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Alcohol abuse and disorder of granulopoiesis

Xin Shi [a], Angelo L. DeLucia [a], Jianxin Bao [b], Ping Zhang [a,c,*]

[a] Department of Integrative Medical Sciences, Northeast Ohio Medical University College of Medicine, Rootstown, OH 44272, United States of America
[b] Department of Anatomy and Neurobiology, Northeast Ohio Medical University College of Medicine, Rootstown, OH 44272, United States of America
[c] Department of Surgery, Northeast Ohio Medical University College of Medicine, Rootstown, OH 44272, United States of America

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ABSTRACT

Granulocytes are the major type of phagocytes constituting the front line of innate immune defense against bacterial infection. In adults, granulocytes are derived from hematopoietic stem cells in the bone marrow. Alcohol is the most frequently abused substance in human society. Excessive alcohol consumption injures hematopoietic tissue, impairing bone marrow production of granulocytes through disrupting homeostasis of granulopoiesis and the granulopoietic response. Because of the compromised immune defense function, alcohol abusers are susceptible to infectious diseases, particularly septic infection. Alcoholic patients with septic infection and granulocytopenia have an exceedingly high mortality rate. Treatment of serious infection in alcoholic patients with bone marrow inhibition continues to be a major challenge. Excessive alcohol consumption also causes diseases in other organ systems, particularly severe alcoholic hepatitis which is life threatening. Corticosteroids are the only therapeutic option for improving short-term survival in patients with severe alcoholic hepatitis. The existence of advanced alcoholic liver diseases and administration of corticosteroids make it more difficult to treat serious infection in alcoholic patients with the disorder of granulopoieis. This article reviews the recent development in understanding alcohol-induced disruption of marrow granulopoiesis and the granulopoietic response with the focus on progress in delineating cell signaling mechanisms underlying the alcohol-induced injury to hematopoietic tissue. Efforts in exploring effective therapy to improve patient care in this field will also be discussed.

Contents

Abbreviations: ADH, alcohol dehydrogenases; AKT, protein kinase B; ALDH, Aldehyde dehydrogenase; AML, acute myeloid leukemia; AP-1, activating protein-1; ARNT, aryl hydrocarbon receptor nuclear translocator; BKCa, big conductance calcium-activated potassium channels; BMP, bone morphogenetic protein; BrdU, 5-bromo-2-deoxyuridine; CAR, CXC chemokine ligand 12-abundant reticular; CDK, cyclin-dependent kinase; CEBP–β, CCAAT enhancer-binding protein-β; CFU-G, granulocytic progenitor cells; CFU-GM, granulocyte/macrophage colony forming unit; c-kit, stem cell growth factor receptor; CMPs, common myeloid progenitors; cMyc, c-Myc oncogene; CXCL12, CXC chemokine ligand 12; DAMPs, damage-associated molecular patterns; DEAB, diethylaminobenzaldehyde; ER, endoplasmic reticulum; ERAD, endoplasmic reticulum-associated protein degradation; ERK, extracellular-signal-regulated kinase; G-CSF, granulocyte-colony stimulating factor; G-CSFR, granulocyte colony-stimulating factor receptor; Gli1:g, lioma-associated oncogene 1; GM-CSF, granulocyte-macrophage colony-stimulating factor; GM-CSFR, granulocyte-macrophage colony-stimulating factor receptor; GMPs, myelomonocytic progenitor cells; GPI, glycosyl phosphatidylinositol; HSCs, hematopoietic stem cells; HSPCs, hematopoietic stem/progenitor cells; IL-1β, interleukin-1β; IL-18, interleukin-18; JNKs, c-Jun N-terminal kinases; lin, lineage; LED, Lieber-DeCarli low-fat liquid alcohol diet; LKS, lin-c-kit+Sca-1+; LPS, lipopolysaccharide; LTR-HSCs, long-term repopulating HSCs; MAPK, mitogen-activated protein kinase; MDS, myelodysplastic syndrome; MEK, mitogen-activated protein kinase ERK kinase; MELD, model for end stage liver disease; MMP-8, matrix metalloproteinase-8; MMP-9, matrix metalloproteinase-9; MMPs, matrix metalloproteinases; MPPs, multipotent progenitor cells; MSCs, mesenchymal stem cells; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-κB; PAMPs, pathogen-associated molecular patterns; PBMCs, peripheral blood mononuclear cells; PLCD, paraptosis-like cell death; PMNs:, polymorphonuclear leukocytes; PPARγ, Peroxisome proliferator-activated receptor γ; PU.1, Spi-1 proto-oncogene; RA, retinoic acid; RAR, retinoic acid receptor; ROS, reactive oxygen species; Sca-1, stem cell antigen-1; SCF, stem cell factor; SDF-1, stromal cell-derived factor 1; SHH, sonic hedgehog; siRNA, small interfering RNAs; Spi-1, Spi-1 proto-oncogene; STAT3, transcription factors signal transducer and activator of transcription 3; STR-HSCs, short-term repopulating HSCs; TGF-β, transforming growth factor-β; TLR, toll-like receptor; TNF-α, tumor necrosis factor-α; TNFR, TNF receptor; TRIF, TIR-domain-containing adapter-inducing interferon-β (TLR4-toll-like receptor adaptor molecule 1).

* Corresponding author at: Department of Integrative Medical Sciences, College of Medicine, Northeast Ohio Medical University, 4209 State Route 44, Room F339, Rootstown, OH 44272, United States of America.
E-mail address: pzhang@neomed.edu (P. Zhang).

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1. Introduction

Granulocytes are a population of leukocytes characterized by the presence of granules in their cytoplasm. This heterogeneous cell population includes neutrophils, eosinophils, and basophils. Representing the front line of innate immune defense, neutrophils are the most abundant type of granulocytes in the body of humans. Since eosinophils and basophils comprise only a very small portion of the population, the term of "granulocytes" is also used to loosely imply neutrophilic granulocytes (or polymorphonuclear leukocytes, PMNs) (Babior & Golde, 2001). Granulocytes are derived from hematopoietic stem cells (HSCs). In adults, HSCs are rare event cells residing in the bone marrow, which possess the unique capacity of self-renewal and differentiation into all types of blood cells (Kondo et al., 2003; Shizuru, Negrin, & Weissman, 2005). Under the homeostatic condition, the process of HSC self-renewal, differentiation, and commitment to granulopoiesis is tightly controlled in order to maintain the optimal pool of HSCs and the balanced production of granulocytes as well as other blood cell types (Akala & Clarke, 2006; Kondo et al., 2003). In humans, HSCs display the lineage $(lin)^{-}CD34^{+}$ surface marker (Bernstein, Andrews, & Rowley, 1994; Chang, Jensen, Quesenberry, & Bertoncello, 2000; Engelhardt, Lübbert, & Guo, 2002; Hogan, Shpall, & Keller, 2002; Murray et al., 1994, 1995; Murray, Tsukamoto, & Hoffman, 1996; Tian & Zhang, 2016). A subpopulation of dormant human HSCs are $lin^{-}CD34^{-}$ (Dao & Nolta, 2000), which appear to be long-term repopulating HSCs (LTR-HSCs) (Sonoda, 2008). These $lin^{-}CD34^{-}$ HSCs can give rise to short-term repopulating HSCs (STR-HSCs) which likely carry the surface marker of $lin^{-}CD34^{+}$. In mice, the bone marrow lin^{-} stem cell growth factor receptor $(c\text{-}kit)^{+}$stem cell antigen-1 $(Sca\text{-}1)^{+}$ (LKS) cell population is enriched with hematopoietic stem cells (Okada et al., 1992; Osawa, Hanada, Hamada, & Nakauchi, 1996). Mouse LTR-HSCs are also CD34 low/negative (Osawa et al., 1996). The CD34 positive LKS cell population consists of relatively downstream STR-HSCs and multipotent progenitor cells (MPPs). More committed progenitors such as common myeloid progenitors (CMPs) derived from MPPs bear $lin^{-}c\text{-}kit^{+}Sca\text{-}1^{-}$ surface marker (Okada et al., 1992; Osawa, Hanada, et al., 1996). CMPs sequentially differentiate into myelomonocytic progenitor cells (GMP or granulocyte/macrophage colony forming unit, CFU-GM) and granulocytic progenitor cells (CFU-G). CFU-G cells then stepwisely differentiate into myeloblasts, promyelocytes, myelocytes, metamyelocytes, and eventually granulocytes. Mitotic capability is lost after the stage of myelocytes. Therefore, cell development along the granulocytic lineage can be roughly divided into two compartments, *i.e.* the mitotic and post-mitotic compartments. Terminally differentiated mature granulocytes are commonly retained in the storage pool of bone marrow before being released into the systemic circulation. The marrow storage pool houses approximately 96–99% of total body neutrophilic granulocytes in humans and in mice (Furze & Rankin, 2008; Strydom & Rankin, 2013; Summers et al., 2010). On average, the transit time for granulocytes to pass through the post-mitotic compartment/storage pool is around 5 days in humans (Summers et al., 2010).

