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 (PM ϕ) from PCA are altered this study examined factors related to receptor-mediated phagocytosis. **Methods:** Twelve PCA were studied. PM ϕ 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' PM ϕ and controls, a human monocytic cell line, was measured pre- and post-IL-4 treatment. Phagocytosis of FITC-labeled yeast particles by patients' PM ϕ was measured pre- and post-IL-4 treatment. Fluorescence values were obtained using a spectrofluorometer. *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. PM ϕ ratio in cell yield was 53.73% (SD 18.1). Mean uptake absorbance of patients' PM ϕ 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' PM ϕ ($P = 0.018$ 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' PM ϕ 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 elucidation. 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)

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 (PM ϕ) 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; PM ϕ : 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 PM ϕ 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/ml 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 exclu-

sion. The ratio of PM ϕ 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/ml penicillin G sodium and 100 μ g/ml streptomycin sulfate (Sigma Chemicals, Poole, UK) and at 37 °C and 5% CO₂ air. The intended assay was then carried out immediately. In experiments where PM ϕ 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 sub-culturing as previously.²³

PHAGOCYTOSIS

Yeast Labeling

Candida albicans particles were prepared and FITC-labeled (Sigma Chemicals) as previously.²⁴ Labeled particles were then re-suspended at 5×10^7 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 method²⁴ 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, Mannheim GmbH, Germany) at an excitation wavelength of 482 nm and emission wavelength of 520 nm. All samples were analyzed in duplicates.

Fluorescence values were expressed as percentages of arbitrary absorbance units and calculations were made using the following formula:

$$\%A = (F_1 - F_2) \div (F_3) \times 100$$

where %A is the percentage of absorbance units, F_1 is the absorbance of the wells containing both cells and labeled particles, F_2 is the absorbance of the wells containing cells only (auto-fluorescence) and F_3 is the absorbance of the wells containing labeled particles only (total fluorescence).

Receptor Specific Uptake Measurement

Uptake measurement of FITC-labeled man-BSA by MR in PM ϕ from PCA and controls was performed as previously.²⁵ Yeast mannan 0.1 M was used as a blank ligand and was added to wells at a concentration of 12 μ g/ml and FITC-labeled man-BSA (Sigma Chemicals) as specific stable ligand and was added to wells at a concentration of 12 μ g/ml. Where an effect of IL-4 treatment was examined, PM ϕ were treated first with this agent at a concentration of 5 ng/ml for 36 h. Fluorescence in cell lysate was obtained using a spectrophotometer at 482- and 520-nm wavelengths.

Specific uptake (F_{sp}) was calculated as follows:

$$\begin{aligned} F_{sp} &= \text{total uptake (both specific and non-specific)} \\ &\quad - \text{non-specific uptake.} \\ &= [F_1 - F_2] - [F_3 - (F_1 + F_4)] \end{aligned}$$

where F_1 is fluorescence of wells containing cells and FITC-labeled man-BSA, F_2 is fluorescence of wells containing cells only (autofluorescence), F_3 is fluorescence of wells containing cells, yeast mannan and FITC-labeled man-BSA and F_4 is fluorescence of wells containing mannan only.

MRC1 ANALYSIS

DNA Extraction

Genomic DNA was purified from EDTA-blood samples taken from twelve PCA and eight healthy volunteers using QIAamp Blood Midi Kit (spin protocol) (Qiagen GmbH, Hilden, Germany) and as recommended by the manufacturers.²⁶ Samples were processed within an hour of their withdrawal. Volume eluted from the column was <200 μ l which was kept at -20°C till PCR analysis which was normally carried out the following day. This way 200 μ l of whole blood yields 3–12 μ g of DNA, and the DNA concentration was 34.0 ng/ μ l.

