Molecules and Cells

Minireview

Updates on the Immune Cell Basis of Hepatic Ischemia-Reperfusion Injury

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Liver ischemia-reperfusion injury (IRI) is the main cause of organ dysfunction and failure after liver surgeries including organ transplantation. The mechanism of liver IRI is complex and numerous signals are involved but cellular metabolic disturbances, oxidative stress, and inflammation are considered the major contributors to liver IRI. In addition, the activation of inflammatory signals exacerbates liver IRI by recruiting macrophages, dendritic cells, and neutrophils, and activating NK cells, NKT cells, and cytotoxic T cells. Technological advances enable us to understand the role of specific immune cells during liver IRI. Accordingly, therapeutic strategies to prevent or treat liver IRI have been proposed but no definitive and effective therapies exist yet. This review summarizes the current update on the immune cell functions and discusses therapeutic potentials in liver IRI. A better understanding of this complex and highly dynamic process may allow for the development of innovative therapeutic approaches and optimize patient outcomes.

Keywords: crosstalk, immune cells, inflammation, ischemia-reperfusion injury, liver disease

INTRODUCTION

Liver transplantation is the only effective therapeutic option for end-stage liver diseases, liver cancer, and liver-based metabolic disorders (O'Leary et al., 2008). Liver ischemia-reperfusion injury (IRI) is one of the major complications during surgical procedures, which has been known as a risk factor for primary graft dysfunction as well as acute and chronic rejection (Lentsch et al., 2000).

Liver IRI shows a biphasic pattern. In the ischemia stage, it induces reactive oxygen species (ROS) production due to ATP depletion and metabolic disturbances, which leads to DNA and tissue damage. Damaged hepatocytes release damage-associated molecular patterns (DAMPs) such as HMGB1 (high mobility group box-1), free fatty acids, and heat shock proteins (Dar et al., 2019). These DAMPs are recognized by immune cells and activate pro-inflammatory signaling to activate the complement system (Hirao et al., 2022). Once the blood flow, oxygen, and nutrients are restored (called the reperfusion stage), excessive ROS induction and circulation DAMPs exaggerate the innate immunity and sterile inflammatory response, which further accelerates hepatocyte damage. This sterile immune response is mediated, in part, by the pattern recognition receptor system such as the activation of Toll-like receptors (TLRs) and recruitment of immune cells (Eltzschig and Eckle, 2011). Kupffer cells (KCs) are mainly responsible for the production of inflammatory chemokines/cytokines as well as ROS. Recruited neutrophils are early responders to mediate local microvascular changes and parenchymal damage. Later, monocyte and macrophage infiltration exacerbate the injury via excess feed-forward activation of inflammatory signaling. In addition, circulating natural killer (NK) cells promote hepatocyte injury by secreting proinflam-

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matory cytokine interferon-gamma (IFN-γ) (Bandyopadhyay et al., 2016) (Fig. 1).

Recent studies demonstrated that blocking the local inflammatory response in the liver could effectively reduce liver IRI (Kadono et al., 2022; Kaltenmeier et al., 2022; Li et al., 2022). Likely, it is important to explore the potential strategies for the normalization of innate immune response and inflammatory response activation during liver IRI, which ultimately improves the clinical outcomes of liver transplantation and expands the donor pools. In this mini-review, we briefly overviewed the most recent updates on the role of immune cells in hepatic IRI.

TECHNOLOGICAL ADVANCES ENABLING GENOME-WIDE ANALYSIS OF IMMUNE CELLS IN HEPATIC IRI

Recent advances in genome-wide transcriptome analyses allow us to understand the cellular and molecular signals of liver IRI. Single-cell sequencing (scRNA-seq) technology becomes the state-of-the-art approach for discovering cellular heterogeneity, seeking novel marker genes/subset of cells, and elucidating cell-to-cell communications, which is also widely used for identifying novel immune subsets in liver diseases (Lee et al., 2021). Recently, several studies investigated the cell atlas under liver IRI using scRNA-seq. For example, in the rat transplantation model, the transition of distinct immune cell subset was comprehensively analyzed and identified two new subsets named CSF3+ KCs and XCR1+ dendritic cells (DCs), potentially mediating the severe IRI in steatotic livers (Yang et al., 2021). In human liver donors, transcriptome profiling of intrahepatic cells revealed the dynamic changes of the transcriptome in immune cell clusters, particularly in mononuclear phagocytes (Wang et al., 2021a). Likely, scRNA-seq, as well as T/B cell receptor repertoire sequencing of human transplanted livers, successfully generated a single-cell atlas of various immune cells such as macrophages, T/B lymphocytes, and NK/NKT cells (Shan et al., 2023). These studies provide an up-to-date collective image of immune cell dynamics during liver transplantation and hepatic IRI. Raw data are available at reservoirs of datasets for further analysis (Table 1).

