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Macrophages and Inflammatory Mediators in Chemical Toxicity: A Battle of Forces

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Abstract

Macrophages function as control switches of the immune system, providing a balance between pro- and anti-inflammatory responses. To accomplish this, they develop into different subsets: classically (M1) or alternatively (M2) activated macrophages. Whereas M1 macrophages display a cytotoxic, proinflammatory phenotype, much like the soldiers of The Dark Side of The Force in the Star Wars movies; M2 macrophages, like Jedi fighters, suppress immune and inflammatory responses and participate in wound repair and angiogenesis. Critical to the actions of these divergent or polarized macrophage subpopulations is the regulated release of inflammatory mediators. When properly controlled, M1 macrophages effectively destroy invading pathogens, tumor cells and foreign materials. However, when M1 activation becomes excessive or uncontrolled, these cells can succumb to The Dark Side, releasing copious amounts of cytotoxic mediators that contribute to disease pathogenesis. The activity of M1 macrophages is countered by The Force of alternatively activated M2 macrophages which release anti-inflammatory cytokines, growth factors and mediators involved in extracellular matrix turnover and tissue repair. It is the balance in the production of mediators by these two cell types that ultimately determines the outcome of the tissue response to chemical toxicants.

1. Introduction (The Dramatis Personae)

For most of my early scientific career, when I considered the role of macrophages in tissue injury, it was their dark side that intrigued me; after all, the movie Star Wars was on everyone's mind and there were increasing numbers of publications supporting the idea that by releasing cytotoxic mediators that contribute to injury and disease, macrophages were very much like the Death Star. But over the last two decades, as more information has accumulated from my own laboratory and others, it has become clear that the contribution of macrophages and the mediators they release to chemically-induced tissue injury is much more complex. There is in fact, another side to macrophage functioning: suppression of inflammation and wound repair. Thus, the outcome of the response to tissue injury depends on the balance between the two opposing forces of macrophages. Furthermore, it appears that the multiplicitous functions of macrophages are not mediated by a single homogeneous population of cells. But in order to set the stage for this discussion, it is first necessary to provide some background on macrophages and inflammatory mediators they release.

Macrophages are mononuclear phagocytes derived from bone marrow precursors. These cells differentiate into monocytes which circulate in the blood. The majority of monocytes (>95%) localize in tissues and mature into macrophages where they develop specialized functions depending on the needs of the tissue. Thus, in the liver, resident macrophages or Kupffer cells develop a high phagocytic capacity, while in the lung, alveolar macrophages acquire the capacity to release large quantities of highly reactive cytotoxic oxidants. Macrophages are key players in the innate immune response. Through the process of phagocytosis, they function as

scavengers, ridding the body of worn-out cells and debris, as well as viruses, bacteria, apoptotic cells and some tumor cells (1). Macrophages are also one of the most active secretory cells in the body releasing a vast array of mediators that regulate all aspects of host defense, inflammation and homeostasis including enzymes, complement proteins, cytokines, growth factors, eicosanoids and oxidants. In addition, they are considered professional antigen presenting cells, one of the major cell types involved in initiating specific immune responses of T lymphocytes.

