Gastroenterology 2019;157:149–162

CLINICAL—LIVER

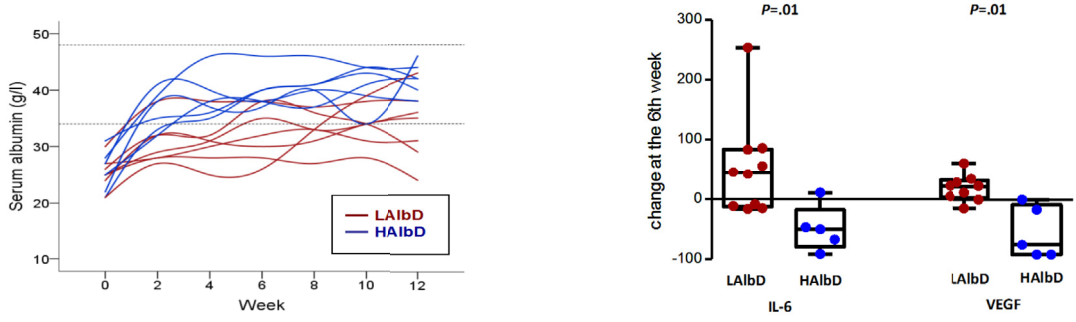
Effects of Albumin Treatment on Systemic and Portal Hemodynamics and Systemic Inflammation in Patients With Decompensated Cirrhosis

Javier Fernández,[1,2,*] **Joan Clària**,[1,2,*] Alex Amorós,[1] Ferrán Aguilar,[1] Miriam Castro,[2] Mireia Casulleras,[2] Juan Acevedo,[3] Marta Duran-Güell,[2] Laura Nuñez,[4] Montserrat Costa,[4] Mireia Torres,[4] Raquel Horrillo,[4] Luis Ruiz-del-Árbol,[5] Cándido Villanueva,[6] Verónica Prado,[2] Mireya Arteaga,[2] Jonel Trebicka,[1,7] Paolo Angeli,[1,8] Manuela Merli,[9] Carlo Alessandria,[10] Niels Kristian Aagaard,[11] German Soriano,[12] François Durand,[13] Alexander Gerbes,[14] Thierry Gustot,[15] Tania M. Welzel,[16] Francesco Salerno,[17] Rafael Bañares,[18] Victor Vargas,[19] Agustin Albillos,[5] Aníbal Silva,[2] Manuel Morales-Ruiz,[2] Juan Carlos García-Pagán,[2] Marco Pavesi,[1] Rajiv Jalan,[20] Mauro Bernardi,[21] Richard Moreau,[1,13,22] Antonio Páez,[4] and Vicente Arroyo[1]

[1]EF Clif, EASL-CLIF Consortium and Grifols Chair, Barcelona, Spain; [2]Hospital Clínic, IDIBAPS and CIBERehd, Barcelona, Spain; [3]South West Liver Unit, Derriford Hospital, Plymouth, United Kingdom; [4]Bioscience Research Group, Grifols, Barcelona, Spain; [5]Department of Gastroenterology, Hospital Ramón y Cajal and CIBERehd, Madrid, Spain; [6]Department of Gastroenterology, Hospital de Sant Pau and CIBERehd, Barcelona, Spain; [7]Department of Internal Medicine, University Hospital of Bonn, Bonn, Germany; [8]Unit of Internal Medicine and Hepatology, Department of Medicine, DIMED, University of Padova, Padova, Italy; [9]Department of Clinical Medicine, Sapienza University of Rome, Rome, Italy; [10]Division of Gastroenterology and Hepatology, San Giovanni Battista Hospital, Torino, Italy; [11]Department of Hepatology and Gastroenterology, Aarhus University Hospital, Aarhus, Denmark; [12]Department of Gastroenterology and Hepatology, Hospital of Santa Creu i Sant Pau and CIBERehd, Barcelona, Spain; [13]Service d'Hépatologie, Hôpital Beaujon, Assistance Publique-Hôpitaux de Paris, Clichy, France; [14]Department of Medicine II, Liver Centre Munich, University Hospital, LMU Munich, Munich, Germany; [15]Liver Transplant Unit, Erasme Hospital (ULB), Brussels, Belgium; [16]Medical Department I, Goethe University, Frankfurt, Germany; [17]Department of Internal Medicine, Policlinico IRCCS San Donato, Milano, Italy; [18]Department of Gastroenterology, Hospital Gregorio Marañon, and CIBERehd, Madrid, Spain; [19]Department of Internal Medicine, Hospital Vall d'Hebron and CIBERehd, Barcelona, Spain; [20]Liver Failure Group, Institute for Liver Disease Health, University College London, Royal Free Hospital, London, United Kingdom; [21]Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy; [22]Inserm, Université Paris Diderot-Paris 7, Centre de Recherche sur l'Inflammation, Paris, France

This article has an accompanying continuing medical education activity, also eligible for MOC credit, on page e17 (https://www.gastrojournal.org/cme/home). Learning Objective: Upon completion of this CME activity, successful learners will be able to (1) discuss the clinical relevance of normalizing serum albumin concentration in patients with decompensated cirrhosis and ascites and (2) identify the main mechanisms of action of albumin in this setting.

Effects of long-term albumin treatment on serum albumin levels and inflammatory cytokines
High albumin dose (HAlbD: 1.5 g/kg every week, blue figures) but not low albumin dose (LAlbD: 1 g/kg every 2 weeks: red figures) normalized serum albumin levels and decreased inflammatory cytokines



Gastroenterology

BACKGROUND & AIMS: We investigated the effect of albumin treatment (20% solution) on hypoalbuminemia, cardiocirculatory dysfunction, portal hypertension, and systemic inflammation in patients with decompensated cirrhosis with and without bacterial infections. **METHODS:** We performed a prospective study to assess the effects of long-term (12 weeks) treatment with low doses (1 g/kg body weight every 2 weeks) and high doses (1.5 g/kg every week) of albumin on serum albumin, plasma renin, cardiocirculatory function, portal pressure, and plasma levels of cytokines, collecting data from 18 patients without bacterial infections (the Pilot-PRECIOSA study). We also assessed the effect of short-term (1 week) treatment with antibiotics alone vs the combination of albumin plus antibiotics (1.5 g/kg on day 1 and 1 g/kg on day 3) on plasma levels of cytokines in biobanked samples from 78 patients with bacterial infections included in a randomized controlled trial (INFECIR-2 study). **RESULTS:** Circulatory dysfunction and systemic inflammation were extremely unstable in many patients included in the Pilot-PRECIOSA study; these patients had intense and reversible peaks in plasma levels of renin and interleukin 6. Long-term high-dose albumin, but not low-dose albumin, was associated with normalization of serum level of albumin, improved stability of the circulation and left ventricular function, and reduced plasma levels of cytokines (interleukin 6, granulocyte colony-stimulating factor, interleukin 1 receptor antagonist, and vascular endothelial growth factor) without significant changes in portal pressure. The immune-modulatory effects of albumin observed in the Pilot-PRECIOSA study were confirmed in the INFECIR-2 study. In this study, patients given albumin had significant reductions in plasma levels of cytokines. **CONCLUSIONS:** In an analysis of data from 2 trials (Pilot-PRECIOSA study and INFECIR-2 study), we found that albumin treatment reduced systemic inflammation and cardiocirculatory dysfunction in patients with decompensated cirrhosis. These effects might be responsible for the beneficial effects of albumin therapy on outcomes of patients with decompensated cirrhosis. ClinicalTrials.gov, Numbers: NCT00968695 and NCT03451292.

Keywords: Liver-Related Complications; Immune Response; Splanchnic Hemodynamics; Interventional Trials.

WHAT YOU NEED TO KNOW

BACKGROUND & CONTEXT

Systemic inflammation is an important issue in acute decompensation of cirrhosis, this is thought to play a major role in the pathophysiology of complications, including multiple organ failure.

NEW FINDINGS

Long-term high albumin treatment (1.5 g/kg every week for 12 weeks) showed significant immunomodulatory effects in an exploratory cohort of patients with decompensated cirrhosis. This finding was validated in a short-term (7 days) investigation in patients with infections.

LIMITATIONS

Low number of patients included in the investigation cohort.

IMPACT

Albumin treatment improves markers of systemic inflammation in patients with decompensated cirrhosis. This effect may underlie the beneficial effects of albumin therapy on clinical outcomes in this setting.

