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Predominantly Pro-Inflammatory Phenotype with Mixed M1/M2 Polarization of Peripheral Blood Classical Monocytes and Monocyte-Derived Macrophages among Patients with Excessive Ethanol Intake

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Abstract: Excessive alcohol consumption impairs the immune system, induces oxidative stress, and triggers the activation of peripheral blood (PB) monocytes, thereby contributing to alcoholic liver disease (ALD). We analyzed the M1/M2 phenotypes of circulating classical monocytes and macrophage-derived monocytes (MDMs) in excessive alcohol drinkers (EADs). PB samples from 20 EADs and 22 healthy controls were collected for isolation of CD14⁺ monocytes and short-term culture with LPS/IFN γ , IL4/IL13, or without stimulation. These conditions were also used to polarize MDMs into M1, M2, or M0 phenotypes. Cytokine production was assessed in the blood and culture supernatants. M1/M2-related markers were analyzed using mRNA expression and surface marker detection. Additionally, the miRNA profile of CD14⁺ monocytes was analyzed. PB samples from EADs exhibited increased levels of pro-inflammatory cytokines. Following short-term culture, unstimulated blood samples from EADs showed higher levels of soluble TNF- α and IL-8, whereas monocytes expressed increased levels of surface TNF- α and elevated mRNA expression of pro-inflammatory cytokines and inducible nitric oxide synthase. MDMs from EADs showed higher levels of TNF- α and CD206 surface markers and increased IL-10 production. LPS/IFN γ induced higher mRNA expression of Nrf2 only in the controls. miRNA analysis revealed a distinctive miRNA profile that is potentially associated with liver carcinogenesis and ALD through inflammation and oxidative stress. This study confirms the predominantly pro-inflammatory profile of PB monocytes among EADs and suggests immune exhaustion features in MDMs.

Keywords: alcohol use disorders; excessive alcohol drinkers; monocyte/macrophage phenotype; M1; M2; innate immunity; flow cytometry; microRNA

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