Accumulating evidence suggests that the diverse biological activity of macrophages is mediated by functionally distinct subpopulations that are phenotypically polarized by their microenvironment and by exposure to inflammatory mediators (Table 1). These divergent macrophage subpopulations are broadly classified into two major groups: classically activated M1 macrophages and alternatively activated M2 macrophages. M1 macrophages are activated by type I cytokines like interferon-γ (IFNγ) and tumor necrosis factor-α (TNFα), or after recognition of pathogen associated molecular patterns or PAMPs (e.g., lipopolysaccharide [LPS], lipoproteins, dsRNA, lipoteichoic acid) and endogenous “danger” signals (e.g., heat shock proteins, HMGB1). Alternatively activated M2 macrophages are further subdivided into M2a (activated by interleukin [IL]-4 or IL-13), M2b (activated by immune complexes in combination with IL-1β or LPS) and M2c (activated by IL-10, transforming growth factor-β [TGFβ] or glucocorticoids). M1 macrophages exhibit potent microbicidal activity, and release IL-12, promoting strong Th1 immune responses. In addition, they exert anti-proliferative and cytotoxic activities, which is due in part to the release of reactive oxygen and nitrogen species and proinflammatory cytokines (e.g., TNFα, IL-1, IL-6) (2,3). It is the M1 population that is thought to contribute to macrophage-mediated tissue injury (2,4–8). In contrast, M2 macrophages support Th2-associated effector functions. M2 macrophages release IL-10 and exert selective immunosuppressive activity, and inhibit T-cell proliferation. M2 macrophages also play a role in the resolution of inflammation through phagocytosis of apoptotic neutrophils, reduced production of pro-inflammatory cytokines, and increased synthesis of mediators important in tissue remodeling, angiogenesis, and wound repair. Similar functions are exerted by tumor-associated macrophages (TAM), which also display an alternative-like activation phenotype and play a detrimental pro-tumorigenic role. It should be noted, however, that classification of macrophages into these two groups (M1 and M2) oversimplifies the complex functional activity of these cells. Macrophage activation is in fact a dynamic process; thus the same cells may initially take part in proinflammatory and cytotoxic reactions and later participate in the resolution of inflammation and wound healing (4,9). This suggests that macrophages undergo progressive functional changes as a result of alterations in their microenvironment (2,10,11).

2. The Dark Side: Exacerbation of Tissue Injury

The concept that macrophages accumulating in tissues in response to injury or infection have a “Dark Side” and can contribute to disease pathogenesis predated the first Star Wars movie by nearly one hundred years. Initially proposed in the late 19th century by one of the “fathers” of modern immunology, Eli Metchnikoff recognized that stimulated phagocytes might be capable of doing harm (12). He described the inflammatory process as a “salutary reaction against some injurious influence” and postulated that “ferments” released by cells at the site of inflammation might be capable of damaging host tissues (13,14). Over the last century, this concept has been refined as the functions of macrophages in many disease processes have been better elucidated. It is now well established that cytotoxic and proinflammatory mediators released by activated macrophages can contribute to the pathophysiological responses initiated by diverse xenobiotics in many different tissues [reviewed in (15)]. Thus, there are numerous examples in the literature describing the contribution of cytotoxic mediators released by macrophages to injury and disease in the liver, lung, skin and brain. For the purposes of this

review, however, the discussion will focus on the liver, the major organ of drug and xenobiotic metabolism.

Some of the earliest experimental evidence linking macrophages with chemically-induced hepatotoxicity is based on histologic examination of livers collected from animals treated with toxic chemicals. Thus, after treatment of rodents with hepatotoxic doses of acetaminophen, carbon tetrachloride, phenobarbital or endotoxin, increased numbers of macrophages are observed in the liver. Moreover, the specific location of the cells in the liver lobule correlates with areas that subsequently exhibit damage (15,16). In a number of experimental models, data clearly demonstrate that macrophages accumulating in tissues following exposure to toxicants become activated, and contribute to liver injury [reviewed in (15,17,18)]. The pathogenic process appears to involve the release of cytotoxic, matrix degrading and proinflammatory mediators by these cells (see further below). That macrophages contribute to tissue injury is most clearly evident from findings that toxicity is directly correlated with their functional status. Accordingly, when macrophage cytotoxic/inflammatory activity is blocked with hydrocortisone or synthetic steroids, hepatotoxicity induced by acetaminophen and carbon tetrachloride is ameliorated (19–22). Similarly, the accumulation of macrophages in the liver and subsequent toxicity of these xenobiotics is abrogated in rodents by pretreatment with macrophage inhibitors such as gadolinium chloride ($GdCl_3$) or dextran sulfate (23–30). Protection against early damage induced by acetaminophen has also been reported in animals depleted of macrophages by pretreatment with liposome-encapsulated dichloromethylene diphosphonate (clodronate) (31). Both $GdCl_3$ and clodronate liposomes also prevent liver damage induced by allyl alcohol, endotoxin, fumonisin, thioacetamide, cadmium chloride, concanavalin A and diethyldithiocarbamate (32–42). The importance of macrophages in the pathogenesis of liver injury is also exemplified by findings that activation of these cells can augment tissue damage induced by hepatotoxicants. Thus, pretreatment of rodents with Toll-like receptor agonists such as LPS or polyinosinic:polycytidylic acid (poly I:C) which induce macrophage accumulation and activation in the liver, results in an exaggerated hepatotoxic response to acetaminophen, carbon tetrachloride, halothane, trovafloxacin, galactosamine and *Corynebacterium parvum* (43–48).

