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MACROPHAGES AND INFLAMMATORY MEDIATORS IN TISSUE INJURY

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ABSTRACT

Tissue injury induced by a diverse group of xenobiotics appears to involve both direct and indirect damage to target cells. Thus, while chemicals may act directly on target cells resulting in toxicity, they may also act indirectly by recruiting and activating resident and inflammatory tissue macrophages. Macrophages are potent secretory cells that release an array of mediators, including proinflammatory and cytotoxic cytokines and growth factors, bioactive lipids, hydrolytic enzymes, reactive oxygen intermediates, and nitric oxide—each of which has been implicated in the pathogenesis of tissue injury. The potential role of macrophages and their mediators in tissue injury has been extensively investigated in the lung and the liver. In both of these tissues, xenobiotics induce localized macrophage accumulation and mediator release. Furthermore, when macrophage functioning is blocked, pulmonary and hepatic injury–induced agents such as ozone, bleomycin, acetaminophen, carbon tetrachloride, and galactosamine are reduced. These data provide direct support for the hypothesis that macrophages and the mediators they release contribute to xenobiotic-induced tissue injury.

INTRODUCTION

Inflammatory macrophages and the mediators they release have been implicated in the pathogenesis of xenobiotic-induced tissue injury. Target organs

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include the lung, liver, skin, and bone marrow. A characteristic response to toxicants in each of these tissues is the accumulation of "activated" macrophages at the site of tissue injury. This observation, together with the discovery that tissue injury can be modified by agents that modulate macrophage functioning, has led to the suggestion that these cells contribute to toxicity. The cytotoxic process most likely involves the release of proinflammatory and cytotoxic mediators, including reactive oxygen intermediates, reactive nitrogen intermediates, cytokines, hydrolytic enzymes, and lipids by macrophages at the site of tissue injury. Whereas some of the mediators have the capacity to exert cytotoxicity directly (i.e. hydrogen peroxide, nitric oxide, peroxynitrite), others degrade the extracellular matrix (i.e. collagenase, elastase) and/or promote inflammatory cell infiltration, proliferation, and activation [i.e. chemotactic factors, colony-stimulating factors, interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), platelet-activating factor (PAF)]. This chapter reviews experimental data implicating macrophages and inflammatory mediators in xenobiotic-induced tissue injury in the lung and the liver.

MACROPHAGES

Mononuclear phagocytes or macrophages are widely distributed bone marrow–derived leukocytes. Although macrophages were initially considered merely scavenger cells that participated in immunologic responses only after B and T lymphocytes exerted their biological activity, more recent evidence suggests that they play a critical role in normal host defense as well as various pathophysiological processes (reviewed in 1). Macrophages are active secretory cells, releasing over 100 different substances that range in molecular mass from 30 (nitric oxide) to 440,000 (fibronectin) and in biologic activity from induction of cell growth to cytotoxicity. Thus, through their abundance, distribution, motility, and responsiveness, macrophages can influence almost every aspect of immune and inflammatory responses (2).

Interaction of macrophages with antigens results in cellular activation. This is associated with alterations in macrophage morphology as well as increased chemotaxis, phagocytosis, cytotoxicity, and mediator production (3). The process of activation appears to be regulated by cytokines and inflammatory mediators (3, 4). Cytokines consist of a broad class of cell-derived proteins that protect the host against inflammatory agents, microbial invasion, or injury. In some instances, this complex defense network successfully restores normal homeostasis. At other times, however, overproduction of cytokines such as IL-1, TNF-α, or PAF, or aberrant regulation of their release, may actually prove deleterious to the host, for example, during endotoxemia, ischemia-reperfusion injury, multiple organ failure, and acute respiratory distress syndrome (5–10). Cytokines regulate the growth and activity of many cells and

appear to act in vivo as amplification factors in a cascade of inflammatory events. They are part of a complex network involving autocrine and paracrine regulation of their synthesis and their actions.

