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LIVER TRANSPLANTATION

IS THE USE OF MACHINE PERFUSION ASSOCIATED WITH A REDUCED RISK OF GRAFT REJECTION AFTER LIVER TRANSPLANTATION?

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Summary

Emerging, albeit controversial, evidence indicates that machine perfusion (MP) is associated with a lower risk of acute graft rejection following adult liver transplantation (LT). This research paper examines how MP affects the immunogenicity of liver grafts and the recipient's immune response, potentially leading to reduced rejection rates. Based on experimental data, preserving the functional phenotype of liver sinusoidal endothelial cells (LSECs) is vital for alloantigen-specific immune responses and tolerance mechanisms. LSECs function as critical regulators of immune responses and utilize unique mechanisms to interact with passenger lymphocytes, provide instructional programming signals, and activate local effector functions of T cells. Despite this mechanistic rationale, the influence of dynamic perfusion on the risk of post-LT graft rejection is overshadowed by the heightened sensitivity of cholangiocytes and hepatocytes to ischemia-reperfusion injury, as well as the intricate orchestration of immune-reactive cell populations within the liver microenvironment. Furthermore, the available evidence stems from studies with insufficient power and limited participant numbers, exhibiting considerable variability in designs, methods, and treatments. Additionally, the clinical significance of the MP-associated reduction in liver graft immunogenicity may remain unnoticed due to the widespread use of tacrolimus-based immunosuppressive regimens and the resulting trend toward minimizing immunosuppression.

Key words: liver transplantation, machine perfusion, rejection, tolerance, outcome

Abbreviations

ACR: acute cellular rejection
ATP: adenosine triphosphate
APC: antigen-presenting cell
cDCD: controlled DCD
CINC: cytokine-induced neutrophil chemoattractant-1

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CIT: cold ischemia time
CRP: C-reactive protein
DAMPs: damage-associated molecular patterns
DBD: donors after brain death
DCD: donors after circulatory death
DGF: delayed graft function
D-HOPE: dual HOPE
EAD: early allograft dysfunction
ECD: extended criteria donors
ENA-78: epithelial neutrophil-activating protein-78
ET: endothelin
FMN: flavin mononucleotide
fWIT: functional warm ischemia time
GM-CSF: granulocyte-macrophage colony-stimulating factor
GRADE: grading of recommendations assessment, development, and evaluation
HBV: hepatitis B virus
HMP: hypothermic machine perfusion
HGMB-1: high mobility group box-protein 1
HOPE: hypothermic oxygenated machine perfusion
IC: ischemic cholangiopathy
ICAM-1: intracellular adhesion molecule-1
IFN-γ: interferon-gamma
IL-1: interleukin-1
IL-6: interleukin-6
IL-8: interleukin-8
IL-12: interleukin-12
IRI: ischemia-reperfusion injury
KC: Kupffer cell
LFA-1: lymphocyte function-associated antigen-1
LSEC: liver sinusoidal endothelial cells
LT: liver transplantation
Mac-1: macrophage-1 antigen
MAIT: mucosa-associated invariant T cell
MIP-2: macrophage inflammatory protein-2
MMF: mycophenolate mofetil
MP: machine perfusion
NADH: nicotinamide adenine dinucleotide-H
NK: natural killer cell
NMP: normothermic machine perfusion
NO: nitric oxide
NRP: normothermic regional perfusion
PAF: platelet-activating factor
PAMPs: pathogen-associated molecular patterns
PD1: programmed death-1
PNF: primary non-function
ROS: reactive oxygen species
SCS: static cold storage
SRR: super-rapid recovery
TAC: tacrolimus
TCMR: T-cell-mediated acute rejection
TCR: T-cell receptor
TGF-β, transforming growth factor-beta
TLR: toll-like receptor
TNF-α: tumor necrosis factor-alpha
TNF-β: tumor-necrosis factor-beta
TXA_2: thromboxane A_2
uDCD: uncontrolled DCD
WIT: warm ischemia time

INTRODUCTION

Liver transplantation (LT) is one of the most complex procedures in modern medicine, providing a life-saving solution for patients with end-stage liver disease [1]. However, this process presents significant challenges, especially in preserving donor organs, preventing transplant-related complications, managing the recurrence of the original disease, and reducing the risk of adverse events associated with immunosuppression (IS) [1]. Recent advancements in organ preservation, particularly machine perfusion (MP) techniques, have emerged as promising alternatives to traditional static cold storage (SCS) methods, with the potential to lessen ischemia-reperfusion injury (IRI) and lower the risk of graft dysfunction [2-4] (Fig. 1). Yet, the potential of MP to decrease the risk of allograft rejection is still not fully understood. This research paper examines whether MP affects the immunogenicity of liver grafts and the recipient's immune response in a manner that reduces rejection rates. Specifically, it examines hypothermic (HMP) and normothermic machine perfusion (NMP) in adult LT involving grafts from brain-dead donors (DBD) or donors after circulatory death (DCD). It also investigates the possible immunological mechanisms behind these effects, such as the modulation of the liver sinusoidal endothelial cell (LSEC) phenotype, the upregulation of a beneficial cytokine profile, the expansion of regulatory T-cell activity, and the reduction of damage-associated molecular patterns (DAMPs). The urgency of this investigation is heightened by the rising demand for donor livers and the reliance on marginal grafts, including those from extended criteria donors (ECDs) and DCDs. These grafts, characterized by their increased vulnerability to IRI due to factors like advanced donor age, steatosis, or circulatory death, underscore the necessity to optimize preservation methods [5]. While traditional organ preservation techniques are limited in mitigating IRI, MP provides a dynamic preservation strategy, offering both therapeutic potential and functional assessment of organs before transplantation [5,6]. Therefore, MP addresses the need for enhanced preservation methods and has implications for alleviating the organ shortage by increasing the viability of marginal grafts [2-6]

The paper synthesizes evidence from the existing literature to provide a comprehensive overview of MP's role in influencing graft rejection rates. It also identifies gaps

in current knowledge and suggests directions for future investigations, particularly emphasizing the need to incorporate immunologic studies into research on MP.

HISTORICAL PERSPECTIVE

The clinical impact of acute rejection

Although most cases of early acute cellular rejection (ACR) have a limited impact on the long-term survival of adult liver graft recipients and can be reversed with increased immunosuppression (IS), several risk factors have been identified, including younger recipient age, HLA-DR mismatch, longer cold ischemic time (CIT), and older donor age; however, their predictive ability varies across studies [7-9]. The liver possesses unique immunobiological traits, as it contains numerous resident immune cells, which foster a pro-tolerogenic environment while maintaining effective immune surveillance against infections and cancer cells [10,11]. This enables the transplanted liver to both shield itself from the host immune response and decrease alloreactivity against other organs transplanted simultaneously [11].

T-cell-mediated acute cellular rejection (TCMR) is the most common form of allograft rejection, mainly occurring within the first three months after liver transplantation (LT) [10-12]. The incidence of TCMR varies from 10% to 30% in studies with clinically driven biopsies and can reach up to 64% in cohorts with protocol liver biopsies [13]. Late rejection episodes are usually associated with poor adherence to immunosuppressive medications [11]. The occurrence and severity of TCMR have significantly declined in recent decades due to the development of more effective immunosuppressive drugs [10-13]. Most TCMR cases are histologically mild, and alloimmune-mediated graft loss is uncommon [10]. However, monitoring late episodes of TCMR is essential, as they tend to be more severe and less responsive to high doses of steroids [14].