Polymerase Chain Reaction (PCR)

MRC1 gene was analyzed by standard PCR technique as previously¹⁶ with some modification. Briefly, genomic DNA was purified as described above. PCR Master Mix

used was GoTaq[®] Green Master mix product (Promega Corporation, Madison, USA) and PCR primers were used. The PCR primers included forward: TTGAGGCTGCAATGAGACAT and reverse: AGTGTAAGGTAGACTGCTCT. Final PCR reaction volume (20 μ l) was composed of 10 μ l GoTaq[®] Green Master Mix, 0.5 μ l forward primer, 0.5 μ l reverse primer, 5 μ l DNA template and 4 μ l distilled water. PCR was performed as follows: denaturing at 95°C for 5 min, 35 cycles at 95°C for 50 s, 63°C for 50 s, and 72°C for 30 s, and a final extension at 72°C for 10 min. PCR products were subjected to agarose gel electrophoresis and visualized by ethidium bromide staining.

Statistical Analysis

Statistical package used to analyzed data was SPSS version 17. Paired and independent sample *t*-tests were used depending on the experiment conducted. Paired sample *t*-test was used to analyze data in the same group pre- and post intervention e.g phagocytosis data and part of uptake data. Independent *t*-test was used to analyze data in patients' group as compared to that in controls, namely the rest of uptake data. The significant level for *P* value was taken as <0.05.

RESULTS

Patients Detail

The number of patients included in this study was 12 PCA. Three other patients were excluded because they did not meet the inclusion criteria. Nine (75%) were males and 3 females (25%) (Table 1). Male to female ratio was 3:1. Mean age was 52.86 (SD 11.1) years. There were 10 patients (83.3%) in Child-Pugh class C and 2 patients (16.7%) in Child-Pugh class B (Table 1). The cause of cirrhosis was HBV-related in 8 patients (66.6%) in whom HBsAg test was positive (Table 1). The co-existence of HBV-related cirrhosis and schistosoma peri-portal fibrosis (PPF) was present in 2 out of those 8 patients (Table 1). PPF was mainly an ultrasound finding made on top of cirrhosis. The other causes of cirrhosis were found as follows: HCV-related in 1 patient (8.3%) in whom HCV test was positive, alcohol-related in 2 patients (16.6%), and autoimmune hepatitis (AIH)-related in 1 patient (4.7%) in whom anti-nuclear antibody test was positive (Table 1). Ultrasound diagnosis of cirrhosis was made in all patients but the liver biopsy diagnosis was obtainable in only 2 patients (16.6%) (Table 1). In terms of occurrence of previous episode(s) of SBP 7 patients (58.3%) reported to have had at least one past episode, no previous episodes in 2 patients (16.6%) and in the remaining 3 patients (25%) the information on whether SBP has actually occurred was not definite (Table 1). None of those patients who have had past episode(s) of SBP were kept on prophylaxis antibiotic treatment.

Table 1 Patients' Clinical Information and Laboratory Results.

	No. of patients	%
Sex:		
Male	9	75%
Female	3	25%
M:F ratio 3:1		
Mean age: 52.8 yrs (SD 11.1)		
Child-Pugh's class:		
C	10/12	83.3%
B	2/12	16.7%
No. of patients with past SBP episode(s) reported	7/12	58.3%
Means of diagnosis		
a) Ultrasound	12/12	100%
b) Histology	2/12	16.6%
Etiology of Cirrhosis:		
HBsAg positive	8	66.6%
HCV positive	1	8.3%
Alcoholic	2	16.6%
Auto-immune	1	8.3%
Total	12	100%

Ascitic Fluid Analysis

The mean volume of sample of ascitic fluid analyzed per patient was 1.3 (SD 0.357) L (Table 2). The mean total number of cells isolated was 23.87×10^6 cells (SD 17.2) per sample, and 53.73% (SD 18.1) of these were PM ϕ as determined by counting under light microscopy (Figure 1a) (Table 2). After overnight incubation the plates were examined in an inverted microscope and striking morphological changes were observed (Figure 1b). These morphological changes were suggestive of cell activation.

Table 2 Results of Ascitic Fluid (AF) Analysis.

