Attenuated antigen-specific T cell responses in cirrhosis are accompanied by elevated serum interleukin-10 levels and down-regulation of HLA-DR on monocytes

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Abstract

Background: Advanced liver disease predisposes to bacterial translocation and endotoxaemia which can contribute to elevated circulating levels of IL-10 and down-regulation of MHC class II on antigen-presenting cells. We sought to evaluate antigen-specific T-cell responses toward common viral antigens in order to investigate defects in cellular immunity in cirrhosis.

Methods: Peripheral blood was obtained from 22 cirrhotic patients with systemic inflammation, 13 cirrhotic patients without systemic inflammation and 14 healthy controls. C-reactive protein was used as an indicator for systemic inflammation using a cut-off of 10 mg/L. Intracellular Th1 cytokines were quantified after T cell-stimulation with the viral peptides EBNA1 and BZLF1 or the bacterial superantigen SEB by flow cytometry. Serum levels of lipopolysaccharide-binding protein (LBP) and IL-10 were quantified by ELISA.

Results: Compared to healthy controls, patients with cirrhosis had higher circulating levels of LBP and IL-10, an expansion of peripheral blood CD14⁺ monocytes with low HLA-DR expression and an increased fraction of CD25-positive CD4⁺ and CD8⁺ T cells. These findings were most pronounced in cirrhotic patients with systemic inflammation but fell short of reaching statistical significance when comparing against cirrhotic patients without systemic inflammation. In the former group TNF-α production in CD4⁺ and CD8⁺ T cells was reduced after stimulation with SEB, whereas there was no significant difference between the total cohort of cirrhotic patients and controls. After stimulation with the overlapping peptide pools for viral antigens EBNA1 and BZLF1, the number of responding T cells and the amount of TNF-α or IFN-γ production did not differ between the three pre-defined groups. However, cirrhotic patients with null-responses to EBV peptides had significantly higher serum IL-10 levels than responders to EBV peptides. Furthermore, TNF-α production in responding T cells was attenuated in patients with a high frequency of CD14⁺ HLA-DR⁻ monocytes.

Conclusion: Our data suggest that bacterial translocation, endotoxaemia, inflammation and T cell activation in cirrhosis are accompanied by an increase in circulating anti-inflammatory cytokines, reduced monocytic MHC class II expression and attenuated cytokine production in T cells. These changes are likely to contribute to altered adaptive immune responses during infection or after vaccination.

Keywords: Cirrhosis, Adaptive immunity, Cellular immunity, Virus-specific T cell responses, Bacterial translocation, Interleukin-10

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Background

Alterations of the immune system are very common in patients with end-stage liver disease and associated with an increased risk of infection and death [1-3]. Functional abnormalities of neutrophils and macrophages [4-8], natural killer cells [9], and the complement system [10] contribute to impaired innate immune responses and have been well described. There is also evidence to suggest that the adaptive immune response is defective in chronic liver disease, and cell-mediated immune responses after vaccination for hepatitis A, hepatitis B, or influenza are frequently attenuated in patients with decompensated liver cirrhosis. [11,12]. Although T cell responses towards hepatitis B virus and hepatitis C virus have been extensively studied [13,14], little is known about the T cell response to persistent other viruses such as Epstein-Barr Virus (EBV) as a marker of cellular immune function in cirrhosis.

In general, unresponsiveness of the adaptive immune system to antigens can be attributed to either a defect in antigen presentation by dendritic cells and macrophages, or to a suppressed or deficient T cell response. Cirrhosis predisposes to recurrent episodes of subclinical translocation of intestinal bacteria and bacterial products resulting in increased levels of endotoxin (lipopolysaccharide [LPS]) and tumour necrosis factor-alpha (TNF-α) [15]. Augmented by TNF-α, LPS is able to induce the secretion of interleukin-10 (IL-10) from Kupffer cells into the circulation [16,17], which can act as an inhibitor of cellular proliferation and T cell-mediated cytokine responses. This occurs in part through a down regulation of major histocompatibility complex (MHC) class II on monocytes/macrophages and inhibition of T-cell co-stimulatory pathways [18,19]. Indeed, reduced expression of MHC class II on monocytes has been observed in critically ill patients with cirrhosis [20,21] and in those with acute-on-chronic liver failure [22] and correlates with increased mortality. However, reduced monocyte HLA-DR expression also occurs in non-cirrhotic patients after trauma, during the systemic inflammatory response syndrome (SIRS) and sepsis [23,24]. SIRS is a frequent finding in patients with cirrhosis and is itself associated with poor outcome. [25] However, it is not known whether these phenotypic alterations occur in less severely ill cirrhotic patients (without SIRS) in association with endotoxaemia, and if present whether they contribute to functional T cell impairment.

