IN INFLAMMATION, AUTOIMMUNITY, INFECTIOUS DISEASES AND SEPTIC SHOCK

Cerami and colleagues [59,60] have studied for many years the severe wasting, called cachexia, which often accompanies chronic parasitic, bacterial or viral infection, and is also all too often a major symptom of cancer patients. Infected cattle may lose up to 50% of their weight. Cachexia can be induced in rabbits by infection with *Trypanosoma brucei*. The animals have a

high triglyceride level in circulation, presumably due to interference of clearance by a depressed lipoprotein lipase activity. A factor was identified in the serum, which not only mediated lipoprotein lipase inhibition in vivo, but also suppressed this enzyme in 3T3-L1 adipocytes in tissue culture. This factor, called cachectin, was purified, partially sequenced and turned out to be identical to TNF. This then led to a series of pathophysiological studies [60–62].

Many of the effects observed in animals with a chronic parasitic burden, could also be mimicked by administration of LPS. Indeed, we have mentioned above that LPS is a major inducer of TNF, both in vitro and in vivo. Infection with Gram-negative bacteria or high doses of LPS can lead to septic shock. That TNF plays a pivotal role in septic shock was demonstrated by pretreating mice or baboons with antibodies against TNF and in this way they became protected against a lethal challenge with LPS [61,62]. Another indication for a key role of TNF in septic shock was reported by Waage et al. [63]. In a retrospective study they found that septic shock with fatal outcome was significantly correlated with high levels of TNF in the serum.

Malaria is undoubtedly the most important parasitic disease of man. About 0.5–1% of the patients develop cerebral malaria, which is often fatal, especially in children. It was found that plasmodium infection results in an increase in circulating TNF levels, and Grau et al. [64,65] reported that treatment of infected mice with anti-TNF antibodies could protect them against the cerebral complications.

But not only parasitic and bacterial infections can become more pathogenic or fatal due to TNF in circulation, this may also be true for some viral infections. For example, $CD4^+$ T-cells latently infected by HIV can be stimulated to active viral replication by TNF. In children with AIDS, elevated serum levels of TNF correlate with progressive encephalopathy [66].

It would of course be difficult to comprehend how a molecule could have been devised in the course of evolution with such detrimental properties. In fact, quite on the contrary, there are numerous indications that the major role of TNF is as an important mediator of protection against parasitic, bacterial and viral infections (reviewed in [63]). The deleterious effects mentioned above should be seen as aberrant situations, such as overreaction of the host or deficiency of a natural, autoregulatory network. There are multiple ways in which TNF contributes to the combat against infection, such as activation of neutrophils and platelets, enhancement of the killing activity of macrophages and NK-cells, activation of the immune system, etc. Remarkably, many cell types infected by viruses or even bacteria become highly susceptible to the cytotoxic effect of TNF.

TNF also plays a role in pathophysiological events occurring in a number of autoimmune diseases (Table I). It should be noted in this respect that the action of TNF may be to some extent locally restricted and due to the membrane-bound form, similar to the situation with IL1. TNF interacts in a complex way with the immune compartment and with the vascular system leading to release of cytokines, expression of new surface determinants, and production of other mediators, such as PAF and eicosanoids [7]. In other cases, such as rheumatoid arthritis, TNF is often present at the site of inflammation, but a causal link has still to be proven [55]. In organ transplantation, ischemia/reperfusion injury is difficult to avoid, but the deleterious effects can clearly be alleviated by pretreament with antibodies against TNF [68]. Furthermore, both organ rejection and graft-versus-host disease can be prevented or diminished by anti-TNF therapy, or by treatments which prevent the synthesis of endogenous TNF. It may also be noted that the immunosuppressive treatment with the monoclonal antibody OKT3, directed against activated T-cells, has a toxic side effect which is due to induction of TNF, presumably triggered by interaction with the Fc-receptors on effector cells [69,70]. This toxicity can be avoided either by using $F(ab')_2$ fragments or by administration of drugs which prevent endogenous TNF synthesis, such as steroids or pentoxifylline.

From the above, it might be concluded that TNF, either endogenously produced or injected as a drug, is a dangerous, sometimes even lethal molecule. But the truth is much more complex and subtle. For example, Kiener et al. [71] described a non-toxic derivative of LPS, viz. monophosphoryl lipid A, which upon injection, nevertheless induced the same level of TNF in cir-

Table I

Diseases in which TNF is/may be involved

Infection		Auto-immunity	
Septicemia, septic shock		Graft versus host	
Toxic shock syndrome		Allograft rejection	
BCG infection/TBC		Systemic vasculitis	?
Lepra		Systemic lupus eryth.	?
Bacterial meningitis		Rheumatoid arthritis	?
Purpura fulminans		Diabetes mellitus (insulin-dependent	??
Flu-syndrome			
Cerebral malaria			
HIV	?		
Hepatitis	?		

Other	
Ischemia/reperfusion injury	
Adult respiratory distress syndrome	
IL2/LAK therapy	
OKT_3 administration	
Chronic lymphocytic leukemia	?
Hodgkins lymphoma	?
Atherosclerosis	?
Cachexia	??

culation. Small doses of TNF can be injected into healthy animals without causing much harm, unless another stimulus is also added, such as a low concentration of LPS which in itself has no effect [72,73]. It is important to stress that upon infection, either parasitic, bacterial or viral, or upon inflammation, not only synthesis of endogenous TNF is induced, but a whole, interconnected gang of cytokines is released. The mortality due to endotoxic shock can be reduced not only by anti-TNF antibodies, but also by an IL1 receptor antagonist [74] or by antibodies against IL6 [75]. Also, PAF antagonists come to the rescue [55].

The pre-eminent symptom of cachexia or wasting is the loss of muscle protein and a negative nitrogen balance. This cannot be reproduced by administration of TNF to animals using various treatment protocols [76]. When TNF is injected daily into mice, there is an anorexic effect; the animals stop eating and drinking, and there is an arrest of bowel movement [77,78]. But after a couple of days the animals recover both their weight and their physical fitness. Cancer patients who show severe wasting have no TNF in their serum (this could of course be a question of detection level). On the other hand, Oliff et al. [79] could induce cachexia in mice by implantation of TNF-producing CHO-cells; but it should be noted that this experiment concerns immune-compromised animals, carrying a large tumor load, and that the heterologous cells not only secrete TNF, but also a number of other cytokines, such as IL6. Indeed, in a syngeneic system, we could not observe a cachectic activity associated with a TNF-producing tumor as compared to animals in which a similar tumor with control cells had been raised [80]. Various physiological and biochemical studies, which as a whole strongly argue that TNF is not the main mediator of cachexia, have been discussed in more detail by Grunfeld and Feingold [81].

6. TNF AND THE CYTOKINE NETWORK

IL1 is, like TNF, a product of activated macrophages (for example, stimulated with LPS). It is most remarkable that IL1 exerts on a wide range of cells the same or a similar effect as TNF, although both monokines act on different receptors, two receptors for TNF and also two different receptors for IL1 [50]. Presumably, at least in some cells, one of the secondary mediators can be induced by either of the two cytokines. However, there are a number of activities which they clearly do not share. The pre-eminent example is the anti-malignant cell cytotoxicity of TNF, especially in combination with IFN, and which cannot be obtained by IL1 treatment. Also, the rapid activation of neutrophils by TNF is not seen with IL1 [49]. On the other hand, IL1 is a growth factor for an early precursor cell of hematopoiesis and is radio-protective, but TNF is not. The prototype assay for IL1 is proliferation of thymocytes in the presence of a mitogen. In this test, mTNF is clearly active, but far below the levels obtainable with IL1. Remarkably, hTNF is inactive and is even to some extent inhibitory [82,83]. Despite the fact that on the vast majority of cells, TNF and IL1 exert the same range of activities, with a few exceptions as cited above, nevertheless upon a single i.v. bolus injection, mTNF is far more lethal as compared to IL1 [84]. Clearly, TNF must be doing something in vivo which does not happen with IL1. It may be stressed here that also hTNF has a fairly low toxicity when injected in mice; the LD_{50} is about 50-fold higher [84,85]. Quite remarkably, the injection of non-lethal doses of TNF together with a small amount of IL1 proved to be lethal; there was a high synergy between these cytokines [85,86]. Could it be that mTNF is more toxic than hTNF in mice, because only the former induces IL1 and this combination then acts synergistically? This is not so, as no protection against mTNF toxicity could be obtained by preatreatment with an IL1 antagonist [87]. Also tumor-bearing animals, which are much more susceptible to TNF treatment as compared to controls, could not be protected from TNF toxicity by treatment with the IL1 antagonist.

