Research Article
Cirrhosis and Liver Failure

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Lysophosphatidylcholines modulate immunoregulatory checkpoints in peripheral monocytes and are associated with mortality in people with acute liver failure

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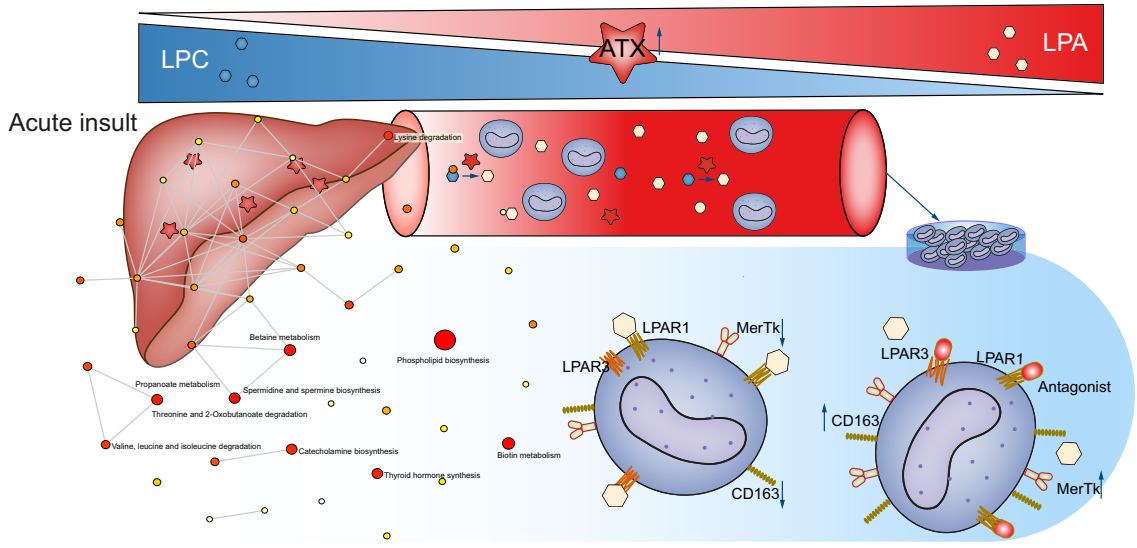
Graphical abstract



Highlights

- Plasma lysophosphatidylcholines are reduced and can be used as prognostic markers of poor outcome in ALF.
- The LPC-ATX-LPA axis appears to modulate innate immune response in ALF via MerTK and CD163 expression.
- LPAR1 and LPAR3 antagonism reverses the effects of LPA on monocyte phenotype.

Impact and implications

We identified a metabolic signature of acute liver failure (ALF) and investigated the immunometabolic role of the lysophosphatidylcholine-autotaxin-lysophosphatidylcholinic acid pathway, with the aim of finding a mechanistic explanation for monocyte behaviour and identifying possible therapeutic targets (to modulate the systemic immune response in ALF). At present, no selective immune-based therapies exist. We were able to modulate the phenotype of monocytes *in vitro* and aim to extend these findings to murine models of ALF as a next step. Future therapies may be based on metabolic modulation; thus, the role of specific lipids in this pathway require elucidation and the relative merits of autotaxin inhibition, lysophosphatidylcholinic acid receptor blockade or lipid-based therapies need to be determined. Our findings begin to bridge this knowledge gap and the methods used herein could be useful in identifying therapeutic targets as part of an experimental medicine approach.

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Lysophosphatidylcholines modulate immunoregulatory checkpoints in peripheral monocytes and are associated with mortality in people with acute liver failure

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Background & Aims: Acute liver failure (ALF) is a life-threatening disease characterised by high-grade inflammation and immunoparesis, which is associated with a high incidence of death from sepsis. Herein, we aimed to describe the metabolic dysregulation in ALF and determine whether systemic immune responses are modulated *via* the lysophosphatidylcholine (LPC)-autotaxin (ATX)-lysophosphatidylcholinic acid (LPA) pathway.
Methods: Ninety-six individuals with ALF, 104 with cirrhosis, 31 with sepsis and 71 healthy controls (HCs) were recruited. Pathways of interest were identified by multivariate statistical analysis of proton nuclear magnetic resonance spectroscopy and untargeted ultraperformance liquid chromatography-mass spectrometry-based lipidomics. A targeted metabolomics panel was used for validation. Peripheral blood mononuclear cells were cultured with LPA 16:0, 18:0, 18:1, and their immune checkpoint surface expression was assessed by flow cytometry. Transcript-level expression of the LPA receptor (*LPAR*) in monocytes was investigated and the effect of LPAR antagonism was also examined *in vitro*.
Results: LPC 16:0 was highly discriminant between ALF and HC. There was an increase in ATX and LPA in individuals with ALF compared to HCs and those with sepsis. LPCs 16:0, 18:0 and 18:1 were reduced in individuals with ALF and were associated with a poor prognosis. Treatment of monocytes with LPA 16:0 increased their PD-L1 expression and reduced CD155, CD163, MerTK levels, without affecting immune checkpoints on T and NK/CD56+T cells. LPAR1 and 3 antagonism in culture reversed the effect of LPA on monocyte expression of MerTK and CD163. MerTK and CD163, but not *LPAR* genes, were differentially expressed and upregulated in monocytes from individuals with ALF compared to controls.
Conclusion: Reduced LPC levels are biomarkers of poor prognosis in individuals with ALF. The LPC-ATX-LPA axis appears to modulate innate immune response in ALF via LPAR1 and LPAR3. Further investigations are required to identify novel therapeutic agents targeting these receptors.

Introduction

Acute liver failure (ALF) is a rare life-threatening disease characterised by acute derangement of liver synthetic function with coagulopathy and altered levels of consciousness from hepatic encephalopathy in people without previous liver disease.[1] ALF is characterised by intense systemic inflammation and parallel impairment of innate and adaptive antimicrobial immune responses (immunoparesis), and is associated with a high incidence of death from sepsis and multiorgan failure.[2] In this context, immune cell function is often suppressed by multiple mechanisms, including inhibitory signalling *via* immune checkpoint pathways, such as programmed cell death 1 (PD-1)[3] and cytotoxic T-lymphocyte-associated protein 4 (CTLA4).[4] However, no selective immune-based therapies exist for ALF.

The association between ALF and sepsis is due to a switch of the innate immune system to a regulatory, anti-inflammatory mode during the first week of illness, as systemic inflammation subsides. Expression of MerTK (mediating the efferocytosis of apoptotic cell) and CD163 (a scavenger receptor) increases in peripheral monocytes and hepatic macrophages favour tissue repair at the expense of a higher risk of sepsis.[5,6] We recently demonstrated that PD-1 blockade restored human monocyte functionality *in vitro* while PD-1-deficient mice and anti-PD-1-treated mice with liver injury showed improved Kupffer cell bacterial clearance and protection from sepsis.[3] However,

Keywords: lysophosphatidylcholine; acute liver failure; immune checkpoint; LPAR; MerTK; CD163; RNA sequencing; Monocytes.
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there are safety concerns regarding the use of immune checkpoint inhibition in these patients given the potential for worsening liver injury.[7] The mechanisms leading to monocyte reprogramming and immune checkpoint overexpression are not yet defined and modulating them to prevent sepsis and to safely promote hepatic regeneration is an unmet clinical need in ALF. Importantly, it was recently proposed that metabolic factors may evoke monocyte reprogramming in acute-on-chronic liver failure (ACLF),[8] but whether this finding extends to ALF remains unknown.

The liver, as a major metabolic organ, is responsible for the metabolism of many exogenous compounds and synthesis of endogenous metabolites. These factors make untargeted metabolomics/lipidomics, as well as more targeted methods (*e.g.* for acylcarnitines, and bile acids[9] as markers of mitochondrial dysfunction and hepatotoxicity, respectively) a useful approach for functional investigations. This was recently highlighted in studies of paracetamol-related ALF by providing a means of gaining a metabolic signature of disease and for highlighting pathways for mechanistic and therapeutic exploration.[9]

More recently the prognostic role of lysophosphatidylcholines (LPCs) in ACLF, and their potential in monocyte functional modulation has been shown.[8] Lysophosphatidylcholinic acid (LPA) and LPC are the most studied lysoglycerophospholipid species modulating acute and chronic inflammatory processes. LPAs originate from the hydrolysation of membrane phospholipids, including phosphatidylcholine (PC), phosphatidylethanolamine, and phosphatidylserine, to lysophospholipids. This process is catalysed by phospholipases A1 (PLA1) and 2 (PLA2). Then a lysophospholipase D, named autotaxin (ATX), converts LPC to LPA.

The LPC-ATX-LPA axis has been reported to exert its effect through LPA1-3 receptors and LPA4-6 receptors that belong to the endothelial gene and non-endothelial gene families respectively.[10]

These receptors are of clinical significance, for example specific LPAR4 deletion improved inflammatory cell recruitment in atherosclerotic lesions.[11,12] Similarly, lysoglycerophospholipids have been reported as active mediators in rheumatoid arthritis, with increased LPA and LPAR1 levels observed in affected patients. Moreover, LPAR1 antagonism modulated synovial inflammation and bone and cartilage damage.[13,14] LPARs are implicated in neuropathic pain,[15] renal, liver and pulmonary fibrosis and intimal hyperplasia, which is responsible for atherosclerosis.[16]

Although well studied in other inflammatory diseases, large clinical studies linking immunometabolic factors and the LPC-ATX-LPA axis in human ALF have not been undertaken. There is evidence in mice that LPA could be protective against paracetamol (acetaminophen)-induced liver injury[17] while LPC induced lobular hepatitis in another animal model.[18] In liver biopsies from organs donated after circulatory death, LPCs were increased and this was associated with ischaemic liver injury.[19] Moreover, LPCs were associated with early allograft dysfunction post-liver transplant[20] but little is known about the role of lysophospholipids in the acute setting that often leads to liver transplantation.