The first studies supporting the use of albumin treatment in cirrhosis were performed in the 1980s and consisted of several randomized controlled trials (RCTs) showing that paracentesis was a rapid, effective, and safe therapy of ascites if performed with intravenous albumin administration (8 g/L of ascitic fluid removed).[1] Sort et al[2] subsequently showed that treatment of spontaneous bacterial peritonitis (SBP) with antibiotics plus albumin (1.5 g/kg body weight at infection diagnosis and 1 g/kg on day 3) was associated with a 60% reduction in the prevalence of type 1 hepatorenal syndrome (HRS), a special form of acute-on-chronic liver failure (ACLF), and in hospital mortality. Ortega et al[3] later showed that the simultaneous administration of terlipressin and albumin (20–40 g/d for 7–14 days) normalized serum creatinine concentration in approximately 50% of patients with HRS) Finally, the ANSWER study recently showed that long-term (18 months) prophylactic administration of albumin (40 g every week) to patients with prior history of ascites is highly effective in preventing follow-up development of new episodes of ascites, refractory ascites, HRS, hepatic encephalopathy, and bacterial infections, reducing hospital admissions and improving survival.[4] This successful research activity on the therapeutic use of albumin in cirrhosis contrasts sharply with the low number of investigations performed on its mechanisms of action.[1]

This article reports the results of the Pilot-PRECIOSA study, which aimed to identify an albumin dosage that normalizes serum albumin concentration and investigate the effects of the administration of this albumin dosage for 12 weeks on hypoalbuminemia, cardiocirculatory hemodynamics, effective blood volume, portal pressure, and systemic inflammation (as estimated by the plasma levels of

* Authors share co-first authorship.

Abbreviations used in this paper: ACLF, acute-on-chronic liver failure; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; EASL-CLIF Consortium, European Association for the Study of the Liver–Chronic Liver Failure Consortium; ELISA, enzyme-linked immunosorbent assay; HAlbD, high albumin dose; HRS, hepatorenal syndrome; IL, interleukin; INFECIR, albumin administration in the prevention of hepatorenal syndrome and death in patients with CIRrhosis, bacterial INFEctions other than SBP and high risk of hospital mortality; LAlbD, low albumin dose; LV, left ventricle; PRA, plasma renin activity; PRECIOSA, PREvention of mortality with long-term administration of human albumin in subjects with decompensated CIrrhOSis and Ascites; PRC, plasma renin concentration; RCT, randomized controlled trial; SBP, spontaneous bacterial peritonitis.

Most current article

0016-5085
https://doi.org/10.1053/j.gastro.2019.03.021

interleukin [IL] 6) in 18 patients with decompensated cirrhosis.

Recent investigations have suggested that systemic inflammation plays a major role in the pathogenesis of acute decompensation and ACLF in cirrhosis.[5] The observation of a marked suppression of the plasma levels of IL-6 during albumin treatment in the Pilot-PRECIOSA STUDY, which suggests an immunomodulatory effect of albumin treatment, prompted us to perform additional investigations to confirm this feature. These investigations consisted of the measurement of a large panel of inflammatory mediators in biobanking material from the Pilot-PRECIOSA study and from the INFECIR-2 study, a RCT aimed at comparing the efficacy of antibiotics alone vs albumin plus antibiotics in patients with decompensated cirrhosis and bacterial infection unrelated to SBP.[6]

Methods

The Pilot-PRECIOSA study and the INFECIR-2 study were approved by the corresponding ethics committees of each hospital involved. The informed consent forms of the 2 studies included the potential use of biobanking material for measuring serum albumin levels and plasma renin and cytokine concentrations.

The Pilot-PRECIOSA Study

The Pilot-PRECIOSA study (IG0802, registered at ClinicalTrials.gov as NCT00968695) is a proof-of-concept, open-label, multicenter, nonrandomized (single-group), prospective, phase 4 safety and dosage exploratory investigation sponsored by Grifols with the aim of getting preliminary information to design a currently ongoing multicenter, randomized, controlled therapeutic trial to assess the efficacy of long-term (1 year) albumin treatment in the prevention of ACLF and mortality in decompensated cirrhosis (PRECIOSA study, ClinicalTrials.gov number NCT03451292).

Investigators of the European Association for the Study of the Liver–Chronic Liver Failure (EASL-CLIF) Consortium from 3 hospitals (Hospital Clinic and Hospital de Sant Pau in Barcelona and Hospital Ramón y Cajal in Madrid, Spain) participated in the design and implementation of the study, which started in July 2009 and was completed in April 2014. These hospitals use the same methodology for cardiocirculatory and hepatic hemodynamic studies and have extensive experience in cooperative hemodynamic, pathophysiological, and therapeutic studies. Nonstandard laboratory measurements (hormones and biomarkers estimating systemic inflammation) were centralized at the Hospital Clinic. The results of the Pilot-PRECIOSA study were submitted to embargo until the start of the PRECIOSA study.

Inclusion and Exclusion Criteria and Patients Evaluated. The study enrolled noninfected patients with decompensated cirrhosis and severe circulatory dysfunction as defined by the presence of ascites, renal dysfunction (serum creatinine $\geq$ 1.2 mg/dL or blood urea nitrogen $\geq$ 25 mg/dL or dilutional hyponatremia [serum sodium $\leq$ 130 mEq/L]), high levels of plasma renin activity (PRA) ($\geq$2 ng/mL·h), and need for diuretic treatment to prevent ascites recurrence (at least 200 mg of spironolactone or 100 mg of spironolactone and 40 mg of furosemide). PRA was used to assess sequential changes in effective arterial blood volume. The exclusion criteria are detailed in the Supplementary Materials.

A total of 135 patients were evaluated; 72 were eligible, and among these, 39 were excluded based on the exclusion criteria. Of the 33 remaining patients, 12 were excluded for data analysis due to (1) lack of abnormal PRA value (<2 ng/mL·h) at enrollment (2 patients), (2) development of complications requiring treatment that interfered with the interpretations of the results (intensive care, liver transplantation, and insertion of a transjugular intrahepatic portosystemic shunt) (3 patients), and (3) discontinuation of albumin treatment (7 patients). Three of the remaining 21 patients died within the study period, and 3 did not give informed consent for cardiocirculatory and hepatic hemodynamic assessment. The clinical characteristics at enrollment and the main complications and causes of death during the study period are presented in Supplementary Table 1.

Chronogram. *Day 0.* Samples were obtained for standard laboratory tests, serum albumin concentration, PRA (as a marker of effective blood volume), plasma concentrations of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) (markers of central blood volume expansion), and IL-6, followed by the hepatic and cardiocirculatory hemodynamic study. The methods for these studies have been previously described (Supplementary Table 2).[7] Immediately afterward, patients received the first albumin dose, and they were followed up for 20 weeks.

Weeks 1–12. The first 10 patients received an albumin dose of 1g/kg body weight every 2 weeks for 12 weeks (a total of 7 albumin treatments). PRA was measured every 2 weeks before each albumin dose in the first 5 patients and ad hoc weekly in the remaining 5 patients. Plasma IL-6 and serum albumin concentrations were measured every 2 weeks. An interim analysis in these first 10 patients showed that this dose of albumin was insufficient to normalize serum albumin concentration throughout the last 10 weeks of the study period in most patients (normal serum albumin concentration, 34–47 g/L). Accordingly, albumin dosage was increased to 1.5 g/kg body weight every week in the remaining patients. Therefore, this second group of patients received a higher albumin dosage per treatment and more albumin treatments (13) within the same time period (on day 0 and then every week for 12 weeks). Samples for PRA were taken ad hoc weekly during treatment. Samples for serum concentration of albumin and plasma levels of IL-6 were obtained every 2 weeks. For the description of the results, the group of patients who received albumin at a dose of 1g/kg every 2 weeks was defined as the low albumin dosage (LAlbD) group and that receiving albumin at a dose of 1.5 g/kg every week as the high albumin dosage (HAlbD) group.

Week 14. Two weeks after the last albumin dose, the cardiopulmonary and hepatic hemodynamic study was repeated.

Post Hoc Measurements of Cytokines, Chemokines, and Other Inflammatory Markers. The post hoc assessment of the effects of albumin treatment on systemic inflammation was performed by assessing a large panel of inflammatory mediators and biomarkers, including 24 cytokines, 10 chemokines, 4 growth factors, and 6 markers of endothelial dysfunction (2), coagulation/platelet dysfunction (2), and monocyte activation (2), in biobanking material (September 2018).

The INFECIR-2 Study

The INFECIR-2 study is an EASL-CLIF Consortium investigator-promoted, phase 4, randomized, open-label, parallel, multicenter trial promoted by the Fundació Clínic (Hospital Clínic, University of Barcelona, Spain). It began in September 2014 and was finished in December 2016 (ClinicalTrials.gov number NCT02034279). The inclusion and exclusion criteria are detailed in the Supplementary Materials. The study aimed to assess the efficacy of short-term albumin treatment in the prevention of ACLF and hospital mortality in 136 patients with decompensated cirrhosis and acute bacterial infections unrelated to SBP. Eighteen patients were considered to have been erroneously included. Therefore, 118 patients were randomly assigned to receive either antibiotics alone (antibiotics-alone group, n = 57), or antibiotics plus 2 albumin doses (ie, 1.5 g/kg at inclusion [day 1] and 1 g/kg on day 3) (albumin-plus-antibiotics group, n = 61). Plasma samples for biobanking were obtained on day 1, before the administration of the first albumin dose; on day 3, before the second albumin dose; and/or on day 7 in 48 patients from the albumin-plus-antibiotics group and 47 patients from the antibiotics-alone group. "On-treatment" values of plasma cytokine levels given in the article represent the average of those obtained on days 3 and 7 (in patients with 2 measurements) or those obtained on day 3 or 7 in patients with only a single measurement. Both groups were similar regarding patient characteristics (except for the combined prevalence of ACLF and kidney dysfunction at baseline, which was higher in the albumin arm), type of infection, and antibiotic therapy. The results of the INFECIR-2 study have recently been reported.[6]

The current study used biobanking aliquots from the INFECIR-2 study for measurement of the serum concentration of albumin, the plasma concentration of renin (PRC), and the plasma concentrations of the same panel of cytokines, chemokines, growth factors, and other inflammatory markers studied in the Pilot-PRECIOSA study. Measurements were performed at baseline and during treatment among 40 patients from the antibiotics-alone group and 38 patients from the albumin-plus-antibiotics group. The prespecified criteria to select these 78 patients were (1) availability of biobanking samples, (2) infection receiving appropriate empirical antibiotic treatment (3) absence of severe complications within the first week of treatment that could affect the interpretation of the results, and (4) completion of 1 week of follow-up.

