Concurrent inflammation as a determinant of susceptibility to toxicity from xenobiotic agents

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Abstract

Sensitivity to the toxic effects of xenobiotic agents is influenced by a number of factors. Recent evidence derived from studies using experimental animals suggests that inflammation is one of these factors. For example, induction of inflammation by coexposure to bacterial endotoxin, vitamin A or Corynebacterium parvum increases injury in response to a number of xenobiotic agents that target liver. These agents are diverse in chemical nature and in mechanism of hepatotoxic action. Factors critical to the augmentation of liver injury by inflammation include Kupffer cells, neutrophils, cytokines such as tumor necrosis factor-alpha (TNF-α) and lipid mediators such as prostaglandins, but these may vary depending on the xenobiotic agent and the mechanisms by which it alters hepatocellular homeostasis. In addition, the timing of inflammation exposure can qualitatively alter the toxic response to chemicals. Inflammation-induced increases in susceptibility to toxicity are not limited to liver. Concurrent inflammation also sensitzes animals to the toxic effects of agents that damage the respiratory tract, kidney and lymphoid tissue. It is concluded that inflammation should be considered as a determinant of susceptibility to intoxication by xenobiotic exposure. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Inflammation; Endotoxin; Sensitivity

1. Introduction

There are numerous factors that can contribute to differences among individuals in their sensitivity to xenobiotic agents. These include variations in age, gender, xenobiotic metabolism, immunologic responses, reserve and repair capacity of tissues, xenobiotic absorption, coexisting disease, coexposure to additional xenobiotic agents and nutritional status, as well as underlying inflammation. Several of these determinants of sensitivity are likely to coexist and exert influences in concert in any individual. Moreover, both genetic and environmental factors have the potential to exert important influences on most of these determinants. Emerging evidence suggests that coexisting inflammation can markedly enhance toxic responses and may be an important determinant of individual susceptibility. The following is a brief review of data supporting this hypothesis.
2. Inflammation is commonplace

Humans experience episodes that expose one or more organs to factors that cause inflammation. The most obvious causal agents are bacteria and viruses, exposure to which usually precipitates inflammation as a host response aimed at eliminating pathogens. In addition, specific immune reactions against antigens typically culminate in an acute or chronic inflammatory response. Moreover, there are many disease conditions that are associated with tissue inflammation, such as arthritis, hepatitis, atherosclerosis, asthma and others. Finally, studies over the last two decades indicate that exposure to bacterial endotoxin, a potent initiator of inflammation, is a commonplace occurrence in people.

Endotoxin comprises proteins in association with lipopolysaccharide (LPS), a major constituent of the outer cell wall of Gram negative bacteria. Such bacteria normally inhabit the intestinal tract in numbers that exceed the number of eukaryotic cells in the human body (Henderson et al., 1996). When these bacteria divide or are injured, large amounts of LPS are released in the intestinal lumen. Although it was once thought that the gastrointestinal (GI) mucosa was a perfect barrier to LPS and other microbial products, research over the last two decades has indicated that LPS can translocate from the GI lumen into the blood, and thereby the liver and other organs become exposed (Fig. 1). The degree to which this happens in a normal, unstressed GI tract remains a matter of debate. Some investigators have detected plasma levels of LPS in healthy, unstressed people, whereas others have not (Nolan, 1981; Jacob et al., 1977; Prytz et al., 1976). Recently it has been proposed that gut-derived bacterial products may regulate the expression of vascular adhesion molecules in other organs (Komatsu et al., 2000), suggesting a constitutive flux of these products across the GI epithelium into the circulation and raising the possibility that this flux may participate in regulating tissue homeostasis.

It is clear that many conditions can lead to an increase in plasma LPS concentration, probably as a result of enhanced GI translocation (Fig. 1). In people, these conditions include disease, surgery, trauma and ischemia that affect the GI tract, acute and chronic liver diseases, Reye's syndrome, and acute or chronic alcohol consumption (Roth et al., 1997). In one study of long distance runners, the runners whose GI tracts were stressed by the run experienced increases in plasma LPS (Brock-Utne et al., 1988). Even the process of anesthetizing a patient has been reported to increase plasma LPS concentration (Berger et al., 1997). In experimental animals, alterations in diet lead to enhanced plasma LPS, as does exposure to certain xenobiotic agents (Spaeth et al., 1990; Rutenburg et al., 1957; Delich, 1995; Hurley, 1995). In addition to exposure from the GI route, considerable LPS exposure can occur via inhalation. This occurs especially in occupations in which LPS-laden dusts or aerosols are generated, but exposure in the home environment is also common (Rylander and Haglind, 1984; Dosman et al., 1980; Pernis et al., 1961; Mattsby-Baltzer et al., 1989; Peterson et al., 1964).

