Bacterial DNA in Patients with Cirrhosis and Noninfected Ascites Mimics the Soluble Immune Response Established in Patients with Spontaneous Bacterial Peritonitis

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Bacterial infections and severity of associated inflammatory reaction influence prognosis in patients with advanced cirrhosis. We compared the innate immune response to bacterial DNA (bactDNA) translocation with that caused by viable bacteria translocation in patients with spontaneous bacterial peritonitis and the relationship between the cytokine response and serum levels of bactDNA. The bactDNA translocation was investigated in 226 patients with cirrhosis and noninfected ascites, 22 patients with spontaneous bacterial peritonitis, and 10 patients with ascites receiving continuous norfloxacin. Serum and ascitic fluid tumor necrosis factor α, interferon-γ, interleukin-12, and nitric oxide metabolites were measured via enzyme-linked immunosorbent assay. Bacterial genomic identifications were made via amplification and sequencing of the 16S ribosomal RNA gene and digital quantization with DNA Lab-on-chips. The bactDNA was present in 77 noninfected patients (34%) and in all cases of spontaneous bacterial peritonitis, even in those with culture-negative ascitic fluid. No patient receiving norfloxacin showed bactDNA translocation. Levels of all cytokines were similar in patients with bactDNA translocation or spontaneous bacterial peritonitis and significantly higher than in patients without bactDNA or in those receiving norfloxacin. Serum bactDNA concentration paralleled levels of all cytokines and nitric oxide in a series of patients with bactDNA translocation or spontaneous bacterial peritonitis followed during 72 hours. Antibiotic treatment in the series of patients with spontaneous bacterial peritonitis did not abrogate bactDNA translocation in the short term. *Conclusion:* bactDNA translocation-associated cytokine response is indistinguishable from that in patients with spontaneous bacterial peritonitis and is dependent on bactDNA concentration. Norfloxacin abrogates bactDNA translocation and cytokine response. (HEPATOLOGY **2008;47:978-985.**)

Spontaneous bacterial peritonitis (SBP) is the most relevant infection in patients with cirrhosis and ascites.[1,2] The accepted pathogenic theory of SBP postulates that viable bacteria of enteric origin cross the intestinal wall in a process known as bacterial translocation, reach the mesenteric lymph nodes, and from there reach the systemic circulation and ascitic fluid (AF).[3-5]

We have recently reported the presence of circulating bacterial genomic fragments in blood and AF in a significant number of patients with decompensated cirrhosis without bacterial infections, indicating the existence of bacterial DNA (bactDNA) translocation.[6,7] Peritoneal macrophages from these patients were found to be primed to synthesize proinflammatory cytokines and nitric oxide (NO) *in vitro*.[6] There is no study, however, assessing the circulating serum levels or the AF concentration of cyto-

Abbreviations: AF, ascitic fluid; bactDNA, bacterial DNA; IFN-γ, interferon-γ; IL-12, interleukin-12; NO, nitric oxide; NOx, index of NO generation; SBP, spontaneous bacterial peritonitis; TNF-α, tumor necrosis factor α.

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kines and NO in relation with presence of bactDNA. Therefore, the potential relevance of bactDNA translocation is unknown. Such investigation is of clinical interest, because cytokines and NO are thought to be involved in the pathogenesis of several complications of cirrhosis, including circulatory dysfunction and hepatorenal syndrome.[7,8] Our recent observation that serum and AF complement activation products are increased to a similar degree in noninfected patients with cirrhosis and bactDNA than in patients with SBP[9] suggests that bactDNA translocation is associated with a great host response.

The aim of the present study was to prospectively investigate the relationship between bactDNA translocation and the inflammatory status that characterizes patients with decompensated cirrhosis. Specifically, we investigated: (1) if bactDNA translocation is associated with increased circulating serum levels and AF concentration of cytokines and NO metabolites in noninfected patients with cirrhosis and ascites; (2) if the degree of activation of these compounds correlates with the intensity of bacterial DNA translocation, as estimated by the serum levels of amplified bactDNA; and (3) if, as occurs with complement activation, the inflammatory response associated with bactDNA translocation is comparable to that observed in patients with viable bacterial translocation and SBP. The potential effect of selective intestinal decontamination with norfloxacin on bactDNA translocation and inflammatory response was also assessed.

