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Bone marrow-derived cells repopulate the human peritoneal cavity after allogeneic stem cell transplantation

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In end-stage liver disease, disruption of the gut–liver axis leads to intestinal dysbiosis and impaired barrier function, promoting translocation of bacterial products into the peritoneal cavity and contributing to spontaneous bacterial peritonitis (SBP). The peritoneal cavity harbors a distinct immune landscape dominated by tissue-resident peritoneal macrophages (PMs), which are crucial for pathogen clearance. Severe infections or medical interventions can drastically alter this landscape. While strong evidence from previous studies suggests that PMs originate from infiltrating monocytes, direct experimental proof is still lacking. Here, we studied a patient with allogeneic stem cell transplantation and decompensated cirrhosis. Genetic chimerism enabled tracking of immune cell origin. One year post-transplant, PMs were almost entirely donor-derived yet displayed all features of mature tissue-resident macrophages. A minor fraction of patient-derived small PMs persisted, indicating resilience. Additionally, peritoneal fluid supported macrophage differentiation, underscoring the role of environmental milieu. Our findings provide direct evidence that circulating monocytes can fully replenish PMs.

The gut–liver axis represents a bidirectional communication network linking the intestine, its microbiota, and the liver through metabolic, immunological, and vascular pathways. This axis plays a central role in maintaining host homeostasis and contributes to the pathogenesis of various liver and intestinal diseases when disrupted[1,2]. Positioned at the interface between the gut and the liver, the peritoneal cavity (PerC) serves as an immunologically responsive compartment, particularly in patients with decompensated liver cirrhosis (DLC).

DLC is accompanied by increased intestinal epithelial permeability and microbial dysbiosis, resulting in bacterial translocation[1,3,4]. Cirrhosis-induced portal hypertension further promotes the development of ascites and the translocation of microbial components into the peritoneal cavity, where they are neutralized by resident immune cells[3]. The PerC plays a pivotal role as an immune compartment[3,5,6], harboring a diverse range of immune cells including macrophages, T and B lymphocytes, natural killer (NK) cells, unconventional T cells, and dendritic cells (DCs)[7–12]. Among these, macrophages represent the predominant and functionally most versatile population, contributing to tissue repair, recognition and phagocytosis of microbial pathogens, antigen presentation, initiation and resolution of inflammation, and the recruitment and activation of other immune cells, thereby maintaining peritoneal immune surveillance in decompensated cirrhosis[13–18].

The long-held belief of myeloid precursors giving rise to circulating monocytes, which migrate into the peritoneal cavity to differentiate into organ-resident macrophages, has been challenged[19]. Within the last years, it has been established that peritoneal macrophages (PMs) originate from embryonic hematopoietic precursors and can be maintained under non-inflammatory conditions independently of invading monocytes[20–23]. Furthermore, accumulating evidence indicates that factors within the tissue microenvironment play a dominant role in tissue-specific differentiation of the cells, surpassing the impact of their developmental origin[24–27].

Within the PerC, at least two distinct PM populations have been described: large PMs (LPM) and small PMs (SPM)[28]. In mice, LPM were found to be the predominant, resident macrophage population that self-

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renew through proliferation, whereas SPM originate from infiltrating monocytes[19,29]. In the human PerC, the distribution of macrophage subpopulation exhibits a high degree of variability, with SPM generally being represented more frequently than LPM[7,8,30,31]. Notably, in contrast to mice, not only SPM, but also LPM exhibit a proinflammatory phenotype when challenged with bacterial components and express MHC class II molecules. Moreover, both subpopulations were found to have actively proliferating cells, indicating that the self-renewal capabilities are not restricted to a single macrophage population[32,33].

During mild infections of the abdominal cavity, a depletion of tissue macrophages and a strong influx of monocytes have been observed in murine models. After a period of three days, a recovery of the original tissue macrophages occurred. Under these conditions, monocyte-derived cells also migrated and showed differentiation towards a tissue-resident phenotype, exhibiting self-renewal potential. However, these cells could still be distinguished from original macrophages by differences in the expression of MHCII, TIM4 or GATA6. A different picture emerged following severe infections: original tissue macrophages were markedly diminished and failed to recover, whereas migrated macrophages differentiated into tissue-resident cells resembling the original peritoneal macrophage phenotype[27].

Comparable dynamics have been reported in humans: during infections, such as spontaneous bacterial peritonitis (SBP), a massive influx of monocytes into the PerC in combination with a depletion of LPM was observed. Throughout recovery, LPM rose back to normal levels about three days after therapy start[32]. Whether this restoration results from migrated, bone marrow-derived cells differentiating into tissue macrophages or from proliferation of original macrophages that replenish the pool of tissue macrophages remains unresolved.

