Tissue Determinants of Antiviral Immunity in the Liver

Gewebespezifische Faktoren zur Regulation der antiviralen Immunität in der Leber

OPEN ACCESS

Authors

Dirk Wohlleber[1], Percy A. Knolle[2, 3]

Affiliations

1 Institute of Molecular Immunology, School of Medicine and Health, Technical University of Munich, Munich, Germany

2 Institute of Molecular Immunology, School of Life Science, Technical University of Munich, Munich, Germany

3 German Center for Infection Research, Munich, Germany

Keywords

Liver, Hepatitis, CD8 T cells, HBV

Schlüsselwörter

Leber, Hepatitis, CD8 T Zellen, HBV

received 4.9.2024

accepted after revision 13.11.2024

Bibliography

Z Gastroenterol 2025; 63: 65–72

DOI 10.1055/a-2365-3900

ISSN 0044-2771

Georg Thieme Verlag KG, Oswald-Hesse-Straße 50, 70469 Stuttgart, Germany

Correspondence

PD Dr. Dirk Wohlleber

Institute of Molecular Immunology, School of Medicine and Health, Technical University of Munich, 80333 Munich, Germany

dirk.wohlleber@tum.de

ABSTRACT

The liver is an organ bearing important metabolic and immune functions. Hepatocytes are the main metabolically active cells of the liver and are the target of infection by hepatotropic viruses. Virus-specific CD8 T cells are essential for the control of hepatocyte infection with hepatotropic viruses but may be subject to local regulation of their effector function. Here, we review our current knowledge of the tissue determinants of antiviral immunity in the liver. Liver Sinusoidal Endothelial Cells (LSECs) not only allow through their fenestrations the access of circulating virus-specific CD8 T cells to engage in direct contact with infected hepatocytes without the need for extravasation but also cross-present viral antigens released from infected hepatocytes to these CD8 T cells. Two important features of LSECs and hepatocytes contribute to antiviral immune surveillance and liver failure. First, CD8 T cell immunity targeting LSECs leads to widespread endothelial cell death and results in sinusoidal microcirculation failure, causing fulminant viral hepatitis, whereas immune-mediated loss of hepatocytes is rapidly compensated by the regenerative capacity of the liver. Second, virus-infected hepatocytes support clearance of infection by responding to TNF, which is released from virus-specific CD8 T cells, with the selective induction of apoptosis. This increased sensitivity for TNF-induced death is caused by reduced mitochondrial resilience in virus-infected hepatocytes and may assist antiviral immunity in preferential targeting of virus-infected hepatocytes. Thus, hepatocytes and LSECs actively contribute to the outcome of antiviral CD8 T cell immunity in the liver. The knowledge of the mechanisms determining CD8 T cell control of hepatotropic viral infection will help to improve strategies to increase antiviral immune surveillance.

ZUSAMMENFASSUNG

Die Leber ist ein Organ mit wichtigen Stoffwechsel- und Immunfunktionen. Hepatozyten, die Parenchymzellen der Leber, sind die stoffwechselaktiven Zellen und das Ziel der Infektion von hepatotropen Viren. Virus-spezifische CD8-T-Zellen sind für die Kontrolle dieser Virus-Infektionen unerlässlich, ihre Effektorfunktion ist jedoch einer lokalen Regulation unterlegen. In diesem Review fassen wir den aktuellen Wissenstand über die Mechanismen der Regulation der Immunantworten zusammen. Sinusoidale Endothelzellen der Leber (LSEC = Liver Sinusoidal Endothelial Cells) ermöglichen durch ihre Fenestrae nicht nur den Zugang zirkulierender virus-spezifischer CD8-T-Zellen zu Hepatozyten, ohne dass eine Extravasation erforderlich ist. Sie kreuz-präsentieren auch virale Antigene, welche von infizierten Hepatozyten sezerniert wurden, an CD8-T-Zellen. Zwei wichtige Eigenschaften von LSEC und Hepatozyten tragen zur Immunüberwachung und zum Leberversagen bei. Erstens führt eine gegen LSEC gerichtete CD8-T-Zell-Immunantwort zu einem Absterben der Endothelzellen und in dessen

Verlauf zu einem Versagen der hepatischen Mikrozirkulation, was zu einer fulminanten Virushepatitis führt, während der immunvermittelte Verlust von Hepatozyten durch die Regenerationsfähigkeit der Leber kompensiert wird. Zweitens unterstützen Virus-infizierte Hepatozyten die Beseitigung der Infektion, indem sie auf TNF, welches von Virus-spezifischen CD8-T-Zellen sezerniert wird, mit der spezifischen Induktion von Apoptose reagieren. Diese erhöhte Sensitivität gegenüber TNF-induziertem Zelltod virusinfizierter Hepatozyten wird durch eine verringerte mitochondriale Resilienz verursacht und kann die Effizienz der antiviralen Immunität fördern. Hepatozyten und LSEC spielen somit eine aktive Rolle während der antiviralen Immunität in der Leber. Eine genaue Kenntnis über die Mechanismen, welche die CD8-T-Zell-Effektorfunktion gegen eine Virusinfektion der Leber regulieren, kann dazu beitragen, Strategien zur Verbesserung der antiviralen Immunität zu verbessern.