Patients and Methods

Study Design. We conducted a prospective study in 258 patients with cirrhosis and ascites. Two hundred twenty-six patients had no bacterial infections and were not receiving selective intestinal decontamination with norfloxacin. They were admitted to the hospital for treatment of an uncomplicated ascites episode. Twenty-two additional patients had SBP. Finally, 10 patients admitted for the treatment of uncomplicated ascites were receiving long-term selective intestinal decontamination with norfloxacin for secondary prophylaxis of SBP. No patient included in the study had a bacterial infection other than SBP, upper gastrointestinal bleeding in the preceding 2 weeks, hepatocellular carcinoma, portal thrombosis, alcoholic hepatitis, previous liver transplantation, or previous transjugular intrahepatic portosystemic shunt. SBP was defined as the presence of $\geq$250 polymorphonuclear cells/μL in AF. The ethics committee of the hospital approved the study protocol, and all patients gave informed consent to participate in the study.

Blood and AF were obtained from all patients at admission and analyzed for routine biochemical and cytological studies. In patients with SBP, samples were obtained at the time of SBP diagnosis. Blood and AF cultures were performed in all cases as described.[10] Finally, aliquots of blood and AF were inoculated under aseptic conditions in sterile, rubber-sealed Vacutainer SST II tubes (BD Diagnostics, Belgium) that were never exposed to free air for qualitative analysis of the presence of bactDNA and determination of cytokine and NO metabolite levels.

Quantitative determination of amplified bactDNA concentration as well as measurement of cytokines and NO metabolites in serum were performed in 8 patients for 3 consecutive days—4 with uncomplicated ascites not receiving norfloxacin who were positive for bactDNA and 4 with SBP—to assess the relationship between the degree of inflammatory response and bactDNA levels.

Quantification and Identification of Amplified Bacterial DNA Fragments. To detect and identify the origin of bactDNA fragments in both blood and AF, a broad-range polymerase chain reaction and nucleotide sequencing analysis was performed according to the methodology previously described[11] in all patients. Briefly, DNA was isolated with QIAmp DNA Blood Mini Kit (Qiagen, Hilden, Germany) and a broad-range polymerase chain reaction amplification of the bacterial 16SrRNA gene conserved region was performed using the following primers: 5′-TTCCGGTTGATCCTGCCGGA-3′ as forward, and 5′-GGTTACCTTGTTACGACTT-3′ as reverse. Polymerase chain reaction amplicons were loaded into DNA Lab-on-chips (Agilent Technologies, Palo Alto, CA) and analyzed in an Agilent 2100 BioAnalyzer (Agilent Technologies). bactDNA fragments were purified with a QIAquick purification kit (QIAgen), and purified amplicons were used for the sequencing reactions with a Big Dye Terminator version 3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA). The same reverse oligonucleotide used for polymerase chain reaction amplification was used as a sequencing primer. The final product was purified via precipitation with ethanol-acetate and analyzed in the ABI-Prism 310 automated sequencer (Applied Biosystems). Sequences obtained were compared with the database from the National Center for Biotechnology Information (NCBI, www.ncbi.nih.gov) using the advanced BLAST search tool.

Quantification of Serum and Ascitic Fluid Cytokine Levels. Enzyme-linked immunosorbent assays for the quantitative measurement of tumor necrosis factor α (TNF-α), interferon-γ (IFN-γ), and interleukin-12 (IL-12) levels as representative cytokines of the proinflammatory immune response were performed in basal serum and

AF of patients using Human Quantikine kits (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. All samples were tested in triplicate and read at 490 nm in a Thermomax microplate reader (Molecular Devices, Sunnyvale, CA). The lower limits of detection of all cytokine assays were 5-10 pg/mL. Standard curves were generated for each plate, and the average zero standard optical densities were subtracted from the rest of standards, controls, and samples to obtain a corrected concentration for all cytokines.

NO Metabolite Levels in Serum and Ascitic Fluid. The sum of the NO metabolites nitrite (NO_2^-) and nitrate (NO_3^-) is widely used as an index of NO generation (NOx) and is expressed as NOx levels.[12] NOx levels in serum samples were calculated by measuring conversion of NO_3^- to NO_2^- by the enzyme nitrate reductase via enzyme-linked immunosorbent assay (R&D Systems) based on the Griess reaction that absorbs visible light at 540 nm. All samples were tested in triplicate; standard curves were generated for each plate, and the average zero standard optical densities were subtracted from the rest of standards, controls, and samples to obtain a corrected NO_x concentration.