To investigate the cellular immune status in cirrhosis, we studied T cell responses in vitro and their association with markers of bacterial translocation, serum IL-10, monocyte HLA-DR expression and T cell subsets in cirrhotic patients without SIRS. Healthy volunteers served as our controls.

Methods

Setting and participants

35 patients with liver cirrhosis presenting to our department between October 2009 and March 2010 and 14 self-declared healthy individuals were included. All subjects provided informed consent and local ethics committee approval was obtained. Liver cirrhosis was confirmed histologically, or through a combination of clinical, biochemical, and imaging data at the discretion of the investigator. Individuals were excluded if they met two or more criteria for systemic inflammatory response syndrome (SIRS) [26], if they were receiving immunosuppressive therapy, underwent surgical intervention within the last month, consumed alcohol within the last three days, experienced an episode of gastrointestinal bleeding, or underwent any endoscopic intervention within the last three days before inclusion. C-reactive protein (CRP) concentrations were measured as an indicator for systemic inflammation by routine laboratory analysis. Using the cut-off of 10 mg/l for cirrhotic patients defined by Papp et al. [27].

Blood sampling and flow cytometry

9 ml heparinised whole blood and 9 ml EDTA whole blood samples (Sarstedt AG & Co, Nümbrecht, Germany) were collected, stored at 4°C, measured within 2 hours for cell surface receptors and stimulated with peptide pools within 3 hours. Briefly, 100 μl EDTA whole blood was stained for 20 min with fluorochrome-labelled monoclonal antibodies against HLA-DR (FITC, F7266; Dako, Hamburg, Germany), CD14 (PE, F0844; Dako), CD4 (FITC; F0766; Dako), CD8 (PE; R0806; Dako) and or CD25 (APC; 17–0259; eBioscience, Frankfurt, Germany) and washed in FACS buffer solution (PBS with 0.25% BSA and 0.02% sodium azide). When indicated, red blood cells were lysed using FACS lysing solution (BD Biosciences, Heidelberg, Germany) prior to staining. More than 50,000 PBMC were collected on a BD LSR II flow cytometer (BD Biosciences). Monocytes and lymphocyte populations were identified by the use of forward and right angle light scatter and by CD14, CD4 or CD8, respectively. The percentage of HLA-DR-expressing monocytes was calculated as the percentage of HLA-DR+ cells of total CD14+ cells; the percentage of CD25-expressing T cells was calculated as the percentage of CD25+ T cells of the CD4+ or CD8+ T cell population.

For intracellular cytokine staining, 500 μl heparinised whole blood was stimulated with peptide mixtures for 6 h at 37°C and 5% CO₂ in the presence of 1 μg/ml co-stimulatory monoclonal antibodies to CD28 (L293) and CD49d (L25; BD Biosciences), and 10 μg/ml Brefeldin A (Sigma-Aldrich, Hamburg, Germany) as golgi-stop. The peptide pools (15-mers with 11 amino-acid overlaps) for Epstein-Barr nuclear antigen 1 (EBNA1) (130-093-613; Sigma-Aldrich, Hamburg, Germany) and for the other viruses such as Epstein-Barr Virus (EBV) as a marker of cellular immune function in cirrhosis.
of the instrument described [28]. Cytometer setup and daily quality control stability was performed by the calculation of non-discrete variables. Statistical analysis for nonparametric Spearman’s correlation coefficient \( R_s \) or by Pearson’s linear correlation coefficient \( R \). Statistical calculations were performed using SPSS v.16 (SPSS Inc., Chicago, IL, USA) and Graphpad Prism v. 5 (La Jolla, CA, USA).

**Results**

**Patient characteristics**

22 (63%) patients with cirrhosis had elevated CRP levels >10 mg/l. Causes were bacterial infections in 5 patients (2× SBP, 3× culture-positive urinary tract infections), inflammatory skin lesions in 3 patients, sterile pyuria in 4 patients and oedematous pancreatitis in 1 patient. An inflammatory focus could not be detected in 9 patients. None of the 13 cirrhotic patients with low CRP levels presented with clinically manifested bacterial infection.

