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FRONTIER

Recent insights into the characteristics and role of peritoneal macrophages from ascites of cirrhotic patients

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Abstract

Macrophages are a diverse myeloid cell population involved in innate and adaptive immune responses, embryonic development, wound repair, and regulation of tissue homeostasis. These cells link the innate and adaptive immunities and are crucial in the development and sustainment of various inflammatory diseases. Macrophages are tissue-resident cells in steady-state conditions; however, they are also recruited from blood monocytes after local pathogen invasion or tissue injury. Peritoneal macrophages vary based on their cell complexity, phenotype, and functional capabilities. These cells regulate inflammation and control bacterial infections in the ascites of decompensated cirrhotic patients. Our recent work reported several phenotypic and functional characteristics of these cells under both healthy and pathological conditions. A direct association between cell size, CD14/CD16 expression, intracellular level of GATA-6, and expression of CD206 and HLA-DR activation/maturation markers, indicate that the large peritoneal macrophage $CD14^{high}CD16^{high}$ subset constitutes the mature phenotype of human resident peritoneal macrophages during homeostasis. Moreover, elevated expression of CD14/CD16 is related to the phagocytic capacity. The novel large $CD14^{high}CD16^{high}$ peritoneal subpopulation is increased in the ascites of cirrhotic patients and is highly sensitive to lipopolysaccharide (LPS)-induced activation, thereby exhibiting features of inflammatory priming. Thus, phosphorylation of ERK1/2, PKB/Akt, and c-Jun is remarkably increased in response to LPS *in vitro*, whereas that of p38 MAPK is reduced compared with the monocyte-derived macrophages from the blood of healthy controls. Furthermore, *in vitro* activated monocyte-derived macrophages from ascites of cirrhotic patients secreted significantly higher levels of IL-6, IL-10, and TNF-α and lower amounts of IL-1β and IL-12 than the corresponding cells from healthy donor's blood. Based on these results, other authors have recently reported that the surface expression level of CD206 can be used to identify

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mature, resident, inflammatory peritoneal macrophages in patients with cirrhosis. Soluble CD206 is released from activated large peritoneal macrophages, and increased concentrations in patients with cirrhosis and spontaneous bacterial peritonitis (SBP) indicate reduced odds of survival for 90 d. Hence, the level of soluble CD206 in ascites might be used to identify patients with SBP at risk of death. In conclusion, peritoneal macrophages present in ascites of cirrhotic patients display multiple phenotypic modifications characterized by reduced ratio of cells expressing several membrane markers, together with an increase in the ratios of complex and intermediate subpopulations and a decrease in the classic-like subset. These modifications may lead to the identification of novel pharmaceutical targets for prevention and treatment of hepatic damage.

Key Words: Cirrhosis; Inflammation; Peritoneal macrophages; Phenotypic markers; Activation routes

Core Tip: This frontier article is based on a summary of recent relevant publications on the biology of mouse, as the main animal model used, and human peritoneal macrophages under the perspective of its future clinical translation to the role that these cells can play on several human liver diseases. Concretely, we have reviewed recent findings on several characteristics of human peritoneal macrophages obtained from the ascites of cirrhotic patients compared with those obtained from healthy donors. Featured article: Role of MAP kinases and PI3K-Akt on the cytokine inflammatory profile of peritoneal macrophages from the ascites of cirrhotic patients.

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BIOGRAPHY

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Her subjects of interest include the immunopathology of hepatic cirrhosis and endometriosis, the physiology of human NK cells and peritoneal macrophages, and theoretical models of biological systems, especially the immune system.

INTRODUCTION

Liver cirrhosis is the end stage of various different chronic hepatic diseases, characterized by a gradual substitution of the liver structure by fibrotic tissue[1]. The role of monocytes and macrophages in the physiopathology of liver cirrhosis has been extensively reported[2-5]. Rapid mobilization of these cells to peritoneum or hepatic tissue is an important mechanism of defense against incidental bacterial infection translocated from the gut[6]. Pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), peptidoglycan, mannan, glucans, bactDNA, and many