Statistical Analysis. Continuous variables are expressed as the mean ± standard deviation; categorical variables are expressed as frequency or percentages. Statistical differences of basal characteristics between groups were analyzed using the Fisher's exact test for categorical data and the Mann-Whitney *U* test for quantitative data. Bivariate correlations between continuous variables were calculated using the Spearman test. Statistical differences between cytokines were analyzed via analysis of variance with Bonferroni correction for multiple comparisons. A multiple regression analysis was performed to evaluate the effect of independent variables (group of study, timepoint of sample collection, and amount of serum bactDNA) on TNF-α, IFN-γ, IL-12, and NO. All reported *P* values are 2-sided; a *P* value of less than 0.05 was considered statistically significant. All calculations were performed using SPSS version 12.0 software (SPSS Inc., Chicago, IL).

Results

Classification and Clinical and Laboratory Characteristics of Patients. Patients were divided into 4 groups according to bactDNA and clinical characteristics at admission. Group I comprised 149 patients with noninfected AF not receiving norfloxacin who were negative for bactDNA. Cytokines and NO metabolites were determined in serum and AF from a random sample of 50 patients from this group. Group II comprised 77 patients with noninfected AF not receiving norfloxacin but who were positive for bactDNA. Group III and group IV comprised the 22 patients with SBP and the 10 patients with uncomplicated ascites receiving norfloxacin, respectively. All patients from group III were positive for bactDNA in serum and AF. In contrast, none of the patients in group IV showed bactDNA either in serum or AF. The period of norfloxacin administration was shorter than 14 months in all patients from group IV.

The clinical and laboratory characteristics of all 4 groups of patients are detailed in Table 1. Total white blood cells and polymorphonuclear cells in serum and AF were statistically increased in patients with SBP. There were no significant differences between groups in relation to the remaining measurements.

Bacterial species origin of the detected bactDNA were coincident in serum and AF for a given patient in all cases. Four species were identified in group II patients (*Escherichia coli* in 41, *Klebsiella* in 10, *Staphylococcus aureus* in 13, and *Enterococcus faecalis* in 13) and group III patients (*E. coli* in 12, *Klebsiella* in 3, *S. aureus* in 4, *Streptococcus pneumoniae* in 2, and *E. faecalis* in 1). All 8 patients from groups II and III included in the sequential study showed the same bacterial species in serum during the 72 hours of follow-up (*E. coli* in 5, *S. aureus* in 2, and *S. pneumoniae* in 1).

Nine patients with SBP were culture-positive and 13 were culture-negative. The clinical and analytical characteristics of these 2 subsets of patients are shown in Table 2. Model for End-Stage Liver Disease score and total white blood cell and polymorphonuclear cell counts in AF were significantly higher in patients with culture-positive SBP. bactDNA identification revealed no differences between species found in patients with culture-negative SBP (*E. coli* in 8, *Klebsiella* in 2, *S. aureus* in 2, and *S. pneumoniae* in 1) and culture-positive SBP (*E. coli* in 4, *Klebsiella* in 1, *S. aureus* in 2, *S. pneumoniae* in 1, and *E. faecalis* in 1). In 8 patients with culture-positive SBP organisms isolated in microbiological cultures corresponded to these identified by nucleotide sequencing. In the only case with discrepant results, *S. aureus* was identified via sequencing analysis and *S. pneumoniae* via microbiological culture.

Blood and Ascitic Fluid Levels of TNF-α, IFN-γ, and IL-12. Results are shown in Table 3. All cytokines studied (TNF-α, IFN-γ, and IL-12) were significantly increased in serum and AF in patients from group II and III as compared with patients from groups I and IV, there being no differences between groups II and III or between groups I and IV. Patients with culture-positive SBP showed values of cytokines similar to patients with culture-negative SBP (Table 3). In these patients, no correlation was observed between AF polymorphonuclear cell count and AF concentration of cytokines.