It has long been known that mice treated with galactosamine become highly sensitive to LPS, and this is also true regarding the susceptibility to TNF [88]. The LD_{50} for mTNF decreases almost 20-fold and under these conditions there is no longer a difference between mTNF and hTNF. Galactosamine is a quite specific hepatotoxin; it converts all the UTP and in this way blocks the metabolism in the hepatocytes. The effect is readily reversible with uridine. Clearly, the fact that a well-functioning liver is so important for protection against TNF toxicity means that either this organ produces a protective substance or else is involved in detoxification of a product made as a result of TNF action. Remarkably, pretreatment of animals with small doses of IL1, optimally at about -12 h, significantly protects against a subsequent TNF challenge, and this protection is liver-mediated [89,90]. Possibly, a protective substance is induced by IL1 and needs some time to accumulate.

The adrenal glands are another organ which is essential for protection against TNF toxicity. Indeed, here, upon induction, glucocorticoids are made which on the one hand interfere with endogenous TNF synthesis, and on the other hand protect cells, including tumor cells, from the various actions of TNF. The protective role of the glucocorticoids can be demonstrated by adrenalectomy [91] or by treatment with the glucocorticoid antagonist RU486 [92]. This drug renders TNF much more toxic, but the target where the protective effect of the glucocorticoids normally occurs, is not known. It may also be mentioned here that TNF causes abortion in pregnant mice [93]. Possibly, this may be related to the fact that normal embryonic cells are killed by the

combination of TNF and IFN-γ [94]. Therefore, it is conceivable that the abortiogenic effect of RU486 is also related to sensitization towards endogenous TNF, in addition to its action as a sex steroid hormone antagonist.

Both TNF and IL1 are potent inducers of IL6. A variety of cells are able to produce IL6: macrophages, lymphocytes, endothelial cells, fibroblasts, etc. An infection or an inflammation may be locally restricted, but the IL6 which is produced, circulates through the body and sets in motion a variety of reactions. It cooperates with colony-stimulating factors in the bone marrow, it acts on T- and B-lymphocytes, it causes differentiation of myeloid cells, it acts on the hypothalamus and causes fever, it stimulates nerve cells, etc. But presumably the most important action of IL6 is as an inducer of acute phase proteins. The activation of some of these genes may require, in addition, glucocorticoids and/or IL1 (reviewed in [95–97]). One might believe that these activities are beneficial, for example some acute phase proteins are anti-proteases, which can neutralize the excessive proteolytic activity generated at the infection/inflammation site, and the ACTH-mediated induction of glucocorticoids protects cells from the action of TNF, as mentioned above.

Very high levels of IL6 are induced by the action of LPS. The peak value is obtained after about 3 h, but by 6 h the level is back to normal [98]. Also after injection of IL1 or hTNF in mice, a peak level of IL6 was obtained at about 3 h, which then decreased to background. However, when mTNF was injected which, as mentioned above, finally leads to death, then the IL6 level did not decrease, but stayed high, even after 8 h [98,99]. We found two other conditions where lethality was correlated with a continuous high level of IL6 at late times, namely when there was a synergistic toxicity by combining hTNF either with IL1 or with RU486 [92]. These results could in fact mean that (too much) IL6 is deleterious for the organism, a conclusion which is confirmed by the results of Starnes et al. [75], who, as mentioned above, could protect mice from TNF-induced lethality by administration of antibodies to IL6.

We have reported that mTNF is much more toxic for mice as compared to hTNF [50,84,85,99], although when cytotoxicity is measured in tissue culture, both species are almost equivalent [100]. However, there are cell systems where a marked difference is observed between mTNF and hTNF. We found that the induction of cytotoxicity and IL2 receptor expression in a rat/mouse T-cell hybrid could be obtained not only with IL1, but also with mTNF, but not with hTNF [101]. Later on, as referred to above, other species-specific effects on TNF were reported [82,83], such as the proliferation of thymocytes in the presence of a mitogen or the proliferation of some T-cell lines [102]. We have also mentioned above that mTNF, but not hTNF, interacts with the murine TNF-R75 ([34]; our own unpublished results), precisely the receptor which occurs on activated T-cells, as well as on cell types of the myeloid lineage. Taking into account the species-specific toxicity of TNF in the mouse, these results strongly suggest that there is an important contribution from TNF-R75-carrying cells (lymphocytes, macrophages, NK-cells, etc.) to the lethal effects.

Of course TNF not only induces new antigens on effector cells, but it also stimulates the release of cytokines, such as IL6, IL1, GM-CSF, and a variety of others. Of special importance among the latter may be the IL8-type cytokines, which cause migration of neutrophils and other white-blood cells to the site of inflammation or to the tumor (reviewed in [103]).

7. TNF AS AN ANTITUMOR AGENT

The assay system which gave TNF its name, is the rapid necrosis observed in transplantable, methylcholanthrene-induced sarcomas in mice [2]. These tumors, however, are atypical and hardly a model for cancer in man. The Meth-A sarcoma is immunogenic and the system is so sensitive that not only hemorrhagic necrosis, but also complete elimination of the tumor can be obtained with a single injection of a low, non-toxic concentration of TNF. It was originally a surprise, however, to find that Meth-A sarcoma cells in tissue culture were completely resistant to the action of TNF. Hence the effect in vivo is entirely host-mediated. The endothelium plays a key role in exerting these effects. Newly vascularized tumors, about 9 days after the original tumor cell inoculation, respond very well, while there is no effect at all on the same cells when these were injected into the peritoneum. The antitumor effect can also be interfered with by administration of anticoagulants. Moreover, the immune system is important, as tumor necrosis cannot be obtained in syngenic nude mice. T-helper cells play an essential role in the elimination of the tumor and cured mice become resistant to a challenge with the same type of tumor cells [104].

In our animal model experiments we have chosen a system which is closer to the course of events in human cancer, namely B16BL6 melanoma tumor in syngenic C57BL6 mice. This is a fairly aggressive tumor which has low immunogenicity. The B16BL6 cells in tissue culture are not sensitive to TNF as such, either human or murine, but become highly sensitive in the presence of murine IFN-γ [100]. A series of experiments were carried out in which the in vivo action of mTNF was compared with hTNF, given either paralesionally (p.l.), which means injected in the proximity of the tumor, or intraperitoneally (i.p.) [105]. These were also the first studies in which the synergy between TNF and IFN was tested in vivo. A major result was that, using the appropriate protocol, elimination of the tumor and com-

plete curing could be obtained. But surprisingly, there was a very major difference between hTNF and mTNF (at that time it was believed that the two TNFs had no species specificity); hTNF could only cure when the treatment was combined with IFN administration, while mTNF was effective as such. We would now interpret these data as indicating that the action of mTNF was mainly host-mediated, while that of hTNF (which acts only on TNF-R55, present, amongst others, on tumor cells) was to a large extent directly on the tumor cells when these were sensitized by IFN. The aggressive, but effective protocol used was, however, toxic. More than two-thirds of the animals died. Surprisingly, this was also true for animals treated with hTNF alone, which otherwise is fairly non-toxic; this and several later studies revealed that tumor-bearing animals are much more sensitive to TNF than controls, and this constitutes another major hurdle which has to be overcome in order to develop TNF into a useful anti-cancer agent. The combination of IFN plus TNF as compared to the latter alone does not seem to very significantly increase the toxicity. The main message for the future then, was that there was a need to increase the therapeutic index, either by decreasing the toxicity or by increasing the effectiveness.