Figure 1 Pilar García-Peñarrubia, MD, PhD is a Professor in the Departments of Biochemistry and Molecular Biology B and Immunology in the School of Medicine, at the University of Murcia, Spain. She received her MD degree from the School of Medicine in the University of Murcia, Spain in 1975. She certified as MD specialist in Microbiology and Parasitology in 1980. She received her PhD degree from the School of Medicine in the University of Murcia, Spain in 1979 with honor "cum laude". From 1986 to 1989 she was a Research Associate with the Department of Medicine, School of Medicine, University of New Mexico, Albuquerque, United States, and a Fellow of the Fulbright Foundation (1986-1987). She was Vice Dean of the School of Medicine (University of Murcia) since 1999 to 2002. Her subjects of interests include the immunopathology of hepatic cirrhosis and endometriosis, the physiology of human NK cells and peritoneal macrophages, and theoretical models of biological systems, especially the immune system.

others, induce the secretion of cytokines from myeloid-derived monocytes and macrophages[7-10]. Chronic inflammation and fibrosis are crucial features associated with macrophage accumulation in the liver[2,10]. Moreover, marked hepatic and systemic damage in cirrhotic patients is associated with high secretion of proinflammatory cytokines such as IL-1β, TNF-α, and IL-6, as well as anti-inflammatory cytokines such as IL-10 and TGF-β[11]. In this sense, recent large-scale observational studies have pointed to systemic inflammation as a hallmark of acute decompensation of cirrhosis. Hence, a recent hypothesis proposes that systemic inflammation is the key mechanism in the progression from compensated to decompensated cirrhosis, as well as in the development of acute episodes of decompensation, which are associated with generalized dysfunction or even with extrahepatic multiorgan failure[4].

The study of human tissue-resident macrophages presents several challenges. First, it is necessary to carry out surgical interventions to obtain these macrophages; second, the cell count obtained is low; and third, these cells are difficult to grow *in vitro*. Thus, accumulated research on resident macrophages in both homeostasis and pathology has been performed in animal models, particularly in mice. Nevertheless, it is not always possible to extrapolate from mice to man, in particular in this topic[12,13]. Thus, it is essential to verify whether the tissue factors and macrophage transcription molecules identified in murine models play similar roles in the origin and biology of human tissue-resident macrophages. Additionally, research on the role of the immune system in human inflammatory diseases is principally carried out with peripheral blood leukocytes; however, the study of macrophages from an inflammatory setting can provide relevant information for a better understanding of the physiopathology of numerous human diseases[8,14]. Hence, human peritoneal macrophages (pMϕ) are a potential option for studying the biological characteristics of this cell type[15]. Peritoneal leukocytes play a crucial role in the defense against microbial infections within the peritoneal cavity, and they also contribute to endometriosis and cancer pathologies. Thus, while knowledge about the inflammatory status in peripheral blood and liver of cirrhotic patients has increased dramatically in the last few years, less is known about its correlation with the inflammatory status in ascites fluid (AF), and limited information is available on differences related to cirrhotic etiology[16]. Recent studies have demonstrated that pMϕ are crucial in regulating inflammation and controlling peritoneal infections in decompensated cirrhotic patients. The accurate phenotypic characterization of macrophages obtained from AF of cirrhotic patients helps us to understand and presumably prognosticate the risk of experiencing episodes of spontaneous bacterial peritonitis (SBP), which impairs the outcome in this clinical condition[17,18]. Furthermore, as macrophages are implicated in the physiopathology associated with hepatic cirrhosis, these cells are also studied as targets of new therapies expected to avert the progression of hepatic damage[2,3,14].

Very recently, novel findings on the ontogeny, phenotype, function, specific transcription factors, migratory activity[19-21] and sex differences[22], of mouse resident pMϕ have been reported. These findings open new avenues for the study of human pMϕ in both health and disease conditions.

ORIGIN OF HUMAN PERITONEAL MACROPHAGES

Macrophages are a heterogeneous myeloid cell population involved in innate and adaptive immune responses, as well as in embryonic development, regulation of tissue homeostasis, and wound repair. These immune cells link innate and adaptive immunities by acting as antigen-presenting cells and are crucial in the development and persistence of various inflammatory conditions.