Cirrhotic patients that presented with signs of systemic inflammation more often had advanced liver disease indicated by ascites (Table 1). As expected, LBP serum levels were elevated in cirrhosis, but they did not differ with regards to the inflammation status as indicated by elevated CRP levels (Figure 1A). Concomitantly, IL-10 serum concentrations were elevated in patients with cirrhosis (Figure 1B) showing a trend of a positive linear correlation between serum IL-10 and LBP (Figure 1C).

**MHC class II expression on CD14\(^+\) monocytes is reduced in cirrhosis**

The fraction of circulating monocytes within leukocytes was 6% (range: 3–12%) in controls, 8% (range: 3–20%) in cirrhotic patients without inflammation and 9% (range: 5–23%) in patients with cirrhosis and inflammation \( (P<0.008; \) Kruskal-Wallis test). In patients with cirrhosis, HLA-DR expression on CD14\(^+\) monocytes was reduced in cirrhosis (median 90.6%; range: 29.3%–100%) compared to controls (median 99.9%; range: 93.7%–100%) \( (P<0.0001) \), whereas there was no significant difference with respect to the inflammation status in cirrhosis (Figures 2A and 2B).

Accordingly, the surface HLA-DR expression on CD14\(^+\)HLA-DR\(^-\) monocytes was markedly reduced in patients with cirrhosis as shown by a decreased mean fluorescence intensity (MFI; Figure 1C). However, there was no significant correlation between the fraction of HLA-DR\(^+\) monocytes or HLA-DR MFI on CD14\(^+\) monocytes with serum IL-10, serum LBP, Child-Pugh score or MELD score \( (P>0.4) \).

The fraction of CD25\(^+\) T cells is increased in cirrhosis

Inversely to the monocyte fraction, circulating lymphocytes were lower in patients with cirrhosis and inflammation (median 26%; range 14%–79%) than in patients with cirrhosis without inflammation (38%; range 21%–95%) and in control subjects (51%; range 26%–66%) \( (P=0.014; \) Kruskal-Wallis test). Although the CD4/CD8 ratio did not differ

**Measurement of circulating interleukin-10 (IL-10) and lipopolysaccharide-binding protein (LBP)**

Determination of serum concentrations of IL-10 and LBP was performed in 36 and 39 subjects, respectively. Serum LBP concentrations was measured in duplicate with the HK315 human LBP sandwich ELISA (Hycult Biotech, Uden, Netherlands; lower limit of detection 1 ng/ml) after dilution according to the manufacturer’s instructions. The standard curve was created from a 6-fold series of dilutions of a 50 \( \mu \)g/ml standard in duplicate. Serum IL-10 was determined using the ELISA MAX human IL-10 assay (Biologend, San Diego, CA, USA; lower limit of detection 1 ng/ml) in duplicate according to manufacturer’s instructions. The standard curve was created from a 6-fold series of dilutions of a 250 pg/ml stock concentration. Measurements were performed using a photometric plate reader (VICTOR, Wallac, USA) at 460 nm.

**Statistical analysis**

Baseline patient characteristics were reported as median and range for continuous variables, or as a frequency for discrete variables. Statistical analysis for nonparametric data was performed by using Mann–Whitney \( U \) test, Kruskal-Wallis test with post hoc Dunn’s test or Jonckheere-Terpstra test as appropriate. Bivariate correlation was either performed by the calculation of non-parametric Spearman’s correlation coefficient \( R_s \) or by Pearson’s linear correlation coefficient \( R \).
between the three groups (P=0.439), patients with cirrhosis had a significantly increased fraction of CD25+ CD4+ T cells and CD25+ CD8+ T cells, a finding most pronounced in patients with evidence of inflammation (Figures 3A, 3B and 3C). The fractions of CD25-positive CD4+ and CD25+ CD8+ T cells correlated significantly in a linear fashion (R=0.433; P=0.01) (Figure 3D). Moreover, the fraction of CD25+ CD4+ cells positively correlated with serum IL-10 (Rs=0.508; P=0.011) and tended to correlate with serum LBP (Rs=0.366; P=0.060) (Figures 3E and 3F).

