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Patients With Acute-on-Chronic Liver Failure Have Increased Numbers of Regulatory Immune Cells Expressing the Receptor Tyrosine Kinase MERTK

Christine Bernsmeier,[1] Oltin T. Pop,[1] Arjuna Singanayagam,[1] Evangelos Triantafyllou,[1,2] Vishal C. Patel,[1] Christopher J. Weston,[2] Stuart Curbishley,[2] Fouzia Sadiq,[3] Nikhil Vergis,[3] Wafa Khamri,[3] William Bernal,[1] Georg Auzinger,[1] Michael Heneghan,[1] Yun Ma,[1] Wayel Jassem,[1] Nigel D. Heaton,[1] David H. Adams,[2] Alberto Quaglia,[1] Mark R. Thursz,[3] Julia Wendon,[1] and Charalambos G. Antoniades[1,2,3]

[1]Institute of Liver Studies, King's College Hospital, King's College London, United Kingdom; [2]Centre for Liver Research and National Institute for Health Research, Biomedical Research Unit, University of Birmingham, United Kingdom; [3]Section of Hepatology, St. Mary's Hospital, Imperial College London, United Kingdom

BACKGROUND & AIMS: Characteristics of decompensated cirrhosis and acute-on-chronic liver failure (ACLF) include susceptibility to infection, immuneparesis, and monocyte dysfunction. MER receptor tyrosine kinase (MERTK) is expressed by monocytes and macrophages and contributes to down-regulation of innate immune responses. We investigated whether MERTK expression is altered on monocytes from patients with liver failure. **METHODS:** We analyzed blood and liver samples collected from patients admitted to the liver intensive therapy unit at King's College Hospital in London from December 2012 through July 2014. Patients had either ACLF (n = 41), acute decompensation of cirrhosis without ACLF (n = 9), cirrhosis without decompensation (n = 17), or acute liver failure (n = 23). We also analyzed samples from healthy individuals (controls, n = 29). We used flow cytometry to determine the level of innate immune function, and associated the findings with disease severity. We developed an assay to measure recruitment and migration of immune cells from the tissue parenchyma. Immunohistochemistry and confocal microscopy were used to determine levels of MERTK in bone marrow, liver, and lymph node tissues. We performed immunophenotype analyses and measured the production of tumor necrosis factor and interleukin 6 and intracellular killing of *Escherichia coli* by monocytes and peritoneal macrophages incubated with lipopolysaccharide, with or without an inhibitor of MERTK (UNC569). **RESULTS:** The number of monocytes and macrophages that expressed MERTK was greatly increased in the circulation, livers, and lymph nodes of patients with ACLF, compared with patients with stable cirrhosis and controls. MERTK expression (mean fluorescence intensity) correlated with the severity of hepatic and extrahepatic disease and systemic inflammatory responses. Based on immunophenotype, migration, and functional analyses, MERTK-expressing monocytes migrate across the endothelia to localize into tissue sites and regional lymph nodes. Expression of MERTK reduced the response of cultured monocytes to lipopolysaccharide; the addition of UNC569 restored production of inflammatory cytokines in response to lipopolysaccharide. **CONCLUSIONS:** Patients with ACLF have increased numbers of immunoregulatory monocytes and macrophages that express MERTK and suppress the innate immune response to microbes. The number of these cells correlates with disease severity and the inflammatory response. MERTK inhibitors restore production of inflammatory cytokines by immune cells from patients with ACLF, and might be developed to increase the innate immune response in these patients.

Keywords: ALF; SIRS; Immune Regulation; Bacterial Infection.

Patients with cirrhosis show a marked susceptibility to infections, which develop in 35% of hospitalized patients compared with 5%–7% of the general population.[1] Furthermore, infection accounts for more than 50% of admissions of cirrhotic patients to the hospital and is the main precipitant for the rapid decompensation referred to as *acute-on-chronic liver failure* (ACLF), which is associated with the development of multiple organ failure. Once established, ACLF carries a prohibitively high mortality rate and a significant burden on critical care services and health care resources.[2,3] Impaired peripheral immune responses to microbial challenges, termed *immuneparesis*, is postulated to be responsible for the development of secondary infections, and is an independent predictor of mortality in patients with ACLF.[3] Despite advances in organ failure support, there are no targeted strategies to combat susceptibility to infection in patients with cirrhosis and ACLF.

Abbreviations used in this paper: **ACLF, acute-on-chronic liver failure; AD, acute decompensation with no cirrhosis; ALF, acute liver failure; CCR, chemokine receptor; CD, cluster of differentiation; CLD, chronic liver disease; CLIF, Consortium on Chronic Liver Failure; HC, healthy control; HUVEC, human umbilical vein endothelial cell; IL, interleukin; LPS, lipopolysaccharide; LITU, Liver Intensive Therapy Unit; LN, lymph node; MELD, model of end-stage liver disease; MERTK, MER receptor tyrosine kinase; NACSELD, North American Consortium for Study of End-stage Liver Disease; pMφ, peritoneal macrophage; SIRS, systemic inflammatory response syndrome; SOFA, Sequential Organ Failure Assessment; TNF-α, tumor necrosis factor α.**

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Monocyte dysfunction in ACLF, characterized by low HLA-DR and attenuated proinflammatory responses to microbial challenge, is associated with an adverse outcome and may account for the predisposition to infectious complications.[4,5] These observations in ACLF echo our recent findings of monocyte dysfunction and immuneparesis in acute liver failure (ALF).[6]

