

Antigen-presenting cells under the influence of alcohol

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The negative influence of alcohol (ethanol) and its metabolites on innate and adaptive immunity is well-recognized. Much attention has recently been focused on the impact of acute and chronic alcohol exposure on antigen-presenting cells (APC). In particular, insights have been gained into how the properties of human blood monocytes and rodent macrophages are influenced by alcohol *in vitro* and *in vivo*. Here, we review the impact of alcohol on various aspects of APC function and the underlying mechanisms, including its effects on intracellular signaling events. We also discuss new information regarding the influence of alcohol on various APC populations in the liver, a primary site of alcohol metabolism.

Adverse effects of alcohol on immune reactivity

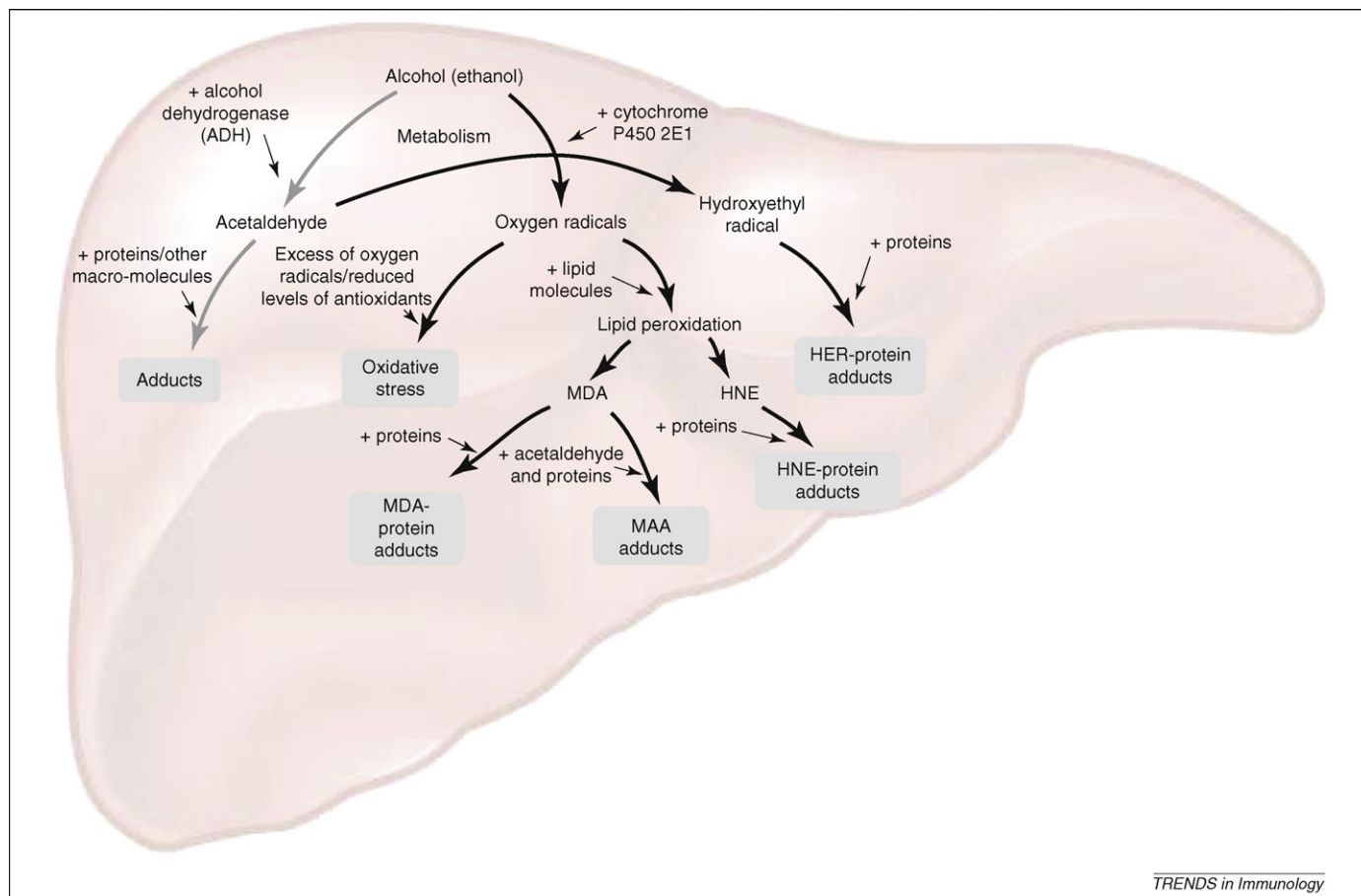
Alcohol (ethanol) is the most commonly abused substance in the US and contributes to well-described changes in inflammatory and immune responses. Many studies have demonstrated an association between excessive alcohol use and increased susceptibility to infectious diseases, including bacterial pneumonia and tuberculosis and progression of hepatitis C virus (HCV) infection [1,2]. Alcoholic patients also have increased incidences of autoimmune diseases [3,4], and in trauma and burn patients, acute excessive alcohol intake can increase the incidence of sepsis [5–7].

Acute or chronic alcohol consumption alters serum immunoglobulin (Ig) levels [8,9] and the numbers and function of lymphocytes (T, B and natural killer [NK] cells), monocytes and neutrophils (reviewed in Ref. [10]). Abnormalities in cell function include altered endocytic and phagocytic activity, migration [11–13] and cytokine expression (reviewed in Ref. [14]). In rodents, chronic alcohol feeding increases nuclear factor (NF)κB activation, which affects leukocyte function (i.e. increased maturation, cytokine and chemokine secretion) [15,16]. Also, in mice, and in cell culture systems, alcohol affects other cell signaling pathways, such as reduced activation of signal transducer and activator of transcription (STAT)1 and STAT3 [17,18]. Furthermore, products of alcohol metabolism have been linked to autoimmunity (e.g. systemic lupus erythematosus [SLE]). Thus, in SLE, anti-malondialdehyde (MDA) antibody (Ab) titers correlate strongly with Ab markers for this disease, such as anti-nuclear Abs [19].