Spatial transcriptomics is the other cutting-edge method that gives information on transcriptomics at distinct spatial locations by quantifying the mRNA expression of many genes within the spatial context of tissues and cells (Giolai et al., 2019; He et al., 2023). Particularly, it is very useful to understand spatial differences of gene expressions in liver IRI, depending on liver zonation. One pioneering work has characterized zone-specific injury-related DEGs, cellular composition changes, and functional pathways, revealing that hepatic IRI mainly targets pericentral zone (Zone 3) (Xin et al., 2023). These technological advances may provide novel insights for selecting new therapeutic targets/biomarkers and developing therapeutic strategies.

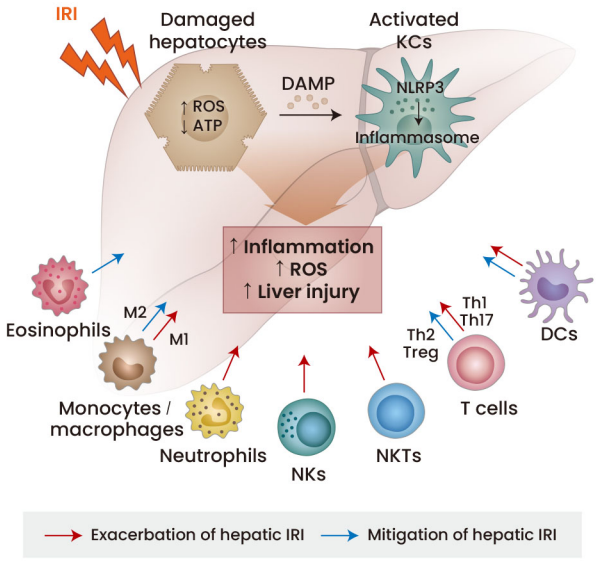

Fig. 1. The Immune cell functions in liver IRI. Liver IRI leads to hepatocyte dysfunction and death. Damaged hepatocytes release ROS and DAMPs, which trigger immune response. This response involves the activation of KCs and DCs, which in turn release inflammatory cytokines and chemokines. They drive the recruitment and activation of various leukocyte cells, including T cells, monocytes, neutrophils, and macrophages. The delicate balance between pro-inflammatory and anti-inflammatory immune cells plays a crucial role in the regulation of hepatic IRI. The red arrow indicates an exacerbation of hepatic IRI, indicating amplification of immune responses. The blue arrow represents the mitigation of hepatic IRI by limiting excess inflammation and facilitating the resolution of liver injury. IRI, ischemia-reperfusion injury; ROS, reactive oxygen species; DAPMs, danger-associated molecular patterns; KCs, Kupffer cells; DCs, dendritic cells; NK, natural killer cells; NKT, natural killer T cells; Th, T helper; Treg, regulatory T.

LIVER IMMUNE CELL FUNCTIONS IN LIVER IRI

Macrophages

Liver macrophages are a key player of hepatic IRI. They sense the initial damage-associated signals for priming inflammation and recruiting immune cells and contribute to inflammatory resolution and tissue repair. They are categorized into liver-resident macrophages (i.e., KCs) or infiltrated bone marrow-derived monocytes/macrophages (Guillot and Tacke, 2019). KCs maintain tissue homeostasis by removing pathogens and regulating hepatic iron metabolism. Under liver IRI, KCs are first activated during the ischemic phase and further intensified after reperfusion. Activated KCs produce ROS and secrete pro-inflammatory cytokines including TNFα, IL-1β, and chemokines, collectively contributing to liver damage (Abu-Amara et al., 2010).

