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Review

Peritoneal Immunity in Liver Disease

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Abstract: The peritoneum represents a confined microenvironment that has an emerging role as a distinct immunological compartment. In health, this niche is mainly populated by a heterogenous group of macrophages and T lymphocytes but also Natural Killer cells and B lymphocytes. Together they are crucial for immunological surveillance, clearance of infection and resolution of inflammation. Development of ascites is a defining feature of decompensated liver cirrhosis, and spontaneous bacterial peritonitis is the most frequent bacterial infection occurring in this patient group. Recent studies of ascitic fluid have revealed quantitative, phenotypic and functional differences in both innate and adaptive immune cells compared to the healthy state. This review summarises current knowledge of these alterations and explores how the peritoneum in chronic liver disease is simultaneously an immunologically compromised site and yet capable of provoking an intense inflammatory response. A better understanding of this might enable identification of new therapeutic targets aimed to rebalance the peritoneal immunity and reduce the reliance on antimicrobials in an era of increasing antimicrobial resistance.

Keywords: peritoneum; ascites; cirrhosis-associated immune dysfunction (CAID); spontaneous bacterial peritonitis (SBP)

1. Introduction

The peritoneum is a distinct immunological compartment and of particular interest in chronic liver disease as the development of ascites (the pathological accumulation of fluid in the peritoneal cavity) is a defining feature of decompensated cirrhosis [1]. SBP is infection of this fluid and is the most common bacterial infection in patients with decompensated cirrhosis [2]. It occurs in up to 10% of cirrhotic patients in hospital, and once established it significantly worsens prognosis with the development of multi-organ failure and increased mortality [3]. By definition, SBP occurs in the absence of an overt intra-abdominal source (such as perforated viscus or malignancy) and is therefore characterised by a disparity between the low microbial burden in ascitic fluid and the intensity of the inflammatory response in the peritoneum, indicating a markedly disordered immunity. Patients with cirrhosis in fact have a spectrum of immune alterations termed cirrhosis-associated immune dysfunction (CAID) that are characterised by two key components: high-grade systemic inflammation and immune deficiency [4]. While immune deficiency in CAID is better understood with regard to functional paralysis of circulating and liver-resident immune cells, this cannot necessarily be extrapolated to the distinct immune compartment of the peritoneum.

In this review, we briefly discuss the composition of the peritoneal immune cell population in health and the role of innate and adaptive peritoneal immune cells in response to peritoneal infection. We then focus on each immune cell subset and describe known

quantitative and qualitative immunological changes in cirrhosis and SBP. We also consider the role of pathological bacterial translocation and the ascitic microbiome on altering peritoneal immune cell function. Finally, we discuss the potential therapeutic avenues to manipulate and augment the peritoneal immune response in cirrhosis to improve patient outcomes from SBP.

2. Peritoneum in Healthy and Infectious State

The peritoneum is a serous membrane consisting of a single layer of mesothelial cells over a basement membrane. It covers the abdominal wall and visceral organs, and the cavity it forms is called the peritoneal cavity. Under physiological conditions, the peritoneal cavity contains a small amount of peritoneal fluid (between 20 to 100 mL), which acts to lubricate the visceral organs but also contains a significant number of immune cells essential for maintaining tissue homeostasis and protecting from peritoneal infections and injury.

The immune cell composition of the peritoneal cavity in health has most comprehensively been assessed in peritoneal lavage fluid of patients undergoing laparoscopic procedures for benign gynaecological conditions (e.g., laparoscopic tubal sterilisation) [5–7]. All measures are only of relative abundance but clearly demonstrate that the mix of immune cells in the peritoneum is different to peripheral blood. Monocytes/macrophages account for the majority of peritoneal leukocytes, followed by T lymphocytes, Natural Killer (NK) cells, dendritic cells, B lymphocytes and a small proportion of polymorphonuclear cells (Table 1). While some peritoneal macrophages are considered long-lived resident cells [8], the majority of peritoneal immune cells are mobile and constantly circulate in and out of the peritoneum via small pores in between the mesothelial cells called stomata. These stomata either open to lymphatic vessels and drain to regional lymph nodes or open directly to aggregates of leukocytes in the omentum known as milky spots.

Table 1. Estimated relative abundance of immune cell subsets in the peritoneum in healthy state [5–7].

Cell Type	Relative Abundance (% of All Peritoneal Leukocytes)
Monocyte/macrophages	35–90
T lymphocytes	35–45
NK cells	8–10
Dendritic cells	2–6
B lymphocytes	2
Neutrophils	5–8
Eosinophils	4
Basophils	<1

Intra-abdominal infections are usually secondary to disruption of gastrointestinal tract integrity and result in exposure to polymicrobial insult. The typical response to bacterial infection in the peritoneum (reviewed elsewhere [9]) is broadly similar to the innate and adaptive immune response to bacterial infections in other body compartments. Peritoneal macrophages (and dendritic cells) recognise conserved microbial products through different families of pathogen recognition receptors (PRRs). Pathogens binding to these receptors leads to release of pro-inflammatory cytokines and chemokines (e.g., IL-1B, IL-6, Tumour Necrosis Factor-α, and IL-10) that recruit leukocytes from the milky spots and circulation to the peritoneum. First to arrive in the peritoneal cavity are neutrophils. They are able to phagocytose and kill bacteria, while also releasing further pro-inflammatory cytokines (e.g., IL-8, TNF-α) and extruding neutrophil extracellular traps (NETs). Monocytes are recruited into the peritoneal cavity and differentiate into macrophages. They help eliminate infection by phagocytic killing but also clear apoptotic debris and resolve inflammation. Dendritic cells that have phagocytosed pathogens in the peritoneum may move to lymph

nodes to present processed antigens to T lymphocytes. This leads to the differentiation and proliferation of T helper and cytotoxic T lymphocytes primed to peritoneal antigens.