The picture that emerges from these clinical and experimental studies is that mild endotoxemia in people is commonplace and episodic (Fig. 2). The frequency and magnitude of increases in plasma LPS probably vary a lot among individuals and even within an individual, depending on lifestyle, disease and other factors. Except in extreme cir-
Fig. 2. Hypothetical variation in plasma LPS concentration. Recent evidence suggests that a variety of factors can influence the concentration of LPS in plasma. Therefore, systemic exposure to inflammasens such as LPS occurs commonly and intermittently. The likely consequence of this enhanced exposure is the initiation of modest inflammatory responses that can alter tissue homeostasis, thus rendering tissues more sensitive to toxic effects of xenobiotics. These periods of increased sensitivity would coincide with inflammatory events that arise from exposure to LPS or other inflammasens.

3. Inflammation and sensitivity to xenobiotic agents

Reports in people of the relationship between coexisting inflammation and toxic responses to xenobiotic agents are lacking. This may be due in part to difficulties in timing of assessment of inflammation in patients at risk or to a lack of awareness of the influence of inflammation on responses to xenobiotics. Nevertheless, studies in experimental animals indicate that modest inflammation can heighten the sensitivity of individuals to the toxic effects of a wide variety of xenobiotic agents and that these effects can be observed in several tissues (Table 1). Emerging evidence suggests that inflammation induced by some xenobiotics contributes to their toxicities. For example, hepatotoxicity from acetaminophen is associated with inflammation (Blazka et al., 1995–96; Lawson et al., 2000b) and is dependent on nitric oxide, chemokines, and Kupffer cells (Gardner et al., 1998; Hogaboam et al., 2000; Laskin et al., 1995; Michael et al., 1999, for review, see Luster et al., 2001). Accordingly, it is reasonable to expect that sensitivity to these chemicals may be altered in the face of an ongoing inflammatory response.

3.1. Effects in liver

Perhaps the greatest body of literature on this subject pertains to responses in liver, and the notion that inflammation influences the response of the liver to insult is not new. Many of the supporting studies have used LPS as a tool to induce inflammation, although other agents have also been used. Another approach has been to examine the effects of reducing exposure to endogenous LPS. The results from these studies demonstrate that both endogenous LPS and administered LPS can influence hepatotoxic responses.

3.1.1. Contribution of endogenous LPS to hepatotoxic responses

In early studies, it was observed that hepatic necrosis induced by feeding rats a diet deficient in choline was prevented by treatment with antibiotics and did not develop in germ-free rats (Nolan, 1975). This observation led to the hypothesis that liver injury in this model resulted from exposure to gut-derived bacteria.
Table 1
Xenobiotics for which inflammatory stimuli increase tissue injury