Many more studies regarding TNF as an anti-cancer agent in animal tumor model systems have been reported (reviewed in [55,106]). It should be noted that in many animal studies hTNF has been used as this was available due to the efforts of a number of biotechnology companies which have developed and produced high-grade hTNF for clinical trials. But there are two deficiencies which should be highlighted. hTNF in mice does not interact with the mTNF-R75, such that an important compartment regarding both indirect host effects as well as toxicity is not switched on. Furthermore, as in tumor studies, repeated treatments have to be given, an immune response in the mice against the hTNF starts to obscure the picture after a few days.

As regards the direct action in vivo, the efficacy of TNF can be enhanced not only by IFN, but also by some chemotherapeutic drugs [107,108], and especially the combination with topoisomerase inhibitors may be promising [109,110]. It should also not be overlooked that the cytotoxic activity of TNF on malignant cells is considerably increased at higher temperatures (the healing effect of fever?), suggesting that the combination of TNF therapy with thermotherapy makes sense [111]. We have found that the cytotoxic activity of TNF on a number of cell lines is dramatically increased in the presence of lithium ions [112]. The biochemical reason for this effect is not yet clear. As Li^+ has low toxicity (it has been used for many years to treat manic depression) and also does not appreciably increase the toxicity of TNF, it may offer interesting possibilities for therapy. With some tumors at least, the treatment with the combination TNF plus Li^+ was highly effective, while either agent alone had no or low activity, and this occurred without loss of animals [112].

New approaches can also be developed to reduce the toxicity of TNF. We have seen above that a considerable reduction of toxicity can be achieved by a pretreatment with IL1, presumably by activation of a protective liver function [89,90]. The toxic effects seen after injection of TNF are at least partially due to release of prostaglandins, and this can be prevented by cyclo-oxygenase inhibitors, such as indomethacin [113]. Although this gives good protection in a single-injection experiment, it is, however, counter-productive when the treatment has to be repeated for longer times on consecutive days (our unpublished experiments).

It has long been known that when experimental animals are treated for a number of days with a low concentration of endotoxin, they become refractory to a much higher, normally lethal dose of LPS. A similar induction of tolerance (called tolerization or tachyphylaxis) can be obtained by injecting small doses of TNF for 5–6 days. Tolerance is induced against certain effects of TNF, most importantly the lethality, but not against some others. The key question is, how would the anti-tumor activity be affected? Fraker et al. [114] reported that TNF-tolerized mice carrying an MCA sarcoma also had become refractory to a TNF antitumor treatment. On the other hand, we found with mice carrying a B16BL6 melanoma, that induction of tolerance allowed us to apply an effective treatment with mTNF plus mIFN-γ [78]. Using p.l. administration, a high cure rate was obtained with minimal lethality. The difference in response between the two studies is presumably again that in the former mainly host-mediated processes were involved, while in the latter the protocol was aimed at the direct anti-malignant cell activity. The studies on induction of TNF tolerance are promising. But before they can be transposed to the clinic, much more should be known about the underlying mechanisms at the cellular and the molecular level.

A step closer to human cancer is the study of human tumor xenografts in nude mice. As this is human material, usually these studies are carried out with hTNF. It should be kept in mind therefore that, because hTNF does not interact with the mTNF-R75 and because nude mice lack TNF-R75-carrying T-lymphocytes, these model systems do not cover a major part of the host-mediated processes. Also, we have seen above that after a successful treatment immunity develops, and this is not possible in a nude mouse. For the same reason, also the toxicity may be much reduced. Subcutaneously xenografted tumors derived from human breast or bowel carcinoma showed a good response, often complete regression, by intratumoral (i.t.) treatment with TNF [115]. Intraperitoneal (i.p.) treatment was only marginally effective with some tumors, but the combination with IFN again gave a considerable improvement. Ovarian carcinoma

development is rather restricted in the peritoneum, and the same is true after xenografting in nude mice. Hence i.p. injection allows a fairly local treatment. All four human ovarian carcinoma xenografts responded, but in quite different ways [116]. One was sensitive to TNF alone, another to IFN-γ alone, but still another one was uniquely sensitive to a combination of TNF plus IFN-γ [116]. It should be noted that in view of the high species specificity of IFN-γ, this was clearly a direct effect on the malignant cells. Further studies with this tumor model, however, also revealed a potential complication. In the mice treated with TNF, the tumor cells adhered to the peritoneal surface, and there was even evidence of micro-metastases [117,118]. Possibly, one should combine the TNF treatment with inhibitors of neo-angiogenesis [119]. With subcutaneous tumors, however, we found that endogenous expression of TNF reduced the spreading of tumor cells and seemed to induce a host-mediated encapsulation of the tumors [80].

The first studies regarding the use of hTNF for treatment of cancer patients started already in 1985. A considerable number of clinical trials have now been reported (reviewed in [120–122]). In most of these, TNF was administered i.v., either as a bolus injection or by continuous infusion. The maximum tolerated dose was often in the range of 200 $\mu g/m^2$, and it is fair to say that no significant anti-tumor responses were noted with these systemic treatments. Of course, considering the animal tumor model results, one would not have expected positive results at these moderate concentration levels. The dose-limiting toxicity was often hypotension, and occasionally hepatoxicity. There is evidence that the former may be due to induction of endothelium-derived relaxing factor (EDRF), which means NO; in animals, this hypotension can be alleviated by adjuvant treatment with inhibitors of NO-synthesis [123]. Also the hepatoxic effects may be remediable by appropriate adjuvant therapy.

A few clinical studies have so far been reported involving the combination of TNF plus IFN-γ. The maximum tolerated dose of TNF is about 3-fold lower in the combination, and again the limiting toxicity is hypotension. Also in these combination studies no significant anti-tumor activity was observed.

In view of the results in animal model systems, one should use on the one hand a more aggressive therapy (combinations with IFN, with chemotherapeutic drugs, with lithium, with thermotherapy), but at the same time reduce the toxicity (appropriate adjuvant treatment, tolerization, etc.). But considering the potential lethality of TNF, one can only proceed with utmost caution. On the other hand, the animal model systems (and the earlier results of W. Coley) also showed that a loco-regional treatment has a higher chance of success. Of course, for the majority of cancer cases, local treatment is not an option. But there are also many cancer indications where local treatment can be used, and in the studies carried out so far, the response rate is good to excellent [122]. Another route of administration, although not so widely applicable, but which certainly merits more study, is by way of the lymphatic system [106].

8. TNF: BIOCHEMICAL MECHANISM OF ACTION

The variety of effects exerted by TNF on different cell types, and especially the cytotoxic action on many malignant cells (mostly in synergy with IFN) as compared to normal, diploid cells, are intriguing enigmas which can only be satisfactorily explained when the (presumably multiple) signalling pathways of TNF become known in molecular terms. So far, some pieces of the puzzle have been put into place, but these are patches of knowledge which still cannot be linked up to one another. One can distinguish events directly connected with the TNF receptor action, early secondary reactions, and then the two major cellular responses, on the one hand, the nucleus-independent cytotoxicity, and on the other hand selective transcriptional activation.