3. Peritoneal Innate Immunity

3.1. Macrophages

The peritoneal cavity contains a heterogenous population of macrophages that under normal conditions make up 35–90% of all peritoneal leukocytes [5–7]. Two broad subsets of macrophages were first described in the mouse peritoneum. The majority (90%) are large peritoneal macrophages (LPMs), which express high levels of canonical macrophage surface markers CD11b and F4/80. The rest are small peritoneal macrophages (SPMs), which express lower levels of CD11b and F4/80 but high levels of Major Histocompatibility Complex (MHC)-II [10]. In mice, LPMs selectively express the zinc finger transcription factor GATA-binding protein 6 (GATA6) which is essential for their differentiation [11]. LPMs are largely maintained in the peritoneal cavity through self-renewal but are also replenished from the bone marrow. Using a combination of fate mapping techniques, this replenishment has been shown to be sexually dimorphic: high in males and low in females, likely driven by changes in the local peritoneal microenvironment that arise on sexual maturation [12]. LPMs reside in a less activated state but once activated undergo rapid proliferation with co-expression of M1 and M2 markers suggesting roles in tissue repair as well as response to infection. In contrast, SPMs derive from blood monocytes that rapidly enter the peritoneum in a CCR2-dependent manner after lipopolysaccharide (LPS) stimulation and differentiate into SPM where they phagocytose bacteria and make large amounts of nitric oxide [13].

In an elegant experiment using whole-mount immunofluorescence and confocal microscopy of the peritoneal wall in a murine intraperitoneal *Escherichia coli* (*E. Coli*) infection, Ardavín et al. showed one way in which LPMs and SPMs orchestrate defence against infection and promote resolution of infection. At the point of infection, LPMs adhered to the mesothelium, forming multi-layered cellular aggregates within fibrin-dependent immune cell scaffolds. During the resolution of infection, LPMs then promoted blood monocyte recruitment to the aggregates that control inflammation through fibrinolysis-driven disaggregation [14]. However, LPMs have also been implicated in providing a haven for certain bacteria once phagocytosed, such as *Staphylococcus aureus*, and allowing their subsequent dissemination [15].

More recently attempts have been made to characterise the human counterparts of these murine peritoneal macrophages by studying abdominal washouts from women undergoing laparoscopic gynaecological surgery. In general, human peritoneal macrophages express higher levels of phagocytic markers (e.g., CD16, CD11c), cytokine receptors (e.g., CD115 and CD119), antigen-presentation markers (e.g., Human Leukocyte Antigen-DR isotype, HLA-DR) and co-stimulatory molecules (e.g., CD40 and CD80) when compared to blood monocytes, suggesting that peritoneal macrophages are primed for pathogen defence. They can be divided by their CD14/CD16 expression into classic-like CD14^{++}CD16^{-}, intermediate CD14^{++}CD16^{+} and large CD14^{high}CD16^{high} subsets. The CD14^{high}CD16^{high} subset has no corresponding blood counterpart and based on their intracellular content of GATA6, activation/maturation markers like CD206 and HLA-DR and expression of IL-10 are thought to represent mature M2 polarised steady-state human resident peritoneal macrophages [16], akin to murine LPM. However, they are still immunologically primed, with high phagocytic potential to LPS [16]. Furthermore, GATA6 expression does not seem to be as essential for their differentiation [17] as it is for murine LPM. Whether replenishment of human LPM is sexually dimorphic, as it is for murine LPM, is unknown.

In decompensated cirrhosis, similar subsets of peritoneal macrophages based on CD14/CD16 expression have been identified in ascites [18] and display a pre-activated status at baseline [19]. Although some macrophages are pre-activated in the healthy peritoneum [16], the proportion of these peritoneal macrophage subsets alters in cirrhosis with

pre-activated CD14^{high}CD16^{high} cells increasing to 33% in ascites (compared to 15% in health), with a corresponding decrease in the CD14^{++}CD16$^-$ classical subset [18]. Furthermore, peritoneal macrophage activation depends on the aetiology of liver disease with higher inflammatory status in ascitic macrophages of alcohol-related cirrhosis than in Hepatitis C virus related cirrhosis [20]. Despite being pre-activated and able to generate a vigorous respiratory burst, peritoneal macrophages from cirrhotic ascites have reduced phagocytic activity compared to those from healthy peritoneum [21].

Other groups have described different macrophage subsets in the ascitic fluid of patients with decompensated cirrhosis. Irvine et al. segregated human peritoneal macrophages by expression of C-C chemokine receptor 2 (CCR2) and complement receptor for immunoglobulin (CRIg). They found that CRIg^{high}CCR2$^-$ macrophages were larger and showed enhanced phagocytic and anti-microbial activity, compared to CRIg^{low}CCR2$^+$ macrophages [22]. Although it has yet to be shown if CRIg^{high}CCR2$^-$ peritoneal macrophages are present in non-cirrhotic patients, having a higher proportion of CRIg^{high} macrophages in cirrhotic patients was associated with reduced liver disease severity (as measured by MELD scores) [22]. Stengel et al. identified a distinct population of CD206$^+$CCR2$^-$ large peritoneal macrophages in ascitic fluid of patients with cirrhosis and in the dialysate fluid of patients undergoing continuous ambulatory peritoneal dialysis (CAPD), which they used as a control group [23]. Prior studies of CD206$^+$ macrophages in other organ compartments of mice and humans suggested that they are typically anti-inflammatory, immunosuppressive or reparative [24]. In contrast, Stengel et al. found that when activated CD206$^+$ peritoneal macrophages were more likely to produce pro-inflammatory cytokines (e.g., TNF-α) and showed resistance to endotoxin tolerance. Although the CD206$^+$ peritoneal macrophages from cirrhotic ascites and from CAPD dialysate fluid displayed a similar phenotype, in cirrhosis they made up a higher proportion of peritoneal macrophages and released more inflammatory cytokines [23].