Recently, several studies have focused on the DAMP-activated intracellular signaling in KCs to modulate liver IRI. DAMPs released from dead hepatocytes directly activate inflammasome signaling in KCs via different pattern-recognition receptors and produce pro-inflammatory cytokines, exacerbating liver IRI (Shan and Ju, 2020). In detail, ROS-activated

Table 1. scRNA-seq and spatial transcriptomics in liver IRI

Technique	Condition	Species	Platform	Main findings	Data accession	Reference
scRNA-seq	Immune features of the donor's liver during liver transplantation	Human	10× Genomics Chromium	• Profiled various immune cell types using TCR/BCR repertoire to explore the role of immune cells in inflammation and immune rejection	NGDC Genome Sequence Archive (HRA003896)	(Shan et al., 2023)
	Pre-procurement, end of preservation, and 2 h post-reperfusion sample analysis during liver transplantation	Human	10× Genomics Chromium	• Provided annotations of mononuclear phagocyte, endothelial cell, NK/T, B, and plasma cell cluster. • Identified protective TNIP3 expression in KC after reperfusion	GEO database (GSE171539)	(Wang et al., 2021a)
	Liver IRI in steatotic liver transplantation	Rat	BD Rhapsody	• Identified 11 different cell types • Discovered proinflammatory KCs with high CSF expression in transplanted steatotic liver	Genome Sequence Archive in National Genomics Data Center (CRA004061)	(Yang et al., 2021)
	Liver NPC atlas in 5 different liver diseases	Mouse	10× Genomics Chromium	• Analyzed NPCs in alcoholic liver disease, nonalcoholic steatohepatitis, drug-induced liver injury, cholestasis, and liver IRI	GEO database (GSE166178)	(Wang et al., 2021d)
Spatial transcriptomics	Zone-dependent liver IRI murine model	Mouse	NanoString	• Discovered 191 differentially expressed genes between zone 1 and zone 3 • Pericentral zones (Zone 3) are most sensitive to IRI	GEO database (GSE217936)	(Xin et al., 2023)

scRNA-seq, single-cell RNA-sequencing; IRI, ischemia-reperfusion injury; TCR/BCR, T cell receptor/B cell receptor; NGDC, National Genomics Data Center; NK/T, natural killer T cell; GEO, Gene Expression Omnibus; TNIP3, TNFAIP3-interacting protein 3; KCs, Kupffer cells; CSF, colony-stimulating factor; NPCs, non-parenchymal cells.

inflammasome component NLRP3 and AIM2 in KCs leads to liver damage (Kim et al., 2015). This is also modulated by TXNIP and its downstream signaling, which is associated with another proinflammatory signaling, the STING/ TBK1 pathway (Zhan et al., 2022). In addition, autophagy in KCs antagonizes NLRP3-dependent inflammasome activation during liver IRI (Wang et al., 2021c). A dietary antioxidant Fisetin treatment activates GSK3β/AMPK and inhibits NLRP3-inflammasome pathway, showing a protective effect against hepatic IRI (Pu et al., 2021).

KCs have pleiotropic effects. In contrast to the pro-inflammatory and deleterious effects, KCs can be protective against liver IRI by producing nitric oxide and decreasing oxidative stress (Hsu et al., 2002; Zhou et al., 2019). Moreover, KCs expressing heme oxygenase-1 or treated with IL-10 attenuate liver IRI (Ellett et al., 2010; Kobayashi et al., 2002). Two bile acid receptors are also implicated in modulating KC functions. Nuclear receptor farnesoid X receptor (FXR, NR1H4) upregulates its target small heterodimer partner in KC, which suppresses inflammatory immune response during liver IRI (Jin et al., 2020). Membrane receptor G protein-coupled bile acid receptor (GPBAR1, also known as TGR5) ameliorates inflammation and hepatocellular apoptosis against liver IRI via regulating Keap1-Nrf2 signaling in KCs (Zhuang et al., 2021).

