# Macrophages from Patients with Cirrhotic Ascites Showed Function Alteration of Host Defense Receptor

Abdel Motaal M. Ahmed*, Abdel Gadir Y. Kadaru†, Ibtihal Omer‡, Ahmed M. Musa§, Khalid Enan‖, Isam M. El Khidir¶, Roger Williams#

*Department of Medicine, The National University for Medical and Allied Sciences, †Department of Medicine, Faculty of Medicine, University of Khartoum, ‡Microbiology Department, Ribat University, §Institute of Endemic Diseases, University of Khartoum, ‖Molecular Biology Unit, The National Research Laboratory Centre, ¶Virology Department, Faculty of Medicine, University of Khartoum, Khartoum 11123, Sudan and #Institute of Hepatology, The Foundation for Liver Research, London, UK

***Background:*** **Patients with cirrhotic ascites (PCA) are susceptible to spontaneous bacterial peritonitis (SBP) which has increased morbidity and mortality. Since some host defense aspects of peritoneal macrophages (PMϕ) from PCA are altered this study examined factors related to receptor-mediated phagocytosis.** ***Methods:*** **Twelve PCA were studied. PMϕ were isolated from ascitic fluid (AF) samples removed from these patients. Uptake of mannose receptor (MR)-specific ligand, fluorescein isothiocyanate-mannosylated-bovine serum albumin (FITC-man-BSA), by patients' PMϕ and controls, a human monocytic cell line, was measured pre- and post-IL-4 treatment. Phagocytosis of FITC-labeled yeast particles by patients' PMϕ was measured pre- and post-IL-4 treatment. Fluorescence values were obtained using a spectrofuorometer.** ***MRC1*** **gene was analyzed in blood samples from PCA and controls, healthy donors, using standard polymerase chain reaction (PCR) technique.** ***Results:*** **Past SBP episode(s) were reported in 58.3% of patients. Mean AF volume analyzed per patient was 1.3L. PMϕ ratio in cell yield was 53.73% (SD 18.1). Mean uptake absorbance of patients' PMϕ was 0.0841 (SD 0.077) compared to 0.338 (SD 0.34) of controls,** ***P*** **= 0.023. Following IL-4 treatment absorbance increased to 0.297 (SD 0.28) in patients' PMϕ (*P* = 0.018 on paired sample *t*-test), and to 0.532 (SD 0.398 in controls (*P* = 0.053 on independent sample *t*-test). Mean phagocytosis absorbance of patients' PMϕ was 0.1250 (SD 0.032) before IL-4 treatment compared to 0.2300 (SD 0.104) after (*P* = 0.026). PCR analysis for *MRC1* gene was negative in all PCA samples compared to positive results in all controls.** ***Conclusion:*** **Since decreased phagocytosis and MR uptake were enhanced post-IL-4 treatment MR downregulation pre-treatment is plausible. Negative PCR results for *MRC1* might suggest an anomaly, but this awaits further ellucidation. These altered host defense findings are relevant to infection pathophysiology, and their relevance to SBP susceptibility in PCA is worth verifying.** **(J Clin Exp Hepatol 2014;4:279–286)**

Ascites

Patients with liver cirrhosis and ascites (PCA) have a low threshold to develop bacterial infection, which is associated with increased morbidity and mortality.[1] Spontaneous bacterial peritonitis (SBP), for instance, is considered the prototype infection in cirrhosis and occurs in 30–50%.[2,3] With regarding infection pathophysiology host defense of circulating phagocytes from patients with cirrhosis was shown to be impaired.[4,5] However, little is known about host defense of tissue-type phagocytes in PCA. The tissue-type peritoneal macrophages (PMϕ) play a pivotal role in pathophysiological processes in the peritoneal milieu,[6,7] and a function anomaly in these cells could be relevant to SBP pathophysiology. Mannose receptor (MR) is a novel example of a host defense receptor that has recently been focused. It is a C-type lectin endocytic receptor with extra-cellular domains that co-ordinate sugar binding of ligands.[8,9] Ligand binding and internalization are referred to as receptor-mediated endocytosis.[10,11] Mannose-containing glyco-conjugates constitute part of the structure of microorganisms responsible for infection.[11] MR expression is upregulated by interleukin-4 (IL-4) and dexamethasone,[12,13] and is downregulated by in vivo activation state in which MR gets internalized.[14] *MRC1*, the gene encoding the human MR, is located on chromosome 10p12 and consists of 30 exons.[15] More recently *MRC1* polymorphism has been associated with susceptibility to certain