Parameter	Result
Fluid volume	1.3L (SD 0.357)
Mean fluid protein level	2.25 mg/dl (SD 0.57)
Mean total cell no. isolated	23.87×10^6 cells (SD 17.2)
Mean percentage of PM ϕ in total cells no. isolated	53.73% (SD 18.1).
Cell viability	93% (SD 2.8)
Fluid appearance was:	
clear in	8/12 patients (66.6%)
hazy in	3/12 patients (25%)
cloudy/slightly blood-stained in	1/12 patients (8.3%)

The mean cell viability was 93% (SD 2.8). Fluid appearance was described as clear in 8 (66.6%) patients, as hazy in 3 (25%) patients and as cloudy or slightly blood-stained in one patient (Table 2). Fluid protein level was 2.25 mg/dl (SD 0.57) (Table 2), which was in keeping with a clear fluid appearance in the majority of samples.

Phagocytosis Results

Phagocytosis measurement was made in six samples (A, B, C, D, E and F) of patients' PM ϕ before and after treatment with IL-4 (Figure 2). The absorbance increased in patients' PM ϕ samples as follows: from 0.175 to 0.365 in sample A, from 0.08 to 0.17 in B, from 0.10 to 0.15 in C, from 0.14 to 0.24 in D, from 0.10 to 0.15 in E and from 0.125 to 0.18 in sample F (Figure 2). These values gave a mean absorbance of 0.2300 (SD 0.104) in IL-4-treated PM ϕ as compared to 0.1250 (SD 0.032) in untreated PM ϕ , $P = 0.026$ on paired sample t -test (Figure 2). The vertical axis shows absorbance values and the letters on the horizontal axis represent the samples tested (Figure 2). The black diamond symbols represent absorbance value before IL-4 treatment and the light square symbols represent absorbance values after IL-4 treatment (Figure 2).

Receptor Uptake Results

The mean absorbance of specific uptake of PM ϕ from 6 PCA was 0.0841 (SD 0.077) (dark bars) as compared to 0.338 (SD 0.34) of controls (light bars), $P = 0.023$ on independent sample t -test (Figure 3). Following treatment with IL-4 the mean uptake absorbance increased from 0.0841 to 0.297 (SD 0.28) (dark bars) and from 0.338 to 0.532 (SD 0.398) (light bars) in patients' PM ϕ and in controls respectively, $P = 0.053$ on independent sample t -test and $P = 0.018$ on paired sample t -test (Figure 3).

Specific uptake absorbance values were calculated using the formula in Material and Methods.

Experimental conditions were identical and experiments were carried out in duplicates.

Polymerase Chain Reaction Results for MRC1

There were no bands in lanes 1, 2, 3 and 5 (Figure 4a). These were negative results in four out of twelve PCA's samples tested. The only positive result (visible band) was in lane 4 which represents a sample from a healthy donor (Figure 4a). Likewise no bands were visualized in the remaining 8 patients' samples (film not shown). Positive results (visible bands) in lanes 1–7 were present in the remaining seven control samples from healthy donors. 550 bp on 1.5% agarose gel M: 50 bp DNA size marker (Figure 4b).

DISCUSSION

In this study we examined the host defense function of PM ϕ from PCA. Since these cells are tissue-resident

Figure 1 Photographs of stained sections of patients' PM ϕ at high magnifications (A & B) and phase contrast views of tissue culture plates (C & D). Slide A & B were stained using May Grunwald Giemsa stain. Observe the triking morphological changes following overnight incubation (D). No stimulus was added to these cells.

phagocytes they are expected to play a pivotal role in the pathophysiology of SBP, namely host defense aspect of pathophysiology. The function of circulating phagocytes has already been extensively studied in cirrhosis patients,²⁷⁻³⁰ but only little information is available on the function of PM ϕ from these patients. Ninety percent of our patients were in Child-Pugh class C indicating an advanced stage of cirrhosis. Because of the study's nature