MACROPHAGES AND INFLAMMATORY MEDIATORS IN PULMONARY TOXICITY

Alveolar macrophages are the first line of cellular defense in the lung and, like other mononuclear phagocytes, are highly phagocytic (5, 11–13). This process is facilitated by surface receptors for complement and the Fc fragment of IgG, IgA, and IgE (5). Following exposure to inhaled or blood-borne antigens, alveolar macrophages release mediators that recruit and activate inflammatory cells in the lung, thus amplifying their role in host defense (14, 15). Alveolar macrophages also release reactive oxygen and nitrogen intermediates, lysosomal enzymes, cytokines, bioactive lipids, and polypeptide growth factors (5, 16–20). The capacity of alveolar macrophages to mobilize large numbers of inflammatory leukocytes and to release secretory products suggests that these cells may also be major mediators of tissue damage. In this regard, alveolar macrophages have been implicated in tissue injury induced by a number of different pulmonary toxicants (Table 1). However, whether their participation in this process results from the macrophages responding appropriately to the xenobiotic or from abnormal regulation of the release of potentially cytotoxic mediators remains to be determined (21).

The lung is exposed to a variety of environmental pollutants in inspired air, including ozone, nitrogen dioxide, and acid aerosols (sulfuric acid). Inhalation

Table 1 Toxicants whose pathophysiology is thought to be associated with macrophages and inflammatory mediators

Pulmonary toxicants	Hepatotoxicants
Ozone	Acetaminophen
Sulfuric acid	Carbon tetrachloride
Nitrogen dioxide	*Corynebacterium parvum*
Cigarette smoke	Galactosamine
Hyperoxia	1,2-Dichlorobenzene
Bleomycin	Cadmium
Amiodarone	Allyl alcohol
Titanium dioxide	Endotoxin
Silica	
Asbestos	
Cadmium chloride	

of toxic levels of these gases results in damage to Type I alveolar epithelial cells and hyperplasia and hypertrophy of Type II cells. These cellular changes are associated with an accumulation of macrophages in the alveoli (18, 22). Recent evidence suggests that the pathogenesis of injury induced by these environmental pollutants is mediated in part by macrophage-derived reactive oxygen and nitrogen intermediates and inflammatory cytokines (Table 2). Thus, following inhalation of ozone, nitrogen dioxide, or sulfuric acid, alveolar macrophages release increased amounts of hydrogen peroxide, nitric oxide, IL-1, TNF-α, and fibronectin, which have the capacity to induce or amplify tissue injury. The lung is also a target of orally administered drugs, such as bleomycin and amiodarone. Pulmonary toxicity associated with these agents includes lung fibrosis, parenchymal cell injury, and thickening of the alveolar walls. Eventually, the alveolar architecture is destroyed and multiple air-filled cystic spaces are formed (23–26). Experimental data suggest that exaggerated release of cytokines and oxidants from alveolar macrophages also plays a major role in the pathogenesis of these processes (Table 2).

Fibrosis, asbestosis, mesothelioma, and bronchogenic carcinoma induced by inhalation of mineral dusts such as asbestos and silica are also characterized by macrophage accumulation in the alveoli. These cells are activated to release oxygen-derived free radicals and proinflammatory cytokines such as IL-1 and TNF-α. Inhaled silica particles and asbestos fibers are phagocytized by alveolar macrophages. However, because these inert particles cannot be digested intracellularly, the macrophages rupture, releasing proteolytic enzymes and chemoattractants, such as macrophage inflammatory protein-2 (MIP-2), which cause infiltration of neutrophils into the lung. These events initiate an acute inflammatory response. Alveolar macrophages have also been reported to release a chemotactic factor for interstitial lung fibroblasts (27), as well as growth-promoting factors for Type II alveolar epithelial cells and fibroblasts (28, 29). Thus these cells have the capacity to contribute to fibrotic lung diseases associated with chronic mineral dust exposure.

Several human pulmonary diseases with a macrophage-associated inflammatory component have gained attention over the past few years. Two of these diseases are sarcoidosis and hypersensitivity pneumonitis. Sarcoidosis is a chronic, multiorgan disorder of unknown etiology that is characterized by an accumulation of macrophages and T lymphocytes in the lung, which form granulomas (21, 30–32). Interferon-γ released by T lymphocytes can activate alveolar macrophages to produce excess amounts of reactive oxygen intermediates and cytokines (i.e. IL-1, TNF-α, MIP-1). Thus both cell types appear to participate in the development of sarcoid granulomas (21, 32). Hypersensitivity pneumonitis is also a granulomatous interstitial lung disorder with unknown etiology. It is thought to be caused by organic dusts derived from mold such as those found in hay (farmer's lung), in humidifiers, and in air condi-