Table 1. Clinical and Analytical Characteristics of All 4 Groups of Patients Included in the Study

	Group I (n = 50)	Group II (n = 77)	Group III (n = 22)	Group IV (n = 10)
Age (years)	60.05 ± 11.60 (35–78)	63.22 ± 11.53	57.14 ± 11.42*	64.62 ± 4.47
Male sex, n (%)	14/7	36/41	12/10	6/4
Alcohol ethiology, n (%)	33 (66.0%)	30 (38.9%)	8 (38%)	6 (60.0%)
Previous episodes of ascites, n (%)	5 (24.0%)	29 (37.6%)	6 (27.2%)	10 (100.0%)
Child-Pugh A/B/C (n)	0/32/18	1/41/35	0/15/7	2/6/2
MELD mean score	12.9 ± 4.5	16 ± 8	19 ± 10	12.7 ± 5.1
Mean arterial pressure (mm Hg)	85.5 ± 15.9	83.03 ± 9.47	83.90 ± 14.91	82.7 ± 12.2
Bilirubin (mg/dL)	3.3 ± 2.1	3.33 ± 3.60	3.82 ± 3.23	2.5 ± 1.3
Albumin (g/dL)	2.8 ± 0.6	2.75 ± 0.56	3.12 ± 1.07	2.6 ± 0.8
Quick (%)	61.2 ± 14.6	59.19 ± 15.80	52.16 ± 21.47	60.0 ± 10.9
Serum creatinine (mg/dL)	0.8 ± 0.4	1.02 ± 0.60	1.24 ± 1.03	0.8 ± 0.2
Serum sodium (mEq/L)	135.0 ± 5.1	134.20 ± 5.44	131.26 ± 8.84	133.2 ± 4.1
Blood WBC/mm^3	5576.4 ± 2389.3	6205.57 ± 3409.48	8626.19 ± 5234.98*	4883.7 ± 2210.6
Total blood PMNs/mm^3	4920 ± 2995	4471.15 ± 2915.83	7018.11 ± 4732.41*	3214 ± 1818
Total AF proteins (g/dL)	1.7 ± 0.8	1.58 ± 0.76	1.74 ± 1.17	1.64 ± 1.2
AF WBC/mm^3	207 ± 185.9	165 ± 180	4395.88 ± 5583.27*	135.0 ± 123.7
Total AF PMNs/mm^3	39.2 ± 42.9	46.4 ± 79.2	3917.41 ± 5322.22*	34.6 ± 33.5

Values are expressed as the mean ± standard deviation. Group I: patients without BT. Group II: patients with bacterial DNA translocation. Group III: SBP patients. Group IV: SID patients.

Abbreviations: MELD, model for end-stage liver disease; PMN, polymorphonuclear cell; WBC, white blood cell.

*$P < 0.05$ compared with the remaining groups.

Results of the Sequential Studies Performed in Patients from Groups II and III. Figures 1 and 2 show 2 representative patients from groups II and III, respectively. bactDNA was detectable in the blood of all 8 patients during the 72-hour period of follow-up. There was a parallel behavior between serum bactDNA concentration and serum levels of all cytokines that was also observed with respect to the serum levels of NO metabolites. Cytokine and NOx serum levels and the corresponding bactDNA values at all time points in patients included in the sequential studies are scattered in Fig. 3. A close direct correlation was found between the serum levels of cytokines and NO metabolites and the serum concentration of bactDNA. There were no significant differences in serum concentration of bactDNA between patients included in the sequential analysis from groups II and III (35.9 ± 12.29 ng/μL versus 33.23 ± 10.31 ng/μL).

Table 2. Clinical and Analytical Characteristics of SBP Patients, Distributed According to the Result of Microbiological Culture

	SBP		
	AF negative-culture (n = 13)	AF positive-culture (n = 9)	*P* Value
Age (years)	59.77 ± 1.50	52.88 ± 10.59	NS
Male sex, n (%)	7/6	5/4	NS
Alcohol ethiology, n (%)	4 (30.7%)	4 (50%)	NS
Previous episodes of ascites, n (%)	4 (30.7%)	2 (25%)	NS
Child-Pugh A/B/C (n)	0/9/4	0/6/3	NS
MELD mean score	16 ± 9	25 ± 12	0.02
Mean Arterial Pressure (mm Hg)	94.32 ± 7.60	68.33 ± 11.79	0.01
Bilirubin (mg/dL)	2.41 ± 2.14	6.24 ± 3.47	NS
Albumin (g/dL)	2.90 ± 1.26	3.55 ± 0.64	NS
Quick (%)	62.35 ± 20.26	35.17 ± 9.47	0.02
Serum creatinine (mg/dL)	1.29 ± 1.19	1.18 ± 0.78	NS
Serum sodium (mEq/L)	131.22 ± 9.60	131.34 ± 8.06	NS
Blood WBC/mm^3	9627.69 ± 5474.69	6998.75 ± 4693 ± 56	NS
Total blood PMNs/mm^3	7694.50 ± 4851.87	5858.57 ± 4641.74	NS
Total AF proteins (g/dL)	1.94 ± 1.32	1.36 ± 0.76	NS
AF WBC/mm^3	1450.90 ± 1294.90	9795.00 ± 6506.83	0.01
Total AF PMNs/mm^3	1190.35 ± 1232.80	8917.00 ± 6422.40	0.01

Values are expressed as the mean ± standard deviation.