In SBP, peritoneal macrophages express lower levels of CD16, CD86, CD11b, CD206 and HLA-DR compared to macrophages from sterile ascites, with corresponding impaired phagocytic function. Treatment of SBP with antibiotics reverted these markers to levels seen on macrophages from sterile ascites and restored (in culture-negative SBP only) their phagocytic function [25]. In keeping with this, a reduction in the proportion of CRIg^{high} peritoneal macrophages (which have enhanced phagocytic and anti-microbial activity) was observed at the time of SBP, but only in a small number of patients [22]. V-set and immunoglobulin containing domain 4 (VSIG4) is membrane protein belonging to the CRIg superfamily. Interestingly, peritoneal macrophages shed VSIG4 during SBP, and the level of soluble VSIG4 in ascites is correlated with disease severity and mortality from SBP. However, soluble VSIG4 does not directly affect peritoneal macrophage lipopolysaccharide (LPS)-induced TNF-α release or phagocytic activity [26]. Other groups have found that during SBP episodes, LPMs change to a more inflammatory phenotype characterised by low CD206, low MER Proto-Oncogne Tyrosine Kinase (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 in vitro in response to LPS and *E. coli*. In fact, ascitic fluid sCD206 is an independent predictor of death in patients with SBP [23]. The rapid loss of CD206$^+$ LPMs in cirrhotic patients in response to SBP is akin to the process of macrophage depletion in murine models of peritonitis [27] and might allow for infiltration of blood monocytes and their differentiation into more inflammatory SPM.

In summary, the majority of peritoneal macrophages display a pre-activated status, even more so in cirrhotic ascites. In cirrhosis, they are prone to a massive release of proinflammatory cytokines upon PRR stimulation with resistance to endotoxin tolerance and yet have impaired phagocytic activity (Table 2). This results in systemic inflammation from spill-over of inflammatory cytokines and organ-failure, without effective bacterial clearance.

Table 2. Summary of changes to innate peritoneal immune cells between health, decompensated cirrhosis and spontaneous bacterial peritonitis (SBP).

Immune Cell	Health	Decompensated Cirrhosis	SBP
Macrophages	3 subsets based on CD14/CD16 expression [16] Immunologically primed with high phagocytic potential to LPS [16] CRIg macrophages with high phagocytic and antimicrobial activity [22]	↑ CD14^{++}CD16^{++} subset [18] that have a pre-activated state [19] ↓ Phagocytic activity [21] ↑ Pro-inflammatory CD206$^+$ large peritoneal macrophage subset [23] ↓ CRIg^{high} macrophages with increased cirrhosis severity [22]	↓ Activation markers and impaired phagocytosis [25] ↓↓ CRIg^{high} macrophages [22] ↓ CD206$^+$ macrophages [23] ↑ sCD206 in ascites, which predicts mortality in SBP [23]
Neutrophils	Rapid influx into peritoneum during infection [28]	↓ Phagocytosis and oxidative burst [29]	↑↑ PMN count ↓ Activation markers (CD69, CD80) [30] ↓ NET generation [30] ↓ Oxidative burst activity [25]
NK cells	Not available	↑ NK cell frequency compared to blood due to migration towards CXCL10 [31]	Mixed activation/deactivation [31]

↑ Increase; ↓ Decrease; ↑↑ Large increase; ↓↓ Large decrease; LPS, lipopolysaccharide; CRIg, complement receptor of the immunoglobulin superfamily; SBP, spontaneous bacterial peritonitis; PMN, polymorphonuclear leukocytes; CXCL10, C-X-C motif chemokine ligand 10; NET, neutrophil extracellular traps.

3.2. Dendritic Cells

Dendritic cells (DC) are a heterogenous population of antigen presenting cells (APC) that regulate the balance between immunity and tolerance, and they can be classical, plasmacytoid or monocyte-derived. In humans, they make up 2–5% of peritoneal leukocytes [6]. Although in mice classical DC seem to predominate in the peritoneum [32], in humans myeloid-derived DC have been identified in peritoneal lavage of patients undergoing laparoscopic surgery [33]. These myeloid-derived DCs were important in response to Gram-positive bacteria and imparted a Th1 profile on T cell responses.

In malignant ascites, plasmacytoid dendritic cells have been identified and induce CD8$^+$ regulatory T cells [34], although there is no association between their presence in ascites and survival in ovarian cancer [35]. To date there have been no studies on DC in ascites of patients with cirrhosis.