Herein, we discuss published work concerning the influence of alcohol on various antigen (Ag)-presenting cell (APC) populations, that include ‘classical’ APCs, such as monocytes, macrophages and dendritic cells (DC), and Kupffer cells (KC), Langerhans cells and other cell types that present Ag under different conditions, including liver sinusoidal endothelial cells (LSEC). ‘Acute’ alcohol consumption in healthy human studies usually refers to achievement of maximal blood alcohol levels of 0.1 g/dL and acute alcohol treatment *in vitro* to exposure of cells (from 10 min up to 24 h) to 25 mM alcohol (approximately equivalent to a blood alcohol level of 0.1 g/dL) or higher. Acute ethanol exposure of mice is designed to achieve similar blood levels. Seventy-two hour alcohol exposure *in vitro* has been designated as ‘chronic’ [20,21]. Several isocaloric models of chronic alcohol consumption (>6 weeks) have been described in mice that lead to some features of alcoholic liver disease in humans.

Alcohol metabolism

There are three oxidative pathways of alcohol metabolism (Ref. [22]; Figure 1), the main process for alcohol elimination. The majority of metabolism is completed by the liver, via the enzyme alcohol dehydrogenase (ADH) in hepatocytes, leading to generation of acetaldehyde. With high alcohol concentrations or with chronic alcohol consumption, the microsomal ethanol-oxidizing system (MEOS) becomes the alternate pathway of metabolism, utilizing the enzyme cytochrome P450 (CYP)2E1. A minor pathway of oxidative alcohol metabolism is through catalase, located in peroxisomes within hepatocytes, that catalyzes the conversion of alcohol in the presence of hydrogen peroxide-generating systems. In all pathways, the oxidative metabolism of ethanol yields acetaldehyde and acetate as metabolites. Notably, ethanol oxidation by CYP2E1 also results in the production of reactive oxygen species (ROS), including superoxide anion and hydroxyl radicals, which are highly reactive and harmful, resulting in lipid peroxidation [23]. Two prevalent lipid peroxidation products are MDA and 4-hydroxynonenal (4-HNE) [24]. These products, in addition to acetaldehyde, can interact with or haptenate many proteins and other molecules to form hybrid compounds called adducts, which are important in alcohol-induced liver injury [24]. In particular, mixed MDA-acetaldehyde-protein adducts (MAA) have been the most studied to date. Immune effects resulting



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Figure 1. Metabolism of alcohol (chemically known as ethanol). The principal alcohol-metabolizing enzymes are alcohol dehydrogenase (ADH) and cytochrome P450 2E1. ADH converts alcohol to acetaldehyde, which reacts with other proteins to generate hybrid molecules or 'adducts.' Cytochrome P450 2E1 also generates acetaldehyde, in addition to reactive oxygen-containing molecules (oxygen radicals), including the hydroxyethyl radical molecule. Abbreviations: MDA, malondialdehyde; HNE, 4-hydroxy-2-nonenal; HER, hydroxyethyl radical; MAA, mixed MDA-acetaldehyde-protein adducts. Modified from Ref. [97].

from such protein adduction include alterations in Ig production, T-cell activation, cytokine and chemokine production [25].

Two non-oxidative pathways for alcohol metabolism exist, but contribute minimally [22]. However, the products of these pathways might also affect immune function and, thus, have clinical relevance. Briefly, one pathway leads to formation of fatty acid ethyl esters, which remain in plasma and tissues for extended periods, and the effects of which are currently unknown. The second pathway requires the enzyme phospholipase D, and converts phospholipids to phosphatidic acid, an important cell signaling component. The presence of ethanol in the reaction leads to the formation of phosphatidyl ethanol, which probably can interrupt or affect many cell signaling pathways. The question of whether alcohol alone exerts its effects on APCs or it does so in combination with its metabolites, requires further investigation.

Monocytes: alterations in cytokine production

Table 1 outlines observations on human monocytes exposed to alcohol *in vitro*, whereas Table 2 summarizes reported *in vivo* effects of alcohol on these cells. Monocytes from healthy volunteers who consumed moderate levels of alcohol acutely (i.e. 0.8 mg/kg within 30 min) showed reduced T-cell allostimulatory capacity 4–18 h later, com-

pared to control monocytes from the same volunteers obtained before drinking [26–28]. This could not be ascribed to changes in cell surface expression of major histocompatibility complex (MHC) class II which remained the same before and after alcohol intake [27,28]. By contrast, consistently reduced expression of the costimulatory molecules CD80 and CD86 was observed on monocyte-derived DC exposed to alcohol (25 mM) during their differentiation *in vitro* [26,28]. In addition, alcohol-treated DCs showed reduced IL-12 and increased IL-10 production [26].

