<Review Article>

Current Understanding of Alcoholic Liver Disease: A prevalent “hidden” disease of increasing clinical importance

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Alcoholic liver disease (ALD) continues to be a major metabolic liver disease in the world and is responsible for at least one third of 1.8 million annual deaths caused by cirrhosis and liver cancer. It is a disease caused by alcohol addiction but its initiation and progression are also predicted by secondary genetic and environmental factors. Its pathologic spectra are manifested by diverse and complex layers of cellular perturbation caused by metabolism of a simple molecule, ethyl alcohol. Alcoholic hepatitis defined as acute on chronic liver failure, is a very unique entity with high short-term mortality and limited therapeutic options. However, last three decades have witnessed remarkable advancements in our understanding of molecular mechanisms underlying the disease which even shaped basic science at the most fundamental level. Indeed, the ALD field has always been a forefront of research on oxidant stress, organelle stress, gut dysbiosis, endotoxin, inflamasome, macrophage and hepatic stellate cell biology, tumorigenesis, and metabolic reprogramming. Based on these new findings and better stratification of patients, novel therapeutic approaches have emerged and are being tested. This article will review the current understanding of molecular mechanisms of ALD and important challenges our society faces with the disease.

Key words: CYP2E1 ER stress mitochondria stress gut dysbiosis inflamasome pyroptosis

1. Epidemiology

Most ancient history of alcohol drink dates back to the Neolithic period of 7,000-6,650 BC in the Henan province of northern China where a fermented alcohol drink from rice, honey, grapes, and berries was shown to be produced and consumed through archeological and chemical analysis. Primitive forms of barely beer and grape wine began in the Mesopotamia region in 5,400 BC. Since then, alcohol beverages have widely been produced and consumed throughout the world and have been part of culture, religion, medicine and feast. In 2010, the worldwide per capita consumption of pure alcohol by person aged 15 years or older for a year, was 6.2 liters based on the WHO report, roughly equivalent to one drink (12 g ethanol) per person a day. Russia and some of the East European nations which rank top 10 nations for per capita consumption, consume 13-15 liters (2.3-2.6 drinks per person a day), U.S.A. and Japan rank at the 48th and 71th and consume 9.2 and 7.2 liters (1.6 and 1.2 drinks per person per day), respectively.

Considering this prevalent consumption of alcohol, its misuse is expected to have a global impact. Indeed, 33 million deaths were caused by alcohol misuse in 2012, corresponding to 5.9% of all deaths in the world. Excessive alcohol intake which causes more than 200
diseases including alcohol use disorder (AUD) and liver cirrhosis, was the 5th leading risk factor for premature death and disability and responsible for 5.1% of the burden of disease and injury around the globe in 2010. This means alcohol misuse is reducing healthy life years of people by 5.1% worldwide. In the U.S., 15.1 million adults aged 18 and older (6.2% of this age group population), have AUD with the male to female gender ratio of 2:1. Youth drinking is becoming an important issue worldwide, and in the U.S. approximately 0.6 million adolescents aged 12-17, have AUD with the gender ratio of roughly 1:1. This is an alarming statistic because youth drinking is closely associated with eventual addiction to alcohol. Alcohol is the 3rd leading preventable cause of death and responsible for 88,000 deaths in the U.S. including its 11% caused by drunk driving. In 2010, alcohol misuse cost the U.S. $249 billion. More specifically for liver disease, in 2015, 76,529 deaths were caused by liver disease, and 47% were due to alcohol. Similarly, 47.9% of cirrhosis deaths were alcohol-related. In 2009, one third of liver transplantations performed in the U.S. was due to alcohol-related liver disease. In 2016, 1.2 million deaths were caused by cirrhosis and chronic liver disease around the globe, and 27% were due to alcohol. In addition, 245,000 HCC deaths (30% of all HCC) were associated with alcohol. Collectively, more than half million lives are being lost due to cirrhosis or HCC associated with alcohol every year in the world.

2. Conceptual and Historical Perspective of ALD

2.1. Life-style disease

ALD is a disease caused by excessive intake of alcohol ranging from 50-90 g/kg/day over 20-40 years. Clinically significant ALD rarely develops due to modest or moderate drinking. In essence, it is a disease of alcohol addiction. This is a simple but yet important reminder when we discuss the pathogenesis and therapy of ALD. But not all heavy drinkers develop ALD. Thus, although heavy drinking is required for ALD, it alone is not sufficient; leading to a prevailing notion that ALD is a multifactorial disease, the expression of which is predicated by interactions of genetic and environmental risk factors much like other chronic adult-onset diseases such as diabetes and neurodegenerative diseases. In short, ALD is a life-style metabolic disease initiated by addiction to alcohol and promoted by secondary risk factors.

2.2. Nutrition vs. hepatotoxicity

In early 1930’s, experimental evidence started mounting to incriminate nutritional deficiencies in the pathogenesis of ALD. Compelling studies demonstrated that rats given 20% alcohol in drinking water and a diet low in protein and choline developed liver cirrhosis but not when these nutritional deficiencies were corrected. To allow experimental dissection of nutrition vs. alcohol, Best et al introduced for the first time in 1949, isocaloric pair feeding by substituting ethanol consumed by ethanol diet-fed mice with equal calories of sucrose. Using this approach, fatty liver developed in ethanol-fed mice was shown to be prevented by supplementation of a methyl donor such as methionine, choline, or casein. Subsequent studies, particularly a series of studies by Porta and Hartroft, established the role of lipotropic deficiency in experimental ALD and cirrhosis. In 1963, Lieber et al developed an ethanol containing liquid diet which contained all required nutrients in adequate concentrations and a control diet in which ethanol was isocalorically substituted by carbohydrate. This diet achieved daily ethanol intake of 12-18 g/kg, 36% of dietary calories from ethanol, and postprandial blood alcohol concentrations (BAC) of 100-150 mg/dl in young growing rats. Fatty liver developed in these rats after one month of feeding serving as the basis for a claim that ethanol exerts toxic effects to cause liver pathology despite adequate nutrition. This hepatotoxicity hypothesis was enforced by the MEOS (microsomal ethanol oxidizing system) that the Lieber laboratory reported which was later identified to be CYP2E1 and CYP reductase. This diet commonly called “Lieber-DeCarlie (L/D) diet” has become a main stream of ethanol feeding method to experimental animals. However, the diet was criticized for providing inadequate nutrition and suboptimal growth in growing rats mainly because limited intake of ethanol-containing diet due to the animal’s aversion to ethanol. Indeed, if ethanol content in the diet was di-
luted to 26% of calories from 36%, rats consumed 50% more volume of diet, achieved a comparable daily ethanol intake as that of the 36% Cal ethanol diet but grew much better26 and fatty liver was prevented in these mice27.