This present work addresses a gap in our understanding of immunometabolism in ALF. The aim of this research was to (i) investigate the metabolic signature and the immunometabolic role of the LPC-ATX-LPA pathway in order to find a mechanistic explanation for monocyte behaviour in ALF and (ii) identify possible therapeutic target(s) to modulate the systemic immune response in ALF.

Materials and methods

Study population

The exploratory cohort included individuals with ALF recruited at King's College Hospital in London between December 2012 and July 2015 ("Gut-Liver Axis in Acute and Chronic Liver Failure Syndromes and Transplantation"; London -Westminster Research Ethics Committee No.: 12/LO/1417; IRAS No.: 104301). The validation cohort included patients admitted from May 2013 to December 2021 to King's College Hospital using the same exclusion criteria (pregnancy, disseminated malignancy, pre-existing immunosuppressive states including drugs and HIV infection and chronic granulomatous diseases). Patients were screened and approached for recruitment as part of the "Monocyte and macrophage phenotype and function in sepsis, acute hepatic failure and chronic liver disease" (London-Westminster Research Ethics Committee No.: 12/LO/0167, IRAS No.: 87203) and the "Immunometabolism in Sepsis, Inflammation and Liver Failure Syndromes/IMET" (North West Haydock Research Ethics Committee No.: 19/NW/0750, IRAS No.: 244089) studies within 24 h of admission. Patients or family consultees in case of lack of capacity provided written informed consent. Clinical data and laboratory parameters were collected, and disease severity and prognostic scores were calculated, including model of end-stage liver disease (MELD), Child-Pugh, and Sequential Organ Failure Assessment (SOFA)[21] (see Tables S1 and S2).

Isolation of plasma and human peripheral blood mononuclear cells

Blood was drawn into lithium heparin Vacutainers (BD, Franklin Lakes, NJ) and peripheral blood mononuclear cells (PBMCs) isolated as per supplementary materials and methods.

Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy

^{1}H NMR spectroscopy was initially used to profile plasma and identify the main metabolite groups perturbed in ALF. Please see supplementary materials and methods for details.

Untargeted UPLC-MS lipid analysis

Ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS) was employed using an untargeted method to generate lipid profiles from serum in both positive and negative ionisation modes.[22] Please see supplementary materials and methods for details.

UPLC-MS - BIOCRATES p180 assay

We utilised the AbsoluteIDQ® p180 kit (Biocrates Life Sciences, Austria) to quantify 180 known metabolites as previously described.[8] Please see supplementary materials and methods for details.

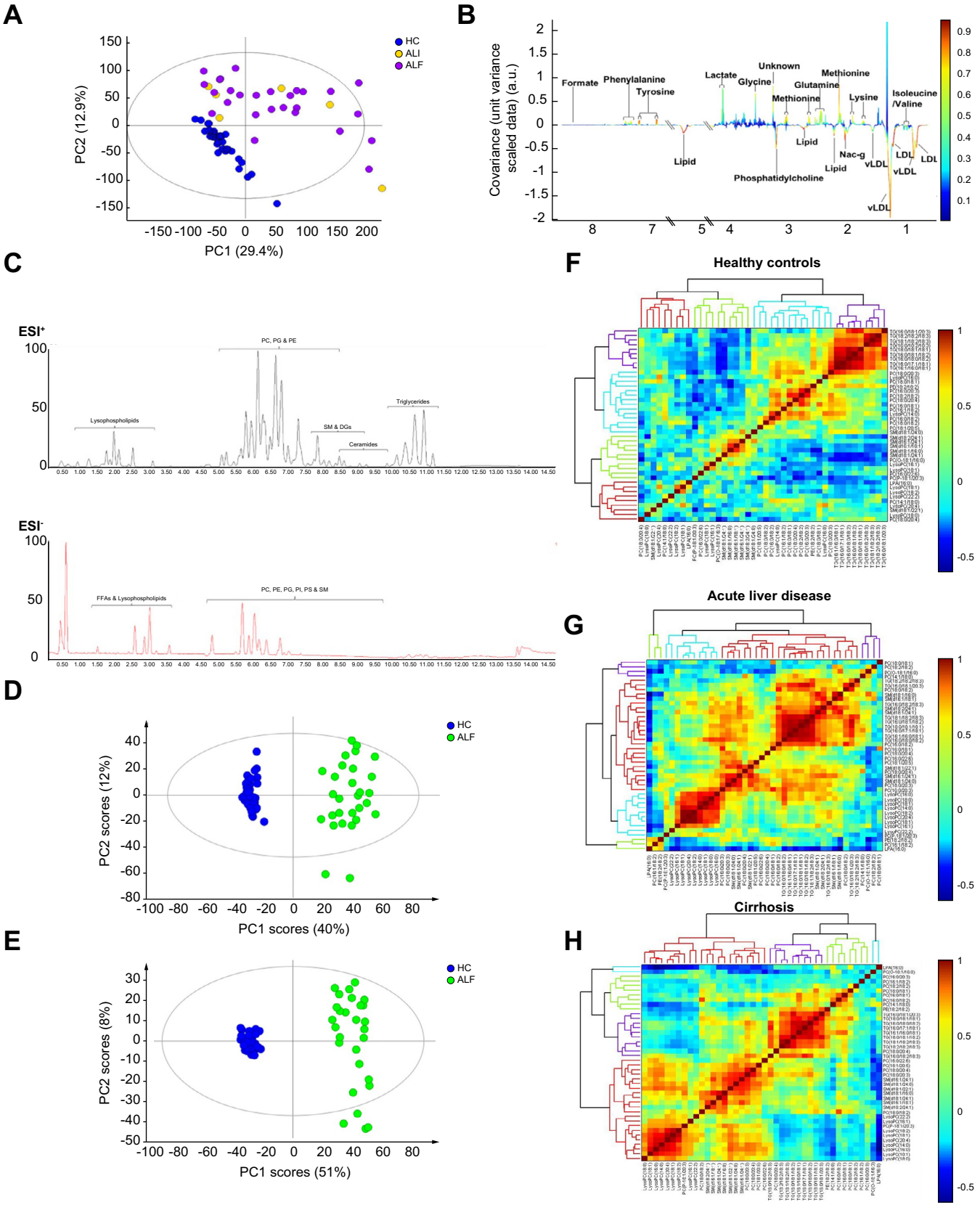

Fig. 1. ^{1}H NMR spectroscopic and chromatographic data. (A,B) Multivariate analysis of ^{1}H NMR spectroscopic data: ALI and ALF are metabolically different from HC. In the exploratory cohort, a three-component PCA model of acute liver disease and HCs (R2 = 0.48; Q2 = 0.38), showed a clear separation between HC and ALF scores in PC2 which explains 29% of total variance for the model. The PCA loading revealed a decrease in lipid resonances (LDL, VLDL and phosphatidylcholine), as well as a decrease in the BCAAs isoleucine and valine and raised amounts of lactate and formate and the amino acids lysine, glycine, glutamine and the AAAs tyrosine and phenylalanine (B)

Cytokine analysis and ELISAs

Plasma cytokines (*IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNFa*) were measured using V-PLEX Proinflammatory Panel 1 Human Kit (Meso-Scale Discovery,Rockville, MD). ATX, PLA1, PLA2, OPN and M30 were quantified with ELISA as per supplementary materials and methods.

Single-epitope enzymatic immunohistochemistry for detection of ATX-positive cells

Liver tissue from explants was stained with primary antibody ENPP2 (Abcam # ab77104, UK). Please see supplementary materials and methods for details.

Cell culture

Isolated PBMCs from both HCs and individuals with ALF were incubated with different lipids (LPA 16:0, LPA 18:0, LPA 18:1, LPC 16:0, LPC 18 - Avanti Polar Lipids, Alabaster, AL) and LPAR antagonists H2L518630 (Sigma-Aldrich), Ro 6842262(Bio-techne) or Ki 16425 (Sigma-Aldrich). Please see supplementary materials and methods for details.

Immunophenotyping

Monocyte phenotype was determined by flow cytometry on PBMCs using the antibodies in Tables S3 (see supplementary materials and methods).

Monocyte total RNA isolation and mRNA sequencing

Please see supplementary materials and methods for details.

Statistical analysis

Please see supplementary materials and methods for details.

Results

As detailed in the Methods section, the exploratory cohort included 34 individuals with acute liver disease (7 acute liver injury [ALI] and 27 ALF), 40 HCs, 13 with stable cirrhosis, and 50 with acute decompensation of cirrhosis (AD). Transplant-free survival rates at 28 and 90 days and 1 year for ALF were 67%, 56% and 52%, respectively (Tables S1). The validation cohort consisted of 62 individuals with ALF, 31 HCs, 41 individuals with stable decompensated cirrhosis (according to PREDICT definition[23]), and 31 with sepsis (according to SEPSIS-3 criteria).[21] A sub-cohort was used for metabolomics/lipidomics (Tables S2). The transplant-free survival rate of individuals with ALF was 56% at both 28 and 90 days.

AD and ALF are metabolically different from HC according to multivariate analysis of 1H NMR spectroscopic data

In the exploratory cohort, a three-component principal component analysis (PCA) model of acute liver disease and HCs (R^2 = 0.48; Q^2 = 0.38), produced from the data obtained from the ^{1}H NMR spectroscopic analysis of plasma, showed a clear separation between HC and ALI/ALF scores in PC2 which explained 29% of the total variance for the model (Fig. 1A,B). The PCA loading revealed a decrease in lipid resonances (LDL, VLDL and phosphatidylcholine), as well as a decrease in the branched-chain amino acids isoleucine and valine and increased lactate, formate and the amino acids lysine, glycine, glutamine and the aromatic amino acids tyrosine and phenylalanine (Fig. 1B) compared to HCs.

The ALF samples that lay outside the Hoteling's ellipse, and that were separated from all other samples in PC1, also revealed the presence in the plasma ^{1}H NMR spectra of drug-related compounds, such as sedative and anaesthetic agents, being administered to critically ill patients receiving intensive care.