Laboratory Methods

Hormones and IL-6 were measured by radioimmunoassay (PRA), chemiluminescent immunoassay (PRC), immunoassay (ANP and BNP), and enzyme-linked immunosorbent assay (ELISA) (IL-6). Measurement of the panel of cytokines, chemokines, and other inflammatory mediators in patients from the Pilot-PRECIOSA and INFECIR-2 studies were performed by using 2 multiplex immunoassays based on Luminex multianalyte profiling technology (Luminex Corp, Austin, TX). The plasma levels of sCD163 and sMR/sCD206 were determined by ELISA. Methods are detailed in the Supplementary Materials.

Statistical Methods

In the Pilot-PRECIOSA study, for a given patient receiving albumin treatment, there were several available results for serum albumin, PRA, and plasma IL-6. We averaged all the available values within the last 10 weeks of treatment to obtain a single on-treatment value for comparison with the corresponding baseline value.

Results are presented as median and interquartile range. For univariate analysis, Mann-Whitney test and Wilcoxon signed rank test were used for non-normally distributed variables. In all statistical analyses, significance was set at $P < .05$. Analyses were performed with SAS, version 9.4 (SAS Institute, Cary, NC) statistical packages. Graphs were performed with GraphPad Prism, version 5.00 (GraphPad Software, San Diego, CA).

Results

Baseline Clinical Characteristics of the Patients Included in the Pilot-PRECIOSA Study

All of the 18 patients included were admitted to the hospital for the treatment of ascites; 3 had diabetes mellitus, 1 had hepatocellular carcinoma, and 2 had minimal hepatic encephalopathy. Other characteristics at enrollment are presented in Supplementary Table 1.

Effect of Long-Term Albumin Treatment and Its Dosage on Serum Albumin Concentration (Pilot-PRECIOSA Study)

Thirteen out of the 18 patients included who completed the sequential measurement of plasma albumin concentration had baseline hypoalbuminemia (serum albumin concentration < 34 g/L). The effect of albumin treatment on serum albumin concentration was related to 2 factors. The first factor was albumin dosage. Although patients of the LAlbD group with baseline hypoalbuminemia (n = 7) exhibited increases in serum levels of albumin during treatment, only 1 achieved normalized the serum albumin concentration, that is, had an increase in albumin level to a value ≥34 g/L in all measurements. In contrast, all patients of the HAlbD group with baseline hypoalbuminemia (n = 6) achieved on-treatment normalized serum albumin concentration ($P < .001$) Figure 1*A*). The median increases in serum albumin among patients receiving HAlbD or LAlbD are detailed in Table 1 for all patients and in Figure 1*B* specifically for patients with baseline hypoalbuminemia. Four of the 5 patients with normal baseline serum albumin concentration (3 from the LAlbD group and 2 from the HAlbD group) showed relatively stable serum albumin concentration (always within the normal limits of 34–47 g/L) throughout the study (Figure 1*C*). The fifth patient exhibited an on-treatment increase in serum albumin, but this was also within normal limits.

The second factor influencing the effect of albumin treatment was hypoalbuminemia grade at baseline. There was a significant inverse correlation between the baseline serum albumin concentration and the median change in serum albumin during treatment in both the HAlbD and the LAlbD groups (Figure 1*D*): the lower the baseline albumin concentration, the higher the median increase in the serum albumin levels achieved during treatment. The response to albumin treatment at each level of serum albumin concentration was higher in patients receiving HAlbD.

Effect of Long-Term Albumin Treatment and Its Dosage on Plasma Renin Activity (Pilot-PRECIOSA Study)

Long-term albumin treatment was not associated with significant suppression in PRA in patients receiving HAlbD or LAlbD, suggesting a minor effect on the effective blood volume (Table 1). Figure 1*E* and *F* shows the individual time-course changes of PRA in patients receiving LAlbD and HAlbD, respectively. An intriguing observation was the extreme instability of effective blood volume, as indicated by the development of acute, high, and transient positive peaks of PRA (increase in PRA >100% to levels over 10 ng/mL·h) in a significant number of patients. Peaks were observed more frequently in the LAlbD group (6 patients, 60%) than in the HAlbD group (1 patient, 12.5%) ($P = .04$), suggesting that although albumin treatment was not effective in improving mean effective blood volume, it was capable of stabilizing circulatory function.

Effect of Long-Term Albumin Treatment and Its Dosage on Plasma Interleukin 6 Levels (Pilot-PRECIOSA Study)

To explore the possibility that albumin treatment can affect systemic inflammation, we sequentially measured the plasma levels of IL-6 on day 0 and every 2 weeks after day 0 in the Pilot-PRECIOSA study. IL-6 is a paradigmatic proinflammatory cytokine whose plasma levels are increased in most patients with cirrhosis and systemic inflammation.[5] Nine patients from the LAlbD group and 7 from the HAlbD group had measurable levels of IL-6 at baseline and during treatment. The effect of albumin treatment on systemic inflammation in each patient could then be estimated as the absolute or percent change of IL-6 between baseline value and on-treatment value (Table 1). The median baseline value for plasma IL-6 levels in the 16 patients was well above the normal range, consistent with the existence of systemic inflammation in this group of patients. We arbitrarily defined a patient as having developed significant immunomodulatory response to albumin treatment when the on-treatment IL-6 level decreased by more than 20% below the baseline level. An outstanding finding of the current study was that the majority of patients receiving HAlbD (6 of 7 patients, 85.7%) but only 1 of 9 patients receiving LAlbD (11%) ($P = .003$ for between-group comparison) had a reduction of plasma IL-6 of >20%, suggesting that long-term treatment with HAlbD, but not with LAlbD, induces significant immunomodulatory effects in patients with decompensated cirrhosis. Consistent with these findings, we found that the median reduction from baseline for IL-6 was significantly greater among patients receiving HAlbD than among those receiving LAlbD, whether reduction was expressed by percentage or absolute values (Table 1).

A second important finding was that systemic inflammation was unstable in a significant number of patients (1 of 7 receiving HAlbD and 4 of 9 receiving LAlbD), with acute, high, and reversible peaks of the plasma IL-6 (ie, increases by at least 100% to levels over 100 pg/mL) during albumin treatment (Figure 2*A*). The remaining 11 patients showed small changes (mainly patients receiving LAlbD) or marked reductions (mainly patients receiving HAlbD) of on-treatment IL-6 (Figure 2*B*).

Effect of Long-Term Albumin Treatment and Its Dosage on a Large Panel of Plasma Cytokines (Pilot-PRECIOSA Study)

The finding that elevated baseline plasma IL-6 levels, as determined by ELISA, can be reduced by albumin therapy in a dose-dependent manner prompted us to investigate the effects of this treatment on the plasma levels of a large number of cytokines (n = 24) in biobanking samples obtained at baseline and at week 6 of albumin treatment in 10 patients from the LAlbD group and 5 patients from the HAlbD group. In addition, we measured the plasma levels of the 24 cytokines in 25 healthy donors recruited at the Hospital Clínic Blood Bank.

Among the 24 cytokines measured, 11 were not detectable in any patient/healthy individual. Baseline values of all but 2 of the remaining 13 cytokines included in the panel were significantly higher among patients with decompensated cirrhosis than among healthy individuals, confirming the existence of full-blown systemic inflammation in decompensated cirrhosis (Table 2). In the next tables, only changes in relevant cytokines are presented. Patients receiving LAlbD experienced only a small reduction or moderate increase during treatment in the plasma levels of these cytokines, a feature that contrasts sharply with the marked suppression of most cytokines in patients receiving HAlbD (Table 3 and Figure 2*C* and *D*). These results strongly suggest that long-term albumin treatment, if given at high dosage, has a significant immunomodulatory effect in decompensated cirrhosis, reducing the degree of systemic inflammation.