We have seen above that TNF interacts with its receptor and causes clustering (at least in the case of TNF-R55), the complex becomes internalized and finally is degraded in the lysosomes [35]. There is no evidence for recycling of the receptor; in the absence of protein synthesis the receptor does not reappear on the surface [124]. The receptor is metabolically labile, with a half-life of 30 min [125] to 2 h [124]. The cellular response correlates with the number of TNF/TNF-R complexes internalized, but the number of receptors on the cell surface is usually not rate-limiting [124]. The TNF receptor can be down-modulated by activation of protein kinase C, for example with phorbol ester; it is not known whether the receptor itself or an accessory protein becomes phosphorylated. On the other hand, at least in some cell types, protein kinase A up-regulates the receptor [126]. Internalization of the complexes is presumably required for signalling as chloroquine protects cells against cytotoxicity [43]. We have seen above that triggering the receptor can also be obtained by cross-linking by means of monoclonal antibodies. This has only been shown for the TNF-R55, but as the latter is present on tumor cells and can mediate both cytotoxic responses as well as nuclear activation, most of what is reported in this section refers in fact to TNF-R55-mediated events. It is not yet clear what physiological effects the TNF-R75 might have [127].

Activation of neutrophils [47,49] and phosphorylation of a 27 kDa protein [128] are events which occur within 5 min after TNF addition, and they are protein synthesis-independent. The latter phosphorylation is not observed in all cells, and might be a side phenomenon. As the cytotoxic action is inhibited by a pertussis toxin treatment, there might be a G-protein in-

volved in the signal transduction [43,129]. But this has not yet been directly verified.

Sensitive cells treated with TNF release arachidonic acid into the medium, and this is already significant from 1 h onwards [14,43,44,130]. When the cells contain the appropriate enzymes, this arachidonic acid may be converted to prostaglandins [45] and other eicosanoids. As arachidonic acid is mainly present in the 2-position of phospholipids, this TNF-induced release suggests the activation of a phopholipase A_2. Studies with various inhibitors, such as quinacrine and steroids, also point in the same direction [43,129]. However, none of these are highly specific and furthermore the steroids act by indirect mechanisms (but we found no evidence for a lipocortin involvement [131]). It has been reported that treatment of chondrocytes with IL1 actually releases phospholipase A_2 into the medium, and the same is true for TNF treatment [132]. It seems very likely that the activation of a phospholipase is an essential step in the cytotoxicity: it occurs only in TNF-sensitive cells, inhibitors of phospholipase activity also protect the cells from the cytotoxic action, and vice versa activators, such as Li^+ ions, enhance the arachidonic acid release as well as the cytotoxicity [44]. There is no reason to believe that arachidonic acid or its metabolites play a role in killing the cells, but rather it is the activation of a phospholipase itself which is essential. It may be that at least in some cells phospholipase C also becomes activated, but this is presumably not an essential step. There is no evidence for a crucial role of intracellular calcium influx. Some genes which are activated by TNF can also become activated by phorbol ester (through activation of the transcription factor NF$\varkappa$B, see below); but cells treated for a long time with phorbol ester such that the protein kinase C is inactive, still respond to TNF both with respect to gene activation [133], as well as with respect to cytotoxicity (our unpublished results). The link between the activation of a phospholipase and the next step, which is the actual generation of lethal products, is completely unknown.

Matthews [134] was the first to report that sensitive cells treated with TNF show abnormalities in their mitochondria; they looked swollen and had fewer cristae. On the other hand, we have proposed that reactive oxygen generated in the target cells is the basis for lethal events and leads, amongst others, to lipoxygenation and to DNA breakdown [14]. That reactive oxygen species are involved is further shown by the decreased TNF cytotoxicity in anaerobiosis [130]. There was even direct evidence for lipid peroxidation, as Matthews et al. [130] document the formation of malonyldialdehyde (this was only detectable some 20 h after the start of the TNF treatment, but this may be due to the low sensitivity of the detection method). That superoxide generated in the mitochondria is a key step in the cytotoxic action of TNF is also strongly confirmed by the results of Wong et al. [135]. They found that TNF, which is known to induce protecting proteins (see below), induces manganese superoxide dismutase (MnSOD). This is a mitochondrial enzyme which deactivates the superoxide. Overexpression of MnSOD conferred resistance to TNF, while a decrease of enzyme (by means of antisense RNA) enhanced the sensitivity. It is also of considerable interest that cancer cells are often deficient in MnSOD [136]. Of course, this enzyme is not the only determinant for (inducible) TNF resistance, but at least in some cell lines it is a key factor. The hypothesis then is that TNF-induced events subvert part of the normal electron flow in the mitochondria, and redirect it to form oxygen radicals. Recently, direct evidence for such disturbances in the electron transfer pathway has been obtained [137,138]. As a result of these reactive oxygen species, oxidation of lipids and of proteins occurs, followed by their degradation; also the fragmentation of DNA would be an expected consequence.

TNF induces the transcription and activation of a number of genes in a variety of cell types [55]. Which genes become induced depends of course on the particular combination of positively and negatively acting transcription factors present in that particular cell. The prominently induced genes can be revealed by comparative two-dimensional gel electrophoresis [139]. Quite often, the set largely overlaps with those inducible by IL1 [50,140]. But this is not always the case. The same protein may be uniquely inducible in one cell type by TNF and in another cell type by IL1 [140]. It has long been thought that the explanation why cells resistant to TNF become sensitive when TNF is combined with actinomycin, is that TNF itself induces protective proteins [54]. One of these protective proteins, at least in some cells, seems to be MnSOD, as mentioned above. It may be that the dramatic sensitization of many cell types by IFN is due to interference by the latter cytokine with the induction of protective proteins by TNF. One of the better studied proteins induced in many cell types by TNF is IL6. There is no correlation between sensitivity of the cell to the cytotoxic action of TNF and the inducibility of the IL6 gene; the IL6 gene can readily be induced not only in normal diploid fibroblasts, but also in TNF-resistant tumor cell lines, such as MG63 [141]. On the other hand, the IL6 gene cannot be induced in the very sensitive WEHI164 or in the human transformed cell lines BT20 or MCF7. But it can be induced in the prototype sensitive cell line L929 [140–142]. TNF induces a rapid, but transient induction of c-fos and c-jun, and a more lasting activation of NF$\varkappa$B [143–145]. It is of interest that the c-fos and c-jun genes may be induced by different signalling pathways [144]. There are responsive elements both for the c-fos/c-jun complex as well as for the NK$\varkappa$B complex and the cyclic AMP-dependent transcription factor in the promoter of the IL6 gene [146]. Both TNF and LT activate NF$\varkappa$B within minutes after binding to the recep-

tor [145,147,148]; furthermore, triggering either the TNF-R55 or the TNF-R75 leads to NFκB activation (as mentioned above, the biological effects which result from TNF-R75 activation, are so far unknown). One way to activate NFκB is by stimulation of protein kinase C, which presumably phosphorylates the binding protein IκB, and in this way releases the p65 NFκB subunit (Fig. 1). The TNF-mediated activation of NFκB, however, is protein kinase C-independent [149]. Several groups have shown by promoter manipulation that the induction by TNF of the MHC class I genes and of the IL6 gene does indeed involve the NFκB responsive element [148,150-152]. However, although NFκB activation may be necessary, it might not be the major limiting nuclear transcription factor; in L929 cells, we found a surprising correlation between cytoplasmically determined cytotoxicity and nucleus-dependent IL6 gene activation, suggesting that both signalling pathways have many steps in common ([142]; our unpublished results).

Some of the different reaction pathways discussed in this section are illustrated in Fig. 1.