Static cold storage-associated events

While SCS is the traditional method for preserving liver grafts before transplantation, it has several limitations that can increase the risk of graft dysfunction and rejection [15] (Fig. 1). The basis of this preservation technique is to induce hypothermic conditions that reduce cellular activity, theoretically decreasing the consumption of mitochondrial ATP substrate stores while increasing anaerobic metabolism [15]. Although SCS slows metabolic processes and reduces the risk of ischemic injury during the liver's period without a blood supply, it introduces various physiological challenges that can affect graft quality and increase the risk of rejection [15,16]. The most significant risk associated with SCS is IRI (Fig. 1). IRI results from

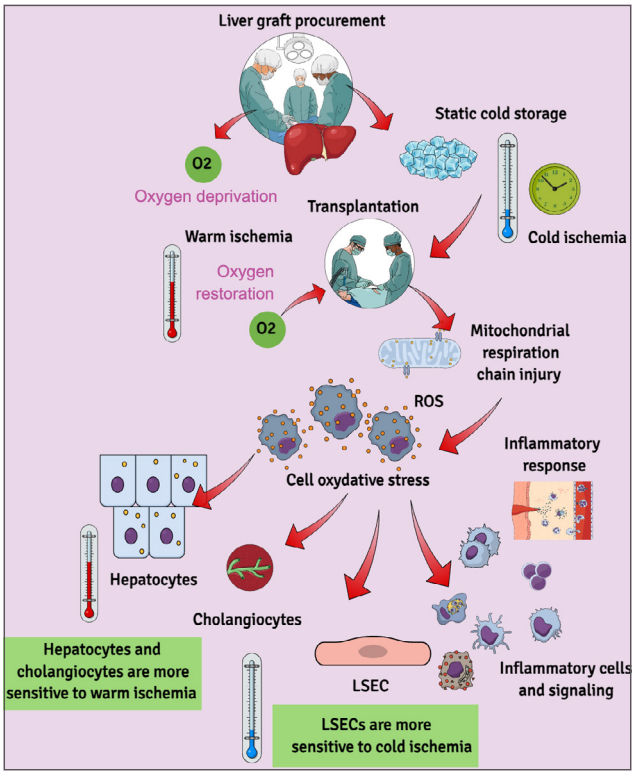

Figure 1. Schematic representation of the cascade of ischemia-reperfusion injury (IRI) events in liver transplantation. The process of liver graft procurement and transplantation involves oxygen deprivation under hypothermic (cold ischemia) and normothermic (warm ischemia) conditions, followed by its restoration. Oxygen deprivation causes damage to the mitochondrial respiration chain and saturation of its reducing capacity. After oxygen restoration post-graft implantation, reactive oxygen species (ROS) are produced both within and outside mitochondria, spreading through intracellular and extracellular spaces, which contributes to cellular dysfunction and cell death. Damage-associated molecular patterns (DAMPs) released from injured cells have strong inflammatory properties, attracting innate and adaptive immune cells and creating a pro-inflammatory environment affecting various liver cell types: hepatocytes, cholangiocytes, and sinusoidal endothelium. The intensity of inflammatory responses depends on the type of ischemia (cold versus warm), its duration, the methods of restoration, and the biological features of the liver graft (such as age, steatosis, fibrosis), leading to different clinical outcomes. Liver cells exhibit varying levels of sensitivity to ischemia-reperfusion injury (IRI), with hepatocytes and cholangiocytes being more susceptible to warm ischemia than liver sinusoidal endothelial cells (LSEC). The activation of the innate immune response triggered by IRI initiates adaptive (antigen-specific) immune reactions, which may explain the higher rates of post-transplant acute rejection seen in some clinical studies. LSEC: liver sinusoidal endothelial cell; ROS: reactive oxygen species.

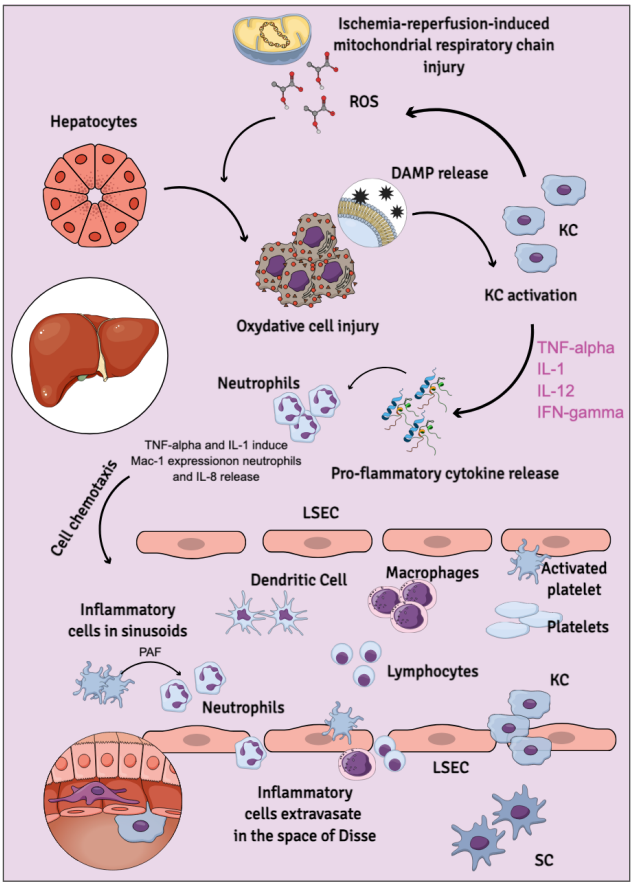

Figure 2. Schematic depiction of the inflammatory process driven by ischemia-reperfusion injury (IRI) within liver grafts. O2 deprivation causes mitochondrial injury, leading to the release of reactive oxygen species (ROS) in liver cells. Damage-associated molecular patterns (DAMPs) are released due to the toxic effects of ROS on cell organelles and structures. DAMPs activate Kupffer cells (KC), which in turn increase the release of ROS and pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), interferon-gamma (IFN-γ), and interleukin-12 (IL-12). TNF-α and IL-1 enhance the expression of Mac-1 (CD11b/CD18) adhesion proteins on neutrophils and stimulate IL-8 production, promoting neutrophil chemotaxis within the liver sinusoids. The TNF-α-driven expression of intracellular adhesion molecule-1 (ICAM-1) on the intraluminal side of liver sinusoidal endothelial cells (LSEC) facilitates neutrophil and inflammatory cell rolling, binding, and extravasation in the Space of Disse and hepatic plates. Additionally, IL-1 and TNF-α recruit and activate CD4+ T (Th1) lymphocytes, which interact with antigen-presenting cells (APCs), including inflammatory dendritic cells (DCs) and LSECs, promoting MHC-II and MHC-I-restricted antigen-specific immune responses under stimulation of INF-γ, IL-2, and TNF-β. Platelets play a vital role in IRI by releasing platelet-activating factor (PAF), cytokines, and growth factors. PAF primes neutrophils for ROS production, further amplifying the neutrophil response. Activated platelets adhere to LSEC and induce their programmed cell death. DAMP: damage-associated molecular pattern; IFN: interferon; IL: interleukin; KC: macrophage-1 antigen; Kupffer cell; PAF: platelet-activating factor; ROS: reactive oxygen species; SC: stellate cells. TNF: tumor necrosis factor.

initial oxygen deprivation followed by the restoration of normal oxygenated blood flow to the liver and involves the dysregulation of the healthy phenotype of all liver cellular components, leading to substantial cellular damage, inflammation, and oxidative stress, which may heighten the risk of ACR (Fig. 1) [16,17]. Although the liver's microenvironment is normally hypoxic, it is particularly vulnerable to IRI due to its complex vascular structure and high metabolic demands [17]. IRI can cause hepatocyte and cholangiocyte injury, endothelial dysfunction, and activation of the inflammation/innate immune system, shifting to a pro-inflammatory, pro-thrombotic, pro-apoptotic, and vasoconstrictive phenotype, thereby increasing the risk of early graft dysfunction, delayed graft function (DGF), and rejection (Figs. 1,2) [16]. DGF may lead to prolonged inflammation and innate immune activation, further increasing the risk of ACR or cholestasis [16-18].

Hepatocyte injury

Most initial changes in anoxic hepatocytes occur within the mitochondria (Figs. 1,2) [19]. The absence of O_2 immediately disrupts electron flow, causing the respiratory chain to become reduced. As mitochondria can no longer accept electrons from substrates, there is a decline in pyridine nucleotides, leading to an increased intracellular NADH/NAD+ ratio. The disruption of oxidative phosphorylation leads to cellular ATP depletion, accelerated glycolysis, increased lactate production, and alterations in ion balance (H+, Na+, and Ca2+), all of which severely harm hepatocytes. Reperfusion injury primarily results from the generation of toxic reactive oxygen species (ROS) when oxygen is reintroduced to ischemic tissues. ROS are produced from both intracellular and extracellular sources, with mitochondria being the primary source in liver cells [19]. In turn, ROS and toxins accumulated in mitochondria during oxygen deprivation are released upon reoxygenation, leading to the breakdown of cellular organelles and the production of DAMPs, such as high-mobility group box-1 protein (HMGB-1). These DAMPs promote inflammation by activating antigen-presenting cells (APCs), including dendritic cells, resident immune cells, and Kupffer cells (KC) [19].