Upon bacterial infection or host confrontation with an inflammatory challenge, mobilization of granulocytes from the bone marrow is enhanced. The level of circulating granulocytes can increase over 10 fold in hours with the concomitant reduction of granulocyte storage in the bone marrow (Furze & Rankin, 2008; Strydom & Rankin, 2013). In the meantime, marrow quickly initiates the granulopoietic response to enhance production of granulocytes at the expense of other blood cell lineage development (Barthlen et al., 1999; Ueda, Kondo, & Kelsoe, 2005). During the granulopoietic response, hematopoietic stem/progenitor cells (HSPCs) are activated for proliferation. Reprograming of these hematopoietic precursors at the transcriptional and post-transcriptional levels takes place to promote their commitment toward granulocytic lineage development (Melvan et al., 2011; Shi et al., 2013, 2018; Shi, Lin, Gao, & Zhang, 2017; Zhang et al., 2008; Zhang, Bagby, Happel, Raasch, & Nelson, 2008). With these enhanced activities, the transit time for granulocytic lineage cells to pass through both the mitotic and post-mitotic compartments is markedly shortened (Terashima, Wiggs, English, Hogg, & van Eeden, 1996). The substantial capacity of bone marrow for producing large amount of granulocytes through the granulopoietic response is critically important for reinforcing host immune defense against invading pathogens.

Alcohol is the most frequently abused substance in human society (Merikangas & McClair, 2012). Excessive alcohol consumption injures the bone marrow and impairs homeostasis of granulopoiesis as well as the granulopoietic response (Melvan et al., 2011, 2012; Raasch et al., 2010; Shi, Lin, et al., 2017; Zhang et al., 2009). Alcohol exposure also impairs functional activities of granulocytes (Szabo & Mandrekar, 2009; Zhang, Bagby, Happel, Summer, & Nelson, 2002). The resulted defects in immune defense significantly increase the host susceptibility to serious infections, particularly pneumonia and septicemia (Cook, 1998; de Wit, Jones, Sessler, Zilberberg, & Weaver, 2010; MacGregor & Louria, 1997; Mehta, 2016; O'Brien Jr. et al., 2007; Zhang et al., 2002; Zhang, Bagby, et al., 2008; Zhang, Nelson, et al., 2008). Clinical studies on large cohorts of patients treated in the ICU and emergency medical settings have shown that 12–26% of total cases are alcohol dependent or have a high blood alcohol level at admission (O'Brien Jr. et al., 2007; Plurad et al., 2010). Alcoholic patients have significantly higher rates of septic complications including septic shock. One third to half of hospitalized patients with pulmonary infections are alcoholics (Dorff, Rytel, Farmer, & Scanlon, 1973; Goss et al., 2003; Winterbauer, Bedon, & Ball Jr., 1969). Lung infections in alcohol abusers are frequently complicated with septicemia (Fernández-Solá et al., 1995; Hammond, Lyddell, Potgieter, & Odell, 1993; Musher et al., 2000; Ruiz et al., 1999; Saitz, Ghali, & Moskowitz, 1997). A striking feature of alcoholic patients with septic infection is that they often present with granulocytopenia (Jong, Hsiue, Chen, Chang, & Chen, 1995; MacGregor & Louria, 1997;). Mortality rates in alcoholic patients with septic infection and granulocytopenia continue to be exceedingly high at 83–100% (Jong et al., 1995; Perlino & Rimland, 1985). Excessive alcohol consumption causes severe injury in the liver. Patients with alcoholic liver diseases are also frequently complicated with serious infections, which are the main cause of death in these individuals (Bruns, Zimmermann, & Stallmach, 2014; Karakike, Moreno, & Gustot, 2017). This review will focus on discussing the recent development in studies on alcohol-induced injury to marrow granulopoiesis and the granulopoietic response. The progress in delineating the underlying cell signaling mechanisms will be highlighted. Efforts in exploring effective therapy to improve patient care in this field will also be addressed.

2. Alcohol injures hematopoietic stem/progenitor cells

2.1. Cell toxicity of alcohol

Excessive alcohol consumption causes damage to hematopoietic tissue (Ballard, 1997; Heermans, 1998; Michot & Gut, 1987). Morphological examinations have revealed that bone marrow samples

from alcohol abusers exhibit a significant reduction of mature granulocytes with vacuolization in myeloid progenitor cells (Ballard, 1980; Yeung, Klug, & Lessin, 1988). This alcohol-induced vacuolization in hematopoietic precursor cells is not restricted in the granulocytic lineage, the erythroid and megakaryocytic lineages are also involved (Latvala, Parkkila, & Niemelä, 2004; Roselle, Mendenhall, Muhleman, & Chedid, 1986; Yeung et al., 1988). Ultrastructural examination discloses that the vacuoles are present in the cytoplasm and free of organized structure (Yeung et al., 1988). *In vitro* culture of marrow cells from normal individuals in nutrient medium containing alcohol can induce cytoplasmic vacuolization (Yeung et al., 1988). The critical alcohol concentration for inducing vacuolization is 62.5 mg/dl. The proportion of cells developing vacuoles appears correlating with the concentration of alcohol. In the clinic, vacuolization in peripheral blood leukocytes including granulocytes and lymphocytes has also been observed in patients with acute alcohol intoxication (Davidson & McPhie, 1980). In addition to causing vacuolization in hematopoietic precursor cells, alcohol exposure also leads to formation of vacuolar inclusions in a variety of other cell types, including neurons (Goldstein, Maxwell, Ellison, & Hammer, 1983), inner ear hair cells (Nordemar, 1988), ovary granulosa and theca cells (Laura, Martinez, Padovani, & Martinez, 2003), myocardial cells (Rajbanshi & Pandanaboina, 2014), pancreas acinar cells (Werner et al., 2002), as well as uterine tube epithelial cells (Martinez et al., 1999).

Alcohol-induced formation of vacuoles in hematopoietic precursor cells is a sign of cell stress. At the present time, however, knowledge about the effects of alcohol-induced vacuolization on functional activities of hematopoietic cells remains limited. Cytoplasmic vacuolization is a morphological change frequently occurring in cells following exposure to various natural and artificial low-molecular-weight compounds as well as infection with bacterial or viral pathogens (Aki, Nara, & Uemura, 2012; Shubin, Demidyuk, Komissarov, Rafieva, & Kostrov, 2016). Vacuolization may primarily reflect an adaptive response for cell survival (Henics & Wheatley, 1999), which subsequently has the potential to lead to distinctive forms of cell death subsequently (Aki et al., 2012; Henics & Wheatley, 1999; Shubin et al., 2016). Recent studies have revealed that a variety of inducers can cause cell vacuolization leading to specific types of cell death through different pathways (Aki et al., 2012; Shubin et al., 2016). Exposure to weakly basic amine-containing lipophilic compounds can induce cell vacuolization (Marceau et al., 2012; Shubin et al., 2016). These lipophilic bases are uncharged in neutral extracellular fluid, allowing them to enter into cells *via* simple diffusion and/or active transportation. After entering acidic endosomal-lysosomal organelles and Golgi apparatus in the cell, they become positively charged through protonation rendering them unable to diffuse out through the organelle membrane. The trapped weak bases with positive charge increase the osmotic pressure, which drives diffusion of water into the organelles to form vacuoles. Ethanol is a slightly charged water-soluble polar molecule diffusible to cytoplasmic membrane. At the present time, nevertheless, there is no evidence to suggest if any alteration of osmotic pressure in the organelles occurs due to physicochemical interactions of ethanol during the process of cell vacuolization. Disruption of various metabolic pathways can induce formation of vacuoles in different cellular compartments irrelevant to their acidic/basic environments. Vacuolization of endoplasmic reticulum (ER) and swelling of mitochondria are associated with paraptosis-like cell death (PLCD) (Shubin et al., 2016). Impairment of either endoplasmic reticulum-associated protein degradation (ERAD) or ER-localized big conductance calcium-activated potassium channels (BKCa) mediates PLCD. Oxidative stress, impairment of protein folding in the ER, and disruption of ubiquitin-proteasome system cause ER stress and vacuolization. Excessive production of reactive oxygen species (ROS) interrupts the function of BKCa system leading to mitochondrial swelling. Alcohol has been shown to evoke oxidative stress (Das & Vasudevan, 2007), disrupt protein folding in the ER (Ji, 2015), and inhibit ubiquitin-proteasome activity (Donohue Jr. & Thomes, 2014) in cells. These negative effects of alcohol on cell functional processes may potentially contribute to the formation of vacuoles. Methuosis is a type of cell death associated with vacuolization of macropinosomes (Maltese & Overmeyer, 2014), during which, failure of macropinosomes to fuse with other organelles of the endocytic pathway leads to macropinosome accumulation in the cytoplasm, fusing with each other to form vacuoles. Ultrastructural examinations of bone marrow samples from subjects with alcohol intoxication have shown that surface invagination of the cell membrane in erythroblasts leads to endocytosis and consequently vacuole formation (Yeung et al., 1988). It remains to be defined if this type of alcohol-induced vacuole formation shares a similar mechanism as seen in abnormal macropinocytosis during methuosis.