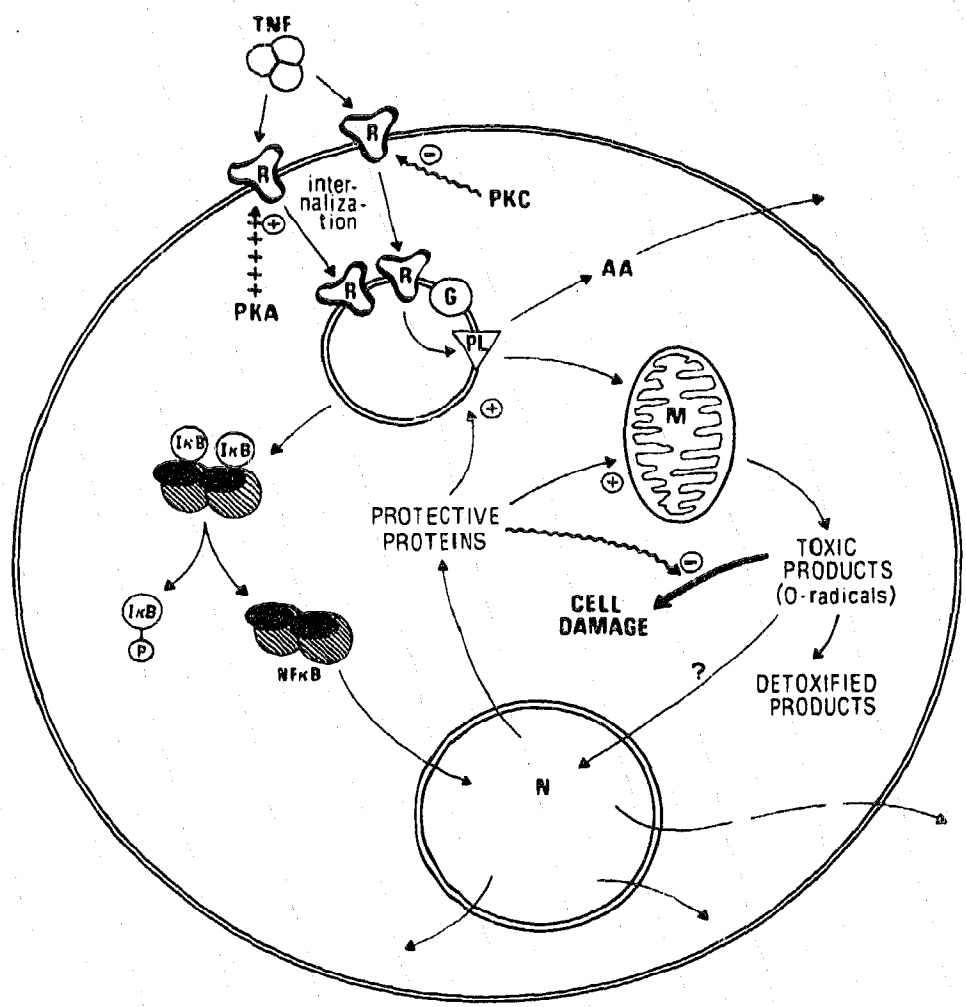

Fig. 1. Model for the mechanism of action of TNF. The trimeric TNF (or LT) interacts with its receptor (TNF-R55) and by clustering causes internalization. The receptor itself is negatively regulated by protein kinase C and positively by protein kinase A. Internalized, clustered TNF receptor complexes transmit one or more signals. There is activation of a phospholipase (PL), presumably A_2, and this step may be mediated by a G-protein (G). As a result of the phospholipase action, arachidonic acid (AA) is released (which in some cells is converted to prostaglandins, prostacyclins, etc.). A signal is transmitted to the mitochondria (M), where the electron transport system is subverted and radicals start to form. These reactive oxygen species may eventually kill the cell (oxidation of enzymes, lipids, and degradation of DNA). Another signal, very rapid, leads to activation of the transcription factor NFκB (it is not known whether also in this case phosphorylation of the negative regulatory element IκB is involved). Transcriptional activation of a number of genes in the nucleus (N) depends on active, nuclear NFκB, but perhaps also on signals from the nucleus-independent cytotoxic pathway. Amongst the induced proteins are protective factors which either prevent the formation of toxic products, or else detoxify these (e.g. mitochondrial MnSOD). Other activated genes include cell surface antigens (e.g. MHC class I) and secreted proteins (e.g. IL6). Not all reactions shown here occur necessarily in the same cell type. Several observations are not yet included in the model, such as the strong synergistic action of Li^+ and the essential role of a serine-type protease (protection against the cytotoxic effect by serine protease inhibitors [153]).

9. CONCLUSION

There is presumably no other cytokine which has such a range of activities on so many cell types as TNF. Most probably, its evolutionary raison d'être is a defense against viral, bacterial and parasitic infections. However, due to inappropriate expression, either in amount or in time or in localization, it may have very detrimental effects on the host. It is becoming very clear that TNF plays a key role, most often that of a villain, in a variety of infectious and inflammatory autoimmune diseases. Due to the complexity of interconnected cytokine networks, it is not always easy to distinguish which is the main perpetrator and which effects are due to the accomplices - it is a story of Dr. Jekyll and Mr. Hyde. Clearly, an antagonist of TNF, such as for example soluble TNF receptor, might have many clinically valuable applications.

One should also not underestimate the positive potential. Amongst all the cytokines or low-molecular weight drugs, there is none which even vaguely resembles the selective toxic effect of the combination TNF plus IFN on a variety of malignant cells, while leaving most normal cells unharmed. At the very least, a biochemical understanding of the underlying phenomena may give us valuable insights as to the difference between malignant and normal cell metabolism and/or growth control. TNF, in synergy with other drugs, has already proven to hold a real potential in locoregional cancer therapy. This, I would predict, is only the beginning as studies involving animal tumor systems have provided many indications for improved therapy. In the end, we may come to a scientific rationale for effective cancer therapy starting from observations made two-hundred years ago.