Studying the origin and phenotypic differentiation of immune cells in human tissues remains a major challenge, as experimental approaches developed in animal models cannot be applied directly to humans for ethical reasons. In this study, we examined the peritoneal immune cell compartment in a patient who had undergone allogeneic stem cell transplantation and subsequently developed decompensated liver cirrhosis accompanied by ascites. This unique clinical constellation offered a rare opportunity to trace the origin of peritoneal immune cells and evaluate their phenotypic reconstitution. Our findings reveal that, one-year post-transplantation, the majority of peritoneal macrophages and all peritoneal T cells were donor-derived and closely resembled the immune cell landscape observed in non-transplanted individuals. Notably, a small fraction of host-derived small peritoneal macrophages persisted, suggesting intrinsic resilience. Furthermore, we observed that peritoneal fluid facilitates macrophage differentiation, underscoring the importance of local tissue signals in shaping immune cell identity.

Results

Longevity and replacement of tissue immune cells within the peritoneal cavity

Our study was initiated by a distinctive clinical case identified at our hepatology unit: a 72-year-old woman with cirrhosis and ascites who had received an allogeneic stem cell transplant approximately one year earlier due to myelodysplastic syndrome (for patient history see Fig. 1 and Supplementary Data). The transplant was obtained from a male HLA-identical unrelated donor, which enables cell tracing studies of bone marrow-derived cells. After paracentesis, cells from ascitic fluid and peripheral blood of the patient were isolated and assessed.

To determine the proportion of donor- and recipient-derived cells in peripheral blood, T cells (CD3+) and monocytes (CD14+) were isolated from PBMCs by fluorescence-activated cell sorting and analyzed for their origin using a clinically validated chimerism PCR assay for short tandem repeats (STR), approved for stem cell transplantation diagnostics at our institution. Doing so, we found that both T cells and monocytes carried 100% of the donor's STR profile (Fig. 2A, B), which indicates complete

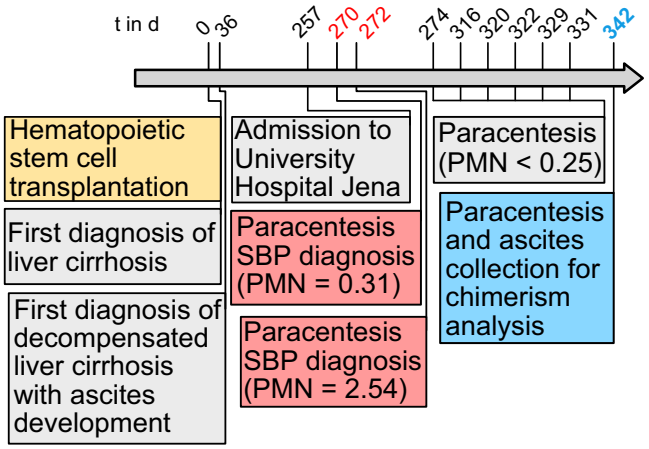


Fig. 1 | Chimeric patient history. History of the chimeric patient starting with stem cell transplantation on day 0. Time points in days after transplantation. Time points of spontaneous bacterial peritonitis are highlighted in red, time of sampling for chimerism examination in blue. Further paracenteses after day 342 are not indicated. PMN: polymorphonuclear neutrophils expressed in giga-particles per liter (10^9 cells/L).