The liver as metabolic organ and site of infection by viral pathogens

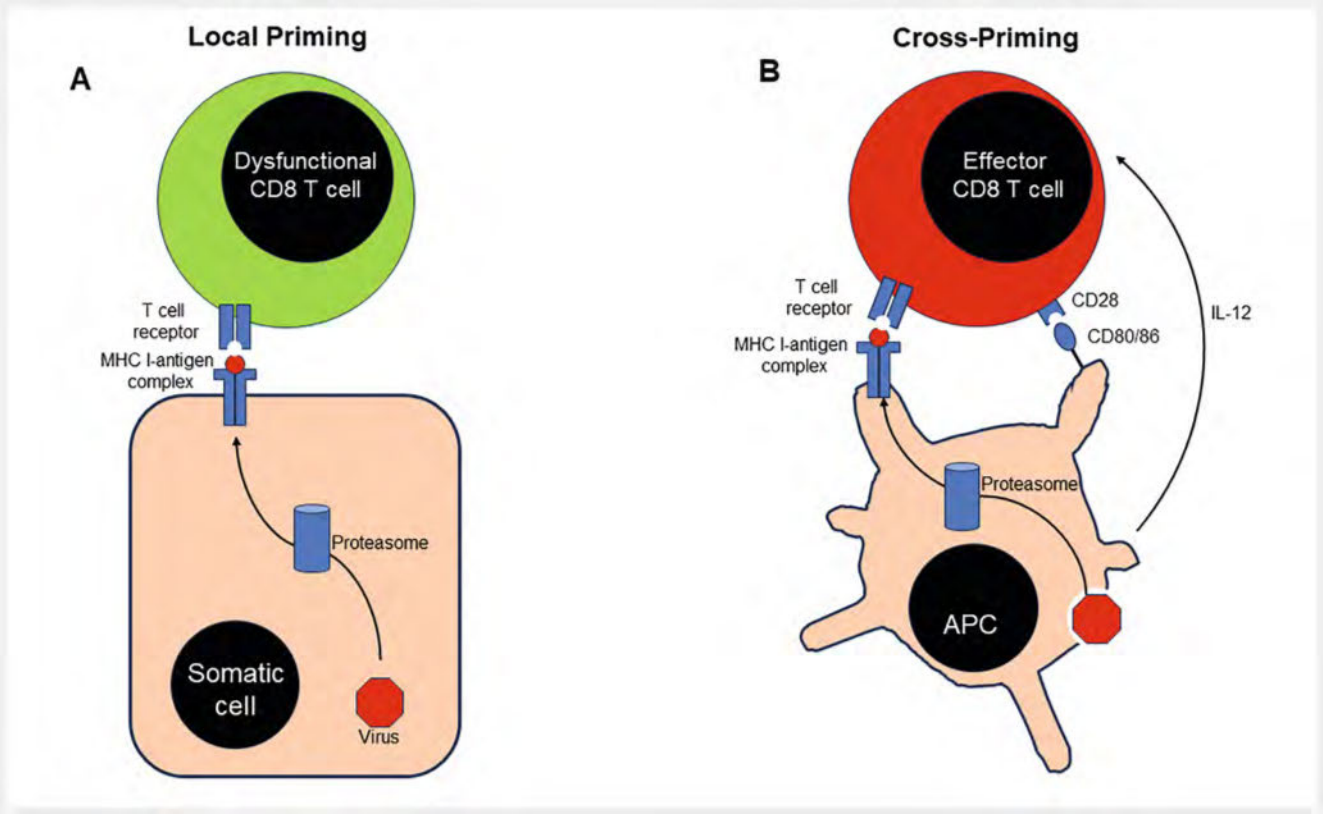
A key task of the liver is to metabolize nutrients from the gut and provide carbohydrates, lipids and amino acids. Nutrients from the gut are transported via the bloodstream of the portal vein to the liver, which is not only low in oxygen tension but also harbors bacterial degradation products. To prevent continuous immune stimulation by toxins and bacterial degradation products that serve as pattern recognition receptor (PRR)-ligands, the liver has evolved numerous strategies to dampen those immune immunostimulatory signals [1, 2, 3]. Besides soluble mediators like transforming growth factor beta (TGF-beta), interleukin-10 (IL-10) and prostaglandins, all cell populations of the liver, i. e. hepatocytes, the parenchymal cells, liver sinusoidal endothelial cells (LSEC), Kupffer cells, the liver resident Macrophages, and hepatic stellate cells (HSC) are capable of shaping immune responses in the liver. This situation, i. e. dampened immune responses combined with a surplus of metabolic products, makes the liver an ideal target for pathogens, especially viruses with hepatotropism, that find ideal conditions for their own replication.

The main viruses that target the liver are so-called hepatitis viruses. There are five known hepatitis viruses, i. e., Hepatitis A virus (HAV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Hepatitis D virus (HDV) and Hepatitis E virus (HEV). These viruses are not genetically related and do not consist of a family of viruses but share the unique feature of hepatotropism. Other viruses, like Cytomegalovirus (CMV), Epstein-Barr-virus (EBV) or adenoviruses (AdV), can also infect the liver, but those infections are less frequent compared to hepatitis virus infections [4, 5, 6]. From the hepatitis viruses, HBV and HCV have been considered the most clinically relevant because of the number of infections and the development of chronic infections leading to liver cirrhosis and hepatocellular carcinoma. Whereas, for chronic hepatitis C, a curative directly acting antiviral therapy exists, chronic hepatitis B can currently only be treated with nucleo(s)tide analogues, suppressing viral replication but not leading to a final cure from infection because of HBV establishes an extrachromosomal persistence form, the cccDNA [7]. It is estimated that one-third of the world's population is infected with HBV during lifetime. This leads currently to approx. 300 million chronic hepatitis B patients worldwide [8, 9]. While most HBV-infected adults clear the infection (90 %) in a CD8 T cell-dependent manner, it is still not entirely known why some patients fail to eradicate the virus [10, 11, 12, 13, 14]. The fact that most adults clear the infection and most chronic HBV cases result from perinatal infection of newborns after birth argues that host factors decide whether an HBV infection is cleared or develops into chronic hepatitis B.