After the onset of injury, bone marrow-derived monocytes/macrophages invade and regenerate the resident macrophage pool in liver (Guillot and Tacke, 2019; Yue et al., 2017), which generally contribute to the liver repair after IRI (Ohkubo et al., 2014). There is a growing recognition that alteration of metabolic state affects the polarization of macrophage in liver disease including IRI which shows metabolic disturbance (Dixon et al., 2013). PPAR-γ agonists ameliorated liver injury in hepatic IRI model, with decreased proinflammatory M1 macrophages and increased anti-inflammatory M2 macrophages (Linares et al., 2018). Another metabolic hormone glucagon-like peptide-1 also plays a protective role by inhibiting M1 polarization in liver IRI (Li et al., 2022). Therefore, these new updates have further validated multi-functional aspects of KCs and infiltrated macrophages in hepatic IRI and defined cellular signaling of tissue inflammation.

Dendritic cells

DCs are very heterogeneous innate immune cells that have been newly identified to play crucial roles in liver transplantation (Nakano et al., 2021). TIM4-expressing DCs are infiltrated into the liver after IRI, promoting hepatic injury and inflammatory cytokine secretion (Li et al., 2015). In addition, plasmacytoid DC are known to secrete type 1 interferon (i.e., IFNα) during IRI. Depletion of plasmacytoid DC inhibited hepatic IFNα production, resulting in protection of the liver from IRI (Castellaneta et al., 2014). Likely, inhibition of DC activation by CD47-enriched extracellular vesicles ameliorates liver IRI (Yuan et al., 2021), suggesting a pro-inflammatory and damage-inducing role of DCs. However, beneficial roles have been also reported. Prostaglandin E receptor, EP3 (PTGER3) signaling is important for monocyte-derived DC function. Loss of PTGER3 in DCs delayed liver repair, along with increased inflammatory macrophages in liver IRI (Nakamoto et al., 2020). Furthermore, a subtype of DCs, FLT3/FLT3L-dependent CD103+ DCs, mitigates IRI and hepatocyte apoptosis by the modulation of Treg cells, suggesting the interactions among immune cells in liver IRI (Zhou et al., 2019).

Lymphocytes

T cell

T cells are subcategorized into CD4+ effector or CD8+ cytotoxic cells (Parker and Picut, 2005). CD4+ T cells play a key role in proinflammatory immune response under liver IRI and depletion of CD4+ T cells attenuates inflammatory response and liver damage (Zwacka et al., 1997). In human transplant patients, CD4 transcript levels were positively correlated with the expression of inflammatory genes (Kageyama et al., 2021). In response to hepatic IRI, CD4+ T cells infiltrate the liver and increase IR-induced platelet adherence and neutrophil migration through CD40-CD40L (CD154) as well as CD28-B7-dependent pathway, exacerbating liver injury (Khandoga et al., 2006). Blocking CD154 reduces tissue inflammation and injury in hepatic IRI (Shen et al., 2009). Furthermore, mesenchymal stem cell-derived exosomes modulate the CD4+ T cell CD154 expression via delivering chaperonin containing TCP1 subunit 2, thereby alleviating liver IRI (Zheng et al., 2020).

CD4 T cells further differentiate into several subtypes including T helper1 (Th1), Th2, Th17, or regulatory T (Treg) cells (Nakayamada et al., 2012). Each subtype mediates a very specific immune response (Wang et al., 2022). Th1 cells help eliminate intracellular pathogens by mainly secreting IFN-γ and recruiting immune cells. Th2 cells activate eosinophils by secreting IL-4, IL-5, and IL-13. Th17 cells generally stimulate an inflammatory response by producing IL-17A. Treg cells are involved in immunosuppressive responses by secreting inhibitory cytokines IL-10 and TGF-β. In addition to Th1 and Th2, several functional studies have characterized the role of Th17 and Treg cells in hepatic IRI. The levels of Th17 cytokine, IL17A, and its receptor IL17RA were upregulated, whereas Treg marker, Foxp3 levels were downregulated after IRI (Ren et al., 2022). Buffering of the acidic microenvironment in liver IRI alleviated liver injury by Treg cell infiltration through the PI3K-mTOR pathway (Gan et al., 2020). As the IRI-induced stress results in an imbalance of Th17/Treg, FOXO1 activation corrects the balance and mitigates the inflammation and tissue injury (Ren et al., 2022). In addition, the other transcription factor NRF2 suppresses CD4+ T cell differentiation into the proinflammatory subtypes, while promotes immune-modulatory function of Treg cells (Kojima et al., 2023).