IRI-INDUCED DYSREGULATION OF THE LIVER SINUSOIDAL MICROENVIRONMENT

Liver sinusoidal endothelial cells (LSEC) form the lining of the hepatic sinusoids, lacking an organized basal membrane, with their cytoplasm penetrated by open fenestrations, leading to a discontinuous hepatic microvascular endothelium (Figs. 2,3) [20]. LSEC plays a vital role in protecting

and regulating vascular homeostasis, inflammation, vascular tone, toxic clearance, and immune response [16,20]. LSECs are particularly susceptible to IRI and experience notable changes during cold storage, including disruptions in plasma membranes, vacuolization of the nuclear membrane, and rounding of cell shape [16]. These changes contribute to impaired graft microcirculation, platelet activation, persistent vasoconstriction, increased expression of adhesion molecules, oxidative stress, activation of KCs, neutrophil infiltration, and hepatocyte death (Tab. I) [16,21]. Several mechanisms contribute to the disruption of the sinusoidal microenvironment during IRI (Tab. I) [16]. The shortage of energetic substrates, combined with an imbalance between low nitric oxide (NO) bioavailability and increased production of endothelin (ET) and thromboxane A2 (TXA2), causes the narrowing of the sinusoidal lumen, leading to microcirculatory dysfunction. The decreased NO levels in the liver result from both reduced production and increased scavenging by higher levels of ROS, which ultimately worsen IRI injury by affecting neutrophil adhesion, platelet aggregation, and contraction of hepatic stellate cells (HSC) [16]. Interestingly, the absence of biomechanical stimuli during SCS has been shown to deteriorate LSEC protective phenotype by downregulating the expression of the transcription factor Kruppel-like Factor 2 (KLF2), which regulates the transcription of a variety of protective genes including the endothelial synthase of NO (eNOS), the anti-thrombotic molecule thrombomodulin, or the antioxidant transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) [16].

Activation of inflammation/innate immune response

DAMPs released by hepatic cells trigger the activation of KCs [22,23] (Tabs. I,II, Fig. 2). In turn, activated KCs significantly increase their release of ROS and pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), interferon-γ (IFN-γ), and interleukin-12 (IL-12) [24,25]. Both TNF-α and IL-1 enhance the expression of Mac-1 (CD11b/CD18) adhesion proteins on neutrophils and stimulate IL-8 production, further promoting neutrophil chemotaxis within the parenchyma [26]. IL-1 may stimulate ROS release from neutrophils, which in turn increases TNF-α synthesis by KCs [27]. TNF-α promotes the expression of intracellular adhesion molecule-1 (ICAM-1) on the intraluminal side of LSEC, aiding neutrophil rolling, binding, and parenchymal extravasation [27]. Additionally, TNF-α induces P-selectin expression in LSEC, which is essential for neutrophil recruitment [28]. It has been shown that TNF-α enhances the release of several molecules, including interleukin-6 (IL-6), macrophage inflammatory protein-2 (MIP-2), epithelial neutrophil-activating protein-78 (ENA-78), cytokine-induced neutrophil chemoattractant-1 (CINC), and various CXC motif chemokines (including CXL-1, -2, and -3) [16]. Furthermore, IL-1 and TNF-α

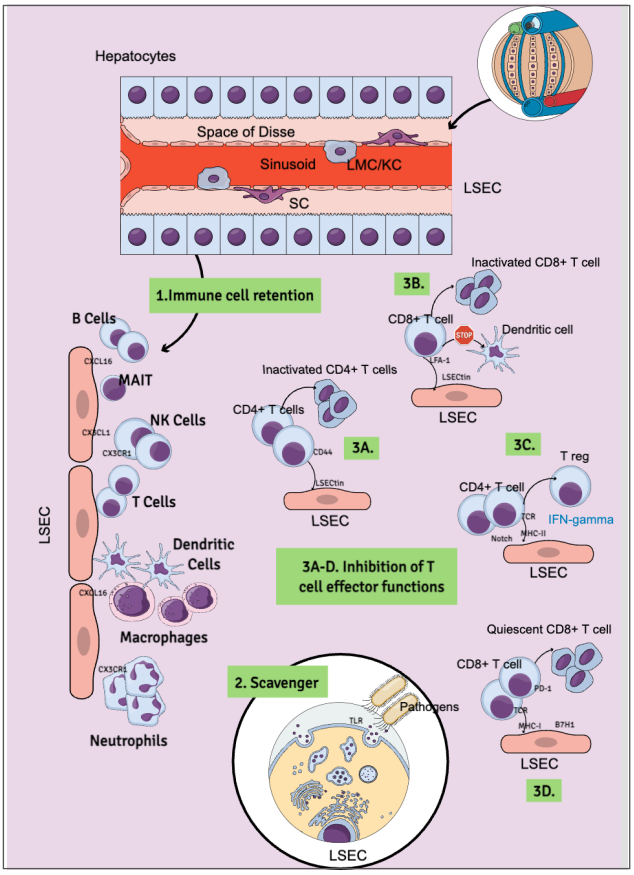

Figure 3. Schematic representation of the immunomodulatory functions of liver sinusoidal endothelial cells (LSEC). 1) LSECs help retain inflammatory and immune cells in the liver sinusoids, creating an environment for both innate and adaptive immune responses; 2) LSECs exhibit high scavenger activity via their Toll-like receptors (TLR), aiding in the clearance of intestinal and blood-borne pathogens and molecules; 3A-D) LSECs display unique modulatory functions by: A) inactivating CD4+ T cells through the interaction of the LSECtin receptor with CD44 (on CD4+ T cells); B) diverting the activation of CD8+ T cells from engaging with antigen-presenting cells (APC), such as dendritic cells (DC), via the interaction of the LSECtin receptor with lymphocyte function-associated antigen-1 (LFA-1); C) inducing the formation of antigen-specific regulatory CD4+ T cells by the interaction of MHC-II-restricted antigens with the T cell receptor complex (TCR) in the presence of LSEC-produced interferon-gamma, Notch signaling activation, and the absence of IL-12 co-stimulation (which is poorly expressed on LSECs); D) inducing quiescence in CD8+ T cells due to the combined effect of low blood flow in the sinusoidal space. In the unique microenvironment of liver sinusoids, antigen-specific, MHC-I-restricted cross-presentation by LSECs, in the presence of B7H1-to-programmed death-1 receptor (PD-1) co-stimulation, triggers the generation of quiescent CD8+ T cells. Therefore, LSECs play a vital role in orchestrating the liver microenvironment's innate and adaptive immune responses against intestinal and blood-borne alloantigens and pathogens. IFN: interferon; KC: Kupffer cells; LFA-1: lymphocyte function-associated antigen-1; LMC: liver monocytic cells; LSEC: liver endothelial sinusoid cells; MAIT: mucosa-associated invariant T cells; NK: natural killer; PD-1: programmed death-1; SC: stellate cell; TCR: T-cell receptor complex; TLR: toll-like receptor.

Table I. Dysregulation of the sinusoidal microenvironment in ischemia-reperfusion injury (IRI). IRI shifts the sinusoidal phenotype to a pro-inflammatory, pro-thrombotic, pro-apoptotic, and vasoconstricted environment (based on references [16-21,23,24,26-30,32-43,45-48]).