Alterations in cytokine production, depending on the duration of alcohol exposure, have been reported in mixed leukocyte reaction (MLR) cultures with alcohol-exposed monocytes as stimulators. However, selective exposure of T cells and not monocytes to alcohol resulted in normal cell proliferation, indicating that the effects of alcohol are mediated principally via the monocyte stimulators [29]. These studies showed that acute alcohol exposure reduced interferon (IFN) γ production and elevated IL-10 and IL-13 production, but did not affect IL-4 expression [29]. Monocytes stimulated with bacterial lipopolysaccharide (LPS) during acute alcohol (25 mM for the duration of LPS treatment) showed suppressed tumor necrosis factor (TNF) α and IL-1 β , but enhanced IL-10 production [20,30–33]. By contrast, the effects of chronic alcohol exposure have varied from study to study. Most reports

Table 1. *In vitro* effects of alcohol or its metabolites on functions of human monocytes and monocyte-derived DC

Function	Alcohol exposure	Effect	Refs
Phagocytic activity and microbial (<i>Candida</i>) killing function	1hr–7days	Reduced (but stimulated after several days exposure)	[12,72]
ROS production	45 min	Enhanced	[95]
DC differentiation from blood monocytes	7 days	Inhibited	[26]
IRAK-1 and ERK 1/2 kinases	16h	Inhibited	[43]
IRAK-M levels	16h	Increased	[43]
LPS-induced NFκB activation (phosphorylation of p65)	1 h	Impaired	[20,40]
IKβα-independent NFκB activity	15 min–1hr	Modulated ^a	[40]
Costimulatory molecule (CD80 and CD86) expression	7 days	Reduced	[26,28,31]
IL-12 production	7 days	Reduced	[26,31]
IL-10; TGFβ production	7 days	Enhanced	[26,31]
TNFα; IL-1β production (LPS or staphylococcal enterotoxin B induced)	1.5–3hr (mRNA), 16hr (protein)	Reduced; but enhanced with prolonged <i>in vitro</i> exposure	[20,31,32]
TNFα-TACE interactions	1–3 h	Inhibited	[32]
LPS-induced redistribution of TLR4 complex components in lipid rafts	1hr	Disrupted	[44,45]
HO-1 (activation reduces TNFα)	40hr	Activated	[38]
DNA binding capacity of Sp-1 and AP-1 transcription factors	4–24 h	Augmented	[37]

^aA dual effect has been proposed: (i) an inhibitory effect of NFκB binding via decreased IKK activity and p65 phosphorylation and transcriptional activity and, (ii) promotion of proteolytic degradation of IκBα.

indicate that monocytes exposed chronically to alcohol upregulate TNFα production in response to LPS *in vitro* [34,35]. Others document reduced TNFα [36]. This discrepancy might arise from differences in the cells investigated (i.e. one study used a cell line, whereas in the others, human monocytes from chronic alcoholics were used). In these latter studies, chronic alcoholics without liver disease (AWLD) were examined. Monocytes stimulated with LPS alone showed reduced TNFα and IL-12, but enhanced IL-1β production [36]. Interestingly, these cytokine effects, with the exception of IL-12, were normalized after two weeks abstinence. In a more recent study [35], monocytes from AWLD showed increased spontaneous production of IL-1β, IL-6, IL-12 and TNFα. When stimulated with LPS or IFNγ, these monocytes showed increased overall cytokine production [35]. However, when the ratio of induced to spontaneous cytokine production was determined, AWLD monocytes had lower ratios compared to controls, indicating overall suppression of monocyte function in these patients.

Recently, the mechanism by which acute alcohol exposure causes enhanced production of the immunosuppressive cytokine IL-10 by human monocytes has been examined [37]. *In vitro* studies have demonstrated increased levels of STAT3, in addition to upstream Src kinase activation and binding to DNA after acute alcohol

exposure and further enhancement with LPS stimulation [37,38]. Augmented DNA binding capacity by the transcription factors Sp-1 and AP-1 was also reported. Heme oxygenase (HO)-1, a stress-induced protein, is also known to be involved in IL-10 and TNFα production. The same investigators also found that HO-1 RNA levels were increased with acute alcohol exposure and that, when HO-1 was inhibited, IL-10 production by monocytes was also suppressed. Collectively, these data indicate that Src kinase and STAT3, HO-1, AP-1 and Sp-1 pathways are important in modulation of IL-10 production by human monocytes after acute alcohol exposure.

Blood monocytes: alterations in molecular regulators and signaling

There is evidence that acute alcohol exposure *in vitro* (25 mM) preferentially induces the inhibitory dimer of NFκB, p50/p50, rather than the activating heterodimer, p65/p50 [39]. Furthermore, acute alcohol exposure inhibits LPS-induced NFκB by decreasing DNA binding of the p65/p50 heterodimer [20,40]. When IκB, an inhibitor of NFκB, was investigated, a specific decrease in phospho-specific IκBα was observed, indicating that alcohol interferes directly with NFκB activation and subsequently its regulation of other genes. Additional studies also show that IκBα degradation in monocytes is not affected by acute

Table 2. *In vivo* effects of alcohol on monocyte and macrophage function (acute or chronic alcohol intake)

Species and cells	Function	Exposure	Effect	Refs
Rat KC, alveolar macrophages, microglia	Phagocytic ability (through C3b and FcR)	Acute and Chronic	Impaired	[16,64,72,96]
Human monocytes, monocyte-derived DC	Costimulatory molecule expression, IL-12 production, T cell allostimulatory ability	Acute	Reduced	[26–28]
Human monocytes	NFκB activation (through CD14, TLR4, IL-1 and TNF receptors)	Acute	Inhibited	[33,84]
Murine macrophages	TNFα, IL-1β, IL-6, IL-12 production	Acute	Reduced	[33,54]
Human monocytes		Chronic	Reduced ^a	[35]
Human monocytes	IL-10 production	Acute	Enhanced	[33]
Murine macrophages	TLR2-, 3-, 4- and 9-mediated inflammatory responses (p35 and ERK1/2 MAPK activation)	Acute	Reduced	[55,56]
Rat KC	Chemotaxis	Chronic (32 weeks)	Suppressed	[16]
Rat alveolar macrophages	NO and hydrogen peroxide production	Acute or chronic	Reduced	[49,50]

^aAlthough 'spontaneous' production of these cytokines is enhanced in AWLD, an overall suppressive effect of alcohol consumption on LPS- or IFNα-induced production is observed.

alcohol treatment, indicating that an I κ B α -independent pathway exists for NF κ B suppression [33]. Thus, acute alcohol exposure *in vitro* inhibits I κ B kinase (IKK)-mediated phosphorylation of the p65 component of NF κ B in human monocytes [40].