NK and NK T cells (NKT)

NK cells account for up to 40% of total intrahepatic lymphocytes and play an important role in regulating liver immunity (Tian et al., 2013). Hepatic NK cells are divided into circulation NK (c-NK) originating in the bone marrow and liver-resident NK (lr-NK) cell subtypes in mice (Huang et al., 2022). NK cells produce many cytokines such as IFN-γ and directly interact with other liver cells. Depletion of NK cells significantly decreased IL-17 secretion, hepatic CXCL-2 expression, and neutrophil infiltration, attenuating hepatic IRI (Feng et al., 2012). In addition, distinctive protective role of tumor necrosis fac-

tor-related apoptosis-inducing ligand (TRAIL) on NK cells was described as the adoptive transfer of TRAIL-deficient NK cells elevated liver injury (Fahrner et al., 2014).

Natural killer T (NKT) cells express NK cells surface receptors (e.g., NK1.1 [mouse] or CD161+/CD56+ [human]) as well as conventional T cell receptors, which play a crucial role in regulating innate and adaptive immunity by producing cytokines such as IFN-γ and IL-4 (Gao et al., 2009; Kumar, 2013). NKT cells are divided into two distinct types and they have opposing roles during liver IRI. Type I NKT (also known as iNKT cells) promoted liver damage whereas type II NKT (also known as vNKT cells) protected against hepatocellular damage (Arrenberg et al., 2011). It is reported that activation of NKT causes liver injury after reperfusion (Shimamura et al., 2005). NKTs directly induce hepatic IRI via CD1d-dependent activation of their TCRs, while depletion of NKT cells attenuated liver injury (Kuboki et al., 2009). In addition, obesogenic diet increases NKT cells and IFN-γ levels, which exacerbate liver damage in IRI (Liggett et al., 2022). At the same time, the beneficial role of iNKT was also described. Pre-activation of iNKT ameliorates the liver damage *via* IL-13 production in hepatic IRI. Similarly, iNKT promotes tissue repair by both IL-4-and IFN-γ-mediated acceleration of macrophage polarization (Cao et al., 2009; Goto et al., 2021).

Granulocytes

Neutrophil

Neutrophils are the first line of surveillance leukocytes to eliminate pathogens (Kolaczkowska and Kubes, 2013). Neutrophils are one of the central factors that contribute to liver damage by inducing an excessive acute inflammatory response after liver injury including hepatic IRI (Jaeschke and Smith, 1997). In the liver, unlike other organs, neutrophil activation in the sinusoids does not induce tissue damage. However, migration of neutrophils close to the hepatocytes causes tissue damage by inducing oxidative stress (Schofield et al., 2013). Neutrophil elastase secreted by activated neutrophils decreases endothelial prostacyclin production and induces hepatic injury (Okajima et al., 2004). At the molecular levels, MAPK-activated protein kinase 2 (MK2) in neutrophils induces ROS production with increased p47phox phosphorylation and myeloid-specific MK2 deletion attenuates hepatic IRI (Sun et al., 2018). Recently, ERK signaling was also reported to regulate oxidative stress and inflammatory response in neutrophils (Wang et al., 2023).

Another major mechanism of neutrophil-induced inflammation is the formation of neutrophil extracellular traps (NETs). NETs are extracellular scaffolds of DNA fibers decorated with granule-derived antimicrobial peptides, and enzymes, such as neutrophil elastase, cathepsin G, and MPO (Kaplan and Radic, 2012). NET formation is induced by DAMP through the TLR4/9 signaling, inducing liver damage and inflammatory responses during liver IRI (Huang et al., 2015). IL-33/ST2-dependent NET formation exacerbates inflammation and hepatotoxicity under hepatic IRI (Yazdani et al., 2017). Recently, the new form of cell death mainly mediated by neutrophil activation called NETosis has been extensively explored. In hepatic IRI, human thrombomodulin ameliorates hepatocellular damage by inhibiting NET formation and NETosis (Liu et al., 2022). In addition, others reported that loss of carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1, CC1, or CD66a) on neutrophil accelerates hepatic IRI by promoting NETs (Hirao et al., 2023).