Abbreviations: MELD, model for end-stage liver disease; PMN, polymorphonuclear cell; WBC, white blood cell.

Table 3. Serum and AF Cytokine Levels in All 4 Groups of Patients Included in the Study and in the Subgroups of SBP Patients, According to the Result of the AF Microbiological Culture

	Group I (n = 50)	Group II (n = 77)	Group III (n = 22)	Group IV (n = 10)
Patients included in the study				
Serum TNF-α (pg/mL)	141.0 ± 41.9	342.5 ± 107.3*	372.0 ± 100.0*	143.7 ± 36.5
Serum IFN-γ (pg/mL)	191.0 ± 49.2	322.5 ± 78.6*	335.2 ± 76.3*	210.0 ± 47.6
Serum IL-12 (pg/mL)	380.7 ± 89.2	614.2 ± 197.8*	518.2 ± 134.8	331.2 ± 92.3
AF TNF-α (pg/mL)	160.9 ± 38.3	430.2 ± 144.9*	533.5 ± 116.9*	206.3 ± 10.31
AF IFN-γ (pg/mL)	240.5 ± 65.3	370.3 ± 32.2*	404.9 ± 76.6*	255.5 ± 75.3
AF IL-12 (pg/mL)	429.4 ± 112.5	794.4 ± 225.3*	885.2 ± 110.3*	358.0 ± 125.1
	Group III (SBP) (n = 22)	**Culture-negative SBP (n = 13)**		**Culture-positive SBP (n = 9)**
Subgroups of SBP patients†				
Serum TNF-α (pg/mL)	372.04 ± 100.07	362 ± 69.86		382.09 ± 127.86
Serum IFN-γ (pg/mL)	335.23 ± 76.37	323.99 ± 66.44		346.46 ± 86.28
Serum IL-12 (pg/mL)	518.24 ± 134.87	553.72 ± 147.3		482.76 ± 94.45
AF TNF-α (pg/mL)	533.57 ± 116.91	521.38 ± 104.61		553.4 ± 139.87
AF IFN-γ (pg/mL)	404.95 ± 76.64	394.48 ± 66.66		421.95 ± 92.89
AF IL-12 (pg/mL)	885.24 ± 110.36	870 ± 112.71		909.99 ± 109.04

All values are expressed as the mean ± standard deviation.

*$P < 0.05$ compared with groups I and IV.

†P value not significant.