Table 2 Inflammatory mediators implicated in toxicity

Mediator	Toxicant (selected references)	
	Lung	Liver
Reactive oxygen intermediates	Ozone (18) Asbestos (80) Amiodarone (78) Bleomycin (79)	Endotoxin (59, 81, 115) Acetaminophen (43, 68) *Corynebacterium parvum* (61, 64) Galactosamine (71, 77, 82) Carbon tetrachloride (63) 1,2-Dichlorobenzene (76) Phenobarbital (44)
Reactive nitrogen intermediates	Ozone (18, 102) Endotoxin (103) Silica (104)	Endotoxin (96–99, 114) Acetaminophen (97) Carbon tetrachloride (194)
Hydrolytic enzymes	Silica (22, 150)	*C. parvum* (58, 64) Endotoxin (69)
Lipids	Ozone (122) Endotoxin (147) Hyperoxia (123) Silica (124, 125) Bleomycin (132) Mineral dusts (125, 133)	Endotoxin (126, 127, 147–149) Galactosamine (126–129)
IL-1	Ozone (19) Cigarette smoke (157) Mineral dusts (159–161) Bleomycin (132) Amiodarone (158)	Endotoxin (60, 155) Acetaminophen (156)
TNF-α	Ozone (19) Endotoxin (166) Mineral dusts (159, 161) Bleomycin (168)	Endotoxin (151, 165, 192) Acetaminophen (156) Galactosamine (67, 164, 193) Alcohol (151)
Fibronectin	Ozone (19) Amiodarone (78) Mineral dusts (160) Cadmium chloride (170) Bleomycin (173)	Acetaminophen (156) Carbon tetrachloride (174)

tioners (21). An early influx of neutrophils into the lung is thought to precede macrophage and lymphocyte recruitment. The pathogenesis of hypersensitivity pneumonitis, like that of sarcoidosis, is associated with increased production of alveolar macrophage–derived inflammatory mediators. Hyperproduction of

eicosanoids (i.e. leukotriene B_4, leukotriene C_4, prostaglandin E_2, thromboxane B_2) and cytokines (i.e. TNF-α, granulocyte- and macrophage-colony–stimulating factor) by alveolar macrophages has been observed in experimentally induced hypersensitivity in mice (33–36). Taken together, these findings suggest that activated alveolar macrophages and their secretory products may contribute to a variety of lung disorders induced by xenobiotics.

MACROPHAGES AND INFLAMMATORY MEDIATORS IN HEPATOTOXICITY

Kupffer cells constitute 80 to 90% of all the macrophages in the body and represent about 29% of the sinusoidal cells in the liver. They are predominantly localized in the lumen of the hepatic sinusoids in periportal and central regions of the liver lobule and are anchored to the endothelium by long cytoplasmic processes (37). The major function of Kupffer cells is to clear particulate and foreign materials from the portal circulation, primarily through phagocytosis. Kupffer cells possess both Fc and C3 receptors and are known to phagocytize a wide variety of both opsonized and nonopsonized particles (38). Kupffer cells play a central role in the uptake and detoxification of endotoxin from the portal circulation (39). Like other mononuclear phagocytes, they have the capacity to act as antigen-presenting cells for the induction of T lymphocyte responses (40). When activated by antigens or inflammatory stimuli, Kupffer cells release superoxide anion, hydrogen peroxide, nitric oxide, hydrolytic enzymes, and eicosanoids that aid in antigen destruction (41, 42). Kupffer cells also release a number of different immunoregulatory and inflammatory cytokines, including IL-1, IL-6, TNF-α, PAF, transforming growth factor–β and interferon-γ (41, 42).

Treatment of experimental animals with a number of different hepatotoxicants, including acetaminophen, endotoxin, carbon tetrachloride, phenobarbital, allyl alcohol, or galactosamine, is associated with the accumulation of macrophages in the liver (38, 43–46). Although the macrophage accumulation is relatively rapid, typically occurring within 48 to 72 h, the specific location of these cells within the liver varies with the chemical agent. Thus, whereas treatment of rats with acetaminophen or carbon tetrachloride results in accumulation of macrophages in centrilobular regions of the liver, macrophages that accumulate in the liver following endotoxin, phenobarbital, carbon tetrachloride, or galactosamine treatment of rats are scattered in clusters throughout the liver lobule (38, 43, 45, 47). These patterns of macrophage localization appear to be correlated with areas of the liver that subsequently exhibit signs of injury (48–50). Macrophages isolated from livers of hepatotoxicant-treated animals have been reported to display morphologic and functional properties of activated mononuclear phagocytes. These cells, which

consist of resident Kupffer cells and inflammatory macrophages, appear larger and more stellate than cells from untreated rats, are highly vacuolated, and display an increased cytoplasmic:nuclear ratio (38, 43, 44, 51).