### Table 1 Baseline characteristics

| Characteristic                              | Controls (N=14) | Patients with cirrhosis without inflammation (N = 13) | Patients with cirrhosis and inflammation (N = 22) | P value |
|---------------------------------------------|-----------------|------------------------------------------------------|---------------------------------------------------|---------|
| Years of age – median (range)               |                 |                                                      |                                                   | <0.001  |
| Male sex – no. (%)                          |                 |                                                      |                                                   | n.s.    |
| Positive Epstein-Barr virus serology – no. (%) |                 |                                                      |                                                   | n.s.    |
| LBP serum concentration (μg/ml) – median (range) |                 |                                                      |                                                   | 0.002   |
| IL-10 serum concentration (pg/ml) – median (range) |                 |                                                      |                                                   | 0.001   |
| Alcoholic aetiology of cirrhosis – no. (%)  |                 |                                                      |                                                   | n.s.    |
| Child-Pugh stage A/B/C – no.                |                 |                                                      |                                                   | n.s.    |
| Child-Pugh score – median (range)           |                 |                                                      |                                                   | 0.005   |
| Ascites – no. (%)                           |                 |                                                      |                                                   | 0.007   |
| MELD score – median (range)                 |                 |                                                      |                                                   | n.s.    |

| Laboratory values - median (range)          |                 |                                                      |                                                   |         |
| Total serum bilirubin - μmol/l              |                 |                                                      |                                                   | n.s.    |
| International normalized ratio              |                 |                                                      |                                                   | 0.029   |
| Creatinine - μmol/l                         |                 |                                                      |                                                   | n.s.    |
| Aspartate aminotransferase - μmol/bxs       |                 |                                                      |                                                   | n.s.    |
| Alanine aminotransferase - μmol/bxs         |                 |                                                      |                                                   | n.s.    |
| White blood cell count – Gpt/l              |                 |                                                      |                                                   | n.s.    |
| C-reactive protein – mg/l                   |                 |                                                      |                                                   | <0.001  |
| Serum Albumin – g/l                         |                 |                                                      |                                                   | n.s.    |

* P values in Kruskal-Wallis test or Mann–Whitney U test for continuous data or Fisher’s exact test for discrete data are indicated. * indicates a statistical significant difference between cirrhotic patients with and without elevated C-reactive protein levels.

**MELD:** Model for end-stage liver disease score, n.s.: not significant (P ≥ 0.05).

![Figure 1](http://www.biomedcentral.com/1471-230X/13/37)

**Figure 1** Serum levels of lipopolysaccharide-binding protein (LBP) and interleukin-10 (IL-10) are increased in cirrhosis. Box-plots of serum levels of (A) LBP and (B) IL-10 in healthy controls (cntrl) and cirrhotic patients (cirrh) are indicated (left). Scattered dot plots and medians are indicated for cirrhotic patients when stratified for inflammation (+/-infl) as evidenced by elevated CRP levels (right). P values in Mann–Whitney U test are indicated; n.s. = not significant. (C) Serum concentrations of IL-10 and LBP in cirrhotic patients tend to correlate in a linear fashion. Linear regression curve, Pearson product–moment correlation coefficient R and P value are indicated.
The SEB-induced cytokine response is attenuated in cirrhotic patients with on-going inflammation

After stimulation of whole blood with the superantigen SEB, which cross-links the T cell receptor to MHC class II, all control subjects and all cirrhotic subjects without inflammation had a positive cytokine response for TNF-α and IFN-γ in both CD4+ and CD8+ T cell subsets (2-fold increase of responding cells), whereas 5 of 22 (23%) cirrhotic patients with inflammation had not (Figures 4A and 4B). The fraction of IFN-γ-producing or TNF-α-producing CD4+ and CD8+ cells after SEB differed significantly between cirrhotic patients with inflammation than in cirrhotic patients without inflammation (Figure 4C). Furthermore, TNF-α production in responding CD4+ and CD8+ T cells was lower in cirrhotics with inflammation than in cirrhotic patients without evidence of inflammation (Figure 4D). The number of TNF-α-producing CD4+ T cells and CD8+ T cells after SEB stimulation negatively correlated with CRP levels ($R_s=-0.439 \ [P=0.008]$ and $R_s=-0.443 \ [P=0.008]$, respectively) but not with IL-10 ($P=0.709; P=0.408$), MELD score ($P=0.499; P=0.264$) or Child-Pugh score ($P=0.545; P=0.243$) in non-parametric correlation, indicating cellular exhaustion in patients with severe acute phase reaction.