In addition to inducing vacuolization-associated injury, alcohol causes cell death through the apoptosis pathway. Studies have shown that alcohol exposure promotes apoptosis in stem/progenitor cells of both the embryonic and adult tissue origins, including human and murine embryonic stem cells (Arzumnayan, Anni, Rubin, & Rubin, 2009; Nash, Krishnamoorthy, Jenkins, & Csete, 2012), human placental trophoblast cells (Bolnick et al., 2014), human neural stem cells (Hao, Parker, Zhao, Barami, & Lyman, 2003), and human bone marrow-derived mesenchymal stem cells (MSCs) (Chen et al., 2013). HSCs appear to be more resistant to alcohol than neural stem cells in the induction of apoptosis. *In vitro* incubation of human neural stem cells with 5 mM of ethanol causes apoptosis, whereas HSCs are unaffected by even 20 mM ethanol (Hao et al., 2003). However, blood alcohol concentration can reach much higher levels than 20 mM in alcohol abusers with acute or binge intoxication. Further studies are warranted to define if alcohol at high levels frequently seen in the blood of heavy drinkers would induce apoptosis in HSCs. In addition, it seems also worthwhile to further verify if long-term exposure to low levels of ethanol would exert any negative effects on HSC survival as well as other functional activities.

2.2. Toxic products from alcohol metabolism

Except for the direct toxicity of alcohol to hematopoietic precursor cells (Guthrie Jr & Beckman, 1983; Liu, 1980), cellular metabolites of ethanol can exert negative effects on HSPCs. Nucleated bone marrow cells metabolize ethanol (Bond & Wickramasinghe, 1983; Wickramasinghe, Bond, Sloviter, & Saunders, 1981; Xu, Jennett, Smith, Sorrell, & Tuma, 1989). Acetaldehyde, the immediate metabolite from ethanol through both the alcohol dehydrogenase (ADH) and cytochrome P450 pathways (Smith, Gasparetto, Jordan, Pollyea, & Vasiliou, 2015; Lu & Cederbaum, 2008), is cytotoxic. It can bind and form covalent adducts with macromolecules, including proteins and nucleic acids, either on cell surface membrane or in intracellular constituents (Latvala, Parkkila, Melkko, & Niemelä, 2001; Niemelä, 2001; Xu et al., 1989; Yu et al., 2010). Alcohol exposure causes oxidative stress in cells. ROS generated during metabolism of ethanol cause damage to cell membrane lipids as well as cellular proteins and nucleic acids (Koch et al., 2004; Niemelä, 2001; Wu & Cederbaum, 2003; Wu, Zhai, & Shi, 2006). Molecular modifications of lipids, proteins, and nucleic acids mediated by metabolites of alcohol can subsequently lead to disruption and/or impairment of HSPC structural integrity, signaling regulation, metabolism, survival, proliferation, as well as differentiation (Garaycoechea et al., 2018; Latvala et al., 2001; Van Wassenhove, Mochly-Rosen, & Weinberg, 2016). In addition, DNA damage caused by cellular accumulation of acetaldehyde is carcinogenic, which may play a role in inducing the malignant transformation of HSPCs (Smith et al., 2015; Yu et al., 2010). Fig. 1 illustrates generation of harmful metabolites from ethanol metabolism.

The capacity of tolerance to alcohol-induced disruption in cell functional activities may vary among HSPCs at different stages of differentiation. HSCs and the upstream multipotent progenitors are relatively more resistant to the negative effects exerted by ethanol and

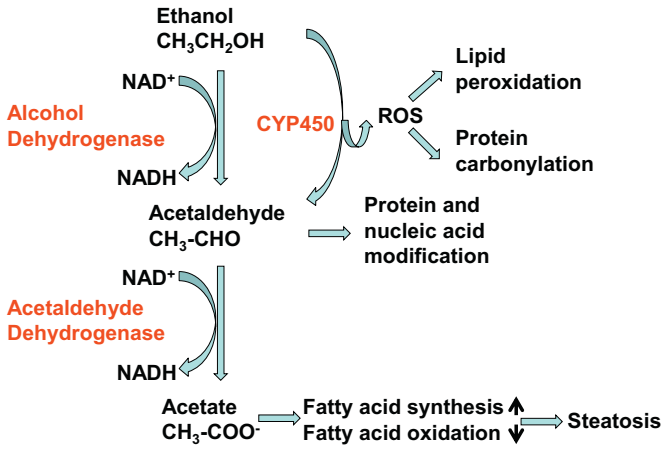

Fig. 1. Generation of harmful metabolites from ethanol metabolism. EYP450: cytochrome P450.

acetaldehyde in comparison to committed myeloid and the downstream progenitor cells (Smith et al., 2015). Aldehyde dehydrogenase (ALDH) catalyzes the conversion of acetaldehyde to acetate, which is a key step in alcohol metabolism. ALDH activity is higher in HSCs than in the relatively downstream progenitors and most differentiated types of hematopoietic cells (Storms et al., 1999). This uniqueness in high ALDH activity seems beneficial for detoxification of reactive aldehydes in order to maintain the functional integrity of primitive hematopoietic precursor cells. The physiologic role of ALDH activity in modulation of HSC function has been observed across species ranging from zebrafish to humans (Chute et al., 2006; Garaycoechea et al., 2012; Gasparetto et al., 2012; Ma, Chung, Liang, & Leung, 2010; Muramoto et al., 2010). The ALDH family contains 19 isoenzyme members with different substrate specificities, subcellular localizations, and patterns of expression (Jackson et al., 2011). In mice with deficiency in both ALDH1A1 and ALDH3A1, a wide range of hematopoietic abnormalities have been observed, including reduction in HSC number along with changes in cell cycling, intracellular signaling, as well as gene expression (Gasparetto et al., 2012). In this respect, however, inconsistent observations also exist. Studies on competitive repopulation have reported that inhibition of ALDH in 34^-LKS cells with diethylaminobenzaldehyde (DEAB) causes an increase in repopulating activity of these LTR-HSCs in lethally irradiated mice (Muramoto et al., 2010). Further investigation on ALDH isoforms involved in the process will provide critical information for understanding the current controversy. Mice with combined inactivation of aldehyde catabolism (ALDH2 knockout) and the Fanconi anemia DNA-repair pathway (Fancd2 knockout) display a predisposition to leukemia, and are susceptible to the toxic effects of ethanol (Langevin, Crossan, Rosado, Arends, & Patel, 2011). In humans, deficiency in ALDH2 expression and function caused by a single nucleotide substitution (ALDH2*2) is associated with marrow failure (Van Wassenhove et al., 2016). People with polymorphisms in genes responsible for metabolism of alcohol derived reactive aldehydes and repair of their DNA adducts in HSCs and other hematopoietic cells have been postulated to be specifically vulnerable to ethanol-induced disorders of hematopoiesis (Smith et al., 2015).

Enzymes involved in the metabolism of alcohol have certain overlap with those catalyzing the synthesis of retinoic acid (RA), a small lipophilic molecule playing an important role in the regulation of HSPC activities. RA regulates cell function through its entering the nucleus to activate nuclear RA receptors (RAR). Binding of RAR/retinoid X receptor heterodimer to the RA response element triggers transcriptional activation of retinoid-responsive genes (Vassalli, 2019). RA is derived from retinol (vitamin A). The initial step of reversible retinol oxidation to retinaldehyde is catalyzed by either alcohol dehydrogenases (ADH1, ADH3, and ADH4) or retinol dehydrogenases (Kumar, Sandell, Trainor, Koentgen, & Duester, 2012). Retinaldehyde is then irreversibly metabolized to RA by cytosolic ALDH isozymes, such as ALDH1A1, ALDH1A2, and ALDH1A3, in a tightly regulated manner (Duester, Mic, & Molotkov, 2003). HSCs from human and murine origins express high levels of ALDH1A1 (Smith et al., 2015; Vassalli, 2019). Studies have shown that inhibition of ALDH1A1 delays RA-mediated differentiation of murine HSCs (Muramoto et al., 2010). RA plays a central role in initiation of RARα-cyclin-dependent kinase-activating kinase signaling to mediate granulocytic differentiation (Luo et al., 2007). Inhibition of ALDH activity impedes granulocytic differentiation of hematopoietic precursor cells in the *in vitro* culture system. In alcohol metabolism, cytosolic ADH, such as ADH1 and ADH4, catalyzes the conversion of ethanol to acetaldehyde that is subsequently converted to acetate by mitochondrial ALDH2 and cytosolic ALDH (Cheung, Davies, Hoog, Hotchkiss, & Smith Pease, 2003; Holmes, 1994; Sladek, Manthey, Maki, Zhang, & Landkamer, 1989). Under the normal condition, enzyme forms most efficient for retinol metabolism are different from those most efficient for ethanol metabolism (Duester, 1998). In the ADH family, for example, ADH1 is the most efficient one for catalyzing ethanol oxidation, while ADH4 serves as the best candidate for a retinol dehydrogenase. Among ALDH family members, similarly, oxidation of acetaldehyde is performed most efficiently by ALDH2, whereas ALDH1 functions efficiently as a retinal dehydrogenase. Nevertheless, the overlap of enzymatic activities involved in both ethanol metabolism and RA synthesis implies the possibility that the intoxicated level of ethanol may competitively inhibit the activity of RA synthetic pathway (Duester, 1998). Indeed, *in vitro* experiments have shown that ethanol competitively inhibits ADH-catalyzed retinol oxidation (Julià, Farrés, & Parés, 1986; Mezey & Holt, 1971). Exposure to high level (100 mM) of ethanol leads to a significant decrease in RA detection in 7.5-day-old embryos (Deltour, Ang, & Duester, 1996). Therefore, further delineating the potential interplay between alcohol metabolism and RA signaling in the circumstance of cell exposure to the intoxicated level of ethanol will be helpful for improving knowledge about molecular mechanisms underlying alcohol-induced disruption of HSPC commitment to homeostatic granulopoiesis and the granulopoietic response.