In addition, macrophages from rats treated with hepatotoxicants such as phenobarbital, acetaminophen, or endotoxin adhere to and spread on culture dishes more rapidly than resident Kupffer cells. These properties are characteristic of morphologically activated macrophages. Macrophages from animals treated with hepatotoxicants also exhibit enhanced phagocytic, chemotactic, cytotoxic, and metabolic activity, as well as increased release of superoxide anion, hydrogen peroxide, nitric oxide, and its oxidation products—proteolytic enzymes, IL-1, IL-6, and TNF-α (38, 43, 44, 50, 52–62). These data suggest that macrophages in the liver become functionally activated following exposure to hepatotoxicants. Evidence has accumulated over the last few years to support the hypothesis that these cells can promote hepatic damage (Tables 1 and 2).

MACROPHAGE-DERIVED CYTOTOXIC AND PROINFLAMMATORY MEDIATORS

Activated macrophages release a variety of mediators that have been implicated in tissue injury, including reactive oxygen and nitrogen intermediates, hydrolytic enzymes, lipids, and cytokines. These mediators probably act in concert to promote tissue injury.

Reactive Oxygen and Nitrogen Intermediates

Superoxide anion is largely produced by membrane-associated NADPH oxidases in macrophages activated by inflammatory stimuli. This radical rapidly dismutates to hydrogen peroxide. In the presence of divalent cations, hydrogen peroxide and superoxide anion form hydroxyl radical and molecular oxygen. Reactive oxygen intermediates such as superoxide anion, hydrogen peroxide, and hydroxyl radical have been linked to membrane, protein, and DNA damage, to lipid peroxidation, and to cytotoxicity (72–74). Peroxidation of membrane lipids by reactive oxygen intermediates can also induce the formation and release of a number of other vasoactive agents, including prostaglandins, thromboxanes, and leukotrienes. Reactive oxygen intermediates are thought to be primary mediators of macrophage-induced cytotoxicity, of reperfusion and ischemic tissue injury, and of injury associated with both acute and chronic inflammatory diseases (72, 75). Macrophages that accumulate at sites of tissue injury induced by toxicants have been reported to be activated to release hydrogen peroxide and superoxide anion (8, 43, 44, 59, 61). Stimulation of these cells to produce additional reactive oxygen intermediates augments tissue injury in the liver induced by agents such as *Corynebacterium parvum* and

galactosamine and injury in the lung induced by ozone, amiodarone, bleomycin, and asbestos (18, 61, 63, 76–80). Conversely, administration of antioxidants such as superoxide dismutase, allopurinol, or quinone derivatives is hepatoprotective (63, 68, 76, 77, 81, 82). Similarly, pretreatment of rats with vitamin A reportedly attenuates bleomycin-induced lung injury by a mechanism that involves inhibition of alveolar macrophage superoxide anion production (79). Taken together, these studies suggest that oxygen-derived free radicals produced by macrophages contribute to the pathogenesis of tissue injury (42, 61, 76, 82).

Activated macrophages are also known to release relatively large amounts of nitric oxide. This highly reactive mediator is produced via the NADPH- and L-arginine–dependent enzyme nitric oxide synthase (83–85), and its activity is increased following macrophage activation (83, 84). Nitric oxide is now widely recognized as playing an important role in a variety of physiological processes, including the regulation of vascular relaxation and blood flow, airway responsiveness, and bronchiole relaxation (85–87). Nitric oxide is also involved in macrophage-mediated cytotoxicity and in the regulation of cellular proliferation (17, 83, 85, 88–91). Thus overproduction of nitric oxide may be significant not only in tissue injury, but also in the wound healing process. The diverse actions of nitric oxide appear to be due to the activities of two major classes of nitric oxide synthases. In vascular endothelium, neural tissue, platelets, and neutrophils, the enzyme is expressed constitutively (83, 85). In contrast, the macrophage enzyme, which has also been identified in smooth muscle cells, endothelial cells, hepatocytes, fibroblasts, and certain epithelial cells, is only induced after activation of these cells by bacteria or cytokines (83).