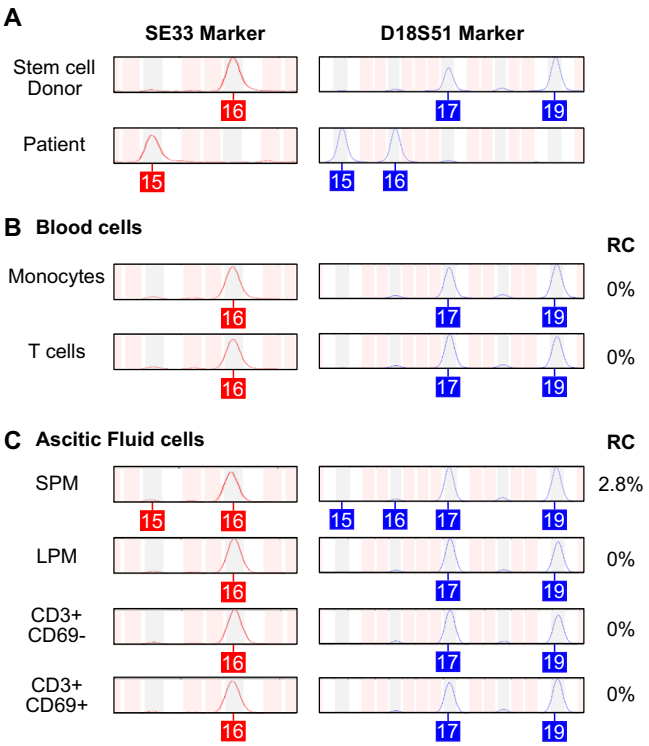


Fig. 2 | Chimerism analysis of blood and ascites T cell and macrophage populations using the PowerPlx ESX 17 Multiplex PCR Assay. Chimerism results are shown as electropherograms of two alleles (15 and 16) of the SE33 marker set and four alleles (15, 16, 17, 19) of the D18S51 marker set. Marker peaks within the electropherogram were called and quantified using GeneMapper software (ABI). **A** Marker distribution of the stem cell donor and the recipient before transplantation. **B** Marker profile of recipient whole blood monocytes and T cells at day 342 after transplantation. Quantified recipient chimerism (RC) values are indicated on the right side of the electropherogram as percentages. **C** Marker profile of recipient peritoneal macrophage populations and peritoneal T cell populations. Quantified recipient chimerism (RC) values are indicated on the right side of the electropherogram as percentages. Patient alleles for the markers SE33 (15) and D18S51 (15 and 16) were only found for the population of small peritoneal macrophages.

donor hematopoiesis and provided the basis for chimerism analysis of the abdominal cavity sample. For the characterization of peritoneal immune cells, resting T-cells (CD3+CD69-), tissue-resident memory T cells (CD3+CD69+)[34,35], and small and large peritoneal macrophages (CD14+CD206- and CD14+CD206+ respectively)[32] were isolated by cell sorting of mononuclear cells from ascitic fluid. The STR analysis indicated that the T cell populations originated entirely from the donor. Furthermore, all cells within the LPM population were found to originate from the donor's bone marrow (Fig. 2C). Interestingly, for cells of the SPM population, the STR profile revealed a recipient-derived origin of 2.8%. These results demonstrate that, within a relatively short time frame, the majority of cells were replaced by bone marrow-derived donor cells, while only a small fraction of patient SPM persisted in the peritoneal cavity for about one year.

Peritoneal immune cell composition is fully reestablished after one year of chemotherapy, paracentesis, and spontaneous bacterial peritonitis

Stem cell transplantation and chemotherapy, paracentesis, and SBP are drastic events that can affect the composition and phenotype of the immune cells of the peritoneal compartment. The finding that the majority of the patient's peritoneal T-cell and macrophage populations originated from bone marrow-derived cells raised the question of to what extent migratory monocytes were able to mature into tissue-resident peritoneal macrophages and whether the composition of other immune cells of the abdominal cavity was altered.

To answer these questions, a reference cohort of 20 patients with liver cirrhosis, matched to the disease severity and the age of the chimeric patient, was enrolled and analyzed for PerC immune cell composition and the immune phenotype of peritoneal macrophages. In these patients, peritoneal macrophages (CD14+ cells) accounted for the majority of mononuclear immune cells with a median fraction of 62.9%. The median proportions of SPM and LPM among PM were 54.1 and 45.9%, respectively. T cells made up the second largest population with 12.3%, with equal median amounts of CD4+CD8- and CD4-CD8+ T cells. NK cells accounted for 12% of the AF mononuclear cells, while B cells and DCs were significantly less present with 1.5 and 0.83%, respectively (Fig. 3A). These data are in good agreement with findings from other studies[9] and further elaborate on the characterization of the peritoneal immune cell landscape, particularly in the context of liver cirrhosis.

Interestingly, almost identical results were found for the chimeric patient. Peritoneal macrophages accounted for 61.5% of the AF mononuclear cells (SPM: 38%; LPM: 62%). T cells were present to a lesser extent in comparison to the control cohort (6.6% vs. 12.3%), whereof the ratio of CD4/CD8 T cells was considerably shifted towards CD4-CD8+ T cells. The fraction of NK cells and B cells was slightly above average (14.4% and 8.9%, respectively). DC cells were not present in significant numbers (Fig. 3A – red circle). In conclusion, the patient's peritoneal immune cell composition was found to correspond to the peritoneal immune cell composition of patients with liver cirrhosis but without stem cell transplantation.