REFERENCES

[1] Nauts, H.C. (1989) Cancer Surveys 8, 713-723.
[2] 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.
[3] 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.
[4] Matthews, N. (1978) Br. J. Cancer 38, 310-315.
[5] Männel, D.N., Moore, R.N. and Mergenhagen, S.E. (1980) Infect. Immun. 30, 523-530.
[6] Ruff, M.R. and Gifford, G.E. (1981) Lymphokines 2, 235-272.
[7] Bonavida, B. (1991) Biotherapy 3, 127-133.
[8] Pennica, D., Nedwin, G.E., Hayflick, J.S., Seeburg, P.H., Derynck, R., Palladino, M.A., Kohr, W.J., Aggarwal, B.B. and Goeddel, D.V. (1984) Nature 312, 724-729.
[9] Fiers, W. (1991) in: Tumor Necrosis Factors: Structure, Function, and Mechanism of Action (Aggarwal, B.B. and Vilček, J. eds.) in press, Marcel Dekker, New York.
[10] McGraw, R.A., Coffee, B.W., Otto, C.M., Drews, R.T. and Rawlings, C.A. (1990) Nucleic Acids Res. 18, 5563.
[11] Spies, T., Morton, C.C., Nedospasov, S.A., Fiers, W., Pious, D. and Strominger, J.L. (1986) Proc. Natl. Acad. Sci. USA 83, 8699-8702.
[12] Granger, G.A. and Williams, T.W. (1968) Nature 218, 1253.
[13] Seow, H.F., Goh, C.R., Krishnan, L. and Porter, A.G. (1989) Bio/Technology 7, 363-368.
[14] 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*, pp. 587-595, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[15] Kriegler, M., Perez, C., DeFay, K., Albert, I. and Lu, S.D. (1988) Cell 53, 45-53.
[16] Wingfield, P., Pain, R.H. and Craig, S. (1987) FEBS Lett. 211, 179-184.
[17] Lewit-Bentley, A., Fourme, R., Kahn, R., Prangé, T., Vachette, P., Tavernier, J., Hauquier, G. and Fiers, W. (1988) J. Mol. Biol. 199, 389-392.
[18] Jones, E.Y., Stuart, D.I. and Walker, N.P.C. (1989) Nature 338, 225-228.
[19] Eck, M.J. and Sprang, S.R. (1989) J. Biol. Chem. 264, 17595-17605.
[20] Ysebaert, M., Van Emmelo, J. and Fiers, W. (1980) J. Mol. Biol. 143, 273-287.
[21] Yamagishi, J., Kawashima, H., Matsuo, N., Ohue, M., Yamayoshi, M., Fukui, T., Kotani, H., Furuta, R., Nakano, K. and Yamada, M. (1990) Protein Eng. 3, 713-719.
[22] Van Ostade, X., Tavernier, J., Prangé, T. and Fiers, W. (1991) EMBO J. 10, 827-836.
[23] Gray, P.W., Aggarwal, B.B., Benton, C.V., Bringman, T.S., Henzel, W.J., Jarrett, J.A., Leung, D.W., Moffat, B., Ng, P., Svedersky, L.P., Palladino, M.A. and Nedwin, G.E. (1984) Nature 312, 721-724.
[24] Tavernier, J., Van Ostade, X., Hauquier, G., Prangé, T., Lasters, I., De Mayer, M., Lewit-Bentley, A. and Fourme, R. (1989) FEBS Lett. 257, 315-318.
[25] Aggarwal, B.B. (1991) Biotherapy 3, 113-120.
[26] Hohmann, H.P., Remy, R., Brockhaus, M. and Van Loon, A.P.G.M. (1989) J. Biol. Chem. 264, 14927-14934.
[27] Brockhaus, M., Schoenfeld, H.J., Schlaeger, E.J., Hunziker, W., Lesslauer, W. and Loetscher, H. (1990) Proc. Natl. Acad. Sci. USA 87, 3127-3131.
[28] Loetscher, H., Pan, Y.E., Lahm, H.W., Gentz, R., Brockhaus, M., Tabuchi, H. and Lesslauer, W. (1990) Cell 61, 351-359.
[29] Schall, T.J., Lewis, M., Koller, K.J., Lee, A., Rice, G.C., Wong, G.H.W., Gatanaga, T., Granger, G.A., Lentz, R., Raab, H., Kohr, W.J. and Goeddel, D.V. (1990) Cell 61, 361-370.
[30] Smith, C.A., Davis, T., Anderson, D., Solam, L., Beckmann, M.P., Jerzy, R., Dower, S.K., Cosman, D. and Goodwin, R.G. (1990) Science 248, 1019-1023.
[31] Engelmann, H., Novick, D. and Wallach, D. (1990) J. Biol. Chem. 265, 1531-1536.
[32] Aiyer, R.A. and Aggarwal, B.B. (1990) Lymph. Res. 9, 333-344.
[33] Wallach, D., Engelmann, H., Nophar, Y., Aderka, D., Kemper, O., Hornik, V., Holtmann, H. and Brakebusch, C. (1990) 3rd International Conference on Tumor Necrosis Factor and Related Cytokines, Makuhari (Chiba, Japan), Abstr. no. P3-36.
[34] Lewis, M., Tartaglia, L.A., Lee, A., Bennett, G.L., Rice, G.C., Wong, G.H.W., Chen, E.Y. and Goeddel, D.V. (1991) Proc. Natl. Acad. Sci. USA 88, 2830-2834.
[35] Mosselmans, R., Hepburn, A., Dumont, J.E., Fiers, W. and Galand, P. (1988) J. Immunol. 141, 3096-3100.
[36] Espevik, T., Brockhaus, M., Loetscher, H., Nonstad, U. and Shalaby, R. (1990) J. Exp. Med. 171, 415-426.
[37] Espevik, T. and Nissen-Meyer, J. (1986) J. Immunol. Methods 95, 99.
[38] Fransen, L., Van der Heyden, J., Ruysschaert, R. and Fiers, W. (1986) Eur. J. Cancer Clin. Oncol. 22, 419-426.

