BASIC AND TRANSLATIONAL—LIVER

Abnormal Plasma Microparticles Impair Vasoconstrictor Responses in Patients With Cirrhosis

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BACKGROUND & AIMS: Circulating membrane-shed microparticles (MPs) participate in regulation of vascular tone. We investigated the cellular origins of MPs in plasma from patients with cirrhosis and assessed the contribution of MPs to arterial vasodilation, a mechanism that contributes to portal hypertension. **METHODS:** We analyzed MPs from blood samples of 91 patients with cirrhosis and 30 healthy individuals (controls) using flow cytometry; their effects on the vascular response to vasoconstrictors were examined in vitro and in vivo. **RESULTS:** Circulating levels of leuko-endothelial ($CD31^+/41^-$), pan-leukocyte ($CD11a^+$), lymphocyte ($CD4^+$), and erythrocyte ($CD235a^+$) MPs were higher in patients with cirrhosis than in controls. Plasma of patients with cirrhosis contained hepatocyte-derived MPs (cytokeratin-18^+), whereas plasma from controls did not. The severity of cirrhosis and systemic inflammation were major determinants of the levels of leuko-endothelial and hepatocyte MPs. MPs from patients with advanced cirrhosis significantly impaired contraction of vessels in response to phenylephrine, whereas MPs from healthy controls or from patients of Child–Pugh class A did not. This effect depended on cyclooxygenase type 1 and required phosphatidylserine on the surface of MPs. Intravenous injection of MPs from patients with cirrhosis into BALB/C mice decreased mean arterial blood pressure. **CONCLUSIONS: Cirrhosis is associated with increases in circulating subpopulations of MPs, likely resulting from systemic inflammation and liver cell damage. The overall pool of circulating MPs from patients with advanced cirrhosis impairs vasoconstrictor responses and decreases blood pressure, contributing to the arterial vasodilation associated with portal hypertension.**

Keywords: Microvesicle; COX-1; Hypocontractility; Hepatitis.

In patients with cirrhosis, abnormal persistent vasodilation of arterial vessels leads to increased portal venous inflow and contributes to portal hypertension. Indeed, portal hypertension is determined by both the intrahepatic resistance to portal blood flow, related to liver architecture changes, and the extent of inflow into the portal venous system from the splanchnic bed. Portal hypertension is a major factor in the development of complications of cirrhosis, and because arterial vasodilation associated with portal hypertension is potentially reversible, understanding its mechanisms is of great therapeutic interest.[1]

Vasodilation is associated with both enhanced formation of vasodilators and vascular hyporesponsiveness to vasoconstrictors, also referred as to "vascular hypocontractility." Overproduction of nitric oxide cannot fully account for this effect, and several studies, including those in endothelial nitric oxide synthase (NOS-3) knockout mice, have shown that other factors are involved in the pathogenesis of arterial vasodilation. However, little is known about these factors, and more importantly, the mechanisms leading to vascular hypocontractility have not yet been elucidated.[1]

Microparticles (MPs) are membrane vesicles with a diameter ranging from 0.1 to 1 μm, released in extracellular space following cell activation or apoptosis.[2] MPs harbor at their surface most of the membrane-associated proteins of the cells they stem from and are characterized by the loss of plasma membrane asymmetry resulting in the exposure of phosphatidylserine on their outer leaflet.[2] MPs are present in the blood of healthy subjects, and their levels are increased in pa-

Abbreviations used in this paper: CFSE, carboxyfluorescein succinimidyl ester; COX, cyclooxygenase; DMEM, Dulbecco's modified Eagle medium; HUVEC, human umbilical vein endothelial cell; MELD, Model of End-Stage Liver Disease; MP, microparticle; NOS, nitric oxide synthase.