<table>
<thead>
<tr>
<th>Agent</th>
<th>Inflammagen</th>
<th>Tissue affected</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choline-deficient diet</td>
<td>Endogenous bacterial products (LPS)</td>
<td>Liver</td>
<td>Antibiotics protect</td>
<td>Nolan, 1975</td>
</tr>
<tr>
<td></td>
<td>CCl₄</td>
<td>Liver</td>
<td>LPS tolerance protects</td>
<td>Nolan, 1975</td>
</tr>
<tr>
<td></td>
<td>Endogenous bacterial products (LPS)</td>
<td>Liver</td>
<td>Polymyxin B protects</td>
<td>Shvarts et al., 2000</td>
</tr>
<tr>
<td></td>
<td>Endogenous LPS</td>
<td>Liver</td>
<td>LPS antibody protects</td>
<td>Chamulitrat et al., 1995</td>
</tr>
<tr>
<td></td>
<td>Vitamin A</td>
<td>Liver</td>
<td>Response depends on reactive oxygen species, Kupffer cells</td>
<td>Czaia et al., 1994</td>
</tr>
<tr>
<td>Galactosamine</td>
<td>Endogenous bacterial products (LPS)</td>
<td>Liver</td>
<td>Colloctomy protects</td>
<td>Badger et al., 1996; elSisi et al., 1993</td>
</tr>
<tr>
<td></td>
<td>Endogenous LPS</td>
<td>Liver</td>
<td>LPS antibody protects</td>
<td>Nolan and Camara, 1985</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>Liver</td>
<td>Response depends on Kupffer cells, TNF, reactive oxygen species, adhesion molecules, neutrophils, NF-κB</td>
<td>Czaia et al., 1994</td>
</tr>
<tr>
<td></td>
<td>C. parvum</td>
<td>Liver</td>
<td>LPS tolerance or polymyxin B protects</td>
<td>Buhrami et al., 1994; Xu et al., 1994; Komatsu et al., 1996; Chosay et al., 1997; Stachlewitz et al., 1999; Jaeschke et al., 1998, 1999; Takayama et al., 1999; Lawson et al., 2000a; Nowak et al., 2000; Radford and Thugpen, 1994; Calcagno et al., 1992; Honchel et al., 1991; Cebula et al., 1984; Adachi et al., 1994, 1995</td>
</tr>
<tr>
<td>α-Naphthyl-isothiocyanate</td>
<td>Endogenous bacterial products (LPS)</td>
<td>Liver</td>
<td>Inactivation of Kupffer cells protects against acute exposure; antibodies protect against long-term exposure</td>
<td>Hansen et al., 1994; Brown et al., 1996</td>
</tr>
<tr>
<td>Lead</td>
<td>LPS</td>
<td>Liver</td>
<td>LPS tolerance or polymyxin B protects</td>
<td>Honchel et al., 1991</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>LPS</td>
<td>Liver</td>
<td>LPS tolerance or polymyxin B protects</td>
<td>Cebula et al., 1984</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Endogenous bacterial products (LPS)</td>
<td>Liver</td>
<td>Inactivation of Kupffer cells protects against acute exposure; antibodies protect against long-term exposure</td>
<td>Adachi et al., 1994, 1995</td>
</tr>
<tr>
<td>Polychlorinated biphens</td>
<td>LPS</td>
<td>Liver</td>
<td>Response depends on Kupffer cells, neutrophils, COX-2</td>
<td>Hansen et al., 1994; Kinser et al., 2000; Ganey et al., 2001</td>
</tr>
<tr>
<td>Allyl alcohol</td>
<td>LPS</td>
<td>Liver</td>
<td>Response depends on Kupffer cells, neutrophils, COX-2</td>
<td>Barton et al., 2000a,b, 2001</td>
</tr>
<tr>
<td>AFB₁</td>
<td>LPS</td>
<td>Liver</td>
<td>Response depends on Kupffer cells, neutrophils, TNF</td>
<td>Yee et al., 2001</td>
</tr>
<tr>
<td>Monocrotaline</td>
<td>LPS</td>
<td>Liver</td>
<td>Response depends on Kupffer cells, TNF</td>
<td>Sauer et al., 1995</td>
</tr>
<tr>
<td>2-Nitro-</td>
<td>Vitamin A</td>
<td>Liver</td>
<td>Response depends on Kupffer cells</td>
<td>Wueweera et al., 1996</td>
</tr>
<tr>
<td>1-naphthalene</td>
<td>Vitamin A</td>
<td>Liver</td>
<td>Response depends on Kupffer cells</td>
<td>Yaman et al., 1998; Juggio et al., 1998, 1998; Schwartz et al., 1995; Monti and Koren, 1999</td>
</tr>
<tr>
<td>Vinyldiene</td>
<td></td>
<td></td>
<td>Response depends on platelet-activating factor</td>
<td>Peavy and Fairchild, 1987</td>
</tr>
<tr>
<td>chloroide</td>
<td></td>
<td></td>
<td></td>
<td>Funacchi et al., 1998; Cho et al., 1999, 2000; Zhou et al., 1999, 2000; Rumi et al., 2000a,b</td>
</tr>
<tr>
<td>Cerulien</td>
<td>LPS</td>
<td>Liver and others</td>
<td>Response depends on platelet-activating factor</td>
<td>Yamano et al., 1998; Juggio et al., 1998, 1998; Schwartz et al., 1995; Monti and Koren, 1999</td>
</tr>
<tr>
<td>Grain dust</td>
<td>LPS (adsorbed)</td>
<td>Lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Particulate matter</td>
<td>LPS (adsorbed)</td>
<td>Lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ozone</td>
<td>LPS</td>
<td>Lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPS</td>
<td>LPS</td>
<td>Lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>LPS</td>
<td>Lymphoid tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mercuric chloride</td>
<td>LPS</td>
<td>Kidney</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
sequently, it was demonstrated that rats made tolerant to LPS developed less liver injury than naïve rats in response to carbon tetrachloride (CCL₄) (Nolan, 1975) or α-naphthylisothiocyanate (Calcamuggi et al., 1992), suggesting that endogenous LPS enhanced the hepatotoxic response to these chemicals. This was supported for CCl₄ by the observation that administration of an antibody directed against LPS decreased liver injury (Czaja et al., 1994). More recent evidence suggests that fibrosis induced by CCl₄ may also be related to LPS exposure. Treatment with polymixin B, an inactivator of LPS, reduced fibrosis in response to administration of CCl₄ (Shvarts et al., 2000). Endogenous LPS may also contribute to injury from administration of galactosamine since prior colectomy (Nolan and Camara, 1985) or administration of anti-LPS antibody (Czaja et al., 1994) protected against galactosamine-induced hepatic necrosis. Like CCl₄, liver injury from acute ethanol consumption has been linked to increased exposure to endogenous LPS. Blood LPS concentration increases in rats after administration of ethanol (Enomoto et al., 1998), and inhibition of inflammation protects against liver damage in this model (Adachi et al., 1994). With respect to more chronic exposure to ethanol, it is interesting that increased concentrations of blood LPS are often observed in alcoholic people (Bode et al., 1987). Furthermore, antibiotics protect against hepatic injury in animals from longer duration treatment with ethanol (Adachi et al., 1995). Accordingly, emerging evidence suggests that exposure to endogenous LPS may contribute to hepatotoxicity of certain xenobiotic agents.