Differential effects on NF κ B activation in monocytes and macrophages are observed, depending on the duration of alcohol exposure. Acute alcohol exposure leads to decreased NF κ B activation, whereas chronic alcohol treatment enhances NF κ B activation. Monocytes from alcoholic patients show enhanced constitutive and LPS-induced NF κ B activation and TNF α production [41]. A follow-up study using a rat model of alcoholic liver disease, in which hepatic macrophages (i.e. KC) were investigated, revealed a decrease in cellular adenosine 3'-5'-cyclic monophosphate (cAMP) related to increased LPS-inducible TNF α and NF κ B activation [42]. When cAMP was enhanced chemically in these cells, abrogation of cytokine production ensued, with no effect on transcription factor activation. Instead, decreases in the transcriptional activity of NF κ B were observed.

Toll-like receptor (TLR) signaling pathways in human monocytes have also been examined after acute alcohol exposure *in vitro* [43] that differentially affects downstream TLR2 and TLR4 signaling. Alcohol attenuated TLR4-, but not TLR2-induced, production of TNF α in addition to NF κ B activation. However, with concomitant TLR2 and TLR4 activation, alcohol augmented TNF α production. The kinase IL-1R-associated kinase (IRAK-1) is normally activated after TLR ligation. However, after acute alcohol exposure *in vitro* (25 mM), IRAK-1 activity downstream of TLR4 is reduced, whereas its activity is augmented after engagement of both TLR2 and TLR4. This differential effect of alcohol exposure was demonstrated by decreased IRAK-1:TNF receptor-associated factor (TRAF) 6 association in TLR4-activated cells, but sustained IRAK-1:TRAF6 association in monocytes stimulated via TLR2 and TLR4. Furthermore, consistent with previous reports, mitogen-activated protein kinase (MAPK) phosphorylation was inhibited by acute alcohol exposure *in vitro*. By contrast, acute alcohol treatment augmented c-Jun NH $_2$ -terminal kinase (JNK) phosphorylation and activator protein-1