Recent studies have suggested that nitric oxide released by macrophages may contribute to inflammation and tissue injury (85, 87, 92–96). Nitric oxide has also been implicated in the hepatotoxicity of chemicals such as acetaminophen and endotoxin (97, 98), as well as in carrageenin-induced increases in epidermal vascular permeability and edema (99). Each of these pathophysiologic processes is linked to macrophages. Mulligan et al (100, 101) have suggested that IgG- and IgA-mediated immune complex–induced injury to rat lung and to skin is mediated by nitric oxide. Acute exposure of rats to ozone has also been reported to result in increased production of nitric oxide by alveolar as well as interstitial macrophages (18, 102). This increase in nitric oxide is associated with increased expression of inducible nitric oxide synthase protein and mRNA, which is observed in vitro in isolated cells and in vivo in histologic sections. Wizemann et al (103) and Blackford et al (104) have described similar increases in nitric oxide in the lung following exposure of animals to endotoxin and silica, respectively. Nitric oxide may play a role in tissue injury induced by these pulmonary irritants. Numerous pathophysiological conditions, including atherosclerosis, ischemia-reperfusion injury, acute

hypertension, and endotoxemia, are associated with abnormal production of nitric oxide (85). A common feature of these conditions is localized generation of excess reactive oxygen intermediates in particular superoxide anion and hydrogen peroxide (105).

Superoxide anion reacts rapidly with nitric oxide, forming peroxynitrite, a relatively long-lived cytotoxic oxidant that has been implicated in stroke, heart disease, and immune complex–stimulated pulmonary edema (106–110). Peroxynitrite may initiate lipid peroxidation and can react directly with sulfhydryl residues in cell membranes (111, 112). In the presence of transition metals, peroxynitrite becomes an effective nitrating agent, with reactivity similar to nitronium ion (113). Paradoxically, the reaction of superoxide anion and nitric oxide may also function as a defense against oxidant stress by reducing intracellular levels of these reactive intermediates (107, 108). In this regard, inhibition of nitric oxide synthesis has been reported to augment oxidant-dependent tissue injury induced by *C. parvum*, and it has been proposed to play a protective role in hepatotoxicity induced by endotoxin (98, 114–116). Thus nitric oxide or secondary oxidants generated from nitric oxide (i.e. peroxynitrite) may be cytotoxic or protective depending on the levels of superoxide anion present in the tissue and on the extent to which tissue injury is mediated by reactive oxygen intermediates (107).

Lipid Mediators

Eicosanoids are a heterogenous family of 20-carbon fatty acid derivatives formed from the oxygenation of arachidonic acid (reviewed in 117). Eicosanoids are classified as either products of the cyclooxygenase pathway (prostaglandins and thromboxanes) or the lipoxygenase pathway (leukotrienes, hydroxy fatty acids) of arachidonic acid metabolism. A variety of eicosanoids are released by activated macrophages (2); however, the precise role of these reactive species in toxicity is unknown. Leukotrienes and prostaglandins have proinflammatory activity and play pivotal roles both in normal host defense and in the pathogenesis of a wide range of immune and inflammatory diseases, including asthma, airway hyperresponsiveness, and acute allergic reactions, as well as persistent and late-onset responses to allergens (21, 118, 119). In addition, leukotriene B_4 is known to be a potent polymorphonuclear leukocyte chemoattractant and to induce monocyte IL-1, TNF-α, and hydrogen peroxide production (120, 121). Thus release of leukotriene B_4 may constitute a local control mechanism for the recruitment and activation of inflammatory cells. Leukotriene B_4 has been reported to be elevated in the lung following exposure of rats or humans to ozone, hyperoxia, or silica (122–125), and in the liver following exposure of rats to galactosamine (126). In addition, recent studies have demonstrated that administration of lipoxygenase inhibitors or antagonists protected mice against galactosamine-induced hepatitis (126–129).

Thromboxanes and prostaglandins, in particular prostaglandin E_2, are products of both immune and nonimmune cells whose actions include inhibition of neutrophil chemotaxis and the release of oxygen radicals and lysosomal enzymes (130). Prostaglandin E_2 also decreases macrophage proliferation, adhesion, migration, and expression of TNF-α and IL-1 (130, 131). Enhanced release of prostaglandins and thromboxanes has been documented following exposure of animals to ozone (122), bleomycin (132), silica, and coal dust (125, 133). These data suggest that eicosanoids may also be involved in the resolution of inflammatory tissue damage.