3.1.2. Influence of exogenous LPS on hepatotoxic responses

Hepatotoxicity caused by small doses of galactosamine is exacerbated by cotreatment with a small dose of LPS (Bahrami et al., 1994). Numerous reports confirm the requirement for inflammatory mediators for this response (Xu et al., 1994; Komatsu et al., 1996; Chosay et al., 1997; Jaeschke et al., 1998, 1999; Takayama et al., 1999; Stinchlweitz et al., 1999; Nowak et al., 2000; Lawson et al., 2000a). Likewise, coadministration of LPS with lead (Honchel et al., 1991), ethanol (Hansen et al., 1994), benzyl alcohol (Cebula et al., 1984), CCI₄ (Chamulitrat et al., 1995) or polychlorinated biphenyls (Brown et al., 1996) results in markedly enhanced liver injury in rats. Cerulein normally produces pancreatitis in rats, however, LPS coadministration to cerulein-treated rats results in liver injury, as well as damage to several other organs (Yamano et al., 1998). Thus, accumulated evidence from several laboratories indicates that LPS exposure can enhance the effects of numerous hepatotoxins including drugs, food-borne toxins and environmental contaminants (see Roth et al., 1997).

3.1.2.1. Allyl alcohol. For many of the above-mentioned xenobiotics, elucidation of dose-response relationships and studies of critical inflammatory mediators that drive these effects have not been performed. Efforts to address these issues have begun for allyl alcohol, a chemical used in the manufacture of food flavorings and other agents. Very small, nontoxic doses of LPS convert a nontoxic dose of allyl alcohol into one that produces marked periporal injury (Sneed et al., 1997). In this model, LPS doses as small as 1/1000th of that which causes liver injury by itself enhanced the toxicity of allyl alcohol (Sneed et al., 1997). This increased response was not reproduced in isolated hepatic parenchymal cells, ruling out an effect of LPS on bioactivation of allyl alcohol and suggesting an indirect action (see below).