3.3. Neutrophils

Neutrophils provide a first line of defence in peritoneal infection by phagocytosis, killing and digestion of bacteria. They are the most abundant immune cell type in the blood, but they do not reside in the peritoneal cavity in large numbers [5]. Rather, in the event of peritonitis they are rapidly recruited [28]. In cirrhosis, ascitic neutrophils show impaired phagocytic activity and oxidative burst activity compared to peripheral neutrophils from the same patient [29]. Although circulating neutrophils in cirrhosis patients are already functionally impaired compared to healthy controls [36,37], this may be exacerbated once they enter the peritoneum by soluble factors in the ascites. In support of this, the observed functional impairment of ascitic neutrophils could be partially restored by incubation with autologous plasma [29]. One such factor could be resistin, which is produced by ascitic neutrophils and in turn down-regulates their inflammatory responses (e.g., impaired NETosis and oxidative burst) [38].

Neutrophil numbers increase in the peritoneum during SBP, and an ascites polymorphonuclear cell (PMN) count of >250/mm^3 is diagnostic of SBP [39]. Even in non-infected ascites, a higher PMN count is associated with a worse prognosis [40]. Furthermore, those who do not have an adequate reduction in their PMN count after antibiotic treatment for SBP have a worse prognosis than those that do [41]. Compared to neutrophils from non-infected ascites, in SBP ascitic neutrophils express lower levels of the activation markers CD69 and CD80, have impaired NET generation [30] and have impaired oxidative burst activity [25]. It is unclear, however, whether this is a consequence of the ongoing bacterial infection or represents part of the pathogenesis for developing the infection in the first place.

3.4. Myeloid-Derived Suppressor Cells

Myeloid-derived suppressor cells (MDSC) represent a heterogenous population of immature myeloid cells, including precursors of macrophages, granulocytes and dendritic cells. There are two major groups: mononuclear MDSC (defined phenotypically as CD33$^+$CD14$^+$HLA-DR$^-$/lowCD15$^-$ cells) and polymorphonuclear MDSC (CD33$^+$ CD14$^-$CD15$^+$ or CD33$^+$CD14$^-$CD66b$^+$ cells) [42]. They suppress immune responses through the production of arginase 1 (Arg1), inducible nitric oxide synthase (iNOS) and generation of reactive oxygen species (ROS) or secretion of IL-10 [43]. Although they are not normally seen in the peritoneum under homeostatic conditions, they accumulate in malignant ascites and are correlated with a worse prognosis [44]. They are attracted by the tumour-associated inflammatory mediator prostaglandin E2 [45] but can also be induced by malignant ascitic fluid, dependent on IL-10 and IL-6 [44].

Their important role in hepatic injury and repair is being increasingly appreciated [46], and the proportion of MDSC in the peripheral circulation is raised in cirrhosis with hepatocellular carcinoma [47] and acute-on-chronic liver failure [48]. However, only one group have evaluated the presence of MDSC in ascites of patients with cirrhosis and found very low proportions (under 1% of all peritoneal leukocytes) with no difference in proportion between patients with and without hepatocellular carcinoma [49].

3.5. NK Cells

Natural killer (NK) cells are an innate-type lymphocyte that classically are associated with anti-viral and anti-tumour immunity. However, NK cells can recognise microorganisms through pattern recognition receptors and exert anti-bacterial activity, either directly through secretion of cytotoxic molecules (e.g., granzymes, granulysins and perforins) or indirectly by interaction with other immune cells (e.g., by secreting TNF-α and Interferon-γ which activates macrophages). In health, a small number of NK cells reside in the human peritoneum [6]. In mice, peritoneal NK cells are proficient pro-inflammatory cytokine producers [50], and in peritonitis there is a rapid influx of mature splenic NK cells in a CCR2-dependent manner [51], but little is known about their human counterparts.

Lutz et al. [31] compared NK cells in the blood, ascites and liver explants of patients with advanced liver disease, with and without SBP. NK cell frequency was increased in uninfected ascites (compared to blood), and ascitic NK cells were predominantly CD16$^+$, expressed more of the inhibitory receptor NKG2A and less of the activating receptor NKG2D. Blood NK cells were shown to migrate towards ascites containing high levels of CXCL10, suggesting the enrichment of CD16$^+$ NK cells in the ascites may be related to their high expression of chemokine receptor CXCR3. During SBP, there was a mixed picture of higher levels of the activation marker CD69 but lower levels of the activating receptor NKG2D. Furthermore, simulation of mononuclear cells with *E. coli* led to downregulation of NKG2D expression and IL-12 and IL-18 mediated secretion of INF-γ by ascitic, but not blood, NK cells. The authors postulated that ascitic NK cells may play a dual role in SBP, being protective in the early phase but then driving excess immune activation in the later phase. Clearly more work is needed to elucidate the phenotypic and functional characteristics of peritoneal NK cells in health and cirrhosis.

4. Peritoneal Adaptive Immunity

4.1. Effector T Cells

The peritoneum in health contains both cytotoxic CD8^{+} T cells and a smaller number of helper CD4^{+} T cells [6]. These T cells can be activated in local lymph nodes and milky spots by DCs (and other APCs) bearing antigens from the peritoneum. During peritonitis, peritoneal T cells exhibit a Th2-like phenotype and produce less pro-inflammatory cytokines (IFN-γ and TNF-α) compared to patients with localised intra-abdominal infections [52]. In peritoneal-dialysis related peritonitis, the CD4/CD8 T cell ratio increases, and the T helper phenotype is also predominantly Th2 [53].