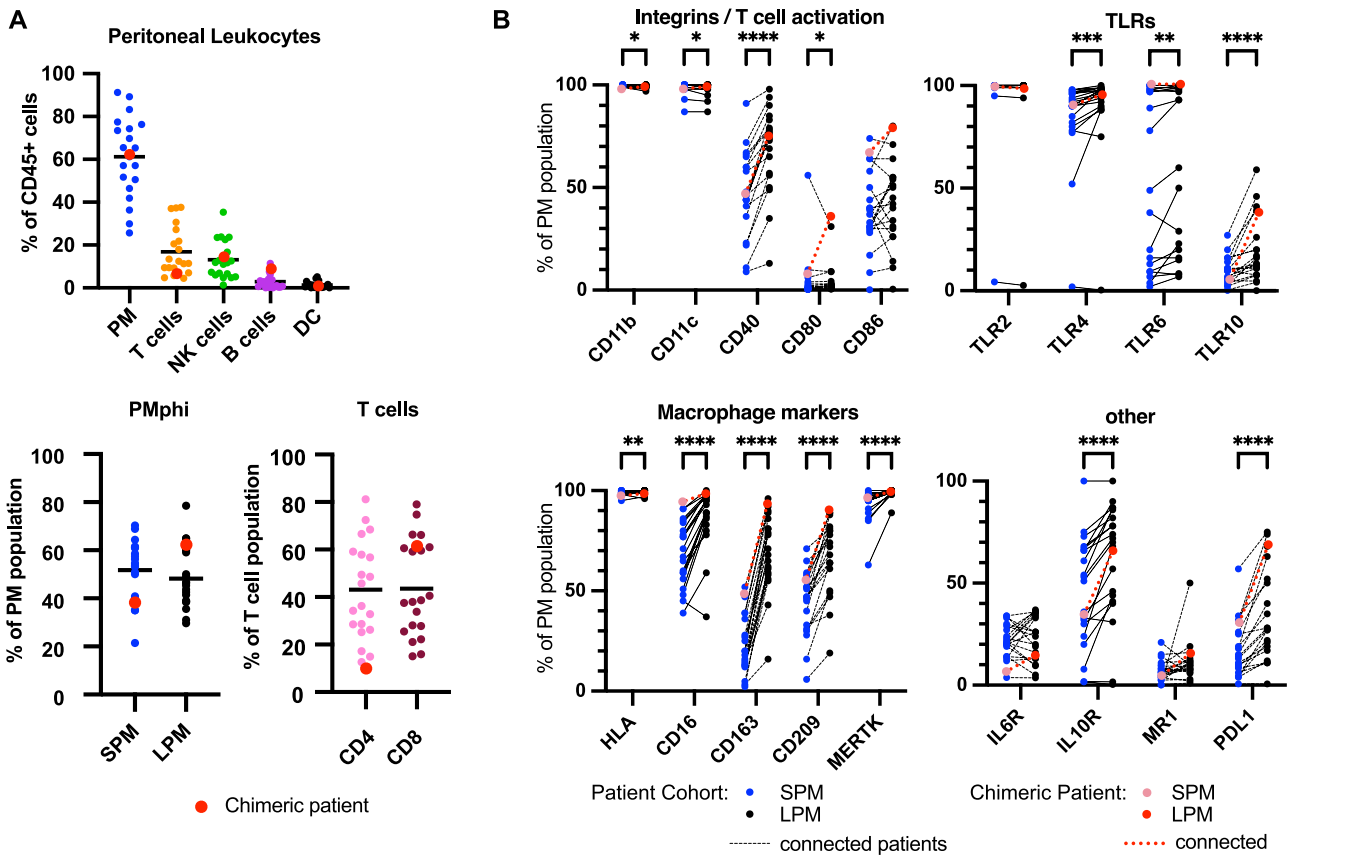


Fig. 3 | Peritoneal immune cell composition and immunophenotyping of peritoneal macrophages. A Ascitic fluid cells of patients with liver cirrhosis ($n = 20$) were isolated and analyzed for the expression of cell surface markers to discriminate PM, T and B cells, NK cells and DC, as well as PM subpopulations (SPM/LPM) and T cell subpopulations (CD4/CD8 positive cells). Bone marrow transplanted patient marked with a red dot. **B** Peritoneal macrophage subpopulations were further investigated for the expression of immunoreceptors and macrophage surface markers using FACS. To identify informative and robust markers that distinguish SPM from LPM, statistical comparisons between the two subsets were performed using Wilcoxon tests. Multiple Wilcoxon tests were applied across all markers. p-value: <0.05 (*), < 0.01 (**), < 0.001 (***), < 0.0001 (****). Blue and black dots represent expression values of SPM and LPM from the control cohort, respectively. Pink and red dots represent expression values of SPM and LPM from the chimeric patient, respectively. Paired SPM–LPM samples from individual patients are connected by dotted lines (black for the cohort, red for the chimeric patient).