3. Alcohol disrupts homeostasis of granulopoiesis

Leukopenia is common in heavy alcohol drinkers, which can occur along with other hematological abnormalities including lymphopenia, anemia, and thrombocytopenia (Latvala et al., 2004; Liu, 1980; Panasiuk & Kemona, 2001). In patients referred for bone marrow examinations due to occult abnormalities in peripheral blood cells, 40% of them excessively consume alcohol (Latvala et al., 2004). Further observations have unveiled that the effects of alcohol consumption on leukocyte level in the peripheral circulation may vary depending on the quantity, duration, and pattern of alcohol consumption. Under certain circumstances, alcohol abusers may even exhibit a transient increase in granulocyte counts in the circulation. In a report of 45 healthy volunteers and 300 chronic drinkers with or without recent excessive drinking, alcoholics with recent excessive drinking have higher levels of circulating neutrophils as compared to healthy controls or alcoholics without recent drinking (Li et al., 2017). In another prospective study on 88 patients with alcoholic liver cirrhosis, model for end stage liver disease (MELD) scoring is used to divide them into five different groups, in which group 1 to 5 constitute patients with MELD scores of 1–9, 10–19, 20–29, 30–39 and >40, respectively. (Jain, Aggarwal, Rao, Dahiya, & Singla, 2016). All patients in group 1 have normal leukocyte count. Leukocytosis predominates in MELD group 2 and 3 patients. In group 4, leukopenia is more prevalent. All patients in group 5 have leukopenia. Concomitantly, a progressive fall in hemoglobin level and an increase in the instance of thrombocytopenia have been observed in patients with the increase in their MELD scores. In experimental studies, a reduction of leukocyte counts in the blood along with decreased levels of hematocrit and hemoglobin has been detected in dogs fed on diet

containing ethanol (Beard, Barlow, & Tuttle, 1963) In an study on rats with oral administration of alcohol at a high dose (2 mL per animal per day) for 10 to 22 weeks, it has been observed that despite overall leukopenia following alcohol ingestion, the absolute neutrophil count in the blood is increased along with a significant reduction of absolute lymphocyte count in alcohol-treated animals (Kanwar & Tikoo, 1992).

The level of granulocytes in the systemic circulation can be affected by multiple factors, including the granulopoietic activity of hematopoietic precursor cells, the storage capacity for mature granulocytes in the marrow storage pool, the mobilization of granulocytes from bone marrow into the systemic circulation, the removal of granulocytes from the circulation by macrophages in the liver, bone marrow stroma, and marginal zone of the spleen under the homeostatic circumstance, as well as the exit of granulocytes from the blood stream into tissue sites of inflammation during the inflammatory reaction. Alcohol may exert diverse effects on each of these processes.

3.1. Disturbance of homeostatic granulopoiesis

As mentioned previously, bone marrow samples from individuals with heavy alcohol consumption frequently exhibit hypocellularity with few mature granulocytes (Ballard, 1980; Liu, 1980; Nakao, Harada, Kondo, Mizushima, & Matsuda, 1991). This morphological feature implies the existence of impaired granulopoietic activity of hematopoietic precursor cells. Alcohol and its metabolites are toxic to hematopoietic precursor cells, particularly myeloid progenitors. Bone marrow cells from alcohol abusers with osteonecrosis exhibit a significantly reduced activity of CFU-GM in comparison to those from normal volunteers and patients with bone-marrow grafting for a non-union orthopedic problem (Hernigou & Beaujean, 1997). However, observations about the *in vitro* effect of alcohol on the granulocyte/macrophage colony forming activity in normal bone marrow cells appear inconsistent. In an investigation on human marrow cells, culturing cells in the soft agar medium containing alcohol has shown that ethanol at concentrations commonly seen in the blood of intoxicated patients (>200 mg/100 ml) sharply inhibits CFU-GM activity (Tisman & Herbert, 1973). While studies from other groups have reported that the CFU-GM activity in human marrow cells cultured in either the soft agar or methylcellulose medium system is resistant to the negative effect of alcohol even at the high concentration of ethanol (>600 mg/100 ml) (Imperia, Chikkappa, & Phillips, 1984; Meagher, Sieber, & Spivak, 1982). In animal experiments carried out by our group, we have used 5-bromo-2-deoxyuridine (BrdU) incorporation to determine the activity of proliferation in mouse bone marrow LKS cells. Neither acute alcohol intoxication (*via* intraperitoneal injection with 20% alcohol in saline at a dose of 5 g alcohol/kg body weight) alone nor chronic feeding on the Lieber-DeCarli low-fat liquid alcohol diet (LED supplies 36% of calories as ethanol) for 5 weeks plus binge alcohol intoxication causes significant change in marrow LKS proliferative activity under the homeostatic condition (Shi, Lin, et al., 2017; Zhang et al., 2009). In addition, *in vitro* exposure to ethanol at concentrations of 50 and 100 mM does not affect marrow c-kit$^+$Sca-1$^+$ proliferation in the culture system as reflected by their BrdU incorporation (Shi, Lin, et al., 2017). LKS cells are rare premitive precursors containing enriched HSCs in mice (Okada et al., 1992). Under the homeostatic condition, most of HSCs are in the dormant status (Kohli & Passegué, 2014). Only a small fraction of them enter into cell cycle for self-renewal and/or lineage commitment. In addition, the high cellular activity of ALDH may also help HSCs to resist the negative effect of alcohol on proliferation. Another possible reason for the inconsistency of observations from *ex vivo* cultures and animal experiments in relation to the morphological feature of bone marrow in alcoholic patients may be the duration of alcohol exposure. As described earlier, the typical hypocellularity of bone marrow biopsy and reduced CFU-GM activity are usually seen in individuals with chronically heavy alcohol consumption.

In adults, the hematopoietic niche environment in the bone marrow provides critical signals for regulating HSPC activities. The hematopoietic niche consists an interdependent network of osteolineage cells, endothelial cells, pericytes, CXC chemokine ligand 12 (CXCL12)-abundant reticular (CAR) cells, MSCs, sympathetic nerve fibers, nonmyelinating Schwann cells, and other hematopoietic cells (Calvi & Link, 2015; Wei & Frenette, 2018). Excessive alcohol consumption has been shown to cause structural damage and functional interruption of the marrow niche environment, which may serves as a potential mechanism underlying the disturbance of homeostatic granulopoiesis following alcohol exposure. In humans, long term heavy alcohol consumption causes decrease in bone mass and bone mineral density (Maurel, Boisseau, Benhamou, & Jaffre, 2012). An experimental study on rats fed with a diet containing high content of alcohol (35% *v*/v) for 17 weeks has shown a significant decrease in bone mineral density and trabecular thickness (Maurel et al., 2011). Human, animal, and cell culture studies have demonstrated that alcohol has a dose-dependent toxic effect on osteoblast activity. Alcohol exposure causes increase in osteocyte apoptosis and accumulation of lipid droplets within the osteocytes, leading to the development of osteopenia and decrease in bone formation (Chakkalakal, 2005; Maurel et al., 2012). Alcohol also enhances adipogenic activity in the bone marrow and in the cortical bone microvessels (Maurel et al., 2014; Maurel, Pallu, et al., 2012; Wezeman & Gong, 2001). Marrow MSCs are multipotent and capable of differentiation into osteoblasts as well as adipocytes. Studies on MSCs have suggested that signaling pathways regulating adipogenic and osteogenic differentiations of MSCs may exist a potentially inverse relationship, such that cell signaling promotes adipogenesis at the expense of osteogenesis and *vice versa* (Yuan et al., 2016). Peroxisome proliferator-activated receptor γ (PPARγ) is a master transcriptional regulator of adipocyte differentiation, which inhibits osteoblast differentiation. While bone morphogenetic protein (BMP) and Wnt signals promote osteogenic differentiation, which inhibit the function of PPARγ transactivation during MSC differentiation toward adipocytes. *In vitro* culture of human MSCs with alcohol at 50 mM promotes their differentiation toward adipocytes (Wezeman & Gong, 2004). Alcohol up-regulates PPARγ2 at the point of MSC lineage commitment and increases adipocyte P2 synthesis for cell lipid transport as well as storage. It has been reported that mice chronically fed on diet containing high dose of alcohol, bone marrow MSC osteogenic differentiation is reduced with the enhancement of adipogenic differentiation, leading to osteopenia in these animals (Liu et al., 2016). This biased osteo/adipogenic lineage differentiation of marrow MSCs is correlated to the activation of phosphatidylinositide 3-kinase/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling, resulting in downregulation of runt-related transcription factor 2 and upregulation of PPARγ *via* activation of p70 ribosomal protein S6 kinase. Transient transfection of human bone marrow MSCs with small interfering RNAs (siRNA) targeting PPARγ in the culture system containing 50 mM alcohol significantly represses the adipogenic effect of alcohol on MSCs as reflected by lower adipocyte number, increased matrix mineralization, reduced adipogenic gene markers, up-regulated osteogenic gene marker, and enhanced bone matrix protein synthesis (Huang et al., 2010). Simultaneous downregulation of PPARγ and upregulation of calcitonin gene-related peptide, a neuropeptide gene closely associated with bone regeneration, can also efficiently suppress adipogenic differentiation of marrow MSCs and promoting their osteogenic differentiation (Li, Wang, Li, Sun, & Zhao, 2014). In addition, *in vivo* blockage of the mTOR pathway by rapamycin treatment has been shown to ameliorate alcohol-induced osteopenia by rescuing impaired osteo/adipogenic lineage differentiation of marrow MSCs (Liu, Kou, et al., 2016).