Low efficacy of local priming in the liver for virus-specific CD8 T cell immunity

Protective immunity against intracellular pathogens, like viruses but also intracellular bacteria or parasites, requires activated cytotoxic CD8 T cells [15]. Activation of CD8 T cells needs the presentation of endogenous peptides, which are degraded within the cell in a proteasome-dependent manner and loaded on major histocompatibility complexes I (MHC) in a TAP-dependent manner (▶ **Fig. 1A**). This, however, does not allow the presentation of exogenous antigens on MHC I complexes, which in turn is required to prime CD8 T cells by professional antigen-presenting cells (APC). The process of presenting exogenous antigens on MHC I molecules to induce protective immunity is restricted mainly to APCs and is termed cross-priming [15, 16, 17] (▶ **Fig. 1B**). Cross-priming of CD8 T cells occurs mainly in secondary lymphoid tissue by specialized subsets of dendritic cells (DC). These DCs are characterized by the expression of CD8, CD102 and XCR1 and rely on the transcription factors basic leucine zipper ATF-like transcription factor 3 (BATF3) and interferon regulatory factor 8 (IRF8) [18, 19]. Proper priming of naïve CD8 T cells includes, besides T cell receptor – MHC I interaction, the co-stimulation via CD80/86-CD28 and IL-12 signaling.

Within the liver, the liver sinusoidal endothelial cells (LSEC) represent the most prominent non-parenchymal cell population lining the smallest blood vessels, the sinusoids. LSECs possess an extraordinary scavenger function and can cross-present exogenous antigens to CD8 and CD4 T cells via MHC I and MHC II complexes, respectively [20, 21]. In contrast to professional APCs, LSECs do not express co-stimulatory molecules like CD80/86 or IL-12, which impairs the proper priming of CD4 and CD8 T cells with potent cytotoxic effector function. CD4 T cells rather develop into regulatory CD4 T cells that can suppress organ-specific autoimmunity, highlighting the role of hepatic immune regulation for systemic immune responses [22, 23]. CD8 T cells that are cross-primed by LSEC in the absence of co-stimulation develop into a memory-like state, which can be reactivated by T cell receptor-MHC I interaction and co-stimulation via CD28 and IL-12 sig-



▶ **Fig. 1** Schematic drawing of priming of CD8 T cells. (**A**) Priming of CD8 T cells by somatic cells, like hepatocytes, leads to activation and differentiation into functionally impaired CD8 T cells. (**B**) Cross-priming of CD8 T cells by professional antigen-presenting cells (APC) with exogenous antigen leads and co-stimulatory signals leads to functional maturation into effector CD8 T cells.

nalling [24]. Interestingly, priming of CD8 T cells by virus-infected LSEC leads to a cytotoxic CD8 T cell phenotype in vitro [25]. Thus, antigen cross-presentation by LSEC towards lymphocytes leads to a rather tolerogenic phenotype of immune cells.

Hepatocytes, the functional units of the liver, do not only function as a metabolic powerhouse but do also shape liver-specific immunity. First reports have shown that hepatocytes can prime naive CD8 T cells with the outcome of BCL-2 interacting meditator of cell death (BIM)-dependent cell death [26, 27]. Furthermore, hepatocytes have also been shown to remove CD8 T cells by priming followed by engulfment of those, a process called suicidal emperipolesis [28]. Those events have been reported in the context of the presentation of autoantigens and thereby contribute to immune tolerance induction to prevent auto-immune disease. The role of hepatocyte-specific priming of virus-specific CD8 T cells is getting clearer in recent years. In patients, virus-specific CD8 T cells against HCV or HBV occur between 1–3 months after infection, which is a rather long incubation period compared to other viral infections. This has led to the speculation that HCV-specific CD8 T cells might be primed by HCV-infected hepatocytes rather than by professional antigen-presenting cells [29]. Recent publications have shown that upon HBV infection, virus-specific CD8 T cells can be primed by HBV-infected hepatocytes [14, 30]. Local priming of HBV-specific CD8 T cells leads to proliferation and activation but finally results in dysfunctional CD8 T cells. These dysfunctional CD8 T cells do not respond to classical checkpoint inhibition with anti-PD-L1 antibodies but respond to IL-2 signaling, which might indicate strategies to overcome CD8 T cell dysfunctionality and lead to viral eradication in infected hepatocytes [14]. In contrast, successful antiviral immunity was associated with an influx of HBV-specific cytotoxic CD8 T cells into the liver of HBV-infected chimpanzees, which might be primed in secondary lymphoid tissue [31]. Moreover, regulatory immune cell populations such as myeloid-derived suppressor cells downregulate the function of CD8 T cell immunity locally in the liver [11, 32].

In conclusion, naive virus-specific CD8 T cells can be primed in the liver, which leads, in most cases, to dysfunctional CD8 T cells. An efficient antiviral immunity requires the priming of virus-specific CD8 T cells in secondary lymphoid tissues by professional antigen-presenting cells and the migration of cytotoxic effector CD8 T cells to the infected organ.

The effect of antiviral CD8 T cell immunity on the integrity of the infected liver

In most cases, viral infections of the liver are controlled by the immune system. Effective immune surveillance is believed to result from MHC I-dependent recognition of virus-infected hepatocytes by patrolling cytotoxic CD8 T cells that have been primed in secondary lymphoid organs, as outlined above. The small diameter and the low blood flow in the liver sinusoids facilitate the adhesion

of CD8 T cells to sinusoidal cell populations [33]. Because of these unique conditions, adhesion of CD8 T cells does not require the expression of the otherwise needed selectins [34]. The efficacy of immune surveillance in the liver is enhanced by a unique property of LSECs. While lining the liver's smallest blood vessels, LSECs possess holes with a 50 to 200 nm diameter, so-called fenestrae [33]. As the liver sinusoid has no basal membrane, circulating lymphocytes can directly access and contact underlying hepatocytes through these fenestrae [35]. Thereby, virus-specific CD8 T cells can recognize viral antigens presented on MHC I molecules on hepatocytes while staying within the sinusoidal vascular lumen without the requirement of transmigration across the layer of sinusoidal cells. It is important to note that these interactions do not require local inflammation, which is of special interest, as, for example, HBV infection does not induce any inflammation during viral infection or replication [36]. During the antiviral immune response in HBV infection, cytotoxic CD8 T cells attach to platelet aggregates forming in the liver sinusoids and are then triggered to perform effector function, i. e. killing of HBV-infected hepatocytes through their cellular protrusions reaching through the fenestrae [12].

