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Tumor Necrosis Factor α and Toxicology

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ABSTRACT: The molecular cloning of a group of proteins, collectively referred to as cytokines, and including interleukins, chemokines, growth factors, colony stimulating factors, and tumor necrosis factors, has allowed for the increased understanding of the mechanisms for many disease processes as well as provided strategies for the development of novel therapies. Conceptually similar to hormones and peptides, this group of phylogenetically related molecules are also involved in various toxicological processes, including apoptosis, cell repair, and in particular inflammation. In this review, we offer a description of what many believe represents the primary regulatory cytokine, tumor necrosis factor (TNF)α and its role in toxicological processes. For over a decade it has been suspected that this molecule helps mediate the shock state induced by bacterial endotoxin and the wasting diathesis that typifies chronic diseases. Advances in molecular biology that have provided tools to modulate TNFα regulation and synthesis have allowed for the identification of additional roles for TNFα in homeostasis, cellular damage, and repair. This review provides a brief summary of our understanding of TNFα biology followed by a discussion of its role in toxicological responses. This is followed by specific examples of organ-specific and tissue-specific responses to chemical damage where TNFα has been implicated. The review concludes with a review of its implication in human risk assessment, particularly as it relates to genetic polymorphisms of TNFα expression and disease susceptibility.

KEY WORDS: tumor necrosis factor and toxicity, proinflammatory cytokines and chemical damage.

I. INTRODUCTION

Tumor necrosis factor (TNF)α, a 17-kDa polypeptide, was first identified as causing a wasting syndrome in tumor-bearing mice, hemorrhage reduction in the size of some tumors, and necrosis in normal tissues.[1–3] Although the cytokine is initially synthesized in a 31-kDa precursor form, the N-terminal sequence is removed prior to secretion in a 17-kDa form.[4] The protein sequence is well conserved as human TNFα shows 80% homology with mouse or rabbit. A variety of cell types produce TNFα. In the pathological processes, tissue fixed macrophages, such as Langerhan's cells, Kupffer cells, and astroglia, are believed to be the major sources of TNFα.[6] However, other cell types, including endothelial cells, epithelial cells, and fibroblasts, secrete significant amounts when treated with appropriate stimuli. Endotoxin is the major stimulator for TNFα but other agents, including certain cytokines themselves, are also effective. Mitochondrial radical production and a variety of enzymes, including phospholipases,

S mases, and protein kinases, can also participate in the cellular activation of TNFα.[5] As a number of toxic chemicals possess prooxidant activities and modulate cellular phospholipases, it is conceivable that some toxic agents, such as ultraviolet radiation, may act directly to stimulate TNFα activity. At the transcriptional level, TNFα is regulated by a TATA box but promoted through a series of regulatory sequences, including binding sites for nuclear factor-kappa β (NF-κB), nuclear factor of activated T cells (NFAT), cAMP-responsive element (CRE), activation transcription factor-2 (ATF-2)/Jun, two SV40 promoter-1 (SP-1), activating protein-1 (AP-1) and AP-2.

Like other cytokines, TNFα confers its signals to target cells through binding to specific cell surface membrane receptors. These receptors are found on almost all nucleated cells. Two distinct receptors mediate the biological activities of TNFα, one of a molecular mass of 55 kDa (p55, R1) and the other of 75 kDa (p75, R2). The p55 receptor is constitutively expressed and historically has been considered the main mediator for TNFα responses (Figure 1). The p75 receptor is inducible and provides for ligand passing and induction of soluble TNF receptor molecules as well as limited responses. In addition, two distinct families of proteins, the TNF receptor-associated factors (TRAFs) family of adaptor proteins and the death domain homologs, help coordinate cell- and tissue-specific responses in this complex pathway by interacting with the intracellular unit of the TNF receptors and initiating cell-signaling pathways.[7] For noncytotoxic processes, TNFα serves primarily to induce the synthesis of biologically active proteins by causing changes in gene expression. This occurs through a complex signaling pathway referred to as mitogen-activated protein (MAP) kinase/extracellular signal-regulated kinase (ERK), collectively referred to as MAPK. Phosphorylation of these kinases results in activation of nuclear transcription factors, arguably, the most important being NF-κB.[8] NF-κB is a dimer of the Rel family of proteins that is activated following dissociation of an inhibitor protein belonging to the IκB family. Once dissociated, an NF-κB dimer can translocate to the nucleus and alter the expression of genes such as IL-6.[9]