A question arises, however, as to the nature of the macrophage population mediating the hepatotoxic response. As indicated above, recent studies suggest that these cells possess a classically activated or M1 macrophage phenotype. Consistent with this idea are findings that M1 macrophages are activated to release reactive oxygen species (ROS), reactive nitrogen species (RNS), hydrolytic enzymes, lipid mediators, and proinflammatory cytokines, each of which has been implicated in hepatotoxicity [reviewed in (15)]. Moreover, abrogating the production of these proinflammatory mediators by depleting cytotoxic liver macrophages using $GdCl_3$ or dextran sulfate correlates with protection against liver injury induced by a variety of hepatotoxicants (24–26,30,31,33,36,37,49–54).

3. The Energy of the Dark Side

In parallel to the forces of The Dark Side, classically activated M1 macrophages contribute to tissue injury by unleashing a deadly barrage of dark side energy which is in the form of cytotoxic and proinflammatory mediators. Most notable are ROS and RNS, which have been implicated in tissue injury induced by a variety of toxicants. ROS and RNS are produced in significant quantities by macrophages via enzyme catalyzed reactions and during mitochondrial respiration. Whereas the generation of low levels of ROS and/or RNS under tonic conditions functions to regulate a number of cellular signaling pathways including kinases, transcription factors, metabolic enzymes and proteases, during acute inflammatory responses, these mediators function to destroy invading pathogens and foreign materials. Evidence suggests that uncontrolled or excessive production of ROS and/or RNS by resident

macrophages and inflammatory leukocytes contributes to oxidative and nitrosative stress and consequent tissue injury. Many biological molecules including lipids, proteins, and DNA are targets for modification by reactive species resulting in diverse pathologic consequences. For instance, peroxidation of membrane lipids by ROS can lead to the release of arachidonic acid and the generation of additional proinflammatory mediators including prostaglandins, thromboxanes, and leukotrienes. ROS can also react with cellular lipids to generate lipid peroxides and cytotoxic reactive aldehydes (55). Recent studies have also identified several novel products generated as a consequence of ROS and RNS modification of biological molecules, including nitrated alkenes, nitrosothiols, *S*-glutathionylation, and nitrotyrosine [reviewed in (56,57)]. Elucidating the signaling properties of these new biomolecules currently represents an area of intense investigation with reference to a wide range of pathologies.