CD4^{+} and CD8^{+} T cells are slightly enriched in the peritoneum of cirrhotic patients compared to blood as a proportion of all leukocytes [54], and there is evidence that they are phenotypically and functionally distinct (Table 3). For example, in patients with decompensated cirrhosis without SBP, ascitic CD8^{+} T cells expressed more HLA-DR and Program Death receptor-1 (PD-1) and had a diminished capacity to induce proliferation of autologous PBMCs than paired peripheral CD8^{+} T cells [55]. Some of these differences might depend on the aetiology of liver disease as patients with alcoholic liver disease displayed lower numbers of CD3^{+}CD4^{+} cells in ascitic fluid than hepatitis C virus positive subjects [56].

Table 3. Summary of changes to adaptive peritoneal immune cells between health, decompensated cirrhosis and spontaneous bacterial peritonitis (SBP).

Immune Cell	Health	Decompensated Cirrhosis	SBP
Effector T cells	CD8^{+} T cells > CD4^{+} T cells [6] Predominantly Th2 response during infection [53]	↑ PD-1 and HLA-DR on CD8^{+} T cells, with reduced capacity to induce PBMC proliferation [55]	↓ [57] or ↑ [54] CD4^{+} T cells ↑ PD-1, CTLA-4 and LAG-3 on CD8^{+} T cells with ↓ cytotoxicity [58]
γδ T cells			↑ Frequency [57]
MAIT cells		Competent MAIT cells present [54]	↑ Frequency competent MAIT cells [54,59]

↑ Increase; ↓ Decrease. SBP, spontaneous bacterial peritonitis; PBMC, peripheral blood mononuclear cells; MAIT, mucosal associated invariant T cells; HLA-DR, human leukocyte antigen–DR isotype; PD-1, programmed cell death protein 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; LAG-3, Lymphocyte-activation gene 3.

During SBP, the peritoneal CD4/CD8 ratio may decrease due to a diminished CD4^{+} cell population, particularly from loss of CD45RA^{+} naïve CD4^{+} T cells [57]. However, others have reported that the peritoneal CD4^{+} T cell count increases during SBP [54]. Yang et al. have recently shown that ascitic CD8^{+} T cells, but not peripheral CD8^{+} T cells, from patients with SBP, have an exhausted phenotype as indicated by higher expression of the immune checkpoints PD-1, Cytotoxic T lymphocyte-associated Antigen 4 (CTLA-4) and Leukocyte Activating Gene-3 (LAG3). The ascitic CD8^{+} T cells exhibited decreased cytotoxic molecule expression in response to target cells and decreased pro-inflammatory cytokine secretion, compared to those from uninfected ascites [58]. Whether this represents adaption in the presence of infection to prevent damage from an over-exuberant pro-inflammatory response or a predisposition to infection in patients with exhausted CD8^{+} T cells remains to be determined.

4.2. T Regulatory Cells

T regulatory (T$_{regs}$) cells are a specific population of suppressor CD4^{+} T cells that terminate normal immune responses and help avoid auto-immune disease. They make up 5–10% of peripheral CD4^{+} T cells, and in the peritoneum they make up 1–2% of all lymphocytes [60]. Their importance in peritoneal immunity has been highlighted by murine studies. Adoptive transfer of in vitro stimulated T$_{regs}$ greatly increased survival

of T$_{reg}$ deficient mice with peritonitis [61]. Importantly, though, T$_{regs}$ are not required for mounting early inflammation in peritonitis in mice but are essential for late recovery and survival from severe sepsis [62]. Related to this is the observation that mice lacking T$_{regs}$ have increased numbers of large peritoneal macrophages with a pro-inflammatory state and simultaneous signs of excessive activation and exhaustion [63].

In cirrhosis, peripheral Tregs are increased in Hepatitis C cirrhosis [64] and in acute-on-chronic liver failure are triggered by hepatitis B reactivation [65]. Only one group has reported on T$_{regs}$ in ascites of cirrhotic patients, finding them to make up 5.7% of all ascitic CD45$^+$ cells, which was less than in the periphery [66]. Their role in malignant ascites is more widely studied, where they are more abundant, highly activated and correlate with worse clinical outcomes [67,68].

4.3. Gamma-Delta T Cells

Human gamma-delta ($\gamma\delta$) T cells represent a unique population of T cells that have characteristics of both innate and adaptive immunity. Unlike conventional $\alpha\beta$ T cells, they are major histocompatibility complex (MHC)-unrestricted and can be activated by T cell receptor (TCR)-dependent (e.g., by phosphorylated antigens) and TCR-independent (e.g., by stress-induced proteins such as MHC class I chain related protein A/B, MICA/B, or Natural Killer Group 2 member D, NKG2D) pathways. They account for 0.5 to 10% of T cells in the peripheral blood but are enriched in different epithelial tissues such as the skin or gut. In mouse models of peritoneal infection, $\gamma\delta$ T cells increase in number [69,70] and are able to discriminate between infecting pathogen types to produce cytokines that direct appropriate T-helper cell responses [71]. In human peritonitis in peritoneal dialysis patients, there is also a rapid increase in $\gamma\delta$ T cells [72].

Although they are found in cirrhotic ascites and their numbers increase in SBP [57], the pathophysiological and prognostic significance of this is yet to be elucidated. In malignant ascites, $\gamma\delta$ T cells are abundant, clonally expanded (suggesting activation in a TCR-dependent manner), highly functional and associated with better clinical outcomes. However, they also express high levels of inhibitory receptors (e.g., T cell immunoreceptor with Ig and ITIM domain [TIGIT] and T cell Ig and mucin domain-3 [TIM-3]) compared to intra-tumoural $\gamma\delta$ T cells, suggesting they could become immunosuppressive over time [73,74].