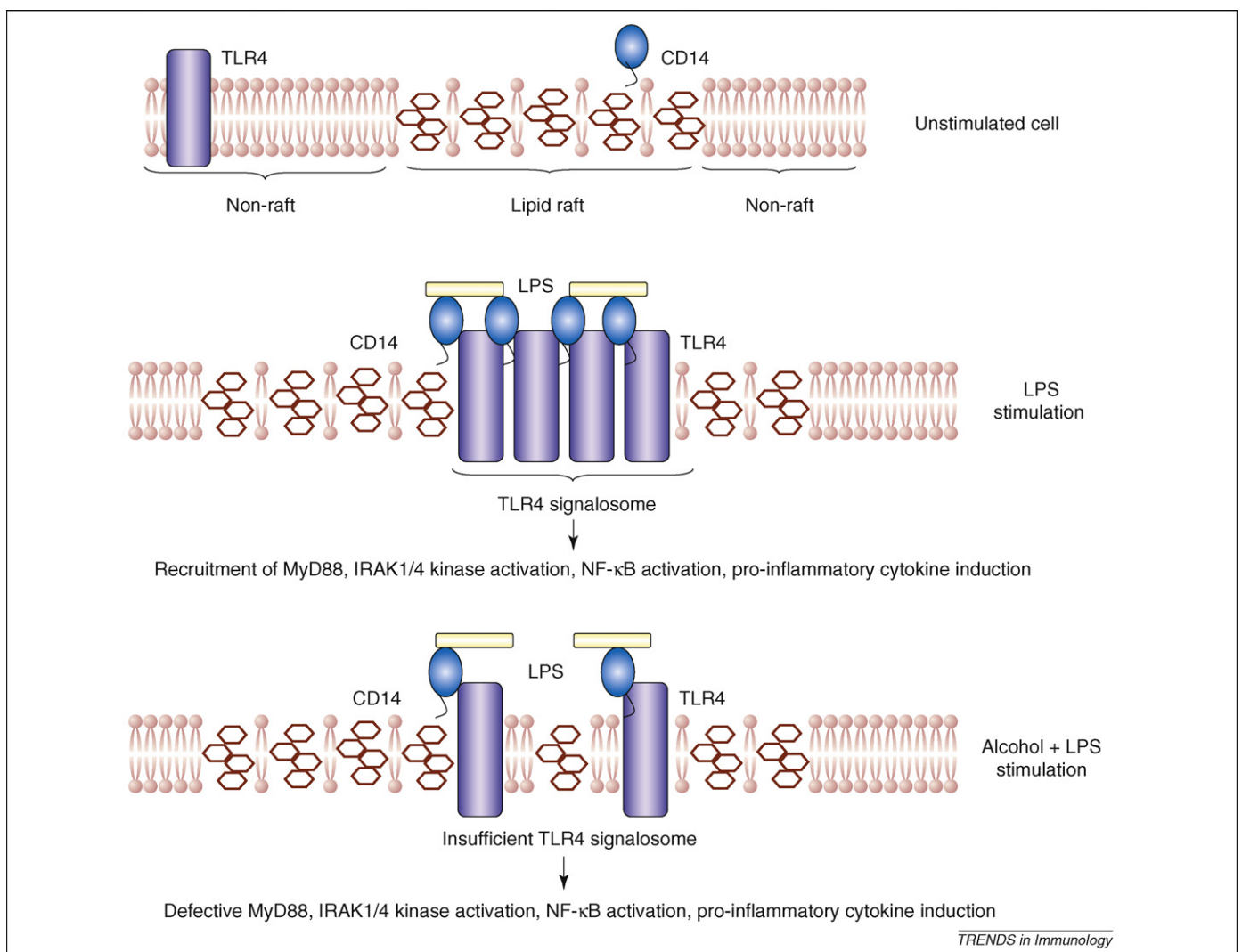


Figure 2. Influence of alcohol on incorporation of TLR4 and CD14 into lipid rafts. Stimulation with the TLR4 ligand, LPS, results in recruitment of TLR4 and CD14 into lipid rafts, which triggers downstream TLR4 signaling events via a TLR4 signalosome. Alcohol inhibits LPS-induced recruitment of TLR4 and CD14 into the rafts, resulting in defective MyD88 and inhibitory effects on IRAK1/4 kinase activation, NF κ B activation and consequent reduction of pro-inflammatory cytokines in monocytes and macrophages (e.g. IL-6).

(AP-1) nuclear binding with TLR2 plus TLR4 activation [43]. Thus, both differential and complex effects of acute alcohol exposure on cell signaling in monocytes have been reported, which might determine how inflammatory responses are affected by alcohol.

Several studies have investigated the influence of alcohol on lipid rafts in monocytes and macrophages (Ref. [44]; Figure 2). After activation by specific ligands, TLR2 and TLR4 are recruited into lipid rafts where they form complexes with an array of signaling molecules. Recent studies with human monocytes and mouse macrophages have shown that alcohol alters components of the TLR4 complex within lipid rafts subsequent to activation [45–47]. Furthermore, these studies have revealed altered reorganization of the actin cytoskeleton because of changes in actin distribution and/or synthesis (which is required for cell activation by LPS), reduced receptor clustering and subsequently disrupted signal transduction in alcohol-exposed human monocytes [46]. Acute alcohol exposure has been reported to increase membrane fluidity and depleted cholesterol, without affecting cell viability [45]. This same study furthermore showed that alcohol changed the protein content of lipid rafts and modulated cell membrane fluidity, consistent with previous findings in brain cells and hepatocytes.

Tissue macrophages: widely studied targets of alcohol exposure

The influence of acute and chronic alcohol exposure on cell signaling pathways and APC function have been studied most extensively in macrophages (Table 2). Additionally, KC of the liver are capable of alcohol oxidation and production of acetate from acetaldehyde, the significance of which remains unknown [48].

The production of ROS is an important readout of macrophage function. Acute or chronic alcohol exposure *in vivo* decreases nitric oxide [49], superoxide anion (although differentially, depending upon the stimulus) [49,50] and hydrogen peroxide [50] production by rat alveolar macrophages *in vitro*. In the liver, however, overproduction of oxygen radicals might be a mechanism of alcohol-induced liver damage. Accordingly, KC have been identified as the source of increased production of alcohol-induced superoxide anion after both acute and chronic alcohol exposure [16,51–53]. Thus, depending on the location of the resident macrophage, alcohol might have differential effects on superoxide anion production.

The differential effects of alcohol on cytokine production by macrophages have been shown to depend on the extent of alcohol exposure (acute versus chronic) and the cytokine of interest. Acute alcohol exposure has been shown to result in post-transcriptional and post-translational suppression of TNF α in rodent alveolar macrophages [32]. Impaired IL-6 production [54–56] and NF κ B activation [57,58] have also been observed. The inhibitory effect of acute alcohol exposure on TNF expression seems to be mediated partly by alcohol interfering directly with the interaction of TNF α with TACE (TNF α converting enzyme), a disintegrin and metalloproteinase protein in the cell membrane of human monocytes [32]. Furthermore, this inhibition is reversible upon metabolism or cessation

of alcohol [59]. Like monocytes, however, macrophages and KC increase TNF α production in response to chronic alcohol exposure [30,60]. Such increases in TNF α production might promote a ‘semi-mature’ DC phenotype associated with tolerogenic properties of these cells [61]. Recently, it has been shown that chronic alcohol exposure enhances TNF α transcription via increased IFN regulatory factor (IRF)-3 binding to and transactivation of the TNF promoter [62]. An important checkpoint role for heat shock proteins and their regulator, heat shock factor-1, in alcohol-induced TNF α regulation has also been suggested [21]. Augmented TNF α production has been shown to be crucial for development of alcoholic liver disease [14,60]. These observations, together with increased pro-inflammatory cytokine production by hepatic KC in response to ingestion of apoptotic bodies (produced as a result of alcohol-induced tissue injury) prompted a recent study [63] which showed that chronic alcohol exposure, concurrent with apoptotic particle stimulation, led to increased TNF α and IL-6 production by KC compared to control macrophages.

Additional functional changes in macrophages include impaired phagocytosis [64–67] observed in KC, and splenic, peritoneal and alveolar macrophages of alcohol-treated rodents. This reduced phagocytic activity might reflect modifications in actin organization in alcohol-exposed macrophages [44,68]. Chemotactic responses of rodent macrophages (i.e. KC) are also suppressed by chronic *in vivo* alcohol exposure [16]. Alcohol can selectively modulate signal transduction pathways associated with inflammatory activation of microglia, the resident macrophages within the central nervous system (CNS). Thus, ethanol pretreatment of mouse BV2 microglial cells or rat primary microglia cultures decreased LPS-induced nitric oxide and IL-1 β expression. Moreover, the associated selective modulation of signal transduction pathways in microglia might promote the well-known adverse effects of alcohol on the CNS [69].