Alcohol exposure not only changes the cell corporation of hematopoietic niche, but alters the cue of marrow niche environment as well. Marrow niche cells express a variety of cell membrane bound molecules as well as soluble factors to regulate hematopoietic precursor cell activities by providing either stimulatory or inhibitory signals. These cell

membrane bound and soluble mediators including ligands for receptors associated with Wnt and hedgehog signaling pathways, stem cell factor (SCF, c-kit ligand, or steel factor), transforming growth factor-β (TGF-β), granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), as well as CXCL12 (stromal cell-derived factor 1 or SDF-1). The complexity of niche signaling regulation is denoted by the feature that a specific signaling molecule can be expressed by a wide range of niche cell types at different extent. Similarly, each niche cell type can express different signaling molecules either simultaneously or dynamically. Currently, knowledge about the integrated niche regulation of granulopoiesis under the homeostatic condition remains insufficient. Information regarding the effects of excessive alcohol consumption on the niche signaling regulation of granulopoiesis remains limited and superficial. It has been reported that cell-free conditioned media from the culture of normal human T cells exposed to ethanol at the concentration >100 mg% exhibit a significant reduction in capacity to stimulate proliferation of CFU-GM in human or rat bone marrow cells (Imperia et al., 1984). In a nonhuman primate model of ethanol self-administration for 12 months, it has been observed that ethanol exposure inhibits activation-induced production of G-CSF along with ethanol-dependent upregulation of distinct microRNAs (miR-181a and miR-221) in peripheral blood mononuclear cells (PBMCs) (Asquith et al., 2014). Alcohol-induced up-regulation of these microRNAs appears being involved in mediating the reduction of G-CSF production by PBMCs through inhibiting the expression of transcription factors signal transducer and activator of transcription 3 (STAT3) as well as aryl hydrocarbon receptor nuclear translocator (ARNT). Further investigations on the effects of alcohol on the marrow niche signaling regulation of HSPC function will provide critical insight into mechanisms underlying the disruption of granulopoiesis under homeostatic conditions in hosts who excessively consume alcohol.

3.2. Impediment of marrow capacity for storage of granulocytes

Alcohol negatively affects the marrow capacity of storing mature granulocytes, which may serve as another mechanism underlying the development of hypocellularity with the loss of mature granulocytes in the bone marrow in alcohol abusers (Ballard, 1980; Liu, 1980). In this respect, the specific pattern of chronic plus binge alcohol consumption appears to cause the most striking disturbance. Alcoholics with recent excessive drinking have significantly higher levels of circulating neutrophils compared to healthy controls or alcoholics without recent drinking (Li et al., 2017). Experimental studies on mice with chronic plus binge alcohol administration have also shown substantial increases in the number of circulating granulocytes 9–24 h after the binge intoxication (Li et al., 2017; Shi, Lin, et al., 2017). This chronic plus binge alcohol administration induced elevation of circulating granulocyte levels include increases in both percentage of neutrophils in white blood cells and absolute counts of neutrophils in the systemic circulation (Shi, Wei, Gao, & Zhang, 2017). Nevertheless, chronic feeding with alcohol diet for 5 weeks alone does not alter the granulocyte level in the blood. Binge alcohol intoxication alone only causes a moderately increase in the granulocyte level in the circulation. Analysis of the bone marrow has revealed that chronic plus binge alcohol administration substantially reduces the marrow storage pool of granulocytes without any significant change in hematopoietic stem/progenitor cell populations. These observations indicate that excessive alcohol consumption, particularly chronic plus binge drinking, reduces the marrow storage capacity for granulocytes by promoting release of them into the systemic circulation.

At the present time, knowledge about mechanisms by which excessive alcohol consumption reduces marrow storage pool of granulocytes and increases granulocyte release from the bone marrow into the circulation remains limited. Chronic plus binge alcohol exposure causes exacerbated tissue injury in the liver (and likely in other organ systems) with a strong inflammatory reaction (Bertola, Park, & Gao, 2013; Li et al., 2017; Maricic et al., 2015; Xu et al., 2015). The generated proinflammatory cytokines and chemokines apparently promote marrow release of granulocytes into the circulation as well as infiltration of granulocytes into tissue sites of inflammation. In addition to the effects on generating inflammatory mediators, alcohol exposure also exerts an impact on activities of granulocytes and other nucleated cell types either in the circulation or in the bone marrow. Studies have shown that feeding with liquid alcohol diet for 5 weeks alone is able to increase caspase-1 activation in blood neutrophils in mice (Shi, Wei, et al., 2017). This increase in caspase-1 activation is further enhanced in mice with chronic-plus-binge alcohol administration. Both chronic alcohol diet feeding alone and chronic-plus-binge alcohol administration cause a significant increase in caspase-1 activation in neutrophils in the bone marrow. In addition, either chronic alcohol diet feeding alone or chronic-plus-binge alcohol administration causes a significant increase in caspase-1 activation in non-neutrophilic cell types in both the systemic circulation and the bone marrow. Alcohol-induced inflammatory reaction and activation of the caspase-1 inflammasome pathway in marrow cells likely participate in mediating the release of granulocytes from marrow storage pool into the circulation. In the bone marrow, immature myeloid cells bind to the stromal network through interactions between oligosaccharides and lectins as well as between adhesion molecules. During the maturation process, cell surface molecules are altered and the ability for multivalent binding to stroma components or cells reduces gradually. Mobilization of marrow cells involves the remodeling of marrow matrix and the basement membranes of the bone marrow sinuses by activities of marrow proteases, particularly matrix metalloproteinases (MMPs) (Pelus, Bian, King, & Fukuda, 2004; Starckx, Van den Steen, Wuyts, Van Damme, & Opdenakker, 2002; Yu & Han, 2006). Mature granulocytes can secrete considerable amounts of latent MMPs, including neutrophil procollagenase (pro-matrix metalloproteinase-8 or proMMP-8) and progelatinase B (pro-matrix metalloproteinase-9 or proMMP-9) (Opdenakker, 2001). Activation of these MMPs is achieved *via* proteolysis, induced by extracellular proteinases including plasmin and urokinase (Yu & Han, 2006). Activated MMPs can further activate other pro-MMPs, constituting a feedforward mechanism in the loop of activation. Proinflammatory cytokines interleukin-1β (IL-1β) and IL-18 produced through activation of the caspase-1 inflammasome pathway may mediate the local inflammatory reaction (Jha, Brickey, & Ting, 2017; Kopitar-Jerala, 2017), which would activate related extracellular proteinases to facilitate the activation of MMPs *via* proteolysis in the marrow hematopoietic environment (Borkowska et al., 2014; Del Rosso et al., 2011; Del Rosso, Fibbi, Pucci, Margheri, & Serrati, 2008; Syrovets, Lunov, & Simmet, 2012). Further investigations on alcohol-induced inflammatory reaction in relation to the functional alteration of molecules regulating retention of granulocytes in the marrow storage pool will improve understanding about mechanisms underlying marrow release of granulocytes following excessive alcohol exposure, particularly following chronic plus acute alcohol consumption. The adverse effects of alcohol on homeostasis of granulopoiesis and marrow storage of granulocytes are summarized in Fig. 2.

4. Alcohol impairs the granulopoietic response

In response to infection, particular acute bacterial infection, the bone marrow can quickly release a large amount of granulocytes from its storage pool into the systemic circulation. In the meantime, activation of HSPCs occurs to promote granulocyte generation in order to reinforce host defense for eliminating invading pathogens. Any defects in this process will compromise host immunity. Alcohol abuse, especially acute intoxication or chronic plus binge drinking, suppresses the granulopoietic response. Clinical investigations have shown that the occurrence of serious infection in alcoholic patients is frequently preceded by an episode of very heavy drinking (Dorff et al., 1973). Bone marrow analysis of alcoholic patients during the neutropenic stage has demonstrated that virtually none of the neutrophil precursors has matured

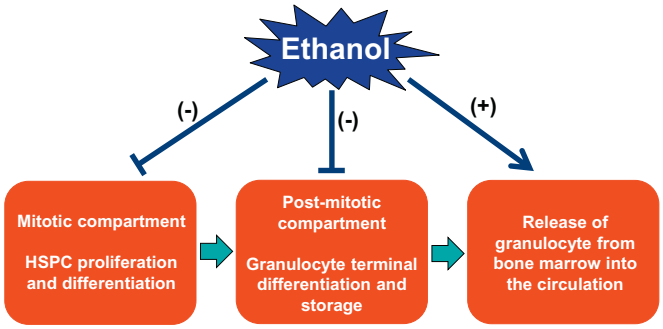

Fig. 2. The adverse effects of alcohol on homeostasis of granulopoiesis and marrow storage of granulocytes.

beyond an early developmental stage (Ballard, 1997). Further, the neutrophil storage in the bone marrow is depleted more rapidly in active alcoholics than in healthy control subjects. Activation of HSPC proliferation and transcriptional reprograming of primitive hematopoietic precursor cells for enhancing their commitment toward granulocytic lineage development are critically important in the granulopoietic response (Shi et al., 2013, 2018; Zhang, Bagby, et al., 2008; Zhang, Nelson, et al., 2008). Alcohol intoxication disrupts several key signaling mechanisms regulating the activation of HSPCs during the granulopoietic response.