Macrophage-derived ROS and RNS have been implicated in the pathogenesis of liver injury induced by hepatotoxicants such as acetaminophen, galactosamine, endotoxin, carbon tetrachloride, 1,2,-dichlorobenzene and alcohol (25,43,52,58–73). Macrophages accumulating in the liver of animals treated with various hepatotoxicants have been reported to release excessive quantities of ROS and RNS (34,58,62,67,70,74–76). Moreover, stimulation of macrophages to produce additional oxidants exacerbates liver injury. This has been observed in rodents administered vitamin A, *Corynebacterium parvum*, latex beads or poly I:C, which activate macrophages in the liver to produce ROS and augment injury induced by hepatotoxicants such as endotoxin, acetaminophen, carbon tetrachloride and galactosamine (43,47,60,65,77,78). Conversely, hepatotoxicity induced by galactosamine and 1,2-dichlorobenzene, as well as carbon tetrachloride and vitamin A, is abrogated by methyl palmitate, an effective inhibitor of oxidative metabolism in liver macrophages (43,60,61,64). Protection is also observed in various models of hepatotoxicity using agents that function to reduce levels of ROS and oxidative stress including allopurinol, hemin, ethyl pyruvate, glutathione, N-acetylcysteine, chondroitin-4-sulfate, ascorbate, N-acetyl-l-cysteine, Cu, Zn-superoxide dismutase (SOD1) and oleanolic acid (30,35,59,60,68,69,71,79–85). Moreover, mice over-expressing antioxidants such as SOD1 or extracellular glutathione peroxidase (GPX1) are protected from liver injury induced by acetaminophen (86,87). Similar hepatoprotection has also been produced by administration of a nonpeptidyl mimetic of manganese SOD (SOD2), as well as by extracellular SOD (SOD3) gene therapy (88–90). Surprisingly, SOD1−/− mice have also been reported to be resistant to acetaminophen- induced hepatotoxicity (86); however, this appears to be due to reduced CYP2E1 activity and altered cellular redox balance. A question arises as to the nature of the ROS involved in hepatotoxicity and its cellular origin. The findings that mice lacking NADPH oxidase, the major enzyme mediating the generation of superoxide anion by macrophages, do not display altered sensitivity to acetaminophen, suggest that this reactive oxygen intermediate is not a critical mediator of macrophage induced hepatotoxicity in this model (91). It may be that the contribution of macrophage-derived superoxide anion to tissue injury is dependent on the hepatotoxicant and the extent to which other inflammatory mediators are produced in the tissue.

The role of RNS in hepatotoxicity also appears to depend on the toxicant. Thus, whereas with some toxicants, hepatoprotective effects are observed in mice with a targeted disruption of the inducible nitric oxide synthase (iNOS) gene or in mice treated with an iNOS inhibitor (30, 52,62,63,66,73,92–94), with others, liver injury is exacerbated (95–98). A comparable protective effect has been observed in the liver during ischemia/reperfusion by blocking arginase activity which raises nitric oxide levels (99). It has also been reported that nitric oxide donors protect against hepatotoxicity induced by acetaminophen (100). Thus, it appears that nitric oxide, or secondary oxidants generated from nitric oxide (e.g., peroxynitrite), may be cytotoxic or protective depending on quantities of these mediators produced in the tissue, as well as levels of superoxide anion present, and the extent to which tissue injury is mediated by ROS (101).

Another group of mediators that contribute to macrophage-mediated cytotoxicity and tissue injury are proinflammatory cytokines including TNFα, IL-1, and IL-6, as well as chemokines such as CXCL8 (IL-8), CXCL2 (MIP-2) and CCL2 (MCP-1) [reviewed in (15)]. These proteins can induce damage directly in target tissues and/or indirectly by recruiting and activating additional leukocytes, a process that amplifies the inflammatory response. Most notable among the pro-inflammatory cytokines is TNFα which has been implicated not only in the pathogenesis of septic shock and inflammatory tissue injury, but also in the regulation of apoptosis, acute-phase protein gene expression, and cytochrome P450 activity (15,102–105). TNFα also stimulates the release of other cytotoxic and immunoregulatory mediators including IL-1, IL-6, platelet activating factor, colony-stimulating factor, prostaglandins, ROS and RNS from macrophages and neutrophils which can augment tissue injury (12,106,107). Hepatic injury induced by alcohol, endotoxin, acetaminophen, carbon tetrachloride, cadmium, galactosamine and aflatoxin is characterized by excessive production of TNFα(73,84,97, 108–118). Moreover, hepatotoxicity induced by a number of these agents is abrogated by administration of antibodies to TNFα or a TNF receptor antagonist, and is suppressed in mice lacking TNFα or TNFR1 (45,97,108–111,116,119–122). These findings demonstrate that TNFα is indeed a critical mediator of macrophage-induced liver injury.