Alcohol metabolites have been shown to affect macrophage function through altered protein internalization and cytokine or chemokine expression. It has been reported [70] that MAA-haptenated proteins are bound preferentially by scavenger receptors in a macrophage cell line. A separate study also found increased TNF α , macrophage chemotactic protein-1 (MCP-1) and macrophage inhibitory protein (MIP) production by rat KC stimulated by MAA-haptenated protein in the presence of LPS [71]. Interestingly, this increased secretion was not affected by chronic alcohol consumption, indicating that the *in vivo* roles of MAA-haptenated proteins are more complex.

Impact of alcohol on macrophage cell signaling pathways

The effects of acute alcohol exposure on signaling pathways has been extensively studied in macrophages [72]. This work has focused mainly on the serine-threonine protein kinases, such as the MAPK family, including p38, extracellularly regulated kinases 1 and 2 (ERK1/2) and the JNK family, all of which are important in regulating immune responses [54,55]. These pathways are activated by various factors, including inflammation, growth factors, cytokines and viral infection. Importantly, these protein kinases are

involved in the regulation of NF κ B-dependent gene expression. Acute alcohol exposure *in vivo* (22 mM) inhibits mouse macrophage IL-6 production, and this corresponded with a reversible inhibition of p38 and ERK1/2 phosphorylation [54]. Inhibition of p38 and ERK1/2 during LPS stimulation showed them to be crucial for the production of LPS-induced IL-6.

There is recent evidence that alcohol concentration can differentially regulate TLR4 recruitment into the lipid rafts of murine macrophages (the RAW 264.7 cell line) [73]. Thus, whereas low alcohol concentrations (10 and 50 mM) increase TLR4 localization, high concentrations (200 mM) disrupt TLR4 and signaling molecule recruitment to lipid rafts (Figure 2). Furthermore, some signaling molecules associated with TLR4 and IL-1R activation, such as myeloid differentiation factor 88 (MyD88) and phosphorylated ERK, are also recruited into lipid rafts at low alcohol concentrations, indicating activation and signaling through TLR4 by alcohol exposure alone. Indeed, one study reported that 1 h after alcohol exposure, rat KC exhibited reduced expression of IRAK and reduced LPS-induced activation of NF κ B [74]. However, 21 h post-exposure, KC instead expressed IRAK and increased NF κ B activation in response to LPS stimulation.

Macrophages: impact on chemokine production

Most studies of the influence of alcohol on chemokine production have been made using KC [75]. Levels of various chemokines are elevated in patients with alcoholic hepatitis, alcoholic cirrhosis and experimental models of chronic alcohol consumption. The pattern of chemokine production differs based on the stage of alcoholic disease. Early on in the disease course, CXC and α -chemokines predominate, and are associated with neutrophilic infiltration. However, later in alcoholic disease, there is a shift in which CC and β -chemokines are increased and there is mononuclear cell infiltration [75]. In most cases, basal levels of circulating chemokines are higher in rodents fed alcohol compared to controls, and further elevations are observed with exogenous stimulation, such as LPS [75]. Such studies support the hypothesis that elevated chemokine production might partly be because of increased endotoxin release from the gut into the portal circulation, as the result of disrupted gut epithelium resulting from alcohol consumption. It has also been shown that increased ROS, increased protein kinase C and subsequent NF κ B activation contribute to chemokine elevation. In contrast to these studies, recent work has shown that acute alcohol exposure decreases CXC chemokine production by mouse alveolar macrophage cell line cells after LPS stimulation [76].

Dendritic cells: adverse effects on distribution, maturation and function

Early work on DC and alcohol was confined to human peripheral blood monocyte-derived DC propagated *in vitro* [26,77]. When cultured for 7 days with a low concentration (25 mM) of alcohol, monocyte-derived DC showed reduced CD80 and CD86 expression in addition to altered cytokine production and displayed reduced allostimulatory capacity in MLR, compared to control DC [26,77]. Surprisingly,

NF κ B activation, which is normally upregulated during DC maturation, was not affected by *in vitro* alcohol exposure [26]. These alcohol-exposed DC induce anergy in naïve T cells, thereby preventing their proliferation. When humans consume alcohol acutely, blood monocyte-derived DC exhibit reduced ability to present tetanus toxoid and impaired T-cell allostimulatory capacity [26–28]. In AWLD patients, increased secretion of pro-inflammatory cytokines (i.e. IL-1 β , IL-6, IL-12 and TNF α) by circulating DC has been reported [35], whereas subjects with alcoholic liver cirrhosis and at least 1 yr after alcohol withdrawal, and patients with active alcohol intake, showed decreased numbers of circulating DC and reduced secretion of these cytokines.

Prolonged alcohol exposure has been shown to inhibit the development and function of mouse myeloid (m) and plasmacytoid (p)DC derived from bone marrow precursors *in vitro*, in a dose-dependent manner [78]. Furthermore, the function of murine alcohol-treated mDC and pDC, when stimulated with herpes simplex virus or the TLR9 ligand CpG, was inhibited compared to control DC, as determined by maturation marker expression in addition to a reduced capacity to stimulate naïve allogeneic T-cell proliferation. *In vivo*, alcohol-exposed DC were poorer at priming naïve T cells after adoptive transfer to recipients. Interestingly, alcohol-exposed DC primed T cells that produced more IL-10 when restimulated with alloAg *ex vivo*. The reduced allostimulatory capacity of alcohol-exposed DC can be ascribed to their immature phenotype and perhaps also to their higher inhibitory molecule expression (i.e. PD-L1, programmed death ligand-1) relative to costimulatory B7 molecule expression [78].