Attenuated T cell responses to EBNA1 and BZLF1 are associated with higher interleukin-10 levels and low monocyte HLA-DR expression in cirrhotic patients

All cirrhotic patients and 12 of 14 control subjects were seropositive for EBV antibodies against EBNA as determined by immunoblot, indicating previous EBV infection. After stimulation of whole blood with overlapping peptide pools for the viral antigens EBNA1 and BZLF1, a broad spectrum of responses was observed in patients with cirrhosis and controls (Figure 2A). The overlay histogram (right) demonstrates HLA-DR expression on CD14+ monocytes from three representative individuals from the control group (cntrl, grey area), from the cirrhosis group without inflammation (cirrh –infl, black line) and from the cirrhosis group with inflammation (cirrh +infl, grey line). Panels B and C display distribution and median of fraction of HLA-DR-expressing CD14+ monocytes (B) and of mean fluorescence intensity of HLA-DR+ CD14+ monocytes (C) from healthy controls and patients with cirrhosis (left) and from patients with cirrhosis when stratified for elevated CRP (right). P values in Mann–Whitney U test are indicated; n.s. = not significant.
patients and control subjects. Figure 4B demonstrates the number of positive cytokine (TNF-α, IFN-γ) responses in CD4+ and CD8+ cells, represented as the sum of a positive response of each combination (score 0–4). The median number of positive responses after EBNA1 and BZLF1 exposition did not differ significantly between the three groups, which held also true in analyses performed separately for CD4 and CD8 T cell responses. Furthermore, intracellular cytokine production in responding T cells was not altered with respect to the three observation groups (data not shown). However, the fraction of patients with a null-response (score 0) towards EBNA1 or BZLF1
Figure 4 (See legend on next page.)
in critically ill cirrhotic patients [21]. In our cohort, monocytes is increased on circulating classical and non-
typical inflammation although did not reach statistical
significance. We were not able to demonstrate significantly
attenuated T cell responses to the SEB were more
often observed in patients with cirrhosis and on-going in-
flammation. It remains unclear whether a reduction of
MHC class II directly contributes to impaired antigen
presentation to CD4 cells because even a low number of
MHC class II molecules on antigen-presenting cells is
sufficient to generate an effective T cell response [35] and
a compensatory up-regulation of co-stimulatory molecules
CD80 and CD86 has been observed on monocytes from
cirrhotic patients [36]. Responses to SEB are dependent
on HLA expression, since the superantigen cross-links
MHC class II on monocytes with the T-cell receptor
leading to a strong induction of TNF-α in T cells via pro-
tein kinase C [37]. Despite increased phenotypical markers

tended to be higher in patients with cirrhosis and inflam-
ation (9/22 [41%] and 7/22 [32%], respectively) than in
 cirrhosis without inflammation (3/13 [23%] and 3/13
[23%]) or in controls (3/12 [25%] and 0/12 [0%]). Cirrhotic
null-responders to any of the viral antigens EBNA1 or
BZLF1 had higher IL-10 levels than cirrhotic patients with
at least one positive response (P=0.005) (Figure 4E). IL-10
serum levels in cirrhosis significantly increased from
responders through null-responders towards one of the
viral antigens to null-responders to both viral antigens
(P for trend = 0.003; Jonckheere-Terpstra test) (Figure 4E).
Furthermore, patients with a low fraction of HLA-DR*
CD14+ monocytes <70% had a lower number of TNF-
α-producing CD4+ T cells after stimulation with EBNA1
(median 0.02% vs. 0.05%; P=0.019). No correlation was
found between low HLA-DR expression on CD14+
monocytes and TNF-α response in CD8+ T cells towards
EBNA1 or BZLF1 peptides in cirrhotic patients (P=0.817
and P=0.976, respectively).

Discussion
In this study we determined T cell responses towards EBV
as a model to investigate the cellular immune function
in patients with cirrhosis. Cirrhotic patients displayed
increased markers of bacterial translocation and increased T
cell activation as well as reduced monocyte MHC class II
expression and increased IL-10 serum levels. This pheno-
type was more pronounced in cirrhotic patients with sys-
temic inflammation although did not reach statistical
significance. We were not able to demonstrate significantly
decreased antigen-specific T cell responses in the pre-
defined patient cohorts. However, patients with diminished
T cell responses towards the aforementioned viral proteins
had significantly higher serum concentrations of IL-10
than patients with unaltered T cell responses.

