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Can Macrophages in Cirrhotic Ascites Fluid Predict Clinical Outcome in Spontaneous Bacterial Peritonitis?

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Spontaneous bacterial peritonitis (SBP) is an infection that constitutes a major decompensating event in patients with cirrhosis. Nearly 60% of patients with cirrhosis develop ascites within 10 years,[1,2] with SBP occurring in 10%–28% of those patients.[3,4] There is a 70% risk of SBP recurrence within 1 year without antibiotic prophylaxis.[5] Rapid diagnosis and targeted antimicrobial/adjunctive therapy[6] remain the cornerstone of disease management to mitigate high SBP-related mortality.[7] Predictive biomarkers of SBP-related mortality are in development, but little is known about the characteristics of the peritoneal immune reaction to this type of infection in cirrhotic patients. In this issue of *Gastroenterology*, Stengel and Quickert[8] report on a much-needed characterization and functional evaluation of peritoneal macrophages in SBP, with interesting cellular findings that contrast with prior studies. The authors identify a population of large peritoneal macrophages (LPM) in cirrhotic ascites fluid (AF) that may not only drive SBP severity but also contribute a cleaved protein, soluble CD206 (sCD206), the concentrations of which in AF may have future applicability as a prognostic tool.

Numerous prior studies of $CD206^+$ macrophages in the peritoneum or other organ compartments in mice and humans have generated evidence to suggest that macrophages bearing CD206, a scavenger receptor recognizing mannose, *N*-acetylglucosamine and fucose residues on glycoproteins, are typically anti-inflammatory,[9] immunosuppressive,[10,11] or reparative,[12,13] with rare exception.[14] The current study, in contrast, demonstrates that CD206-bearing LPM may be proinflammatory in situations of altered homeostasis, as in decompensated cirrhosis, thereby serving as a novel target for inhibition to abate SBP-related mortality. This study also serves as a cautionary note for investigative drugs[15,16] or cell-based therapies[17] that enrich for $CD206^+$ macrophages to treat hepatic fibrosis, warning that these cells may have plasticity to adopt a proinflammatory profile in decompensated cirrhosis.

Several elements strengthen the authors' central claims. The first relates to their use of a control group. The authors recognize that $CD206^+$ peritoneal macrophages in cirrhotic patients may not represent those found in healthy controls. They therefore use AF LPM from continuous ambulatory peritoneal dialysis (CAPD) patients with end-stage renal disease

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Conflicts of Interest

The author discloses no conflicts.

(without concurrent liver disease) to demonstrate the functional capacity of LPM in cirrhotic AF. Although CAPD patients are not ideal controls like normal, healthy subjects, they are reasonable, with ≥2 advantages: (1) CAPD AF cells can be easily accessed and analyzed and (2) CAPD AF immune profiles may be closer to normal than AF from other control patients, such as those with infections, cardiac disease (which can cause passive hepatopathy), or cancer. In this study, LPM in cirrhotic AF were less abundant than in CAPD AF, yet had higher ex vivo proinflammatory activity. This is a critical finding confirming that cirrhosis is an immune dysregulated state,[18] where excessive inflammation in the peritoneum, as may be promoted by AF LPM, can lead to catastrophic systemic inflammation and multiorgan failure.

The authors next use numerous techniques to prove the proinflammatory nature of LPM, utilizing surface markers, ex vivo transcriptomic analysis in the presence or absence of lipopolysaccharide stimulation, as well as ex vivo cytokine production and response to live *Escherichia coli (E coli)*. Table 1 summarizes key findings pertinent to AF LPM and these data demonstrate that cirrhotic AF LPM have distinct morphometric and cell surface markers. They respond to lipopolysaccharide and *E coli* stimulation with the upregulation of type 1 interferon-related genes and inflammatory metabolic genes. They exhibit evidence of escape from ligand-induced tolerance, and produce a dose-dependent release of sCD206 in response to lipopolysaccharide and *E coli.* AF sCD206 was an independent risk factor for mortality in SBP in the primary cohort after adjusting for age and MELD score. Specifically, concentrations of AF sCD206 of >0.53 mg/L were predictive of a lower 90-day survival, and strongly correlated with laboratory evidence of severe immune activation, particularly increased serum tumor necrosis factor. The breadth of data presented supports the authors' hypothesis that cirrhotic AF $CD206^+$ LPMs are proinflammatory and pathologic in SBP.