Another important lipid mediator is PAF, which has recently been directly implicated in tissue injury (134–139). PAF is released by a variety of cell types, including macrophages, and is thought to act in a paracrine and autocrine manner to amplify and propagate early stages of the inflammatory response. Thus PAF released from inflammatory phagocytes stimulates macrophage and neutrophil chemotaxis and oxidative metabolism (140–144). Exposure of macrophages to ozone in vitro results in increased release of PAF (145). Pendino et al (146) reported that in rats, ozone inhalation induces up-regulation and functional activation of receptors for PAF on alveolar macrophages. This may represent an important mechanism by which these cells become activated and contribute to tissue injury. Endotoxemia is also associated with increased production of PAF in both the lung and the liver (147, 148), suggesting that this lipid mediator may participate in tissue injury induced by bacterially derived toxins. Yue et al (149) reported that administration of PAF receptor antagonists reduces the toxicity associated with endotoxemia (149).

Hydrolytic Enzymes

Macrophage activation is also associated with increased release of a variety of proteolytic and lysosomal enzymes, including plasminogen activator, collagenase, elastase, gelatinase, acid phosphatase, and cathepsin D, that can act directly on cellular membranes, inducing damage. Proteases released in the liver following hepatotoxicant exposure have been shown to play a role in macrophage-mediated target cell destruction as well as in altered hepatocyte functioning (41, 42, 58, 69). Increased production of lysosomal enzymes by alveolar macrophages has also been observed following exposure of mice to silica (22, 150), and these may play a similar role in the lung.

Inflammatory Cytokines and Growth Factors

Macrophages release a number of different cytokines and growth factors that have the capacity to promote tissue injury, inflammation, and fibrosis (2, 137). These include IL-1, TNF-α, fibronectin, and colony-stimulating factors that can act directly on target tissues and cells or that may indirectly activate infiltrated leukocytes, thus amplifying the inflammatory response (137, 151).

IL-1 is a low-molecular weight protein that mediates a wide variety of biologic effects (152, 153). IL-1 induces proliferation and activation of T and B lymphocytes, macrophages, endothelial cells, synovial cells, and epithelial cells (152, 153). IL-1 also augments collagenase and prostaglandin production by macrophages as well as cytotoxicity, and in conjunction with IL-6, induces hepatocyte production of acute-phase proteins (152–154). Both IL-1 and IL-6 depress hepatic albumin synthesis and cytochrome P450 activity (152–155), suggesting that they participate in hepatotoxic reactions. In this regard, increased IL-1 production by hepatic macrophages and increased protein expression in the liver have been described following exposure of rats to acetaminophen or endotoxin (60, 156).

In the lung, IL-1 is proinflammatory, and augmented release of this cytokine by activated macrophages has been well documented in xenobiotic-induced lung injury. For example, IL-1 secretion by alveolar macrophages increases following exposure of rats to ozone (19). Furthermore, although release of this mediator by alveolar macrophages is decreased following inhalation of cigarette smoke, intracellular accumulation of IL-1 in these cells is increased (157). Fibrosis associated with bleomycin exposure has also been linked to increases in alveolar macrophage–derived IL-1 production (132), as has lung injury induced by amiodarone (158). In addition, lung inflammation subsequent to mineral dust exposure is associated with enhanced IL-1 release (159, 160), and chronic asbestos exposure resulting in asbestosis has recently been reported to involve increases in mRNA and protein for IL-1 in alveolar macrophages (159, 161).

TNF-α is another secretory product of activated macrophages (121, 162). It has been implicated not only in the pathogenesis of septic shock and inflammation, but also in the regulation of acute-phase protein gene expression, of cytochrome P450 activity, and of cellular proliferation, and in apoptosis (121, 162, 163). TNF-α also stimulates the release of other immunoregulatory and cytotoxic mediators, including IL-1, IL-6, PAF, colony-stimulating factor, prostaglandins, and nitric oxide from macrophages (90, 121, 162); it may act in concert with these mediators to augment tissue injury. TNF-α has been implicated in the hepatotoxicity of a number of xenobiotics, including acetaminophen, galactosamine, alcohol, and endotoxin (151, 156, 164, 165), and is also thought to mediate bronchial hyperresponsiveness in rats following exposure to aerosolized endotoxin (166) and to play a key role in the allergic reaction in human airways (167). Recent studies have also suggested that alveolar macrophage–derived TNF-α is involved in tissue injury and airway hyperresponsiveness observed following inhalation of ozone (19). Alveolar macrophage production of TNF-α is augmented in experimental models of tissue injury induced by inhaled particulates, such as silica, titanium dioxide, and asbestos (159, 161). In addition, alveolar macrophage–derived TNF-α is