Figure 2 Effect of IL-4 on phagocytosis in patients' macrophages. The bottom curve (dark diamond symbols) represents phagocytosis measurements before IL-4 treatment and the top curve (square symbols) represents measurements after IL-4 treatment. Letters in horizontal axis denote samples of patients' PM ϕ . Values in vertical axis represent absorbance.

and size we were uncertain if a convincing correlation of data would be achieved betdifferent aetiological subgroups or other clinical parameters of cirrhosis. At this stage of cirrhosis ascites is present in about 50-70% of cases and SBP and its complications are known to be prevalent.³¹⁻³³ This high risk group of cirrhotic patients was targeted because of the dismal outcome of infection in them. Depending on what type of new information about infection pathophysiology is gained such knowledge might help in reducing susceptibility to infection.

In a previous study we demonstrated for the first time that quantitative phagocytosis measurement in PM ϕ from PCA was lower than that in controls ($P = 0.0001$).⁵ In the same study PM ϕ showed vigorous respiratory burst (RB) and we hypothesized that this heightened RB could be associated with decreased phagocytosis.⁵ MR internalizes rapidly in the presence of reactive oxygen intermediates released in RB and MR also internalizes in the presence of other inflammatory mediators like trypsin and antigenic products.^{11,14,34,35} In such an environment clearance of products from inflammatory milieu by host defense receptors is reduced.¹⁴

This study showed a significantly lower MR specific uptake in patients' PM ϕ than that in controls ($P = 0.023$). One plausible explanation to this finding is receptor

Figure 3 Mannose receptor (MR) specific uptake measurement of PMφ from patients and controls. The panel on the left represents uptake measurements in patients' PMφ (dark bar) and controls (light bar) before IL-4 treatment $P = 0.023$ on independent sample t -test. The panel on the right represents uptake measurements after IL-4 treatment in both groups $P = 0.053$ on independent sample t -test and $P = 0.018$ on paired sample t -test. The figures on the vertical axis represent absorbance.

downregulation in an activated peritoneal environment in cirrhosis. In a few studies IL-4 has upregulated MR expression in healthy macrophages.^{12,35–37} In this study we examined the effect of this anti-inflammatory cytokine and we found uptake measurements in both groups were higher in IL-4-treated cells than that in untreated cells. However, this increase in uptake did not reach statistical significance on independent sample t -test which compared data in the two groups ($P = 0.053$). However, the increase in uptake was significant on two-tailed sample t -test which compared patients-only data pre- and post-IL-4 treatment ($P = 0.018$).

We then measured phagocytosis before and after treatment of cells with IL-4, in the patients' group only. Phagocytosis increased significantly following IL-4 treatment as compared to pre-treatment levels, $P = 0.026$ paired sample t -test (Figure 3).

The increase in specific uptake and in phagocytosis following IL-4 supported our claim that IL-4 had an enhancing effect on a previously downregulated MR, but more specific MR expression studies in PMφ from PCA are required to substantiate this claim. However, an increase of up to four fold in phagocytosis has been reported before in IL-4-treated macrophages from healthy volunteers and also in macrophages from patients with chronic obstructive airway disease.³⁸ IL-4-enhancing effect on MR expression both in health and in certain disease states is possibly through its anti-inflammatory properties. IL-4 serum levels are expected to show wide variations, particularly in cirrhosis and these may cause limitation in interpretation. However, since our data were essentially obtained from in vitro experiments it is perhaps too early to discuss in depth facts about clinical or in vivo applications. Dexamthasone, is another agent that has been shown to increase MR activity by increasing receptor

Figure 4 a. PCR Results of *MRC1* gene in patients with cirrhosis. In lanes 1, 2, 3 and 5 no bands for *MRC1* gene were visualized (negative PCR results) in four out of twelve samples from PCA. In lane 4 a clear band is visualized (positive PCR result) for *MRC1* gene in a healthy donor's sample, serving as a normal reference. In the remaining eight patients' samples the results were also negative (film not shown). 550 bp on 1.5% agarose gel M: 50 bp DNA size marker. b. PCR Results of *MRC1* gene in normal subjects. In lanes 1–7 clear bands were visualized for *MRC1* gene (positive PCR results). No patients' samples were included here. M: 50 bp DNA size marker. 550 bp on 1.5% agarose gel.