TNFα was originally characterized as a protein inducing necrosis of methylcholanthrene-induced sarcomas. Its cytostatic and cytotoxic activities are associated primarily with certain transformed cell lines, tumor cells, and, to a lesser extent, normal cells. Depending on the target cell, TNFα can induce necrotic or apoptotic cell death.[10] Cytotoxicity by TNFα, unlike its other functions, can occur independently of *de novo* gene transcription and translation and involves mitochondrial production of oxygen radicals generated primarily at the semiquinone site. This requires ceramide, a sphingolipid generated in cells following stimulation with TNFα, which generates H_2O_2 from the mitochondrial electron transport chain. The major function attributed to TNFα is not cytotoxicity, but rather synthesis of gene products that influence the cell as in differentiation, growth stimulation, antiviral activity, immunomodulation, and inflammation.[5,11] For example, TNFα has been shown to be mitogenic for a number of normal cell types such as fibroblasts, hepatocytes, smooth muscle cells, and lymphocytes. It also increases activities of natural killer (NK) cells, endothelial cells, macrophages/monocytes, neutrophils, and lymphocytes. TNFα stimulates the production of several hormones, including ACTH and thyroid-stimulating hormones.[12] In muscle cells, TNFα stimulates glycolysis and glycogenesis,[13] whereas in synovial fluid it stimulates the synthesis of PGD_2, plasminogen activator, collagenase, and hyaluronic acid.[14] Because of the influence chronic inflammation plays in disease processes, recent attention in toxicology has focused on its role in regulating inflammatory processes.[1–3] This occurs through the

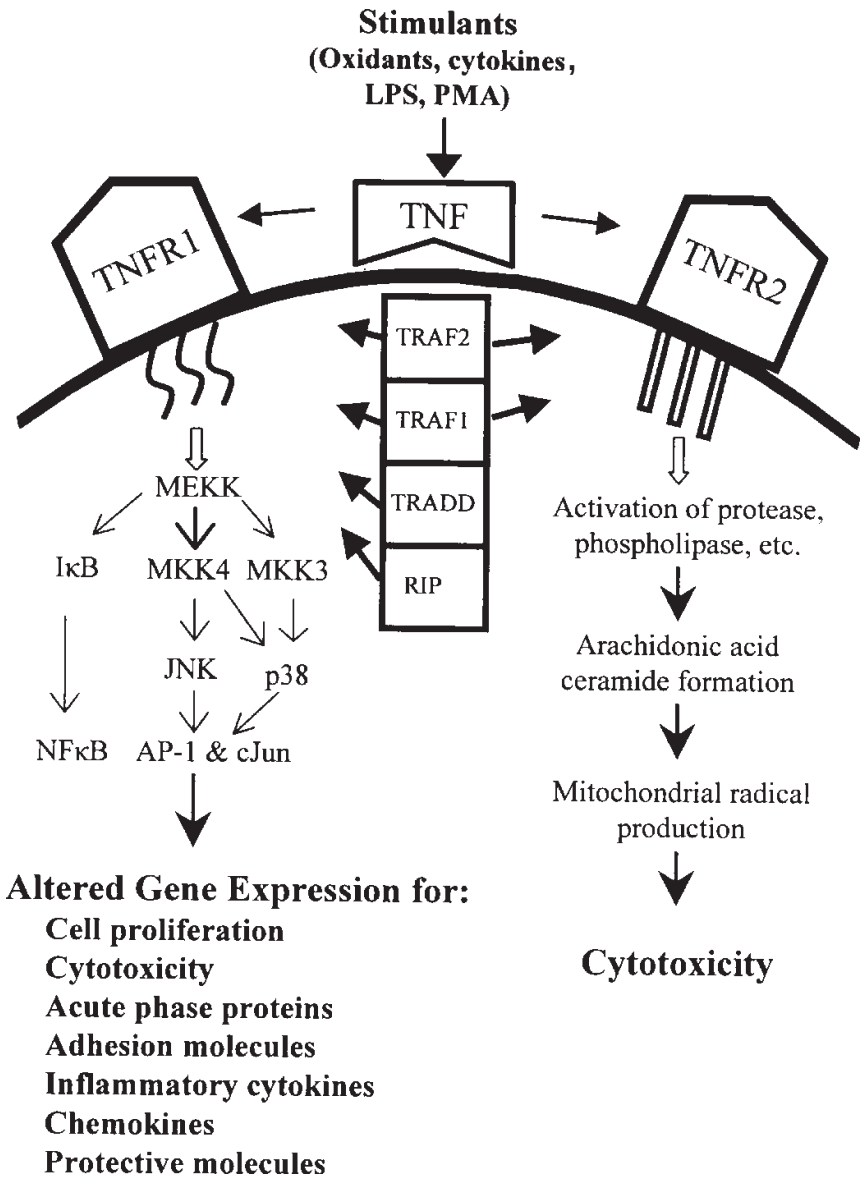

FIGURE 1. Model for TNFα action. TNFα, which is induced by a variety of stimulants, can bind to either the TNFR1 (p55) or TNFR2 (p75) receptor. Although not yet fully established, TNFR1 is the major inducer of biological effects, whereas TNFR2 appears to be confined mainly to cells of the immune system and is responsible for cytotoxic effects. TNF cell signaling, which is highly regulated and complex, occurs via the MEK-MAP kinase kinase (MEKK) pathway, resulting in the activation of the NF-κB and AP-1 transcription factors. These transcription factors regulate genes involved in cell proliferation, apoptosis, and inflammation. In addition, there is a ceramide-related pathway that leads to cytotoxicity independent of gene transcription. Regulation is also provided through a family of adaptor proteins and death domain homologs (TRAFs) that interact with TNF receptors to produce a cell signal (other abbreviations in text).