[39] Sugarman, B.J., Aggarwal, B.B., Hass, P.E., Figari, I.S., Palladino Jr., M.A. and Shepard, H.M. (1985) Science 230, 943-945.
[40] Schmid, D.S., Tite, J.P. and Ruddle, N.H. (1986) Proc. Natl. Acad. Sci. USA 83, 1881-1885.
[41] Dealtry, G.B., Naylor, M.S., Fiers, W. and Balkwill, F.R. (1987) Eur. J. Immunol. 17, 689-693.
[42] Robaye, B., Mosselmans, R., Fiers, W., Dumont, J.E. and Galand, P. (1991) Am. J. Pathol. 138, 447-453.
[43] Suffys, P., Beyaert, R., Van Roy, F. and Fiers, W. (1987) Biochem. Biophys. Res. Commun. 149, 735-743.
[44] Suffys, P., Beyaert, R., De Valck, D., Vanhaesebroeck, B., Van Roy, F. and Fiers, W. (1991) Eur. J. Biochem. 195, 465-475.
[45] Dayer, J.M., Beutler, B. and Cerami, A. (1985) J. Exp. Med. 162, 2163-2168.
[46] Bussolino, F., Camussi, G. and Baglioni, C. (1988) J. Biol. Chem. 263, 11856-11861.
[47] Gamble, J.R., Harlan, J.M., Klebanoff, S.J. and Vadas, M.A. (1985) Proc. Natl. Acad. Sci. USA 82, 8667-8671.
[48] Klebanoff, S.J., Vadas, M.A., Harlan, J.M., Sparks, L.H., Gamble, J.R., Agosti, J.M. and Waltersdorph, A.M. (1986) J. Immunol. 136, 4220-4225.
[49] Bevilacqua, M.P., Wheeler, M.E., Pober, J.S., Fiers, W., Mendrick, D.L., Cotran, R.S. and Gimbrone, M.A. (1987) in: Leukocyte Emigration and Its Sequelae (Movat, H.Z. ed.) pp. 79-93, Karger, Basel.
[50] Fiers, W., Beyaert, R., Brouckaert, P., Everaerdt, B., Grooten, J., Haegeman, G., Libert, C., Suffys, P., Takahashi, N., Tavernier, J., Van Bladel, S., Vanhaesebroeck, B., Van Ostade, X. and Van Roy, F. (1989) in: Vectors as Tools for the Study of Normal and Abnormal Growth and Differentiation (Lother, H., Dernick, R. and Ostertag, W. eds.) pp. 229-240, Springer-Verlag, Berlin.
[51] Van Hinsbergh, V.W.M., Kooistra, T., Van den Berg, E.A., Princen, H.M.G., Fiers, W. and Emeis, J.J. (1988) Blood 72, 1467-1473.
[52] Fiers, W., Brouckaert, P., Guisez, Y., Remaut, E., Van Roy, F., Devos, R., Fransen, L., Leroux-Roels, G., Marmenout, A., Tavernier, J. and Van der Heyden, J. (1986) in: The Biology of the Interferon System 1985 (Schellekens, H. and Stewart II, W.E. eds.) pp. 241-248, Elsevier, Amsterdam/New York/Oxford.
[53] Vilček, J., Palombella, V.J., Henriksen-DeStefano, D., Swenson, C., Feinman, R., Hirai, M. and Tsujimoto, M. (1986) J. Exp. Med. 163, 632-643.
[54] Wallach, D. (1984) J. Immunol. 132, 2464-2469.
[55] Balkwill, F.R. (1989) Cytokines in Cancer Therapy, Oxford University Press, Oxford.
[56] Aggarwal, B.B. (1990) in: Tumor Necrosis Factor: Structure, Mechanism of Action, Role in Disease and Therapy (Bonavida, B. and Granger, G. eds.) pp. 49-54, Karger, Basel.
[57] Porter, A.G. (1990) FEMS Microbiol. Immunol. 64, 193-200.
[58] Browning, J. and Ribolini, A. (1989) J. Immunol. 143, 1859-1867.
[59] Cerami, A., Ikeda, Y., Le Trang, N., Hotez, P.J. and Beutler, B. (1985) Immunol. Lett. 11, 173-177.
[60] Tracey, K.J., Lowry, S.F. and Cerami, A. (1987) in: Tumour Necrosis Factor and Related Cytotoxins, pp. 88-108, John Wiley & Sons, Chichester.
[61] Cerami, A. and Beutler, B. (1988) Immunol. Today 9, 28-31.
[62] Tracey, K.J. and Cerami, A. (1989) Curr. Opin. Immunol. 1, 454-461.
[63] Waage, A., Halstensen, A. and Espevik, T. (1987) Lancet i, 355-357.
[64] Grau, G.E., Fajardo, L.F., Piguet, P.F., Allet, B., Lambert, P.H. and Vassalli, P. (1987) Science 237, 1210-1212.
[65] Grau, G.E. (1990) Eur. Cytokine Network 1, 203-210.
[66] Mintz, M., Rapaport, R., Oleske, J.M., Connor, E.M., Koenigsberger, M.R., Denny, T. and Epstein, L.G. (1989) Am. J. Dis. Child. 143, 771-774.
[67] Rook, G.A.W., Taverne, J. and Playfair, J.H.L. (1991) Biotherapy 3, 167-175.
[68] Kunkel, S.L., Strieter, R.M., Chensue, S.W., Campbell, D.A. and Remick, D.G. (1991) Biotherapy 3, 135-141.
[69] Alegre, M., Vandenabeele, P., Flamand, V., Moser, M., Leo, O., Abramowicz, D., Urbain, J., Fiers, W. and Goldman, M. (1990) Eur. J. Immunol. 20, 707-710.
[70] Alegre, M.-L., Vandenabeele, P., Depierreux, M., Florquin, S., Deschodt-Lanckman, M., Flamand, V., Moser, M., Leo, O., Urbain, J., Fiers, W. and Goldman, M. (1991) J. Immunol. 146, 1184-1191.
[71] Kiener, P.A., Marek, F., Rodgers, G., Lin, P.F., Warr, G. and Desiderio, J. (1988) J. Immunol. 141, 870-874.
[72] Rothstein, J.L. and Schreiber, H. (1988) Proc. Natl. Acad. Sci. USA 85, 607.
[73] Neilson, I.R., Neilson, K.A., Yunis, E.J. and Rowe, M.I. (1989) Surgery 106, 439-443.
[74] Ohlsson, K., Björk, P., Bergenfeldt, M., Hageman, R. and Thompson, R.C. (1990) Nature 348, 550-552.
[75] Starnes Jr., H.F., Pearce, M.K., Tewari, A., Yim, J.H., Zou, J.-C. and Abrams, J.S. (1990) J. Immunol. 145, 4185-4191.
[76] Mullen, B.J., Harris, R.B.S., Patton, J.S. and Martin, R.J. (1990) Proc. Soc. Exp. Biol. Med. 193, 318-325.
[77] Stovroff, M.C., Fraker, D.L., Swedenborg, J.A. and Norton, J.A. (1988) Cancer Res. 48, 4567-4572.
[78] Takahashi, N., Brouckaert, P. and Fiers, W. (1991) Cancer Res. 51, 2366-2372.
[79] Oliff, A., Defeo-Jones, D., Boyer, M., Martinez, D., Kiefer, D., Vuocolo, G., Wolfe, A. and Socher, S.H. (1987) Cell 50, 555-563.
[80] Vanhaesebroeck, B., Mareel, M., Van Roy, F., Grooten, J. and Fiers, W. (1991) Cancer Res. 51, 2229-2238.
[81] Grunfeld, C. and Feingold, K.R. (1991) Biotherapy 3, 143-158.
[82] Ehrke, M.J., Ho, R.L.X. and Hori, K. (1988) Cancer Immunol. Immunother. 27, 103-108.
[83] Ranges, G.E., Zlotnik, A., Espevik, T., Dinarello, C.A., Cerami, A. and Palladino Jr., M.A. (1988) J. Exp. Med. 167, 1472-1478.
[84] Brouckaert, P., Libert, C., Everaerdt, B. and Fiers, W. (1991) (submitted).
[85] Everaerdt, B., Brouckaert, P., Shaw, A. and Fiers, W. (1989) Biochem. Biophys. Res. Commun. 163, 378-385.
[86] Waage, A. and Espevik, T. (1988) J. Exp. Med. 167, 1987.
[87] Everaerdt, B., Brouckaert, P. and Fiers, W. (1991) (submitted).
[88] Lehmann, V., Freudenberg, M.A. and Galanos, C. (1987) J. Exp. Med. 165, 657-663.
[89] Wallach, D., Holtmann, H., Engelmann, H. and Nophar, Y. (1988) J. Immunol. 140, 2994-2999.
[90] Libert, C., Van Bladel, S., Brouckaert, P., Shaw, A. and Fiers, W. (1991) J. Immunol. 146, 2625-2632.
[91] Bertini, R., Bianchi, M. and Ghezzi, P. (1988) J. Exp. Med. 167, 1708-1712.
[92] Brouckaert, P. and Fiers, W. (1991) (in preparation).
[93] Parant, F., Tavernier, J., Fiers, W. and Parant, M. (1990) Microb. Pathogen. 8, 143-149.
[94] Suffys, P., Beyaert, R., Van Roy, F. and Fiers, W. (1989) Anticancer Res. 9, 167-172.
[95] Hirano, T., Akira, S., Taga, T. and Kishimoto, T. (1990) Immunol. Today 11, 443-449.
[96] Van Snick, J. (1990) Annu. Rev. Immunol. 8, 253-278.
[97] Matsuda, T. and Hirano, T. (1990) Biotherapy 2, 363-373.
[98] Libert, C., Brouckaert, P., Shaw, A. and Fiers, W. (1990) Eur. J. Immunol. 20, 691-694.
[99] Brouckaert, P.G., Everaerdt, B., Libert, C., Takahashi, N. and Fiers, W. (1989) Agents Actions 26, 196-198.
[100] Fransen, L., Ruysschaert, M.R., Van der Heyden, J. and Fiers, W. (1986) Cell. Immunol. 100, 260-267.