Macrophages are found in all tissues as resident cells in steady-state conditions, and also as immigrant foreign cells derived from peripheral blood monocytes in response to microbial invasion, tissue injury, or inflammation[7,8]. The contribution of monocytes to resident macrophages is highly tissue-dependent, and until recently it was admitted that most of the homeostatic murine pMϕ are terminally differentiated and replenished by blood monocytes[8]; however, groundbreaking findings revealed that the majority of tissue-resident macrophages do not arise from hematopoietic progenitors, as they directly originate from embryonic precursors (yolk sac and fetal liver) and are able to proliferate and self-renew[19]. Thus, local proliferation reestablishes the normal macrophage number after inflammation-induced loss of resident pMϕ[20]. Moreover, it has been reported that the expression of transcription factor GATA-binding protein 6 (GATA-6) is mostly limited to the long-lived murine F4/80^{hi}CD11b^{hi} large peritoneal macrophages (LPMs) of embryonic origin, whereas the subpopulation of F4/80^{low}MHC-II^{hi}, namely small peritoneal macrophages (SPMs), arise from inflammatory monocytes[21]. Recently, Louwe *et al*[23] reported that the fate and function of inflammation-activated pMϕ seem to be regulated by environmental changes. Thus, moderate inflammation-elicited murine pMϕ survive for 5 mo, although they do not acquire the GATA-6^{hi} resident signature. In contrast, high inflammation results in depletion of resident macrophages for a sustained period, although ultimately, stimulated cells achieve a mature GATA-6^{hi} expression.

Bain *et al*[22] reported that murine pMϕ exhibit sexual dimorphism, determined by different microenvironmental cues and a differential replenishment rate from the bone marrow. After sexual maturity, the time of residency and local tissue factors seem to result in increased expression of immune function-related genes in F4/80^{hi}CD102+ macrophages, particularly CD209b, in female mice, which more efficiently control the peritoneal infection with *Streptococcus pneumoniae*. In contrast, the rate of replenishment from the bone marrow is higher in male animals. In this regard, Oh *et al*[24] identified the mTORC2-FOXO1 axis as crucial for integrating microenvironmental signs to regulate metabolic reprogramming, differentiation, and activity of peritoneal tissue-resident macrophages.

PERITONEAL MACROPHAGES CAN MIGRATE VIA A NONVASCULAR ROUTE TO THE INJURED LIVER

Studies conducted in mice have revealed the crucial role played by pMϕ in homeostasis as well as in the physiopathology of multiple systemic or abdominal diseases[25,26]. In this regard, Wang and Kubes[27] reported that murine mature pMϕ F4/80^{hi}GATA-6^{+} rapidly (within 12 h post-injury) infiltrate the injured liver through a non-vascular route across the mesothelium layer, thereby adopting an alternatively activated phenotype with an increased expression of arginase 1, and protect against acute liver damage. The recruitment of pMϕ toward the sites of liver injury was dependent neither on chemokine receptor signaling nor on β1 or β2 integrins, which indicates that this mechanism differs from that of the recruitment of immune cells *via* an intravascular route. Recruitment guidance was dependent on ATP and hyaluronan in the injured tissues, as well as on macrophage expression of CD44, which is a known receptor for the last molecule.

These findings challenge the present assumption that tissue-resident macrophages are stationary cells, and suggest that rapid mobilization of pMϕ, with ability to induce tissue repair, into the damaged liver, can be an important defense mechanism against infections, trauma, metabolic diseases, fibrosis, and tumor diseases.

CHARACTERISTICS OF HUMAN PERITONEAL MACROPHAGES IN HOMEOSTASIS

Human pMϕ are the best choice for carrying out studies on the biological properties of tissue-resident macrophages under homeostatic conditions; moreover, these macrophages can be used as a healthy control group to compare data from individuals suffering from various pathologies affecting the peritoneal cavity, such as cirrhotic or cancer ascites. For this purpose, peritoneal fluid (PF) samples must be obtained either from healthy people or from individuals whose disease does not affect the peritoneal compartment. The most frequent control samples referred to in the corresponding literature were collected from patients on continuous ambulatory peritoneal dialysis (CAPD), not affected by SBP, as well as from exploratory gynecological laparoscopies/laparotomies performed in healthy women[28-31].