Effects of Long-Term Albumin Treatment on Systemic and Splanchnic Hemodynamics, Natriuretic Peptides, and Liver and Renal Function (Pilot-PRECIOSA study)

Treatment with HAlbD, but not with LAlbD, was associated with a significant increase in cardiac index, systolic volume, and left ventricular (LV) stroke work index, indicating an increase in LV function (Table 4). There were no changes in most parameters estimating cardiac preload, including atrial pressure, pulmonary capillary wedged pressure, and plasma concentrations of ANP and BNP. There was, however, a significant increase in mean pulmonary artery pressure in patients receiving HAlbD, although it might be related to improvement in right ventricular function. All patients had severe portal hypertension at enrollment. HAlbD and LAlbD treatment, however, was not associated with significant changes in hepatic venous pressure gradient, a sensitive marker of portal pressure. There were also no major changes in other relevant standard laboratory parameters in either group.

Effect of Short-Term Albumin Treatment on Serum Albumin and Plasma Levels of Renin and of a Large Panel of Inflammatory Cytokines in Patients With Infections (INFECIR-2 Study)

Next, we asked whether albumin therapy could have a reducing effect on plasma cytokine levels in patients with bacterial infections included in the INFECIR-2 study.

Bacterial infections are known to result in an enhancement of the systemic inflammation already present in patients with decompensated cirrhosis.[5,7] This explains why baseline levels of tumor necrosis factor α, IL-4, IL-6, and IL-10 were significantly higher among patients included in the INFECIR-2 study than among those included in the Pilot-PRECIOSA study (Table 2). As expected for a randomized trial, in the INFECIR-2 study, the baseline plasma cytokine levels were similar among patients assigned to receive antibiotics alone and among those assigned to receive albumin plus antibiotics (Table 5).

Treatment with antibiotics alone was not associated with significant changes in most cytokines. Only 1 patient showed a significant suppression (tumor necrosis factor α) during treatment. In contrast, patients treated with albumin plus antibiotics had, during treatment, a significant decrease or a clear trend for a reduction in most cytokines (Table 5), suggesting that albumin associated with antibiotics was

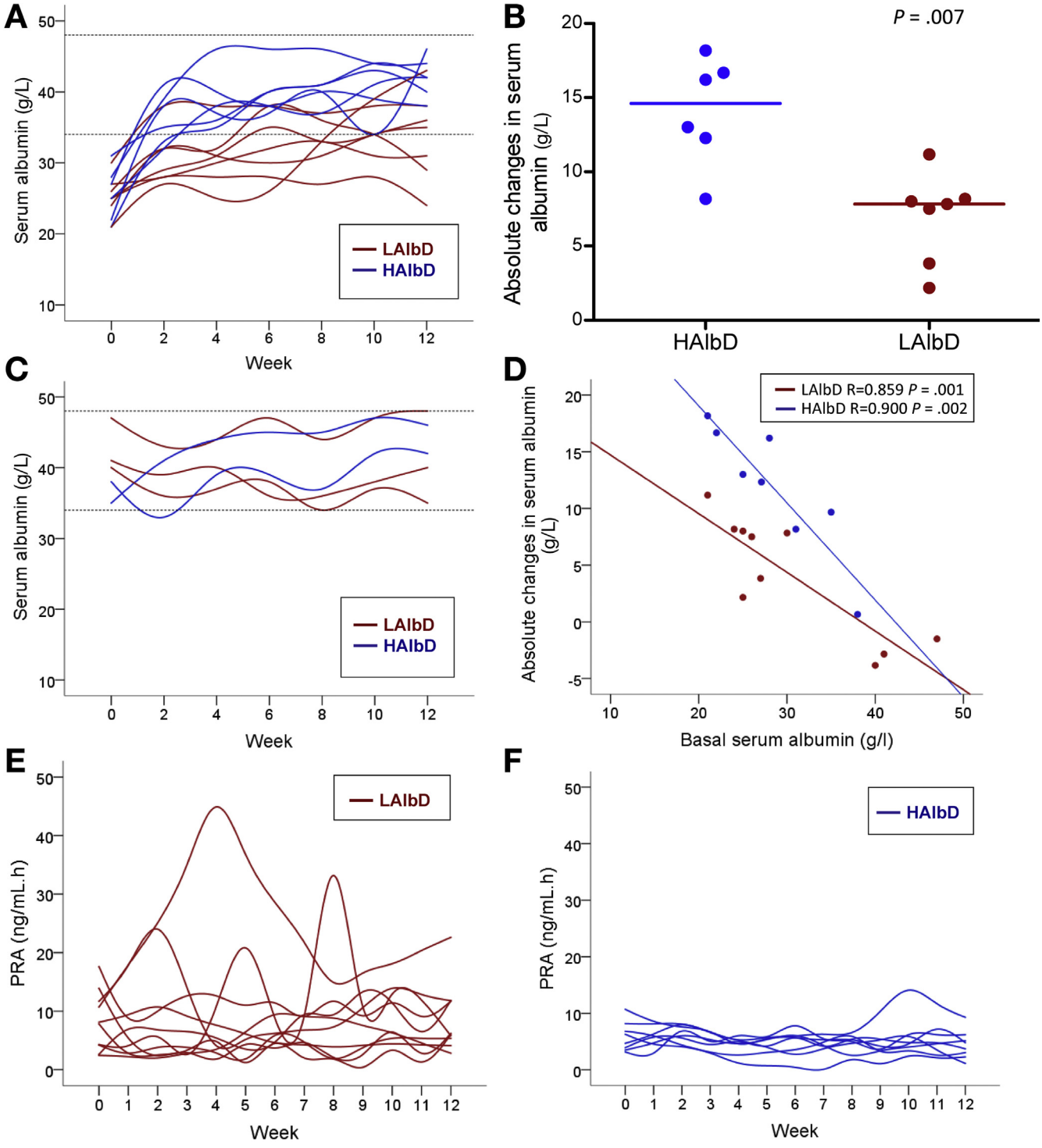


Table 1. Serum Albumin, PRA, and Plasma Levels of IL-6 at Baseline and During Albumin Treatment in 18 Patients With Decompensated Cirrhosis Unrelated to Bacterial Infection Who Were Enrolled in the Pilot-PRECIOSA Study and Divided Into 2 Groups Depending on Whether They Received LAlbD or HAlbD Treatment

Parameter	Patient Group		*P* value[a]
	HAlbD group (n = 8)	LAlbD group (n = 10)	
Serum albumin concentration			
Baseline, *g/L*	27.6 (22.7 to 34.0)	26.5 (24.8 to 40.3)	.83
On-treatment average value, *g/L*	39.2 (38.7 to 43.0)[b]	33.3 (31.8 to 37.9)[c]	**.004**
Absolute change, *g/L*	12.7 (8.5 to 16.6)[b]	5.7 (–1.8 to 8.0)[c]	**.01**
Percent change, %	48.7 (26.7 to 71.3)[b]	20.2 (–4.1 to 32.5)[c]	**.04**
Plasma renin activity			
Baseline, *ng/mL·h*	5.5 (3.6 to 7.9)	7.9 (3.8 to 12.3)	.41
On-treatment average value, *ng/mL·h*	4.9 (3.9 to 5.8)	6.9 (3.8 to 11.3)	.17
Absolute change, *ng/mL*	0.2 (–4.2 to 1.3)	–0.4 (–5.5 to 5.8)	.64
Percent change, %	2.0 (–44.4 to 36.2)	–6.7 (–45.3 to 146.2)	.69
Plasma IL-6 concentration			
Baseline, *pg/mL*	123.5 (51.5 to 151.5)	41.5 (25.8 to 75.0)	**.02**
On-treatment average value, *pg/mL*	62.5 (24.5 to 93.6)[b]	57.5 (30.0 to 79.2)	.76
Absolute change, *pg/mL*	–53.0 (–108.0 to –18.0)[b]	–3.2 (–11.1 to 30.0)	**.04**
Percent change, %	–56.0 (–68.8 to –24.2)[b]	–7.6 (–15.7 to 79.7)	**.04**

NOTE. Values are reported as median (interquartile range). For each variable in each patient, the average value during treatment was obtained by using all on-treatment values of this variable available.
[a]*P* value for the between-group comparison. Values in bold indicate $P < .05$.
[b]$P < .05$ for the within-group comparison with baseline values.
[c]$P = .05$ for the within-group comparison with baseline values.

more effective than antibiotics alone in attenuating baseline systemic inflammation in patients with bacterial infections.

In the INFECIR-2 study, baseline values for serum albumin concentration were similar between patients of the antibiotics-alone group (26 [20–30] g/L) and those of the albumin-plus-antibiotics group (25 [19–30] g/L) ($P = .91$). The baseline activity of the renin-angiotensin system, estimated by PRC, was greater among patients in the albumin-plus-antibiotics group than among those in the antibiotics-alone group, although the difference was not statistically significant (241.6 [46.3–903.0] μIU/mL and 125.0 [34.0–398.6] μIU/mL, respectively; $P = .25$). Antibiotics alone were not associated with significant changes from baseline for serum albumin concentration (0 [–20 to 1.0] g/L) or PRC (–1.2 [–26.5 to 129.6] μIU/mL). In contrast, albumin-plus-antibiotics treatment significantly increased serum albumin concentration (7.0 [4.0–10.0] g/L, $P < .0001$) and suppressed PRC (–40. 5 [–272.9 to –4.5] μIU/mL; $P = .002$).