[101] Plaetinck, G., Declercq, W., Tavernier, J., Nabholz, M. and Fiers, W. (1987) Eur. J. Immunol. 17, 1835–1838.
[102] Ranges, G.E., Bombara, M.P., Aiyer, R.A., Rice, G.G. and Palladino Jr, M.A. (1989) J. Immunol. 142, 1203–1208.
[103] Matsushima, K. and Oppenheim, J.J. (1989) Cytokine 1, 2–13.
[104] Palladino, M.A. and Figari, I.S. (1988) Ann. Inst. Pasteur Immunol. 139, 299–301.
[105] Brouckaert, P.G. G., Leroux-Roels, G.G., Guisez, Y., Tavernier, J. and Fiers, W. (1986) Int. J. Cancer 38, 763–769.
[106] Scheringa, M. and Marquet, R.L. (1990) Biotherapy 2, 275–281.
[107] Fiers, W., Brouckaert, P., Goldberg, A., Kettelhut, I., Suffys, P., Tavernier, J., Vanhaesebroeck, B. and Van Roy, F. (1987) in: Tumour Necrosis Factor and Related Cytotoxins (Bock, G. and Marsh, J. eds) pp. 109–123, John Wiley & Sons, Chichester.
[108] Marquet, R.L., Eggermont, A.M.M., De Bruin, R.W.F., Fiers, W. and Jeekel, J. (1988) J. Interferon Res. 8, 319–323.
[109] Baloch, Z., Cohen, S. and Coffman, F.D. (1990) J. Immunol. 145, 2908–2913.
[110] Utsugi, T., Mattern, M.R., Mirabelli, C.K. and Hanna, N. (1990) Cancer Res. 50, 2636–2640.
[111] Watanabe, N., Niitsu, Y., Umeno, H., Sone, H., Neda, H., Yamauchi, N., Maeda, M. and Urushizaki, I. (1988) Cancer Res. 48, 650–653.
[112] Beyaert, R., Vanhaesebroeck, B., Suffys, P., Van Roy, F. and Fiers, W. (1989) Proc. Natl. Acad. Sci. USA 86, 9494–9498.
[113] Kettelhut, I.C., Fiers, W. and Goldberg, A.L. (1987) Proc. Natl. Acad. Sci. USA 84, 4273–4277.
[114] Fraker, D.L., Sheppard, B.C. and Norton, J.A. (1990) Cancer Res. 50, 2261–2267.
[115] Balkwill, F.R., Lee, A., Aldam, G., Moodie, E., Thomas, J.A., Tavernier, J. and Fiers, W. (1986) Cancer Res. 46, 3990–3993.
[116] Balkwill, F.R., Ward, B.G., Moodie, E. and Fiers, W. (1987) Cancer Res. 47, 4755–4758.
[117] Malik, S.T.A., Griffin, D.B., Fiers, W. and Balkwill, F.R. (1989) Int. J. Cancer 44, 918–925.
[118] Malik, S.T.A., Griffin, D.B., Naylor, M.S., Fiers, W., Oliff, A. and Balkwill, F.R. (1990) in: Cytokines and Lipocortins in Inflammation and Differentiation, pp. 393–403, Wiley-Liss, New York.
[119] Ingber, D., Fujita, T., Kishimoto, S., Sudo, K., Kanamaru, T., Brem, H. and Folkman, J. (1990) Nature 348, 555–557.
[120] Jones, A.L. and Selby, P. (1989) Cancer Surveys 8, 817–836.
[121] Spriggs, D.R., Sherman, M.L., Frei III, E. and Kufe, D.W. (1990) in: Tumor Necrosis Factor: Structure, Mechanism of Action, Role in Disease and Therapy (Bonavida, B. and Granger, G. eds.) pp. 233–239, Karger, Basel.
[122] Taguchi, T. and Sohmura, Y. (1991) Biotherapy 3, 177–186.
[123] Kilbourn, R.G., Gross, S.S., Jubran, A., Adams, J., Griffith, O.W., Levi, R. and Lodato, R.F. (1990) Proc. Natl. Acad. Sci. USA 87, 3629–3632.
[124] Scheurich, P., Thoma, B., Unglaub, R. and Pfizenmaier, K. (1988) in: Tumor Necrosis Factor/Cachectin and Related Cytokines (Bonavida, B., Gifford, G.E., Kirchner, H. and Old, L.J. eds) pp. 38–44, Karger, Basel.
[125] Baglioni, C., Ruggiero, V., Latham, K. and Johnson, S.E. (1987) in: Tumour Necrosis Factor and Related Cytotoxins (Bock, G. and Marsh, J. eds.) pp. 52–63, John Wiley & Sons, Chichester.
[126] Scheurich, P., Kobrich, G. and Pfizenmaier, K. (1989) J. Exp. Med. 170, 947–958.
[127] Thoma, B., Grell, M., Pfizenmaier, K. and Scheurich, P. (1990) J. Exp. Med. 172, 1019–1023.
[128] Hepburn, A., Demolle, D., Boeynaems, J.M., Fiers, W. and Dumont, G.E. (1988) FEBS Lett. 227, 175–178.
[129] Hepburn, A., Boeynaems, J.M., Fiers, W. and Dumont, J.E. (1987) Biochem. Biophys. Res. Commun. 149, 815–822.
[130] Matthews, N., Neale, M.L., Jackson, S.K. and Stark, J.M. (1987) Immunology 62, 153–155.
[131] Beyaert, R., Suffys, P., Van Roy, F. and Fiers, W. (1990) FEBS Lett. 262, 93–96.
[132] Suffys, P., Van Roy, F. and Fiers, W. (1988) FEBS Lett. 232, 24–28.
[133] Pober, J.S., Lapierre, L.A., Stolpen, A.H., Brock, T.A., Springer, T.A., Fiers, W., Bevilacqua, M.P., Mendrick, D.L. and Gimbrone, M.A. (1987) J. Immunol. 138, 3319–3324.
[134] Matthews, N. (1983) Br. J. Cancer 48, 405–410.
[135] Wong, G.H.W., Elwell, J.H., Oberley, L.W. and Goeddel, D.V. (1989) Cell 58, 923–931.
[136] Oberley, L.W. and Buettner, G.R. (1979) Cancer Res. 39, 1141–1149.
[137] Lancaster Jr., J.R., Laster, S.M. and Gooding, L.R. (1989) FEBS Lett. 248, 169–174.
[138] Schulze-Osthoff, K., Beyaert, R., De Valck, D., Grooten, J., Vanhaesebroeck, B., Bakker, A. and Fiers, W. (1991) Submitted.
[139] Beresini, M.H., Lempert, M.J. and Epstein, L.B. (1988) J. Immunol. 140, 485–493.
[140] Beyaert, R., Schulze-Osthoff, K., Van Roy, F. and Fiers, W. (1991) Cytokine 3 (in press).
[141] Defilippi, P., Poupart, P., Tavernier, J., Fiers, W. and Content, J. (1987) Proc. Natl. Acad. Sci. USA 84, 4557–4561.
[142] Vandevoorde, V., Haegeman, G. and Fiers, W. (1991) Submitted.
[143] Lin, J.-X. and Vilček, J. (1987) J. Biol. Chem. 262, 11908–11911.
[144] Haliday, E.M., Ramesha, C.S. and Ringold, G. (1991) EMBO J. 10, 109–115.
[145] Osborn, L., Kunkel, S. and Nabel, G.J. (1989) Proc. Natl. Acad. Sci. USA 86, 2336–2340.
[146] Yasukawa, K., Hirano, T., Watanabe, Y., Muratani, K., Matsuda, T. and Kishimoto, T. (1987) EMBO J. 6, 2939–2945.
[147] Hohmann, H.P., Remy, R., Pöschl, B. and Van Loon, A.P.G.M. (1990) J. Biol. Chem. 265, 15183–15188.
[148] Israël, A., Le Bail, O., Hatat, D., Piette, J., Kieran, M., Logeat, F., Wallach, D., Fellous, M. and Kourilsky, P. (1989) EMBO J. 8, 3793–3800.
[149] Hohmann, H.P., Lötscher, H.R., Brockhaus, M., Remy, R., Baeuerle, P.A. and Van Loon, A.P.G.M. (1991) (submitted).
[150] Zhang, Y., Lin, J.-X. and Vilček, J. (1990) Mol. Cell. Biol. 10, 3818–3823.
[151] Shimizu, H., Mitomo, K., Watanabe, T., Okamoto, S. and Yamamoto, K. (1990) Mol. Cell. Biol. 10, 561–568.
[152] Libermann, T.A. and Baltimore, D. (1990) Mol. Cell. Biol. 10, 2327–2334.
[153] Suffys, P., Beyaert, R., Van Roy, F. and Fiers, W. (1988) Eur. J. Biochem. 178, 257–265.