It has been reported that HLA-DR expression on
monocytes is increased on circulating classical and non-
classical monocytes in patients with cirrhosis in the ab-
bence of bacterial infections and SIRS [29] but is reduced
in critically ill cirrhotic patients [21]. In our cohort,
of T cell activation in cirrhosis, we observed a reduced fraction of T cells with cytokine responses to SEB as well as reduced TNF-α production in CD4+ and CD8+ T cells after stimulation with SEB. The cytokine response toward the viral antigens EBNA1 and BZLF1 was not as distinct as the response to SEB with high inter-individual variability and no conclusive evidence for overall impaired T cell responses in cirrhosis. Besides down-regulation of MHC class II on monocytes, circulating IL-10 may also directly attenuate T cell responses by inhibition of the co-stimulatory CD28 signalling pathway [19]. Notably, we observed higher serum IL-10 concentrations in patients with a null response towards EBNA1 and/or BZLF despite the presence of anti-EBNA antibodies indicative of past EBV infection and immunosuppressive memory.

In addition to the observed pattern of attenuated T cell responses in patients with low monocyctic HLA-DR expression or high circulating IL-10 levels, one can speculate that the presence of immune-regulatory subsets may also have contributed to attenuated T cell responses. Márquez et al. [36] reported that in patients with decompensated cirrhosis, an increase in CD25-positive effector CD4+ T cells was also accompanied by an increase of CD4+ CD25high Foxp3+ regulatory T cells that may suppress T cell responses. This assumption, however, could not be corroborated in our study since we did not prove these cells to be CD127low Foxp3-positive. Moreover, a subset of CD14+ cells with low HLA-DR expression have recently been reclassified as myeloid-derived suppressor cells, and expand during infection or inflammation where they are capable of suppressing T cell responses and induce CD4+ CD25+ Foxp3+ regulatory T cells [38,39].

A further limitation of our study is that we did not correct for HLA restrictions as being responsible for non-response to stimulation with viral peptides. However, among the EBV antigens several epitopes of the latent protein EBNA1 and the immediate early lytic peptide BZLF1 are rather promiscuous in the MHC class I and II context [40]. Furthermore, our observations are in line with clinical observations: although cirrhosis may worsen the course of viral infections and vice versa [41,42], there is no strong evidence that cirrhosis itself predisposes to viral infections or other T cell defect-associated infections such as tuberculosis [43].

Conclusions
Although this ex vivo study failed to provide evidence for a disturbed T cell response in cirrhotic patients in general, we did observe a pattern of attenuated responses towards viral antigens in patients that display low monocyctic HLA-DR expression and/or increased serum IL-10 levels. Our data suggest that bacterial translocation, endotoxaemia, inflammation and T cell activation in cirrhosis are also accompanied by an increase in circulating anti-inflammatory cytokines, reduced monocyctic MHC class II expression and attenuated cytokine production in T cells, which are likely to contribute to altered adaptive immune responses during infections or after vaccination in vivo.