Function	Signals/pathways	Effect
Patency of the sinusoidal lumen	↓ NO ↑ET ↑ TXA_2	Sinusoidal vasoconstriction and microcirculatory dysfunction
	↓ KLF2 ↓ eNOS ↓ thrombomodulin ↓ Nrf2	Sinusoidal lumen narrowing and pro-thrombotic stimulation
Intra-sinusoidal cell adhesion	↑ ROS	Increased neutrophil chemotaxis and adhesion within the sinusoidal lumen, activation of platelet aggregation and HSC contraction
KC activation	DAMPs PAMPs	DAMP and PAMP-activated KCs release ROS, IL-1, IL-12, TNF-α, and INF-γ IL-1 and TNF-α upregulates neutrophils adhesions within sinusoids TNF-α upregulates ICAM-1 expression on LSECs leading to neutrophils rolling, binding and extravasation in the liver parenchyma
Activation of native and acquired immune response	IL-1 TNF-α GM-CSF TNF-β	Recruitment of neutrophils Activation of CD4+ T-cells Extravasation of immune cells into the liver parenchyma

DAMPs: damage-associated molecular patterns; eNOS: endothelial NO synthase; ET: endothelin; GM-CSF: granulocyte-macrophage colony stimulating factor; KLF2: Kruppel-like Factor 2; HSC: hepatic stellate cells; IL-1: inteleukin-1; IL-8: interleukin-8; IL-12: interleukin-12; NO: nitric oxide; Nrf2: nuclear factor erythroid 2-related factor 2; PAMPs: pathogens associated molecular patterns; TNF-α: tumor necrosis factor alpha; TNF-β: tumor necrosis factor beta; TXA_2: thromboxane A2.

recruit and activate CD4+ T lymphocytes, which produce granulocyte-macrophage colony-stimulating factor (GM-CSF), INF-γ, and tumor necrosis factor-beta (TNF-β). These cytokines enhance KC activation, facilitating neutrophil recruitment and adhesion to the liver sinusoids [29]. Platelets play a crucial role in IRI and produce and release several factors involved in inflammation, hepatic cell injury, and regeneration (Fig. 2) [30]. These include platelet-activating factor (PAF), cytokines, and growth factors – such as nitric oxide (NO), TGF-β, serotonin – and calcium-dependent proteases (calpains). PAF primes neutrophils for ROS generation, further amplifying the neutrophil response [16]. Platelets also adhere to hepatic sinusoids and induce programmed LSEC death through NO production, which ultimately leads to the formation of the potent toxicant peroxynitrite ($ONOO^-$) [30]. Peroxynitrite is a reactive nitrogen species created by the reaction between nitric oxide (NO) and superoxide anion ($O2^-$). It can modify many biomolecules, including amino acids, proteins, enzymes, and cofactors. Excessive reactive nitrogen species production causes nitrosative stress, leading to structural damage and the loss of cell function, which further amplifies inflammatory and innate immune responses [31].

To note, hepatocytes and LSECs demonstrate differential sensitivity to ischemia, with hepatocytes more sensitive to warm ischemia and LSECs to cold ischemia (Fig. 1) [32]. Although most hepatocytes remain viable for 48h after cold preservation and warm reperfusion in rats, LSECs rapidly suffer from severe damage in prolonged cold ischemia (> 6h) (up to 50% of them become non-viable cells) [32]. However, considering the intimate cellular crosstalk between LSECs and hepatocytes, prolonged cold storage-induced LSEC injury may adversely impact hepatocyte viability and vice versa. Hepatocytes from cold-stored livers exhibit disturbances in key hepatocellular functions, such as solute transport and drug metabolism, which contribute to liver graft dysfunction [33].

THE IMMUNE FUNCTIONS OF LSECS

LSECs are vital regulators of immune responses in the liver due to their strategic position within liver sinusoids and their large surface area for interactions with passenger leukocytes and other resident immune cells (Fig. 3, Tab. II) [34]. Compared to professional APCs like dendritic cells, LSECs use unique mechanisms to interact with passenger lymphocytes, transmit instructional signals, and activate local effector functions of antigen-specific CD8 T cells (Tab. II, Fig. 3). Recognizing these specific immune features of LSECs is crucial for understanding the role of MP-based strategies in modulating liver immune functions.

Four immunologic functions are relevant to the immunomodulatory role of LSECs. They are affected during IRI: immune cell residency, antigen uptake, T-cell activation, and the induction of regulatory or quiescent T cells (Tab. II, Fig. 3).

Immune cell residency

LSECs play a crucial role in liver immune-modulatory functions and exhibit immunomodulatory properties (Tab. II, Fig. 3). The liver sinusoids separate the intravascular space from hepatocytes while providing a platform for various immune cell populations to reside in the liver [34]. Consequently, specialized natural killer (NK) cell populations, innate lymphocytes – including so-called MAIT (mucosa-associated invariant T) cells – and myeloid cells (such as neutrophils and Kupffer cells) adhere to the surface of LSECs to establish liver residency [34]. The expression of chemokine receptors CXCR6 and CX3CR1 appears to facilitate immune cell retention in the liver sinusoids, working alongside the production of CXCL16 and CX3CL1 by LSECs [35]. Since these receptor-ligand interactions are not unique to the liver, it remains an open question whether there are liver-specific recruitment processes [34].

Table II. Immunomodulatory functions of liver endothelial sinusoidal cells (LSECs).

Function	Target cells	Receptors/signals	Effect
IMMUNE CELL RESIDENCY			
Retention of immune cell populations in liver sinusoids [34,35]	NK cells Innate and activated T cells MAIT APC (KC, DC)	CXCR6 CX3CR1 CXCL16 CX3CL1	Combined with the low blood flow in sinusoids, this function allows for immune cell circulation and residency in the liver parenchyma
SCAVENGER FUNCTION			
Antigen uptaking [34,36]	Blood-borne molecules and pathogens	TLR	Intestinal and blood borne pathogens and molecules are removed from circulation
INHIBITION OF T-CELL ACTIVATION			
Silencing of CD4+ T-cell effector function [37,38]	Activated CD4+ T cells	LSECtin (LSECs) CD44 (T cells)	Inhibition of CD4+ T-cell activation into CD4+ helper
Silencing of CD8+ T-cell activation [34,39,40]	CD8+ T cells	LFA-1	Inhibition of CD8+ T-cell activation of effector functions
INDUCTION OF T-REG/QUIESCENT T-CELLS			
Induction of regulatory T cells through MHC II-restricted antigen presentation [41-45]	CD4+ T cells	MHC-II IFN-γ TGF-beta NOTCH	Due to low expression of MHC-II and the absence of IL-12 production by LSCEs, antigen-specific presentation to T cells results in the generation of regulatory cells
Cross-presentation of soluble antigens on MHC I molecules to CD8+ T cells [46-48]	CD8+ T cells	MHC-I (on LSECs) B7H1 (on LSECs) PD1 (on T cells)	Due to low blood flow in sinusoids, LSEC MHC-I restricted antigen stimulation results in differentiation of quiescent CD8+ T cells

APC: antigen-presenting cell; CD: cluster differentiation; IFN-γ, interferon-gamma; IL-12: interleukin-12; KC: Kupffer cell; LFA-1: lymphocyte function-associated antigen-1; LSEC: liver sinusoidal endothelial cell; MAIT: mucosa-associated invariant T cell; NK: natural killer; PD1: programmed death 1; TLR, toll-like receptor.

Scavenger function

LSECs have endocytic uptake functions and surpass professional APC populations, such as dendritic cells or myeloid cells, in their ability to uptake circulating antigens (Fig. 3, Tab. II) [36]. Additionally, the low perfusion pressure in the liver sinusoids facilitates the endocytic uptake of blood-borne molecules by LSECs. LSECs also express pattern recognition receptors, such as Toll-like receptors (TLRs), located on the plasma membrane at the cell surface or within endocytic compartments. This highly efficient scavenger function, combined with TLR expression, enables LSECs to act as sentinel cells, instructing parenchymal hepatocytes to initiate the liver's acute phase response through IL-6 secretion. In turn, IL-6-activated hepatocytes enhance systemic innate immune effector functions by supplying increased levels of complement, C-reactive protein (CRP), and other acute-phase proteins [34].

Inhibition of T-cell activation and induction of regulatory/quiescent T cells

Beyond scavenger receptors, LSECs also play a key role in tolerance induction through C-type lectin receptors such as L-SIGN and LSECtin (Fig. 3, Tab. II) [37,38]. These receptors participate in the uptake of molecules containing mannose residues and aid in the removal of pathogens from the bloodstream. The T-cell surface molecule CD44, found on activated T cells, has been identified as a natural ligand for LSECtin. The interaction between LSECtin and CD44 inhibits T-cell activation, proliferation, and effector function, emphasizing the importance of LSECtin expression in regulating local T-cell responses.