Untargeted UPLC-MS lipidomic analysis showed reduced LPCs in individuals with ALF

A previous ^{1}H NMR spectroscopy and UPLC-MS study from our group identified LPCs as the main lipid class driving change seen in survivors *vs.* non-survivors in those with AD.[24] Based on these findings, an untargeted UPLC-MS lipid profiling method[22] was applied to sera from the exploratory cohort of patients (Fig. 1C). Data were acquired in both positive and negative ESI modes and both sets of data were subjected to multivariate statistical analysis.

PCA modelling of HC and ALF-derived MS results revealed a distinct separation between HC and ALF for both the positive (Fig. 1D, two component model, cumulative R^2 = 0.59; Q^2 = 0.52) and negative ESI data (Fig. 1E, two component model, cumulative R^2 = 0.59; Q^2 = 0.52). Multivariate modelling using OPLS-DA (orthogonal projections to latent structures discriminant analysis) was performed comparing survival at 30 and 90 days and 1-year post admission. All analysis performed on ALF and ALI groups produced invalid models to predict mortality with low or negative Q^2Y values for 30, 90 and 1-year post admission (data not shown).

Following multivariate statistical analysis, 34 discriminating features (Tables S4), selected from S-plots and VIP (variable importance in projection) plots for ALF *vs.* HC, were subjected to tandem mass spectrometry (MS/MS). Furthermore, 70 features with the highest overall intensities were also subjected to MS/MS (Tables S5). Fragmentation data generated by MS/MS were used to confirm the tentative IDs matched to database searches.

compared to HCs. (C) BPI chromatograms of QC serum in ESI+ and ESI- ionisation modes. (E,D) PCA modelling of HC and ALF for both positive and negative ESI data, revealed a distinct separation between HC and ALF for both positive (D, two component model, cumulative R2 = 0.59; Q2 = 0.52) and negative mode data (E, two component model, cumulative R2 = 0.59; Q2 = 0.52). (F–H) Correlation heatmaps from the identified positive ESI lipid. The lipids detected in positive ESI were further assessed to determine correlation patterns in acute (ALI and ALF) liver disease, as performed previously for the ^{1}H NMR spectral data and the UPLC-MS-detected amines. Correlation heatmaps were computed from the identified positive ESI lipid $\log_{10}$ transformed data, using Pearson correlation r2 colour scale of -0.5-1.0, separately for HC (F), acute liver disease (G) and cirrhosis (H). ^{1}H NMR, proton nuclear magnetic resonance; AAA: aromatic amino acid; BCAA: branched-chain amino acids; BPI, base peak intensity; DG, diacylglycerol; ESI, electrospray ionization; FFAs, free fatty acids; PC, phosphatidylcholine; PCA, principal component analysis; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; QC, quality control; SM, sphingomyelin. (This figure appears in color on the web.)

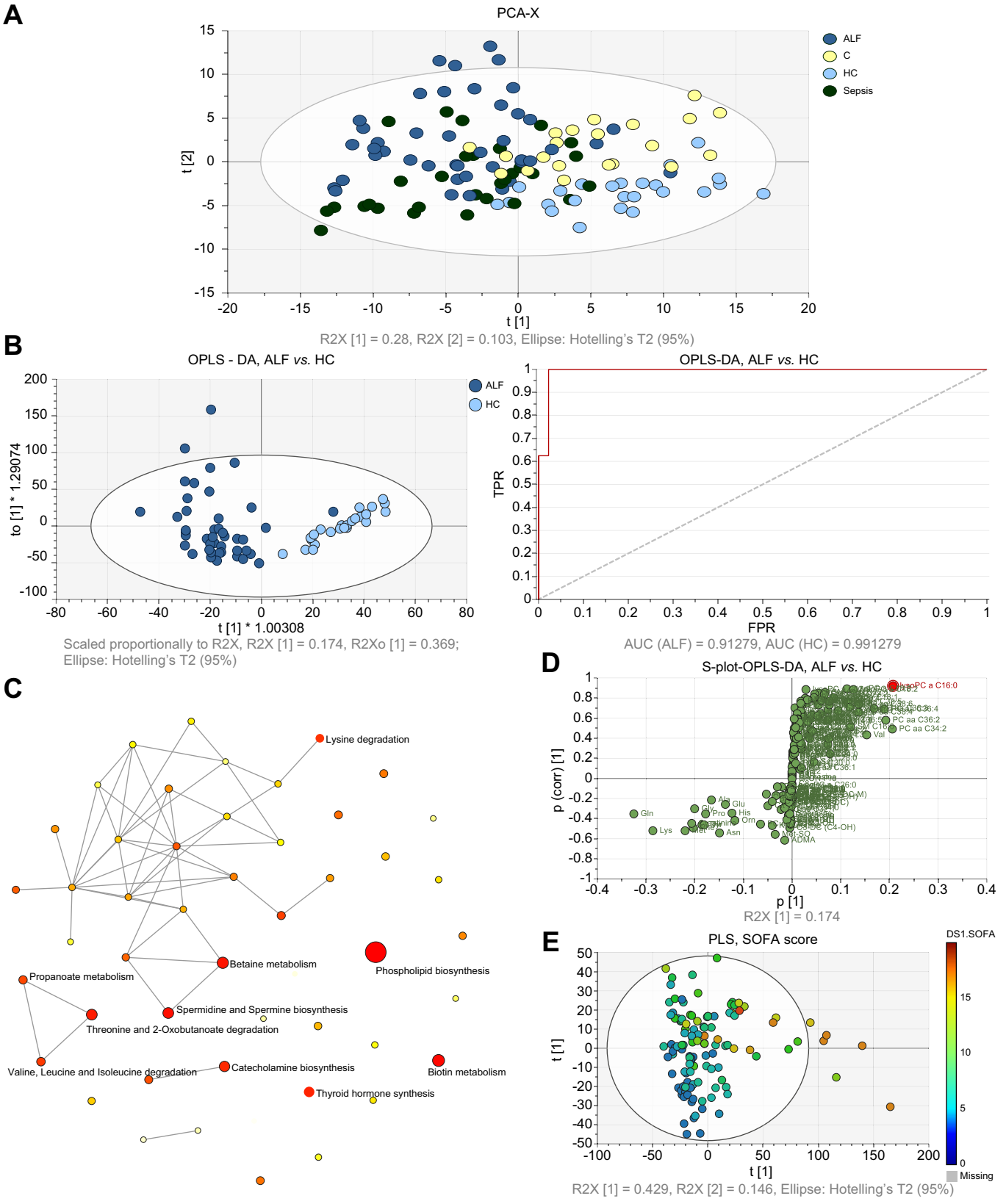

Fig. 2. Discriminative power of LPCs. (A,B) Validation cohort 180-metabolite panel (Biocrates) confirmed the discriminating power of LPCs. In order to confirm our preliminary data, targeted metabotyping was performed on plasma from the validation cohort including 43 individuals with ALF, 24 HCs, 21 individuals with cirrhosis, and 31 with sepsis (as proinflammatory control). PCA and OPLS-DA identified LPC 16:0 as highly discriminant between ALF and HCs together with glutamine, alanine, phosphatidylcholine 34:2, 36:2, 36:3 (R2X 0.592; R2Y 0.666, Q2 0.613, AUROC 0.969961). (C) Enrichment analysis showed phospholipid biosynthesis as the principal pathway differentiating ALF from HC. (D) S-Plot confirmed LPC 16:0 as highly discriminant between HCs and ALF. (E) LPC 16:0 is related to the severity of ALF. When samples were analysed using SOFA score as Y in a PLS model, LPC 16:0 remained in the top 10 metabolites identified with VIP, highlighting the correlation with disease severity. ALF, acute liver failure; HC, healthy control, LPC, lysophosphatidylcholine; OPLS-DA, orthogonal projections to latent structures discriminant analysis; PCA, principal component analysis; PLS, partial least squares; VIP, variable importance in projection. (This figure appears in color on the web.)