3.1.2.2. Aflatoxin B₁. The augmentation of the acute hepatotoxicity of aflatoxin B₁ (AFB₁) by LPS has also been studied recently. People and animals are exposed to AFB₁ as a fungal metabolite that contaminates grains and nut products. Administration of a small, nontoxic dose of LPS markedly enhanced periporal parenchymal cell and bile duct injury in rats exposed to AFB₁ (Barton et al., 2000b). Acute aflatoxicosis in humans is thought to result from AFB₁ doses that are similar to those that cause liver injury in rats (Chao et al., 1991). Accordingly, the results in rodents raise the possibility that coexisting inflammation may in part determine the severity of aflatoxicosis in human cases. In addition to caus-
ing acute hepatotoxicity. AFB₁ is a human and animal carcinogen. Several epidemiological studies have found that people with hepatitis are at greater risk for hepatocellular carcinomas from dietary aflatoxin (Groopman et al., 1993; Jacobson et al., 1997; Qian et al., 1994; Ross et al., 1992). This suggests that hepatic inflammation may predispose people to the carcinogenic effects of this fungal toxin.

3.1.2.3. Monocrotaline. Monocrotaline is one of many toxic pyrrolizidine alkaloids found in a wide variety of plant species, some of which are used in nutritional supplements and herbal teas (Huxtable, 1980; Huxtable et al., 1986; Ridker et al., 1985). Exposure of people to these alkaloids has resulted in venoocclusive disease of the liver. Similarly, rats given large doses of monocrotaline experience acute, centrilobular hepatocellular injury accompanied by destruction of endothelium in sinusoids and central veins and hemorrhage (DeLeve et al., 1999). Small doses of monocrotaline and LPS, which are noninjurious when given by themselves, resulted in pronounced liver injury when given together (Yee et al., 2000). Dose–response studies using plasma alanine aminotransferase activity as a marker of hepatotoxicity indicated that LPS cotreatment reduced the threshold for liver injury from monocrotaline to between 13 and 33% of that from monocrotaline given alone. For this plant toxin, the lesion morphology suggested that exposure to each of the agents potentiates the hepatotoxicity of the other. That is, the cotreatment results in a pyrrolizidine-like, centrilobular lesion and a midzonal lesion resembling that which occurs after larger, hepatotoxic doses of LPS.

The observations that inhibition of either Kupffer cell function or synthesis of TNF protected against hepatotoxicity suggest a role for inflammation in LPS enhancement of monocrotaline hepatotoxicity (Yee et al., 2001). Inasmuch as pyrrolizidine-containing herbal supplements are consumed in part to combat illnesses of various sorts, some of which may be associated with inflammation, it is tempting to speculate that people who consume these alkaloids during an episode of inflammation may be putting themselves at risk of a toxic response.

3.1.3. Influence of other inflamagens on hepatotoxic responses

The ability of inflamagens to enhance chemical toxicity is not limited to LPS. Other agents that induce inflammation or stimulate inflammatory cells also increase injury from hepatotoxins. Vitamin A activates Kupffer cells, resulting in increased phagocytic activity and release of reactive oxygen species, TNF, and prostaglandins (Hoglen et al., 1997). A single administration of vitamin A increased the hepatotoxicity of CCl₄ in rats (Badger et al., 1996). Partial protection from this effect was observed with either inhibition of Kupffer cell function or depletion of neutrophils, suggesting a role for inflammatory mediators. Similarly, this regimen of vitamin A also increased the hepatotoxicity of vinylidene chloride, although it is unclear whether the augmentation was mediated by increased bioactivation of vinylidene chloride or by inflammatory mediators (Wuweera et al., 1996). A longer-term treatment with vitamin A (7 days) enhanced the hepatotoxic response of rats to CCl₄ through a mechanism involving Kupffer cells and reactive oxygen species (cSisi et al., 1993). In mice, however, vitamin A protected against CCl₄-induced hepatotoxicity, perhaps through an effect on metabolism of this agent (Sauer et al., 1995). In rats, 7-day treatment with vitamin A augmented the hepatotoxicity of 2-nitronaphthalene, and inhibition of Kupffer cell function afforded protection against liver damage (Sauer and Sipes, 1995). Interestingly, vitamin A protected against lung injury from 2-nitronaphthalene, perhaps through an anti-inflammatory effect on pulmonary macrophages.