mRNA levels,¹³ but again this was only at an in vitro level. Phagocytosis of alveolar macrophages increased with in vitro treatment of azithromycin more than the increase in untreated cells. Of interest, this increase in phagocytosis measurement was associated with MR upregulation.³⁸ However, when comparing the enhancing effect of IL-4 on phagocytosis shown in this study to that of azithromycin reported in another study³⁸ the IL-4 effect is less than that of azithromycin. The increase is <2 fold vs. 2–4 fold respectively. It is prudent to add that macrophage heterogeneity could account, at least in part, for such a difference in effect because the two macrophage populations were from different habitat.

Novel receptor characterization and expression studies have applied radioisotope techniques.^{11,12,34–37} Unfortunately we were unable to perform these techniques. However, in order to back-up the initial results we obtained from fluorescence technique we opted for PCR analysis.

Needless to say PCR has been used in host defense research and it provided valuable information in various disease settings including cirrhosis.^{16,17,39,40} For instance, in a recent study the investigators demonstrated molecular evidence of bacterial antigenic material in cirrhotic ascites in the absence of a microbiological evidence for SBP.⁴⁰ MR studies, in particular, identified an association between polymorphic allele of *MRC1* and susceptibility to certain infections including tuberculosis and leprosy.^{16,17} In those studies standard PCR technique was used. This study has tested the gene encoding MR and in all patients' samples we were unable to visualize a band representative of *MRC1* on agarose gel electrophoresis (Figure 4a). In contrast, clear bands for the gene were visualized in all control samples (Figure 4b). The negative PCR finding in the patients' group is interesting.

First, it is in line with the finding of decreased fluorescence uptake measurement reported above. Second, it might suggest the presence of an anomaly such as polymorphism, but this needs further elucidation. Given that the results in control samples were consistently positive it is fair to say that the standard PCR technique is adequate for gene detection and that patients' results are true negatives. In a previous study the initial PCR results were not shown separately as we did in this study, instead the investigators proceeded to sequence analysis using a sophisticated sequencer.¹⁶ According to this study's protocol the sequence analysis was meant to be dependent on obtaining positive PCR results beforehand. Since patients' results were negative we were therefore unable to undertake sequence analysis. We presume that a larger study size is required if a suitable material was required for sequencing, and this is our next task in a future study.

CONCLUSION

Decreased phagocytosis could be attributed to by the finding in this study of reduced MR uptake. The increase in phagocytosis following IL-4 treatment suggests that MR was possibly downregulated prior this treatment, and hence uptake was reduced. The negative PCR result for *MRC1* might also suggest a possible mutation but this awaits further verification. Since the factors examined in this study relate mainly to pathophysiology the findings have provided useful information that could help in a better understanding of the complex issue of infection pathophysiology. Whether this could help in reducing the increased susceptibility to infection in PCA remains to be seen.

FUNDING

The research was partly funded by a grant from Research Directorate at Ministry of Higher Education (grant number 462/2010).

CONFLICTS OF INTEREST

All authors have none to declare.