The influence of chronic alcohol consumption on DC, freshly isolated from mice, has also been assessed. Reduced numbers of DC in the spleen, but increased numbers in the thymus, are seen in mice given 20% ethanol in drinking water for up to 28 weeks [79]. These changes could not be ascribed to altered DC precursor numbers, differentiation or turnover rate. In a similar manner, liver and spleen DC were affected differentially by alcohol exposure, with alcohol exerting a less marked inhibitory effect, as determined by phenotypic and functional characteristics [78] on liver DC (the latter are inherently more resistant to maturation than splenic DC [80]). Although classic DC phenotypic maturation markers, such as CD40, CD80 and CD86, were all expressed at low levels on both control and alcohol-exposed, freshly isolated DC, alcohol-exposed liver and spleen DC were both poorer stimulators of naïve allogeneic T cells in MLR compared to control, freshly isolated liver and spleen DC. These data indicate that alcohol uses another mechanism, independent of classic costimulatory pathways, to affect the T-cell stimulatory capacity of immature liver and spleen DC.

Interestingly, when immature hepatic and splenic DC were tested for their capacity to prime naïve T cells *in vivo*, hepatic DC from chronic alcohol-exposed mice displayed increased ability to prime T cells compared to control hepatic DC. By contrast, splenic alcohol-exposed DC had reduced capacity to prime naïve T cells compared to control splenic DC that corresponded with *in vitro* phenotypic maturation and functional data [81]. However, the

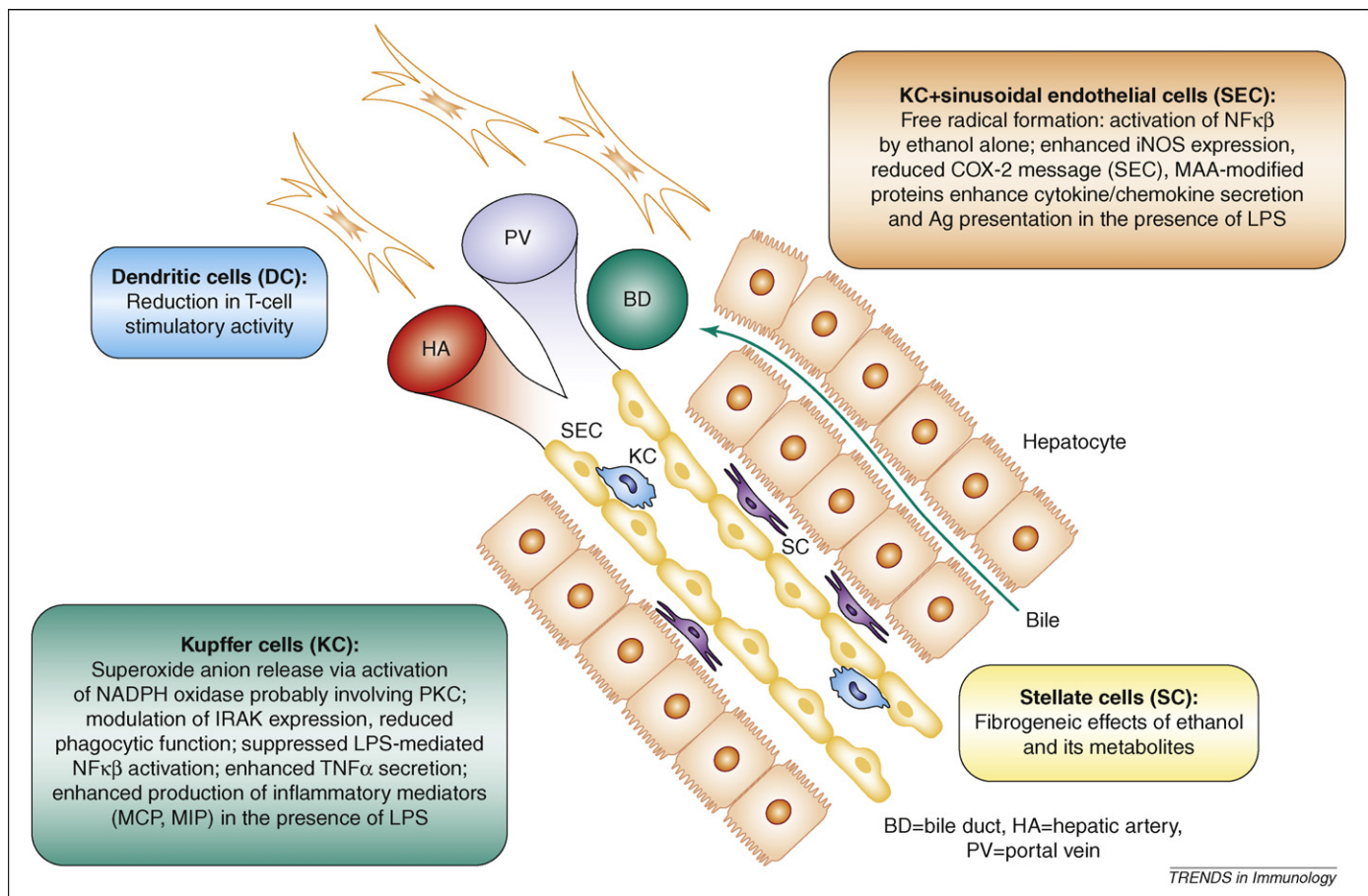


Figure 3. Reported effects of alcohol and its metabolites on the function of various liver cell populations with antigen-presenting capacity. Various liver-resident cells (KC, DC, sinusoidal endothelial cells and stellate cells) are affected by exposure to alcohol, as summarized in each of the panels associated with a specific cell type. Abbreviations: DC, dendritic cell; KC, Kupffer cell; SC, stellate cell; SEC, sinusoidal epithelial cell.

enhanced priming ability of alcohol-exposed hepatic DC did not correspond with and, in fact, conflicted with phenotypic and *in vitro* functional data. Examination of DC migration to draining lymph nodes *in vivo* revealed enhanced migration of alcohol-exposed hepatic DC [81]. The altered migration of hepatic alcohol-exposed DC was found to be independent of both CC chemokine receptor 7 and CD11a expression.