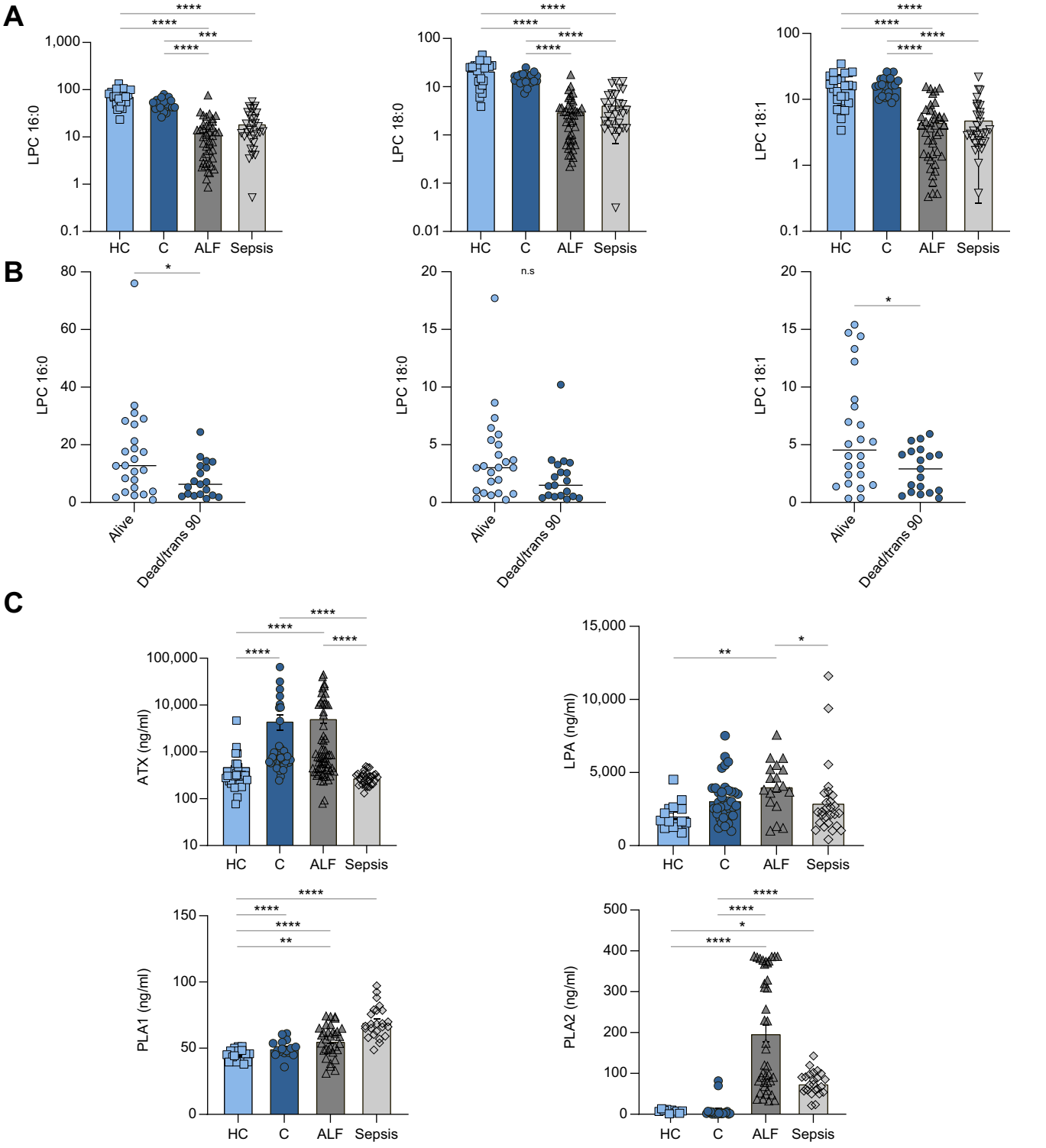

Fig. 3. Prognostic significance of LPC. (A) LPC is significantly reduced in ALF compared to HC and C but not sepsis. Kruskall-Wallis test: HC n = 24, C n = 21, ALF n = 43, Sepsis n = 31. HC *vs.* ALF ****p <0.0001. Graphs show mean +SEM. (B) LPC 16:0, LPC 18:0 and LPC 18:1 were reduced in those with poor prognosis (dead or transplanted at 90 days post admission). Alive n = 24, dead/transplanted n = 19. Mann-Whitney test: LPC 16:0, *p = 0.0339; LPC 18:0 p = 0.06; LPC 18:1 *p = 0.0434. Graphs show mean + SEM. (C) Autotaxin and PLA2 are significantly increased in ALF. ATX: HC n = 31, C = 41, ALF = 62, Sepsis = 31, Kruskal Wallis, HC *vs.* ALF ****p <0.0001. LPA: HC n = 14, C n = 38, ALF n = 18, Sepsis n = 27, Kruskal Wallis, HC *vs.* ALF ** p = 0.0022. PLA1: HC n = 16, C n = 16, ALF n = 32, Sepsis n = 24, one way ANOVA, HC *vs.* ALF ***p = 0.0006. PLA 2: HC n = 10, ALF n = 40, n = 20, sepsis n = 24, Kruskal Wallis, HC *vs.* ALF ****p <0.0001. Graphs show mean + SEM. *p <0.05, **p <0.01, ***p <0.001, ****p <0.0001. ALF, acute liver failure; ATX, autotaxin; C, cirrhotic patients, HC, healthy control; LPA, lysophosphatidylcholinic acid; LPC, lysophosphatidylcholine, PLA, phospholipase A.

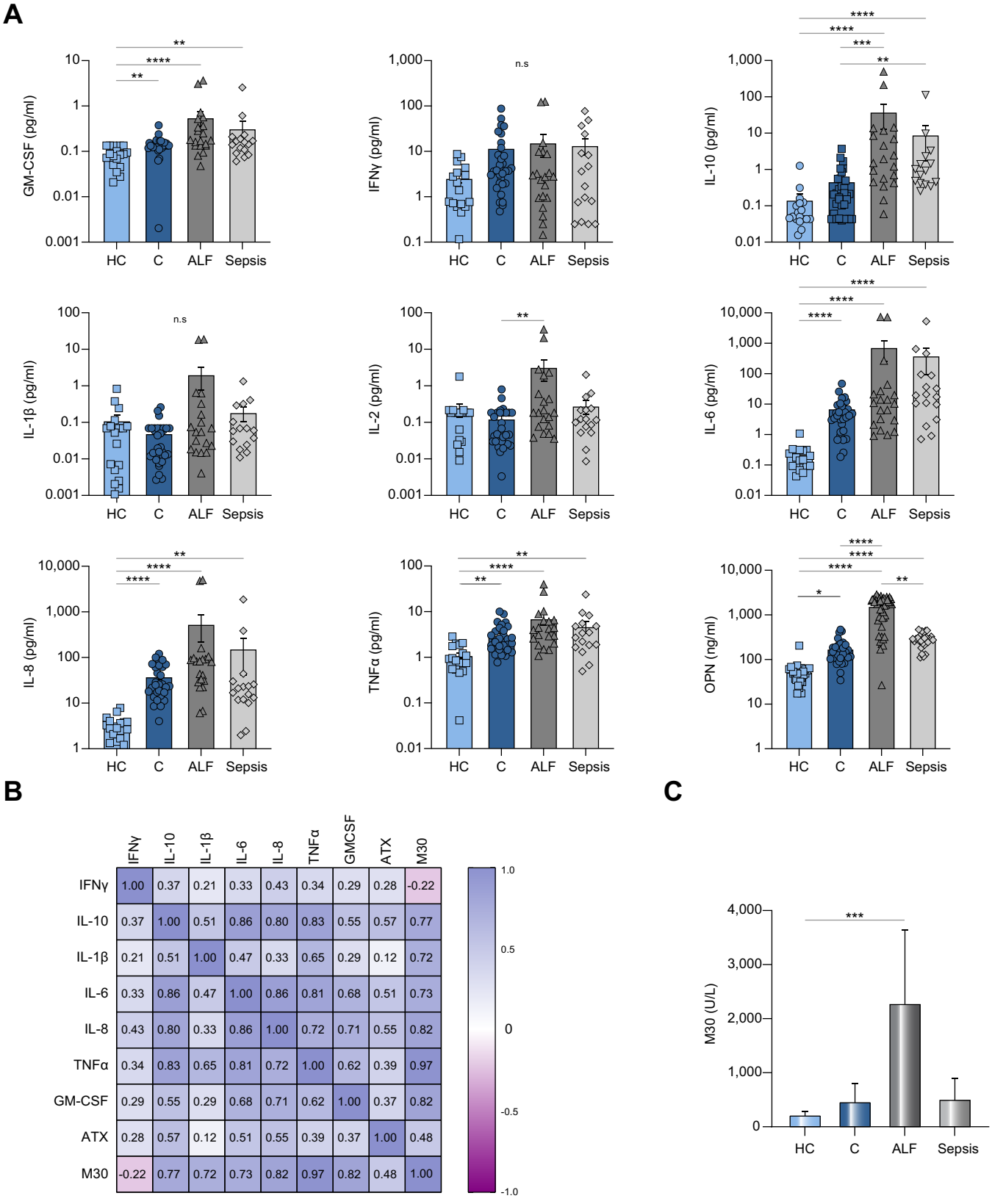

Fig. 4. Increased inflammatory cytokine plasma levels correlate with LPC-ATX-LPA dysregulation in acute liver failure. (A) Individuals with ALF had increased plasma proinflammatory cytokines (validation cohort). HC n = 18, C n = 30, ALF n = 20, sepsis n = 16. Graphs show mean + SEM. Kruskall-Wallis. Most relevant comparisons are shown in detail: HC *vs.* ALF: GM-CSF ****p <0.0001, IFNγ p >0.9999, IL-10 ****p <0.0001, IL-1β p >0.9999, IL-2 p = 0.7178, IL-6 ****p <0.0001, IL-8 ****p <0.0001, TNFα ****p <0.0001, OPN ****p <0.0001. (B) Correlations Matrix: M30 is directly correlated with proinflammatory cytokines in particular with TNFa. Soluble Mer does not correlate with other immunological markers. Correlation matrix, Spearman's Correlation, correlation coefficients (rho) are shown per pair in the

In this way, the identities of four of the discriminating features (LysoPC(14:0) $[M+H]^+$, LysoPC(18:1) $[M+H+H_2O]^+$, LysoPC(16:1) $[M+H+H_2O]^+$) were confirmed. Pairwise comparisons of ALF *vs.* HC show a decrease in LysoPC(14:0), LysoPC(18:0), LysoPC(18:1), LysoPC(18:2), PC(18:0/20:3), PC(18:1/20:5), sphingomyelin (SM) (d16:1/18:1), SM(d16:1/24:1), SM(d18:1/16:0), SM(d18:1/22:1), SM(d18:1/24:0) and TG(18:2/18:2/18:3) and an increase in PC(14:1/18:0), PC(16:1/18:2) and LPA(18:0e/0:0) in individuals with ALF compared to HCs. A decrease in LysoPC(16:0) was common to all liver diseases (stable cirrhosis, AD, ALI and ALF) compared to HCs.

Hierarchical cluster analysis of lipids identified from UPLC-MS data

Lipids were further assessed to determine correlation patterns in ALF. Correlation heatmaps were computed from the identified positive ESI lipid $\log_{10}$ transformed data, using Pearson correlation r^2 (colour scale of -0.5-1.0) separately for HCs (Fig. 1F), acute liver disease (Fig. 1G) and cirrhosis (Fig. 1H).

Four main clusters of lipids were determined from metabolite-metabolite correlation analysis of the HC group. In the purple cluster, eight triglycerides (TGs) showed a strong intercorrelation (r^2 >0.7). In the blue cluster there was a strong correlation between phosphocholines (r^2 >0.6). Furthermore, PC(16:0/18:1), LysoPC(14:0), PC(16:0/18:2) were correlated with PC (18:0/20:3), TG (16:1/16:0/18:1) and TG (16:0/17:1/18:1) (r^2>0.7). Within the green cluster there was a strong correlation between SMs including SM(d16:1/24:1), SM(d16:1/18:1), SM(d18:1/24:1) (r^2 >0.6).