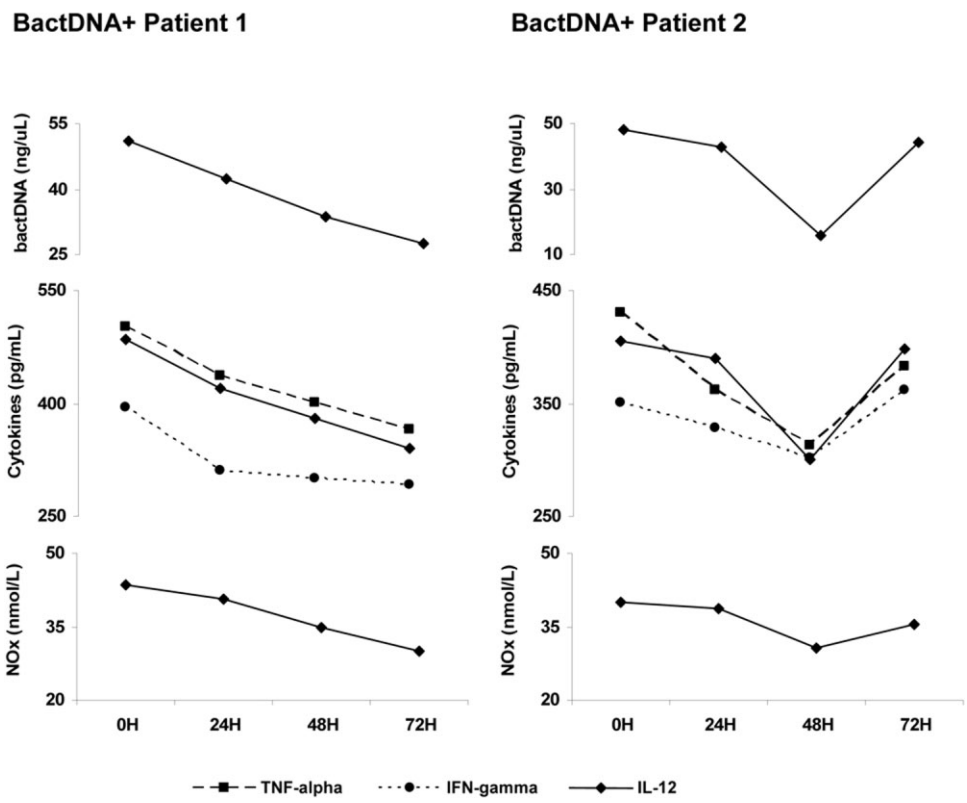

Fig. 1. Results of a 3-day study performed in 4 consecutively admitted patients with bactDNA translocation. Serum bactDNA concentrations were measured and correlated with serum TNF-α, IFN-γ, IL-12, and NO expression levels. The results of 2 representative patients are shown.

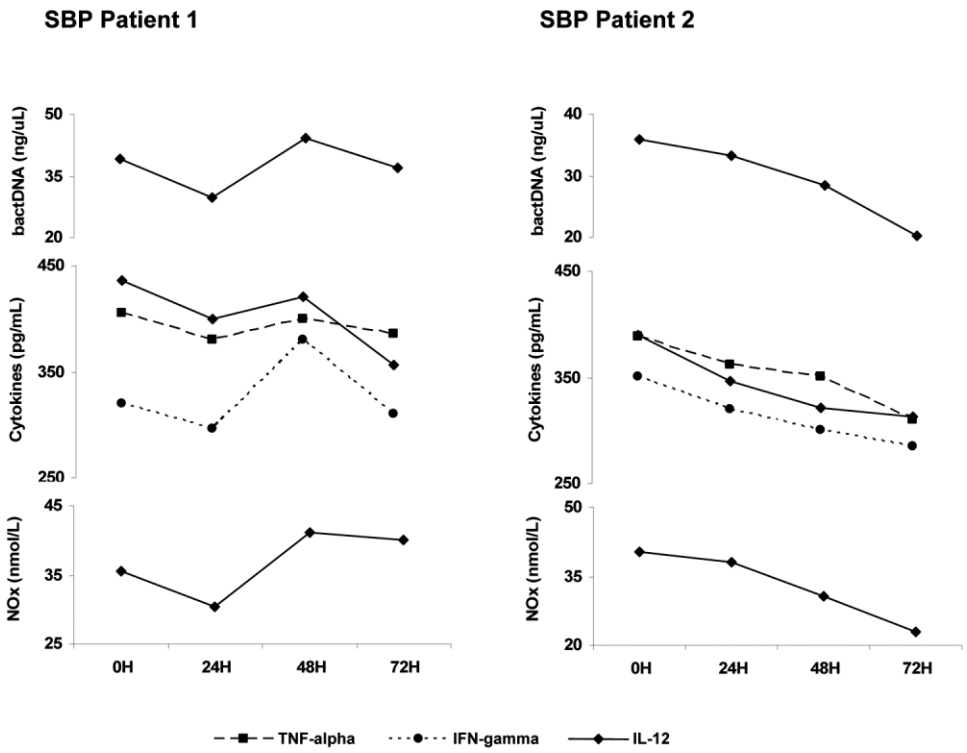

Fig. 2. Results of a 3-day study performed in 4 consecutively admitted patients with SBP. Serum bactDNA concentrations were measured and correlated with serum TNF-α, IFN-γ, IL-12, and NO expression levels. The results of 2 representative patients are shown.

Discussion

The present study shows that translocation of bactDNA is a frequent event in patients with cirrhosis and ascites. The prevalence of bactDNA in serum and ascitic fluid in the 226 patients without bacterial infections or treatment with norfloxacin included in our investigation was 34%. This high incidence of translocation of bactDNA confirms previous studies from our laboratory and is in keeping with the results of a recent study by Albillos et al.[13] assessing serum levels of LBP, an endotoxin transport protein that accurately estimates endotoxemia, in this type of patient. LBP was increased in approximately 30% of cirrhotic patients with ascites without bacterial infection. Translocation of products of bacterial origin is, therefore, frequent in decompensated cirrhosis.