Whereas the outcome of the interaction of LSECs with naive lymphocytes has been described before to result in a memory-like CD8 T cell phenotype, the effects of the interaction of LSEC with already differentiated cytotoxic CD8 T cells differ substantially. In an adenoviral model of liver infection, LSECs have been demonstrated to take up and cross-present hepatocyte-derived viral antigens to cytotoxic CD8 T cells. Thus, CD8 T cells may not only recognize their cognate antigen on virus-infected hepatocytes but also on LSECs cross-presenting viral antigens released from infected hepatocytes. This may serve to expand the possibility of virus-specific CD8 T cells to achieve immune control of viral infection in the liver. Since LSECs serve as a physical platform for CD8 T cells to adhere in the liver sinusoids, circulating antigen-specific CD8 T cells easily engage with antigen-presenting LSECs. Such antigen-specific activation of effector CD8 T cells by cross-presenting LSECs leads to the induction of effector function and, consequently, to the killing of the antigen-presenting LSECs.

Dissecting the relevance of antigen presentation by infected hepatocytes as compared to cross-presenting LSECs revealed a very different outcome. CD8 T cells recognizing their antigen on virus-infected hepatocytes caused liver damage through the elimination of infected hepatocytes and contributed to the control of viral infection. Even at very high numbers of antigen-specific CD8 T cells in combination with infection of most hepatocytes, antiviral immunity leads only to transient liver damage followed by control of infection. These data from a preclinical model of hepatotropic infection do not reflect the situation observed during fulminant infection with hepatotropic viruses, such as HAV and HBV, where liver failure is observed in the presence of high numbers of virus-specific CD8 T cells. However, when antigen recognition in the preclinical model of hepatotropic viral infection is restricted to cross-presenting LSECs, fulminant liver failure develops. Notably, fulminant liver failure from CD8 T cells recognizing their cognate antigen on LSECs cross-presenting viral antigens from infected hepatocytes is caused by widespread failure of sinusoidal blood flow [37]. Sinusoidal perfusion failure is only detected when high numbers of antigen-specific CD8 T cells are present and most hepatocytes are infected. Damage to endothelial cells is known to result in thrombosis causing tissue damage. The widespread ramifications of the vascular sinusoidal network of the liver may operate to prevent devastating microvascular perfusion failure. In the presence of a high antigen load with most hepatocytes being infected in combination with a high number of antigen-specific CD8 T cells, however, the killing of cross-presenting LSECs may become a widespread event and lead to a critical microvascular perfusion failure. Since a functioning blood flow is required for the proverbial regeneration potential of the liver, immune-mediated sinusoidal perfusion failure may explain the sudden loss of liver regeneration during fulminant viral hepatitis. While cutting off the blood supply from areas where hepatocytes are infected may provide a means to contain the infection, it comes at the risk of critical liver damage. Notably, the selective killing of virus-infected hepatocytes by antigen-specific CD8 T cells does not elicit fulminant liver failure [37], indicating that hepatocyte-directed immunity does not impair liver regeneration because it does not interfere with sinusoidal blood perfusion.

These insights into the effects of CD8 T cell immunity in the virus-infected liver demonstrate that the target cell population attacked by virus-specific CD8 T cells in the liver has a key influence on the outcome and liver integrity during antiviral immunity. Identifying the mechanisms responsible for critical immune-mediated liver damage may be important for designing and developing future therapies to prevent fulminant liver damage in patients with hepatotropic viral infections.

Amplification of antiviral CD8 T cell immunity by infected hepatocytes

The number of virus-specific CD8 T cells in the liver increases after infection of hepatocytes. Numbers can be as high as 10^6 virus-specific CD8 T cells per g liver tissue in preclinical models of hepatotropic virus infection [38]. However, the number of hepatocytes infected by hepatotropic viruses in such preclinical models is in the range of 10^7–10^9 per g of liver tissue [38]. This raises the question of how specific CD8 T cells can control infection in the liver if they are outnumbered by a factor of 100 to 1.000 by infected hepatocytes. CD8 T cells have been demonstrated to be capable of serial killing of target cells [39]. However, hepatocytes are large cells that may require several CD8 T cells to achieve killing [40], pointing towards mechanisms that may help virus-specific CD8 T cells to kill virus-infected hepatocytes.

We have recently discovered that virus-infected hepatocytes display a unique sensitivity to undergo apoptotic cell death and that this unique death sensitivity helps virus-specific CD8 T cells to selectively eliminate infected hepatocytes. Effector CD8 T cells that are activated through their T cell receptor while recognizing their cognate antigen on virus-derived peptides in the context of MHC I molecules on the surface of hepatocytes are not only triggered to execute effector functions to kill their target cell but at the same time also release effector cytokines like tumor necrosis factor (TNF) and Interferon-gamma. Notwithstanding the important role of Interferon-gamma in improving the cytotoxic effector

function of CD8 T cells [41], it does not have a direct death-inducing effect on virus-infected hepatocytes. In contrast, TNF released from activated virus-specific CD8 T cells binds to TNF receptor 1 (TNFR1) on hepatocytes, and this TNFR1 stimulation leads to pro-apoptotic cell death signaling selectively in virus-infected hepatocytes, whereas in non-infected hepatocytes, TNFR1 stimulation only leads to pro-survival NF-kB-signalling [42].