Two main clusters were revealed in the correlation heatmap from the acute liver disease (ALI and ALF) groups. In the red cluster, 6 TGs were highly correlated (r^2 >0.8). Also, in the red cluster, SM(d18:1/24:1), SM(d18:2/24:1), SM(d16:1/18:1), SM(d18:1/16:0) were correlated (r^2 >0.7). In addition, SM(d16:1/24:1), SM(d18:1/24:0), PC(18:0/20:3) and PC(16:0/20:3) also from the red cluster were correlated (r^2 >0.8). Seven LPCs in the blue cluster were correlated (r^2 >0.8).

Three main clusters were found in the correlation heatmap from the cirrhosis group. Eight triglycerides were correlated (r^2 >0.8). Furthermore, 7 LPCs were correlated (r^2 >0.8). In addition, PC(18:0/20:3), SM(d16:1/24:1), SM(d18:1/24:0), SM(d18:1/22:1) and SM(d18:1/16:0) were also highly correlated (r^2 >0.8) and SM(d18:1/16:0), SM(d18:1/24:1), SM(d16:1/18:1) and SM(d18:2/24:1) were highly correlated (r^2 >0.8) as well.

Overall, metabolite correlations within the LysoPC and SM groups intensified in both cirrhosis and acute liver disease compared to HCs.

LPC 16:0 was highly discriminant between ALF and HC

In order to confirm our preliminary data, targeted metabotyping (Biocrates Absolute IDQp180) was performed on plasma from the validation cohort, including 43 individuals with ALF, 24 HCs, 21 individuals with stable decompensated cirrhosis, and 31 with sepsis (as proinflammatory control). Unsupervised multivariate analysis using PCA and supervised OPLS-DA identified LPC 16:0 as highly discriminant between ALF and HC together with glutamine, alanine, and the PCs 34:2, 36:2 and 36:3 (R2X 0.592; R2Y 0.666, Q2 0.613, AUROC 0.969961) (Fig. 2A,B,D). From enrichment analysis, phospholipid biosynthesis was the principal pathway differentiating ALF from HC (Fig. 2C) and cirrhosis but not from sepsis (Fig. S2). Moreover, LPC 16:0 remained relevant (within the top 10 metabolites identified with VIP) when samples were analysed using SOFA score as Y in a PLS model (R2X 0.645; R2Y 0.677; Q2 0.599; p = 4.17616e-22), highlighting the correlation with the severity of the disease (Fig. 2E).

LPCs are prognostic markers of poor outcome in ALF

Univariate analysis showed a reduction of LPC 16:0, 18:0 and 18:1 in individuals with ALF compared to HCs and those with cirrhosis (Fig. 3A). Moreover, individuals with poor prognosis (dead or transplanted at 90 days) had the lowest relative amounts of these lipids, *e.g.* for LPC 16:0 and LPC 18:1 p <0.05 (Fig. 3B). Furthermore, the amounts of these LPCs were negatively correlated with MELD score (Fig. S1). Both for ALF and sepsis, no difference was found in the quantities of LPCs observed according to the presence of one particular organ failure (namely renal, circulatory and respiratory) (Fig. S1F).

ATX and PLA2 were increased in the plasma of individuals with ALF

In order to explore the source of reduced LPC concentrations in plasma, we studied the main enzymes involved in their metabolism. There was an increase in ATX (ALF *vs.* HC p <0.001) and its product LPA in ALF compared to both HCs and those with sepsis (ALF *vs.* HC p <0.01). PLA1 was also increased in all diseases (cirrhosis, ALF and sepsis), however PLA2 was only increased in ALF *vs.* cirrhosis, and *vs.* HCs (p <0.0001) (Fig. 3C). Taken together these findings suggest a potential role for ATX-mediated conversion of LPC into LPA in the liver.

Hepatic ATX expression was increased in ALF

In explants from individuals with ALF of different aetiologies, ATX expression was observed in areas of viable hepatocytes sparing necrotic areas (Fig. S2). This suggests a role for hepatocytes in the conversion of LPC to LPA.

Individuals with ALF showed a markedly enhanced cytokine response and increased circulatory makers of cell death

Similarly to sepsis, plasma from individuals with ALF contained increased amounts of both proinflammatory (IL-6, IL-8, TNFa) and anti-inflammatory cytokines (IL-10) compared to that from HCs and those with cirrhosis (Fig. 4 and Table S1). Interestingly OPN, a highly modified integrin-binding extracellular matrix glycophosphoprotein produced by the cells of the immune

corresponding cell. (C) M30, a caspase-cleaved cytokeratin 18 fragment produced during apoptosis, was increased in ALF compared to HC. Graphs show mean + SD, Kruskall-Wallis test, HC *vs.* ALF ***p = 0.0006. *p <0.05, **p <0.01, ***p <0.001, ****p <0.0001. ALF, acute liver failure; ATX, autotaxin; C, cirrhotic patients; HC, healthy control; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; LPA, lysophosphatidylcholinic acid; LPC, lysophosphatidylcholine; OPN, osteopontin; TNF, tumor necrosis factor. (This figure appears in color on the web.)

system and a mediator of hepatic macrophage infiltration,[25] was increased in ALF compared to sepsis (p <0.01), confirming a role in hepatic inflammation. M30, a caspase-cleaved cytokeratin 18 fragment produced during apoptosis, was increased in individuals with ALF compared to HCs (Fig. 4C) and directly correlated with proinflammatory cytokines (Fig. 4B). The LPCs 16:0 and 18:0 were directly correlated with lymphocyte count, IL-10, IL-6, IL-8 and IL-1β and inversely with monocyte and neutrophil counts (Fig. S1D).

CD14$^+$ monocytes in ALF have a pro-restorative profile with increased MerTK and PD-L1 expression

Phenotypic characterisation of PBMCs by flow cytometry showed increased expression of MerTK and PD-L1 in CD14+ monocytes from individuals with ALF compared to HCs (Fig. 5A-B, complete phenotyping gating strategy is shown in Fig. S3A). Both markers were highly expressed in the intermediate CD14$^+$CD16$^+$ and classical CD14$^+$CD16$^-$ monocyte subsets (Fig. S3B). Immune checkpoint expression on natural killer (NK) and CD56+ T cells did not show a statistically significant difference between study groups (Fig. S4). Notably, we observed an increasing trend of CTLA4-, PD-1- and PD-L1-positive CD56+CD4+T cells (%) in individuals with ALF compared to those with cirrhosis and HCs (Fig. S4B). The percentage of TIGIT expression was increased in ALF CD56+CD8+T and NK cells compared to other groups but again this was not statistically significant.

PBMCs from individuals with ALF were hypo-responsive to LPS/LPC/LPA co-culture

We next decided to test *in vitro* cytokine production and its modulation following lipid treatment (Fig. 5C); therefore, PBMCs were cultured for 24 h and then stimulated with lipopolysaccharide (LPS: 100 ng/ml). Cells from individuals with ALF produced less IL-6 compared to those from HCs (p <0.05). Moreover, LPA 16:0, LPA 18:0, LPA 18:1, LPC 16:0 and LPC 18:0 were also tested as potential modulators of the immune response. The addition of LPAs/LPCs failed to reverse cytokine levels in ALF-derived PBMCs and did not affect HC cytokine production.

LPA 16:0 increases and restores PD-L1 expression and reduces MerTK, CD163 and CD155 expression in monocytes

We next questioned whether *in vitro* LPA treatment of PBMCs from HCs and individuals with ALF would alter their phenotypic characteristics. Following incubation with LPA 16:0, the percentage of CD14$^+$CD16$^+$ and CD14$^+$CD16$^-$ cells within the monocyte pool was reduced (Fig. 5D). Moreover, after culture with LPA 16:0, LPA 18:0 and LPA 18:1, CD14$^+$ monocytes showed immunophenotypic changes (Fig. S5). LPA 16:0 treatment increased monocyte PD-L1 expression (% of positive cells, p <0.05) in ALF samples, that was reduced in untreated conditions compared to HCs (p <0.05), and reduced monocyte MerTK, CD163 and CD155 expression in ALF (% of positive cells and mean fluorescence intensity, p <0.05). Interestingly, no similar effects were observed in T-cell subsets (CD8, CD4, and regulatory) which showed unaltered expression of immune checkpoints following LPA treatment (Fig. S5).

Expression of LPARs in monocytes was upregulated during ALF and their reduction was linked to poor prognosis

Given the potential lipid-mediated modulation of monocyte function, we next sought to examine whether hepatic and monocyte expression of LPA receptors (LPARs1-6) was potentially upregulated. Monocyte expression of LPARs in publicly available microarray data sets was therefore explored. Having already demonstrated increased hepatic expression of ATX and LPAR6 (Gene Expression Omnibus dataset GDS4389, series GSE28619/30) in alcoholic hepatitis and HBV-related ALF,[8,26] we observed a trend toward increased expression of LPAR2, 3 and 6 (GSE120652) in those with ALF resulting from paracetamol overdose.

In vitro stimulation of monocytes with LPS increased LPAR1, LPAR3 and reduced LPAR6 (GSE61298)[27] expression (Fig. S6A). Moreover CD16 -ve monocytes expressed more LPAR1, and LPAR6, and reduced LPAR3 compared to CD16+ve monocytes, confirming a different activation according to monocyte subsets (GSE16836)[28] (Fig. S6B). Also, LPARs were no differently expressed in PBMCs according to the outcome of patients with an acute flare of hepatitis B on chronic infection (GSE168049)[29](data not shown) and in monocyte from patients with paracetamol-induced acute liver failure (GSE80751)[30](Fig. S6C).

LPAR identification with monocyte stimulation by receptor antagonists

From our genetic research, it emerged that LPAR1, 3 and 6 could be involved in the monocyte phenotype changes observed in ALF. To explore which LPAR played a role in the observed effects, fresh PBMCs were stimulated with the antagonists for the three most common receptors (LPARs 1-3); currently there are no commercially available LPAR 4-6 antagonists.

A preliminary study was conducted to decide targets and optimal concentrations of the available antangonists. PBMCs from HCs were cultured with antagonists for LPAR1 (Ro 6842262), LPAR2 (H2L5186303), and LPAR1/3 (Ki 16425). We observed that CD155, CD163, MerTK, PD-1 and PD-L1 were reduced by the LPAR1/3 antagonist (at 1uM) compared to LPA 16:0 (30 uM). This suggests a key role of LPAR1/3 in monocyte phenotype modulation (Fig. S7).