Nevertheless, CAPD patients are not healthy people; moreover, it has been reported that the fluid flow through the rat omentum increases with peritoneal dialysis, thereby leading to a dramatic enlargement of the leukocyte aggregates called "milky spots", which are rich in macrophages, lymphoid B and T cells, mast cells, and stromal cells [32,33], and promoting omental fibrosis[34]. Thus, due to these objections, peritoneal cells from CAPD do not really qualify as representative of homeostatic peritoneal cells to be used as healthy control.

We have recently described an optimized method for obtaining human pMϕ from the PF of healthy women[35], and studied several characteristics of healthy human pM ϕ compared with the well-known CD14/CD16 blood monocyte subsets, in order to analyze common properties or tissue-specific differences[36]. Hence, PF from 79 healthy women was acquired from the Gynecological Unit of the HCUVA, Murcia, Spain. Cell samples from blood and PF were obtained during exploratory or therapeutic laparoscopies for benign gynecological pathology (simple ovarian cysts or uterine fibroids) or tubal ligation. Under physiological conditions, a small amount of 5-20 mL PF is present in the peritoneal cavity. It is produced by mesothelial cells and contains a mix of plasma transudate, ovarian exudates, tubal fluid, and macrophages' secretions[37-39]. The physiological functions of PF include lubricating the friction of the intestinal loops and other organs contained in the peritoneal cavity, allowing the exchange of nutrients, repairing injured tissues, and eliminating detritus and microorganisms. In our experience, the first PF obtained by the endoscopic aspirator is quite scarce; with a mean of 6.85 ± 2.6 mL (range 5-8.7 mL). Moreover, this fluid has practically no polymorphonuclear (PMN) leukocytes, which is indicative of the absence of local inflammatory signals. Among human peritoneal leukocytes, macrophages are the predominant cell type (45-90%) followed by T lymphocytes (predominantly T effector/memory cells, CD45RO) (45%), NK cells (8%), dendritic cells (2-6%), B lymphocytes (2%) and less than 5% of PMN cells[31,35,40-43].

Our results revealed that primary human pMϕ have phagocytic and oxidative activities, and they respond to activation of the main proinflammatory routes such as Toll-like receptors and inflammasomes, which further results in the secretion of different proinflammatory cytokines[35]. Furthermore, we demonstrated that pMϕ are heterogeneous with respect to their morphology and CD14/CD16 cell expression. This peritoneal population is made up of akin proportions (approximately 42%) of classic ($CD14^{++}CD16^{-}$) and intermediate ($CD14^{++}CD16^{+}$) small cells, and a novel subset of complex $CD14^{high}CD16^{high}$ cells (approximately 16%), which are not found in the peripheral blood. In contrast, nonclassical blood monocyte-like cells are not detected in the peritoneal cavity[36]. Moreover, pMϕ reveal higher expression of CD14 and CD16 than blood monocytes, which makes them more competent or available for phagocytosis in the presence of LPS or microorganisms. Notably, the percentages of these cell subpopulations are modulated under inflammatory processes. Thus, besides describing the presence of a novel human $CD14^{high}CD16^{high}$ LPM subpopulation (33% ± 2.4%) in the ascites of decompensated cirrhotic patients for the first time, we also found that the percentage of intermediate $CD14^{++}CD16^{+}$ subset was predominant (49% ± 2.0%), whereas the classic $CD14^{++}CD16^{-}$ subset revealed lowest values (18% ± 1.3%) [44]. These modifications in pathological versus steady-state conditions strengthen the importance of these results.

We also analyzed the expression of several monocyte/macrophage-associated membrane receptors implicated in phagocytosis of IgG-opsonized (CD64, high affinity FcγRI) and complement-opsonized microorganisms (CD11b and CD11c, the α chains of Complement receptors, CR3 and CR4); adhesion to activated endothelial cells and tissue recruitment (CR3, CR4, CD62L, and 6-sulfo LacNAc (Slan)), antigen presentation (MHC class II molecule HLA-DR), costimulatory markers (CD80, CD86, CD40),