ability of TNFα to provide cell signals and regulate genes that code for inflammatory mediators, such as IL-1, IL-6, IL-8, macrophage inflammatory protein (MIP)-2, granulocyte macrophage-colony stimulating factor (GM-CSF), intracellular adhesion molecule (ICAM)-1, and endothelial leukocyte adhesion molecule (ECAM)-1. In con-

cert, these mediators locally enhance vascular permeability, stimulate the expression of adhesion molecules on endothelial cells, and serve as leukocyte activators and chemoattractants (i.e., chemokines). Most genes that code for inflammatory mediators, including acute phase proteins, contain binding sites for the NF-κB and, to a lesser extent, AP-1 transcription factors in the cis-acting elements of the promoter region that helps regulate their expression.

II. ROLE IN TOXICOLOGICAL PROCESSES

The role of TNFα in chronic diseases is viewed by many immunologists and toxicologists with considerable interest as more diseases, ranging from arthritis and Alzheimer's to idiopathic pulmonary fibrosis and chronic hepatitis, manifest an inflammatory component that exacerbates, albeit to varying degrees, disease severity.[5,11] The general hypothesis that links these pathologies is summarized in Figure 2 and is as follows: initial injury, whether initiated by an infectious, chemical, or environmental agent, produces focal tissue necrosis in the target organ through any one of several toxic mechanisms (e.g., lipid peroxidation, mitochondrial damage). As a result of this damage, tissue-fixed macrophages along with adjacent endothelial and epithelial cells become activated and secrete products that cause additional cell damage or induction of inflammatory products. Some of these products are short-lived, such as reactive oxygen species (ROS) and the nitrogen-centered radical, nitric oxide (NO·).[15] Other products, such as arachidonic acid and proinflammatory cytokines, regulate the production of additional inflammatory mediators and, thus, amplify as well as propagate these responses. The recruitment and activation of neutrophils and circulating monocytes into the site are believed to be important in the toxicological processes, as these cells cause additional damage via degranulation and release of neutral proteinase or the generation of superoxide anions (O_2^-) via NADPH-oxidase during the respiratory burst. O_2^-, which itself mediates tissue damage, can be reduced further to other cell-damaging reactive oxygen intermediates (ROIs), including H_2O_2 and hydroxyl radical (OH·).[6] The ultimate toxicity of O_2^- may depend on its ability to bring about the reduction of Fe^{+3} to Fe^{+2}, providing for the generation of OH· from H_2O_2, and to interact with NO· to generate peroxynitrite (ONOO·) and subsequently OH·. The overall effect of this complex series of events is a chronic inflammatory response resulting in tissue damage that may either lead to fibrotic changes or recovery.[16] TNFα is also associated with the activation of repair mechanisms following xenobiotic damage. Although these particular mechanisms have not been well defined, they appear to involve the ability of TNFα to control the replication of various cell types, such as fibroblasts and hepatocytes, by acting as a mitogen as well as stimulating apoptosis.[5,11]

Support that TNFα can mediate organ-specific toxic or repair responses stems from several observations: (1) elevated levels of TNFα can be found in target organs following exposure to certain occupational and environmental agents; (2) administration of TNFα in experimental animals mimics many of the pathophysiological responses associated with the toxic response; (3) organs in which these types of responses occur contain resident (tissue-fixed) macrophages or macrophage-like cells capable of producing high levels of TNFα; and (4) inhibition of TNFα prevents many of the pathophysiological or repair responses from occurring. The use of transgenic mice that overexpress TNFα or have a nonfunctional TNF gene (knockouts) and the development of specific pre- and posttranscriptional TNF inhibitors have