LSECs also inhibit the antigen-specific activation of CD8 T cells in the liver (Fig. 3, Tab. II) [34,39]. T-cell activation by antigen-presenting dendritic cells is suppressed when LSECs are in direct contact with T cells [34]. This role of LSECs on other APC populations in the liver can be understood through the diversion of the crucial signaling molecule lymphocyte function-associated antigen-1 (LFA-1) on T cells away from the antigen-presenting cell to LSECs, thus depriving the immunological synapse of a key signal component [40]. Therefore, it is likely that these two veto mechanisms (i.e., the interaction of LSEC with CD44 and LFA-1) prevent local antigen-specific activation of CD8 T cells and the execution of effector functions [34].

Because LSECs express only low levels of co-stimulatory molecules and do not produce IL-12, which is essential for inducing effector functions in cells, they do not promote the differentiation of naive CD4 T cells into helper CD4 T cells (Fig. 3, Tab. II) [41,42]. The outcome of antigen-specific stimulation of naive CD4 T cells by antigen-presenting LSECs, through transforming growth factor-beta (TGF-β) or Notch-dependent signaling pathways, is their differentiation into T cells with regulatory potential [34,43]. It is important to note that the liver does not contain many regulatory T cells, identified by the transcription factor Foxp3; instead, these cells are recruited during inflammation by similar chemokines that attract effector T cells [44]. However, LSEC-induced regulatory T cells may not always express Foxp3, which could lead to an underestimation of the number of regulatory T cells in the liver [45].

Finally, in the slow flow of liver sinusoids, the recruitment of antigen-specific naïve CD8+ T cells by LSCE cross-antigen presentation often leads to the formation of quiescent T cells (Fig. 3, Tab. II) [46]. LSEC-induced retention of antigen-specific MHC-I–restricted CD8+ T cells begins a complex dialogue resulting in mutual activation, characterized by increased expression of MHC I and B7H1 on LSECs and PD1 on CD8 T cells [47]. Co-inhibitory signaling through the B7H1-PD1 axis causes CD8 T cells to revert to a quiescent state [47]. In this state, LSEC-activated CD8 T cells do not perform effector functions, secrete cytokines, or display cytotoxic activities against tumor cells [18].

MACHINE PERFUSION IN LIVER TRANSPLANTATION

Hypothermic perfusion

MP has emerged as a significant advancement in LT, offering a transformative approach to organ preservation and optimization [49,50]. Techniques used in studies of ACR include HMP (both HOPE and D-HOPE), NMP, and normothermic regional perfusion (NRP). HOPE is recognized for its ability to improve graft preservation by reducing IRI and maintaining mitochondrial function [50-64]. By lowering oxidative stress and inflammation, HOPE effectively mitigates the harmful effects that often compromise graft functionality [50-58], particularly in the context of DCD liver grafts [58]. This technology is especially beneficial for ECD livers, which are more vulnerable to IRI due to factors like advanced donor age and steatosis [59]. HOPE's mechanism of preserving mitochondrial integrity while decreasing ROS production may also directly and indirectly influence the risk of post-transplant ACR. By interrupting the cascade connecting innate and adaptive immune responses, HMP could alter antigen-specific effector T-cell responses, potentially making the liver graft less reactive or more tolerogenic. Additionally, the targeted regulation of metabolic activity during preservation helps maintain energy balance and improves the graft's postoperative performance. Liver graft dysfunction early after transplant is a known factor that increases the risk of ACR [7,12]. Although the data on HOPE's impact on LT outcomes is promising for reducing graft dysfunction and ischemic cholangiopathies [56-58], a more detailed understanding of its effect on post-transplant ACR remains a critical area

for further research, along with investigating how donor quality (DCD versus DBD), recipient health, and perfusion protocols influence current clinical outcomes.

Normothermic perfusion

NMP offers additional benefits by keeping the donor liver in a metabolically active state [49,65]. This method enables real-time assessments of viability, therapeutic interventions, and improved tissue preservation [65-68]. By maintaining continuous metabolic activity, parameters such as bile production and enzyme levels can be monitored, which helps determine the liver's functional viability before transplantation and lowers the risks associated with using suboptimal grafts [49]. NMP's ability to support glycogen repletion, regenerate ATP stores, and stabilize coagulation pathways by reducing hyperfibrinolysis leads to better post-transplant results, especially with marginal grafts [67]. Its capacity to maintain normothermic conditions minimizes immune activation and may improve rejection rates [49]. However, like HOPE, there is a need for standardized protocols to ensure consistency and reproducibility, particularly when applying specific therapies such as stem cell delivery or anti-inflammatory agents [67,69-71].

Sequential hypothermic and normothermic perfusion

The integration of sequential HMP and NMP offers an alternative method for preserving ECD livers [72,73]. This approach combines the advantages of both hypothermic and normothermic conditions, tailored to the specific preservation needs of high-risk grafts [73]. Laboratory analyses show that enzyme levels (AST, ALT), bilirubin, and graft function remain stable across groups using advanced perfusion techniques, demonstrating the reliability and safety of these methods in transplant applications [73]. Sequential approaches improve graft preparation by applying different perfusion conditions, potentially reducing early allograft dysfunction (EAD) and enhancing post-transplant survival [73]. While promising, challenges related to implementing sequential methods, such as the technical complexities of dual-system setups, must be addressed to enable broader clinical adoption and research on the immunologic benefits they provide.

Normothermic regional perfusion

The role of NRP in reducing the risk of post-transplant ACR has been addressed only minimally. The need to lower the higher complication rates associated with transplantation from DCD liver grafts, especially when using uncontrolled DCD (uDCD), has led to the adoption of stricter donor and graft selection criteria in some contexts and the use of NRP in others to overcome the limitations of super rapid retrieval (SRR) [74,75]. Many retrospective studies have shown that NRP treatment effectively prevents IC compared to SRR [76], but randomized trials are lacking. A recent retrospective, observational cohort study compared LT from controlled DCD (cDCD) donors recovered by NRP (=106) *vs* SRR (136) [77]. EAD was higher in the SRR group (56.1% *vs* 36.4%; p = 0.007), while IC-free survival improved in NRP recipients, and patient and graft survival were similar between groups [77]. However, the inflammatory and immunomodulatory effects of NRP alone or combined with hypothermic perfusion techniques before and after SCS have been limitedly studied [78]. In a rodent LT model, DBD livers exhibited minimal inflammation during the 2-hour NRP period. In contrast, DCD livers with 30- and 60-minute donor WIT experienced greater mitochondrial damage and inflammation, indicated by increased perfusate lactate, flavin mononucleotide (FMN), and high mobility group box protein-1 (HMGB-1) levels, leading to subsequent TLR activation during NRP. D-HOPE, compared to NRP, resulted in significantly less mitochondrial complex I injury and inflammation [78]. Overall, these findings indicate that DCD donation is linked to mitochondrial and DAMP-related inflammatory injuries in grafts, which can be partially reversed when O2 is administered hypothermically before organ procurement [78].

Donor type

Both DBD and DCD trigger systemic inflammatory responses through different pathways and can cause organ injury and dysfunction [79,80]. Brain death is associated with a systemic inflammatory response like sepsis-induced cytokine release, marked by elevated levels of pro-inflammatory cytokines, including IL-6, IL-8, and IL-10, compared to DCD donors [81]. The duration of brain death may influence the severity of the inflammatory response; however, longer durations do not necessarily lead to more severe inflammatory reactions in abdominal organs [80]. Brain death also activates the complement and coagulation cascades, leading to a pro-thrombotic state that can further affect graft survival [79-81]. The implications of brain death on the risk of post-transplant immune activation are mainly mediated by SCS, with prolonged cold ischemia time (CIT), older donor age, and liver graft steatosis contributing to cell injury and the activation of native and acquired immune responses [7-9].

DCD is generally associated with lower pro-inflammatory gene expression and cytokine activation, leading to a milder inflammatory response compared to DBD [82]. However, DCD may impact organ viability due to the activation of the IRI cascade associated with prolonged warm ischemia times (WIT) when compared to DBD. Given the heightened sensitivity of cholangiocytes to oxygen deprivation overall, and WIT, compared to hepatocytes and LSECs, the introduction of MP has provided clinical benefits in patients at high risk of post-transplant biliary lesions [58].