cytokines receptors (CD116, GM-CSFR and CD119, IFNγR1 or IFNγ chain α receptor), and the mannose receptor (CD206), reported as a M2 polarized marker and denotative of activation/maturation[45]. In comparison with the complete population of blood monocytes, CD86, CD64, and CD11b revealed similar expression on pMϕ; whereas, small significant differences were observed for a higher expression of HLA-DR, CD116 and CD119 on pMϕ. The most compelling differences were found for CD40, CD80, CD11c, CD206, Slan, and CD62L, of which CD62L was the only receptor expressing higher levels of blood monocytes. These findings suggest that human pMϕ could exert remarkable antimicrobial (also high phagocytic and oxidative capacity), antigen-presenting, and T-cell costimulatory capacities; however, this remains to be further explored. Conversely, the steady increase in the percentages and density of CD206 expression from 28.2% in $CD14^{++}CD16^{-}$ to 60.3% in $CD14^{++}CD16^{+}$ and 92.8% in $CD14^{high}$ $CD16^{high}$ suggested that human pMϕ may also exhibit features and functional characteristics of M2 macrophages, as previously described in CAPD[46,47] and endometriosis patients[48]. Nevertheless, the most remarkable differences between blood and pMϕ subsets were detected on selectin CD62L expression, that is, percentages of pMϕ expressing CD62L in each subpopulation increase in parallel with the expression of CD16, whereas the corresponding expression of CD62L in blood monocytes diminishes as CD16 increases. Moreover, it was observed that the percentages of cells expressing Slan were statistically higher in the peritoneal subset[49]. These differences in adhesion molecules could be associated with a differential pattern of cell-tissue recruitment (endothelium/mesothelium)[27]. Expression of GATA-6 in the three subsets of pMϕ was similar, whereas it was absent in blood monocytes. Nevertheless, we found a high correlation between the increment of GATA-6 and the cell membrane expression of CD14 and CD16; suggesting that monocyte migration to the peritoneal compartment in steady-state is scarce, or that the GATA-6 expression in recently arrived peritoneal monocytes is rapid. The homeostatic state of this cell population was confirmed by the low percentages of cells exhibiting intracellular IL-6, TNF-α, and IL-10 cytokines. Notably, the intermediate subset revealed the highest level of intracellular cytokines, whereas the $CD14^{high}CD16^{high}$ LPM subset presented a higher number of IL-10 positive cells related to the named proinflammatory cytokines, supporting the hypothesis related to its M2 polarization tendency. Eventually, we found a linear relationship between CD14/CD16 cell expression and activation/maturation markers, such as CD206 and HLA-DR, intracellular level of GATA-6, phagocytic/oxidative capacity, and intracellular level of IL-6, TNF-α, and IL-10. These data suggest that the population of LPM $CD14^{high}CD16^{high}$ could act as the phenotypic marker of mature differentiated human-resident pMϕ in homeostasis, whereas the intermediate $CD14^{++}CD16^{+}$ subset could be a transitional cell type also integrated by newly recruited blood monocytes.

CHARACTERISTICS OF HUMAN PERITONEAL MACROPHAGES FROM THE ASCITES OF CIRRHOTIC PATIENTS

In the last decade, our group has also focused on the study of pMϕ characteristics in patients with decompensated cirrhosis and culture-negative ascites. We found that these pMϕ display a preactivated status at baseline, with elevated expression of HLA-DR, CD86 and CD54 membrane markers, increased phosphorylated levels of PKB (Akt), ERK1/2 and c-Jun intracellular signaling molecules, and high secretion of IL-6 [50]. These findings presumably indicate that repeated events of bacterial translocation (BT) promote a sustained immune response, even in the temporary absence of PAMPs. This primed state could enhance an IL-6-regulated fast response to intermittent BT events[50]. Further studies performed *in vitro* with pMϕ from ascites of cirrhotic patients revealed that the secretion of proinflammatory cytokines TNF-α, IL-1β, and IL-6 are regulated by the MAPK signaling intracellular cascades, whereas the PI3K-Akt pathway plays an important role in regulating the anti-inflammatory activity of IL-10[51,52].