Although this study had several strengths, some areas merit further investigation. First, replication of these findings in more varied etiologies of cirrhosis would be optimal, because this study was enriched for males with alcoholic cirrhosis. Second, the authors report that AF resident LPM and infiltrating small peritoneal macrophages express *GATA-6*. Prior studies have reported *GATA-6* expression restricted to tissue or cavity resident macrophages,[19–21] so it was surprising to find expression in the AF small peritoneal macrophages, some of which are likely bone marrow-derived macrophages (given CCR2 positivity). Future studies are warranted to ascertain if *GATA-6* and dependent gene expression from both resident and nonresident macrophages is a phenomenon limited to human peritonitis. Third, despite the abundance of proinflammatory AF LPMs in cirrhotic patients without SBP, consecutive AF samples from SBP patients revealed a depletion of $CD206^+$ LPMs on days 1 and 3 of peritonitis, followed by recovery to baseline after SBP resolution. The authors prove that SBP-induced depletion of LPMs is not due to cell death or egress into the systemic circulation or viscera, particularly because LPMs exhibit poor movement in transwell experiments and few migration markers. However, the unexpected absence of $CD206^+$ LPMs during early SBP correlated with an increase in AF sCD206, suggesting that cleavage of CD206, the salient protein identifying these cells in AF without SBP, prevented their subsequent identification by flow during SBP. The recovery of $CD206^+$ LPMs by day 3 of SBP further suggests that the cells may continue to be present in AF, but need several days to resynthesize and replenish CD206 on their surfaces. Identifying the transcriptomic

signature or alternative markers of AF LPM that shed their surface CD206 during SBP will be needed in future studies.

In all, Stengel and Quickert[8] present compelling data that AF LPMs in cirrhotic patients have an inflammatory phenotype that sheds surface bound CD206 as sCD206 in response to bacterial peritonitis. These data should be reproduced in cohorts of cirrhotic patients with more varied etiologies, as well as in recurrent SBP, but underscore the pathogenic and proinflammatory potential $CD206^{+}$ macrophages in the AF of patients with cirrhosis. AF sCD206 is a novel biomarker with excellent clinical potential to prognosticate mortality risk from SBP. If validated in various ESLD cohorts with primary and recurrent SBP, AF sCD206 concentrations can be used to target high-risk patients for primary or secondary antimicrobial prophylaxis.

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Table 1.

Summary of Characteristic Features of Peritoneal Macrophages in Ascites Fluid

	Cirrhosis			CAPD
	No SPB		SBP	No SBP
	LPM	SPM	LPM	LPM
Cell surface markers	$CD14^+$, $CD16^+$, $CD206^+$, $CD163^+$, $MERTK^+$, $CD40^+$, $CCR2^-$	$CD14^+$, $CD16^+$, $CD206^-$, $CD163^+$, $MERTK^+$, $CCR2^+$,	Low CD206, Low MERTK, normal CD163	$CD14^+$, $CD16^+$, $CD206^+$, $CD163^+$, $MERTK^+$, $CD40^+$, $CCR2^-$
Gene expression in absence of LPS	TCA cycle, gluconeogenesis (*PDK2, PDK3, PDK4*) genes; *VSIG4 (CRIg), CD163, MARCO, MSRI (CD204), GATA-6*	*IL6R, LGALS3, IRAK3, BACH1, GATA-6*		
Gene expression response to LPS	Higher expression of interferon signaling, IL-12 signaling, IL-23 mediated signaling (*IFNB1, OAS1, STAT1*); Lower gene expression of tolerance mediators (*IRAK3, TNF-AIP3*)	IL-1 signaling, TRAIL signaling, TNF receptor signaling (*IL1A, IRAK2, NFKB1, NFKB2*)		
TNF response to escalating doses of LPS	10–100 ng/mL	5–10 ng/mL		3–6 ng/mL

LPM, large peritoneal macrophages; LPS, lipopolysaccharide; SBP, spontaneous bacterial peritonitis; TNF, tumor necrosis factor.