Eosinophil

Eosinophils are one of the innate immune granulocytes. It is involved in host defense mechanisms against viral and bacterial infections but also mediates in regulating inflammation, maintaining epithelial barrier function, and affecting tissue remodeling (Shamri et al., 2011). Very recently, it has been reported that infiltrated eosinophils showed a protective role in acute liver injury models through cross-talk with macrophages (Xu et al., 2022). Accumulation of eosinophils during liver IRI induced IL-33/ST2–dependent IL-13 secretion, which showed the hepatoprotection against IRI by suppressing neutrophils (Wang et al., 2021b).

IMMUNE-TARGETING THERAPEUTICS FOR LIVER IRI

Ameliorating liver IRI after liver transplantation has been evaluated in preclinical models but so far, there is no consensus for pharmaceutical treatment for preventing or treating liver IRI due to the limited information about the molecular and cellular basis of the disease as well as mixed results on the efficacy. However, technological and conceptual advances continue to improve our understanding of liver IRI, which makes possible to discover new therapeutic strategies.

In recent years, several experimental therapeutic strategies have been proposed to target hepatic immune cells to ameliorate liver IRI. For example, antibiotics pretreatment shows protective effect against liver IRI by altering gut microbiota and α-ketoglutarate levels, which promote macrophage M2 polarization (Lu et al., 2023). A mitochondria-targeted antioxidant peptide SS-31 directly acts on macrophages to inhibit M1 polarization and tissue inflammation (Shang et al., 2021). Curcumin, isolated from turmeric, shows diverse beneficial effects in many diseases, including hepatic IRI (Zhu et al., 2023), particularly on macrophage polarization (Liu et al., 2018). In addition, pharmacological targeting of RNAase activity of endoplasmic reticulum stress sensor in KC reduces unfolded protein response and attenuates inflammatory damage during hepatic IRI (Cai et al., 2022). Furthermore, co-culturing with mesenchymal stem cells restrains M1 macrophage polarization, whereas boosts M2 polarization potentially through the control of mitochondrial homeostasis, attenuating liver IRI (Shang et al., 2023).

In addition to the KC, neutrophils and other immune cells are also suggested for novel therapeutic targets. Reparixin, which acts as allosteric antagonist of the chemokine receptors CXCR1 and CXCR2, targets neutrophil infiltration to suppress liver IRI (de Oliveira et al., 2018). Similarly, formyl-peptide receptor 1 blockade inhibits the accumulation of neutrophils in liver IRI (Honda et al., 2017). Moreover, blocking IL-17A or administration of DNase, a NET inhibitor, decreases neutrophil infiltration, NET formation, and liver necrosis in hepatic IRI (Tohme et al., 2019). Likely, tetramethylpyrazine and platinum nano-antioxidant have shown to inhibit neutrophil

infiltration and activation, and protects liver against IRI (Liu et al., 2022; Mu et al., 2022).

Although therapeutic potential of macrophage- or neutrophil-depletion remains questionable due to their pleiotropic effects at various stages, these preclinical findings have highlighted the potential for immune or inflammation-targeting therapeutics in liver IRI through the deeper understandings on homeostatic regulation of tissue inflammation by diverse immune cells.

CONCLUSION AND PERSPECTIVES

In conclusion, liver IRI poses a significant challenge to organ transplantation and allograft function, emphasizing the need of comprehensive understandings underlying mechanisms for the development of effective therapeutics in liver transplant surgeries. The recruitment of macrophages, DCs, neutrophils, NK cells, NKT cells, and cytotoxic T cells through the activation of inflammatory signaling pathways collectively contribute to the pathogenesis of liver IRI. A holistic perspective that considers the intricate interplay among these immune cells, rather than focusing solely on individual cells or molecules, is crucial for gaining deeper insights. Although the precise mechanisms of liver IRI remain complicated and poorly understood, recent technological advancements, the accumulation of knowledge, and ongoing investigations focusing on these immune cells hold great promise for the development of novel clinical treatment strategies aimed at preventing liver IRI.

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AUTHOR CONTRIBUTIONS

M.J.H. and K.H.K. conceptualized the scope of the review. M.J.H., J.H.S., and K.H.K. researched recent publications. M.J.H. drafted the manuscript. J.H.S., K.L.P., C.J., and K.H.K. revised the manuscript.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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