Corynebacterium parvum also activates the reticuloendothelial system, of which Kupffer cells comprise a major part. Cotreatment with C. parvum increased liver injury from galactosamine, whereas hepatotoxicity caused by acetaminophen or CCl₄ was diminished (Raiford and Thigpen, 1994). The latter effect may be attributed to decreased bioactivation of acetaminophen and CCl₄ because C. parvum decreased activity of some isoforms of cytochrome P450. Interestingly, C. parvum and LPS in combination produce severe liver injury (Sato et al., 1993; Wu et al., 1993).
3.2. Extrahepatic tissues

The studies described above demonstrate that the ability of underlying inflammation to augment hepatotoxicity is not specific to a chemical class or to chemicals that act by a specific mechanism. Moreover, the ability of concurrent inflammation to influence toxicity applies to extrahepatic organs. In the respiratory tract, LPS may be largely responsible for airflow obstruction due to grain dust inhalation in agricultural workers (Jugielo et al., 1996, 1998; Schwartz et al., 1995). Similarly, there is interest in the role of LPS in exacerbation of cardiopulmonary diseases by ‘‘inert’’, ambient particulate matter (Monn and Koren, 1999). Acute treatment of mice with LPS prior to exposure to ozone led to increased edema in the lungs and lethality (Peavy and Fairchild, 1987). Similarly, treatment of rats with LPS before or after exposure to ozone augmented ozone-induced mucous cell metaplasia in nasal epithelium (Fanucchi et al., 1998; Cho et al., 1999). Although both LPS and ozone had effects when given alone, cotreatment resulted in a greater number of mucous cells, more intraepithelial mucous substances and more persistent increases in mucin specific mRNA. Mucous cell metaplasia in response to ozone was neutrophil-dependent (Cho et al., 2000), and the LPS/ozone cotreatment resulted in a greater number of neutrophils in the nasal transitional epithelium compared with ozone alone (Cho et al., 1999), thus suggesting a potential mechanism for the increase in injury.

Similarly, LPS acts synergistically with the trichothecene fungal toxin, deoxynivalenol, to cause B-cell apoptosis in lymphoid tissue (Zhou et al., 2000). Cotreatment with LPS caused a synergistic increase in TNF mRNA in the spleen and in circulating TNF, as well as an additive increase in interleukins-6 and -1β (Zhou et al., 1999). Additionally, LPS increased mortality in response to deoxynivalenol: within 40 h of exposure all cotreated mice died whereas all animals survived when they were treated with either agent alone.

Cotreatment of mice or rats with LPS and mercuric chloride increased nephrotoxicity (Rumbaieha et al., 2000a,b). In both species, the amount of mercury in the renal tissues was increased in LPS-treated animals. In this model, it is not known with certainty that inflammation is the cause of the increased sensitivity, but this seems likely based on the known effects of LPS.

The results of these studies demonstrate that the ability of LPS and other inflammmagens to enhance toxic responses is likely a phenomenon applicable to a wide variety of xenobiotic agents that affect many tissues and act by various mechanisms. Such results have led to the idea that human exposure to LPS and other agents (e.g. pathogens) in the environment that precipitate a modest inflammatory response may act as a determinant of susceptibility to environmental, foodborne and other toxic, xenobiotic agents. That is, people undergoing an inflammatory response may represent a subset of the population that is likely to respond to xenobiotic exposure with a toxic outcome.

4. Timing of inflammation relative to xenobiotic exposure may determine the nature of the response

The temporal relationship between the occurrence of inflammation and exposure to a xenobiotic agent is likely to be an important determinant in whether a toxic response is augmented. The reason for this is that initial proinflammatory events are eventually counteracted by downregulatory mediators such as prostaglandin E2 and interleukin-10. This results in the subsequent development of tolerance to the effects of LPS and other inflammmagens. Such tolerance reduces the ability of cells to produce proinflammatory mediators that likely drive the increased organ injury observed during inflammation. Typically, tolerance develops a day or two after exposure to inflammmagens such as LPS. This development of LPS tolerance can afford protection against tissue injury, as has been observed in animals for liver damage induced by acetaminophen or CCl4 (Liu et al., 2000) and for ischemia-reperfusion injury in the kidney (Heemann et al., 2000). By contrast, augmented responses are observed when LPS is administered closer in time to the exposure to the xenobiotic agent (e.g. within a few hours).
example, acute exposure to LPS increased pulmonary toxicity of ozone, whereas treatment with LPS for 5 days diminished the response (Peavy and Fairchild, 1987). With respect to vitamin A, although treatment with this agent for 7 days enhances hepatotoxic responses, Kupffer cells isolated from animals using this treatment regimen are still in an 'inflammatory' state in that they respond to stimuli with increased production of mediators (Hoglen et al., 1997). Thus, depending on the nature of the inflammation and the temporal relationship between inflammation and xenobiotic exposures, enhanced or diminished toxic responses to xenobiotic agents can occur.