4.4. MAIT Cells

Mucosal-associated invariant T (MAIT) cells are an innate-like T cell subset, defined by the co-expression of the semi-invariant T-cell receptor Vα7.2 chain and CD161. MAIT cells are present in peripheral blood, enriched in mucosal tissues and highly abundant in the liver, with critical roles in the response to bacterial infections. They are able to recognise riboflavin metabolites of bacterial or fungal origin presented by the MHC I-like molecule MR1 on APCs and following activation can release pro-inflammatory cytokines (TNF-α, INF-γ and IL-17) and lyse target cells.

Peripheral MAIT cells are depleted and exhausted in end-stage liver disease [75], but two groups have recently shown they are increased in ascites and remain highly functional. Niehaus et al. [54] compared MAIT cells in the ascites and periphery of patients with decompensated liver disease and found that MAIT cells were significantly increased in ascites. The peritoneal MAIT cells displayed an activated phenotype and increased functional responses following stimulation with *E. Coli* or IL-12 and IL-18. During SBP, peritoneal MAIT cell frequency increased most of all immune cell subsets, suggesting active homing of MAIT cells to the site of infection. This finding was corroborated by Ibidapo-obe et al. [59] who went on to show that MAIT cells expressed high levels of the chemokine receptors CCR6, CCR5 and CXCR3, enabling them to migrate along gradients of CCL20, CCL5 and CXCL10. The ascitic fluid in SBP contained higher levels of these chemokines compared with uninfected ascitic fluid and induced preferential migration

of blood-derived MAIT cells in transwell assays, suggesting a mechanism for MAIT cell redistribution into the peritoneum during SBP.

4.5. Natural Killer T Cells

Natural Killer T (NKT) cells are another type of innate-like T cells that express semi-invariant αβ TCR. They recognise self and foreign glycolipids presented in complex with CD1d, an MHC I-like molecule. Like MAIT cells, following activation, NKT cells produce a broad range of cytokines and play protective roles against microbial infections and cancers [76].

One group have shown that in patients with ovarian cancer and ascites, NKT cells are enriched in the peritoneum compared to the periphery [66]. However, these results need to be interpreted with caution as they identified NKT cells simply as $CD3^+CD56^+$ cells, which is not specific as identifying them by their expression of the canonical Vα24 TCR [77]. Despite potentially higher numbers of NKT cells, it seems that malignant ascites inhibit the ability of CD1d expressing cells to stimulate/activate NKT cells [78].

NKT cells are present in cirrhotic ascites and make up around 1% of all peritoneal lymphocytes. This is a lower proportion than NKT cells in periphery of the same patients. The aetiology of liver disease also relevant, as the proportion of NKT in ascites of patients with alcohol-related liver disease is lower than those with cirrhosis associated with hepatitis C virus infection [56]. Further studies looking at the function of NKT cells in cirrhotic ascites and their role in SBP are needed.

4.6. B Cells

B cells are divided into two major subsets. Conventional B2 cells generate specific antibodies against foreign antigens in secondary lymphoid organs. In contrast, B1 cells spontaneously secrete natural antibodies against mainly non-protein antigens, providing immediate defence to counteract microbial infection. B1 cells are found predominantly in the peritoneal and pleural cavities and consist of around 2% of all peritoneal leukocytes [5]. Although the phenotype and function of peritoneal B cells are well studied in mice [79] they are less well characterised in humans. In patients with ovarian cancer and ascites, IL-10 producing B cells are observed in the ascites [80], and those with a high frequency of B cells had a worse prognosis [81]. In cirrhosis, other than being identified at very low numbers in ascites [22], there have been no detailed investigations into peritoneal B cells.

5. Peritoneal Soluble Immune Factors

The complement system, natural antibodies, acute phase proteins, cytokines/chemokines and antimicrobial peptides all play a role in peritoneal immunity [9]. Historically it has been appreciated that non-cirrhotic ascites (e.g., in congestive cardiac failure or renal failure) have anti-bacterial activity similar to normal peritoneal fluid, whereas cirrhotic ascitic fluid has a marked reduction of both bactericidal and opsonic activities [82]. This seems to be due to a deficiency in particular complement proteins (e.g., C3 and C4) in cirrhotic ascites [83], although this could be simply secondary to a dilution effect from large ascites volumes. Lower levels of C3 and the lectin-pathway molecule ficolin-1 in ascites have been shown to be risk factors for all-cause mortality or liver transplantation independently of liver function in patients with cirrhosis and ascites [84].

With regard to the cytokine profile in ascites, both pro- and anti-inflammatory cytokines are increased, illustrating the complexity of the peritoneal immune environment. The pleotropic pro-inflammatory cytokine IL-6 is consistently reported to be higher in the ascites of cirrhotic patients than in paired serum, although its level does not correlate with liver disease severity [85,86]. The anti-inflammatory cytokine IL-10 is significantly elevated in ascites of cirrhotic patients compared to plasma, whereas there was no difference in IL-10 levels between patient and control plasma [87].