Monocyte-derived macrophages differentiate and develop regularly within the peritoneal cavity

Macrophages make up the largest proportion of immune cells in the human PerC. For immunophenotyping of PM, cell surface proteins such as integrins, TLRs, phagocytosis-relevant proteins, and proteins with functions in T cell interaction and activation were investigated. Expression was examined on both SPM and LPM, and significant differences were found between the two macrophage populations, particularly for phagocytosis-relevant proteins such as CD16, CD163, CD209 and MERTK, as well as for the integrin CD11c and the T-cell receptor ligand PD-L1 (Fig. 3B - blue and black circles). Thus, underlining functional differences between the two macrophage populations.

A very similar expression profile of these markers was also found for macrophages of the stem cell transplanted patient (Fig. 3B - pink and red circle). Notably, the vast majority of markers (16 out of 18) in the chimeric patient fell within the 95% reference interval derived from the reference cohort (Supplementary Fig. 4A, B). The integrins CD11b and CD11c were strongly expressed on almost all macrophages. Receptors such as IL6R, MR1 and TLR10 were only present on a minority of cells. Strikingly, the most robust markers of SPM and LPM differentiation (p-value < 0.0001), including CD16, CD163, CD209, MERTK, CD40, PD-L1 and IL10-R, were also differentially expressed in the same direction in the chimeric patient's SPM and LPM populations as observed in the cohort, confirming accurate SPM and LPM differentiation. A principal component analysis (PCA) integrating all 18 markers revealed that the chimeric patient's SPM and LPM exhibited global expression patterns highly comparable to those of the reference cohort (Supplementary Fig. 4C, D).

These data demonstrate that donor-derived monocytes successfully differentiated into tissue-resident macrophages within the patient's peritoneal cavity, suggesting that local peritoneal milieu factors may play an important role in macrophage development.

Peritoneal fluid drives differentiation of monocytes towards tissue macrophage phenotype

The importance of the tissue environment for tissue-specific differentiation of macrophages has already been convincingly confirmed in mice. In order to investigate whether soluble factors within the tissue fluid of the human abdominal cavity have an impact on the differentiation of monocytes towards tissue macrophages, monocytes were isolated from healthy donors and treated for three days with paired samples of patient serum or cell-free ascites. Thereafter, the expression of selected PM markers was determined using flow cytometry (Fig. 4). Cells treated with AF showed highly significantly increased expression levels of CD14 and significantly elevated levels of TLR6 and CD209 in comparison to serum-treated cells. There was a trend towards increased CD163 expression when cells were treated with AF. In addition, a weak trend towards increased expression levels of CD206 after AF treatment was noticeable. We also examined the expression of the newly described and unexpected tissue-macrophage marker CD28[36,37]. Unpublished data from our group demonstrate a distinct and robust expression of CD28 predominantly in LPMs, whereas monocytes lack CD28 expression and SPMs show only low levels, making CD28 a highly suitable marker for differentiation analyses. Notably, CD28 expression increased markedly in cells treated with ascites fluid. Altogether this data shows that soluble factors within the peritoneal fluid can stimulate monocyte differentiation towards a tissue-resident macrophage-like phenotype.

Discussion

The gut–liver axis constitutes a bidirectional communication network connecting the intestine, its microbiota, and the liver via metabolic, immunological, and vascular pathways. In the context of liver cirrhosis, disruption of this axis facilitates microbial translocation and promotes inflammation within the peritoneal cavity. This highlights the immunological relevance of the peritoneal compartment and its resident immune cell populations, expanding the classical concept of the gut–liver axis into a broader gut–liver–peritoneum axis. During SBP, the peritoneal cavity undergoes a massive influx of neutrophils and monocytes from the bloodstream. This is accompanied by a substantial loss of LPM, potentially due to dilution effects, cell death, or migration into omental milky spots. Remarkably, three days after initiation of antibiotic therapy, a relative increase in the LPM fraction can be observed, approaching baseline levels. It is hypothesized - analogous to murine models - that infiltrating monocytes serve as precursors for both small and large peritoneal macrophages. This assumption is supported by studies examining changes in surface marker expression on PMs during peritoneal dialysis or peritonitis[8,32], by analogies drawn from mouse models[7,30], and by RNA velocity analyses following single-cell sequencing of peritoneal washouts[12].