Prolonged *in vivo* alcohol exposure does not affect the endocytic capacity of mouse splenic DC [82]. Unlike TLR9 activation, stimulation of splenic DC with TLR3 and TLR4 ligands led to upregulation of the maturation markers CD40 and CD86, with no difference between alcohol and control groups. However, similar to previous findings with TLR9-activated DC, the allostimulatory capacity of alcohol-exposed DC was impaired. Alcohol-exposed splenic DC produced enhanced levels of IL-1 β and IL-10, but decreased TNF α , IL-12, IFN- γ and IL-6. Similarly, another study found decreased production of the same cytokines, in addition to IL-17A, but observed increased IL-13 from mouse splenic DC exposed to alcohol *in vivo* [83].

Epidermal Langerhans cells: defective migration

It has been well-documented that alcohol intake can affect the healing of burn and trauma injury [84], resulting in increased infectious complications. In mice, reduced numbers of baseline epidermal Langerhans cells (LC) occurs within 4 weeks of chronic alcohol feeding and becomes

more pronounced with prolonged alcohol exposure [85]. In addition, migration of LC and dermal DC from the skin to draining lymph nodes after inflammation is delayed in alcohol-fed compared to control mice. LC were more sensitive to the effects of alcohol compared to dermal DC, with a shorter duration of alcohol exposure required to induce migratory changes. Although defective migration of LC might contribute to the immune-compromised state in burn and trauma injuries of chronic alcoholics, impaired LC function (i.e. stimulatory capacity or ability to cross-prime responses) might also contribute to this effect, but this remains to be elucidated.

Liver APC: KC, liver sinusoidal endothelial cells and hepatic stellate cells

In addition to DC, the liver has several unique cell types with Ag-presenting capacity, including KC, liver sinusoidal endothelial cells (LSEC) [86,87] and hepatic stellate cells (HSC) [88,89] that are all affected by alcohol exposure (Figure 3). Many studies on these cells relate to the influence of alcohol metabolites and protein adducts (MAA-adducts) formed in the liver and which might modulate hepatic inflammatory responses.

The influence of chronic alcohol exposure on the transcription factor, early growth response-1 (EGR-1) has been examined in KC. EGR-1 is an important factor in the increased LPS-induced TNF α production by KC from chronic alcohol-consuming rats [90]. Alcohol exposure of

macrophage line cells enhances DNA binding activity of EGR-1 to TNF α promoter sites after LPS treatment.

LSEC from alcohol-fed rats have impaired receptor-mediated endocytosis of MAA-albumin in a post-internalization step, not through binding of the receptor or degradation of the product [91]. When alcohol-exposed LSEC are stimulated with LPS and MAA-albumin, they produce less TNF α compared to controls, but show no differences in the production of MIP-2 and MCP-1 [71].

The influence of alcohol on HSC has been widely examined with regard to fibrogenic mechanisms, as they are important sources of transforming growth factor β TGF β and collagen production. These studies have also shed light on other means by which alcohol affects immune reactivity in HSC (reviewed in Ref. [92]). Increased extracellular matrix protein production by human HSC exposed to alcohol is associated with triggering of ERK1/2, PtdIns-3K and JNK pathways by acetaldehyde, leading to type-1 collagen and fibronectin gene upregulation. AP-1 activation is also increased with alcohol exposure. Production of the pro-fibrotic cytokines TGF β , IL-6 and TNF α is increased by HSC exposed to alcohol or acetaldehyde, although the molecular mechanisms by which this is achieved are largely unknown. The effects of MAA-adducts on HSC responses have also been analyzed. Thus, stimulation of rat HSC with MAA-bovine serum albumin alone enhanced secretion of MIP-2 and MCP-1, compared to protein alone [93]. The adhesion molecule, intercellular adhesion molecule-1 (ICAM-1 or CD54), was also increased in MAA-exposed rat HSC. These findings indicate that alcohol metabolites affect HSC function, to promote liver fibrosis and macrophage infiltration.

Concluding remarks

The studies discussed here have provided insight into the influence of alcohol exposure on various APC, their development, phenotype and function. It is clear that the duration of alcohol exposure has differential effects on APC function. Although recent work has begun to establish an understanding of the mechanisms behind these differences, more work is required to elucidate the complex effects of alcohol exposure on these cells.

The potential consequences of alcohol exposure on APC in the clinical setting are extensive. Not only are these effects important for host innate and adaptive immune responses but they are also important in the combined effects that alcohol might have when used concurrently with other drugs, such as immunosuppressive agents (e.g. corticosteroids, calcineurin inhibitors, rapamycin, mycophenolate mofetil), with diverse inhibitory effects on APC maturation and function [94]. By utilizing the current knowledge of the effects of alcohol on APC, and by studying direct effects of chronic alcohol consumption in bacterial and viral infection models, we are likely to gain further insight into the immune compromised status of alcoholics, especially in the context of hepatic viral infections (e.g. HCV).

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