The nutrition vs. toxicity debate also involved subhuman primate models. Baboons were fed the L/D diet containing ~50% calories from ethanol because this species unlike rodents has less oral aversion to ethanol. A study conducted by Lieber and Rubin showed that these baboons on the diet for 1-4 years, developed fatty liver, steatohepatitis, perivenular and bridging fibrosis and cirrhosis28. However, others using Maccaca radiate monkeys29, Rhesus monkeys30, and baboons31 fed L/D or similar diet containing nearly 50% Cal ethanol failed to observe the development of advanced ALD as reported in Lieber’s studies. The current consensus is that metabolic consequences of chronic and excessive ethanol consumption may exert cytotoxic effects on the liver but these effects are profoundly influenced by nutrition.

2.3. Importance of sustained blood alcohol level and binge intake.

How one takes alcohol is as important as how much alcohol one consumes. As exemplified by the contrasted effects of 26% Cal vs. 36% Cal diet in rats, if ethanol is given diluted with nutrients, it will cause less damage to the liver even with an equally high dose. This is actually known to us as conventional wisdom - “do not drink with empty stomach”; “eat while you drink”. There are scientific explanations for this wisdom and one most accepted mechanism is enhanced metabolism of ethanol by food intake via NADH re-oxidation generating NAD as an obligatory co-factor for alcohol dehydrogenase (ADH) in cytosol and acetaldehyde dehydrogenase 2 (ALDH2) in mitochondria. NADH oxidation is achieved by nutrient-driven oxidative phosphorylation in hepatocyte mitochondria, particularly via NAD-NADH cycling by mitochondrial glutamate/malate dehydrogenases and Complex 1 respiration. As expected, the rats on the 26% Cal ethanol diet had only 12 mg/dl of BAC as opposed to 187.3 mg/dl in those fed the 36% Cal ethanol diet despite a similar alcohol intake30. This brings back to the discussions on the most important behavior of alcoholics predisposed to ALD. ALD occurs in those who reach out to alcoholic drinks every 3-4 hours to avoid most uncomfortable withdrawal symptoms of anxiety, tremor, vomiting, hallucination, and psychomotor agitation resulting in part from reduced responsiveness of GABA receptors in the brain. In a way, alcoholics titrate their ethanol intake to maintain BAC throughout a day, underlying their constant and steady drinking. Naturally, they will eat less food to raise their BAC quickly. This is a most fundamental behavior of alcoholics that has a causal significance for the development of ALD. This behavior is nearly impossible to reproduce in rodents by using ad libitum ethanol intake methods due to their natural aversion to ethanol. For this reason, the intragastric ethanol infusion (iG) model was developed to sustain high BACs and reproduce much more severe liver pathology than any other rodent models321-331. Another unique behavior of ALD patients, particularly those developing alcoholic hepatitis (AH), is a binge alcohol intake that they repeat roughly every 5-7 days on top of steady daily alcohol intake3435. Indeed, weekly alcohol binge in the iG model helped reproduce some of histologic and clinical features of AH in patients36.

Past history of alcohol research which is filled with controversies and debates, has also made significant contributions to advancement of science because the investigators’ rigorous efforts to understand how the cell is perturbed by alcohol led to their discoveries in fundamental biochemistry and cell biology. To make tributes to some of these investigators, I would like to share with you a group photo taken at the Gordon Research Conference on Alcohol: Metabolic Effects and Molecular Mechanisms of Injury, organized by Esteban Mezey and Gary Wand in 1996. Of course, this acknowledgment is inadequate to recognize all scientists who have contributed to historical work in the field of alcohol. This photo is just to give you a snap-shot of renowned investigators in the last 4 decades included in this group (Fig. 1).

3. Molecular pathogenesis of ALD

The pathogenetic mechanisms of ALD take place in two major anatomical compartments: one within hepato-
Fig. 1  A snap-shot of historical investigators in the field of alcohol and alcoholic liver disease from a group photo taken at the Gordon Research Conference on “Alcohol: Metabolic Effects and Molecular Mechanisms of Injury” in Ventura, California in 1996, organized by Esteban Mezey and Gary Wand. Images of twelve notable scientists are enlarged and shown around the group photo. From bottom left clockwise, Charles Lieber (1931-2009), a developer of Lieber-DeCarli diet who has put forth the hepatotoxicity hypothesis via demonstration of MEOS and metabolic consequences of ethanol oxidation in ALD pathogenesis; Marcos Rajkind (1935-2011), an expert on hepatic stellate cells and liver fibrosis who defined molecular mechanisms of acetaldehyde-mediated collagen gene transcription; Ting-Kai Li, the past NIAAA director with ground breaking research in alcohol metabolism and establishment of alcohol preferring rats; Steven Schenker, past Hepatology editor, an expert in natural history and nutritional and hormonal basis of ALD pathogenesis; Charles Halsted, a pioneer in research on alcohol’ effects on methionine and folate metabolism and establishment of miniature pig model of ALD; Ronald Thurman (1941-2001), one of the most prolific investigators in ALD with discoveries of “adaptive swit increase in ethanol metabolism (SIAM)”, a link between gut endotoxin and NOX activation, proinflammatory gene activation in Kupffer cells in experimental ALD, and sensitization mechanism of Kupffer cells in female rats; Yedio Israel, proposed the hypermetabolic state and hypoxia hypothesis in 70’s which has been supported by many and is still considered as one of key mechanisms of ALD. Continues to pursue research on the role of acetaldehyde in alcohol addiction; Dean Tuma, contributed to understanding of many of biochemical consequences of alcohol metabolism including dysregulated protein trafficking and protein adduct formation with the most notable being a discovery of malondialdehyde-acetaldehyde adduct; Hirohisa Ishii (1938-2010), an international leader in ALD field since involvement in MEOS research in Charles Lieber’s laboratory in early 70’s, demonstrated ethanol-mediated sensitization of hepatotoxicity and hepatitis induced in mice by acetaldehyde adduct; Emanuel Rubin, collaborated with Charles Lieber to publish seminal articles on a baboon model of ALD in 1973-74. Later focused on ethanol-mediated changes in membrane lipid composition as the molecular basis for altered cell signaling; Magnus Ingelman-Sundberg, an expert in CYP450 pharmagogenetics who characterized oxygen-dependent, ethanol-inducible CYP2E1 in rat liver and lung in 1987; Arthur Cedarbaum, unequivocally demonstrated both in vitro and in vivo, the critical role of CYP2E1 in mediating oxidative damage in ALD and its synergistic effect with endotoxin. Not shown is Esteban (Steve) Mezey who did seminal research on hormonal regulation of alcohol metabolism and the pathogenesis of ALD.
toocytes and another in an extra-hepatocyte compart-
ment. At least four of the categories of the mechanisms
discussed below occur in hepatocytes and the remain-
ing in the latter compartment.

3.1. Alcohol metabolism and oxidant stress

ALD is a metabolic liver disease, the disease caused
by metabolism of ethanol. ADH and the secondary
metabolic enzyme CYP2E1, produces acetaldehyde
(AA) as an immediate metabolite of ethanol. AA is elec-
trophilic and forms via its CHO-group a covalent bond-
ing with an ε-amino group of lysine residue, eventually
forming a protein adduct and affecting functions of
lysine-enriched proteins. Among numerous proteins
modified by AA, the examples include AA-tubulin ad-
duct impairing microtubule formation37 and AA ad-
duct to delta4-3-ketosteroid-5β-reductase inhibiting its
catalytic activity and causing cytotoxicity by increased
concentration of 7ɑ-hydroxy-4-cholesten-3-one38. Obvi-
ously, many other cellular proteins are targeted and af-
fected by the same mechanism, contributing to cellular
derangement and toxicity.