Effect of Short-Term and Long-Term Albumin Treatment on Chemokines; Growth Factors; and Biomarkers of Macrophage Activation, Endothelial Dysfunction, and Coagulation/ Platelet Function (Pilot-PRECIOSA and INFECIR-2 Studies)

To have a comprehensive view of the effects of albumin treatment in both the Pilot-PRECIOSA and the INFECIR-2

Figure 1. Changes in serum albumin concentration and PRA induced by treatment with HAlbD (*blue color in all panels*) and LAlbD (*red color*) in the 18 patients included in the Pilot-PRECIOSA Study. (*A*) Individual changes in serum albumin concentration among the 13 patients with baseline hypoalbuminemia (serum albumin concentration < 34 g/L). The horizontal lines indicate the upper and lower normal limits of serum albumin. All 6 patients with hypoalbuminemia treated with HAlbD developed a rapid increase (within 2 weeks) in serum albumin concentration up to normal levels, which persisted during the remaining 10 weeks of the study. In contrast, although all 7 patients with baseline hypoalbuminemia treated with LAlbD had increased serum levels of albumin during treatment, only 1 normalized the serum albumin concentration. (*B–D*) Two factors influenced the response to albumin treatment. (*B*) The first factor was the albumin dosage: among the 13 patients with baseline hypoalbuminemia, the individual absolute median increase in serum albumin was almost double in patients receiving HAlbD vs those receiving LAlbD. (*C*) The second factor was the feedback mechanism by which baseline serum albumin concentration influences the hepatic synthesis of albumin. In most patients without hypoalbuminemia, the inhibition of hepatic synthesis of albumin prevented the increase in the serum concentration of albumin to abnormal levels during albumin treatment. (*D*) This feedback mechanism was also reflected by the close inverse correlation between the baseline serum albumin concentration and the mean increase in serum albumin during treatment. The lower the baseline levels of serum albumin, the higher the absolute mean increase in the serum concentration of albumin in both the LAlbD and HAlbD groups. (*E*) Circulatory dysfunction was extremely unstable during albumin treatment in patients receiving LAlbD, with high peaks of PRA in 6 patients. (*F*) Circulatory instability was significantly improved in patients receiving HAlbD, with only 1 patient presenting 1 peak of PRA throughout treatment.

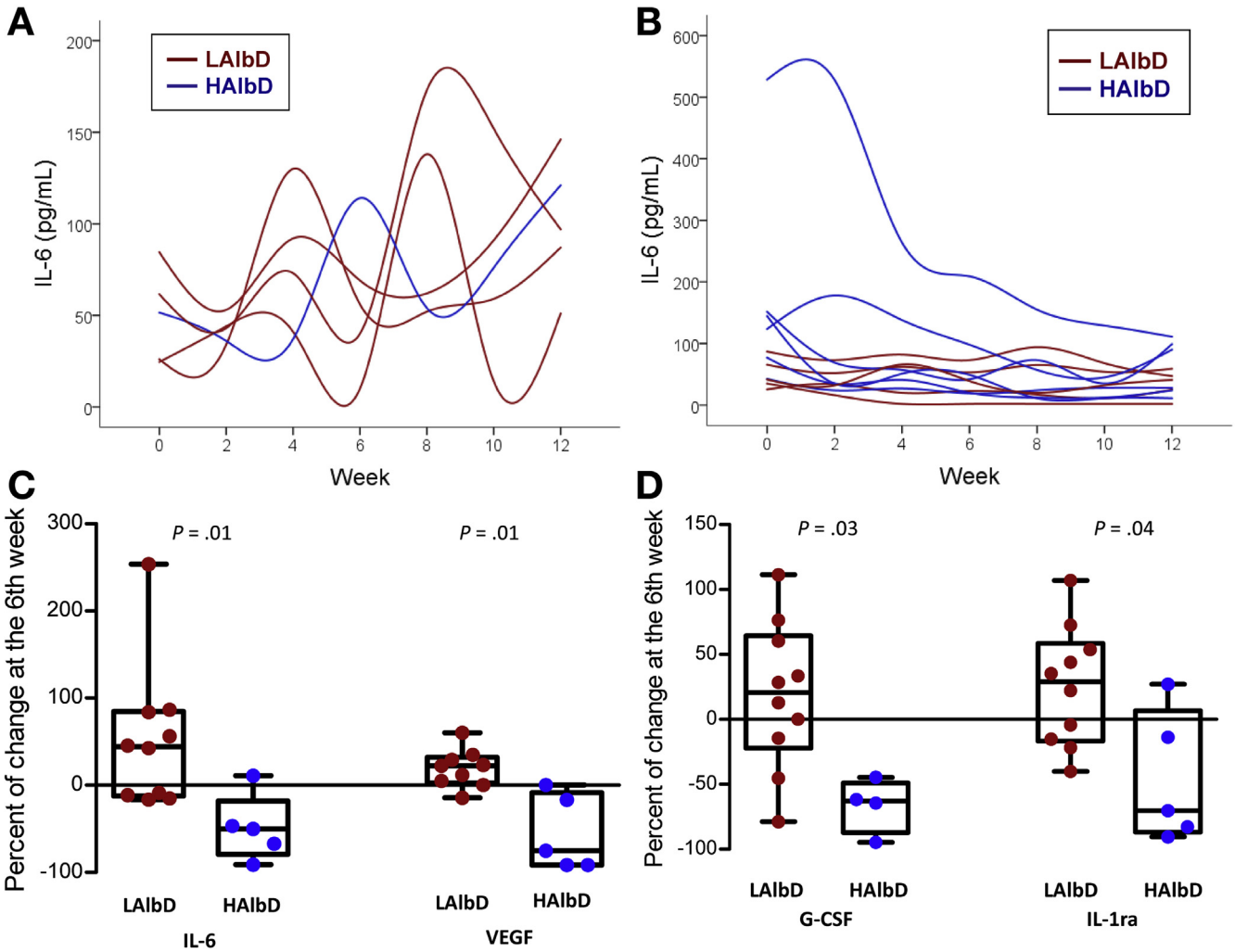


Figure 2. Changes in IL-6 and other cytokines induced by treatment with HAIbD (*blue color in all panels*) and LAIbD (*red color*) in the 15 patients included in the Pilot-PRECIOSA study with sequential cytokines measurements. (*A*) The degree of systemic inflammation, as estimated by repeated measurements of plasma IL-6 in baseline conditions and during treatment, was extremely unstable in 4 patients receiving LAIbD and in 1 receiving HAIbD. (*B*) In the remaining patients, there was a marked suppression of the circulating plasma levels of IL-6 (mainly in patients receiving HAIbD) or no change to minor changes (mainly in patients receiving LAIbD). (*C*, *D*) Data derived from the assessment of a large panel of inflammatory cytokines at baseline and at week 6 showed that plasma levels of IL-6, vascular endothelial growth factor, G-CSF, and IL-1 receptor antagonist had a median reduction from baseline (interquartile range, %) that was significantly greater among patients treated with HAIbD than among those receiving LAIbD. G-CSF, granulocyte colony-stimulating factor; IL1-ra, IL-1 receptor antagonist.

studies, we assessed a broad variety of soluble factors, including chemokines, growth factors, and markers of macrophage activation and endothelial and coagulation/platelet dysfunction. As shown in Supplementary Tables 3 and 4, in both studies, albumin treatment was associated with minor or no changes in most of these factors, suggesting that it exerts its immunomodulatory effect mainly by influencing production and/or release of specific cytokines.

Discussion

Current albumin dosage in cirrhosis is based on empirical assumptions and on the concept that albumin mainly acts as a plasma volume expander.[8] Of note, albumin therapy can have many other important biological effects, because it is able to bind to and inactivate a wide range of endogenous and exogenous ligands.[1] The ability of albumin to bind proinflammatory molecules such as pathogen-associated molecular patterns (e.g., the Gram-negative bacteria byproduct lipopolysaccharide),[9] prostaglandins,[10] nitric oxide,[11] and reactive oxygen and nitrogen species[12] could be of great importance in the context of cirrhosis, because these molecules are involved in the pathogenesis of the systemic inflammation and circulatory and organ dysfunction/failure that characterize decompensated cirrhosis and ACLF.[13] Because the occurrence of these non-osmotic effects of albumin therapy in cirrhosis was elusive, there was an urgent need to address this question, which gave rise to the present study.