5. Inflammatory factors and mechanisms

5.1. Initial hypotheses

Much remains unknown about how LPS and other inflammagens exacerbate toxic responses. In 1975, Nolan suggested three mechanisms by which LPS might influence hepatotoxic responses (Nolan, 1975). One hypothesis was that liver injury induced by chemical exposure leads to decreased removal and detoxification of LPS and, therefore, greater tissue exposure (Table 2). An argument against this hypothesis is that inhibition of Kupffer cell function, which diminishes LPS clearance (Mimura et al., 1995), protected against LPS potentiation of hepatotoxicity due to allyl alcohol, galactosamine or monocrotaline (Sneed et al., 1997; Stachlewitz et al., 1999; Yee et al., 2001).

The second hypothesis was that xenobiotic agents increase intestinal absorption of LPS, thereby enhancing toxicity. As noted above, such increases may play an important role in hepatotoxicity from CCl4 or α-naphthylisothiocyanate (Nolan, 1975; Calcamuggi et al., 1992; Czaia et al., 1994). The third hypothesis related to LPS-induced alterations in endogenous mediators that are either protective or detrimental to hepatic function. Later revision of these hypotheses included a mechanism which invoked release of inflammatory mediators from cells of the reticuloendothelial system, specifically Kupffer cells of the liver, as well as decreased detoxification of LPS (Nolan and Camara, 1985).

5.2. Revision of hypotheses based on new knowledge of mechanisms of inflammation

Since the formulation of these early hypotheses, our knowledge of inflammation and of the mechanisms by which LPS exerts adverse effects on cells has expanded tremendously. Based on this new knowledge, it seems likely that cells, soluble mediators and other signaling molecules activated or expressed during the inflammatory response underlie the heightened sensitivity (Fig. 3). Inflammagens such as LPS cause tissue injury by themselves at large doses, and it seems likely that some of the same factors are at work during augmentation of xenobiotic toxicity by smaller LPS doses. These factors include, but are not limited to, Kupffer cells, cytokines such as TNF and interleukin-1, reactive oxygen species, nitric oxide, proteases, and lipid metabolites such as platelet activating factor and prostaglandin D2 (PGD2) (for reviews, see Hewett and Roth, 1993; Burrell, 1994; Hardie and Kruse-Elliott, 1990; Watson et al., 1994;Jaeschke, 2000). Activation of complement and coagulation systems in the blood may also play critical roles in inflammatory tissue injury (Pearson et al., 1996; Jaeschke et al., 1994). Activation of other cells such as sinusoidal endothelium, stellate cells and platelets may also be important.

The process of cell activation by LPS involves transcription factors such as nuclear factor-kappa B (NF-κB), activation of which results in gene products that amplify and extend the inflammatory response (Abraham, 2000; Wu and Zern,
Fig. 3. Simplified depiction of inflammatory events that can sensitize tissue to chemical-induced injury. Inflammagens such as LPS activate Kupffer cells (macrophages) and other inflammatory cells. This results in release of soluble mediators of inflammation and recruitment of other inflammatory cells that effect downstream events. At large doses of inflammagens, this response can lead to overt injury. At smaller doses, cells become more sensitive to the toxic effects of xenobiotics. These xenobiotics alone at larger doses can also produce cell death. The numerous positive and negative feedback mechanisms that occur during inflammation are not pictured.

