Toll-like receptor 2 activation in monocytes of alcohol use disorder patients contributes to systemic inflammation and alcohol-associated liver disease

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Background and Aims: A minority of alcohol use disorder (AUD) patients develops progressive alcohol-associated liver disease (ALD) potentially linked to gut barrier dysfunction, microbial translocation, and activation of systemic immune responses. Activation of circulating monocytes by microbial products might contribute to systemic and liver inflammation leading to ALD progression. Human data linking monocytes to early stages of ALD are lacking. We explored the links between changes in monocytes, microbial translocation, systemic inflammation, and monocyte-derived macrophages in early human ALD.

Method: We included n=123 AUD patients following a highly standardized rehabilitation program and n=26 healthy controls. We determined the total number of monocytes and proportion of monocytes subsets by FACS. Serum microbial translocation markers and cytokines were measured by ELISA and multiplex assay, respectively. Cytokines reflecting activation of monocytes were assessed by qPCR. Toll-like receptor (TLR) expression in monocytes and activation as well as phagocytosis were assessed in vitro. ALD severity and liver inflammatory responses were analyzed in liver biopsies by histology, qPCR, immunohistochemistry, and ELISA.

Results: In AUD patients, the number of blood monocytes increased ($p<0.0001$). Among the 3 monocyte subpopulations, intermediate and non-classical increased while classical monocytes decreased compared to controls. Monocytes from AUD patients up-regulated IL1β and IL8 together with TLR2, down-stream AP-1 and inflammasome NLRP3. IL1β and IL8 were actively secreted by those monocytes upon stimulation in vitro with the TLR2 ligand Peptidoglycan. Stimulation with E. coli confirmed preserved bacterial phagocytic activity. Systemic levels of cytokines and alterations in monocytes correlated with microbial translocation markers.

In parallel, IL1β and IL8 were increased in ALD livers together with activation of intrahepatic macrophages (CD163+, iNOS+, TREM1+). Liver chemokines (MCP1, CX3CL1) involved in monocytes attraction were induced in liver tissue. IL1β and IL8 correlated with liver chemokines, iNOS+ up-regulation in macrophages and ALD severity markers (e.g., fibrosis, AST/ALT, CK18-M65 and M30).

Conclusion: Our results point to a contribution of activated monocytes to systemic and liver inflammation. Monocytes likely infiltrate the liver, transform into monocyte-derived macrophages and release IL1β and IL8 in response to Peptidoglycan and TLR2 activation, ultimately leading to ALD progression.

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