4. The Jedi Order: Suppression of Inflammation and Initiation of Wound Repair

Just as in Star Wars, where there was a balancing force to counter the machinations of The Dark Side; The Jedi, guardians of peace and justice; so it is that there is a tissue protective role for macrophages. This activity is mediated by M2 macrophages that accumulate at injured sites later in the inflammatory process; these cells function to restore homeostasis by down regulating M1 cells and the production of cytotoxic inflammatory mediators, and by stimulating tissue repair (4,10). Through the release of various cytokines and growth factors, M2 macrophages also stimulate angiogenesis, stabilize new matrix components, and induce fibroblasts and macrophages to synthesize extracellular matrix proteins (4,5,123–126). M2 macrophages have been reported to be immunosuppressive in animal models of multiple sclerosis, rheumatoid arthritis and lung inflammation (127–129). Moreover, in the liver, depleting or blocking activation or recruitment of M2 macrophages into inflammatory sites delays repair and/or exacerbates injury and the development of fibrosis induced by hepatotoxicants such as acetaminophen, carbon tetrachloride and cadmium (6,27,37,118, 130–135).

5. The Jedi Weapons

M2 macrophages, like the Jedi, have specialized field gear for their missions of defense and repair. In macrophages, these include an ability to release inflammatory mediators and growth factors such as TNFα, IL-6, IL-10, IL-18 binding protein and TGFβ, as well as various eicosanoids. These mediators counteract cytotoxic and proinflammatory events and promote tissue regeneration either directly or indirectly by inducing the production of additional anti-inflammatory, growth promoting and angiogenic mediators including IL-4, IL-13, lipoxins, resolvins, protectins and vascular endothelial cell growth factor (VEGF) (27,136,137). Following hepatotoxicant exposure, expression of protective proteins including IL-4, IL-10, IL-13, TNFα TGFβ and VEGF increases in the liver (27,63,73,84,115,138–142). Additionally, upregulation of these mediators protects against chemically-induced hepatotoxicity, while blocking their activity causes an exaggerated response. For example, administration of IL-13 protects mice from lethal endotoxemia, while treatment of animals with anti-IL-13 antibodies exacerbates acetaminophen-induced hepatotoxicity and significantly reduces survival (73, 140). Similarly, in mice treated with IL-10, acetaminophen-induced liver injury is ameliorated, while carbon tetrachloride and acetaminophen induced hepatotoxicity is exaggerated in IL-10

or IL-13 knockout mice, and in IL-4/IL-10 double knockout mice (73,139,141). The exaggerated hepatotoxic response is associated with increased production of cytotoxic mediators including ROS, RNS, TNFα, IFNγ and/or various chemokines.

Within the Star Wars story there were individuals such as Anakin Skywalker who struggled with which side of The Force they chose to follow. TNFα appears to possess a similar dichotomous behavior, as it plays a dual role in hepatotoxicity. For TNFα, this is most likely related to the timing of its release in tissues. Thus, when released early after injury by M1 macrophages, it functions as a proinflammatory and cytotoxic cytokine, while TNFα released later in the inflammatory response by M2 macrophages plays an essential role in antioxidant defense and in the initiation of tissue repair (143). This latter activity is due to the ability of TNFα to function as a potent mitogen, stimulating hepatocyte proliferation following acute injury (144–146). TNFα also stimulates macrophages and other cells to produce mediators important in wound healing, including TGFβ, connective tissue growth factor, VEGF, matrix metalloproteinase-9, IL-6, and chemokines such as CCL2, CXCL8 and CXCL1 (103,147). These findings, together with the observations that knockout mice lacking the gene for TNFα or TNF receptor 1 (TNFR1) are significantly more sensitive to liver injury induced by acetaminophen or carbon tetrachloride than their wild type counterparts, demonstrates the importance of TNFα in repair of damaged tissue (113–116,148).