Cross-presentation of viral antigens, which were initially released from hepatocytes, on MHC I molecules by LSEC and subsequent activation of virus-specific CD8 T cells can thus induce the killing of virus-infected hepatocytes, which we termed non-canonical CD8 T cell effector function [43]. Such killing by effector CD8 T cell can occur in the absence of MHC I-restricted activation by the target cell [43] and, therefore, may also operate in situations where virus-infection leads to downregulation of MHC I molecules, as observed for DNA viruses like herpes virus [44]. Interestingly, the cross-presentation of viral antigens by LSECs and the consequent release of TNF from effector CD8 T cells is not involved in fulminant liver damage [37]. Rather, very high doses of TNF fail to cause liver failure even when more than 80 % of hepatocytes are infected with a hepatotropic virus. This all points towards an important role of TNF in the immunosurveillance against virus infection in hepatocytes that does not threaten liver integrity.

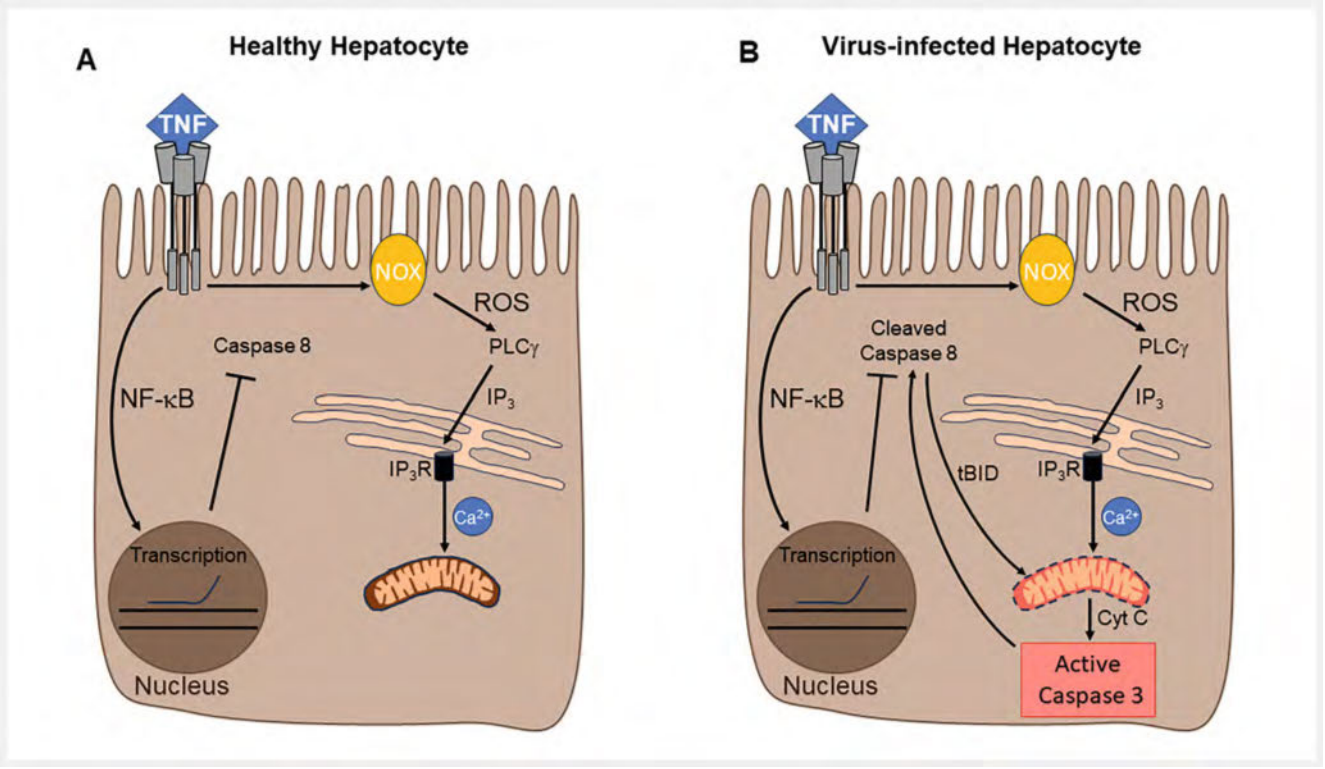
Cell-type specific TNF receptor gene knockout in hepatocytes and TNF knockout in CD8 T cells revealed that the non-canonical CD8 T cell effector function accounts for at least 50 % of the total antiviral CD8 T cell immunity in the liver. This likely increases the efficiency of antiviral immune surveillance, as few CD8 T cells that release high amounts of TNF can induce death in several virus-infected hepatocytes. This mechanism may also counteract viral immune escape strategies, in which viruses interfere with the presentation of viral degradation products on MHC I molecules to prevent the recognition of infected hepatocytes by effector CD8 T cells, as outlined above. The non-canonical CD8 T cell effector function remains operative under these conditions of viral immune escape as the cross-presenting LSEC are not infected themselves. Moreover, since virus infection often spreads from one cell to another, the local death-inducing effect in virus-infected hepatocytes from TNF released by effector CD8 T cells may serve to contain spots of infection in neighboring hepatocytes. Because of the particular features of the replication-deficient hepatotropic viruses used in the preclinical models to discover the non-canonical CD8 T cell effector function, the impact of TNF on the overall antiviral CD8 T cell immunity is probably an underestimation because TNF is likely acting to control the spread of replicating virus from one hepatocyte to the next in the liver. TNF is known to induce activation of immune cells and may, theoretically, contribute to increased killing of hepatocytes by strengthening CD8 T cell responses. However, TNF also induced death in virus-infected hepatocytes in the absence of immune cells [42], suggesting that cell-intrinsic mechanisms were responsible for the increased sensitivity to TNF-induced death. Therefore, the non-canonical effector function of CD8 T cells that exploits the unique sensitivity of virus-infected hepatocytes to undergo TNF-induced apoptosis is presumably highly relevant for antiviral immune surveillance in the liver.