In SBP, these differences are exaggerated with higher IL-6 [88,89] and IL-10 [90] in ascites of patients with cirrhosis and SBP than in uninfected ascites (Figure 1). The level

of TNF-α is also elevated in ascites with SBP and, along with IL-6, was found at higher levels in those with SBP who developed renal and hepatic impairment [88,89]. It could be postulated that levels of the anti-inflammatory IL-10 increase during SBP as a compensatory mechanism to limit immune-pathology and consequences of hemodynamic perturbations (e.g., renal failure). In keeping with this, the level of ascitic IL-10 in SBP falls after antibiotic treatment, and low levels on day 7 of treatment are associated with improved patient survival [90].
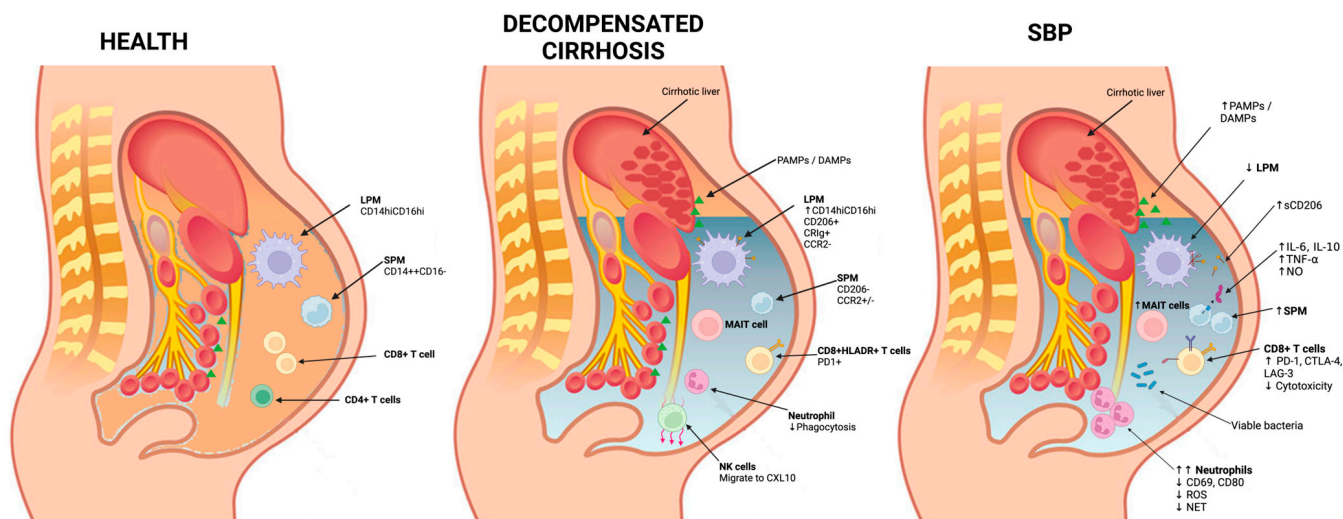
Figure 1. Changes in the peritoneal immunity between health, decompensated cirrhosis and spontaneous bacterial peritonitis (SBP). In health, peritoneal immune surveillance is dominated by peritoneal macrophages (PM), which can be divided into LPM (CD14^{hi}CD16^{hi}CRIg$^+$CCR2$^-$) and SPM (CD14^{++}CD16$^-$). LPMs are immunologically primed, with high phagocytic potential to LPS. A small number of T cells, NK cells and neutrophils circulate in and out of the peritoneum. In decompensated cirrhosis, ascites is enriched with LPM that are CD206$^+$, more likely to produce pro-inflammatory cytokines (e.g., TNF-α) and show resistance to endotoxin tolerance but have impaired phagocytic function. Immunosuppressive subsets of ascitic T cells have been identified (e.g., CD8$^+$HLA-DR T cells expressing PD-1), but MAIT cells remain immunocompetent. In SBP, activation of PM leads to loss of surface CD206 on LPM and the release of soluble CD206 in ascites. LPM numbers in the ascites are reduced in early SBP but recover after treatment. Neutrophils rapidly infiltrate the peritoneum in SBP but have impaired NET generation and oxidative burst capacity. (LPM, large peritoneal macrophages; SPM, small peritoneal macrophages; PAMP; pathogen associated molecular patterns; DAMP, damage associated molecular patterns; CRIg, complement receptor of the immunoglobulin superfamily; CCR2, C-C-motif chemokine receptor 2; HLA-DR, human leukocyte antigen—DR isotype; PD-1, programmed cell death protein 1; CXCL10, C-X-C motif chemokine ligand 10; IL-6, interleukin-6; IL-10, interleukin-10; TNF-α, tumour necrosis factor-α; NO, nitric oxide; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; LAG-3, Lymphocyte-activation gene 3; ROS, reactive oxygen species; NET, neutrophil extracellular traps.).

Levels of the hematopoietic growth factor IL-7 were significantly lower in the ascites of cirrhotic patients complicated with SBP than in uninfected ascites. Furthermore, recombinant IL-7 stimulation promoted the proliferation of CD4$^+$ and CD8$^+$ T cells from ascites and results in elevated cytolytic (increased target cell death) and non-cytolytic (INF-γ production) activity of ascitic CD8$^+$ T cells [91]. The same group reported higher levels of IL-35 in ascites with SBP [58]. IL-35 is released by T$_{regs}$ and is known to suppress effector CD4$^+$ and CD8$^+$ T cell function [92]. In vitro, recombinant human IL-35 stimulation suppressed the cytotoxicity of ascitic CD8$^+$ T cells from cirrhotic patients [58].