The endoplasmic reticulum (ER)-localized CYP2E1
and its reductase, CYP reductase are induced by
chronic alcohol consumption39. Reduced CYP2E1 do-
ates an electron to a dioxygen molecule to generates
superoxide anion which will be sequentially reduced to
hydrogen peroxide by superoxide dismutase and to
the most reactive hydroxyl radical in the presence of
ferrous iron. Hydroxyl radical causes lipid peroxidation
and generates aldehydic lipid metabolites such as 4-
hydroxynonenal (HNE) and malondialdehyde (MDA).
MDA increases the AA adduct formation by 10–30 fold, synergizing the formation of a highly reactive, hy-
brid MDA-AA (MAA) adduct40. These aldehydes mod-
ify proteins and DNA while depleting reduced glu-
tathione, amplifying oxidant stress and cytotoxicity.

Another major site of oxidant stress in ALD is the
mitochondrion. Liver mitochondria exhibit remarkable
plasticity and the ability to increase both respiration
and acetaldehyde metabolism via active biogenesis in
response to sustained BAC41. In a way, this is expected
from enhanced ethanol metabolism by ADH which re-
dquires half molecule of dioxygen to re-oxidize NADH
produced by metabolism of one molecule of ethanol to
regenerate the require co-factor NAD. This adaptive
response of enhanced mitochondrial respiration most
likely increases the absolute amount superoxide anion
which is spontaneously generated at the rate of ~3%
by electron leaking out of complex I and III of the elec-
tron transport chain. In normal hepatocytes, this oxidi-
ant stress will be handled by MnSOD and glutathione
peroxidase. However, in ALD, a mitochondrial pool of
 glutathione is depleted and this makes mitochondria
susceptible to oxidant damage42,43.

Furthermore, the adaptive increase in oxygen con-
sumption renders the centrolobular region susceptible
to hypoxia due to incomplete compensation of en-
hanced oxygen consumption by oxygen delivery44, which
may aggravate oxidant stress. CYP2E1 which
directly requires one molecule of dioxygen to metabo-
lose one molecule of ethanol and is enriched in the zone
3 of the liver acinus, also contributes to centrolobular
hypoxia.

3.2. Organelle stress

ER stress: Rough ER with ribosomes is the cellular
organelle responsible for protein synthesis, folding,
post-translational modifications, and assembly. Smooth
ER is involved in synthesis of lipids including chole-
sterol, steroids, and phospholipids and xenobiotic me-
tabolism by CYPs.

When unfolded or misfolded proteins accumulate in
the lumen of ER, unfolded protein response (UPR) en-
sues to halt protein translation, increase production of
chaperones, and stimulate protein degradation via acti-
vation of three effectors, RNA-dependent protein
kinase (PKR)-like ER kinase (PERK), inositol-requiring
enzyme-1 (IRE1), and activating transcription factor-6
(ATF6).

Sustained, excessive, and unmet UPR causes ER
stress which leads to proteolytic activation of sterol
regulatory element-binding protein-1c (SREBP-1c) con-
tributing to the genesis of fatty liver45. It also induces
apoptotic pathway via JNK activation, C/EBP homolo-
gous protein (CHOP) expression, and downregulation
of Bcl2, leading to cytochrome C release from mito-
ochondria and caspase 3 activation, particularly with
concomitant mitochondrial stress46,47. ER stress partici-
pates in ALD pathogenesis most likely because of accu-
mulation of aldehyde-modified proteins, oxidant stress arising from AA, CYP2E1 or mitochondrial respiratory chain, and increased levels of homocysteine resulting from impaired re-synthesis of methionine from homocysteine by folate and vitamin B12 dependent methionine synthase or betaine-homocysteine methyl transferase. In fact, ER stress is a generalized event in alcohol-induced toxicity of organs including the liver, pancreas, lung, heart and brain.

**ER-mitochondria stress crosstalk:** Mitochondria are connected with ER via a 10-30 nm tether which is termed mitochondrial associated ER membranes (MAMs). MAMs are now recognized to be composed of a group of proteins forming “ER-mitochondria encounter structure (ERMES) complex” through which ER-produced lipids and nuclear-encoded proteins are transferred to mitochondria. How this “channel” functions to disseminate “stress” from ER to mitochondria is an obvious area of active research. MAMs are enriched with PERK which senses ER stress and executes a pro-apoptotic signaling via CHOP upregulation. MAMs also facilitate a transfer of Ca$^{2+}$ released by stressed ER to mitochondria and to initiate mitochondrial Ca$^{2+}$ loading and formation of apoptotic permeability transition pore (PTP), establishing the ER-stress mitochondrial stress link. ER stress along with other stress conditions such as oxidant stress, depletion of amino acids or heme, and viral infection, induces mitochondrial UPR (UPR). This occurs as part of Integrated Stress Response (ISR) initiated by eIF2α phosphorylation by PERK and GCN2 resulting in global translational suppression but preferential translation of mRNAs containing open reading frames in 5’ untranslated region such as those for CHOP, ATF4, and ATF5. These transcription factors stimulate transcription of UPR nuclear genes for expression of chaperones and proteases to facilitate recovery from mitochondrial dysfunction. Here, CHOP is a protective factor capable of inducing the mitokine GDF15. ATF4 upregulates metabolic genes (asparagine synthetase, phosphoenolpyruvate carboxylase 2), cystathionine γ-lyase which generates cysteine from cystathionine, and FGF21, a mitogen capable of regulating glucose and lipid metabolism. ATF5 is a mammalian orthologue of ATFS-1, the first UPR transcription factor discovered in *C. Elegans* uniquely possessing both a nuclear localization sequence and a mitochondrial-targeting sequence. When it is imported into normal mitochondria, it undergoes degradation by the matrix-localized protease LON. Upon mitochondrial damage, it goes to nucleus and stimulates transcription of mitochondrial chaperone (mtHSP70), mitochondrial protease LONP1, and anti-microbial defensin 5 genes. In hepatotoxicity models, ER stress induces activation of JNK which associates with the mitochondrial outer membrane SH3 homology associated BTK binding protein (Sab, or SH3BP5), causing intra-mitochondrial Src inactivation, inhibition of electron transport, increased generation of ROS, sustained JNK activation, and apoptosis. Whether this JNK-Sab mechanism underlies ALD and how UPR is compromised to contribute to ALD are yet to be studied. In pancreatitis model, the reverse direction of crosstalk from mitochondria to ER is suggested by prevention of ER stress by inhibition of cyclophilin D (peptidylprolyl isomerase D) and mitochondrial depolarization. This is yet to be tested in ALD model.

