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Role of hepatic macrophages in alcoholic liver disease

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Abstract

Alcohol consumption can lead to the increase in gut permeability and cause the translocation of bacteria-derived lipopolysaccharides from the gut to the liver, which subsequently activates immune responses. In this process, macrophages play a critical role and involve in the pathogenesis of alcoholic liver disease (ALD). To define the mechanism underpinning the function of macrophages, it is important to conduct extensive studies to further explicate the phenotypic diversity of macrophages in the context of ALD.

In this review, the role of hepatic macrophages in the pathogenesis of ALD is discussed.

INTRODUCTION

The pathogenesis of alcoholic liver disease (ALD) is complex.¹² In addition to the direct cytotoxic and the reactive oxygen species (ROS)-mediated effects of alcohol¹ and its metabolite, acetaldehyde,^{3–5} on hepatocytes, alcohol ingestion can activate the innate and adaptive immune responses,²⁶ which can lead to alcohol-induced liver injury. Alcohol consumption can lead to the increase in gut permeability and cause the translocation of bacteria-derived lipopolysaccharides from the gut to the liver, which subsequently activates immune responses.¹²⁷⁸

Such responses by alcohol involves multiple cell types, including resident macrophages (Kupffer cells, KCs), natural killer cells, natural killer T cells, lymphocytes, neutrophils, and monocytes.⁶ The KC serves as the primary effector cell of the innate immune response within the liver⁹ and plays a key role in the early pathogenesis of alcohol-induced liver injury.^{10–12} As mentioned, the impairment in intestinal permeability by alcohol can lead to endotoxemia.¹²⁷⁸ Endotoxin can bind to CD14 (the KC endotoxin receptor) in association with toll-like receptor 4 to initiate intracellular signaling leading to alcohol-induced liver

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injury.¹¹ Inactivation of KCs by gadolinium chloride ameliorates ALD seen with chronic ethanol administration using the Tsukamoto-French model.¹³ In addition to the KCs, another type of macrophages in the liver, known as infiltrating macrophages (IMs), can be differentiated from the circulating monocytes that are recruited into the liver tissues due to inflammatory reactions during liver injury.⁶

HETEROGENEITY OF MONOCYTES AND MACROPHAGES

Monocytes are circulating innate immune cells originated from the progenitor cells in the bone marrow.¹⁴¹⁵ In humans and mice, monocytes can be divided, depending on the surface marker proteins, into two major subsets—classical and non-classical (see review in ref. 6 and table 1). In humans, all CD115+ monocytes are further divided into two major subsets based on their CD14 and CD16 expression, as well as on their expression of markers called CCR2 and CX3/CR1.⁶ The predominant classical subset, representing 90% of circulating monocytes, is characterized by the marker combination CD14^{hi}CD16⁻ and CCR2 +/CX3CR1^{lo}. The less abundant non-classical monocyte subset can be subdivided into two groups: CD14^{dim}CD16⁺ and CCR2⁻/CX3CR1^{hi}/CCR5^{hi} (non-classical monocytes) or CD14^{hi}CD16⁺ and CCR2⁻/CX3CR1^{hi}/CCR5^{hi} (intermediate monocytes, table 1).

In mouse, the monocyte subsets include classical Ly6C^{hi} monocytes, which are similar to the human CD14^{hi}CD16⁻ monocytes, and non-classical Ly6C^{lo} monocytes, which are analogous to human CD14^{dim}CD16⁺ monocytes.⁶¹⁵ In human and mouse, these monocytes will circulate in the blood vessels until they are recruited to the organs in case of an injury or insult.⁶¹⁵

Macrophages have a unique ability to alter their phenotypes and functions depending on tissue environmental factors, such as the presence of cytokines, growth factors, pathogen-associated molecular pattern molecules, and damage-associated molecular pattern molecules.⁶¹⁵⁻¹⁷ This process leads to two macrophage phenotypes: M1 and M2 macrophages (figure 1). M1, proinflammatory macrophages, help mediate the initial defense against intracellular bacteria and viruses and response to a tissue injury. The M1 macrophages produce proinflammatory and stress mediators and cytokines, such as interleukin (IL)-1, tumor necrosis factor- α (TNF- α), interferon- γ , IL-12, IL-18, nitric oxide, and ROS.⁶¹⁵ Once the infection or injury is controlled, macrophages differentiate into an anti-inflammatory, tissue-restorative phenotype in order to reign in excessive tissue-damaging inflammatory responses.⁶¹⁵¹⁸¹⁹ These cells bear similarities to the alternatively activated macrophages (M2) and help promote the resolution of inflammation as well as tissue repair.⁶¹⁵ The activation of circulating monocytes and accumulation of macrophages in the liver in the pathogenesis of ALD have not been fully elucidated.