Molecular determinants of increased sensitivity for TNF-induced apoptosis in virus-infected hepatocytes

The killing of parenchymal cells may not be easy for effector CD8 T cells because target cells defend themselves from induction of cell death through endosomal sorting complexes required for transport (ESCRT)-mediated membrane repair [45, 46]. Increasing the ability of virus-infected hepatocytes to respond to death-inducing signals, therefore, bears the promise to overcome such inhibitory mechanisms and increase CD8 T cell-mediated immune surveillance. But how could a virus-infected hepatocyte become more susceptible to death-inducing signals via the TNF receptor?

TNF receptor signaling is known to induce apoptosis in the absence of pro-survival NF-kB signalling [47]. Yet, NF-kB signaling is equally present in healthy as well as virus-infected hepatocytes, and TNF receptor levels are not changed after infection [42] (▶ **Fig. 2A+B**). Moreover, signaling downstream of the membrane-proximal TNF receptor signaling complex that involves caspase 8 activation is dispensable for the increased sensitivity for apoptosis induction in virus-infected hepatocytes [42]. In viral infections, induction of type I Interferon by immune sensors such as toll-like receptors or cytosolic immune sensors detecting nucleic acids is an essential part of antiviral immunity [48]. However, neither these immune sensory receptors nor Interferons are involved in the increased sensitivity of virus-infected hepatocytes to TNF-induced death. Many forms of cell death have been identified [49], but TNF-induced death in virus-infected hepatocytes occurred exclusively by apoptosis and not by other forms of cell death such as pyroptosis, necroptosis, ferroptosis or oxeiptosis [42]. Together, these results pointed towards a direct involvement of mitochondria in the increased sensitivity of virus-infected hepatocytes to TNF-induced cell death by apoptosis.

Indeed, a comprehensive analysis of virus-infected hepatocytes identified mitochondria as the central hubs within infected hepatocytes that interconnected metabolic changes from virus replication with apoptotic signaling downstream of the TNF receptor [42]. Hepatocytes contain more than 1000 mitochondria to secure the energy supply for their high metabolic demands [50], and require the involvement of mitochondria for the induction of apoptosis [51]. However, the canonical pathway downstream of TNF receptor signaling involving caspase 8 activation, cleavage of BH3 interacting-domain death agonist (BID) and activation of BCL2 associated X protein (BAX), which together lead to pore-formation in mitochondria, to the release of cytochrome C and finally to the activation of the caspase 3 is dispensable for TNF-induced death in virus-infected hepatocytes. Rather, the differential outcome of TNF receptor signaling results from a previously unknown non-canonical death pathway. Analysis of hepatocyte mitochondria at the single organelle level revealed subtle changes after infection, in particular, a lower membrane potential [52]. Most prominently, however, is the markedly reduced capacity for calcium storage capacity and, resulting from this, an increased vulnerability towards calcium challenges of mitochondria in virus-infected hepatocytes. Mechanistically, reactive oxygen species (ROS) signaling associated with membrane-proximal TNF receptor signaling is associated with the induction of mitochondrial



▶ **Fig. 2** Mechanism of TNF induced cell death in virus-infected hepatocytes. (**A**) Stimulation of virus-infected hepatocytes by TNF leads to triggering of TNF receptor 1 (TNFR1) leading to activation of NF-kB that will lead to transcription of pro-survival genes, blocking e. g. caspase 8 activation. In parallel, TNFR1 stimulation leads to NADPH-dependent reactive oxygen species (ROS) formation that activates Phospholipase C gamma thereby producing IP3. IP3 triggers the IP3 receptor in the endoplasmic reticulum (ER) leading to release of calcium ions that are taken up and stored by mitochondria. (**B**) Virus infection of hepatocytes leads to reduced mitochondrial stress resilience with regards to calcium signaling. Calcium released from the ER triggers mitochondrial permeability transition leading to the release of mitochondrial cytochrome C that activates caspase 3. Caspase 8 might be activated by cleavage in a feedback loop by caspase 3 leading to enhanced apoptosis signaling.

permeability transition in virus-infected hepatocytes [42]. The most likely explanation for this is that increased TNF receptor signaling leads to membrane-proximal ROS production from NADPH oxidase that, in turn, causes the release of calcium from the endoplasmic reticulum, which is normally taken up and buffered by mitochondria. The loss of mitochondrial resilience to such a calcium challenge in virus-infected hepatocytes then triggers a process called mitochondrial permeability transition that leads to the release of mitochondrial constituents into the cytosol, induction of caspase activation and execution of apoptosis (▶ **Fig. 2B**). Notwithstanding the many recognized forms of mitochondrial calcium uptake and storage [53, 54, 55], we still lack a mechanistic understanding of how viral infection of hepatocytes causes the loss of mitochondrial resilience to calcium challenge. This loss of mitochondrial resilience, which is responsible for a distinct outcome of an otherwise unchanged TNF receptor signaling pathway, may constitute a unique feature of hepatocytes to mount cell-intrinsic antiviral immunity in close cooperation with antiviral CD8 T cells.

Conclusions

Antiviral CD8 T cell immunity is key for the control of infection with most hepatotropic viruses but may also cause liver damage and even fulminant viral hepatitis in rare cases. Although antiviral CD8 T cell immunity is directed against virus-infected hepatocytes, cross-presentation of viral antigens released from infected hepatocytes through LSECs is observed in preclinical models of hepatotropic virus infection and leads to the activation of virus-specific effector CD8 T cells. The target cell population of CD8 T cell immunity determines whether liver integrity is preserved with loss of sinusoidal blood perfusion resulting from CD8 T cell-mediated killing of LSECs as the main mechanism of fulminant viral hepatitis, whereas immune-mediated loss of hepatocytes is rapidly compensated by the regenerative capacity of the liver. Hepatocytes support the function of antiviral CD8 T cells by becoming sensitive to induction of apoptotic cell death from TNF receptor signaling. Together, these insights demonstrate the intricate cooperation of liver cell populations in the control of viral infection of hepatocytes.