The MER receptor tyrosine kinase (MERTK) is a transmembrane protein of the tyro-3, Axl, and MER–receptor family expressed on monocytes/macrophages, dendritic cells, epithelial cells, and reproductive and neuronal tissues.[7,8] MERTK is activated by its ligands Gas-6, protein-S, and galectin-3, leading to receptor autophosphorylation and activation of the downstream signaling cascade.[8]

MERTK is an important negative regulator of innate immune responses and plays a central role in the resolution of inflammation through inhibition of proinflammatory responses to microbial challenge and promoting the clearance of apoptotic cells.[8,9] MERTK plays a pivotal role in the regulation of monocyte inflammatory responses in which its activation was shown to inhibit Toll-like receptor activation and cytokine-receptor–induced proinflammatory cytokine production through downstream activation of suppressors of cytokine signaling 1/3.[10] MERTK knock out mice were hypersensitive to lipopolysaccharide (LPS) and developed fatal tumor necrosis factor α (TNF-α)–induced severe septic shock.[11] Moreover, increased monocyte MERTK and Gas-6 levels recently were reported in patients with septic shock with persistent expression associated with an adverse outcome.[12,13]

In view of the recently described role of MERTK in suppressing innate immune responses, we sought to determine whether activation of this immune-regulatory pathway could account for monocyte/macrophage dysfunction, and establish its candidacy as an immunotherapeutic target to reduce susceptibility to infectious complications in patients with ACLF.

Methods

Patients and Sampling

The study was approved by the King's College Hospital Ethics Committee (12/LO/0167). Assent was obtained by the patients' nominated next of kin if they were unable to provide informed consent themselves. Between December 2012 and July 2014, 119 subjects were recruited to the study within 24 hours after admission to the Liver Intensive Therapy Unit (LITU) or liver wards. Patients were categorized into different groups: ACLF (n = 41), acute decompensation of cirrhosis with no ACLF (AD; n = 9; according to the Consortium on Chronic Liver Failure–Sequential Organ Failure Assessment [CLIF-SOFA] classification previously described[2]), patients with cirrhosis with no evidence for acute decompensation (n = 17), patients with ALF (n = 23), and healthy controls (HC; n = 29). Cirrhosis was diagnosed by a previous liver biopsy or clinical presentation with typical ultrasound or computed tomography imaging. Exclusion criteria were as follows: age younger than 18 years; malignancy; immunosuppressive therapy other than corticosteroids, which were accepted if required for the treatment of autoimmune liver disease; alcoholic hepatitis; or suspected relative adrenal insufficiency.

Clinical, Hematologic, and Biochemical Parameters

Full blood count, international normalized ratio, liver and renal function tests, lactate, ammonia, and clinical variables were entered prospectively into a database. The following disease severity scores were calculated: Child–Pugh, model of end-stage liver disease (MELD), CLIF-SOFA,[2] North American Consortium for Study of End-stage Liver Disease (NACSELD),[14] Acute Physiology and Chronic Health Evaluation II (APACHE II), Simplified Acute Physiology Score II (SAPS II), SOFA scores, and infections were documented.

Isolation of Monocytes

Monocytes were isolated using cluster of differentiation (CD)14 microbeads as described[6] or sequential depletion using CD66abce, CD56 microbeads, and the Pan-Monocyte Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany). The purity of monocytes was assessed by flow cytometry (Supplementary Materials and Methods).

Phenotyping of Monocytes/Macrophages and Measurement of Cytokine Responses to LPS

Monoclonal antibodies against CD14, CD16, CD86, CD163, CD64, chemokine receptor (CCR)2, CCR5, CCR7 (BD Biosciences, Oxford, UK); HLA-DR, CD32, CX3CR1 (eBioscience, Hatfield, UK), and hMer (R&D Systems, Abingdon, UK) were used to determine the expression of phenotypic markers on monocytes from peripheral blood mononuclear cells (PBMC) using flow cytometry. Results are expressed as the percentage and/or mean fluorescence intensity (MFI). TNF-α and interleukin (IL)6 levels after a 4- to 6-hour incubation of peripheral blood mononuclear cells or isolated monocytes with LPS were determined by flow cytometry–based intracellular staining as previously described.[6] Flow cytometry data were analyzed using Flowlogic software (Inivai Technologies, Mentone, Australia).

MERTK Ligands and Cytokines

Gas-6 (Abnova, Taiwan) and protein-S (Abcam, Cambridge, UK) were measured in plasma and galectin-3 (eBioscience) in serum samples using enzyme-linked immunosorbent assay. Plasma cytokines were measured using Meso Scale Discovery (Rockville, MD) assay kits as previously described.[6] TNF-α and IL6 in cell culture supernatants were measured as previously described.[6]

Immunohistochemistry and Confocal Microscopy

Explanted liver tissue was obtained from patients undergoing orthotopic liver transplantation for cirrhosis (n = 6) or ACLF (n = 6). Hepatic resection margins of colorectal malignancies (n = 4) served as controls. Lymph nodes were obtained from 5 patients undergoing orthotopic liver transplantation for decompensated cirrhosis and 3 controls (benign stricture of the left hepatic duct, n = 2; and giant hepatic cyst, n = 1). Exemplarily, 1 bone marrow trephine from an ACLF patient was included. Tissues were taken for diagnostic histologic examination, formalin-fixed, and paraffin-embedded.