We then focused on LPAR1 and LPAR1/3 antagonism to stimulate ALF cells. LPA 16:0 reduced MerTK and CD163 expression, the effect was statistically significant in HCs and a clear trend was also evident in ALF (Fig. 6). Treatment of PBMCs with LPAR1 and LPAR1/3 antagonists restored the MerTK and CD163 expression reduced by LPA 16:0 treatment.

Taken together these data suggest that an LPAR1/3 mechanism underpins the effect of LPA on the regulatory phenotype of monocytes and these receptors are a target for immunotherapy in ALF.

RNA sequencing showed potential pathways of interest unique to ALF

Finally, to explore pathways linking monocyte function and lipid metabolism, we performed mRNA sequencing on isolated monocytes from individuals with ALF, with decompensated cirrhosis (DC) and HCs (Fig. 7A). As shown in the Venn diagram,

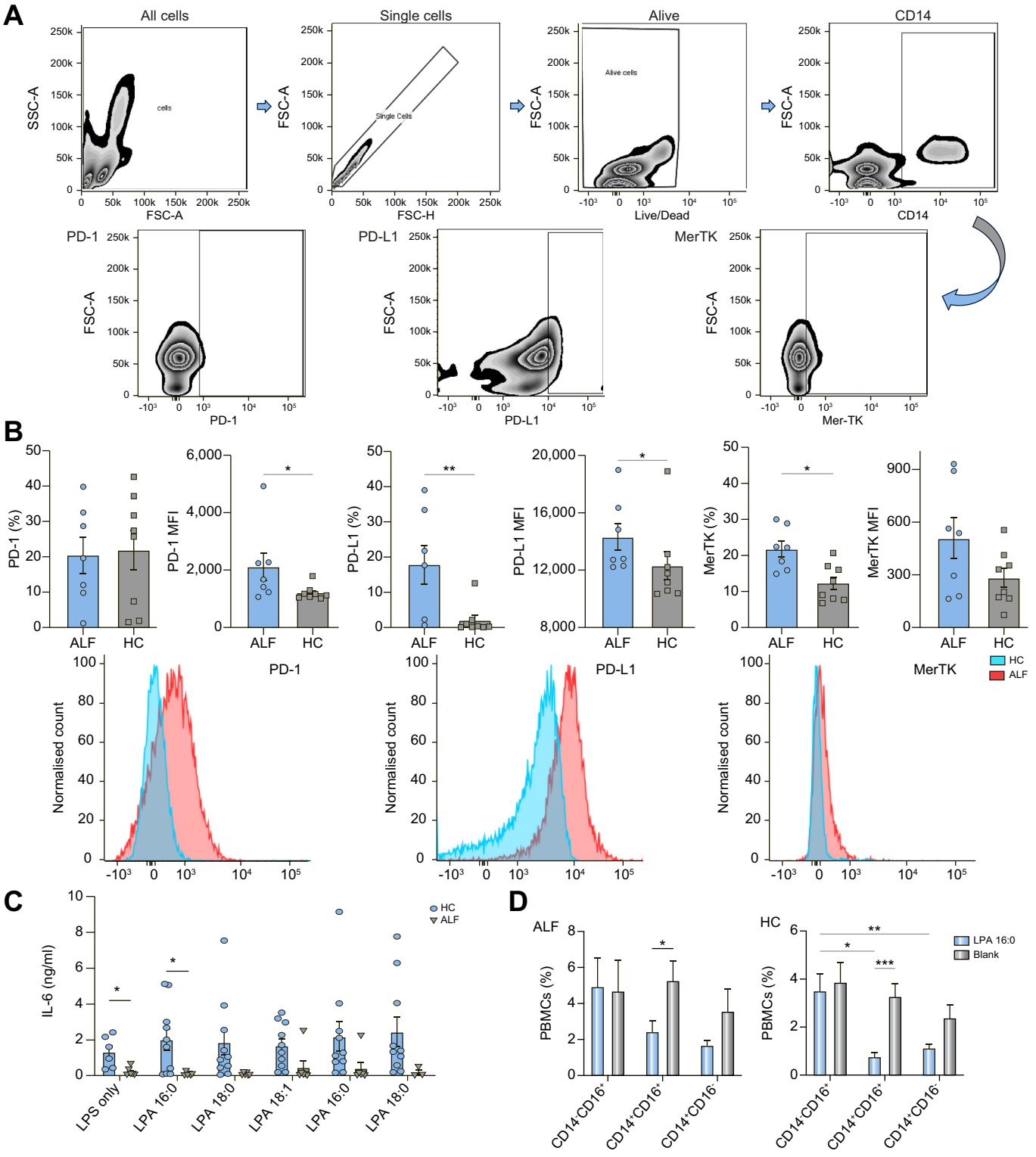

Fig. 5. Monocytes in ALF show a pro-restorative phenotype. (A) Gating strategy, markers including MerTK were assessed on live CD14+ cells. (B) MerTK and PD-L1 expression is increased in fresh CD14+ cells in ALF (n = 7) compared to HC (n = 8). Most relevant comparisons are shown in detail: Mann-Whitney test, PD1% $p = 0.955$, PD1 MFI $^{*}p = 0.0306$, PD-L1% $^{**}p = 0.0059$, PD-L1 MFI $^{*}p = 0.0434$, MerTk % $^{*}p = 0.0140$, MerTk MFI $p = 0.2319$. Graphs show mean +SEM, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. (C) PBMCs from individuals with ALF produced less IL-6 when stimulated with LPS ($p < 0.05$). Moreover, LPA 16:0, LPA 18:0, LPA 18:1, LPC 16:0 and LPC 18:0 were tested as potential modulators of immune response. The addition of LPAs/LPCs failed to reverse the immune dysfunction in ALF PBMCs and did not affect HC function. Mixed effects analysis. Most relevant comparisons are shown in detail: HC *vs.* ALF: LPS only $^{*}p = 0.0135$, LPA 16:0 $^{*}p = 0.0309$. The other comparisons were not statistically significant with $p > 0.05$. Graphs show mean +SEM, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. (D) LPA modifies monocyte phenotype. Percentage of CD14+ cells decreased after 24 h PBMC co-culture with LPA 16:0. Most relevant comparisons are shown in detail: two-way ANOVA, ALF LPA *vs.* blank, CD14-CD16+ $p = 0.9907$, CD14+CD16+ $^{*}p = 0.0125$, CD14+CD16-$p = 0.1336$. Two-way ANOVA, HC LPA *vs.* blank, CD14-CD16+ $p = 0.8709$, CD14+CD16+ $^{***}p = 0.0001$, CD14+CD16-$p = 0.0693$. Graphs show mean + SEM, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. ALF, acute liver failure; HC, healthy control; LPA, lysophosphatidylcholinic acid; LPS, lipopolysaccharides, Mer-Tk, Mer tyrosin kinase; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1. (This figure appears in color on the web.)