Funding Information

Deutsche Forschungsgemeinschaft (272983813 - TRR179) | http://dx.doi.org/10.13039/501100001659

Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Knolle PA, Gerken G. Local control of the immune response in the liver. Immunol Rev 2000; 174: 21–34

[2] Thomson AW, Knolle PA. Antigen-presenting cell function in the tolerogenic liver environment. Nat Rev Immunol 2010; 10: 753–766. doi:10.1038/nri2858

[3] Crispe IN. The liver as a lymphoid organ. Annu Rev Immunol 2009; 27: 147–163. doi:10.1146/annurev.immunol.021908.132629

[4] Gupta P, Suryadevara M, Das A. Cytomegalovirus-induced hepatitis in an immunocompetent patient. Am J Case Rep 2014; 15: 447–449. doi:10.12659/AJCR.890945

[5] Mendez-Sanchez N, Aguilar-Dominguez C, Chavez-Tapia NC et al. Hepatic manifestations of Epstein-Barr viral infection. Ann Hepatol 2005; 4: 205–209

[6] Wang WH, Wang HL. Fulminant adenovirus hepatitis following bone marrow transplantation. A case report and brief review of the literature. Arch Pathol Lab Med 2003; 127: e246–248. doi:10.5858/2003-127-e246-FAHFBM

[7] Gane EJ et al. Nucleotide polymerase inhibitor sofosbuvir plus ribavirin for hepatitis C. N Engl J Med 2013; 368: 34–44. doi:10.1056/NEJMoa1208953

[8] Wiktor SZ, Hutin YJ. The global burden of viral hepatitis: better estimates to guide hepatitis elimination efforts. Lancet 2016; 388: 1030–1031. doi:10.1016/S0140-6736(16)31018-2

[9] Thomas DL. Global Elimination of Chronic Hepatitis. N Engl J Med 2019; 380: 2041–2050. doi:10.1056/NEJMra1810477

[10] Thimme R et al. CD8(+) T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. J Virol 2003; 77: 68–76

[11] Pallett LJ et al. Metabolic regulation of hepatitis B immunopathology by myeloid-derived suppressor cells. Nat Med 2015; 21: 591–600. doi:10.1038/nm.3856

[12] Guidotti LG et al. Immunosurveillance of the liver by intravascular effector CD8(+) T cells. Cell 2015; 161: 486–500. doi:10.1016/j.cell.2015.03.005

[13] Fisicaro P et al. Targeting mitochondrial dysfunction can restore antiviral activity of exhausted HBV-specific CD8 T cells in chronic hepatitis B. Nat Med 2017; 23: 327–336. doi:10.1038/nm.4275

[14] Benechet AP et al. Dynamics and genomic landscape of CD8(+) T cells undergoing hepatic priming. Nature 2019; 574: 200–205. doi:10.1038/s41586-019-1620-6

[15] Kurts C, Robinson BW, Knolle PA. Cross-priming in health and disease. Nat Rev Immunol 2010; 10: 403–414. doi:10.1038/nri2780

[16] Kurts C, Kosaka H, Carbone FR et al. Class I-restricted cross-presentation of exogenous self-antigens leads to deletion of autoreactive CD8(+) T cells. J Exp Med 1997; 186: 239–245. doi:10.1084/jem.186.2.239

[17] Jung S et al. In vivo depletion of CD11c+ dendritic cells abrogates priming of CD8 + T cells by exogenous cell-associated antigens. Immunity 2002; 17: 211–220. doi:10.1016/s1074-7613(02)00365-5

[18] Hildner K et al. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. Science 2008; 322: 1097–1100. doi:10.1126/science.1164206

[19] Eickhoff S et al. Robust Anti-viral Immunity Requires Multiple Distinct T Cell-Dendritic Cell Interactions. Cell 2015; 162: 1322–1337. doi:10.1016/j.cell.2015.08.004

[20] Sorensen KK et al. The scavenger endothelial cell: a new player in homeostasis and immunity. Am J Physiol Regul Integr Comp Physiol 2012; 303: R1217–1230. doi:10.1152/ajpregu.00686.2011

[21] Wittlich M et al. Liver sinusoidal endothelial cell cross-priming is supported by CD4 T cell-derived IL-2. J Hepatol 2017; 66: 978–986. doi:10.1016/j.jhep.2016.12.015

[22] Carambia A et al. Inhibition of inflammatory CD4 T cell activity by murine liver sinusoidal endothelial cells. J Hepatol 2013; 58: 112–118. doi:10.1016/j.jhep.2012.09.008

[23] Carambia A et al. Nanoparticle-based autoantigen delivery to Treg-inducing liver sinusoidal endothelial cells enables control of autoimmunity in mice. J Hepatol 2015; 62: 1349–1356. doi:10.1016/j.jhep.2015.01.006

[24] Bottcher JP et al. Liver-primed memory T cells generated under noninflammatory conditions provide anti-infectious immunity. Cell reports 2013; 3: 779–795. doi:10.1016/j.celrep.2013.02.008

[25] Kern M et al. Virally infected mouse liver endothelial cells trigger CD8 + T-cell immunity. Gastroenterology 2010; 138: 336–346. doi:10.1053/j.gastro.2009.08.057

[26] Bertolino P, Trescol-Biemont MC, Rabourdin-Combe C. Hepatocytes induce functional activation of naive CD8 + T lymphocytes but fail to promote survival. Eur J Immunol 1998; 28: 221–236. doi:10.1002/(SICI)1521-4141(199801)28:013.0.CO;2-F