The inhibitors of MEK1 and c-Jun N-terminal kinases (JNK) decreased the synthesis of TNF-α, IL-1β and IL-6, and could thus be assayed as therapeutic compounds to reduce hepatic damage associated with liver failure[16,35,52]. Conversely, inhibitors of PI3K-Akt blocked the secretion of IL-10 and augmented the production of IL-1β, mainly by inducing the secretion of intracellular IL-1β and caspase-1 to the extracellular compartment (Figure 2). Based on these results, PI3K-Akt inhibitors are excluded as potential drugs for the treatment of hepatic fibrosis, since these agents may enhance the inflammatory status[51].

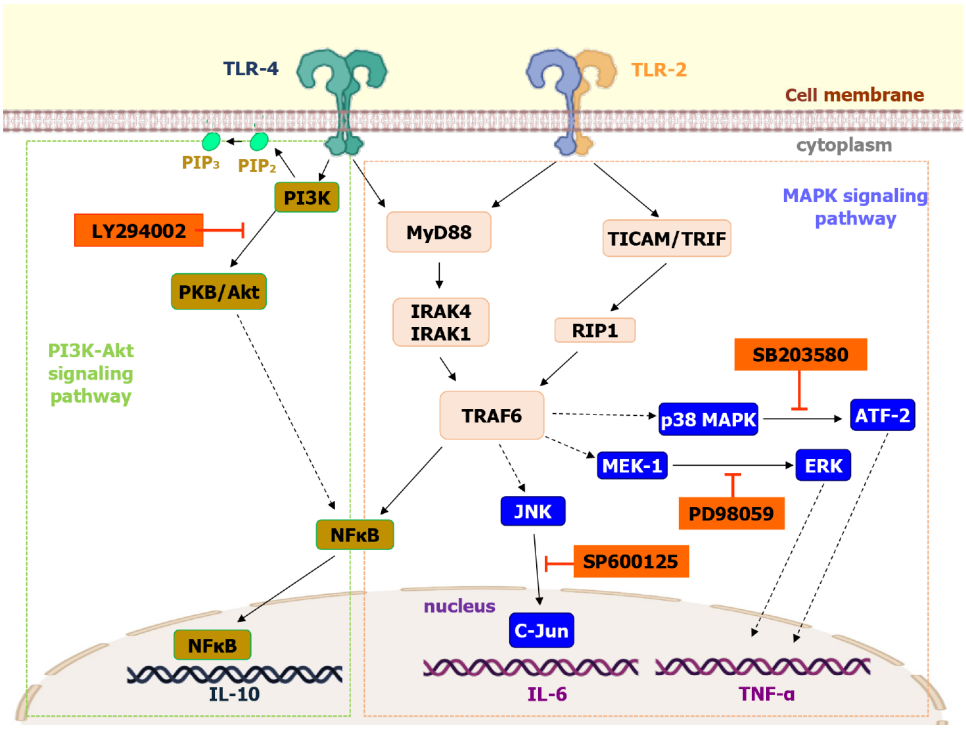

Figure 2 TLR4 and TLR2 cell signaling pathways in normal subjects. TLR2 and TLR4 engagement induce activation of PKB-Akt and MAPK intracellular signaling pathways leading to the phosphorylation of several molecules, which control the expression levels of pro- and anti-inflammatory cytokines. The targets of PD98059, SB203580, SP600125, and LY294002 inhibitors (orange boxes) are indicated by dashed arrows. Adapted from Tapia-Abellán *et al*[51] with permission from John Wiley and Sons, Inc. Citation: Tapia-Abellán A, Ruiz-Alcaraz AJ, Hernández-Caselles T, Such J, Francés R, García-Peñarrubia P, Martínez-Esparza M. Role of MAP kinases and PI3K-Akt on the cytokine inflammatory profile of peritoneal macrophages from the ascites of cirrhotic patients. *Liver Int* 2013; 33: 552-560. Copyright© John Wiley and Sons, Inc.