REFERENCES

1. Sheer TA, Runyon BA. Spontaneous bacterial peritonitis. *Dig Dis*. 2005;23:39–46.
2. Conn HO. Spontaneous peritonitis and bacteraemia in Laennec's cirrhosis caused by enteric organisms: a relatively common but rarely recognized syndrome. *Ann Int Med*. 1964;60:568–580.
3. Conn HO. The prevalence of spontaneous bacterial peritonitis. In: *Spontaneous Bacterial Peritonitis: The Disease, Pathogenesis and Treatment*. New York: Marcel Dekker; 2005:62–70. Print ed, Taylor and Francis.
4. Hassner A, Kietter V, Schlag D, Yedwab M, Aronson M, Shibolet S. Impaired monocyte function in liver cirrhosis. *Br Med J*. 1981;282(12):62–1263.
5. Ahmed AMM, Bomford A, Nouri-Aria KT, Davies T, Smith R, Williams R. Peritoneal macrophages from patients with cirrhotic ascites show impaired phagocytosis and vigorous respiratory burst. *Results Immunol*. 2011;1:53–59.
6. Tanner AR, Arthur MJP, Wright R. Macrophage activation, chronic inflammation and gastrointestinal disease. *Gut*. 1985;25:760–783.
7. Strossel TP. The mechanism of phagocytosis. *J Reticuloendothel Soc*. 1976;19:237–245.
8. Harding C, Levy MA, Stahl P. Morphological analysis of ligand uptake and processing: the role of multivesicular endosomes and CURL in receptor ligand processing. *Eur J Cell Biol*. 1985;36(2):230–238.
9. Stahl P, Schlesinger PH, Sigardson E, Rodman JS, Lee YC. Receptor-mediated pinocytosis of mannose glycoconjugates by macrophages: characterization and evidence for receptor recycling. *Cell*. 1980;19(1):207–215.
10. Kang BK, Schlesinger LS. Characterization of mannose receptor-dependent phagocytosis mediated by mycobacterium tuberculosis lipoarabinomannan. *Infect Immun*. 1998;66(6):2769–2777.
11. Linehan SA, Martinez-Pomares L, da Silva RP, Gordon S. Endogenous ligands of carbohydrate recognition domains of the mannose receptor in murine macrophages, endothelial cells and secretory cells; potential relevance to inflammation and immunity. *Eur J Immunol*. 2001;31(6):1857–1866.
12. Stein M, Keshav S, Harris N, Gordon S. Interleukin 4 potently enhances alternative murine macrophage mannose receptor activity: a marker of immunologic macrophage activation. *J Exp Med*. 1992;176:287–292.
13. Cowan HB, Vick S, Conary JT, Shepherd VL. Dexamethazone upregulates MMR activity by increasing mRNA levels. *Arch Biophys*. 1992 July;296(1):314–320.
14. Shepherd VL, Hoidal JR. Clearance of neutrophil-derived myeloperoxidase by the macrophage mannose receptor. *Am J Respir Cell Mol Biol*. 1990;2(4):335–340.
15. East L, Isacke CM. The mannose receptor family. *Biochim Biophys Acta*. 2002;1572:364–386.
16. Zhang X, Jiang F, Wei L, et al. Polymorphic allele of human *MRC1* confer protection against tuberculosis in a Chinese population. *Int J Biol Sci*. 2012;8(3):375–382.
17. Alter A, de Leseleuc L, Van Thuc N, et al. Genetic and functional analysis of common *MRC1* exon 7 polymorphisms in leprosy susceptibility. *Hum Genet*. 2010;127:337–348.
18. Runyon BA. Management of adult patients with ascites due to cirrhosis. *Hepatology*. 2009;49(6):2087–2107.
19. Human Experimentation. Code of ethics of the World Medical association, declaration of helsinki. *Br Med J*. 1964;ii:177.