There are several studies in patients with decompensated cirrhosis showing increased serum and ascitic fluid levels of cytokines and NO. The mechanisms of this systemic inflammatory reaction are unclear. The results of the present study showing that translocation of bactDNA is associated with a significant inflammatory reaction, as manifested by increased plasma and AF levels of proinflammatory cytokines (TNF-α, INF-γ, and IL-12) and NO metabolites, suggest that translocation of products of bacterial origin could be related to this feature. Interestingly, the cytokine and NO response associated with bactDNA translocation in our patients without bacterial infections was similar to that in patients with SBP, suggesting that translocation of bacterial products has a proinflammatory effect as intense as that induced by translocation of viable bacteria.

A close direct relationship between the serum concentration of cytokines and NO metabolites and the serum concentration of bacterial DNA was found in our patients without bacterial infections. This further supports that the inflammatory reaction observed in patients with decompensated cirrhosis was related to translocation of bacterial products from the intestinal lumen into the systemic circulation. Although bactDNA has powerful proinflammatory activity *in vitro*[6] and in experimental animals,[14,15] other products of bacterial origin such as endotoxin, which are also present at high concentration in serum and AF of patients with decompensated cirrhosis without bacterial infection, could also participate in the inflammatory reaction observed in these patients.

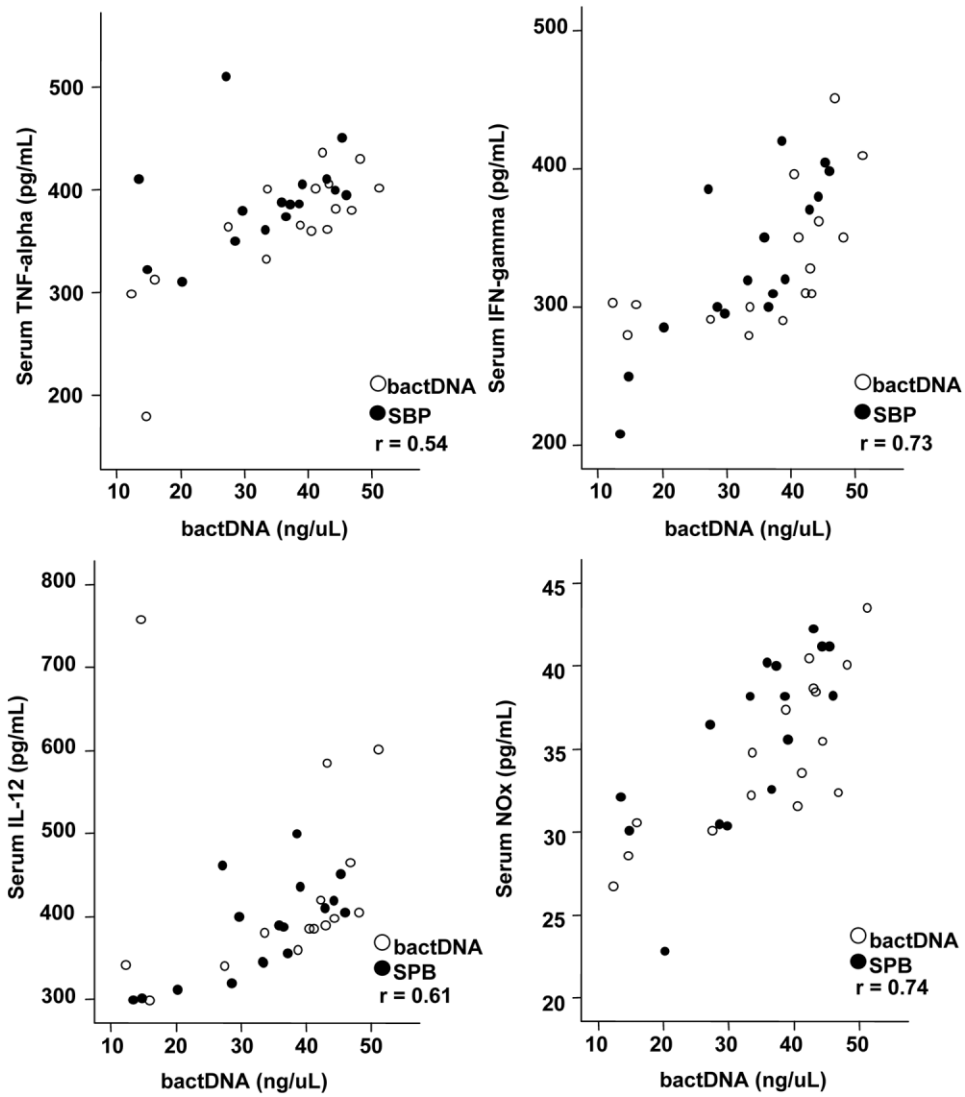

Fig. 3. Analysis of study groups, time points of sample collection, and amount of serum bacterial DNA in each of 3 cytokine and NO levels in serum from the overall series of patients included in the 3-day study.