Peritoneal macrophages from non-infected AF present basal activation of caspase-1 and an increased expression of IL-1β, IL-18, and AIM2 compared to peripheral blood macrophages. The inflammasome activation *in vitro* did not need a priming signal, which supports the preactivated status of these pMϕ[52,53]. As mentioned above, our group reported that a novel $CD14^{high}CD16^{high}$ LPM subpopulation in the AF of cirrhotic subjects is highly sensitive to stimulation with LPS. The $CD14^{++}CD16^{+}$ intermediate subpopulation is augmented in the blood of decompensated cirrhotic patients (from 4% to 11%) and is prevalent in ascites (49%). Baseline hyperactivation of ERK and JNK/c-Jun routes found in ascites pMϕ was associated with cell subsets expressing high levels of CD14/CD16, whereas PI3K/PKB was correlated with CD16 low expressing cells. *In vitro* stimulated pMϕ from ascites of cirrhotic individuals generated statistically higher levels of TNF-α, IL-6, and IL-10, and lower amounts of IL-1β and IL-12 than monocyte-derived macrophages (M-DM) from the blood of controls[44] (Figure 3).

Moreover, Irvine *et al*[54] reported two subsets of pMϕ in AF from decompensated cirrhotic patients: that is, a more phagocytic subset expressing high levels of *VSIG4* (encoding CRIg) and Tim4, and a second less phagocytic subset exhibiting low levels of *VSIG4*, high levels of CCR2a, and responsiveness to retinoic acid. Our unpublished data revealed that these subsets are equivalent to our $CD14^{high}CD16^{high}$ LPMs and $CD14^{++}CD16^{+/-}$, respectively.

More recently, Stengel *et al*[18] have reported that LPMs from the AF of cirrhotic patients present a proinflammatory signature based on the expression of $CD14^{+}$, $CD16^{+}$, $CD206^{+}$, $CD163^{+}$, $MERTK^{+}$, $CD40^{+}$, $CCR2^{-}$, and on *in vitro* transcriptomic analysis and cytokine secretion in the presence and absence of LPS or viable *Escherichia coli* stimulation, respectively. Meanwhile, the corresponding subset of SPMs from AF expresses $CD14^{+}$, $CD16^{+}$, $CD206^{-}$, $CD163^{+}$, $MERTK^{+}$, $CCR2^{+}$. As normal control group, they used macrophages from effluents of CAPD patients with end-stage renal disease, not affected by SBP. These control LPMs displayed a similar phenotype to that of the corresponding subset from cirrhotic patients AF. Interestingly, during SBP episodes, LPMs change to a more inflammatory phenotype characterized by low CD206, low MERTK, and normal CD163 cell surface expression. In particular, LPMs shed surface-bound CD206 as soluble CD206 (sCD206) in response to bacterial peritonitis as well as