Competing interests
The authors who have taken part in this study declared that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Authors’ contributions
JP obtained the patients’ samples and performed the experiments. TB and JP analysed and interpreted the results, conducted literature search, performed statistical analysis and wrote the manuscript. AS conceived the study, supervised the work of co-authors, interpreted the results and revised the manuscript. OF participated in the design of the study and revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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References
1. Borzio M, Salerno F, Piantoni L, Cazzaniga M, Angelè P, Bissoli F, Boccia S, Colloro-Mels G, Corigliano P, Fornaciari G, Marenco G, Pistarà R, Salvagnini M, Sangiovannì A. Bacterial infection in patients with advanced cirrhosis: a multicentre prospective study. Dig Liver Dis 2007, 39:41–48.
2. Arvaniti V, D’Amico G, Fede G, Manouseu P, Tschatzis E, Plegueuleo M, Burroughs AK. Infections in Patients With Cirrhosis Increase Mortality Four-Fold and Should Be Used in Determining Prognosis. Gastroenterology 2010, 139:1246–1256. e5.
3. Gustot T, Durand F, Lebrec D, Vincent JL, Moreau R. Severe sepsis in cirrhosis. Hepatology 2009, 50:2022–2033.
4. Gomez F, Ruiz P, Scheiber AD. Impaired function of macrophage Fc gamma receptors and bacterial infection in alcoholic cirrhosis. N Engl J Med 1994, 331:1122–1128.
5. Bruni T, Peter J, Hagel S, Hermann A, Stallmach A. The augmented neutrophil respiratory burst in response to Escherichia coli is reduced in liver cirrhosis during infection. Clin Exp Immunol 2011, 164:346–356.
6. Fiuza C, Salcedo M, Clemente G, Tellado JM. In vivo neutrophil dysfunction in cirrhotic patients with advanced liver disease. J Infect Dis 2000, 182:526–533.
7. Masini E, Mugnai L, Foschi M, Laﬁgi A, Gentilini P, Mannaioni PF. Changes in the production of nitric oxide and superoxide by inﬂammatory cells in liver cirrhosis. Int Arch Allergy Immunol 1995, 107:197–198.
8. Triffo G, Bechiks Z, Stadlbauer V, Davies N, Frances R, Shah N, Mockejeep RP, Such J, Jalan R. Evidence of neutrophil functional defect despite inﬂammation in stable cirrhosis. J Hepatol 2011, 55:574–581.
9. Laso FJ, Madruga JL, Girón JA, López A, Ciudad J, San Miguel JF, Álvarez-Mon M, Orfao A. Decreased natural killer cytotoxic activity in chronic alcoholism is associated with alcohol liver disease but not active ethanol consumption. Hepatology 1997, 25:1096–1100.
membrane of monocytes by affecting arrival and recycling. *Immunity* 1997, 7:861–871.

33. Hershman M, Appel SH, Wellhausen SR, Scornavacca F, Polk HC Jr: Interferon-gamma treatment increases HLADR expression on monocytes in severely injured patients. *Clin Exp Immunol* 1989, 77:67–70.

34. Vicente-Gutiérrez MM, Diez Ruiz A, Gil Extrema B, Bermúdez García JM, Gutiérrez Gea F: Low serum levels of alpha-interferon, gamma-interferon, and interleukin-2 in alcoholic cirrhosis. *Dig Dis Sci* 1991, 36:1209–1212.

35. Trowsdale J: Antigen Presentation. In Immunology. 7th edition. Edited by Male DK, Brostoff J, Roitt IM, Roth D. London: Mosby; 2006:145–162.

36. Martínez M, Fernández-Gutiérrez C, Montes-de-Oca M, Blanco MJ, Brun F, Rodríguez-Ramos C, Grón-Gonzáles JA: Chronic antigenic stimuli as a possible explanation for the immunodepression caused by liver cirrhosis. *Clin Exp Immunol* 2009, 158:219–229.

37. Han Y, Yang DC, Neill R, Jett M: Production of tumor necrosis factor alpha in human T lymphocytes by staphylococcal enterotoxin B correlates with toxin-induced proliferation and is regulated through protein kinase C. *J Infect Immun* 1999, 67:6611–6618.

38. Hoechst B, Ormaddy LA, Ballmaier M, Lehrer F, Krüger C, Manns MP, Goret TF, Korangy F: A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces CD4(+)CD25(+)Foxp3(+) T cells. *Gastroenterology* 2008, 135:234–243.

39. Gabrilovich DI, Nagaijar S: Myeloid-derived suppressor cells as regulators of the immune response. *Nat Rev Immunol* 2009, 9:162–174.

40. Landais E, Saulquin X, Houssaint E: The human T cell immune response to Epstein-Barr virus. *J Dev Biol* 2005, 49:285–292.

41. Ibáñez M, McGovern B, Dhar R, Stone D, McGowan K, Scheibl R, Snydman DR: Increasing Mortality Due to End-Stage Liver Disease in Patients with Human Immunodeficiency Virus Infection. *Clin Infect Dis* 2001, 32:492–497.

42. Duchini A, Wines ME, Nyberg LM, Hendry RM, Pockros PJ: Hepatic Decompensation in Patients With Cirrhosis During Infection With Influenza A. *Arch Intern Med* 2000, 160:113–115.

43. Wu HP, Pan Y-H, Hua C-C, Sheh W-B, Jiang B-Y, Yu T-J: Pneumococcal and liver cirrhosis are not risk factors for tuberculosis in patients with pulmonary infection. *Respirology* 2007, 12:416–419.