4.1. Inhibition of HSPC proliferative activation

Because in the undifferentiated status, HSCs commonly do not share the same profile of signaling mechanisms with the downstream fully differentiated cell types for the regulation of their functional activities. However, primitive hematopoietic precursor cells express pattern recognition receptors and receptors for most proximal proinflammatory cytokines (Du et al., 2012; McKinstry et al., 1997; Nagai et al., 2006; Sawamiphak, Kontarakis, & Stainier, 2014; Schuettpelz et al., 2014; Takizawa et al., 2017). Therefore, they are able to respond to ligand stimulations including those mediated by pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs), and certain types of cytokines. In mice, challenge with gram-negative bacteria, gram-positive bacteria, or fungal pathogens, causes activation of HSPCs through various toll-like receptor (TLR)-associated signaling cascades (Raasch et al., 2010; Shi et al., 2013; Shi, Zhang, Sempowski, & Shellito, 2011). Lipopolysaccharide (LPS), the cell wall component of gram-negative bacteria, is a potent PAMP ligand. Binding of LPS to TLR4 activates the p44/42 mitogen-activated protein kinase (MAPK) or extracellular-signal-regulated kinase1/2 (ERK1/2) pathway through activation of the Ras-c-Raf-mitogen-activated protein kinase ERK kinase 1/2 (MEK1/2) cascades (Guha et al., 2001; Guha & Mackman, 2001). Phosphorylation of p44/42 leads to its nuclear translocation where it activates several ERK targets (including transcription factors such as Elk-1) and induces expression of the first class of G1 cyclins, cyclin D. Up-regulation of cyclin D-cyclin-dependent kinase 4/6 (CDK4/6) activity promotes S phase entry during cell cycling (Meloche & Pouysségur, 2007; Torii, Yamamoto, Tsuchiya, & Nishida, 2006). Mice with *Escherichia coli* bacteremia show a rapid expansion of marrow LKS cell population (Shi et al., 2013; Shi, Lin, et al., 2017; Zhang et al., 2009; Zhang, Bagby, et al., 2008; Zhang, Nelson, et al., 2008). *E. coli* infection induces activation of p44/42 MAPK and up-regulation of cyclin D1 expression in marrow cells (Shi, Lin, et al., 2017), which are accompanied with enhancement of LKS cell proliferation as reflected by their increase in BrdU incorporation (Shi, Lin, et al., 2017; Zhang et al., 2009; Zhang, Bagby, et al., 2008; Zhang, Nelson, et al., 2008). TLR4 gene knockout impaired the increase in BrdU incorporation into marrow LKS cells during *E. coli* infection (Shi et al., 2013). *In vitro* stimulation of isolated HSPCs with LPS also activates their proliferation in the culture system (Shi et al., 2013; Shi, Lin, et al., 2017). This TLR4-p44/42 MAPK-cyclin D1 signaling mediated activation of cell proliferation plays an important role in the expansion of marrow LKS cell pool during the granulopoietic response. Acute alcohol intoxication or chronic plus binge alcohol administration inhibits the activation of TLR4-p44/42 MAPK-cyclin D1 signaling and suppresses proliferative activation of LKS cells in the bone marrow (Shi, Lin, et al., 2017; Zhang et al., 2009). It is well known that activation of the TLR4-p38 MAPK-nuclear factor-κB (NF-κB) pathway mediates cell expression of proximal proinflammatory cytokine tumor necrosis factor-α (TNF-α) (Hochdörfer et al., 2013; Togbe et al., 2007). Mice with *E. coli* bacteremia show a marked early increase in TNF-α level in the systemic circulation (Zhang et al., 2009; Zhang, Bagby, et al., 2008; Zhang, Nelson, et al., 2008). TNF-α also activates p44/42 MAPK signaling cascades (Sabio & Davis, 2014; Winston, Lange-Carter, Gardner, Johnson, & Riches, 1995; Winston, Remigio, & Riches, 1995). Intravenous challenge with recombinant TNF-α evokes expansion of marrow LKS cell population in mice (Zhang et al., 2009). Alcohol intoxication suppresses both tissue production of TNF-α in response to bacterial infection (Zhang et al., 2009 PMID: 19630706) and TNF-α-induced activation of LKS cells during the granulopoietic response (Zhang et al., 2009). Activation of the TLR4-p44/42 MAPK-cyclin D1 pathway not only promotes proliferation of HSCs, but enhances cell cycling in myeloid progenitor and granulocytic progenitor cells as well (Melvan et al., 2012; Shi, Lin, et al., 2017). Both acute alcohol intoxication and chronic plus binge alcohol administration suppress this signaling regulation in enhancement of proliferation in myeloid/granulocytic lineage-committed progenitor cells during the granulopoietic response to bacterial infection.

4.2. Obstruction of HSPC phenotypic conversion and reprogramming

Recent studies on the HSPC response to bacterial infection in murine models have revealed another crucial process, termed "phenotypic conversion," for the rapid expansion of marrow LKS cell pool. In this process, downstream myeloid progenitor cells baring lin$^-$c-kit$^+$Sca-1$^-$ phenotypic marker re-express Sca-1 to convert themselves back to the upstream LKS (lin$^-$c-kit$^+$Sca-1$^+$) phenotype (Raasch et al., 2010; Shi et al., 2013; Shi, Lin, et al., 2017; Zhang et al., 2009; Zhang, Bagby, et al., 2008; Zhang, Nelson, et al., 2008). Approximately 70–85% of total LKS cells in the expanded marrow LKS cell pool are generated through this phenotypic conversion pathway during the early stage of bacterial infection. At the present time, it remains unclear if LKS cells generated from the phenotypic conversion would regain the same capacity of multipotency for development of all blood cell lineages as the true LKS cells under the homeostatic condition. Regardless, the expanded population of LKS cells evidently serves as a platform for HSPC reprograming to enhance their commitment toward granulocytic lineage development during the granulopoietic response. A unique feature in the activation of HSPCs is the marked upregulation of Sca-1 expression by these precursor cells at both the transcriptional and protein levels (Melvan et al., 2011; Shi et al., 2013). Sca-1 is an 18-kDa mouse glycosyl phosphatidylinositol (GPI)-anchored cell surface protein of the Ly6 gene family, which plays a pivotal role in signaling stem/progenitor cell functional activities (Bradfute, Graubert, & Goodell, 2005; Ito, Li, Bernstein, Dick, & Stanford, 2003). Since lacking either an identified ligand or directly associated intracellular signaling pathway, Sca-1 most likely acts as a co-regulator of lipid raft signal to mediate changes in stem/progenitor cell functional activities. Sca-1 has been proposed to alter lipid raft composition *via* weak protein-protein interactions, sequestering or obstructing key signaling molecules in the vicinity of their receptors or even promoting raft clustering (Holmes & Stanford, 2007). Sca-1 may play a role in several lipid raft-mediated signaling pathways, including those involving receptor tyrosine kinases and Src family kinases (Stefanová, Horejsí, Ansotegui, Knapp, & Stockinger, 1991). Sca-1 and lipid rafts also have close associations with c-kit signaling in HSPCs (Bradfute et al., 2005; Ito et al., 2003). In mice with

systemic *E. coli* infection, the upregulation of Sca-1 expression is in correlation with the activation of p44/42 MAPK signaling and enhancement of proliferation in HSPCs (Melvan et al., 2011, 2012). Transcriptional activation of genes for granulocytic lineage development, such as CCAAT enhancer-binding protein-β (CEBP–β), Spi-1 proto-oncogene (Spi-1 or PU.1), and granulocyte colony-stimulating factor receptor (G-CSFR) occurs in parallel with the increase in Sca-1 expression in HSPCs during the granulopoietic response (Shi et al., 2013; Shi, Lin, et al., 2017). Further, antibody cross-linking Sca-1expressed on hematopoietic precursor cells in response to LPS stimulation in the *in vitro* culture system not only generates a feedforward signal for enhancing Sca-1itself expression, but also upregulates PU.1 expression by these cells (Shi et al., 2013). HSPCs from mice with Sca-1 gene knockout exhibit a decrease in granulocyte colony-forming activity (Ito et al., 2003). Mice with Sca-1 knockout also show impairment of the granulopoietic response to serious bacterial infection (Melvan et al., 2011, 2012; Shi et al., 2013).