Translocation of bacterial products may be an important clinical event in patients with cirrhosis and ascites. As indicated previously, it promotes a marked increase in the circulating levels of cytokines and NO production. In the study of Albillos et al.,[13] the inflammatory reaction observed in patients with increased LBP levels was associated with a significant deterioration of circulatory function, which improved following the diminution of endotoxin translocation by norfloxacin administration. Finally, the findings of a recent randomized placebo-controlled study by Fernandez et al.[16] suggest that the prophylactic administration of norfloxacin to patients with cirrhosis and ascites without bacterial infections prevents hepatorenal syndrome and improves survival mainly by decreasing the translocation of bacterial products. The observation in the current study that norfloxacin abrogates bactDNA translocation and the associated inflammatory response is in line with the studies of Albillos et al. and Fernandez et al.[13,16]

The suppression of translocation of bactDNA and endotoxin during long-term administration of norfloxacin is remarkable, particularly if we consider that a significant proportion of patients in this situation select quinolone-resistant bacteria in the fecal flora.[17] There are two possible explanations for this. First, the development of bacterial infections due to quinolone-resistant gram-negative bacteria appear only after a prolonged period of norfloxacin administration,[18] and in our series, the period of norfloxacin administration was shorter than 14 months in all cases. Second, although long-term norfloxacin administration may promote the selection of quinolone-resistant bacteria in the fecal flora, it may also reduce the

absolute amount of gram-negative bacteria in the intestinal lumen, and, therefore, the release of bacterial products and absorption. In fact, despite the development of quinolone-resistant bacteria in the fecal flora, norfloxacin continues to be extremely effective in the prophylaxis of SBP.[16]

In our study, the serum concentration of bactDNA varied from patient to patient and during time in a given patient. Remarkably, this was closely related to the serum levels of cytokines and NO metabolites. This suggests that the translocation of bacterial products and the secondary inflammatory reaction is a highly dynamic process that may vary with factors affecting intestinal function, including motility, permeability, local defense mechanisms, and bacterial overgrowth.

There are two other important observations of the present study. The first observation is the high concordance between microbiological and molecular identification of bacteria in patients with culture-positive SBP. We cannot provide an explanation for the single case of discrepancy other than a bacterial misidentification by culture. In this case, we consider sequencing data strongly reliable since a 99.94% of sequence alignment was obtained in three different DNA samples from that patient. Molecular identification of bactDNA in patients with culture-negative SBP disclosed similar types of bacteria as in patients with culture-positive SBP, and the amount of amplified bactDNA was similar in both instances. This suggests that the reason why bacteria do not grow in culture in a subset of patients with SBP is more related to their AF characteristics that may better opsonize bacteria, making them incapable of growing in culture than to differences in the intensity of bacterial translocation. The second observation relates to the persistence of bactDNA levels in the serum of patients with SBP after initiating cefotaxime administration. This may explain the persistence of high levels of NO metabolites days following infection resolution in patients with SBP.[19] A low effect of cefotaxime on the intestinal flora due to rapid absorption previously excreted in bile through enterohepatic circle[20] or the liberation of bactDNA from destroyed bacteria by effect of the administered antibiotic are two possible explanations.

In conclusion, the results of the present study show that bactDNA translocation is a frequent event in patients with cirrhosis and ascites without bacterial infections and that it is associated with a significant inflammatory reaction, similar to that observed in patients with SBP. The degree of this inflammatory reaction parallels the serum levels of bactDNA. BactDNA is constantly found in patients with SBP, there being a great concordance between the identification of the causative organisms by automated nucleotide sequencing analysis and microbiological techniques. In patients with culture-negative SBP, the causative organisms identified by automated nucleotide sequencing are comparable to those found in culture-positive SBP. Chronic administration of norfloxacin abrogates bactDNA translocation as well as the associated inflammatory reaction.

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