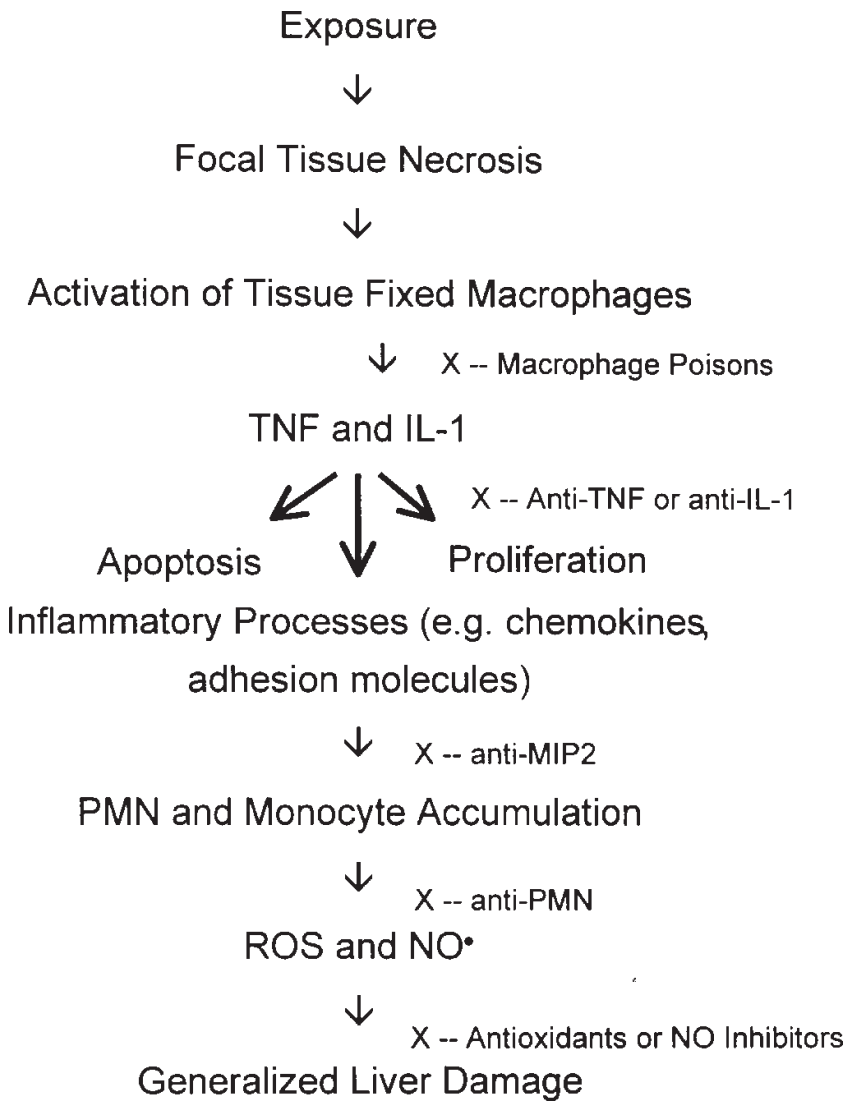

FIGURE 2. Hypothesis of chemical-induced liver injury. Increasing evidence has suggested that inflammation plays a major role in chemical-induced hepatotoxicity and repair. Initial damage produces focal tissue necrosis, resulting in activation of tissue-fixed macrophages. These activated macrophages secrete primary cytokines, including IL-1 and TNFα, which may induce apoptosis, stimulate cell growth, or initiate inflammatory processes. The inflammatory process ultimately results in the recruitment of activated neutrophils and the release of toxic products such as reactive oxygen species (ROS) and nitric oxide. Although exemptions to this scheme exist, the hypothesis is based primarily on the use of different types of inhibitors.

been instrumental in allowing for these observations.

III. TNFα AND ORGAN-SPECIFIC TOXICITY

Although TNF has presumably evolved to provide homeostatic processes by participating in tissue repair and host defense mechanisms, its overproduction can lead to damage of normal cells and lethality.[18] The pathophysiological sequelae associated with its overproduction or underproduction in chemical toxicities are probably similar to the effects in their idiopathic counterparts such as septic shock, inflammatory lung disease, and autoimmune disease. The following section describes the established asso-

ciations between TNF and organ-specific, chemical-induced toxic responses.

IV. LIVER

Traditionally, the study of xenobiotic-induced hepatic injury has focused on the direct actions of hepatotoxins or their metabolites on cellular targets in the liver. However, emphasis has shifted recently toward indirect mechanisms, and, in particular, the role of nonparenchymal cells and their soluble mediators in liver injury. This was stimulated by the observation that the inactivation or depletion of Kupffer cells decreases toxicity from exposure to hepatotoxins such as galactosamine,[19] phalloidin,[20] ethanol,[21] acetaminophen,[22] diethyldithio-carbamate,[23] and carbon tetrachloride.[24] Increasing evidence suggests that mediators released from activated Kupffer cells, including TNFα, are important modulators of this injury. In this respect, TNFα proximally mediates acute phase responses, inflammatory cell infiltration, hyperlipidemia, free oxygen radical generation, fibrogenesis, and cholestasis in the liver.[25–27] Elevated levels of TNFα occur in a variety of acute and chronic liver diseases, including viral or alcoholic hepatitis, biliary obstruction, and ischemia,[27–29] as well as in experimental animals treated with hepatotoxins such as carbon tetrachloride,[30] cadmium chloride,[31] acetaminophen,[32] dimethylnitrosamine,[33] ethanol,[34] plant lectins,[35] and fumonisin B1.[36] A model of xenobiotic-induced inflammatory liver injury has been proposed (see Figure 2) wherein liver damage is thought to occur when a hepatotoxin, such as CCl_4 is metabolized to a highly reactive form (i.e., chloro-trimethyl radical) that is capable of interacting with membrane lipids, resulting in lipid peroxidation and subsequent cellular necrosis.[37] Resident macrophages at the site of damage become activated and secrete inflammatory mediators, including TNFα, which causes infiltration of activated neutrophils and monocytes into the damaged organ. These cells produce additional cytokines, ROS, and reactive nitrogen species that exacerbate the damage. That TNFα is involved in this response can be evidenced by the ability to modulate the pathological sequelae by influencing *in vivo* TNFα synthesis.[34,38–39] An obligatory role for endotoxin in this response has also been suggested.[40]