Both HMP and NMP can reduce inflammatory pathways caused by DBD and DCD. However, the complexity of

current clinical situations may explain the conflicting evidence about which technique is better at lowering the risk of post-transplant ACR in liver grafts (Tab. III). Additionally, the choice of MP strategy is often based on the transplant center's experience and primarily aims to improve early graft dysfunction and protect the biliary tree from injury [83].

Critical appraisal of literature data

Despite existing evidence on the benefits of both HMP [56,58] and NMP [65] regarding specific LT outcomes, particularly in the context of ECD and DCD [58], published studies exhibit significant heterogeneity, as previously discussed in surveys [83], systematic reviews [84], and meta-analyses [56,84]. There is notable variability in indications related to donors and recipients, as well as in techniques (such as hypothermic, normothermic, or sequential combination) and technical parameters (perfusion pressure, flow, duration, perfusate composition, and oxygenation regimen), highlighting the need to determine the best scenarios for each method [83]. Moreover, the assessment of graft viability, utilization criteria, and post-transplant outcome measures remain subjects of ongoing debate [83]. Importantly, ACR has seldom been investigated in most reports [84], mainly due to reduced interest in liver graft rejection in clinical practice, the availability of advanced immunosuppressive drugs, and the relatively limited impact of ACR on graft and patient outcomes [11]. Finally, unlike immunosuppressive medications, whose clinical use depends heavily on pre-clinical research, MP has often been adopted by LT centers worldwide based on clinician discretion before the completion of sufficiently powered randomized trials [56]. As a result, evidence on MP mainly derives from single-center series, with only a small fraction of published experiences coming from randomized controlled trials (RCTs) in the international literature [56,84-89]. Even among RCTs, the risk of bias – stemming from donor and patient selection, the randomization process, deviations from intended interventions, missing outcome data, outcome measurement, and the selection of reported results– can vary considerably [58,84-89].

THE IMMUNOLOGICAL EFFECTS OF MACHINE PERFUSION AND THE REPORTED EVIDENCE

The current body of research provides strong evidence that MP technologies, including HOPE, D-HOPE, and NMP, offer significant advantages in LT. These methods not only improve donor liver preservation but also enhance recipient outcomes by reducing IRI and IRI-related complications like EAD and IC. However, the clinical evidence on the role of MP in modulating post-LT antigen-specific immune responses still requires more robust data (Tab. IV, Fig. 4).

To date, no adequately powered study on the risk of ACR in adult liver transplantation (LT) has been published in the literature. Therefore, the following considerations regarding the role of MP in reducing the risk of liver graft rejection are presented in a narrative format. The main mechanism behind HOPE's biological effects involves mitigating or reversing mitochondrial damage caused by oxygen deprivation and reinfusion. This leads to increased cellular energy storage and decreased accumulation of succinate and nucleotide metabolites [51]. Additionally, it lowers the release of ROS, pro-inflammatory cytokines, and DAMPs, ultimately reducing the severity of IRI metabolic cascades (Tab. IV, Fig. 2) [51]. As demonstrated in experimental models of rodent LT, reduced activation of the innate immune response extends to the adaptive immune system, evidenced by lower expression of costimulatory signals such as CD40 on Kupffer cells (KCs) and CD80 on liver sinusoidal endothelial cells (LESCs), fewer activated CD4+ T cells (CD154+ and CD28+ T cells), and decreased levels of pro-inflammatory IL-2 and IL-17 [52]. In an experimental rat model of LT rejection, 1-hour HOPE combined with low-dose tacrolimus (TAC) successfully prevented ACR at 4 weeks, similar to full-dose TAC [90]. However, this model faces challenges in applying findings to clinical settings, as the proposed full-dose (1 mg/kg) and low-dose (0.3 mg/kg) TAC regimens are ten times higher than those used in human LT [90].

A recent study by Elgosbi et al. compared 14 patients treated with MP to 13 patients who underwent SCS and participated in two randomized controlled trials assessing SCS against HOPE (the HOPE and D-HOPE trials, which used either DBD or DCD, respectively) [91]. Patients who received livers preserved with SCS showed a significantly higher incidence of early ACR (i.e., within the first month after LT) compared to those who received livers treated with HOPE (46% *vs* 7%, p = 0.03) [91]. Using intracellular cytokine staining and an activation-induced marker assay to simultaneously count antigen-specific effector and regulatory T cells (Tregs) [92], the authors showed that HOPE-treated patients had an increase in CD4+Foxp3+CD127lo cells two weeks after transplantation, due to the preferential expansion of alloreactive Treg clones. This explained the reduced expansion of alloreactive CD8+ T cells seen in the HOPE group compared to recipients of SCS livers [91]. The transcriptomic analysis (RNAseq) on liver tissue samples from HOPE-treated grafts revealed upregulation of genes related to cellular responses to heat and hypoxia, as well as overexpression of genes involved in acute inflammatory responses, T-helper cell differentiation, and neutrophil homeostasis and angiogenesis [91]. After reperfusion, HOPE was also linked to the downregulation of genes that

Table III. Summary of two systematic reviews on the impact of machine perfusion (MP) on acute liver graft rejection.

Author	No. of studies	No. of patients	ACR	Limitations
Maspero M et al. 2023 [93]	6 HOPE 1 NMP 1 NRP	226 MP 330 SCS	**Overall** **11%** in MP *vs* **18%** in SCS (p = 0.02) **Sub-analysis** HOPE is superior to SCS for all types of graft MP is superior to SCS for DCD grafts HOPE is superior to SCS for DCD grafts	IS was reported in 6 studies TAC was used in all studies Basiliximab was used in 4 studies Only 4 studies provided a definition of ACR Only 1 study provided classification of ACR based on RAI No study provided information on treatment of rejection
Liang A et al. 2023 [98]	7 HOPE 4 NMP 4 NRP	705 MP 1056 SCS	**Overall** **11.2%** MP *vs* **23%** in SCS (p = 0.31) **Sub-analysis** HOPE and NRP are superior to SCS with DCD grafts only HOPE was not superior to SCS for all types of grafts HOPE was superior to SCS for DCD and ECD-DCD grafts NRP was superior to SCS for DCD grafts HOPE was not superior to SCS for ECD-DBD grafts	Limited sample size of included studies Significant heterogeneities across studies for design, organ perfusion protocols, patient clinical characteristics and post-operative management. Diagnosis of ACR is often ambiguous ACR reporting is often poor

ACR: acute rejection rate; CI: confidence interval; DBD: donation after brain death; DCD: donation after cardiocirculatory death; ECD: extended criteria donors; HOPE: hypothermic oxygenated perfusion; IS: immunosuppression; MP: machine perfusion; NMP: normothermic machine perfusion; NRP: normothermic regional perfusion; RAI: rejection activity index; SCS: standard cold storage; TAC: tacrolimus.

induce oxidative stress and apoptosis [91]. In contrast, SCS-treated livers showed downregulation of genes related to the positive regulation of metabolic processes [91].