20. Eischen A, Duclos B, Schmitt-Goguel M, et al. Human resident peritoneal macrophages: phenotype and biology. *Br J Haematol*. 1994;88:712–722.
21. van Furth R, Raeburn IA, Van Zwet TL. Characteristics of human mononuclear phagocytes. *Blood*. 1979;54:485–498.
22. Mackaness GB. The immunological basis of acquired cellular resistance. *J Exp Med*. 1964;120:105–112.
23. Wright EL, Quenelle DC, Suling WJ, Barrow WW. Use of Mono Mac 6 human monocyctic cell line and J774 murine macrophage cell line in parallel antimicrobial drug studies. *Antimicrob Agents Chemother*. 1996;40(9):2206–2208.
24. Ragsdale RL, Grasso RI. An improved spectrofluorometry assay for quantitating yeast phagocytosis in cultures of murine peritoneal macrophages. *J Immunol Methods*. 1989;123:259–267.
25. Yamamoto Y, Klein TW, Friedman H. Involvement of mannose receptor in cytokine interleukin-1 β (IL-1 β), IL-6, and granulocyte-macrophage colony-stimulating factor responses, but not in chemokine macrophage inflammatory protein 1 β (MIP-1 β), MIP-2, and KC responses, caused by attachment of *Candida albicans* to macrophages. *Infect Immun*. 1997 March;65(3):1077–1082.
26. Hartmann C, Lennartz K, Ibrahim H et al. Stable 16-Year Storage of DNA Purified with the QIAamp® DNA Blood Mini Kit. Qiagen GmbH, Qiagen Straße 1, Hilden, Germany. www.qiagen.com.
27. Rimola A, Soto R, Bory F, Arroyo B, Píera C, Rodes J. Reticuloendothelial system phagocytic activity in cirrhosis and its relation to bacterial infections and prognosis. *Hepatology*. 1984;4(1):53–58.
28. Yousif-Kadaru AGM, Rajkovic IA, Wyke RJ, Williams R. Defects in serum attractant activity in different types of chronic liver disease. *Gut*. 1984;25:79–84.
29. Rajkovic IA, Williams R. Abnormalities of neutrophil phagocytosis, intracellular killing and metabolic activity of alcoholic cirrhosis and hepatitis. *Hepatology*. 1986;6:252–262.
30. Lahnborg G, Friman L, Berghem L. Reticuloendothelial function in patients with alcoholic liver cirrhosis. *Scand J Gastroenterol*. 1981;16:481–489.
31. Wyke RJ. Bacterial infections complicating liver disease. *Bailliere's Clin Gastroenterol*. 1989;3(1):187–210.
32. Caly WR, Strauss E. A prospective study of bacterial infections in patients with cirrhosis. *J Hepatol*. 1993;18(3):353–358.
33. Parsi MA, Atreja A, Zein NN. Spontaneous bacterial peritonitis: recent data on incidence and treatment. *Cleve Clin J Med*. 2004;71(7):569–576.
34. Shepherd VL, Abdolrasulnia R, Stephenson ID, Crenshaw C. Modulation of mannose receptor activity by proteolysis. *Biochem J*. 1990;270:771–776.
35. Abramson SL, Gallin JI. IL-4 inhibits superoxide production by human mononuclear phagocytes. *J Immunol*. 1990;144:625–630. *J Leukoc Biol* 2006; 80(3): 563–571.
36. Crawford RM, Finbloom DS, Ohara J, Paul WE, Meltzer MS. B cell stimulatory factor-1 (interleukin 4) activates macrophages for increased tumoricidal activity and expression of Ia antigens. *J Immunol*. 1987;139:135.
37. Hodge S, Hodge G, Scicchitano R, Reynolds PN, Holmes M. Alveolar macrophages from subjects with chronic obstructive pulmonary disease are deficient in the ability to phagocytose apoptotic airway epithelial cells. *Immunol Cell Biol*. 2003;81:289–296.
38. Hodges S, Hodges G, Jersmann H, et al. Azithromycin improves macrophage phagocytic function and expression of mannose receptor in COPD. *Am J Respir Crit Care Med*. 2008;178:139–148.
39. Frances R, Munoz C, Zapater P, et al. Bacterial DNA activates cell mediated immune response and nitric oxide overproduction in peritoneal macrophages from patients with cirrhosis and ascites. *Gut*. 2004;53:860–864.
40. Such I, Frances R, Munoz C, et al. Detection and identification of bacterial DNA in patients with cirrhosis and culture-negative, non-neutrocytic ascites. *Hepatology*. 2002;36:135–141.