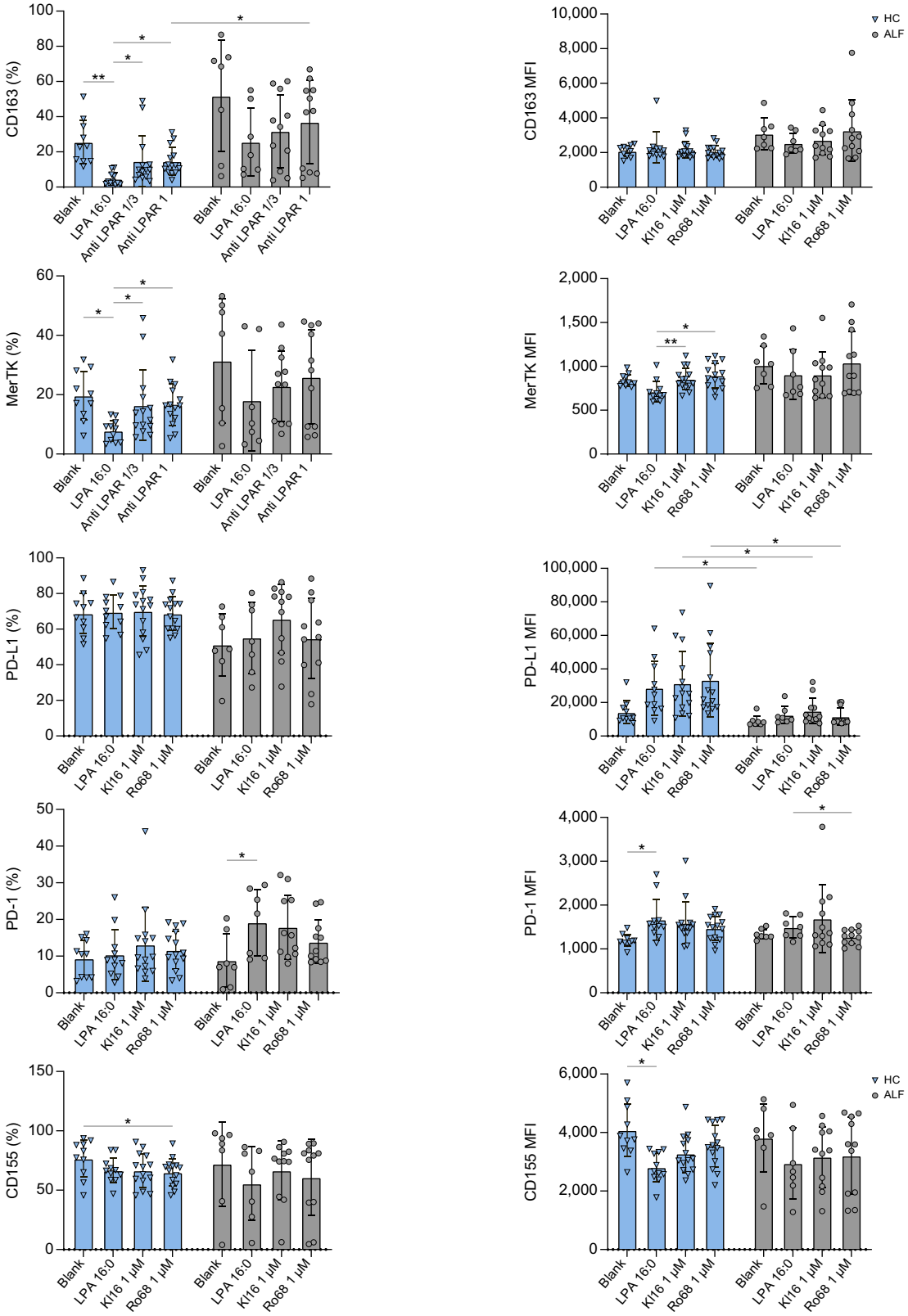

Fig. 6. LPAR1 and 3 were the key receptors leading to LPA-induced monocyte phenotypical changes. In PBMC culture, LPA 16:0 reduced MerTK and CD163 expression in CD14+ cells. Treatment with LPAR1 and LPAR1/3 antagonists restored MerTK and CD163 expression, which had been reduced by LPA 16:0 treatment. Mixed effects analysis. Most relevant comparisons are shown in detail: HC LPA 16:0 *vs.* Ki 16425, CD163% **p* = 0.0215, MerTK% **p* = 0.0276, MerTk MFI **p* = 0.0197. HC LPA 16:0 *vs.* Ro 6842262, CD163% **p* = 0.0225, MerTK% **p* = 0.0266, MerTK MFI **p* = 0.0442. Graphs show mean +SEM, **p* <0.05, ***p* <0.01, ****p* <0.001, *****p* <0.0001. MerTK, Mer tyrosine kinase, PD1, programmed cell death 1, PD-L1, programmed cell death ligand-1.

206 genes were unique to ALF (Fig. 7B). *LPAR1,2,5* and *6* expression levels were not different between study groups (Fig. 7C-D), while *LPAR3* and *LPAR4* genes were filtered out due to low expression levels (data not shown).

Of note, *MerTK* and *CD163* monocyte transcript levels were increased in ALF compared to control while HLA appeared hypo-expressed in ALF, and the antigen processing and presentation pathways were downregulated (Fig. 7E). *HLA-DPA1, HLA-DPB1, HLA-DRA* and *HLA-DMB* were uniquely downregulated in ALF compared to both groups, while *CD163, IFNAR 2, FKBP5, ADM* were upregulated in ALF only (Fig. 7D and F). Moreover, *LAIR1* and *LILRB4*, coding for leucocyte inhibitory receptors, were upregulated in individuals with ALF compared to both HCs and those with DC. Among the main pathways discriminating between ALF *vs.* HCs and *vs.* DC we found genes related to lipid metabolism including fatty acid metabolism and steroid metabolism, in particular *LPCAT2* and *LPCAT3* which were upregulated in ALF.

Ingenuity pathway analysis by Qiagen IPA (Fig. S8) showed the possible relationship between LPARs and monocyte markers. Several pathways were putatively identified, and further research is needed to better explain how to safely modulate monocyte function.

Discussion

Our study is the first to demonstrate the upregulation of the LPC-ATX-LPA axis in both peripheral blood and liver tissue in ALF and its link, via an LPAR1/3-related mechanism, with the immune phenotype modulation of circulating monocytes.

We report a crucial role of lipid metabolism in ALF. LPCs 16:0 and 18:1, which form the most abundant LPC species in plasma, were identified as discriminant in a population of individuals with ALF when compared to HCs following unbiased ^{1}H NMR spectroscopic and LCMS-based analysis of peripheral blood. These lipids were predictive of poor prognosis in the same population (including mortality or liver transplant at 90 days) and the levels were inversely correlated with MELD score. However, no relationship was found with other organ failures, *e.g.* renal, circulatory and respiratory systems.

Inflammation is accepted as the driving mechanism leading to multiorgan failure and death in ALF populations,[31] and monocyte and macrophage dysfunction are central to disease pathogenesis and progression.[2] Initial activation of liver-resident macrophages (Kupffer cells) by damage-associated molecular patterns leads to monocyte recruitment and infiltration. Such effects on monocytes may then contribute to local tissue destruction during the propagation phase and result in the secretion of proinflammatory cytokines.[32,33] In parallel anti-inflammatory cytokines (*i.e.* IL-10) were increased, suggesting the activation of a compensatory anti-inflammatory response syndrome in ALF.[2]

In our cohort, proinflammatory cytokines increased more than sixfold compared to controls (Fig. 4) and were correlated with markers of cell death (M30). Subsequently, the recruited monocytes matured into macrophages following local reprogramming towards resolution responses (to promote tissue integrity).

Intrahepatic events may also affect circulating monocytes, which show numerous acquired defects in ALF syndromes, with dysfunctional antimicrobial activity and increased susceptibility to sepsis.[2] What contributes to such reprogramming has been a matter of debate and many soluble mediators have been proposed.[2,34]

Herein, we have shown that such soluble mediators could include lipids in the LPC and LPA families. The LPCs are lipid metabolites of PC, synthesized by various enzymes including secretory PLA2, HDL-associated lecithin-cholesterol acyltransferase in the reverse cholesterol pathway, as well as hepatic and endothelial lipase.[35].

LPAs are thought to be bioactive molecules and their function is exerted by extracellular signalling through at least six (LPAR1-6) 7-transmembrane G protein-coupled receptors.[36]

There are two major synthetic pathways for LPAs.[36] Lysophospholipids can be converted to LPA via ATX activity or, alternatively, phosphatidic acid is first produced from phospholipids via phospholipase D, or from diacylglycerol through diacylglycerol kinase, and then converted directly to LPA by the actions of either PLA1 or PLA2.

Both pathways appear to be activated in ALF since ATX and PLA2 were increased compared to controls. However, ATX has been found in liver explants obtained from individuals with ALF, confirming the central role of the liver in the dysregulation of the LPC/ATX/LPA axis in ALF. That this is not purely due to systemic inflammation is evidenced by the lack of ATX increases in individuals with severe sepsis.

ALF shares similarities with ACLF, in which cholesteryl esters and lysophospholipids have been considered part of a lipid fingerprint.[37] In addition, PBMC transcriptome analysis has revealed metabolic alterations, including the upregulation of the PPAR signalling pathway, cholesterol metabolism, sphingolipid metabolism and glycosaminoglycan biosynthesis[38] and this was confirmed by our data on monocytes isolated from individuals with ALF.

In this ALF cohort, circulating monocytes showed increased PD-L1 and MerTK expression, confirming a pro-restorative phenotype, previously associated with immunoparesis and increased susceptibility to infections.[2,3,5] Moreover, no significant phenotypic differences were found in blood NK and CD56+T cells in ALF, but this could also be attributed to the early time of sampling. In our recent data from a murine model of paracetamol overdose, PD-1 expression in T-cell subsets remained unaltered during the first 3 days after the acute insult. However, PD-L1 expression in regulatory T and NKT cells was upregulated at 72 h post paracetamol overdose, during the pro-restorative phase.[3]

We demonstrate a switch in monocyte populations, with a reduction in CD14+ cells with LPA *in vitro*. Moreover, among LPA 16:0, 18:0 and 18:1, the former induced profound phenotypic changes *in vitro*, reducing CCR2, CD163, CD155 and MerTK and increasing PD-L1 expression in both HCs and individuals with ALF, without affecting the T-cell population.

In order to identify the LPAR responsible for such an effect, we examined LPAR expression in monocytes through publicly available microarray data sets. *In vitro*, M1 monocytes have increased LPAR1, LPAR3 and reduced LPAR6, suggesting a proinflammatory role for the first two receptors. This is complementary to the previous finding of increased ATX and LPAR1 and 6 in liver tissue from individuals with alcohol-related liver disease and HBV-related ALF.[8] We then demonstrated that