**Impaired autophagy and lysosomal defect:** Autophagy is a vesicular mechanism of containing and targeting damaged organelles or cellular constituents to lysosomal degradation for cell survival and recycling of energy-consuming substrates. In the presence of ER and mitochondrial stress under chronic ethanol consumption, autophagy is expected to be upregulated. If this protective mechanism is impaired, ALD may ensue. Evidence is mounting to support this notion. Chronic ethanol feeding impairs autophagosomes-lysosome fusion, maturation of lysosomal hydrolases, and lysosomal degradation. Lipophagy, a specialized form of autophagy for lipid droplets, is also impaired by ethanol due to suppressed activities of Dynamin 2 and Rab7, both required for appropriate autophagosome trafficking and lysosome fusion, leading to the genesis of fatty liver.

### 3.3 Extracellular vesicles

Cell-derived extracellular vesicles (EVs) emerged as a most efficient mechanism of intercellular communication and are implicated in the pathogenesis of many dis-
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eases including ALD. EVs from alcohol-fed animals or ALD patients contain a distinct set of proteins to promote inflammatory response in macrophages, including CD40L71 and HSP9072, proposed as the effector proteins. A link between ER stress and EV release of CYP 2E1 is shown in hepatocytes from alcohol-fed rats and suggested to trigger proapoptotic signaling in target hepatocytes73. Circulating mitochondrial DNA-enriched EVs are detected in alcohol-fed mice and ALD patients and are suggested to be induced by ER stress in hepatocytes and to mediate neutrophilia and liver injury74. Here, a notion is that ER stress induces mitochondrial stress and DNA damage, causes EV release of mitochondrial DNA, and stimulates neutrophilia via a TLR9-dependent manner. Alternatively, ER stress may trigger a signaling to induce EV release from hepatocytes with mitochondrial damage. EVs from alcohol-fed mice and alcoholic hepatitis patients also contain distinct sets of microRNAs which may serve as potential biomarkers75,76. Among several miRNAs carried by EVs and suggested to have pathogenetic roles in ALD, miRNA-122 has received much attention. It is one of the most abundant miRNAs in the liver and reduced by alcohol consumption. Adenovirus-associated virus-mediated loss or gain of approach for miR-122 in the liver of alcohol-fed mice. worsens or ameliorates liver inflammation and fibrosis caused by superimposing CCl4 challenge via the effects on HIF-1α, its target77. However, exosomes from alcohol-exposed hepatocytes transfer this miRNA to monocytes/macrophages and sensitize them for LPS inflammatory response via inhibition of heme oxygenase-178, seemingly a conflicting effect. Obviously, this area needs to be carefully approached as EVs containing different miRNAs are released by different cell types and cross-affecting them in evolution of ALD. Interestingly, the cells infected with bacteria release exosomes (EVs with the size of up to 150nm) containing pathogen-associated molecular patterns (PAMP) which may incite inflammatory signaling in recipient cells79, the situation relevant to alcoholic hepatitis with bacterial translocation. Constitutively active MyD8880,81 in patients with diffuse large B-cell lymphoma, can be released via EVs to propagate inflammation and promote tumor82, and this exemplifies a possible and important transmission mode of a cellular signaling component via EVs. EVs are also released by prokaryotes (bacterial microvesicles)83, and this opens up potential crosstalk between gut bacteria with intestinal epithelial cells or liver cells if bacterial translocation occurs. What regulates EV release from different liver cell types and how differentially the cells are targeted by EVs in different spectra of ALD, are important outstanding questions.

3.4. Intestinal dysbiosis

Enteric dysbiosis occurs in the evolution of experimental ALD84–86. In the iG model, intestinal bacterial overgrowth is observed in mice fed alcohol for only 3 weeks. Sequencing of 16S ribosomal RNA genes reveal relative increases in Bacteroides and Verrucomicrobia bacteria and a decrease in Firmicutes bacteria compared to pair-fed control mice. Associated with these changes are reduced expression of anti-microbial Reg 3b and Reg3 g lectins in the small intestine87. The deficiency of these REG3 lectins increases numbers of mucosa-associated bacteria and bacterial translocation to the mesenteric lymph nodes and liver, promoting alcoholic steatohepatitis, and correction of this deficiency abrogates these effects88, revealing the importance of dysbiosis with compromised antimicrobial defense in the pathogenesis of ALD. Combined metagenomic sequencing and metabolomics of the cecum content of the iG alcohol model, reveals reduced synthesis of long-chain fatty acids (LCFA) which promote the growth of commensals Lactobacillus, and LCFA supplementation in the model restores eubiosis and gut barrier, and reduced alcoholic liver injury89. Another metabolic consequence of alcohol-induced gut dysbiosis is increased levels of unconjugated bile acids by overexpressed bacterial choloblyicine hydrolase90. This condition leads to lower FXR activity in enterocytes and lower expression of FDF-15, leading to increased hepatic CYP7A1 expression and elevated bile acid concentrations in blood. Treatment of ethanol-fed mice with the intestine-restricted FXR agonist lexaramine or overexpression of a human FGF15 orthologue, stabilizes the gut barrier and attenuates alcoholic steatohepatitis91, suggesting correction of dysbiosis-mediated impair-
ment of the bile acid-FXR-FGF15 pathway is therapeu-
tic. In contrast, gastric acid suppression as see in pa-
tients with proton pump inhibitor treatment, aggra-
vates ALD by promoting overgrowth of intestinal Enterococcus. Fecal transplantation from alcohol-
resistant mouse donor mice to alcohol-sensitive recipi-
ent mice restores gut homeostasis and prevents liver
steatosis and inflammation. Akkermansia Muciniphila,
a gram-negative commensal bacterium which pro-
motes gut barrier function partly by enhancing mucus
production, prevents the development of ALD and ameliorates pre-existing ALD in mice. This beneficial
effect of A. Muciniphila has recently been extended to
overcome the resistance to anti-PD-1 immunotherapy
for carcinomas. Intestinal fungi also overgrow in the
setting of chronic alcohol consumption and fungal β-
glucan translocates to contribute ALD in mice and antifungal agents abrogate these effects, demonstrating
it is not just bacterial diversity affected by alcohol but
fungi are part of intestinal dysbiosis in ALD.