[27] Holz LE et al. Intrahepatic murine CD8 T-cell activation associates with a distinct phenotype leading to Bim-dependent death. Gastroenterology 2008; 135: 989–997

[28] Benseler V et al. Hepatocyte entry leads to degradation of autoreactive CD8 T cells. Proc Natl Acad Sci U S A 2011; 108 (40): 16735–16740. doi:10.1073/pnas.1112251108

[29] Holz LE, Warren A, Le Couteur DG et al. CD8 + T cell tolerance following antigen recognition on hepatocytes. Journal of autoimmunity 2010; 34: 15–22. doi:10.1016/j.jaut.2009.08.005

[30] De Simone G et al. Identification of a Kupffer cell subset capable of reverting the T cell dysfunction induced by hepatocellular priming. Immunity 2021; 54: 2089–2100 e2088. doi:10.1016/j.immuni.2021.05.005

[31] Wieland SF, Spangenberg HC, Thimme R et al. Expansion and contraction of the hepatitis B virus transcriptional template in infected chimpanzees. Proc Natl Acad Sci U S A 2004; 101: 2129–2134

[32] Baumann T et al. Regulatory myeloid cells paralyze T cells through cell–cell transfer of the metabolite methylglyoxal. Nature Immunology 2020; 21: 555–566

[33] Braet F, Wisse E. Structural and functional aspects of liver sinusoidal endothelial cell fenestrae: a review. Comp Hepatol 2002; 1: 1. doi:10.1186/1476-5926-1-1

[34] Wong J et al. A minimal role for selectins in the recruitment of leukocytes into the inflamed liver microvasculature. J Clin Invest 1997; 99: 2782–2790

[35] Warren A et al. T lymphocytes interact with hepatocytes through fenestrations in murine liver sinusoidal endothelial cells. Hepatology (Baltimore, Md) 2006; 44: 1182–1190

[36] Wieland SF, Chisari FV. Stealth and cunning: hepatitis B and hepatitis C viruses. J Virol 2005; 79: 9369–9380. doi:10.1128/JVI.79.15.9369-9380.2005

[37] Welz M et al. Perforin inhibition protects from lethal endothelial damage during fulminant viral hepatitis. Nat Commun 2018; 9: 4805

[38] Manske K et al. In Vivo Bioluminescence Imaging of HBV Replicating Hepatocytes Allows for the Monitoring of Anti-Viral Immunity. Viruses 2021; 13 (11): 2273. doi:10.3390/v13112273

[39] Weigelin B et al. Cytotoxic T cells are able to efficiently eliminate cancer cells by additive cytotoxicity. Nature Communications 2021; 12: 5217. doi:10.1038/s41467-021-25282-3

[40] Halle S, Halle O, Förster R. Mechanisms and Dynamics of T Cell-Mediated Cytotoxicity In Vivo. Trends in Immunology 2017; 38: 432–443. doi:10.1016/j.it.2017.04.002

[41] Zhang N, Bevan MJ. CD8 + T Cells: Foot Soldiers of the Immune System. Immunity 2011; 35: 161–168. doi:10.1016/j.immuni.2011.07.010

[42] Lampl S et al. Reduced mitochondrial resilience enables non-canonical induction of apoptosis after TNF receptor signaling in virus-infected hepatocytes. J Hepatol 2020; 73: 1347–1359. doi:10.1016/j.jhep.2020.06.026

[43] Wohlleber D et al. TNF-induced target cell killing by CTL activated through cross-presentation. Cell reports 2012; 2: 478–487

[44] Gainey MD, Rivenbark JG, Cho H et al. Viral MHC class I inhibition evades CD8 + T-cell effector responses in vivo but not CD8 + T-cell priming. Proceedings of the National Academy of Sciences 2012; 109: E3260–E3267. doi:10.1073/pnas.1217111109

[45] Gong Y-N et al. ESCRT-III Acts Downstream of MLKL to Regulate Necroptotic Cell Death and Its Consequences. Cell 2017; 169: 286–300. e216

[46] Ritter AT et al. ESCRT-mediated membrane repair protects tumor-derived cells against T cell attack. Science 2022; 376: 377–382. doi:10.1126/science.abl3855

[47] Plümpe J et al. NF-κB determines between apoptosis and proliferation in hepatocytes during liver regeneration. American Journal of Physiology-Gastrointestinal and Liver Physiology 2000; 278: G173–G183

[48] Tan X, Sun L, Chen J et al. Detection of Microbial Infections Through Innate Immune Sensing of Nucleic Acids. Annual Review of Microbiology 2018; 72: 447–478

[49] Newton K, Strasser A, Kayagaki N et al. Cell death. Cell 2024; 187: 235–256. doi:10.1016/j.cell.2023.11.044

[50] Wiesner RJ, Ruegg JC, Morano I. Counting target molecules by exponential polymerase chain reaction: copy number of mitochondrial DNA in rat tissues. Biochem Biophys Res Commun 1992; 183: 553–559. doi:10.1016/0006-291x(92)90517-o

[51] Jost PJ et al. XIAP discriminates between type I and type II FAS-induced apoptosis. Nature 2009; 460: 1035–1039

[52] Schneider A et al. Single organelle analysis to characterize mitochondrial function and crosstalk during viral infection. Sci Rep 2019; 9: 8492

[53] De Stefani D, Rizzuto R, Pozzan T. Enjoy the Trip: Calcium in Mitochondria Back and Forth. Annu Rev Biochem 2016; 85: 161–192. doi:10.1146/annurev-biochem-060614-034216

[54] Giorgi C, Marchi S, Pinton P. The machineries, regulation and cellular functions of mitochondrial calcium. Nat Rev Mol Cell Biol 2018; 19: 713–730. doi:10.1038/s41580-018-0052-8

[55] Baughman JM et al. Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. Nature 2011; 476: 341–345. doi:10.1038/nature10234