Although most studies dealing with TNFα-associated hepatotoxicity have addressed necrotic cell death, hepatic apoptosis has been observed following exposure to hepatotoxic chemicals.[41–44] The direct role of TNFα in this response has not been examined, although in the presence of transcriptional inhibitors TNFα induces apoptosis in hepatocyte cell cultures.[45,46] Recently, Leist et al.[47] reported that the hepatotoxins, amanitin, and actinomycin D, both transcriptional inhibitors, mediate liver failure directly through TNFα activity. Furthermore, mice treated with neutralizing antibodies to TNFα, as well as those deficient in p55 expression, are relatively resistant to the toxicity of either chemical. Under other circumstances, TNFα has been implicated in liver repair following chemical damage through its ability to initiate parenchymal and nonparenchymal cell proliferation.[39,48–50] In this respect, TNFα is necessary for liver regeneration following partial hepatectomy[49] and administration of TNFα *in vivo* increases hepatic DNA synthesis within 12 to 24 h.[50,51] The mechanism by which TNFα stimulates hepatic cell proliferation involves both IL-6-dependent[52] and -independent processes.[17] Using a CCl_4-induced hepatotoxicity model in rodents, it has been demonstrated that both TNFα induction and liver repair following chemical damage require the induction of early immediate genes, such as *c-jun* and *c-fos*, and activation of the cEBP, AP-1, and NF-κB transcription factors in hepatocytes.[39–53] DNA binding sites for these transcription factors are found in the promoter region of the TNFα gene.

A number of chemicals are not toxic but mitogenic for hepatocytes, causing an in-

crease in liver mass *in vivo*. These chemicals have been implicated in liver cancer by acting as tumor promoters. Lead nitrate[51,54] and ethylene dibromide[55] both induce TNFα expression in the liver and spleen of experimental animals and stimulate liver hyperplasia without associated necrosis or inflammation. The hyperplasia is temporally similar to that induced by TNFα itself, in that peak DNA synthesis of parenchymal cells is preceded by a wave of nonparenchymal cell proliferation.[52] However, the induction of growth factors normally associated with liver regeneration, such as hepatocyte growth factor (HGF), transforming growth factor (TGF)α, and TGFβ, are not apparent following exposure to these chemicals,[55] and a definitive role for TNFα in carcinogenesis has yet to be described. Furthermore, these substances may not directly stimulate TNFα, but rather enhance the sensitivity of Kupffer cells to endotoxin.[54] WY14643 (WY), a prototype peroxisome proliferator and potent rodent liver carcinogen, also increases liver mass and hepatocyte mitogenesis through a TNFα-dependent pathway.[56] Inhibition of Kupffer cell function by methyl palmitate[56] or treatment of mice with neutralizing antibodies to TNFα[57] prevents hepatic DNA synthesis induced by WY. Although not a peroxisome proliferator, gadolinium chloride ($GdCl_3$) can induce hepatocyte mitogenesis through modulation of TNFα.[58] Whereas $GdCl_3$ has been used to inactivate Kupffer cells, it also induces hepatocyte DNA synthesis[58] and TNFα expression.[59] Similar to WY, administration of neutralizing antibodies to TNFα also prevents the mitogenic response to $GdCl_3$.

V. KIDNEY

Although considerable evidence exists to suggest cytokines play a role in the progression of classic renal diseases, few studies have focused on their role in xenobiotic-induced nephrotoxicity.[60–63] Regarding the former, the overexpression of cytokines, particularly TNFα and IL-1, are involved in many of the structural and functional changes in inflammatory kidney diseases such as Goodpasture syndrome, crescentic glomerulonephritis, proliferative glomerulonephritis, lupus nephritis, IgA nephropathy, as well as renal allograft rejection (reviewed in rRefs.[64–66]) This is due, in part, to the ability of TNFα to induce the production of complement components, ROS and prostanoids, in the kidney.[64] In addition to mediating kidney inflammation, TNFα has been shown to reduce glomerular blood flow, alter glomerular basement membrane permeability, and activate glomerular endothelial and mesangial cells.[64–66] The pivotal role TNFα plays in glomerulonephritis has been demonstrated by the ability of neutralizing antibodies to attenuate glomerular injury in experimental nephrotoxic nephritis.[65] Furthermore, the administration of exogenous TNFα can induce severe proteinuria, glomerular injury, and tubulointerstitial nephritis, thereby mimicking the pathophysiological response in nephrotoxic nephritis.[65]

The importance of TNFα in various classic nephropathies might suggest a similar role in chemical-induced injury. In this respect, suggestive evidence has been presented of a relationship between TNFα, exposure to heavy metals leading to glomerulonephritis and tubulointerstitial lesions, and the development of autoimmune disease.[67] Direct evidence for an association between nephrotoxicity and TNFα was provided in studies of rodents exposed to $CdCl_2$, where significant increases in kidney levels of TNFα and IL-6 occurred.[68] Although a direct role for TNFα in the kidney pathology was not found, IL-6, which is induced by TNFα, was shown to support renal tubular epithelial regeneration, suggesting an involvement in repair processes. A similar role for IL-6 was observed in an experimental model of nephrotoxic nephritis, where IL-6 infusion resulted

in the reduction of TNF and IL-1, along with the inhibition of proteinuria, monocyte infiltration and activation.[69] Corresponding to the decrements in TNFα and IL-1 levels associated with IL-6 treatment, there were significant increases in the level of IL-1 receptor antagonist and soluble TNF receptor, which serve as endogenous cytokine antagonists.