The current article describes 5 important and, to our knowledge, previously unreported observations on the pathophysiology and albumin treatment of decompensated

Table 2. Baseline Plasma Levels of Cytokines Among Healthy Individuals (HS), Patients From the Pilot-PRECIOSA (P-PR) Study, and Patients From the INFECIR-2 (INF-2) Study

Cytokine	HS (n = 25)	P-PR Study (n = 15)	INF-2 Study (n = 78)	*P* value[a]		
				HS vs p-PR	HS vs INF-2	p-PR vs INF-2
TNF-α						
Median level (IQR), *pg/mL*	12.3 (11.5–16.9)	21.8 (16.0–30.6)	32.0 (21.9–49.8)	**.001**	**.0001**	**.04**
Missing variable, n (%)	0 (0)	0 (0)	0 (0)			
G-CSF						
Median level (IQR), *pg/mL*	3.6 (2.4–5.7)	20.0 (8.8–156.9)	74.4 (32.0–155.5)	**.0008**	**.0001**	.11
Missing variable, n (%)	0 (0)	1 (7)	17 (22)			
IL-1ra						
Median level (IQR), *pg/mL*	7.1 (3.8–13.2)	13.1 (9.1–32.8)	29.6 (8.3–76.5)	**.02**	**.0001**	.26
Missing variable, n (%)	0 (0)	0 (0)	0 (0)			
IL-6						
Median level (IQR), *pg/mL*	0.9 (0.9–0.9)	10.5 (8.0–25.1)	37.1(22.6–107.6)	**.0001**	**.0001**	**.0001**
Missing variable, n (%)	0 (0)	0 (0)	0 (0)			
IL-10						
Median level (IQR), *pg/mL*	1.1 (1.1–2.4)	2.7 (0.8–10.8)	10.9 (6.7–19.0)	.20	**.0001**	**.02**
Missing variable, n (%)	0 (0)	3 (20)	13 (17)			
IL-17A						
Median level (IQR), *pg/mL*	0.7 (0.7–3.3)	17.7 (2.3–32.4)	3.2 (1.4–7.2)	**.0002**	**.002**	*.05*
Missing variable, n (%)	0 (0)	3 (20)	0 (0)			
IFNγ						
Median level (IQR), *pg/mL*	3.0 (2.2–4.7)	6.7 (2.1–35.8)	6.8 (1.5–18.4)	.11	**.04**	.49
Missing variable, n (%)	0 (0)	0 (0)	0 (0)			
VEGF						
Median level (IQR), *pg/mL*	24.4 (14.7–45.2)	59.0 (26.3–230.7)	61.0 (32.1–183.0)	**.02**	**.002**	.77
Missing variable, n (%)	0 (0)	1 (7)	32 (41)			

G-CSF, granulocyte colony-stimulating factor; IFN, interferon; IL-1ra, IL-1 receptor antagonist; IQR, interquartile range; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.
[a]Bold type indicates $P < .05$. Italic type indicates $P = .05$.

cirrhosis. The first is that the long-term albumin dosage required to normalize serum albumin concentration is much higher than that used in the therapeutic RCTs so far performed.[4,14] Second, circulatory dysfunction is not a steady state or a slowly progressive process, as it has been traditionally considered, but rather an extremely unstable condition. Third, systemic inflammation in cirrhosis is also unstable, with acute episodes of bursts of circulating cytokines in the absence of any identifiable precipitating event. Fourth, HAlbD, but not LAlbD treatment, is associated with significant improvement in LV function in decompensated cirrhosis, which is currently considered an important mechanism of systemic circulatory dysfunction.[15] Finally, and most importantly, the sequential assessment of the plasma levels of IL-6 during albumin treatment showed, for the first time to our knowledge, that long-term albumin treatment at high dosage has immunomodulatory effects in decompensated cirrhosis. The transcendence of this last finding was the reason to complete the study with 2 additional investigations. The first was aimed at assessing whether the suppressive effect of albumin on IL-6 observed in the patients included in the Pilot-PRECIOSA study also extended to other cytokines and inflammatory molecules. The second was aimed at investigating whether the immunomodulatory effect observed during long-term treatment with HAlbD in patients without bacterial infection also occurs after short-term (1 week) HAlbD treatment in patients with bacterial infections. For these objectives, we leveraged the availability of biobanking material from the Pilot-PRECIOSA and INFECIR-2 studies.

The initial albumin dose evaluated in the Pilot-PRECIOSA study (1 g/kg every 2 weeks for 12 weeks) was based on that used in the pioneer RCT by Gentilini et al[14] (25 g per week) that explored the effect of long-term of albumin treatment on the response to diuretics in patients with cirrhosis ascites and in 2 RCTs that explored the long-term effect of albumin administration on the natural course of decompensated cirrhosis (the ANSWER study, 40 g of albumin every week,[4] and the MATCH study, 40 g of albumin every 2 weeks[16]). The results of the current study indicate that a dose of 1g/kg, which is higher than the MATCH study dose and only slightly lower than the ANSWER study dose, was clearly insufficient to normalize serum albumin concentration in 7 of the 8 patients with hypoalbuminemia included in the LAlbD group. In contrast, our second albumin dosage (1.5 g/kg per week) rapidly normalized serum albumin concentration in all patients with hypoalbuminemia included in the HAlbD group.

The time-course changes of serum albumin concentration during albumin treatment suggest that the homeostatic

Table 3. Plasma Levels of Cytokines at Baseline and at The 6th Week of Treatment in Patients Receiving Either LAlbD or HAlbD in the Pilot PRECIOSA Study

	LAlbD group (n = 10)			HAlbD group (n = 5)			*P* value for between-group comparison[a]	
Cytokine	Undetectable levels, n (%)	Baseline cytokine level, *pg/mL*, median (IQR)	Absolute change from baseline, median (IQR)	Percent change from baseline, median (IQR)	Undetectable levels, n (%)	Baseline cytokine level, *pg/mL*, median (IQR)	Absolute change from baseline, median (IQR)	Percent change from baseline, median (IQR)	Absolute change from baseline	Percent change from baseline
TNF-α	0 (0)	20.3 (13.6 to 28.1)	1.8 (–0.7 to 3.5)	11.0 (–3.5 to 15.9)	0 (0)	30.9 (18.4 to 53.6)	–4.9 (–9.3 to 0.7)	–15.1 (–16.1 to 7.5)	.12	.19
G-CSF	0 (0)	20.0 (8.8 to 156.9)	4.9 (–1.4 to 13.8)	20.5 (–14.5 to 60.3)	1 (20)	47.3 (6.1 to 315.5)	–63.1 (–79.5 to –53.2)	–63.1 (–79.5 to –53.2)	.05	**.03**
IL-1ra	0 (0)	13.1 (10.2 to 35.3)	2.8 (–1.2 to 18.9)	28.8 (–15.2 to 53.6)	0 (0)	8.5 (6.7 to 29.3)	–4.0 (–7.0 to –2.8)	–70.3 (–82.9 to –13.8)	**.03**	**.04**
IL-6	0 (0)	8.9 (6.5 to 24.6)	0.8 (–2.5 to 7.4)	44.1 (–11.7 to 83.9)	0 (0)	10.7 (10.5 to 28.4)	–9.2 (–14.2 to –5.0)	–50.1 (–67.3 to –46.8)	**.01**	**.01**
IL-10	2 (20)	1.8 (0.6 to 10.8)	0.3 (–0.7 to 1.3)	–3.1 (–42.8 to 27.0)	1 (20)	5.6 (2.2 to 27.6)	–3.4 (–14.9 to 0.7)	–24.4 (–66.2 to 21.3)	.35	.43
IL-17A	3 (30)	24.7 (1.4 to 33.7)	0.65 (–0.9 to 9.8)	12.4 (–46.1 to 40.8)	0 (0)	15.4 (2.9 to 19.9)	–9.2 (–16.4 to –1.5)	–59.6 (–82.4 to –51.7)	.07	.14
IFNγ	0 (0)	5.6 (2.6 to 49.0)	0.2 (–0.6 to 2.2)	5.6 (–7.7 to 32.0)	0 (0)	8.7 (1.7 to 22.6)	–4.5 (–14.8 to –0.2)	–51.7 (–65.6 to –15.2)	.12	.12
VEGF	1 (10)	198.0 (56.1 to 230.7)	26.5 (0.0 to 50.8)	11.7 (0.0 to 29.2)	0 (0)	26.3 (23.5 to 50.1)	–8.4 (–17.6 to –4.0)	–75.2 (–91.4 to –16.8)	**.03**	**.01**

G-CSF, granulocyte colony-stimulating factor; IFN, interferon; IL-1ra, IL-1 receptor antagonist; IQR, interquartile range; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.
[a]Bold type indicates $P < .05$. Italic type indicates $P = .05$.

feedback mechanism by which hepatic albumin synthesis is regulated by the serum albumin concentration[17] is fully operative in patients with advanced cirrhosis. Normalization of serum albumin concentration in patients with hypoalbuminemia receiving HAlbD occurred very rapidly (within 2 weeks) after the onset of albumin treatment, but once normalized, it remained within normal limits throughout the study despite the weekly administration of albumin at a concentration of 20 g/dL (5 times higher than the normal serum albumin concentration). This rapid and intense initial increase in serum albumin concentration was probably the consequence of the combination of increased albumin synthesis by the liver secondary to hypoalbuminemia and the effect of the exogenous albumin administrations. In contrast, after normalization of serum albumin, the inhibitory effect of normo-albuminemia on albumin synthesis precluded any further increase in serum albumin concentration, despite continuous albumin treatment. The homeostatic feedback mechanism of serum albumin would also explain why albumin treatment did not increase serum albumin concentration in patients without hypoalbuminemia. For additional explanatory details, see Figure 1 legend.