While these studies provide compelling evidence regarding the monocytic origin of peritoneal macrophages, our findings offer the first direct proof that monocytic cells can fully differentiate into both SPM and LPM. Notably, the vast majority of tissue-resident macrophages in our patient were derived from bone marrow–originating donor cells. Interestingly, we detected a small population of recipient-derived macrophages in the patient's peritoneal cavity, and, unexpectedly, all of these cells belonged to the SPM subset. Remarkably, this residual SPM population had persisted

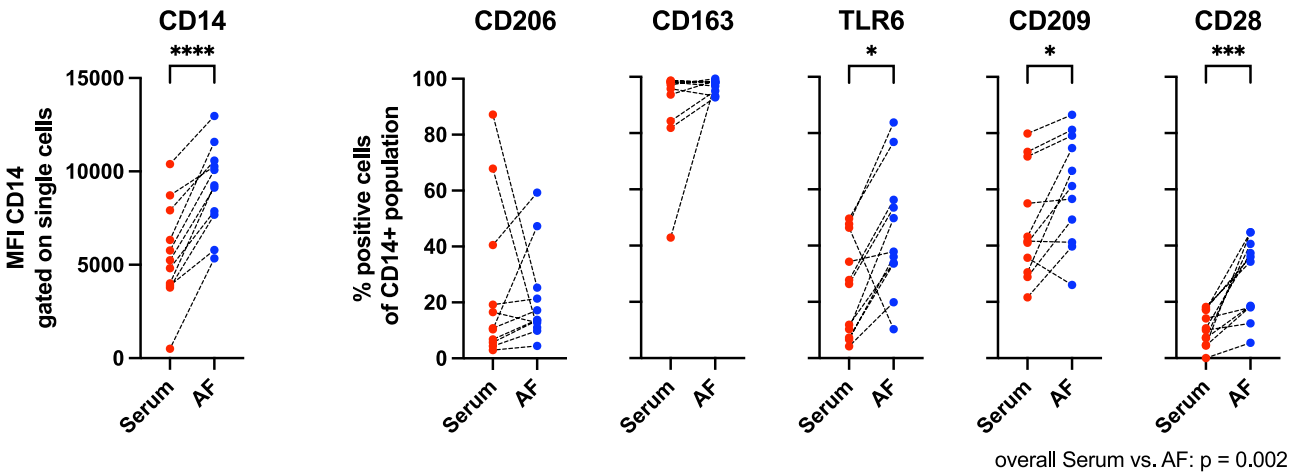


Fig. 4 | Monocyte differentiation towards a peritoneal tissue macrophage-like phenotype by ascitic fluid. Monocytes were isolated from healthy donors and treated with paired samples of serum or ascitic fluid of patients for three days (n = 11). Expression of macrophage surface markers was analyzed by FACS. Statistical comparison was performed by applying paired T-tests. p-value: <0.05 (*), < 0.01 (**), < 0.001 (***), < 0.0001 (****). Statistical testing for differences between Serum and AF treatment regarding the expression of CD206, CD163, TLR6, CD209, and CD28 was performed by Two-Way ANOVA.

in the abdominal cavity for approximately one-year, withstanding chemotherapy, bacterial peritonitis, and multiple paracenteses.

In mouse models, the longevity of peritoneal macrophages is attributed to the large peritoneal macrophage population, which originates embryonically and is maintained through self-renewal during early life. With advancing age or under conditions of severe infection, LPM are progressively replaced by hematopoietic stem cell (HSC)-derived cells. In contrast, small peritoneal macrophages are replenished early after birth and continuously throughout life by HSC-derived progenitors[29,38,39].

The current model of the human peritoneal cavity shows strong parallels to findings from mouse studies. Research by others[8,12,30,32] suggests that human SPM most likely originate from monocytes that migrate into the peritoneal cavity and subsequently differentiate into long-lived LPM. However, the majority of LPM in humans, unlike in mice, appear to represent an intermediate stage, as only a small subset of this main LPM population is capable of further maturing into TIMD4$^+$ GATA6$^+$ SELP$^+$ LPM —the predominant LPM population observed in mice[12].