6. The War between The Forces: Acetaminophen Hepatotoxicity

Over the past few years controversy has arisen over the protective versus pathologic role of liver macrophages in hepatotoxicity. Probably the most notable example of this controversy is related to acetaminophen-induced liver injury. Whereas in some studies it has been reported that blocking macrophages protects against liver injury, in others, exaggerated hepatotoxicity is observed. These divergent findings most likely reflect the distinct macrophage subpopulations responding at different times during the course of liver injury and repair. As described above, evidence suggests that macrophages play a dual role in the pathogenic response to hepatotoxicants such as acetaminophen. Whereas initially, classically activated macrophages displaying an M1 phenotype respond to injury by releasing cytotoxic and proinflammatory mediators which contribute to tissue injury, subsequently, alternatively activated M2 macrophages emigrate into injured sites and release mediators that down regulate inflammation (e.g., IL-10) and initiate tissue repair (e.g., VEGF, TNFα, and TGFβ) Although these macrophage populations are described as phenotypically distinct, they more likely represent extremes on a dynamic continuum of macrophages with varying functional capacities determined by changes in the cytokine milieu in the inflammatory microenvironment. Thus, the extent to which any given macrophage population contributes to or protects against tissue injury depends on the stage in the pathogenic process it encounters, and the specific cytokines and inflammatory mediators generated. In this context, using the same agent to block or delete macrophages may have different consequences depending on when the agent is administered and which macrophage population is targeted.

Another factor that contributes to conflicting findings on the role of macrophages in acetaminophen-induced hepatotoxicity is the method used to eliminate macrophages or to suppress their activity. For the most part, two major approaches have been used: $GdCl_3$ and clodronate containing liposomes. Gadolinium is a rare earth metal that is taken up by macrophages of the reticuloendothelial system (149). Early studies suggested that $GdCl_3$ functions *in vivo* by blocking phagocytosis and preventing macrophages from becoming activated, an effect thought to be due to competitive inhibition of calcium mobilization (150–153). Subsequently, it was shown that $GdCl_3$ exerts its effects by selectively eliminating large highly phagocytic Kupffer cells, and/or provoking a switch in their phenotype or acinar distribution (53,154,155). In control animals, the most active Kupffer cells are located in the

periportal regions of the liver lobule (156,157). After $GdCl_3$ administration, these cells are localized mainly in centrilobular regions of the liver and are primed to participate in tissue repair (154,155). The observation that these macrophages express immature monocyte/macrophage markers suggests that $GdCl_3$ stimulates extrahepatic recruitment of cells from blood and bone marrow precursors (154). It is noteworthy to mention that after $GdCl_3$ treatment of animals, macrophages localized in centrilobular regions of the liver continue to release TNFα which, as described above, plays a key role in repair of damaged liver (138,155,158). Furthermore, these cells are relatively resistant to a second challenge with $GdCl_3$. These findings, together with reports that Kupffer cell production of ROS and RNS is reduced after $GdCl_3$ administration, while IL-10 is unaffected, indicate that $GdCl_3$ targets M1 macrophages, and that macrophages remaining in the liver are of the M2 phenotype (24,25,27,35,53). This is also supported by findings that hepatocyte proliferation is either increased or unaffected by $GdCl_3$ treatment of animals (159,160).

Another method utilized to assess the role of Kupffer cells in chemical toxicity is administration of liposomes containing clodronate. Intravenous administration of these liposomes results in depletion of macrophages in the liver via apoptosis (159). In contrast to the selective depletion of larger macrophages in periportal regions of the liver by $GdCl_3$, both larger Kupffer cells and smaller ones in midzonal and centrilobular regions are eliminated by clodronate liposomes (6,27,118,161–163). Studies on the kinetics of macrophage repopulation in the liver after clodronate liposome administration have shown that macrophages do not begin to reappear for at least 7 days (164). In contrast to macrophages repopulating the liver after $GdCl_3$, these cells originate from a macrophage precursor pool in the liver, rather than directly from bone marrow derived monocytes, and are phenotypically more mature (6,165,166). Furthermore, production of macrophage colony stimulating factor in the liver plays a crucial role in their differentiation, maturation and proliferation (167). The fact that administration of clodronate liposomes prevents acetaminophen-induced increases in protective molecules such as TNFα, IL-6, IL-10, and IL-18 binding protein in the liver supports the idea that M2 cells are a major target of clodronate liposomes (27,118).