considered the major cytotoxic effector in bleomycin-induced fibrosis (168). TNF-α is also known to have deleterious effects on endothelial cells (121). In addition, this mediator sensitizes neutrophils and monocytes to produce reactive oxygen and nitrogen intermediates (90, 121, 169). The fact that inflammatory cytokines such as IL-1 and TNF-α can affect so many different target tissues and that they are produced by a variety of cell types suggests that they are major mediators of inflammatory and immune responses.

Fibronectin is a large dimeric glycoprotein that is found in association with cell surfaces, as well as in blood and other body fluids. It is involved in diverse cellular processes, including cytoskeletal organization, cellular adhesion, spreading, migration, and proliferation. Although much of the fibronectin detected in injured tissue is of plasma origin, this glycoprotein is also synthesized locally by activated macrophages (160, 170, 171). Recent studies have documented increased expression of fibronectin in the lung during adult respiratory distress syndrome, bronchiolitis obliterans, pneumonia, and idiopathic pulmonary fibrosis (172). In addition, increased production of fibronectin by alveolar macrophages and increased expression of this protein in the lung are observed following acute inhalation of ozone (19). Exposure of experimental animals to amiodarone, silica, bleomycin, titanium dioxide, or cadmium chloride is also associated with increased fibronectin in the lung (78, 160, 170, 173). In the liver, up-regulation of fibronectin expression has been documented following exposure of rats to carbon tetrachloride and acetaminophen (156, 174). Thus this mediator may also participate in tissue injury associated with inflammation.

EFFECTS OF MODIFYING MACROPHAGE FUNCTION ON TISSUE INJURY

Probably the best evidence to support the hypothesis that macrophages play a role in tissue injury comes from experiments analyzing the effects of agents known to modify the functioning of these cells on toxicity. Data from these experiments clearly demonstrate that the degree of tissue injury induced by a number of different toxicants is directly correlated with macrophage functioning. Thus agents that depress macrophage functioning reduce toxicity, while compounds that augment macrophage activity enhance tissue injury (Table 3). For example, drugs such as hydrocortisone, certain synthetic steroids, and natural substances such as taurine, which block inflammatory responses, protect against liver injury induced by carbon tetrachloride and acetaminophen and lung injury induced by ozone (175). Similarly, the accumulation of macrophages in the liver and subsequent toxicity of acetaminophen is inhibited by pretreatment of rats with dextran sulfate or gadolinium chloride (176), compounds also known to depress macrophage activity (177, 178). Hepatoprotective effects of gadolinium chloride against allyl alcohol– and carbon

tetrachloride–induced injury as well as ozone-induced pulmonary injury and inflammation have also been described (46, 70, 179).

Several studies have also demonstrated that activation of macrophages and/or stimulation of mediator release augments tissue injury induced by toxic xenobiotics. For example, Chyczewska et al (180) reported that bleomycin-induced fibrotic lung disease was dramatically enhanced by pretreatment of rats with BCG, which causes a marked accumulation of activated macrophages in the lung (180). Similarly, lipopolysaccharide and poly I:C, which are potent activators of liver macrophages (38, 59, 155), have been reported to aggravate the hepatotoxicity of toxicants like acetaminophen, carbon tetrachloride, galactosamine, and *C. parvum* (181–183). In contrast, animals made tolerant to lipopolysaccharide or treated with the antibiotic polymyxin B, a positively charged detergent that binds to and neutralizes lipopolysaccharide, are protected from hepatotoxicity induced by these liver toxicants (183, 184). Pretreatment of rats with lipopolysaccharide prior to ozone or hyperoxia also results in decreased lung injury, possibly mediated by an increase in antioxidant levels (185–188).