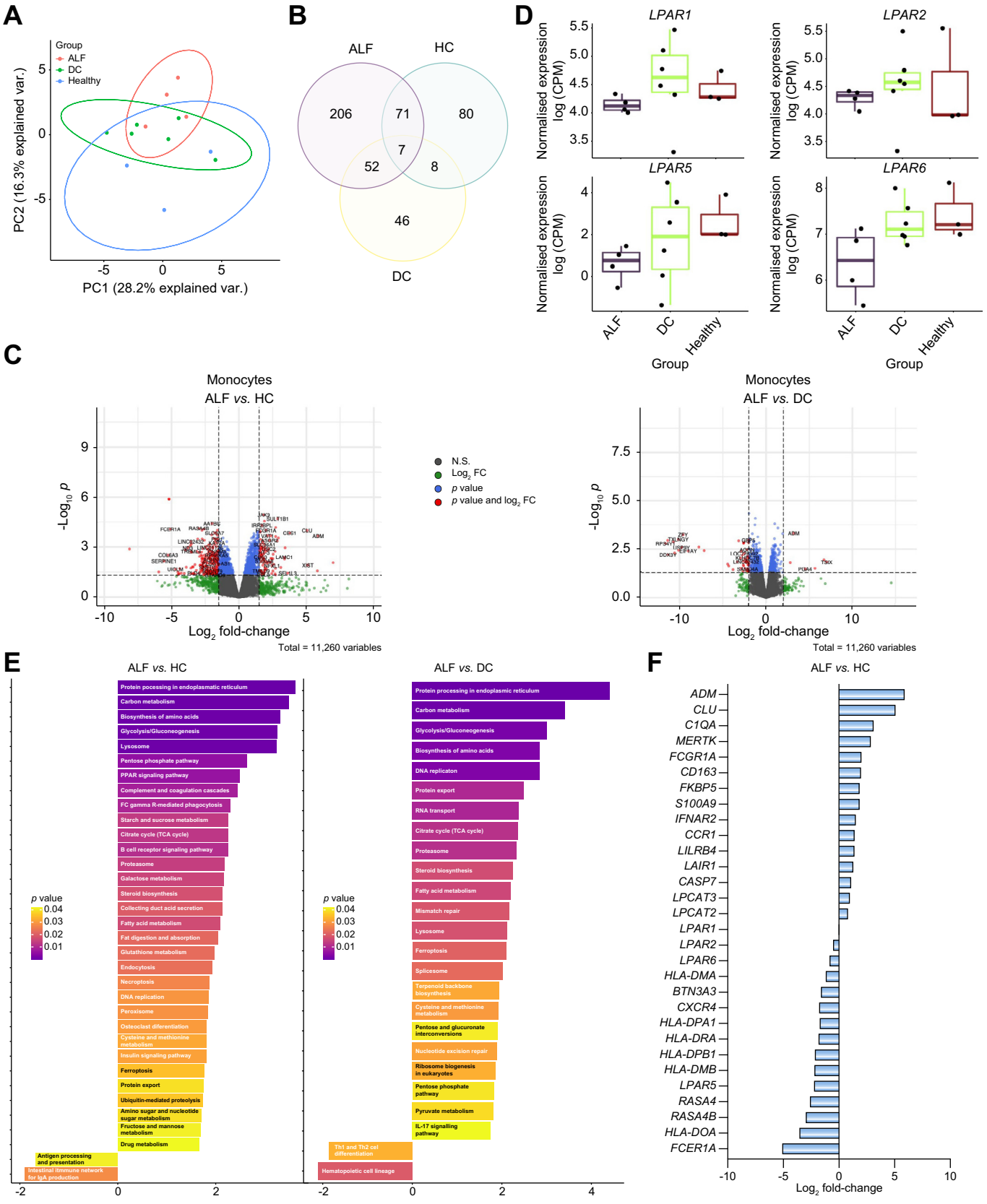

Fig. 7. RNA sequencing of monocytes unveiled pathways of interest in ALF. (A) Principal component analysis for RNA sequencing data showing the three study groups: ALF (n = 4), DC (n = 6) and HC (n = 3). (B) A Venn diagram showing the overlap of differentially expressed genes between the three different pairwise comparisons using p <0.05 and |logFC|>1.5. (C) Volcano plots showing the differential expression in Log_2 fold change of genes between ALF *vs.* HC and ALF *vs.* DC. (D) Box plots of LPAR genes of interest in ALF, DC, HC, LPAR3 and four were detected but filtered out due to low expression levels. Kruskall-Wallis, not significant. LPAR1 p = 0.2250, LPAR2 p = 0.5571, LPAR5 p = 0.4340, LPAR6 p = 0.4058. (E) Pathway analysis plots based upon the GAGE analysis for the pairwise comparisons ALF *vs.*

LPAR1/3 antagonism had a similar effect on both cells from HCs and individuals with ALF, reversing the effect of LPA and increasing CD163 and MerTK.

To evaluate if the phenotypic change was consistent with gene expression, we performed RNA sequencing on isolated monocytes from those with ALF, DC and HCs. *LPAR1, LPAR2, LPAR5* and *LPAR6* expression were not statistically different between groups. Thus, we hypothesised that the circulating lipids stimulate the receptors without inducing differential gene expression.

Our numbers were small to analyse outcome prediction; however, from public datasets, we found that in PBMCs from individuals with ACLF secondary to hepatitis B infection and monocytes from those with paracetamol-induced ALF, LPARs were not differently expressed in patients with poor prognosis.

In our cohort, transcript-level expression of *MerTK*, *CD163, LAIR1, LILRB4, S100A9* in isolated monocytes was increased in those with ALF compared to HCs, while MHC-related genes *(HLA-DPA1*[39]*, HLA-DPB1, HLA-DRA, HLA-DOA* and *HLA-DMB*) appeared hypo-expressed in ALF, confirming an immunosuppressive/pro-restorative profile.

S100A9 is a marker of myeloid-derived suppressor cells, a population of cells that can negatively regulate T-cell function.[40] Their role has been studied in cancer[41] and by our group in ACLF,[42] in which myeloid-derived suppressor cells displayed immunosuppressive properties, decreasing T-cell proliferation and reducing bacterial uptake.

Other genes were upregulated only in ALF including ADM (adrenomedullin, a potent vasodilator peptide[44]), IFNAR2 and FKBP5. The latter encodes FKBP51, which acts as a negative transcriptional regulator of the glucocorticoid receptor and a positive regulator of PPAR signalling. This gene is also related to stress and suicidal behaviour,[43] so it will be interesting to explore its role in the setting of ALF and immune dysfunction from paracetamol overdose.

LPS and IFN-γ increase adrenomedullin production by macrophages *in vitro*.[45] Adrenomedullin strongly inhibits LPC-induced migration of human coronary artery smooth muscle cells in a concentration-dependent manner.[46] Its plasmatic concentration has been correlated with vasopressor requirements, organ dysfunction, and mortality in sepsis.[47] Moreover, pro-adrenomedullin is associated with short-term survival and ACLF development in individuals with DC,[48] but its role in ALF is unknown.

Among the main pathways discriminating between ALF *vs.* HCs and *vs.* DC we also found genes related to lipid metabolism, including fatty acid metabolism and steroid metabolism. Lysophosphatidylcholine acyltransferase (LPCAT) 2 and 3 genes would be of future interest since they are upregulated in ALF and related to the metabolomic signature we discovered. LPCAT2 has been shown to induce macrophage cytokine gene expression and release in response to TLR4 and TLR2[49] and LPCAT3 seems crucial in the arachidonic acid reacylation pathway[50] and ferroptosis,[51] a novel pathway of iron-dependent necrotic cell death characterised by the accumulation of lipid peroxides. Among the various reactive oxygen species, lipid peroxides are direct inducers of ferroptotic cell death.[52] One of the crucial steps in this pathway is indeed performed by LPCAT3,[53] selectively activated by IFN-γ, which incorporates polyunsaturated fatty acid-CoA into phospholipids, using PCs and phosphatidylethanolamine as substrates. Indeed, the links between lipid metabolism and the immune system are multiple and many lipid mediators have been studied in ferroptosis, including specialised pro-resolving mediators that would be an interesting research focus in ALF.[54]

The aim of the present study was to find potential targets for immunomodulation in ALF. Although ATX has been explored as a target for the treatment of individuals with idiopathic pulmonary fibrosis,[55] the heterogenic distribution and expression profiles of LPARs are such that blocking LPA production globally by inhibiting ATX could lead to severe adverse side effects. Thus, drug development needs to focus on the discovery of novel, potent LPAR ligands targeting a specific receptor subtype without negating the physiological roles of other subtypes.[10] Currently, no drugs targeting LPARs have been approved by the FDA, while several clinical trials are testing LPAR1 antagonists for idiopathic pulmonary fibrosis and systemic sclerosis, and a radioligand that targets the LPAR1 receptor has entered a phase I clinical trial for PET imaging of idiopathic pulmonary fibrosis.[10]

Our research has several limitations. Total LPA only was measured by ELISA rather than individually. The reason is that LPA measurement via LCMS techniques is challenging. LPAs can be generated *ex vivo* after sample collection and are affected by several chemical and biological processes, making their accurate measurement in biological fluids difficult.[56] We are currently optimising a technique to efficiently measure LPAs to overcome this limitation. Moreover, we did not measure free and total LPA/LPC, thus the role of albumin binding was not explored in this study.

We used LPS as an immunogenic stimulant and thus only TLR4-mediated inflammation was explored. Other pattern recognition receptors may also be involved, and we could therefore have missed some alternative activation pathways.

We studied LPAR antagonism *in vitro* only, thus the multiple effects of LPAR antagonism need to be elucidated on animal models before applying these findings to individuals with ALF. Currently only LPAR1-3 antagonists are commercially available so more information could be gathered once it is possible for LPAR4-6 antagonism to be explored.

In individuals with ALF, LPCs are reduced and can be used as biomarkers of poor prognosis. The LPC-ATX-LPA axis modulates innate immune responses in ALF through LPAR1 and 3. LPA reduces the pro-restorative phenotypical markers MerTK and CD163 on monocytes and this can be reversed with LPAR antagonism. RNA sequencing unveiled further pathways linking lipid metabolism and immune dysfunction in ALF, distinct from LPAR expression. These preliminary findings highlight the importance of LPARs as possible therapeutic targets in ALF.

HC and ALF *vs.* DC. (F) Differential expression in Log_2 fold change of genes involved in lipid metabolism and immune response. ALF, acute liver failure; DC, decompensated cirrhosis, HC, healthy control; LPAR, lysophosphatidylcholinic acid receptor; PC, principal component. (This figure appears in color on the web.)

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Abbreviations

ACLF, acute on chronic liver failure; ALF, acute liver failure; ALI, acute liver injury; ATX, autotaxin; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; HC, healthy control; LPA, lysophosphatidylcholinic acid; LPAR, LPA receptor; LPC, lysophosphatidylcholine; LPCAT, lysophosphatidylcholine acyltransferase; MELD, model of end-stage liver disease; MS, mass spectrometry; NK, natural killer; PBMCs, peripheral blood mononuclear cells; PC, phosphatidylcholine; PCA, principal component analysis; PD-1, programmed cell death 1; PD-L1, programmed cell death 1 ligand; PLA, phospholipase A; SOFA, Sequential Organ Failure Assessment.

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Conflict of interest

Muireann Coen is an employee of AstraZeneca and has stock ownership and/or stock options or interests in the company. William Bernal is consultant for Versantis AG and Pioneering Medicine VII, Inc.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

FMT, MJWM, study concept and design; FMT, RZ, FA, SM, EJ, AC, MC, SN, AS, CB acquisition of data; FMT,MJWM, RZ, MC, IW, EH, PM, MC, RM, ET, SRA analysis and interpretation of data; FMT, MJWM, drafting of the manuscript; FMT, MJWM, MC, IW, JW, WB, KM, VCP, ET critical revision of the manuscript for important intellectual content; FMT, MJWM, RZ, SA statistical analysis; MJWM, VCP, KM, ET obtained funding; FMT, SN, SM, administrative, technical, or material support; MJWM, study supervision.

Data availability statement

Data can be made available at reasonable request and uploaded on acceptance.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.10.031.

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Author names in bold designate shared co-first authorship

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