3.5. Inflammation, canonical and non-canonical inflammasomes

Inflammation is in large induced by two major
mechanisms in ALD. One involves PAMPs derived
from gut bacteria and another is triggered by damage-
associated molecular patterns (DAMPs) released from
damaged or stressed cells. PAPMs such as LPS are rec-
ognized by TLRs (e.g., TLR4) to activate proinflamma-
tory signaling while DAMPs such as HMGB1, DNA,
RNA, nucleotides (ATP), nucleosides (adenosine), uric
acid are recognized by a different set of TLRs (TLR2,
3, 4, 9) and NOD-like receptor NALP to initiate sterile
inflammation. Gut-derived LPS which has long been im-
plicated in ALD. Ronald Thurman’s laboratory has
published a series of seminal studies using the rat iG
model demonstrating the role of LPS in stimulation of
hepatic macrophages via CD14/TLR4 and their pro-
duction of ROS via NADPH oxidase which signals to
activate NF-κB for transcriptional upregulation of
proinflammatory cytokines (TNFα, IL-1, IL-6) and an array of chemokines. The readers are referred to excellent reviews on nu-
merous pro-inflammatory mediators implicated in
ALD. But most notable are IL-8 (CXCL1 or GRO) and
CXCL2 (MIP2 or GRO2) for neutrophilic inflamma-
tion, and MCP1 (CCL2), MCP2 (CCL8), and MIF which
chemoattract monocytes and promote macrophage
functions as universally recognized for other inflam-
matory diseases. TLR2 and TLR9 are also shown to be
involved in release of CXCL1 by hepatocytes and hepatic
stellate cells (HSCs) to promote transient neutrophilic
infiltration after alcohol binge. TLR4 activation may
also take place in HSCs which not only represses the
TGF-β pseudoreceptor Bambi to activate the fibro-
genic TGF-β pathway but also activates NF-κB for
chemokine and adhesion molecule expression to re-
cruit inflammatory cells. Noteworthy is IL-17 which
is unique in its role in an entire range of ALD
spectra from fatty liver, inflammation, fibrosis and liver
cancer. Inflammasome is a multiprotein oligomer
composed of caspase 1, PYCARD, and NOD-like recep-
tor NALP, which mediates proteolytic activation of
pro-IL-1β and pro-IL-18 by caspase-1 for release of ac-
tive forms. The role of the canonical NLRP3 inflamma-
some to DAMP-mediated activation of pro-IL-1β in
early ASH has been suggested. IL-1β is directly pro-
inflammatory but also stimulates HSC activation via
upregulation and activation of pro-MMP9, an event es-
sential for early matrix remodeling and pro-
inflammatory HSC activation.

There are two outstanding questions with regard to
macrophages and neutrophils in ALD, particularly in
AH. The first question is the source and fate of hepatic
macrophages in ALD. During chronic and early ASH,
the number of hepatic macrophages increases, and in-
filtrating monocyte-derived macrophages are believed
to contribute to this expansion and the pathogenesis of
ASH. This migration and the genesis of monocyte-
derived M1 hepatic macrophages require Notch-1 acti-
vation in ASH. Whether and how these monocyte-
derived M1 macrophages persist or reprogram in the
course of ALD are unknown. Further, a direct entry of
peritoneal macrophages into the liver across its meso-
thelium has been revealed in sterile liver injury, and
this process requires CD44 and the DAMP molecule
ATP. These macrophages replicate rapidly and
switch themselves to the M2 alternatively activated
phenotype to perform reparative functions.
to be studied whether such contribution of the peritoneal macrophages occurs in non-sterile liver injury such as ALD. A recent publication implicates the role of gp91phox in a switch from proinflammatory migratory macrophages to tissue-restorative M2 macrophages in experimental ALD106. Regulation of cell fate of M1 and M2 macrophages in evolution and progression of ALD, is an obvious area of research interest. The second question is on the roles of neutrophil infiltration, a salient feature of AH. It is generally believed that neutrophils infiltrating into the liver are damaging hepatocytes in AH. However, a recent clinical study shows that infiltration of neutrophils is associated with better prognosis in AH, indicating that neutrophilic inflammation may be beneficial in promoting liver repair and controlling bacterial infection in these patients106.

3.6. Pyroptotic cell death in alcoholic hepatitis.

The notion on the beneficial role of neutrophils is supported by a recent observation of activation of the non-canonical inflammasome Caspase (CASP) 4/11-Gasdermin-D (GSDMD) pathway inducing programmed, lytic cell death “pyroptosis” of hepatocytes and macrophages in a mouse model of early AH106. This pathway is activated by increased intracellular, not extracellular levels of LPS as seen in bacterial infection. Intercellular LPS oligomerizes and activates CASP 4/11 (4 in man and 11 in mouse). This leads to proteolytic activation of pro-GSDMD, releasing N-terminal 30kD GSDMD which is recruited to the plasma membrane to form 10-20nm pores111,112. This lytic death releases intracellular bacteria, PAMPS, DAMPs, and cytokines (IL-1/IL-18) and may be protective for infected intestinal epithelial cells as bacteria and these inflammatory mediators are expelled into the gut lumen. However, if cells in internal organs such as hepatocytes undergo pyroptosis, this process locally or systemically disseminates bacteria and PAMPS/DAMPS, and the latter situation may cause endotoxemia and sepsis, or systemic inflammatory response syndrome (SIRS). In fact, the mice lacking CASP11 or GSDMD are protected from lethality caused by injection of a high dose of LPS112. Activation of CASP 11 and GSDMD are not present in chronic early ASH in iG model but become evident when it transitioned to AH by weekly alcohol binge, concomitant with increased bacterial load, hepatocyte death and neutrophilic infiltration in the liver110. Deficiency of CASP 1/11 abrogates these activations, bacterial load, hepatocyte death and neutrophil infiltration. Conversely, the deficiency of IL-18, an important antimicrobial cytokine, aggravates CASP 11-GSDMD activation, liver bacterial load, hepatocyte necrosis, and neutrophilic inflammation101. AAV-mediated expression of active GSDMD in hepatocytes, causes massive hepatocyte necrosis accompanied by intense neutrophilic infiltration in the AH model. More importantly, CASP4 and GSDMD activation are robust in explant livers of AH patients but not evident in normal human livers100. These results collectively establish pyroptosis as the novel and unique type of cell death in AH. Chronic ethanol consumption renders hepatocytes undergo apoptosis triggered by activation of intrinsic or extrinsic proapoptotic pathways mediated by organelle stress or cytokines67,113. They may also undergo necroptosis mediated by receptor-interacting protein kinase (RIP) 1, recruiting RIP3 to form necrosome which in turn phosphorylates, oligomerizes, and activates Mixed Lineage Kinase Domain Like Pseudokinase (MLKL). MLKL is recruited to the plasma membrane like GSDMD but forms ion selective channels instead of non-ion selective pores made by GSDMD to lead to necroptosis114. Pro-apoptotic caspase cleaves RIP3 and suppresses necroptosis which is associated with inflammation. Thus, apoptosis may be present at early steatotic stage of ALD followed by necroptosis in early ASH. After transition to AH, pyroptosis may become predominant as a form of cell death which mechanistically links to neutrophilic inflammation and consequentially leads to endotoxemia and septicemia, the common cause of death from AH (Fig. 2A). Cleaved GSDMD also activates NLRP3-dependent CASP1 activation via a cell-intrinsic pathway112. In fact, CASP1 can still induce plasma membrane damage in GSDMD-deficient cells, but pyroptosis is delayed115. Gram-negative bacteria also secrete LPS-laden outer membrane vesicles (20-150nm) to deliver their contents including LPS to host cells116. Thus, bacteria do not need to invade cells to allow LPS access to cytosol where CASP11/4 become activated to execute GSDMD-
mediated pyroptosis.