The most relevant finding of our study was the observation that both long-term and short-term albumin treatment, if given at high dosage, are associated with significant immunomodulatory effects in decompensated cirrhosis. Three lines of evidence supported this conclusion. The first derived from the sequential measurement of IL-6 during albumin treatment in patients included in the pilot-PRECIOSA study. The median reduction from baseline of plasma IL-6 levels was significantly greater among patients receiving HAlbD than among those receiving LAlbD. This finding is important considering that IL-6 has broad effects on immune and nonimmune cells and often displays hormone-like characteristics that can affect homeostatic processes.[18] The second line of evidence derived from the analysis of the effect of albumin treatment on cytokines other than IL-6 in biobanking material from the Pilot-PRECIOSA study. This investigation confirmed the observations of the first investigation. Treatment with HAlbD, but not with LAlbD, was associated with significant decreases in plasma IL-6 during treatment. Moreover, it showed that this effect also involved other keystone cytokines (eg, granulocyte colony-stimulating factor), confirming that long-term therapy with HAlbD, but not with LAlbD, induces a significant and extensive immunomodulatory effect in decompensated cirrhosis. Finally, the third line of evidence was obtained from the analysis of biobanking plasma samples from the INFECIR-2 study. Treatment with albumin plus antibiotics was associated with a rapid, significant, and widespread suppression of the circulating levels of cytokines, an effect not observed with antibiotics alone. It was interesting to observe that the immunomodulatory effect of albumin in the Pilot-PRECIOSA and INFECIR-2 studies was related mainly to the inhibitory effect of albumin on cytokine production but not to an effect on other inflammatory molecules.

Table 4. Effects of LAlbD and HAlbD Administration on Cardiovascular and Splanchnic Hemodynamics, Cardiac Peptides and Standard Liver and Renal Function Parameters

	LAlbD (n = 10)		*P* value	HAlbD (n = 8)		*P* value
	Baseline	Week 14		Baseline	Week 14	
Systemic hemodynamics	n = 7			n = 8		
RAP, *mm Hg*	6 (4–8)	4 (4–10)	0.87	8 (4–8)	9 (6–9)	.26
MPAP, *mm Hg*	16 (15–17)	15 (12–20)	0.55	15 (11–18)	18 (15–25)	**.02**
PCWP, *mm Hg*	10 (9–11)	8 (7–13)	0.74	11 (8–15)	12 (10–14)	.11
Cardiac index, *L/min/m²*	3.9 (1.8–4.6)	3.8 (2.3–5.3)	0.09	4.2 (3.0–5.0)	5.3 (3.1–6.8)	**.04**
Heart rate, bpm	61 (59–82)	75(62–86)	0.21	69 (59–91)	68 (62–76)	.21
Systolic volume, *mL*	120 (40–127)	90 (52–135)	0.74	125 (85–145)	165 (110–190)	**.04**
LV stroke work index, *g·m/m²*	48 (24–64)	44 (27–68)	0.74	54 (49–69)	82 (51–97)	**.04**
SVRI, *dyn·s/cm⁵/m²*	1158 (1042–3840)	1182 (944–2762)	0.18	1257 (952–1693)	1072 (728–1183)	.09
MAP, *mm Hg*	78 (63–88)	77 (75–85)	0.61	78 (74–85)	77 (66–84)	.48
Cardiac peptides	n = 9			n = 6		
ANP, *fmol/mL*	58 (23–84)	53 (37–64)	0.59	41 (13–87)	65 (29–155)	.14
BNP, *pg/mL*	82 (25–221)	37 (34–126)	0.45	41 (32–69)	49 (18–128)	.46
Splanchnic hemodynamics	n = 7			n = 6		
FHVP, *mm Hg*	15 (9–17)	12 (5–17)	0.45	11 (8–14)	9 (5–10)	.12
WHVP, *mm Hg*	35 (25–38)	30 (26–39)	0.67	32 (29–36)	28 (25–31)	.21
HVPG, *mm Hg*	19 (15–20)	21 (14–22)	0.34	19 (17–27)	22 (18–25)	.89
Liver and renal function	n = 10			n = 8		
AST, *UI/L*	58 (27–78)	53 (26–69)	0.15	45 (40–112)	34 (31–55)	**.02**
ALT, *UI/L*	27 (19–46)	25 (16–34)	0.11	35 (28–56)	24 (21–39)	.13
Serum creatinine, *mg/dL*	1.3 (1.0–1.4)	1.0 (0.9–1.3)	0.06	0.9 (0.8–1.3)	0.9 (0.7–1.2)	.24
BUN, *m/dL*	26 (16–47)	22 (16–32)	0.16	20 (15–31)	24 (17–36)	.25
Serum sodium, *mEq/L*	132 (126–136)	133 (129–137)	0.67	130 (129–135)	132 (131–134)	.87
Serum albumin, *g/L*	27 (25–40)	35 (31–40)	0.06	27 (22–35)	40 (35–41)	**.03**
Serum bilirubin, *mg/dL*	1.8 (1.0–2.1)	1.8 (1.1–2.8)	0.41	3.7 (1.9–13.0)	1.9 (1.6–16.8)	.40
INR	1.3 (1.1–1.6)	1.5 (1.1–1.7)	0.29	1.4 (1.3–2.3)	1.4 (1.3–2.5)	.46
Child-Pugh score, *points*	8 (6–10)	8 (7–9)	0.37	9 (8–11)	7 (6–8)	**.02**
MELD score, *points*	14 (11–17)	14 (9–17)	0.59	16 (13–26)	16 (13–27)	.25

ALT, alanine aminotransferase; ANP, atrial natriuretic peptide; AST, aspartate aminotransferase; BNP, brain natriuretic peptide; BUN, blood urea nitrogen; FHVP, free hepatic venous pressure; HVPG, hepatic venous pressure gradient; LV, left ventricular; MAP, mean arterial pressure; MELD, model for end stage liver disease; MPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; RAP, right atrial pressure; SVRI, systemic vascular resistance index; WHVP, wedge hepatic venous pressure.

NOTE. Values are reported as median (interquartile range). Normal value ranges are as follow: right atrial pressure, 2–10 mm Hg; mean pulmonary arterial pressure, 10–25 mm Hg; pulmonary capillary pressure, 6–14 mm Hg; cardiac index, 2.5–4 L/min/m²; SV, 60–100 mL; LV stroke work index, 45–75 g·m/m²/beat; SVRI, 1970–2390 dyn·sec/cm⁵/m²; MAP, 80–95 mm Hg; ejection fraction, >50%; ANP, 9–24 fmol/mL; BNP, 4–37 pg/mL; HVPG, 1–5 mm Hg.

[a]Bold type indicates $P < .05$.

An intriguing finding of our study was the observation of 1 or 2 acute, intense, and spontaneously reversible peaks of PRA and plasma IL-6 during albumin treatment in many patients included in the Pilot-PRECIOSA study. There are reasons to suggest that the prevalence and frequency of these peaks in the current study are not representative of their actual prevalence and frequency in patients with decompensated cirrhosis. First, we monitored PRA and plasma IL-6 only once every week or every 2 weeks during the study period. However, according to our data, the duration of these peaks may range from less than 1 to 2 or more weeks. Therefore, we could have lost a significant number of peaks in our patients. Interestingly, the prevalence of PRA and IL-6 peaks was lower in patients receiving HAlbD than in those receiving LAlbD, suggesting that treatment with HAlbD may prevent the occurrence of these acute episodes of aggravation of circulatory dysfunction and systemic inflammation in decompensated cirrhosis. Although the current study is, to our knowledge, the first to show these abnormalities, the existence of such episodes of acute circulatory impairment and systemic inflammation had already been anticipated by the systemic inflammation hypothesis as an explanation for the 40% prevalence of ACLF in patients without any identifiable exogenous precipitating event of the syndrome.[13,19] The proposed mechanism of such changes by the systemic inflammation hypothesis is the existence of transient bursts of translocation of viable bacteria or bacterial products from the intestinal lumen to the systemic circulation. Therefore, the potential futility of single measurements of renin and cytokines as surrogate markers of effective blood volume and systemic inflammation in patients with decompensated cirrhosis has to be considered in the design of future studies.