In summary it is apparent that in the acetaminophen-induced hepatotoxicity model, $GdCl_3$ and clodronate liposomes target distinct macrophage subpopulations which likely accounts for conflicting findings on the role of macrophages in the pathogenic process. Thus, while $GdCl_3$ preferentially targets cytotoxic M1 macrophages for elimination, clodronate liposomes mainly target M2 macrophages. This idea is consistent with reports that elimination of macrophages using $GdCl_3$ protects against acetaminophen-induced hepatotoxicity, while liver injury is exacerbated in animals treated with clodronate liposomes (24,25,27,28,30,31,118, 131).

6. Conclusions (The Final Battle)

Popular culture is often used as allegorical material in the teaching of modern philosophy. Indeed, there have been numerous philosophical treatises discussing the ever-present power of The Force and its more seductive Dark Side. The consistent conclusion of these writings is that The Dark Side, as well as opposing the Jedi, is a necessary consequence of The Force in terms of cosmic balance. It is reasonable to extend this allegory to the phenotypic forms of macrophages. In this way, although we may tend to think of the M1 macrophage as evil and the M2 macrophage as good, as they are involved in injury and repair, respectively, it may be more accurate to view them as two sides of the same coin, just as Darth Vader and Anakin Skywalker represent the two sides of The Force in one individual. Thus, it is not so much that M1 and M2 macrophages have opposing actions at inflammatory sites; rather there is a complex interplay between the two phenotypes that is necessary for an appropriate response to a toxic insult. Without doubt, macrophages are an important cellular component of the nonspecific

host defense system. These are the primary cells responsible for protecting the body from the damaging effects of invading pathogens and toxins. Although their presence in the body is clearly essential for appropriate immunological defense and wound repair, an imbalance in macrophage activation may in fact contribute to tissue injury. It is likely that the extent to which macrophages contribute to injury or participate in tissue repair depends on the balance of their phenotypic experience and the timing of their appearance in the liver. Aberrations in the relative responsiveness of these cells leading to an imbalance between production of proinflammatory and anti-inflammatory mediators may be important in determining the final outcome of the pathogenic response to toxicants.

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Table 1

Activated Macrophage Subpopulations

Macrophage Type		Activating Signals	Phenotype
M_1 Classically Activated		IFNγ priming followed by: TNFα GM-CSF LPS Activation of PAMP	-Release pro-inflammatory cytokines (TNFα IL-1, IL-6), IL-12, IL-23, M_1 chemokines -Express MHC-II -Present antigen to T cells -Promote Th1 responses (cell mediated immunity) -Microbicidal activity -Cytotoxicity -Tissue injury/destruction -Tumor resistance
M_2 Alternatively Activated	M_{2a}	IL-4 IL-13	-Phagocytosis -Stimulate proliferation -Promote tissue repair -Express MHC-II -Present antigen to T cells -Promote Th2 responses -Express scavenger receptors
	M_{2b}	Immune complexes + TLR agonists or IL-1β	-Microbicidal activity -Phagocytosis -Express MHC-II -Present antigen to T cells -Promote Th2 responses -Express scavenger receptors
	M_{2c}	IL-10 TGFβ Glucocorticoids	-Release IL-10, TGFβ, PDGF -High levels arginase -Immunosuppressive -Anti-inflammatory (down regulate M_1 responses) -Release extracellular matrix proteins (via TGFβ) -Promote wound repair, tissue remodeling and angiogenesis -Chronic inflammation