Both hepatocytes and hepatic macrophages undergo GSDMD activation in the mouse AH model[10]. Pyroptosis of the latter cell type may be pathophysiologically significant. In the AH mice, liver Cldn5 expression as an indicator for hepatic macrophages, is severely reduced while the expression of neutrophil marker Mpo shows a conspicuous increase, indicating macrophages are depleted due probably to pyroptosis. In the absence of sufficient macrophages, there is no other cell type except neutrophils that fight against translocated bacteria in the liver, causing intense neutrophil infiltration (Fig. 2B).

In integrating hepatocyte cytotoxicity and gut dysbiosis into the mechanisms for inflammation in ALD (Fig. 3), organelle stress and suppressed autophagy in hepatocytes certainly are central to cytotoxicity in ALD and also underlie inflammation via the release of DAMPs by cell death or extracellular vesicles (EVs). Concomitantly, gut dysbiosis, disrupted gut barrier, and intestinal mucosal immune dysfunction, cause translocation of bacteria and PAMPs, initiating TLR-mediated proinflammatory responses in Kupffer cells, hepatocytes, and HSCs which further release chemokines such as CLC11 or CCL2 to promote recruitment of neutrophils and macrophages. TLR4 activation in macrophages primes the cells for activation of canonical NLRP3 inflammasome by DAMPs released by damaged hepatocytes and activates pro-IL-1β. IL-1β upregulates and activates pro-MMP-9 in HSCs to initiate proinflammatory activation of HSCs, amplifying inflammation. Increased bacterial load in the liver in advanced ASH, raises intracellular concentrations of LPS in hepatocytes and hepatic macrophages via infection or uptake of LPS-containing outer membrane vesicles released by bacteria. This activates CASP 11 / 4-GSDMD pathway and causes the lytic cell death pyroptosis of hepatocytes and macrophages releasing bacteria, PAMPs, DAMPs, activated IL-1β, and IL-18 into local microenvironment and aggravating infection and
inflammation.

3.7. Alcoholic liver fibrogenesis

Activated hepatic stellate cells (aHSCs) are a primary cell type responsible for liver fibrogenesis in ALD as generally recognized in any type of chronic liver disease. From the discussions on the pathogenesis of ALD above, key mediators of activation of HSCs can readily be identified including cytokines (TNFα, IL-1β, IL-17), oxidant stress, and endotoxin. The section will review only recent findings relevant to liver fibrogenesis in the setting of alcohol. NADPH oxidase (NOX) generates ROS and this is shown to be a required signaling event for activation of HSCs induced by many of the key known mediators such as angiotensin, PDGF, leptin, TGF-β, and LPS. NOX1, NOX2 and NOX4 are expressed by HSCs. NOX4 mRNA is increased in α-smooth muscle-positive aHSCs in ALD patients, and the liver of HSC-specific NOX4 KO mice fed alcohol express less proinflammatory cytokines and chemokines. NOX4 promoter is stimulated by acetaldehyde and NOX4 upregulates CCR2 and CCL2 mRNA stability while increasing cytoplasmic shutting of ELAV1 (HuR), the mRNA binding protein required for activation of HSC and liver fibrosis. Acetaldehyde is shown to stabilize β-catenin to activate HSCs independent of Wnt but involving inactivation of nucleoredoxin releasing Dishevelled and phosphorylating GSK3β. The DAMP HMGB1 is released by alcohol-treated hepatocytes and stimulates migration of HSC and liver endothelial cells involving Src and ERK activation. IL-22 which is known to promote anti-microbial immunity and tissue repair, is downregulated in AH and pro-

Fig. 3 A schematic drawing depicts how damaged hepatocyte with organelle stress release DAPMs via cell death or extracellular vesicles (EVs) to contribute to inflammation; translocation of bacteria or PAMPs due to gut dysbiosis activating TLR4 in Kupffer cell (KC) or hepatic stellate cells (HSC) which in turn release chemokines such as CXCL1 or CCL2 to recruit polymophonuclear cells (PMN) and migrating macrophages (MM). TLR2 and 9 are also activated in hepatocytes and HSC to contribute to PMN infiltration. Macrophages primed with TLR4 activation release IL-1β via activation of NLRP3-dependent inflammasome and CASP1 by DAMPs released by damaged hepatocytes. Infection with Gram-negative bacteria or uptake of LPS-containing outer membrane vesicles (OMV) from bacteria, increases intracellular LPS and causes pyroptosis by activation of CASP11/4-GSDMD pathway in hepatocytes and macrophages, releasing bacteria, DAMPs, PAMPs, IL-1β, IL-18 and further aggravating PMN inflammation.
posed for AH treatment. This cytokine induces HSC senescence via upregulation of p53 and ameliorates liver fibrosis\(^\text{121}\), further increasing therapeutic potential of this cytokine for ALD. Loss of intracellular vitamin A is a known phenotype of HSC activation. Our own unpublished results show more severely depleted retinoids in activated HSCs isolated from the iG ASH models than the cells isolated from CCl4 hepatotoxic or bile duct ligation model to the extent that a conventional discontinuous gradient ultracentrifugation cannot achieve acceptable isolation from mouse ASH livers. In culture-activated HSCs, retinoids are not only lost but metabolized to retinoic acids\(^\text{121}\). ADH3 which is expressed in HSCs and capable of retinol oxidation, is essential for HSC activation and liver fibrosis\(^\text{124}\). One suggested mechanism for this role of ADH3 is inhibition of NK cell activity including IFN-\(\gamma\) expression\(^\text{120}\).

There are an increasing number of miRNAs identified to regulate HSCs by targeting either activation or quiescence genes. Let-7 family members such as let-7a and let-7b support HSC quiescence and are downregulated in experimental ASH and LPS or TGF-\(\beta\)-treated HSCs while expression of these let-7 members suppresses activation of human HSCs\(^\text{125}\). Col1a2 and Col4a1 are direct targets of let-7b in mesangial cells\(^\text{126}\), and this may be at least in part responsible for the effects seen in HSCs. In addition, Lin28 along with HMGA2 is suggested to be a target of let-7a/b and to establish a negative feedback to let-7\(^\text{125}\), serving as an amplifying fibrogenic effect. Another anti-fibrotic miRNA is miR-214 which targets connective tissue growth factor (CCN2) and is shown to be transcriptionally upregulated by Twist1\(^\text{127}\). Twist expression is suppressed in HSCs in fibrotic livers and cultured HSCs treated with ethanol. Exosomes secreted by quiescent but not activated HSCs contain high levels of Twist1 and transfer Twist 1 among HSCs to upregulate miR-214 and downregulate CCN2 and downstream fibrogenic effectors\(^\text{127}\).