Table 5. Baseline Plasma Levels of Cytokines and Their Changes During the First Week of Treatment, in Patients From the INFECIR-2 Study Who Were Randomly Assigned to Receive Either Antibiotics Alone or Albumin Plus Antibiotics

	Antibiotics Alone (n = 40)						Albumin plus antibiotics (n = 38)					
					P value for change from baseline[a]						*P* value for change from baseline[a]	
Cytokine	Undetectable levels, n (%)	Baseline level, *pg/mL*, median (IQR)	Absolute change from baseline, median (IQR)	Percent change from baseline, median (IQR)	*P* for absolute change	*P* for percent change	Undetectable levels, n (%)	Baseline level, *pg/mL*, median (IQR)	Absolute change from baseline, median (IQR)	Percent change from baseline, median (IQR)	*P* for absolute change	*P* for percent change
TNF-α	0 (0)	37.9 (23.3 to 50.0)	–2.8 (–12.6 to 3.2)	–8.2 (–28.9 to 11.9)	*.05*	**.01**	0 (0)	31.1 (21.2 to 45.3)	–3.4 (–14.7 to 3.1)	–16.2 (–40.5 to 12.8)	**.01**	**.04**
G-CSF	9 (23)	74.4 (19.2 to 185.5)	–3.4 (–55.3 to 11.5)	–21.2 (–91.4 to 21.5)	.33	.85	8 (21)	73.5 (33.6 to 115.0)	–41.5 (–65.1 to 8.4)	–58.6 (–88.8 to 30.2)	**.01**	**.01**
IL-1ra	0 (0)	29.6 (8.3 to 71.5)	–0.6 (–28.0 to 9.0)	–5.9 (–51.5 to 23.6)	.37	.31	0 (0)	29.9 (8.3 to 76.5)	–0.5 (–34 to 2.1)	–3.5 (–73.0 to 8.3)	*.05*	.28
IL-6	0 (0)	37.7 (18.3 to 94.7)	–7.0 (–19.9 to 20.7)	–14.8 (–43.5 to 66.4)	.53	.27	0 (0)	36.9 (23.9 to 158.9)	–7.7 (–33.1 to 0.3)	–23.0 (–55.0 to 3.8)	**.003**	**.005**
IL-10	8 (20)	10.7 (6.3 to 20.9)	–0.2 (–6.7 to 4.4)	–1.8 (–56.7 to 50.1)	.74	.99	5 (13)	11.0 (6.7 to 15.1)	–1.5 (–7.6 to 2.8)	–15.6 (–53.5 to 63.0)	**.03**	**.03**
IL-17A	0 (0)	3.7 (1.2 to 8.2)	0.1 (–1.7 to 1.3)	2.5 (–38.6 to 81.1)	.92	.99	0 (0)	2.7 (1.6 to 7.2)	–0.5 (–2.7 to 0.4)	–15.4 (–52.4 to 29.9)	*.05*	.09
IFNγ	0 (0)	4.8 (1.2 to 15.6)	–0.2 (–6.2 to 1.3)	–7.9 (–51.7 to 37.1)	.17	.52	0 (0)	8.4 (2.0 to 19.7)	–0.5 (–8.6 to 4.3)	–24.8 (–63.3 to 52.9)	.48	.24
VEGF	16 (40)	65.4 (45.6 to 204.0)	–18.5 (–41.4 to 18.2)	–16.4 (–45.3 to 29.4)	.30	.31	16 (42)	50.6 (24.1 to 183.0)	–13.9 (–49.0 to 11.9)	–24.7 (–36.9 to 32.5)	.10	.29

NOTE. Changes during the first week of albumin treatment were assessed between day 3 and day 7 after inclusion. There were no significant between-group differences in cytokine levels at baseline.

G-CSF, granulocyte colony-stimulating factor; IFN, interferon; IL-1ra, IL-1 receptor antagonist; IQR, interquartile range; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

[a]Bold type indicates $P < .05$. Italic type indicates $P = .05$.

Although circulatory dysfunction in cirrhosis has been traditionally attributed to splanchnic arterial vasodilation, there is now evidence that impairment in LV function also plays a major role. In fact, the cardiac index in cirrhosis falls progressively from compensated cirrhosis to decompensated cirrhosis and HRS.[15] Our data show that normalization of serum albumin concentration with long-term HAlbD treatment in noninfected patients with decompensated cirrhosis does not induce significant changes in central blood volume and portal pressure. However, it was associated with a significant improvement in LV function. These observations are important for 2 reasons. First, they explain why treatment with HAlbD is generally not associated with variceal bleeding or pulmonary edema in decompensated cirrhosis without bacterial infections. Second, because systemic inflammation induces direct deleterious effect on heart function, our study supports the concept that the beneficial effect of albumin treatment in the management of organ dysfunction/failure in cirrhosis may be mediated, at least in part, by its immunomodulatory effect. In fact, this has also been observed in rats with carbon tetrachloride-induced cirrhosis, which develop evidence of systemic inflammation and inflammation in the cardiac tissue associated with severe impairment of LV contractibility, which reverses after albumin treatment.[20]

One of the strengths of our study is the use of multiple plasma samples, the prospective collection of which was prespecified in the context of 2 well-designed multicenter controlled trials, 1 of which was randomized. A limitation of our study was the relatively low number of patients included in the Pilot-PRECIOSA study. However, the most important finding of this investigation, the significant immunomodulatory effect of albumin treatment in patients with advanced cirrhosis, was confirmed by assessing the effect of albumin treatment on a large panel of inflammatory cytokines, both in patients included in the Pilot-PRECIOSA study and in a relatively large number of patients included in the INFECIR-2 study, thus offering solid additional arguments supporting our conclusions.

In summary, the current study allowed us to uncover important findings related to the efficacy of albumin treatment in cirrhosis. The most outstanding were that high doses of albumin, but not low doses of albumin, in patients with decompensated cirrhosis have significant immunomodulatory effects, prevent a phenomenon shown by the present study that consists of bursts of circulatory dysfunction, improve LV function, and correct serum albumin levels without inducing albumin overdose, probably because of the preservation of negative feedback mechanisms controlling albumin synthesis, even in advanced liver disease. Because albumin is capable of binding to and inactivating many inflammatory promoters, such as pathogen-associated molecular patterns, bioactive lipid metabolites, reactive oxygen species, and nitric oxide, the immunomodulatory effects of albumin could be related to this scavenging function. However, this explanation may be too simplistic, and further investigations are clearly needed to understand the anti-inflammatory effect of albumin treatment in cirrhosis.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2019.03.021.

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Author names in bold designate shared co-first authorship.

Received November 19, 2018. Accepted March 14, 2019.

Reprint requests
Address requests for reprints to: Javier Fernández, MD, PhD, EF-Clif, EASL-CLIF Consortium and Grifols Chair, Travessera de Gràcia 11, 7th floor, 08021, Barcelona, Spain. e-mail: javier.fernandez@efclif.com.

Acknowledgments
Author contributions: Javier Fernández, Miriam Castro, Juan Acevedo, Luis Ruiz-del-Arbol, Cándido Villanueva, Aníbal Silva, Manuel Morales-Ruiz, and Vincente Arroyo participated in the design and execution of the pilot PRECIOSA study (IG0802); Javier Fernández, Verónica Prado, Mireya Arteaga, Jonel Trebicka, Paolo Angeli, Manuela Merli, Carlo Alessandria, Niels Kristian Aagaard, German Soriano, François Durand, Alexander Gerbes, Thierry Gustot, Tania M. Welzel, Francesco Salerno, Rafael Bañares, Victor Vargas, Agustin Albillos, and Vicente Arroyo participated in the INFECIR-2 study. Javier Fernández, Joan Clària, AA, Ferrán Aguilar, Marco Pavesi, and Vicente Arroyo participated in data analysis and interpretation. Javier Fernández, Joan Clària, Alex Amoros, Ferrán Aguilar, Juan Acevedo, Luis Ruiz-del-Árbol, Cándido Villanueva, Verónica Prado, Jonel Trebicka, Paolo Angeli, Manuela Merli, Carlo Alessandria, Niels Kristian Aagaard, German Soriano, François Durand, Alexander Gerbes, Thierry Gustot, Tania M. Welzel, Francesco Salerno, Rafael Bañares, Victor Vargas, Agustin Albillos, Rajiv Jalan, Mauro Bernardi, Richard Moreau, and Vicente Arroyo participated in the writing group. Laura Nuñez, Miriam Castro, Mireia Torres, Raquel Horrillo, and Antonio Páez from Grifols participated in the design of the pilot PRECIOSA study (IG0802).

Conflicts of interest
These authors disclose the following: Javier Fernández has received research support from Grifols. Mauro Bernardi has received speaker honorarium and act as a consultant for Grifols. Laura Nuñez, Montserrat Costa, Mireia Torres, Raquel Horrillo, and Antonio Páez are full-time employees of Grifols and have no other competing interests to declare. The remaining authors disclose no conflicts.

Funding
The study was supported by European Foundation for the Study of Chronic Liver Failure (EF-Clif), a nonprofit private organization aimed to stimulate research in cirrhosis. EF-Cliff has 2 main activities: the European Association for the Study of the Liver (EASL) Chair, which is mainly devoted to clinical research through the CLIF-Consortium, and the Grifols Chair, which has recently been developed to promote translational research. EF-Clif receives unrestricted donations from Cellex Foundation, Grifols, and the European Union (coordinator center, partner, and contributor in several projects of the European Union Horizon 2020 research program). The funders had no influence on data analysis, decision to publish, or preparation of the manuscript. Jonel Trebicka is an EF-Clif-Cellex Visiting Professor.