Besides the numerical limits of the recruited population, several issues suggest that these data should be interpreted carefully. The incidence of ACR in SCS-treated patients is somewhat higher (46%) than reported in cohorts with clinically diagnosed rejection [11]. The trials' primary endpoints did not emphasize ACR, and the patient count was not based on statistical hypotheses regarding ACR risk. While the diagnosis of ACR depended on histology and the Banff classification system, the criteria for liver biopsies were not specified, and data on rejection severity were not provided. Additionally, the IS protocol referenced by the authors included variability, such as the use of basiliximab or mycophenolate mofetil (MMF) for patients with worsening renal function, which could have affected results. The two patient groups had similar median TAC exposure during the first two weeks post-transplant, but TAC exposure levels in rejecting patients were not compared to those without rejection. Lastly, despite expressing favorable tolerogenic transcriptomic signatures based on methods from a previous publication [92], the authors did not investigate any evidence of

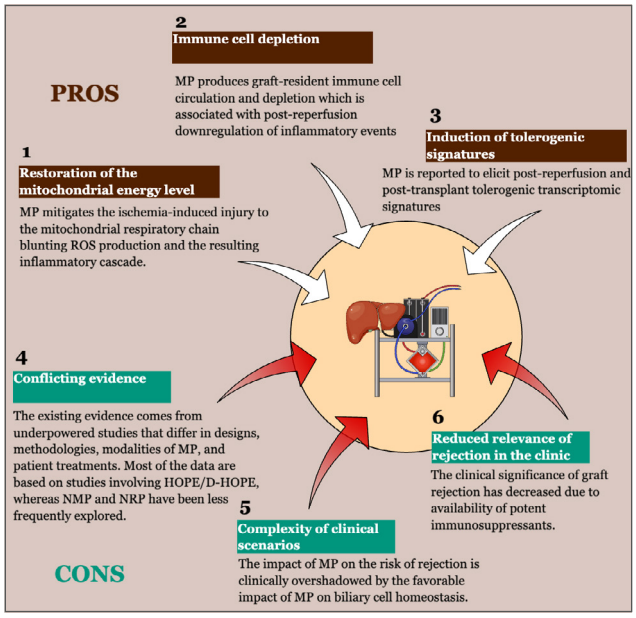

Figure 4. Summary of pros and cons of the impact of machine perfusion on the risk of acute rejection of the liver graft. Abbreviations: D-HOPE: dual-HOPE; HOPE: hypothermic oxygenated perfusion; MP: machine perfusion; NMP: normothermic machine perfusion; NRP: normothermic regional perfusion.

clinical tolerance – at least operational – over a median follow-up of 4.6 years [91].

A recent systematic review by Maspero et al. reported a reduced incidence of ACR after MP compared to SCS by combining data from eight international studies, which included six studies on HOPE and one each on NMP and NRP (Tab. III) [93]. In the pooled analysis, the overall ACR rate was 11% (95% CI, 7%-15%) after MP and 18% (13%-24%) after SCS, which was statistically significant [89]. ACR was significantly lower after HOPE compared with SCS (OR: 0.54, 95% CI, 0.29-1, p = 0.05) and overall after MP compared with SCS alone (OR: 0.55, 95% CI, 33-91, p = 0.02) [93]. In a subgroup analysis of studies that included only DCD grafts, the ACR rate was significantly lower with MP compared to SCS (OR: 0.43, 95% CI: 0.20-0.91, p = 0.03). This effect was also maintained when considering only studies on HOPE with DCD grafts (OR: 0.37, 95% CI, 0.14–1, p = 0.05) [93].

Although compelling, the paper has faced criticism. Liang et al. pointed out that the systematic review missed several studies, including one HOPE study, three NMP studies, and three involving NRP, despite accounting for the data extraction time [94]. Although there was considerable variability in study designs, donor types, and modes of MP, the incidence of ACR in the studies not included in the review was higher than SCS in one HOPE study and two NMP studies [94]. Neil et al. [95] reported that their prior experience with NMP for repeat LT might have been included in the systematic review except for one patient, and their results contrasted with those of Maspero et al., with NMP patients experiencing ACR in up to 61.9% of cases [96]. Given the characteristics of the included studies, Gu et al. recommended using trial sequential analysis to determine whether the number of participants and trials was adequate to draw conclusions and to enhance the review with an assessment of the evidence level according to the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) [97].

Inspired by the findings of Maspero et al., Liang et al. presented the results of a further systematic analysis, which included 7 HOPE studies, 4 NMP studies, and 4 NRP studies (Tab. III) [98]. Their work indicated that HOPE and NRP, but not NMP, may be linked to a reduction in ACR compared to SCS, with this effect observed only in DCD grafts [98]. HOPE did not demonstrate protective effects against decreasing ACR in the ECD-DBD or DBD subgroups, while NMP showed no advantages for ACR in DCD [98].

All the reviews published so far in the literature feature small sample sizes and significant heterogeneity in study design, organ perfusion protocols, clinical characteristics, and perioperative management (Fig. 4) [93-98]. Since the original studies were not designed or powered to detect ACR, the diagnosis of ACR was often unclear, complicating the reporting of ACR events. Only a few studies reported ACR, and the diagnostic criteria varied without a standard biopsy-proven ACR. Therefore, conclusions about the reduction of ACR after LT by MP should be drawn cautiously (Fig. 4) [98].

BALANCING SCIENTIFIC EVIDENCE AND CLINICAL SIGNIFICANCE

Although the basic mechanisms of ACR in liver grafts treated with SCS have been extensively described – especially regarding the modes of alloantigen presentation (direct versus indirect), the role of liver-resident APCs and LSECs, and the chemokine environment that supports both innate and adaptive immune responses in the liver sinusoids [11] — the pathogenesis of ACR in liver grafts treated with MP remains poorly understood [98]. Based on the available evidence in the relevant literature, we propose that ACR should be comparable in SCS-treated and MP-treated grafts. However, this assumption remains unproven, as essential information on the type, severity, and mode of ACR presentation is lacking across studies on MP [93-98]. This can be attributed to the reduced impact of ACR on liver graft prognosis in the current era of TAC-based regimens, along with the increasing focus of MP studies on the mechanisms of graft dysfunction.

Table IV. Proposed immunomodulatory mechanisms of machine perfusion (MP) compared to static cold storage (SCS).

Function	Effect	Open issues
Dynamic perfusion of the liver preserves the phenotype of liver sinusoids by mechanical and molecular mechanisms	The mechanical stimuli during MP may preserve LSEC phenotype upregulating the expression of KLF2 and Nrf2 transcription factors [16] This results in preserved production of eNOS and thrombomodulin within the sinusoidal endothelium and in reduced production of oxidant species [16]	The most appropriate pressure and flow regimens to reduce LSEC deterioration is currently unknown The superiority of combined hepatic arterial and portal perfusion (D-HOPE) over portal-only (HOPE) on the sinusoidal milieu and LSEC phenotype awaits elucidation
Oxygenation of graft perfusate	Ex-vivo O_2 supplementation mitigates the mitochondrial electron chain disruption, ATP depletion, ROS production and DAMP release [22-29,31,50-58] This results in reduced intensity of post-reperfusion inflammation and immune response within the sinusoidal spaces, liver parenchyma and biliary tracts [50-58]	A partial oxygen pressure (pO_2) of at least 70 kPa (500 mmHg) is currently considered necessary to allow for ATP restoration after 1-2 hours of HOPE/D-HOPE [50-58] The optimal pO_2 to mitigate the immune activation and reduce ACR risk is currently unknown LSECs, hepatocytes and cholangiocytes show different sensitivity thresholds to CIT [32,33]
Perfusion temperature	Hypothermic perfusion with adequate pO_2 improves mitochondrial function versus SCS resulting in diminished accumulation of succinate and reduced nucleotide substrates [50-58] Normothermic perfusion preserves cellular and mitochondrial functions and reduces the extent of inflammatory and immune activation [49,65-68]	The T-cell dynamics occurring after hypothermic and normothermic perfusion should be elucidated better Preliminary data suggest that hypothermic perfusion is associated with early expansion of regulatory cells and transcriptomic signatures of cellular responses to acute inflammation injury and downregulation of genes that induce oxidative stress. Longer observation is necessary
Perfusion pressure and flow	Perfusion pressure and flow may contribute to immune cell circulation and clearance, shutting down post-reperfusion immune activation [100,102-104,106] The transition to an exhausted immune phenotype is particularly relevant to cholangiocytes and post-transplant cholangiopathies [98]	MP produce liver-resident inflammatory cells mobilization into the perfusate, but this effect has mainly been described for leukocytes and T cells [55,103,106] Immune cell circulation and depletion may be influenced by additional factors, like the perfusate medium composition, use of blood, nutrients and oxygen carriers [100,101] The dynamics of mobilization of all inflammatory cell populations during MP require clarification
Donor type	Experimental and clinical data suggest that DBD and DCD are associated with activation of pro-inflammatory and pro-thrombotic pathways [79-82] Hypothermic O_2 administration before procurement (i.e., NRP) is associated with reduced inflammatory activation [78]	Matching the type of MP with donor category is currently based on need to assess graft viability prior to transplantation and reduce the risk of ischemic cholangiopathies [83]

ACR: acute cellular rejection; CIT: cold ischemia time; D-HOPE: dual HOPE; eNOS: endothelial NO synthase; HOPE: hypothermic oxygenated perfusion; KLF2: Kruppel-like factor 2; LSEC: liver sinusoidal endothelial cell; MP: machine perfusion; Nrf2: nuclear factor erythroid 2-related factor 2; NRP: normothermic regional perfusion; SCS: static cold storage; WIT: warm ischemia time.