Finally, epigenetic regulation of HSCs in ALD is an important topic. The master regulator for HSC quiescence or differentiation is PPAR-\(\gamma\)\(^\text{128}\) which is epigenetically repressed by the methyl-CpG binding protein MeCP2\(^\text{129}\). MeCP2, by binding to the Ppar\(\gamma\) locus, recruits the transcriptional repressor HP-1 and concomitantly induces EZH2 and H3K27me2/3 at 3\(^\text{rd}\) exons to repress Ppar\(\gamma\)\(^\text{129}\). Upregulated MeCP2 protein expression in activated HSCs is due to canonical Wnt pathway-dependent stabilization of MeCP2 protein\(^\text{130}\). Using HSCs isolated by FACS from Col1a1-GFP mice fed \textit{ad libitum} ethanol-containing diet with weekly alcohol gavage vs. pair-fed controls and performing combinatorial ChIP-seq analysis for enrichment with both MML1, a H3K4 methyltransferase, and H3K4me3, 41 gene loci were shown to be enriched with both by alcohol, opening up investigations of epigenetically activated novel genes in activation of HSCs in ALD\(^\text{131}\).

### 3.8. Alcohol and liver cancer

Alcohol misuse initiates tumorigenesis and also promotes tumor formation. Firstly, ethanol is a Group-1 pro-carcinogen that requires its bioconversion to a primary carcinogenic metabolite, acetaldehyde. Thus, those who have a loss-of-function mutation in \textit{ALDH2} gene (ALDH2\(^*\)), have a significantly increased risk for the development of cancers\(^\text{122,133}\). Acetaldehyde is carcinogenic because it forms an adduct with DNA and interstrand crosslinks via its electrophilic carbonyl group and inhibits the DNA repair enzyme O6-methylguanine-DNA methyltransferase\(^\text{134}\). CYP2E1 induced by chronic alcohol abuse, causes oxidant stress, and generates lipid peroxidation aldehydic metabolites which similarly form adducts while converting other pro-carcinogens such as nitrosamine\(^\text{135}\). Inflammation caused by DAMP and PAMP and oxidant stress by infiltrating neutrophils cause hepatocellular DNA damage and contributes to tumor initiation\(^\text{131}\). TLR4 implicated in the pathogenesis of ASH, also plays an important role in the genesis of tumor-initiating stem cell-like cells (TICs). Ectopic expression TLR4 in hepatocytes by a HCV NS5A transgene plus chronic alcohol feeding which raises endotoxin level, cause liver tumors in mice\(^\text{137}\) via generation of CD 133 + TLR 4-NANOG-dependent TICs which are also isolated from patient HCC tissues\(^\text{138}\).

Alcohol feeding promotes tumor formation and this promotion is associated with activation of canonical Wnt pathway\(^\text{139}\). This link is probably based on the known roles of \(\beta\)-catenin in stimulating tumor cell growth and upregulating CYP2E1\(^\text{138}\). Tumor-
Fig. 4  Alcohol and hepatocarcinogenesis. Acetaldehyde produced by aldehyde dehydrogenase (ADH) or CYP2E1 and aldehydic lipid metabolites (MDA, 4HNE) resulting from lipid peroxidation, form DNA adducts, and reduced level of S-adenosylmethionine (SAMe) may cause aberrant DNA methylation. Induced CYP2E1 converts procarcinogens to carcinogens. Oxidative DNA damage may also result from oxidant stress arising from CYP2E1 and mitochondria, as well as macrophage or polymorphonuclear cell (PMN) inflammation stimulated by DAMPs released by damaged hepatocytes and PAMPs released by gut with dysbiosis. With defective or insufficient DNA repair, this will result in genotoxicity and tumor initiation. Ectopic TLR4 expression and its activation by LPS in hepatocytes or hepatic progenitor cells, generate NANOG-dependent tumor-initiating stem cell-like cells (TICs), contributing to initiation and recurrence of liver tumor. M2 macrophages, activated hepatic stellate cells, and reduced CD8+ T cells, support tumor promotion.

associated M2 macrophages support tumor promotion in part by stimulating HSCs which secrete matrix proteins and growth factors conducive to tumor growth[40]. Tumor-associated myofibroblastic HSCs promote TIC-derived tumor formation or diethylnitrosamine-initiated, alcohol diet-promoted liver tumor development[42]. CYP2E1 and LPS which are two major factors for initiation of liver tumorigenesis, also activate HSCs[90-143] and therefore promote tumor progression. Alcohol-induced immune suppression is an important mechanism of tumor promotion which may involve reduced anti-tumor CD8+ cells[144]. Mucosal-associated invariant T cells (MAIT cells) are recently proposed to link intestinal immunity to antibacterial immune defects in ASH in patients[147]. Further a relative increase in IgA+B cells observed in ALD[146] may facilitate immune tolerance as recently shown for cancer cells[147].

Mechanisms of alcohol-mediated hepatocarcinogenesis are summarized schematically in Fig. 4, depicting how tumor initiation vs. promotion is achieved by hepatocyte intrinsic mechanisms and/or cellular crosstalk between hepatocytes, inflammatory cells, hepatic stellate cells.

4. Cell Fate Regulation and Metabolic Reprogramming

Chronic diseases such as ALD and NAFLD involve multiple cell types including the parenchymal cell type which is a primary target of chronic insult, resident or migrating inflammatory and immune cells whose repertoire and functional characteristics change from acute to chronic, or to acute on chronic phases, and mesenchymal cells responsible for wound healing or fibrosis. These players undergo appreciable alternations in cell fate which are driven by metabolic reprogramming. Fatty liver, the first stage of ALD/NAFLD, can be recognized as hepatocytes undergoing adipogenic regulation rendered by upregulated lipogenic transcription factors such as PPARγ and SREBP-1c[148-150]. Ectopic expression of PPARγ induces adipogenic steatosis in the mouse liver[151] while liver-specific loss of this transcription factor attenuates fatty liver[152-153]. In contrast, HSCs undergo anti-adipogenic regulation as characterized by loss of PPARγ expression to undergo my-
of fibroblastic activation in the same microenvironment as ALD/NAFLD develop perisinusoidal and pericellular “chicken wire” fibrosis, highlighting so-called “Fat Paradox” in steatohepatitis\textsuperscript{[141]}. As hepatocytes begin to undergo dysplastic changes in ALD, TICs are believed to be generated. These TICs can be isolated from rodent or patient HCC tissues by FACS as CD133\textsuperscript{+} /CD49f+/cells\textsuperscript{[127,138]}. A unique feature of this liver TIC is that activation of TLR4 signals to upregulate pluripotent and stemness transcription factors, NANOG, SOX2, and OCT4 which confer TIC’s self-renewing and tumor-initiating activities. And this cell fate regulation is dependent on NANOG-driven metabolic reprogramming such as suppressed mitochondrial oxidative phosphorylation but enhanced fatty acid oxidation to derive energy\textsuperscript{[150]}. Activated HSCs promote liver tumor development as discussed above, and this crosstalk function is mediated by production of monounsaturated fatty acids (MUFAs) catalyzed by steaeryl co-A desaturase (SCD) which is upregulated in a β-catenin-dependent manner\textsuperscript{[142]}. Increased intracellular MUFAs inhibit nuclear import of the mRNA binding protein ELAV 1 (HuR) by Transportin-1/Ran1 and increase its cytosolic level and binding to Lrp5/6 3’ UTR to stabilize the mRNAs and upregulate these Wnt pathway functional receptors\textsuperscript{[142]}. This establishes a novel positive forward loop of β-catenin-SCD-MUFA-ELAV1-LRP5/6 to amplify the canonical Wnt pathway and activate HSCs. Intriguingly, this positive loop also exists in TICs and most likely in the human HCC line Huh7 cells\textsuperscript{[142]}. Thus, the dependence of liver tumor development on HSC’s SCD, suggests that this positive loop mediates tumor promoting tumor microenvironment via generation of MUFA. In fact, SCD is overexpressed in various malignancies including renal cell carcinoma and colon carcinoma and is considered as an emerging pharmacologic therapeutic target\textsuperscript{[156–160]}. This SCD role likely represents a tip of iceberg for a wide range of lipid metabolic reprogramming supporting tumor promotion.