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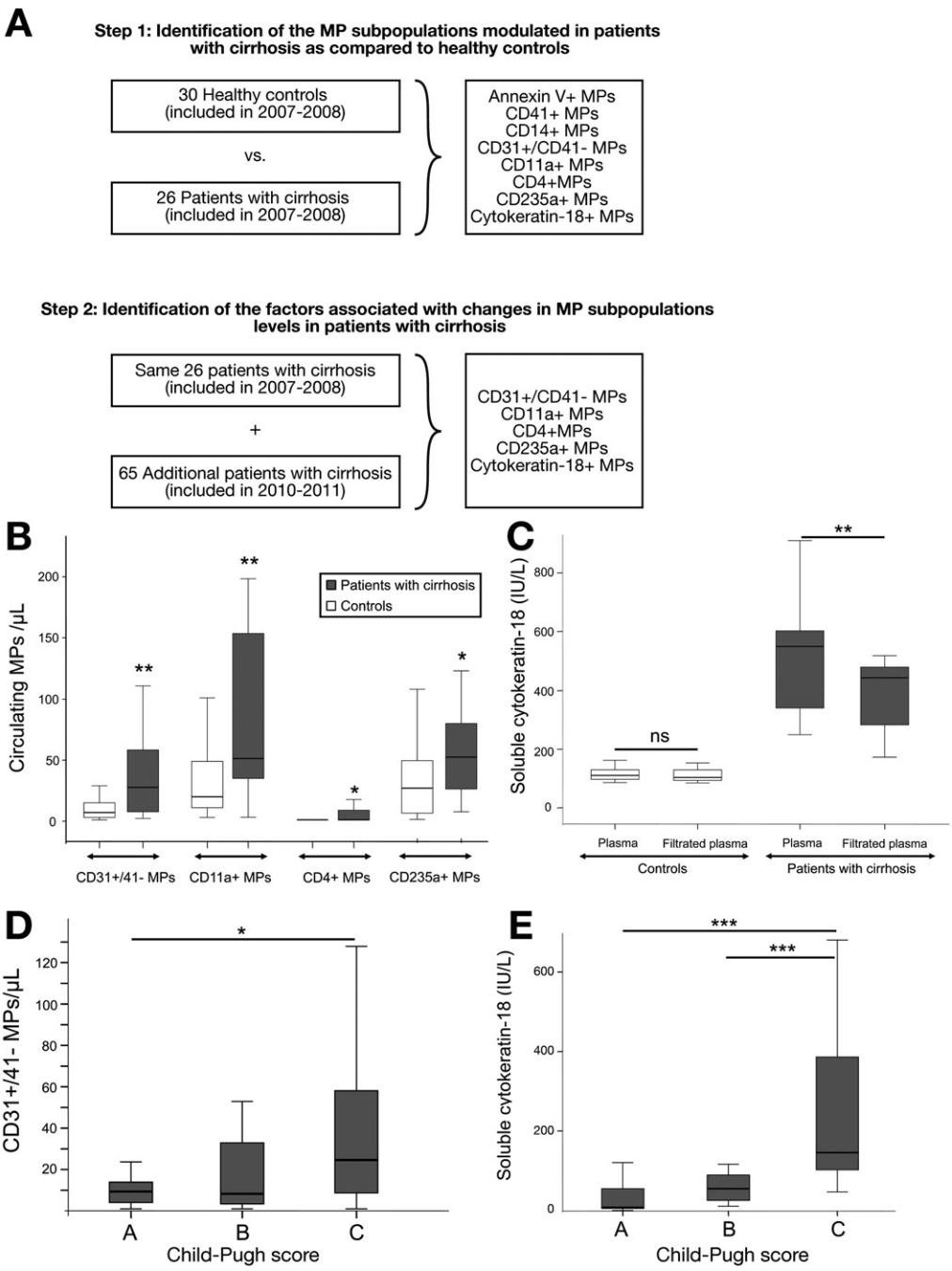

Figure 1. Cellular origin of circulating MPs in patients with cirrhosis and in healthy controls. (*A*) Study flow diagram. (*B*) $CD31^+/41^-$, $CD11a^+$, $CD4^+$, and $CD235a^+$ MPs are of leuko-endothelial, pan-leukocyte, lymphocyte, and erythrocyte origin, respectively (flow cytometry analysis; 26 patients with cirrhosis, 30 healthy controls). (*C*) Soluble native cytokeratin-18 levels (M65 antigen), a marker of hepatocytes, in native and filtrated (to remove MPs) plasmas (enzyme-linked immunosorbent assay; 9 patients with cirrhosis vs 5 controls). The difference between native and filtrated M65 antigen level reflects the level of MPs derived from hepatocytes. (*D*) Circulating levels of $CD31^+/41^-$ in 91 patients with cirrhosis according to Child–Pugh score (A, n = 16; B, n = 18; C, n = 55). (*E*) Levels of cytokeratin-18 (M65 antigen) bound to MPs (obtained by the difference between native and filtrated M65 antigen level) according to Child–Pugh score (A, n = 9; B, n = 9; C, n = 22). Data are given as median (*horizontal bar*), 25th and 75th percentile (*boxes*), and extreme values, which are less than 3 box lengths from either end of the box (*error bar*). ns, not significant; $^*P < .05$; $^{**}P < .01$; $^{***}P < .001$.

tients with high atherothrombotic risk.[2] MPs are not inert by-products. A number of studies point out that MPs can affect several cellular functions, including vascular tone and vascular reactivity.[2] However, these studies were performed using MPs generated in vitro or isolated from patients with cardiovascular diseases or sepsis.[2] Because lipid and protein fractions of MPs, as well as their biological effects, greatly vary depending on the stimulus initiating cell blebbing and MP release,[3–5] we aimed to determine the level and cellular origins of circulating MPs in patients with cirrhosis and test the hypothesis that MPs of patients with cirrhosis contribute to vascular hypocontractility.

Patients and Methods

Patients and Controls

We included 91 patients admitted to the Liver Unit (Hôpital Beaujon, Clichy, France) for alcoholic and/or hepatitis C virus–related cirrhosis during 2 periods: 26 patients between 2007 and 2008 (the pilot cohort) and 65 additional patients between 2010 and 2011 (Figure 1*A*). None of the patients had severe sepsis, hepatocellular carcinoma assessed using serum α-fetoprotein level and computed tomographic scan or ultrasonography, or portal vein thrombosis. The pilot cohort was compared with a group of 30 healthy volunteers. The effect on vascular reactivity of MPs from 6 patients with end-stage renal failure included in a previous study was also analyzed.[6] All patients and controls gave their informed