Adriamycin induces nephrosis characterized by proteinuria, glomerular epithelial damage, and interstitial leukocyte influx.[70] TNFα expression in this lesion corresponds with cellular infiltration and the presence of the chemotactic protein, interferon-inducible protein (IP)-10 within the glomerulus. The levels of both proteins parallel the development of proteinuria and tubulointerstitial nephritis. Nephrotoxicity of adriamycin is presumably due to a direct interaction of the drug with endogenous glomerular cells resulting in the release of TNFα and platelet activating factor (PAF), which in turn initiates a cascade of events leading to renal damage. The antiviral drug puromycin aminonucleoside represents a model for minimal change nephritis characterized by progressive renal fibrosis.[71] ROS are responsible for the changes in the podocytes, and, similar to adriamycin, high levels of PAF and TNFα in the mesangium are associated with a cellular influx, as well as the synthesis of TGFβ, leading to the accumulation of extracelluar matrix proteins in the glomerulus and the interstitium. Taken together, these data at least provide suggestive evidence that TNFα is involved in various aspects of xenobiotic-mediated kidney toxicity.

VI. CENTRAL NERVOUS SYSTEM

The nervous and immune systems show an intimate structural and functional relationship which exhibits extensive bidirectional communication mediated by common regulatory factors and receptors. TNFα is expressed in different brain regions at different stages of development, with the primary cellular sources being astrocytes and glial cells with neurons providing only a minor contribution.[72–74] In addition to locally produced TNFα, peripheral TNFα can penetrate the CNS through a physically or pathologically modified blood-brain barrier.[74–76] The expression and occurrence of TNFα in the brain is associated with changes associated with inflammatory processes, examples of which are listed in Table 1. Several studies have demonstrated that TNFα is involved in physiological sleep regulation and increased non-rapid eye movement sleep.[84] Extensive evidence exists for TNFα involvement in the pathogenesis of inflammatory demyelinating diseases, such as multiple sclerosis and experimental allergic encephalomyelitis (EAE) in mice.[85,86] Recently, it has been demonstrated that the expression of a TNFα transgene in the CNS of mice triggers the development of a chronic inflammatory demyelinating disease, which includes progressive neurological symptoms such as ataxia and seizures.[87,88] The pathological features

TABLE 1
Effects of TNFα in the CNS Possibly Associated with Neurodegenerative and Inflammatory Diseases

Response	Ref.
Cytotoxic to oligodendrocytes	77,78
Stimulates neutral sphingomyelinase	79
Up-regulates class I MHC on astrocytes	80
Enhances INFγ-induced class II MHC on astrocytes	81
Mitogenic for primary astrocytes and astroglioma	82
Induces NO synthase causing cerebral vasodilation	83

of this model, which include astrocytosis, reactive microgliosis, aberrant T-cell entry into the CNS, and myelin disruption, are characteristics of human inflammatory and demyelinating diseases. TNFα is also believed to play a key role in the pathogenesis of some CNS parasitic and bacterial infections, such as meningitis and cerebral malaria, as well as AIDS dementia complex, neurodegenerative diseases, and ischemia.[89–91]

The significance of TNFα in CNS pathophysiology has been studied using TNFα neutralization treatments in established animal disease models or transgenic mice that overexpress or underexpress TNFα. For example, TNFR2-deficient mice are resistant to experimental cerebral malaria when compared with TNFR1-deficient or wild-type mice.[79] This phenomenon has been related to the absence of ICAM expression in brain microvessels of TNFR2-deficient mice. Studies on the role of TNFα in brain ischemic injury are controversial and may be related to differences in the models employed or time-courses studied. TNFα is released during focal ischemia or early reperfusion, and neutralization reduces the injury and improves the neurological outcome.[92] Using TNFR knockout mice, exacerbation of neuronal damage after focal cerebral ischemia occurred.[93]

While substantial evidence is provided for TNFα's role in CNS diseases and trauma, the participation of TNFα in chemically induced neurotoxic responses has been considered only rarely. Overexpression of TNFα in the brain accompanies neurotoxicity in rats and mice associated with trimethyltin exposure, an organotin compound.[94] Recently, these studies have been confirmed and extended to demonstrate that trimethyltin induces TNFα expression and secretion in glial cell cultures.[95] Consistent with neurons as the primary target of trimethyltin intoxication following developmental exposure, it has been shown that trimethyltin induces apoptosis in neuronal/glial co-cultures through TNFα originating from glial cells.[96] In addition to trimethyltin, a broad group of neurotoxicants, including triethyltin, lead, thallium, and tellurium, selectively affect myelination in the adult, although the role of TNFα in these responses has not been studied.