Macrophages which participate in both acute and chronic stages of ASH and NASH, also go through metabolic reprogramming to establish their functional phenotypes. For instance, M1 macrophage activation which signifies early ASH and NASH, requires increased mitochondrial respiration, oxidative phosphorylation, and ROS generation which are orchestrated by NOTCH activation leading to NICD-mediated mitochondrial gene induction and NICD-induced pyruvate dehydrogenase phosphatase, pyruvate kinase activity and pyruvate flux into TCA cycle\textsuperscript{[56]}. In fact, transcriptional upregulation of the proinflammatory gene such as Nos2 is dependent on a direct effect of NICD and the amplifying role of mitochondrial ROS generation stimulated by NICD-mediated mitochondrial metabolic reprogramming\textsuperscript{[156]}. As chronic phase ensues, M2 macrophage activation predominates and this is driven by metabolic reprogramming characterized by aerobic glycolysis, fatty acid oxidation, and α-ketoglutarate production via glutaminolysis\textsuperscript{[161,162]}. By understanding how metabolic reprogramming facilitates the manifestation of respective phenotype of each cell type contributing to different stages of ALD, new therapeutic design will be implemented to manipulate cell fate regulation at the level of metabolism.

5. Major Clinical Challenges

ALD is a difficult disease to treat for three main reasons. Firstly, it is a hidden disease in modern society and is not recognized as an important disease by medical and lay communities due to a stigma attached to it as a “sin disease” or “self-inflicting disease”. Because of this, patients tend to visit community clinics rather than university-affiliated hospitals, particularly so in Japan. Secondly and partly because of the stigma discussed, ALD patients are not screened and diagnosed early, and when they are diagnosed, ALD is often at the advanced stage. Thirdly, the treatment of ADL patients requires concerted efforts of psychiatrists, psychologists, social workers, and hepatologists and a sustained network of collaboration for prolonged periods from initial clinic visits through recovery from alcoholism and ALD. This network is not adequately established in most clinics and hospitals. In fact, primary care physicians or even specialized hepatologists, are not adequately trained or networked for proper diagnosis of alcohol misuse or alcohol use disorders. Based on the recognition of this problem, the university affiliated hospitals must begin pro-active efforts to establish
the network for the aforementioned expertise essential
for ALD patients as required for the treatment of obe-
sity and NASH.

In the U.S. and European nations where orthotopic
liver transplantation is actively practiced, it is being
proven to be most efficacious in severe acute AH
patients, particularly those who do not respond to corti-
costeroids. Acute AH patients who do not respond to
therapies or are not treated, have a ~70% six-month
mortality\textsuperscript{143}. Because of such grave condition, these
patients had to be exempted from the six-month absti-
nence rule. Based on the recommendations of the
French consensus conference of Lyon in 2005\textsuperscript{144}. The
first pilot study with carefully selected steroid-
nonresponder patients was performed by a group in
Lily, France, headed by Philippe Mathurin and achieved a remarkable two-year survival rate of
78%\textsuperscript{145}. Transplantation programs in the U.S. followed
this trial to offer liver transplantation for AH patients.
The program at Johns Hopkins Hospital led by
Andrew Cameron recently reported excellent 6-month
and 1-year patient survival rates of 98% and 97%, and
6-month and 1-year graft survival rates of 98% and 93%,
respectively\textsuperscript{146}. These AH patients had pre-
transplantation abstinence of only 28-85 days.

For alcoholic cirrhosis (AC), patients undergone
more than 6 months of abstinence prior to transplanta-
tion, and 1-year patient survival rates were 100% for
both and graft survival rates for the same periods were
94% and 84%\textsuperscript{147}. Alcohol recidivism after transplanta-
tion was 28% vs. 24% for AH vs. AC patients and alcohol
drinking with harmful patterns was 17% vs. 12% for
AH and AC, depicting similar and relatively low re-
lapse rates of drinking\textsuperscript{148}. These results depicting
highly successful transplantation outcome for both AC
and AH regardless of the pre-transplantation absti-
nence periods, are quite remarkable considering only
20-30% of these AH patients would have survived for
6 months without transplantation. Again, a well-
coordinated team work of multiple professionals (hepato-
ologist, transplant surgeon, psychologist, psychiatrist,
nurse, social worker), is absolutely essential for these
successful programs. Obviously, the acceptance of
brain death and the availability of fresh livers from
these patients are required for orthotopic transplanta-
tion and may present difficulties based on different cul-
tural backgrounds of the society.

The most logical approach to ALD is to catch the dis-
ease at the early stage before it progresses to AC or
AH. Currently, we do not have the means or practice
to effectively screen patients for early chronic ASH. In
fact, we have no clinical information on natural history
of ALD at the stage between fatty liver and AC or AH.
For NASH, early screening is practiced but not for
ALD. I call this black box of the uncharacterized stage
as “early chronic ASH” because based on animal data,
patients’ liver is predicted to show steatosis with bal-
loon cell degeneration, macrophage infiltration and ac-
tivation, and chicken wire fibrosis. This precursor le-
sion is critical for progression to AC or AH. We must
establish a practice to screen for this pathologic stage
with new biomarkers and diagnostic tools. In terms of
assessing liver fibrosis, the liver stiffness by elasto-
graphic techniques has been used as a noninvasive di-
agnostic tool for alcoholic liver fibrosis and cirrhosis
but loses the sensitivity for early fibrosis (F1-F2)\textsuperscript{147}. Se-
rum markers for fibrosis are in general less accurate
than liver stiffness but allow to distinguish F0-1 and F
2-4 and does not require an equipment. The best serum
marker for liver fibrosis to date, appears to be hyaluronic acid\textsuperscript{148,149}. We do not have good biomarkers
for macrophage vs. neutrophil inflammation. The initia-
tive on AH taken by the NIAAA/NIH in the recent
years, has advanced clinical studies on this disease
spectrum, and new biomarkers and treatment options
will hopefully become available through this effort. A
similar effort has to be made on ”early chronic ASH”
that presumably is inflicting a substantially larger
population of alcoholic patients than AH patients.

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Conflict of interests:
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