ROLE OF HEPATIC MACROPHAGES IN ALD

To investigate the effects of alcohol on hepatic macrophage populations, C57BL/6J mice were fed an ethanol-containing Lieber-DeCarli diet for 4 weeks. Interestingly, we observed a marked increase of IMs in the liver of ethanol-fed compared with pair-fed and naive mice. KCs express a high level of F4/80 and low level of CD11b, and they do not express Ly6C

(F4/80^{hi}CD11b^{low}Ly6C⁻). In contrast, IMs express a low level of F4/80 and high level of CD11b, and they are positive for Ly6C (F4/80^{int}CD11b^{hi}Ly6C⁺). It is important to note that two subsets of IMs with high and low expression levels of Ly6C were detected (Ly6C^{hi} and Ly6C^{low} subsets). The Ly6C^{hi} subset is mainly monocytes with horseshoe-shaped or kidney-shaped nuclei. On the other hand, the Ly6C^{low} population contains ~30% neutrophils.²⁰ It is very interesting that the IMs and KCs have different characteristics. For example, the expression levels of matrix metalloproteinase (MMP), such as MMP9 and MMP12, were increased significantly in IMs from ethanol-fed mice compared with KCs from pair-fed mice. MMP9 expression increased >10-fold in IMs, and MMP12 increased nearly 60-fold in KCs.²⁰ The results suggest that IMs and KCs are involved in matrix remodeling during chronic ethanol exposure. We next examined the genes known to be associated with macrophages polarization.²⁰ The M2 markers *Ym1*, *Fizz1*, and *Mrc1* were induced significantly in IMs. However, *Arg1*, a typical M2 gene, is moderately upregulated in KCs but not in IMs.²⁰ Genes known to be expressed as M1 markers, such as *iNOS*, *IL-12p40*, and *CIITA*, were also upregulated significantly in IMs. Lastly, IMs and KCs from ethanol-fed mice expressed elevated levels of proinflammatory cytokines, such as *TNF- α* , *IL-1 β* , and *IFN- γ* .²⁰ These data demonstrated that IMs express M1 and M2 markers, suggesting that there may be two subsets of IMs. In fact, our report showed the distinctive gene expression profiles between Ly6C^{hi} and Ly6C^{low} IMs from ethanol-fed mice (see ref. 20 for more detail). Ly6C^{low} IMs exhibit an anti-inflammatory and tissue-protective phenotype; in contrast, Ly6C^{hi} IMs exhibit a proinflammatory, tissue-damaging phenotype.

CONCLUSION

Taken together, chronic ethanol feeding induces the recruitment of two subsets of hepatic IMs, which play different or even opposite roles in regulating liver inflammation and repair. These findings may not only increase our understanding of the complex functions of macrophages in the pathogenesis of ALD but also help us to identify future novel therapeutic targets for the treatment of ALD.

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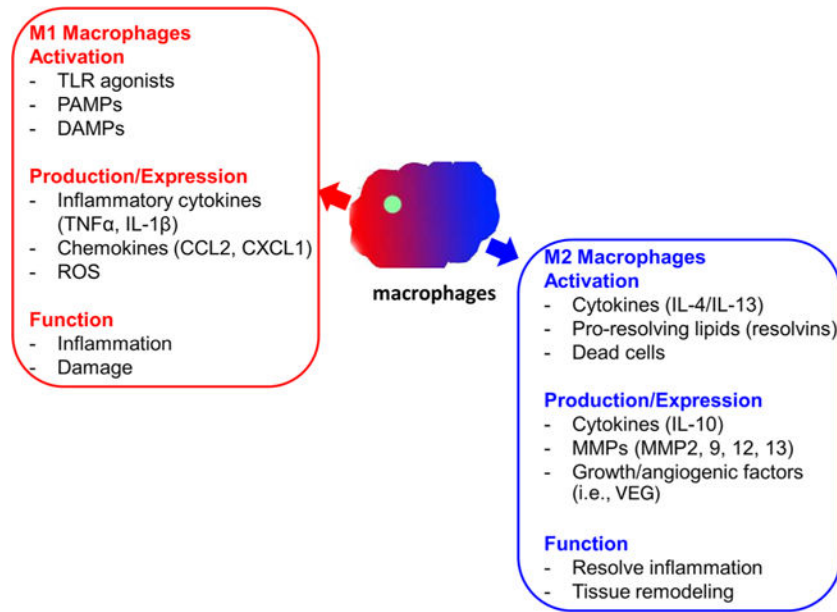


Figure 1. Proinflammatory and tissue-restorative macrophages. DAMP, damage-associated molecular pattern molecule; IL, interleukin; MMP, matrix metalloproteinase; PAMP, pathogen-associated molecular pattern molecule; ROS, reactive oxygen species; TLR, toll-like receptor; TNF- α , tumor necrosis factor-alpha; VEG, vascular endothelial growth factor.

Table 1

Monocyte population of mouse and human as stratified by the protein surface markers (modified from ref. 6)

Species	Subset	Markers	Function
Mouse	Classical	CD11b+ Ly6C ^{hi} CCR2+ CX3CR1-	Inflammatory effectors
	Non-classical	CD11b+ Ly6C ^{lo} CCR2- CX3CR1+	Patrolling, tissue repair
Human	Classical	CD14 ^{hi} CD16- CCR2+ CX3CR1 ^{lo}	Phagocytosis and inflammatory effectors
	Intermediate	CD14 ^{hi} CD16+ CCR2- CX3CR1 ^{hi}	Inflammatory effectors
	Non-classical	CD14 ^{dim} CD16+ CCR2- CX3CR1 ^{hi}	Patrolling, antiviral role

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