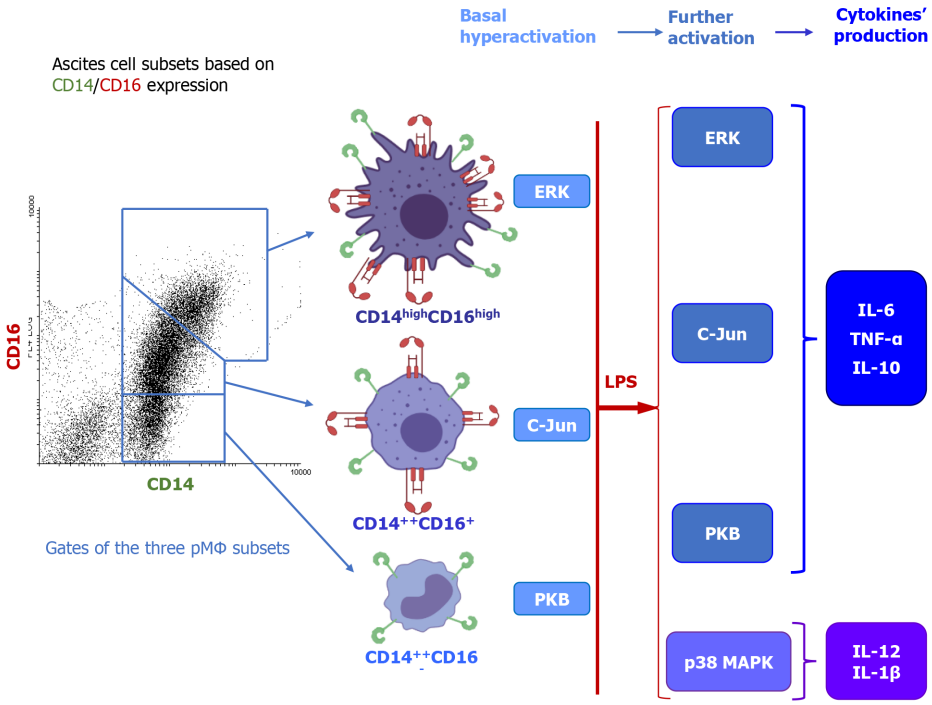

Figure 3 Peritoneal macrophage subsets from cirrhotic patients. The ascitic fluid of cirrhotic patients presents three different subpopulations of peritoneal macrophages based on their cell morphology and CD14/CD16 expression. Baseline hyperactivation of ERK and JNK/c-Jun signaling routes detected in ascites peritoneal macrophage (pMϕ) correlates with CD14/CD16 high expressing subsets, whereas PI3K/PKB correlated with the CD16 low expressing cells. *In vitro* treatment with LPS drastically increases PKB/Akt, ERK1/2, and c-Jun activation, whereas the corresponding p38 MAPK is lowered in pMϕ from ascites cells compared to monocyte-derived macrophages (M-DM) from the control blood. *In vitro* LPS-activated macrophages from cirrhotic ascites also produce statistically higher levels of TNF-α, IL-6, and IL-10, as well as lower levels of IL-1β and IL-12 than the control blood M-DM. Adapted from Ruiz-Alcaraz *et al*[44] with permission from Elsevier. Citation: Ruiz-Alcaraz AJ, Tapia-Abellán A, Fernández-Fernández MD, Tristán-Manzano M, Hernández-Caselles T, Sánchez-Velasco E, Miras-López M, Martínez-Esparza M, García-Peñarrubia P. A novel CD14(high) CD16(high) subset of peritoneal macrophages from cirrhotic patients is associated to an increased response to LPS. *Mol Immunol* 2016; 72: 28-36. Copyright© Elsevier.

in vitro response to LPS and *E. coli*. AF sCD206 is an independent predictor of death in patients with SBP. Concentrations of AF sCD206 of > 0.53 mg/L prognosticate a lower 90-day survival rate. In contrast, the rapid loss of CD206+ LPMs in cirrhotic patients in response to SBP is consistent with a process of macrophage depletion, which could allow for blood monocyte settlement.

Previous studies from episodes of SBP have shown that the ascites from negative-culture SBP and positive-culture SBP patients had significantly more macrophages than those from patients with sterile ascites. Furthermore, pMϕ from positive-culture SBP showed poor bactericidal capacity[55,56] and a tolerant state[57], which is consistent with the hypothesis of systemic inflammation. Moreover, high ascites bacterial burden was associated with reduced pMϕ HLA-DR expression. The presence of pMϕ (CD14+/HLA-DR+) in ascites was associated with a lower number of neutrophils and a tendency towards a lower bacterial burden[58]. Given the scarcity of studies on the role of pMϕ in SBP, new lines of research may be opened in this regard to provide new knowledge about the pathophysiology and potential treatments of liver cirrhosis.

CONCLUSION

These new findings can pave way for several important questions: (1) Are human resident peritoneal macrophages able to migrate through the new described nonvascular route as those cells in mice; (2) Are resident peritoneal macrophages able to migrate to virus or bacterial infected liver; (3) Are human resident peritoneal macrophages able to migrate toward other abdominal organs, such as pancreas, spleen, ovary, or gut; (4) Could omentum comprise a reservoir of mature peritoneal macrophages, ready to move toward other peritoneal organs by detecting danger signs in order to repair tissue damage and maintain health; (5) Are there any differences in GATA-6 expression depending on the type or stage of distinct liver pathologies; (6)

Are there differences in the pattern of cytokine secretion between the three CD14/CD16 cell populations identified in ascites of decompensated cirrhotic patients; (7) Could data of proinflammatory potential of CD206+ LPM in the AF of cirrhotic patients be reproduced in cohorts of cirrhosis from other etiologies; (7) Is AF sCD206 a useful marker to prognosticate mortality risk from decompensated cirrhotic patients; and (8) Could AF sCD206 be used as a useful marker to prognosticate evolution of other peritoneal diseases, such as endometriosis, ovarian cancer, or others?

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