Alcohol intoxication suppresses the upregulation of Sca-1 expression by HSPCs during the granulopoietic response (Shi, Lin, et al., 2017; Zhang et al., 2009). The increase in Sca-1 expression is primarily regulated at the transcriptional level. The promoter region of Sca-1 gene contains multiple binding sites for activating protein-1 (AP-1) transcription factor (Melvan et al., 2011). C-Jun is the most potent transcriptional activator in the AP-1 family (Shaulian & Karin, 2002). Activation of either TLR4 or TNF receptor (TNFR) following ligand (such as LPS or TNF-α) engagement actives c-Jun N-terminal kinases (JNKs) and subsequently enhances the transcriptional activity of c-Jun by phosphorylation of its N-terminal activation domain (Khan et al., 2017; Medvedev et al., 2007; Reinhard, Shamoon, Shyamala, & Williams, 1997; 2007; Song, Régnier, Kirschning, Goeddel, & Rothe, 1997). Systemic bacterial infection in murine models quickly induces JNK activation in bone marrow cells (Melvan et al., 2011). Inhibition of JNK activation with specific JNK inhibitor suppresses up-regulation of Sca-1 expression by cultured hematopoietic precursor cells in response to LPS or TNF-α (Shi, Lin, et al., 2017; Zhang et al., 2009). Alcohol intoxication inhibits JNK activation in marrow cells in response to *E. coli* bacteremia (Melvan et al., 2011), which may serve as the mechanism underlying alcohol-induced suppression of Sca-1 upregulation in HSPCs. Alcohol-induced inhibition of Sca-1 signaling couples with impairment of proliferative activation in HSPCs and disruption of HSPC reprograming for enhancing commitment toward granulocytic lineage development (Melvan et al., 2011, 2012; Raasch et al., 2010; Shi et al., 2011; Shi, Lin, et al., 2017; Zhang et al., 2009). Consequently, the granulopoietic response to bacterial infection is impaired in experimental animals with excessive alcohol exposure. In this context, the chronic plus binge alcohol consumption pattern apparently exerts the most destructive effect on granulocyte-mediated immune defense in hosts. This typical pattern of alcohol consumption exhausts the marrow reserve of mature granulocytes by inducing granulocyte release from the marrow storage pool into the circulation in the homeostatic state. When host confronts the challenge of serious infection, it suppresses the granulopoietic response rendering the host incapable of reinforcing the phagocytic defense against invading pathogens.

4.3. Disruption of major granulopoietic signals

G-CSF is a lineage-specific growth factor stimulating granulopoiesis (Dale, Liles, Summer, & Nelson, 1995). During bacterial infection, tissue cells at sites of infection and inflammation produce G-CSF, leading to the significant increase in G-CSF level in the circulation (Kragsbjerg, Jones, Vikerfors, & Holmberg, 1995; Pauksen, Elfman, Ulfgren, & Venge, 1994; Quinton et al., 2002; Shahbazian et al., 2004). This increase in G-CSF level promotes the granulopoietic response in the bone marrow and enhances mobilization of granulocytes into the circulation (Dale et al., 1995; Semerad, Liu, Gregory, Stumpf, & Link, 2002; Zhang et al., 2005). Binding of G-CSF to its receptor activates the p44/42 MAPK-cyclin D signaling pathway, which mediates activation of HSPC proliferation in both homeostatic and emergency conditions (Marino & Roguin, 2008). Engagement of G-CSF with G-CSFR also triggers activation of the STAT3 signaling cascades (Marino & Roguin, 2008; Zhang et al., 2010). STAT3 directly controls G-CSF-dependent expression of C/EBPβ (Zhang et al., 2010). Both STAT3 and C/EBPβ regulate c-Myc oncogene through interactions with the c-myc promoter during demand-driven granulopoiesis. C-Myc transcription has been identified as a normal event in granulopoiesis linked to proliferative activity as well as to the primitive stage in development (Gowda, Koler, & Bagby Jr, 1986). Activation of the STAT3 pathway also mediates expression of the CDK inhibitor $p27^{Kip1}$ which causes G1 cell cycle arrest to turn into terminally granulocytic differentiation (de Koning et al., 2000; Mangan & Reddy, 2005; McArthur et al., 2002; Rane et al., 2002). Alcohol intoxication disrupts G-CSFR signaling in the regulation of the granulopoietic response at different steps. Alcohol suppresses G-CSF production by tissue cells in response to infectious challenges (Bagby, Zhang, Stoltz, & Nelson, 1998), reducing ligand stimulation for the initial activation of G-CSFR. Further, alcohol exposure significantly increases STAT3 phosphorylation in marrow cells of experimental animals with bacterial infection and enhances G-CSF-induced activation of the STAT3-$p27^{Kip1}$ pathway in cultured murine myeloid progenitor cell line 32D-G-CSFR cells (Siggins et al., 2011). These alcohol-induced disruptions of G-CSFR signaling have been shown to accompany the inhibition of HSPC proliferative activation and marrow production of granulocytes in response to bacterial infection (Bagby et al., 1998; Siggins et al., 2011). Because of impairing host immune defense, alcohol administration causes an increase in bacterial loads in the systemic circulation and vital organ tissues in animal models of bacterial infection (Bagby et al., 1998; Zhang et al., 2009). The infection-associated mortality rates are also significantly increased in animals with alcohol intoxication. Fig. 3 shows the negative effects of alcohol on activation of HSPCs during the granulopoietic response in murine models.

Recent studies have shown that other signaling mechanisms, including the sonic hedgehog (SHH)-glioma-associated oncogene 1 (Gli1) (Shi et al., 2018), TLR4-toll-like receptor adaptor molecule 1 (TRIF)-ROS-p38 MAPK (Takizawa et al., 2017), and granulocyte-macrophage colony-stimulating factor receptor (GM-CSFR)-STAT5A/B (Kimura et al., 2009) signaling cascades are involved in the regulation of granulopoiesis under homeostatic conditions and during the granulopoietic response to stressful challenges. Further investigation on the potential interplays between alcohol and these cell signaling pathways will provide deeper insight into mechanisms underlying alcohol-induced impairment of marrow granulocyte production for maintaining and/or reinforcing host phagocytic defense. It is worth noting that most of

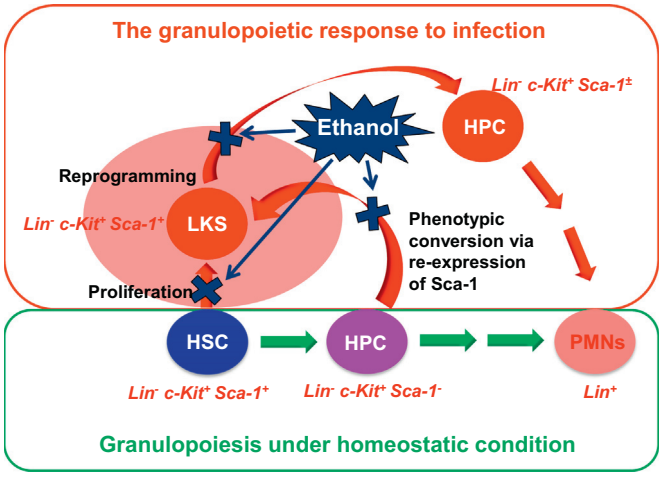

Fig. 3. The negative effects of alcohol on activation of HSPCs during the granulopoietic response in murine models.

studies on alcohol-induced disruption of cell signaling regulation in the granulopoietic response are conducted on animal models thus far, particular in murine models. As described previously, human HSPCs do not share the same set of surface markers with those from the murine origin. Accordingly, signaling regulations in human HSPCs may not be identical to those observed from studies on experimental animals. Further efforts in verification of signaling mechanisms underlying the regulation of functional activities in human HSPCs will be helpful for developing targeted therapy to effectively treat impairment of the granulopoietic response in alcohol abusers.

5. Progress in therapeutic approaches

5.1. Treatment of alcohol addiction and abuse

Generally, alcohol-induced injury to marrow granulocytic lineage in adults is reversible (Michot & Gut, 1987; Nakao et al., 1991). Both in the absence and presence of infection, the reduction of marrow cellularity and impairment of granulocytic lineage development in alcohol abusers can usually return to normal following a short period of abstinence. Granulocytopenia in alcoholic patients with bacterial infection can be transient with a possible "rebound" granulocytosis between 5 and 10 days of hospitalization, if the patient survives (Ballard, 1997; Eichner, 1973; Lindenbaum, 1987). The cytoplasm vacuoles in myeloid precursors disappear following abstinence for 6 to 10 days (Liu, 1973). The marrow granulocyte reserve is also able to return to the normal level following two weeks of abstinence. Along with the injury to granulopoiesis, however, alcohol abuse also causes abnormalities in erythropoiesis and thrombocytopoiesis, leading to pancytopenia in the peripheral circulation (Ballard, 1997; Eichner, 1973; Latvala et al., 2004). There is a concern that long term heavy alcohol consumption may increase the risk of developing myelodysplastic syndrome (MDS), a heterogeneous group of myeloid disorders leading to dysplasia and ineffective hematopoiesis with the characterization of peripheral blood cytopenias (Montalban-Bravo & Garcia-Manero, 2018). Some patients with MDS may even have a transformation into acute myeloid leukemia (AML). Currently, clinical reports about this risk remain highly controversial (Dalamaga, Petridou, Cook, & Trichopoulos, 2002; Du, Fryzek, Sekeres, & Taioli, 2010; Jin et al., 2014; Liu, Holman, Jin, & Zhang, 2016; Pekmezovic et al., 2006; Strom, Gu, Gruschkus, Pierce, & Estey, 2005; Strom, Vélez-Bravo, & Estey, 2008; Ugai et al., 2017). Several sets of case-control studies have shown that alcohol consumption is a risk factor for MDS in adults (Avgerinou et al., 2017; Pekmezovic et al., 2006). Further, a meta-analysis has suggested that alcohol intake may increase the risk of MDS in a dose-dependent manner (Jin et al., 2014). A stronger association of alcohol with MDS has been detected in individuals who consume ≥10 g/day. In contrast, other case-control studies and meta-analyses have failed to identify any role of alcohol intake alone in MDS etiology (Dalamaga et al., 2002; Du et al., 2010). Also, some case-control and cohort studies have reported that alcohol consumption is associated with a decrease in the risk of MDS (Liu, Holman, et al., 2016; Strom et al., 2005; Ugai et al., 2017). These inconsistencies are most likely resulted from various populations of individuals with different drinking patterns and durations included in each study, which suggests the necessity for further strengthening the quality control of investigations on the long-term effect of heavy alcohol consumption on the development of myelodysplastic disorders. Acetaldehyde metabolites generated from alcohol consumption contribute to formation of various DNA adducts, some of which have been demonstrated to be carcinogenic (Yu et al., 2010). As described previously, certain ALDH isoforms may metabolize reactive aldehydes in HSCs. Loss of these ALDHs leads to perturbation of many cell processes which may predispose HSCs to disorders in growth and leukemic transformation. It has been postulated that polymorphisms in genes responsible for metabolizing reactive aldehydes and repairing their DNA adducts in HSPCs may predispose to the development of acute leukemia in heavy alcohol users (Smith et al., 2015). Currently, regardless, reports about the risk of excessive alcohol consumption in developing acute myeloid leukemia remain inconsistent. A recently completed systematic review with meta-analysis of 18 investigations, including 10 case-control and 8 cohort studies carried out in a total of 7142 leukemia cases from America, Europe, and Asia, respectively, fails in defining an increased risk of leukemia among alcohol drinkers (Rota et al., 2014).