6. The Impact of Bacterial Translocation on Peritoneal Immunity

SBP occurs almost exclusively in patients with cirrhotic ascites, rather than in those with other causes of ascites. The classical pathogenic theory of SBP is that portal hypertension promotes intestinal bacteria to translocate across the intestinal wall, reach mesenteric lymph nodes and then obtain access to ascitic fluid [93]. Classical culture techniques fail to identify the causative bacteria in more than 23% of neutrocytic ascites cases [94], which hampers targeted antibiotic choice. More recently, PCR amplification of bacterial 16S ribosomal DNA, with or without subsequent sequencing of the amplified DNA, has been applied to ascites in a bid to increase the detection rate of ascitic bacteria in SBP [95]. Several groups are even now attempting to develop very rapid [96] or even bedside [97] tests for SBP based on 16S PCR technology.

One of the major factors limiting the specificity of these efforts is that even in culture-negative non-neutrocytic ascitic fluid, bacterial DNA can be detected in over 30% of patients [98]. The methods used do not normally distinguish viable bacteria from free bacterial DNA, but nevertheless the presence [99] and amount [100] of bacterial DNA in non-neutrocytic ascites are correlated negatively with patient survival. This finding was challenged by other groups, who found that bacterial DNA in sterile body fluids did not indicate increased mortality in cirrhotic patients with suspected infection [101]. The role of the composition of the microbiome in the ascites is less clear. There is high divergence in bacterial species present in different patients, but there is some association between microbiota similarity and ascitic PMN count and liver disease severity (measured by Child Pugh class) [102].

While the association between bacterial DNA levels in ascites and patient outcomes may just reflect the link between liver disease severity, portal hypertension and bacterial translocation, there is some evidence that bacterial DNA can directly influence the peritoneal immune environment. Bacterial DNA activates immune cells through toll-like receptor 9 (TLR-9) which induces the release of nitric oxide (NO), and it has been observed that the presence of bacterial DNA in ascites is associated with increased levels of NO [103,104]. NO is of clinical relevance as it leads to splanchnic vasodilation and increases the risk of renal dysfunction in SBP [105].

Ascitic macrophages appear to be the main source of this NO as macrophages from ascites with detectable bacterial DNA are primed to synthesise significantly higher amounts of NO than those from ascites without detectable bacterial DNA [106]. This priming of ascitic macrophages by bacterial DNA is mediated via pre-activation of the Absent In Melanoma 2 (AIM2) inflammasome [107]. However, others have shown that levels of the activation marker HLA-DR on ascitic macrophages were inversely correlated with bacterial DNA burden [108], once again indicating the complexity peritoneal macrophage phenotype in cirrhosis.

7. Therapeutic Approaches to Improve Peritoneal Immunity in Chronic Liver Disease

SBP is common, affecting up to 10% of hospitalised patients with decompensated cirrhosis, and associated with a high mortality rate. The mortality during first hospitalisation is 20% [109], and one-year mortality is up to 66% [110]. Compounding the problem is the rapid emergence of multi-drug resistance microorganisms causing infections in patients with cirrhosis [111]; hence, strategies to improve the peritoneal immune response without relying on anti-microbials are needed. Therapeutic approaches must strike a careful balance between augmenting anti-bacterial responses and controlling damaging systemic inflammation. Multiple strategies to modulate CAID in the peripheral immune compartment, by targeting circulating humoral factors, immunometabolism and immune cell signalling, are in experimental or clinical trial stages [4]. Given the interplay between the systemic and tissue immune compartments, it is likely that these will impact the peritoneal immunity as well.

Targeting bacterial translocation will not only tackle the presumed pathogenic path for infecting bacteria in SBP but hopefully reduce immune cell priming in the peritoneum. The poorly absorbed antibiotic norfloxacin has been shown to reduce recurrence of SBP [112] and is now standard care for patients at high-risk of SBP or with a previous episode of SBP [1]. Interestingly, norfloxacin can have direct effects on immune cells, separate to its antibacterial effects. The peripheral T regulatory cell population increases in patients with cirrhosis taking norfloxacin for SBP prophylaxis, and dendritic cells express less CD80 and CD86 co-stimulatory molecules [113]. Norfloxacin is also associated with peripheral neutrophil function (as measured by ROS production) in cirrhotic patients [114]. Whether these changes are recapitulated in peritoneal immune cells requires further study.

Rifaximin has been shown to modulate the intestinal microbiome [115] and could be an alternative as secondary prevention for SBP [116] but is yet to be recommended by international guidelines [1]. Another antibiotic, co-trimoxazole, is the subject of an ongoing clinical trial (Primary Antibiotic Prophylaxis Using Co-trimoxazole to Prevent SBP in Cirrhosis, ASEPTIC, NCT04395365). Long term antibiotic use can, however, drive the development of antibiotic-resistance microorganisms. The intermittent use of ciprofloxacin, for example, has been associated with a higher rate of quinolone-resistant organisms [117].

8. Conclusions

The peritoneal immunity in decompensated cirrhosis is markedly disordered compared to that in health. Innate immune cells are characterised by high expression of activation markers and release of pro-inflammatory cytokines, while having impaired anti-bacterial effector functions. In the adaptive arm, populations of immunosuppressive T cells have been identified, while other cells (e.g., MAIT cells) remain immunocompetent. Patients with decompensated cirrhosis are highly susceptibility to infection in the peritoneal compartment (i.e., SBP), which occurs in the face of low bacterial burden and results in systemic inflammation and organ-failure, without effective bacterial clearance.

Further work is required to fully understand the role of each immune cell subset to the peritoneal immunity in decompensated cirrhosis, and healthy controls should be included in these studies to ensure differences can be attributed as unique to cirrhosis. Through this, strategies to improve the peritoneal immune response that do not rely on anti-microbials could be identified.

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