Considerations regarding MP's role in modulating graft and recipient alloreactivity are rooted in acquired knowledge of IRI-associated inflammation and the subsequent activation of an antigen-specific T cell-mediated immune response, which may then worsen the ACR cascade [99]. However, MP also contributes to changes in the mechanistic processes of IRI that require further analysis [98,100].

Graft immunodepletion and blunting of inflammation – greater benefits for the biliary compartment

Unlike in SCS, where grafts are immersed in perfusion solutions and minimal passenger (i.e., graft-derived) immune cell circulation is typically expected, in dynamic perfusion techniques, inflammatory passenger cells circulate within the graft depending on the cell type (mononuclear cells, T cells, B cells, NK cells, etc.), the graft (e.g., kidney *versus* liver), the perfusion method (HMP *versus* NMP), MP's pressure and flow settings, the duration of perfusion (short *versus* prolonged), and the use of blood, nutrients, and 02 carriers [100,101]. In experimental studies of ex vivo lung and kidney perfusion, many passenger leukocytes mobilize and extravasate into the perfusate, affecting (i.e., downregulating) graft immunogenicity [100-102]. Data indicating the mobilization of passenger leukocytes into the perfusate have also been published in liver NMP [103,104]. The dynamics of inflammatory cell mobilization in kidney grafts during NMP exhibit significant cellular diapedesis of T cells, B cells, NK cells, and monocytes from the kidney into the circuit during perfusion, accompanied by high concentrations of IFN-γ and cell-free donor DNA [100]. In the context of LT, the migration of donor passenger T cells from the donor liver allograft into the recipient's circulation was demonstrated before the clinical use of MP [105].

MP techniques have much greater potential for mobilizing liver-resident inflammatory cells into the perfusate than SCS. This has been demonstrated for T cells and neutrophils during HOPE [55] and NMP [106]. Recent single-cell transcriptome profiling of human donor livers before, during NMP, and after transplantation revealed a significant efflux of passenger leukocytes, mainly neutrophils, in the perfusate [107]. Additionally, during NMP, neutrophils shift from a pro-inflammatory state to an aged, chronically activated, and exhausted phenotype, while anti-inflammatory and tolerogenic monocytes and macrophages increase [103]. Along with O_2 supplementation during perfusion, maintaining the mitochondrial respiration chain, and reducing ROS-driven inflammatory stimulation upon graft reperfusion, the decreased number of liver-resident T cells has been reported to downregulate both innate and adaptive immune responses, leading to less post-transplant cholangiopathy [55] and – presumably – a reduced risk of post-transplant ACR [93,100].

The major liver compartments (i.e., hepatocytes, biliary epithelium, and sinusoidal endothelium) exhibit different sensitivities to O_2 deprivation and inflammatory injury, with cholangiocytes being more vulnerable than both hepatocytes and LSECs [32,33,107]. As a result, the effect of MP on inflammatory cell mobilization and their transition to an exhausted phenotype is more clearly seen in post-transplant cholangiopathy than in reducing ACR across various donor risk levels [98]. This is particularly true for high-risk grafts, especially those involving DCDs (uDCS >> cDCDs), extended donor fWIT, elderly livers, steatosis, and prolonged CIT. All these factors significantly affect the viability of cholangiocytes, followed by hepatocytes [55].

Finally, the current evidence on MP's effects on IRI, immune cell mobilization, priming, and graft metabolic processes lacks the organ-organ and organ-system crosstalk observed in the recipient's body. Interactions between multiple organs are essential to ensure the viability and homeostasis of physically separated organs [108]. To date, the interactions between grafts and lymphoid organs are not reproducible during dynamic perfusion, yet they play a crucial role in the mechanisms of rejection and tolerance [109].

The complexity of the clinical scenario

Despite existing evidence about the role of sinusoidal endothelium and the LSEC phenotype in the liver's immunomodulatory and tolerogenic profile, the impact of MP on the risk of ACR is likely overshadowed by several factors (Fig. 4). These include the significant role of MP in enhancing cholangiocyte and hepatocyte viability [2-6], the complex orchestration of immune responses – both rejection and tolerance – within the liver graft [7-13], as well as the use of more potent immunosuppressants (TAC-based) [14]. Although crucial, the role of LSECs in regulating alloantigen presentation, recognition, and response is not exclusive [34-40]; various liver cell populations contribute to innate and adaptive immune responses, influencing the development of graft tolerance [41-48]. The widespread use of TAC-based IS has reduced the frequency and severity of rejection episodes, facilitating the adoption of minimization protocols to mitigate calcineurin-inhibitor-related adverse events [14]. This has resulted in significant variability in immunosuppressive regimens, shifting from fixed-dose protocols to schedules tailored to individual patient rejection risk profiles [14]. Similarly, the broader application of MP has been accompanied by considerable variability in dynamic perfusion techniques, with different practices varying in the type of MP (e.g., HMP versus NMP), duration, pressure, flow regimens, and their combinations [93,98]. Finally, current understanding suggests that liver graft tolerance is an organ-specific process achieved through antigen-specific stimulation of liver-resident immune-responsive and immune-modulatory cell populations, along with a specific chemokine microenvironment [45-48]. In this context, persistence, rather than elimination, of a biological inflammatory signature is necessary to promote tolerance [45-48].

Proposed immunologic mechanisms of MP

Based on experimental and clinical evidence, the proposed inflammatory and immunologic mechanisms associated with MP are illustrated in Table IV, along with unresolved issues that require clarification for a critical appraisal of the impact of dynamic perfusion on the post-transplant immune response. Exploring these endpoints in clinical practice is challenging due to significant variability in the use of MP and the complexity of current clinical scenarios. Given the low number of RCTs published in the international literature compared to cohort studies and single-center experiences, a preliminary step would be to include protocol biopsies for ACR detection in all studies on MP, focusing on the modification of the LSECs phenotype, T-cell effector functions, and the induction of Treg populations. This requires multidisciplinary involvement of all professionals engaged in LT care.

Several limitations temper the conclusions drawn from this research (Tab. IV). A key constraint is the reliance on secondary data, which introduces variability in donor characteristics, recipient profiles, and perfusion protocols among the studies analyzed [93,98]. Methodological inconsistencies across clinical trials – such as variations in perfusion parameters, perfusate compositions, and durations – further complicate the generalizability of these findings to diverse transplant scenarios. Therefore, the results should be interpreted cautiously, and their applicability to broader clinical contexts warrants further investigation.

CONCLUSIONS

Despite a strong mechanistic rationale, evidence supporting the beneficial effects of machine perfusion in reducing the risk of acute liver graft rejection remains debated. This evidence comes from underpowered studies that show significant variation in their design, methodology, and treatments. In clinical practice, restoring or enhancing the biliary and hepatocyte phenotypes is the most crucial outcome of MP preservation strategies, which outperform SCS, especially when using ECD grafts and for donor grafts at risk of ischemic cholangiopathy. Additionally, the impact of dynamic perfusion on post-transplant graft rejection risk is overshadowed by the different thresholds of the liver cell compartments (i.e., biliary, hepatocytic, and sinusoidal) to IRI and the complex coordination of immune-reactive and immune-modulatory cell populations within the liver microenvironment. Experimental and preclinical data highlight the specific immune features of liver sinusoids and LSECs in regulating the liver immune response, and exploring the impact of MP on LSECs could help understand the role of MP-based strategies in modulating liver immune functions. However, designing and conducting specific trials remains a challenging task. Finally, the idea that the mechanisms of acute rejection in liver grafts preserved with MP are the same as in grafts preserved with static cold storage needs more solid evidence.

Conflict of interest statement

The authors declare no conflict of interest.

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Author contributions

PDS, DP, QL, DC: conceptualized the study; EFK, MG, AR, J D: conducted the literature search; PDS, DP, RD, AR: wrote the preliminary draft; PDS, QL: made critical revisions. All authors prepared the draft and approved the final version.

Ethical consideration

Not applicable

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