An increase in antioxidant levels in the lung may mediate the protection afforded by IL-1 pretreatment of animals in hyperoxic models of lung injury (189). Administration of large doses of vitamin A, which reportedly activates Kupffer cells in vivo (52, 190), augments the hepatotoxicity of carbon tetrachloride as well as endotoxin (48, 191). Tissue injury is postulated to be due to reactive oxygen intermediates released from vitamin A–activated macrophages. In this regard, methyl palmitate, which blocks Kupffer cell oxidative metabolism, abrogates the enhanced hepatotoxicity of carbon tetrachloride induced by vitamin A (191). Methyl palmitate has also been reported to exert a hepatoprotective effect against galactosamine and 1,2-dichlorobenzene (71, 76).

Antibodies to cytokines have been used effectively to modulate macrophage-associated xenobiotic-induced injury. For example, Denis (36) reported that administration of a monoclonal antibody to the antiinflammatory cytokine IL-6 to mice with experimental hypersensitivity pneumonitis increases tissue injury associated with inhalation of organic dusts (36). Conversely, antibodies to the proinflammatory cytokine TNF-α reduce liver injury induced by endotoxin as well as galactosamine (192, 193). Taken together, these observations provide direct support for the hypothesis that macrophages and the mediators they release contribute to tissue injury.

MODEL FOR THE ROLE OF MACROPHAGES IN TISSUE INJURY

Based on current experimental data, it is relatively easy to envision a model of xenobiotic-induced tissue injury that includes a role for macrophages and inflammatory mediators (Figure 1). According to this model, tissues and cells injured by toxicants release factors that attract macrophages to the target organ.

Table 3 Effects of modifying macrophage activity in tissue injury

Toxicant	Pretreatment	Toxicity	References
Lung			
Ozone	Gadolinium chloride	↓	179
	Lipopolysaccharide	↓	185–187
Hyperoxia	Lipopolysaccharide	↓	188
Bleomycin	Vitamin A	↓	79
	BCG	↑	180
Organic dusts	Anti-IL-6 antibody	↑	36
Liver			
Acetaminophen	Gadolinium chloride	↓	176
	Dextran sulfate	↓	176
	Poly I:C	↑	181
Allyl alcohol	Gadolinium chloride	↓	46
Carbon tetrachloride	Gadolinium chloride	↓	70
	Lipopolysaccharide	↑	183
	Polymyxin B	↓	183
	Vitamin A	↑	63, 191
	Methyl palmitate	↓	191
	Hydrocortisone	↓	175
Galactosamine	Methyl palmitate	↓	71
	Superoxide dismutase	↓	82
	Anti-TNF-α antibody	↓	193
	Lipopolysaccharide	↑	65, 182
	Glucan	↑	71
Corynebacterium parvum	Lipopolysaccharide	↑	195
	Gadolinium chloride	↓	195
	Superoxide dismutase	↓	61
Endotoxin	Anti-TNF-α antibody	↓	192
	Gadolinium chloride	↓	196
	Vitamin A	↑	48
1,2-Dichorobenzene	Methyl palmitate	↓	76
	Gadolinium chloride	↓	76

Additional mononuclear phagocytes are also recruited from blood and bone marrow precursors. Once localized in the injured area, the macrophages become activated by cytokines and growth factors derived from inflammatory leukocytes and parenchymal cells and release mediators that contribute to tissue damage.

CONCLUSION

Tissue injury induced by xenobiotics is a complex process that involves a variety of cell types and soluble mediators. Although xenobiotics or their

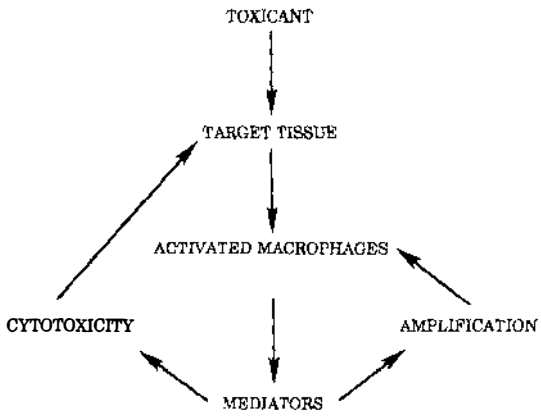

Figure 1 Model for the role of macrophages in tissue injury.

metabolites can directly injure the tissue, they may also activate macrophages and indirectly augment tissue injury. Reactive mediators produced by inflammatory macrophages may act as primary mediators of tissue injury, and/or they may participate in the inflammatory response by initiating a cascade of additional immunologic reactions that result in tissue damage. Further studies on the nature of mediators released from macrophages and their effects on target tissue will be particularly relevant for understanding mechanisms of tissue injury.

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