Since alcohol-induced disorders of granulopoiesis are generally reversible, application of the optimal therapy including the selected or combined pharmacological treatments with psychosocial as well as psychotherapeutic approaches to treat alcohol addiction and abuse should be the primary choice for treatment (Johnson, 2010). Successful maintenance of long-term abstinence will not only allow recovery of marrow injury caused by previous alcohol exposure, but will be helpful for limiting the potential risk for developing MDS as well as other types of myeloid malignances.

5.2. Treatment with G-CSF

Excessive alcohol consumption predisposes the host to develop serious bacterial infection, particularly septic infection. Treatment of alcoholic patients who have septic infection in the presence of granulocytopenia remains a major challenge. Standard treatment of these patients consists of antimicrobial chemotherapy, vitamin supplement, and intensive care support. Even with the standard treatment, the mortality rate remains exceedingly high. Efforts of stimulating marrow granulopoietic activity with G-CSF have been deployed in the treatment of serious infection in hosts with excessive alcohol exposure. In experimental studies, administration of G-CSF for 2 days prior to alcohol intoxication and pulmonary infection with *Klebsiella pneumoniae* has been shown to significantly attenuate alcohol-induced inhibition of granulocyte recruitment into the infected lung (Nelson et al., 1991) G-CSF enhances bactericidal activity in the lung and substantially improves survival of alcohol intoxicated animals following pulmonary infection. In a rabbit model of gram-negative sepsis complicated by leukopenia, administration of G- CSF in combination with penicillin G at 24 h post the infection has been reported to significantly increase the level of circulating leukocytes and improve survival over treating with antibiotics alone (Smith, Sumnicht, Sharpe, Samuelson, & Millard, 1995). The application of G-CSF for treatment of infectious diseases has been approached cautiously because G-CSF promotes both production of granulocytes and functional activities of these immune effector cells. The concern exists about if granulocytes might non-selectively amplify the body's inflammatory response leading to inadvertent tissue injury (Morstyn, Foote, & Nelson, 1997). In a swine model of trauma plus septic infection, intravenous G-CSF administration at the time of resuscitation has been shown to improve survival without potentiating PMN-mediated lung reperfusion injury (Patton Jr et al., 1998). A clinical trial of 756 patients with community-acquired pneumonia has also shown that administration of G-CSF (300 μg/day) to patients for up to 10 days causes a 3-fold increase in the number of circulating PMNs (Nelson et al., 1998). G-CSF treatment is well-tolerated by these patients. Patients treated with G-CSF exhibit a faster resolution of X-ray abnormalities and fewer complications including the adult respiratory distress syndrome and disseminated intravascular coagulation. Another extensive analysis of six clinical studies on G-CSF as an adjunct to antibiotics in the treatment of pneumonia in adults involving a total of 2018 people has demonstrated that G-CSF use appears safe with no increase in the incidence of total serious adverse events or organ dysfunction (Cheng, Stephens, & Currie, 2007). These observations suggest that G-CSF may be useful for treatment of serious infections in immunocompromised patients, such as individuals who abuse alcohol. Indeed, one case report has described that receiving 300 microgram G-CSF daily in adjunction to the standard therapy, a 32-year-old woman with alcoholism, leukopenia, and pneumococcal sepsis is able to recover from her leukopenia and septic infection before being discharged in good condition (Grimsley, 1995).

Excessive alcohol consumption frequently causes serious liver diseases including severe alcoholic hepatitis and alcoholic cirrhosis. Severe alcoholic hepatitis is life-threatening with the acute mortality rate around 30%. Corticosteroids are the only therapeutic option for improving short-term survival at the present time (Gustot et al., 2017; Karakike et al., 2017). Corticosteroids have been shown to exert various effects on marrow HSPC activities and the defense function of immune effector cells (Dror, Ward, Touw, & Freedman, 2000; Family et al., 2018; Hernigou & Beaujean, 1997). Advanced liver diseases are also frequently accompanied with granulocytopenia (Jain et al., 2016; Ng et al., 2002; Qamar et al., 2009) and impairment of neutrophil function (Karakike et al., 2017; Rolas et al., 2013). Infectious complications including septic infection occur in approximately 50% of patients with alcoholic liver diseases, which are the main causes of death in these individuals (Bruns et al., 2014; Karakike et al., 2017). Because of the complexity in pathological changes associated with alcohol abuse, liver damage, corticosteroid therapy, granulocytopenia, and serious infection, treatment of these patients requires a specific caution. Administration of G-CSF in adjunction to the standard therapy has been shown to substantially increase the survival of patients with either severe alcoholic hepatitis or alcoholic liver failure (Chavez-Tapia et al., 2015; Garg et al., 2012; Moreau & Rautou, 2014; Singh et al., 2014). G-CSF treatment increases leukocyte and neutrophil counts, improves liver function, as well as reduces the occurrence of septic infection and scores of sequential organ failure (Garg et al., 2012; Moreau & Rautou, 2014). Mechanisms by which G-CSF therapy improves survival in this patient population remain to be clarified. Experimental and clinical studies have observed that G-CSF administration induces release of HSCs from the bone marrow (Garg et al., 2012; Gordon et al., 2006; Liu et al., 2006; Singh et al., 2014; Spahr et al., 2008). One postulation is that the marrow-derived stem cells may promote liver regeneration (Liu et al., 2006; Pai et al., 2008; Spahr et al., 2008; Yannaki et al., 2005). Regardless, G-CSF stimulates granulopoiesis and promotes marrow production of granulocytes (Basu, Dunn, & Ward, 2002; Bugl, Wirths, Müller, Radsak, & Kopp, 2012; Christopher & Link, 2007). G-CSF also enhances functional activities of mature granulocytes (Pitrak, 1997; Rapoport, Abboud, & DiPersio, 1992; Spiekermann, Roesler, Emmendoerffer, Elsner, & Welte, 1997). Obviously, the beneficial effects of G-CSF on marrow granulopoiesis and neutrophil-mediated phagocytic defense against invading pathogens may play an important role in improving survival of patients with advanced alcoholic liver disease in the presence of bacterial infection (Moreau & Rautou, 2014). Further investigation on the interplays between G-CSF administration and corticosteroid treatment in relation to granulopoiesis and functional activities of granulocytes will provide critical information for developing novel therapeutic strategies to effectively treat life-threatening infection in patients with advanced alcoholic liver diseases and bone marrow injury.

6. Conclusive remarks

Excessive alcohol consumption impairs granulocytic lineage development and marrow production of granulocytes. Ethanol and its metabolites induce disorder of granulopoiesis through their toxicity to HSPCs and disruption of hematopoietic niche environment as well as cell signaling regulations. Alcohol-induced defects of granulocytic defense predispose the host to developing infectious diseases, particularly septic infection. Patients with alcoholism, septic infection, and granulocytopenia have an exceedingly high mortality rate. Alcohol abuse injures the liver, which may lead to developing advanced liver diseases. Treatment of alcoholic liver diseases with corticosteroids may further compromise host immune defense. G-CSF stimulates granulopoiesis and enhances functional activities of granulocytes. G-CSF administration in adjunction to standard therapy has been shown to be beneficial in the treatment of alcoholic patient with septic infection and granulocytopenia. G-CSF treatment can also improve the survival rates of patients with advanced alcoholic liver disease in the absence and presence of infectious complications. Further efforts in exploring protection of granulopoietic activity and the granulopoietic response will promote developing novel approaches for effective prevention and treatment of serious infections in alcohol abusers.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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