1999; Christman et al., 1998). Stellate cell activation in liver can lead to sinusoidal contraction, which may promote arrest of neutrophils in the microvasculature (Jaeschke et al., 1996). Activation of endothelial cells in sinusoids results in the expression of adhesion molecules that are involved in transmigration of neutrophils. This transmigration process places them in contact with parenchymal cells and results in their release of lysosomal proteases and other agents (Essani et al., 1997a,b). These neutrophil-derived products, especially toxic proteases such as cathepsin G and elastase, appear to be important for hepatocellular necrosis during experimental endotoxemia and other acute, inflammatory liver conditions (Ho et al., 1996; Sauer et al., 1996; Yamano et al., 1998). Thus, LPS and other inflammagens initiate a cascade of events which culminates in tissue injury at larger doses and which, at smaller LPS doses, may render tissues more sensitive to the toxic effects of chemicals (Fig. 3). Accordingly, clues about how concurrent inflammation augments toxicity might be found in what is known about mechanisms of LPS-induced tissue injury.

5.3. Mechanisms are complex and not universal

As an example, the interaction between galactosamine and LPS in rodents is the most extensively studied amplification model to date. In this model, many of the factors that are critical to pathogenesis of liver injury from large doses of LPS also play key roles when smaller LPS doses are coupled with galactosamine administration. These factors include Kupffer cell activation, release of TNF and reactive oxygen species, NF-κB activation, expression of adhesion molecules on activated sinusoidal endothelium, and arrest, transmigration and activation of blood neutrophils (Xu et al., 1994; Nowak et al., 2000; Takayama et al., 1999; Stachlewitz et al., 1999; Jaeschke et al., 1999, 1998; Chosay et al., 1997; Komatsu et al., 1996; Lawson et al., 2000a).

Accumulated evidence suggests that the critical inflammatory elements and events may vary depending on the particular chemical to which an individual is coexposed. Models of inflammatory augmentation of hepatotoxicity of allyl alcohol and AFB, illustrate the differences. In a rat model
of potentiation of allyl alcohol hepatotoxicity by LPS, inactivation of Kupffer cells prevented liver injury, suggesting that these cells are critical players (Sneed et al., 1997). Interestingly, however, a neutralizing antibody to TNF, given to rats using a regimen that protects against liver injury from a large dose of LPS, failed to provide protection in this allyl alcohol potentiation model (Sneed et al., 2000). In contrast, the augmentation of AFB$_1$ hepatotoxicity by LPS is prevented by TNF neutralization, suggesting a critical role for this cytokine in this model (Barton et al., 2001).

Another difference in these two models is the role of cyclooxygenase-2 (COX-2). COX-2 is an inducible enzyme that catalyzes synthesis of thromboxane and prostaglandins, especially prostaglandins E$_2$ and D$_2$. The activity of this enzyme is upregulated during inflammation (Liu et al., 1996). Inhibition of COX-2 protected against LPS potentiation of the toxicity of allyl alcohol (Ganey et al., 2001) but not of AFB$_1$ (Barton et al., 2001). Thus, COX-2 products appear to be important in enhancing allyl alcohol toxicity but not AFB$_1$ toxicity. Presumably, the reason for this lies in differences in the mechanisms by which allyl alcohol and AFB$_1$ damage liver, however, which specific differences are important remains unknown.

Prior depletion of neutrophils protected against hepatocellular injury in both of these models (Kinser et al., 2000; Barton et al., 2000a), underlining the importance of this inflammatory cell in augmentation responses. Interestingly, in the AFB$_1$ augmentation model, neutrophil depletion protected only against parenchymal cell injury and not against bile duct epithelial cell injury (Barton et al., 2000a). This result suggests that two different mechanisms, one neutrophil-dependent and the other not, are involved in the augmentation of aflatoxin B$_1$ hepatotoxicity by LPS and that the inflammatory factors that are critical to the response may depend on the target cell.

### 6. Conclusion

In summary, systemic exposure to inflamma-
gen such as LPS appears to be commonplace in people, and the magnitude of episodic exposures can be influenced by a variety of conditions. Studies in animals indicate that exposure to agents that induce inflammation markedly enhances the toxic effects of a wide variety of xenobiotic agents (Table 1). These observations suggest that concurrent inflammation should be considered as a potentially important determinant of susceptibility to intoxication from environmental chemicals, drugs and other xenobiotic agents. That is, when exposure to certain xenobiotics coincides with a period of inflammation, an individual may be at greater risk for adverse effects (Fig. 2). A variety of cells and soluble mediators are likely to play a role in inflammation-enhanced xenobiotic toxicity (Fig. 3). Additional study will be needed to understand the circumstances under which this is relevant to humans and to know which inflammatory factors and mechanisms are important.

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