VII. LUNG

Chronic inflammatory lung diseases, such as idiopathic pulmonary fibrosis, granulomatous diseases, chronic bronchitis, adult respiratory distress syndrome, cystic fibrosis, and asthma, are associated with elevated levels of TNFα in lung fluids.[97–101] Evidence that TNFα is also important in the development of environmental lung diseases stems from both direct and indirect observations. First, a broad spectrum of chemical-induced lung diseases are associated with elevated TNFα level in the lung, including pollutant-induced inflammatory disease,[102–104] fibrotic disease,[105] hypersensitivity pneumonitis, granulomatous disease,[106–108] occupational asthma, and respiratory hypersensitivity (see Table 2 for specific examples). These elevations can be in excess of 100-fold as occurs during fibrotic reactions in animals as well as humans exposed to bleomycin, silica, or asbestos.[106,110,111,116] Secondly, TNFα infusion in experimental animals mimics many of the pathological sequelae observed following exposure to pulmonary toxicants such as the growth of fibroblasts, lung collagen deposition, and inflammation.[125–128] Finally, mice pretreated with products to inhibit TNFα, such as soluble receptors or neutralizing antibodies, are either resistant or show modified responses to pulmonary toxicity from asbestos, silica, bleomycin, quartz, or ozone.[117,122,132,133]

The pathophysiologic events associated with environmentally induced inflammatory lung disease are similar to that which occurs from immune complex-induced lung injury. This involves sequentially, the deposition of pulmonary toxicants resulting in cell damage, activation and release of bioactive products, including TNFα, the upregulation of

TABLE 2
Examples of Pulmonary Toxicants Associated with Elevated Levels of TNFα

Agents	Pathology (Ref.)
Amiodaroe	Pulmonary toxicity[109]
Beryllium	Granulomas[114]
Bleomycin	Fibrosis[106]
Carbon	Monoxide necrosis[105]
Fibers/asbestos, silica	Inflammation/fibrosis/cancer[110,111,116,123,124]
Grain dust	Inflammation[112,113]
Ozone Inflammation/irritation[120,121,122]	
Isocyanates	Respiratory hypersensitivity[115]
Lead oxide	Inflammation/cancer[119]
Urban/diesel particles	Inflammation[117,118]

lung vascular ICAM-1 and E-selectin, the recruitment of blood neutrophils, and ultimately tissue injury and matrix formation by oxidants and proteases from neutrophils and macrophages. As in other organ systems, TNFα, itself does not directly mediate these responses, but rather plays a central role in regulating other mediators. TNFα studies in lung inflammation have focused on its ability to induce chemokines, such as IL-8, MIP-2, and MIP-1α, which are potent neutrophil and monocyte activators and chemo-attractants.[122–124] However, TNFα may also produce direct lung effects such as damage to the pulmonary vascular endothelium and subsequent capillary leakage.[125–128] TNFα is present at low levels in normal lungs, where it may provide a protective role, such as through the expression of superoxide dismutase, a potent antioxidant enzyme.[129] In this respect, TNFα levels in the lung are reduced in smokers, which may account for their increased susceptibility to infections and decreased incidences to some autoimmune and inflammatory diseases. The role of TNFα in lung fibrogenesis is less clear, but it may have a dual role. During injury, when uncontrolled fibroblast proliferation occurs, TNFα production is increased to slow down the fibroproliferative response, whereas during repair, when fibroblasts participate in structural restitution, it stimulates cell proliferation.[130] The major source of pulmonary TNFα is thought to be alveolar macrophages.[131] However, under most conditions lung endothelial and epithelial cells can also contribute significantly to the TNFα pool.

The signaling events responsible for TNFα induction in the lung are relatively complex, involving both direct and indirect events. TNFα itself as well as certain pollutants, such as vanadium pentoxide, asbestos, silica, and ozone, generate ROS. This in turn can signal the Ras pathway,[134] ultimately activating AP-1, NF-κB, and NF-IL-6, members of oxidant-sensitive nuclear transcription factor families, which help regulate genes involved in inflammatory processes, including TNFα.[135] TNFα can also be induced in an autocrine manner, independent of both Ras and ROS, by the activation of NF-κB through phosphatase-dependent IκB activation via members of the TNF receptor-associated factors (see Figure 1).[136]

VIII. SKIN

Keratinocytes, and, to a lesser extent, endothelial cells and resident dendritic cells (Langerhan's cell), are the major sources of dermal cytokines,[139] including TNFα.[132,135–139] In the skin, TNF gene expression occurs in the epidermis within minutes after either physical damage or exposure to ultraviolet light (UV-B), contact allergens, or irritants.[141–147] Irritant contact dermatitis (ICD), caused by agents such as phenol, sodium lauryl sulfate, croton oil, and