The detection of a small fraction of recipient-derived SPM, but no patient-derived LPM, was unexpected. However, this finding suggests that SPM, or at least defined subsets within the SPM population, do not merely represent a transitional differentiation state toward LPM. Instead, they may constitute a distinct macrophage subpopulation. Consistent with this, we and others have previously demonstrated that not only LPM but also SPM express gene sets associated with cell cycle progression and include cells positive for the proliferation marker KI-67, indicating their potential for self-renewal and longevity[7,32].

Interestingly, despite the replacement of PMs and T cells by donor-derived cells, both the overall composition of the immune cells in the patient's peritoneal cavity and the phenotypic characteristics of the two macrophage populations showed no major differences compared to samples from other cirrhosis patients. This observation suggests that the peritoneal immune compartment maintains a remarkably stable composition and function even after hematopoietic reconstitution, underscoring its high regenerative potential. This is consistent with established findings from murine models, demonstrating that macrophage populations in the peritoneal cavity and other tissues can reconstitute and maintain their identity through environmental adaptation[24,25].

The peritoneal fluid is considered an ultrafiltrate of blood[40] and is also partly derived from peritoneal mesothelial cells[41]. However, the soluble factors that influence human macrophage differentiation within the PerC remain largely undefined. In line with this, Irvine et al. reported that expression of the complement receptor of the Immunoglobulin superfamily (CRIg) and phagocytic capacity of the PMs increase when these cells are exposed to autologous ascites fluid[7]. Consistently, our experiments demonstrated that monocytes differentiate into peritoneal macrophage-like cells when cultured in the presence of peritoneal fluid. Interestingly, this was particularly evident by a remarkable increase in the macrophage marker CD14, the expression of which is a defining characteristic of human peritoneal macrophages[9,30,33], and it can be assumed that this increase is associated with an enhanced sensitivity to bacterial components such as LPS, a feature characteristic of large peritoneal macrophages in patients with liver cirrhosis[32]. This is further supported by our observation of increased expression of the C-type lectin receptor CD209 and Toll-like receptor 6 after AF treatment. CD209 is normally absent on monocytes but can be induced during differentiation into monocyte-derived DC in response to GM-CSF and IL-4, typically accompanied by a reduction of CD14 expression[42,43]. CD209 has also been reported to be expressed by tissue macrophages[42,43], and can be expressed following differentiation of the monocyte precursor cell line THP1[44]. Thus, the concurrent increase of CD14, TLR6 and CD209 after AF treatment strongly indicates of monocyte to macrophage differentiation, likely initiated and supported by soluble factors present in the AF. These results are in good agreement with findings from murine studies[24–26].

Investigating a rare clinical case with decompensated cirrhosis who received a hematopoietic stem cell transplantation provided insights into the origin and regenerative capacity of human peritoneal immune cells. We found that even after major events such as chemotherapy, peritonitis, or multiple peritoneal paracenteses, the immune cell compartment of the PerC can fully replenish with the aid of bone marrow-derived stem cells and the peritoneal milieu. As these observations are derived from a single patient, they should be interpreted with appropriate caution. Despite this limitation, these results are clinically significant, as they demonstrate the ability of the peritoneal immune environment to tolerate the aforementioned medical interventions. However, patients undergoing peritoneal dialysis—with daily infusions of dialysis fluid that continuously dilute immune cells and tissue factors—illustrate the limits of its regenerative capacity[8]. Reducing immune cell loss, in combination with the supplementation of microenvironmental factors, may enhance treatment tolerability.

Methods

Patient sample selection and processing

Patients with decompensated cirrhosis who underwent therapeutic paracentesis at the University Hospital Jena were eligible for PM immune phenotyping. Exclusion criteria were evidence of secondary peritonitis, tuberculous peritonitis, peritoneal carcinomatosis, HIV infection, and presence of SBP by using current diagnostic criteria. In a cohort of 20 patients, human PMs and leukocytes from ascitic fluid (AF) were prospectively characterized by flow cytometry. The study was approved by the Internal Review Board of the Jena University Hospital (2880-08/10, 3683-02/3) and was carried out according to the local guidelines and regulations. Written informed consent was obtained from patients or their legal surrogates. The clinical characteristics of the patients are given in Supplementary Table 1.