*Keywords:* cirrhosis, macrophage host defense, mannose receptor, *MRC1* gene, spontaneous bacterial peritonitis
*Received:* 27.5.2014; *Accepted:* 15.8.2014; ; *Available online:* 16.09.2014
*Address for correspondence*: Abdel Motaal M. Ahmed, Consultant Gastroenterologist and Associate Professor, Department of Medicine, The National University for Medical and Allied Sciences, Khartoum 11123, Sudan. Tel.: +249 114624484 (mobile); fax: +249 183789554
*E-mails:* abuzafir46@hotmail.com; am.ahmed@hotmail.com
*Abbreviations:* AF: ascitic fluid; FBS: foetal bovine serum; FITC: fluorescein isothiocyanate; IL-4: interleukin-4; man-BSA: mannosylated bovine serum albumin; MR: mannose receptor; *MRC1*: gene encoding human MR; PCA: patients with cirrhotic ascites; PCR: polymerase chain reaction; PMϕ: peritoneal macrophages; RPMI and DMEM: cell culture media
http://dx.doi.org/10.1016/j.jceh.2014.08.003

infections like tuberculosis and leprosy.[16,17] We have previously demonstrated that PMϕ from PCA produced vigorous respiratory burst, and we speculated that an in vivo activation state might be present.[5] Since uptake measurement of man-BSA, the MR-specific ligand, may indirectly represent MR expression we performed uptake measurement and also analyzed *MRC1* gene in PCA.

## METHODS

### Patients' Samples Collection

Ascitic fluid (AF) was collected from 15 adult patients with established diagnosis of cirrhosis with ascites. Patients were randomly selected and the number was determined arbitrarily. Patients were excluded from the study if they had any of following criteria:

1. malignancy, 2. evidence of current SBP or other infection, 3. an altered level of consciousness, 4. hematemesis within the previous four-weeks to recruitment, 5. a major cardiopulmonary disease, end stage kidney disease or terminal illness, 6. intake of immunomodulator drugs or antibiotics within the previous six-week. These agents could affect some of the functional assays. For the majority of PCA in this study locality secondary prophylaxis with antibiotics is not readily available, and this exclusion criterion was not an obstacle.

An informed consent was obtained in all patients. Patients were then re-assessed where full history was obtained and complete physical examination was performed. History of past episode(s) of SBP was specifically recorded. Patients were originally booked either as day cases or as short-stay admissions for routine therapeutic paracentesis. This procedure was judged indicated by the treating physician. The in-charge physicians have kindly given permission for the use of the aspirated fluid for the study purpose.

Paracentesis procedure was done according to published guidelines.[18] The volume of fluid sample that was sent to the lab was no more than 2.0 L per patient and it was analyzed within an hour. The exact fluid volume for each patient received in the lab was recorded on arrival. Also, fluid appearance, neutrophil cell count and protein level in each sample were obtained and recorded at the outset. If AF appearance was very cloudy or heavily blood-stained or if the neutrophil count was >200 cells/mI the specimen was considered unsuitable for further analysis. The in-charge physician was kept informed of all this information.

Approval for this study was obtained from the Hospital's Research Ethics Committee, according to the Declaration of Helsinki.[19]

### Patients Peritoneal Macrophage Preparation

The cell separation process was done as previously.[5] Cell viability tests were determined by trypan blue dye exclusion. The ratio of PMϕ in cell mixture was determined manually by counting during the microscopic examination of cytocentrifuge preparations. These were stained using May Grunwald Giemsa and acid esterase.[20,21] In experiments where adherent cells were used the cell adherence was allowed to take place first by incubating the plates for 2 h in complete fresh culture media containing 10% RPMI/FBS, 100 u/mI penicillin G sodium and 100 $\mu$g/mI streptomycin sulfate (Sigma Chemicals, Poole, UK) and at 37 °C and 5% $CO_2$ air. The intended assay was then carried out immediately. In experiments where PMϕ were used in suspension the adherent cell layer prepared as above was detached by a quick incubation of plates in trypsin solution for 45 s.[22] The cell harvest was re-counted, re-tested for viability as above and then the intended assaying was performed directly.

### Control Cells Preparation

The Mono Mac 6 (MM6) human monocytic cell line was originally obtained as a commercial product from German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany. This cell line is a human acute monocytic leukemia cell line which was confirmed suitable for in vitro experiments and the cells were prepared by subculturing as previously.[23]

## PHAGOCYTOSIS

### Yeast Labeling

*Candida albicans* particles were prepared and F1TC-labeled (Sigma Chemicals) as previously.[24] Labeled particles were then re-suspended at $5 \times 10^7$ cells/ml in 20% DMEM/FBS (Sigma Chemicals) and stored light-protected at 4 °C. The resulted stock was used within ten weeks of preparation.

### Phagocytosis Measurement

Phagocytosis measurements were done according to previous method[24] with some modification.[5] Labeled yeast particles prepared as above were added to wells at a macrophage-to-particles ratio of 1:40 and phagocytosis proceeded for 30 min. Measurements were performed before and after treatment of cells with IL-4 (Sigma Chemicals). Treatment was given for 36 h at a concentration of 5 ng/ml. Normally cells treated this way were assayed in adherent form. At the end of phagocytosis process plates were thoroughly washed out, cells were lysed in 0.1 N NaOH and fluorescence readings from internalized particles were obtained using a spectrofluorometer (Chem 7, Erba Diagnostics, Manheim GmbH, Germany) at an excitation wavelength of 482 nm and emission wavelength of 520 nm. All samples were analyzed in duplicates.