picryl chloride, results in the immediate induction of TNFα,[148] with the levels of TNFα corresponding to the intensity of the inflammatory response.[149] Multiple lines of evidence indicate that TNFα is required for the development of allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD).[143,145,148,150–152] In this respect, many of the clinical features of ACD or ICD are reduced or prevented in TNF receptor knockout or in wild-type mice administered neutralizing TNF antibodies, whereas intradermal injection of TNFα produces many of their dermal features, including Langerhan's cell migration, epidermal necrosis, leukocyte infiltration, and hemorrhagic necrosis. Furthermore, TNFα can be found at high levels in the skin during acute episodes of irritant or allergic contact dermatitis. TNFα may not directly initiate inflammatory responses by contact irritants. This may occur through the release of preformed IL-1α from injured keratinocytes that serves to stimulate TNFα production. Mechanical disruption of the skin from tape-stripping induces significant levels of TNFα, IL-1, and IL-6 at the site of the disrupted barrier, suggesting that TNFα is also involved in minor irritation.[147] Similarly, pretreatment of mice with agents that reduce circulating levels of TNFα reduce many of the clinical features of minor contact irritant responses such as edema and eosinophil accumulation.[143–148] When irritants, such as phenol and croton oil, are administered to p55 knockout mice, inflammatory responses are markedly reduced in the knockout mice, compared with the wild type.[145] Furthermore, after intradermal injection of exogenous TNFα, inflammation is significantly lower in the p55 mice, suggesting that, as with most TNF responses, p55 is critical.[145] Surprisingly, ear painting with contact sensitizers results in normal Langerhan's cell migration in p55 knockout mice, whereas p75 knockout mice exhibit a 50% reduction in antigen-positive dendritic cells in the draining lymph nodes, suggesting that the p75 receptor is important in Langerhan's cell migration.[152] In ACD, TNFα is responsible for initiating immune responses by signaling antigen-presenting (Langerhan's) cells of the skin to migrate to regional lymph nodes,[153] probably via the induction of adhesion molecules, where they present processed antigen to T lymphocytes.

A large number of specific pathologies and clinical manifestations have been ascribed to the overexpression and release of TNFα in the skin and for brevity are summarized in Table 3. As previously indicated,

TABLE 3
Potential Skin and Histological Changes and Diseases Associated with Overexpression of Dermal TNFα

Characteristic	Disease (example)
Histocytosis	Urticaria
Mucocutaneous Leishmaniasis	Psoriasis
Dermatitis Herpetiformis	Eczema
Contact Dermatitis	SLE
Histological occurrences	
Edema/wheal	Scaling
Hemorrhage	Erythema
Necrosis	Thrombi
Ulceration	Vesiculation
Vasoconstriction	
Inflammatory cell influx (PMNs, monocytes, lymphocytes)	

overexpression of TNFα in the skin may not always be associated with pathological events because TNFα has been implicated in epidermal cell proliferation and wound repair by helping to regulate angiogenesis and keratinocyte differentiation.[154] In psoriasis, however, TNFα has been associated with keratinocyte and dermal endothelial cell hyperproliferation through its ability to induce EGF receptors and TGF.

Skin damage from UV-B irradiation, which presents erythema, epidermal proliferation, leukocyte influx, keratinocyte apoptosis, and proinflammatory cytokine production at the site of exposure, is also associated with overexpression of TNFα.[143] Antigen-specific immunosuppression, which also may occur in response to UV-B, is partially regulated by TNFα.[155,156] Although the mechanism remains to be clarified, it is believed that UV-induced DNA damage signals for excessive cytokine production and that polymorphisms in the TNF gene may be associated with susceptibility to UVB effects.

IX. TNFα AND GENETIC POLYMORPHISMS

The gene for TNFα is located within the class II region of the MHC, between HLA-B and DR. Its expression is tightly controlled at the transcriptional and post-transcriptional levels. Recently, polymorphisms within the TNF loci in humans have been described.[157–158] In particular, two G vs. A transitions in the promoter region at positions -308 and -238 have been shown to influence the level of TNFα expression in response to various stimuli. The presence of these two alleles have been associated with a variety of immune- and inflammatory-associated diseases, such as autoimmune diseases, psoriasis, and periodontitis.[159–160] These studies are of potential importance in risk assessment, as individuals who possess polymorphisms that express high levels of inducible TNFα are at considerably higher risk of developing more severe disease. To date, only limited studies have been conducted in humans examining whether such associations exist with xenobiotic-induced diseases. For example, Zhai et al.[161] indicated that a strong relationship exists between these TNFα polymorphisms and pneumoconiosis in coal miners, whereas similar associations have been observed in a population with sarcoidosis.[162]

X. CONCLUSIONS

In summary, only recently have toxicologists come to appreciate the role inflammation plays in classic toxicological processes. This relationship can be extremely complex, as inflammation may well be only one facet of a time- and dose-dependent continuum of toxicological and repair processes. Although many mediators are responsible for inflammatory processes, TNFα, and to a lesser extent IL-1β, have received the most attention because they represent central mediators involved in regulating this process. Not surprisingly, considerable efforts are being undertaken using our newly found understanding of molecular control to develop specific and safe chemical, biological, and molecular inhibitors of TNFα for potential therapeutic use. This effort has also allowed for a better understanding, not only of the pathological sequelae induced by TNFα, but also its influence in normal physiological and repair processes and has allowed an opportunity to better understand the toxicological processes.

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