Ascitic fluid and whole blood cell isolation

Ascites cells from about 500 ml ascitic fluid (AF) were enriched by centrifugation at 800 *g* for 10 minutes at 4 °C, and cell pellets were resuspended in cell-free ascites. Whole blood (9 ml) was collected from healthy donors in EDTA-monovettes. Following, mononuclear cells from ascites or blood samples were purified by Ficoll density gradient centrifugation using Lympholyte-H (Cedarlane, Burlington, Ontario, Canada), washed and resuspended in phosphate-buffered saline containing 2% heat-inactivated fetal bovine serum and 2 mM EDTA (MACS buffer). For long-term storage, cells were slowly frozen down to −80 °C in freezing medium (RPMI, 10% DMSO, 30% Fetal Calf Serum (FCS)) at a rate of 1 °C per minute. The next day, cells were transferred to liquid nitrogen vapor phase storage. To thaw cell samples for flow cytometry or cell sorting, tubes were removed from the liquid nitrogen and incubated in a water bath at 37 °C for 5 min. Cells were then washed twice in PBS and immediately applied to FACS staining. Cell-free ascitic fluid and serum were obtained from ascitic fluid and whole blood, respectively, by centrifugation at 2,000 g for 10 min at room temperature and stored at −80 °C until use.

Flow cytometry and cell sorting

Staining of AF mononuclear cells was preceded by live-dead staining using the LIVE/DEAD Fixable Near IR chemistry (ThermoFisher Scientific) in accordance with the manufacturer's protocol (4 °C for 30 min). To reduce nonspecific antibody binding, FC-receptor blocking was performed using the Human TruStain FcX Fc Receptor Blocking Solution (BioLegend) as described by the manufacturer. Cell surface staining was performed in MACS buffer with 1:100 to 1:20 dilutions of fluorochrome-conjugated antibodies for 30 minutes at 4°C. Peritoneal leukocytes were stained with the AF-Leukocyte-Panel (Supplementary Table 2) and macrophage phenotyping was performed using the PM-Antibody-Panel (Supplementary Table 3). After extracellular staining, cells were washed twice and resuspended in MACS buffer and analyzed on an LSRFortessa X-20 Cell Analyzer (BD Biosciences). Cells were gated and analyzed as described in Supplementary Figs. 1 and 2. Isotype-matched controls, single-stained samples, and fluorescence-minus-one (FMO) controls were used to perform compensation and to gate on cell populations to be investigated.

Peritoneal macrophage and T cell subpopulation were sorted by flow cytometry on a FACSAria III (BD Biosciences) using staining antibodies listed in Supplementary Table 4 and gating strategy shown in Supplementary Fig. 3. Sorted populations were reanalyzed by FACS to ensure sorting purity.

Macrophage differentiation assay

Monocytes from healthy volunteers were isolated from EDTA whole blood by Ficoll gradient centrifugation (see above), followed by CD14-immunomagnetic cell sorting[32]. Monocytes were cultured in 24-well plates at a density of 250,000 cells per well in 300 µl RPMI-1640 medium supplemented with 10% FCS, 1% L-glutamine-penicillin-streptomycin and treated with paired samples of 25% cell-free ascitic fluid or 25% serum from patients with liver cirrhosis ($n = 11$). After three days, cells were detached by cell scraping and flow cytometry was performed as described above.

Chimerism analysis

For the analysis of donor chimerism in the stem cell transplanted patient, a PCR assay for short tandem repeats (STR) was performed. For this purpose, DNA was extracted automatically (AutoMate Express, Thermofisher) from FACS-sorted cells and quantified by real-time PCR (Investigator Quantiplex Kit, Qiagen). Following, a Multiplex-PCR of 17 STRs, including the gender-specific amelogenin system (PowerPlx ESX 17 Fast, Promega), was conducted to determine the presence of donor cells. Marker peaks within the electropherogram were called and quantified using GeneMapper software (ABI). The analysis was carried out as part of the clinical chimerism screening and monitoring procedure of the Institute of Forensic Medicine Jena and is accredited and annually monitored by independent quality controls.

Statistics

All statistical analyses were conducted using GraphPad Prism 10, as described in the text, and R with the gplots and ggplot2 packages.

Data availability

The raw data generated and analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

Recruited patients and provided samples and clinical data: ST.Q., BE.G., PH.R.; Performed sample collection and preparation: LA.A., MI.R., AR.S., SV.S.; Performed experiments and analyses: LA.A., MI.R., JU.S., TI.S., SV.S.; Assisted with the experimental design: LA.A., MI.R., BE.G., JU.S., GI.M., PE.H., AN.S.; Performed statistical analyses: TO.B., SV.S.; obtained funding: PE.H., AN.S.; Conceived of and supervised the study, obtained funding, and provided guidance with experimental design and writing of the manuscript: TO.B., SV.S.; All authors have read and approved the manuscript.

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Competing interests

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